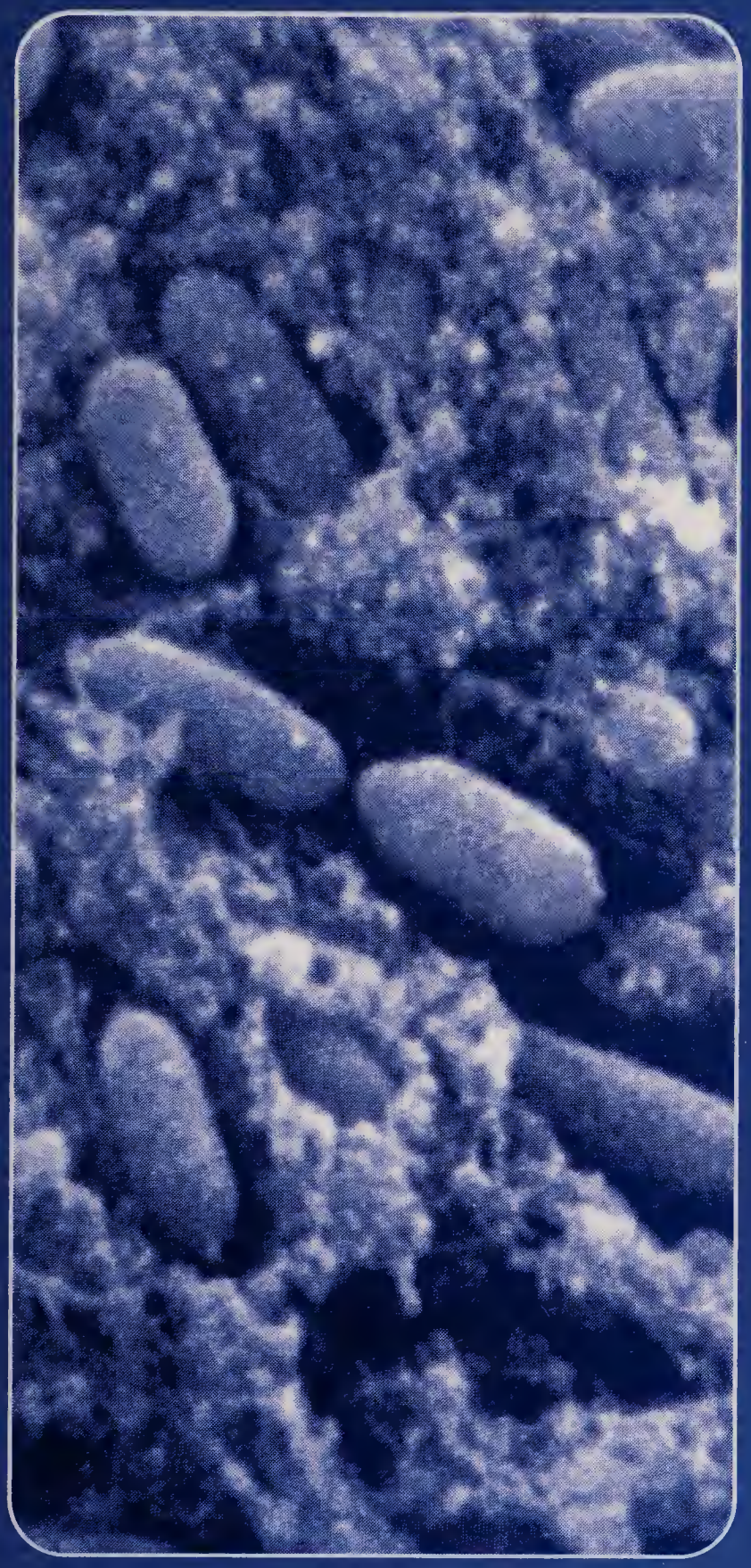
SECOND EDITION

TOXICOLOGICAL CHEMISTRY



- Chemistry of Life Processes
- Environmental Biochemistry
- Microbial Processes

STANLEY E. MANAHAN

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Cover Illustration: Electron micrograph of bacteria on the inner surface of a high density polyethylene milk container impregnated with starch to enhance biodegradability after disposal. Illustration courtesy of Dr. Richard E. Mueller, Research Scientist, Bioprocessing Laboratory, Department of Agricultural Engineering, University of Missouri, Columbia, MO.

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Stanley E. Manahan is Professor of Chemistry at the University of Missouri – Columbia, where he has been on the faculty since 1965. He received his A.B. in chemistry from Emporia State University in 1960 and his Ph.D. in analytical chemistry from the University of Kansas in 1965. Since 1968 his primary research and professional activities have been in environmental chemistry and have included development of methods for the chemical analysis of pollutant species, environmental aspects of coal conversion processes, development of coal products useful for pollutant control, hazardous waste treatment, and toxicological chemistry. He teaches courses on environmental chemistry, hazardous wastes, toxicological chemistry, and analytical chemistry and has lectured on these topics throughout the U.S. as an American Chemical Society Local Section tour speaker. He is also President of ChemChar Research, Inc., a firm working with the development of non-incinerative thermochemical and electrothermochemical treatment of mixed hazardous substances containing refractory organic compounds and heavy metals.

Professor Manahan has written several books. The first of these, *Environmental Chemistry*, has been in print continuously since 1972 and is now in its fifth edition (*Environmental Chemistry*, 5th ed., 1991, Lewis Publishers Inc.). Other books by the author include works on hazardous wastes (*Hazardous Waste Chemistry, Toxicology and Treatment*, 1990, Lewis Publishers Inc.), general chemistry from an environmental perspective (*Environmental Chemistry Fundamentals*, 1992, Lewis Publishers, Inc.), applied chemistry, and quantitative chemical analysis. He has been the author or co-author of approximately 70 research articles.

CONTENTS

PREFACE

The first edition of *Toxicological Chemistry* (1989) was written to bridge the gap between toxicology and chemistry. It defined toxicological chemistry as the science which deals with the chemical nature and reactions of toxic substances; their origins and uses; and the chemical aspects of their exposure, transformation, and elimination by biological systems. It emphasized the chemical formulas, structures, and reactions of toxic substances. The present edition of the book, *Toxicological Chemistry*, second edition, has been approximately doubled in size and significantly increased in scope. It now addresses the topic of environmental biochemistry, which pertains to the effects of environmental chemical substances upon living systems and the influence of life forms on such chemicals. It does so within a framework of environmental chemistry, defined as that branch of chemistry that deals with the origins, transport, reactions, effects, and fates of chemical species in the water, air, terrestrial, and living environments.

Toxicological Chemistry, second edition, has been designed to be useful to a wide spectrum of readers with various interests and a broad range of backgrounds in chemistry. For readers who have had very little exposure to chemistry, Chapter 1, "Chemistry and Organic Chemistry," outlines the basic concepts of general chemistry and organic chemistry needed to understand the rest of the material in the book. The second chapter, "Environmental Chemistry," is an overview of that topic presented so that the reader may understand the remainder of the material in the book within a framework of environmental chemistry. Chapter 3, "Biochemistry," gives the fundamentals of the chemistry of life processes essential to understanding toxicological chemistry and biochemistry. The next four chapters emphasize biochemical processes of environmental chemicals as they occur in living organisms (Chapter 4, "Metabolic Processes"), in microorganisms (Chapter 5, "Microbial Processes"), as biodegradation and biouptake processes (Chapter 6, "Biodegradation and Bioaccumulation") and in the water and soil environments (Chapter 7, "Aquatic and Soil Environmental Biochemistry"). The remainder of the book addresses toxicological chemistry in the same way as was done in the first edition. Chapter 8, "Toxicology," defines and explains toxicology as the science of poisons. Chapter 9, "Toxicological Chemistry," bridges the gap between toxicology and chemistry, emphasizing chemical aspects of toxicological phenomena, including fates and effects of xenobiotic chemicals in living systems. Chapters 10-18 discuss toxicological chemistry within an organizational structure based on classes of chemical substances, and Chapter 19 deals with toxicants from natural sources.

This book is designed to be both a textbook and a general reference book. Questions at the end of each chapter are written to summarize and review the material in the chapter. Literature citations are given on specific points covered in each chapter and supplementary references are cited at the end of each chapter for additional reading about the topics covered.

ACKNOWLEDGMENTS

The author gratefully acknowledges the assistance of Vivian Collier, Kathy Walters, and the rest of the staff of Lewis Publishers for their excellent collaboration in preparing this book. Their unfailing helpfulness and courtesy have contributed substantially to the satisfaction gained in writing this book and other titles published by Lewis Publishers. The assistance of Anne F. Manahan in producing this work is deeply appreciated. Bunny the Beagle also did her part by insisting on invigorating walks after a long day's writing.

The author appreciates the efforts of those reviewers who have carefully and constructively critiqued the book and would very much like to receive copies of their reviews. Feedback in the form of comments and suggestions from users is especially helpful and may be directed to the author at 123 Chemistry Building, University of Missouri, Columbia, Missouri 65211 U.S.A.

SECOND EDITION

TOXICOLOGICAL CHEMISTRY

1

Chemistry and Organic Chemistry

1.1. INTRODUCTION

Chemistry is defined as the science of matter. Therefore, it deals with the air that living organisms breathe, the water they drink, the soil that grows their food, and vital life substances and processes. The cells, organs, and tissues of all living things contain a vast variety of chemical substances and are tremendously sophisticated chemical factories that carry out an incredible number of complex chemical processes.

There is concern today about the uses—and particularly the misuses—of chemistry as it relates to the environment. Ongoing events serve as constant reminders of threats to the environment ranging from individual exposures to toxicants to phenomena on a global scale that may cause massive, perhaps catastrophic, alterations in climate. These include, as examples, alarm over evidence of stratospheric ozone loss greater than previously anticipated, massive petroleum fires in Kuwait in 1991, and air quality in Mexico City so bad that it threatens human health. Furthermore, increasing numbers of employees are involved with dealing with hazardous substances and wastes in laboratories and the workplace.

Problems such as those listed above involve **environmental chemistry**, defined as that branch of chemistry that deals with the origins, transport, reactions, effects, and fates of chemical species in the water, air, terrestrial, and living environments.[1] In addition to its being an essential, vital discipline in its own right, environmental chemistry provides an excellent framework for the study of chemistry. This is because it cuts across all subdivisions of chemistry. In addition to what is commonly regarded as "general chemistry," environmental chemistry must deal with organic chemistry, chemical analysis, physical chemistry, photochemistry, geochemistry, and biological chemistry. By necessity it breaks down the barriers that tend to compartmentalize chemistry as it is conventionally addressed.

This book deals primarily with that particular aspect of environmental chemistry that pertains to the effects of environmental chemical substances upon living systems and the influence of life forms on such chemicals. Such mutual interactions define the subdiscipline of **environmental biochemistry**. The book also addresses the closely related area of **toxicological chemistry**, defined as the chemistry of toxic substances with emphasis upon their interactions with biologic tissue and living systems.

Although many readers will have a good familiarity with basic chemistry, organic chemistry, environmental chemistry and biochemistry, others may not. Therefore, these areas are addressed in the first three chapters. Chapter 1 presents a brief overview of general chemistry and organic chemistry.

1.2. ELEMENTS

All substances are composed of only about a hundred fundamental kinds of matter called **elements**. Elements, themselves, may be of environmental concern. The "heavy metals," including lead, cadmium, and mercury, are well recognized as toxic substances in the environment. Elemental forms of otherwise essential elements may be very toxic or cause environmental damage. Oxygen in the form of ozone, O_3, is the agent most commonly associated with atmospheric smog pollution and is very toxic to plants and animals. Elemental white phosphorus is highly flammable and toxic.

Each element is made up of very small entities called **atoms**; all atoms of the same element behave identically chemically. The study of chemistry, therefore, can logically begin with elements and the atoms of which they are composed. Each element is designated by an atomic number, a name, and a **chemical symbol**, such as carbon, C; potassium, K (for its Latin name kalium); or cadmium, Cd. Each element has a characteristic **atomic mass** (atomic weight), which is the average mass of all atoms of the element.

Subatomic Particles and Atoms

Figure 1.1 represents an atom of deuterium, a form of the element hydrogen. It is seen that such an atom is made up of even smaller **subatomic particles** — positively charged **protons**, negatively charged **electrons**, and uncharged (neutral) **neutrons**.

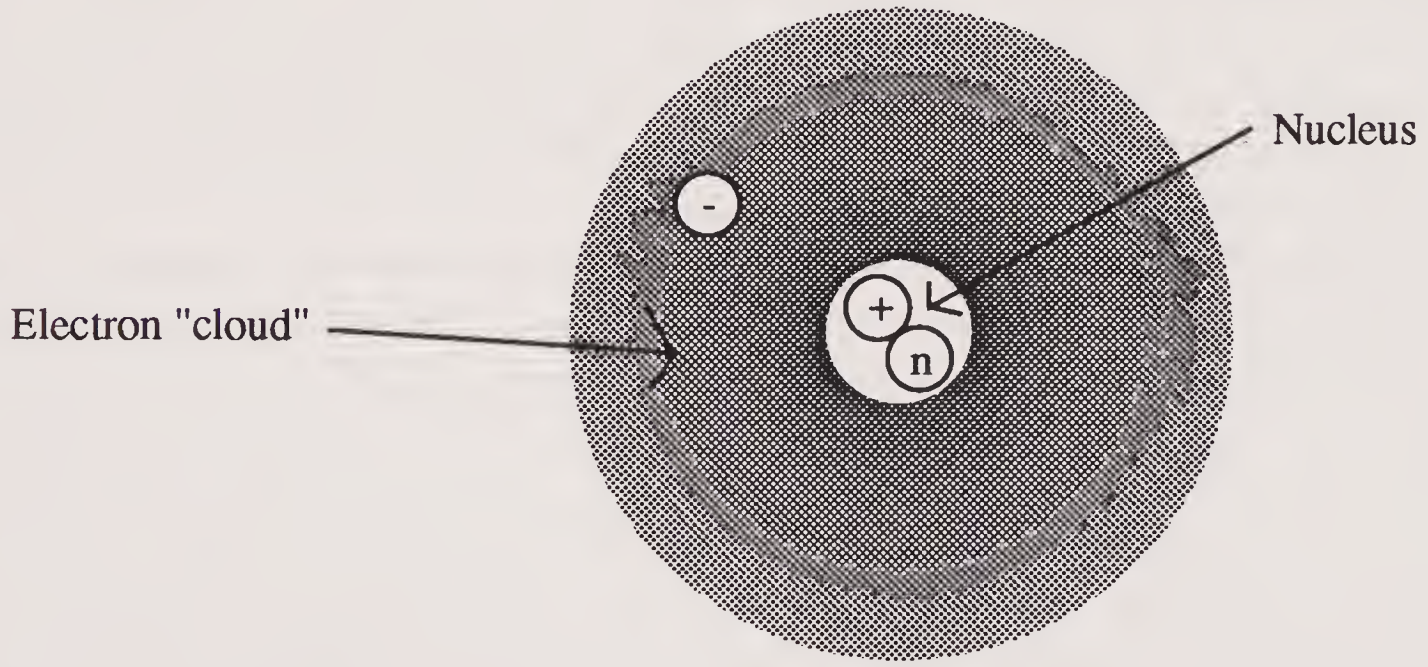


Figure 1.1. Representation of a deuterium atom. The nucleus contains one proton (+) and one neutron (n). The electron (-) is in constant, rapid motion around the nucleus forming a cloud of negative electrical charge, the density of which drops off with increasing distance from the nucleus.

Subatomic Particles

The subatomic particles differ in mass and charge. Their masses are expressed by the **atomic mass unit, u** (also called the **dalton**), which is also used to express the masses of individual atoms, and molecules (aggregates of atoms). The atomic mass unit is defined as a mass equal to exactly 1/12 that of an atom of carbon-12, the isotope of carbon that contains 6 protons and 6 neutrons in its nucleus.

The proton, *p*, has a mass of 1.007277 u and a unit charge of +1. This charge is equal to 1.6022×10^{-19} coulombs, where a coulomb is the amount of electrical charge involved in a flow of electrical current of 1 ampere for 1 second. The neutron, *n*, has no electrical charge and a mass of 1.009665 u. The proton and neutron each have a mass of essentially 1 u and are said to have a *mass number* of 1. (Mass number is a useful concept expressing the total number of protons and neutrons, as well as the approximate mass, of a nucleus or subatomic particle.) The electron, *e*, has an

electrical charge of -1. It is very light, however, with a mass of only 0.00054859 u, about 1/1840 that of the proton or neutron. Its mass number is 0. The properties of protons, neutrons, and electrons are summarized in Table 1.1.

Table 1.1. Properties of Protons, Neutrons, and Electrons

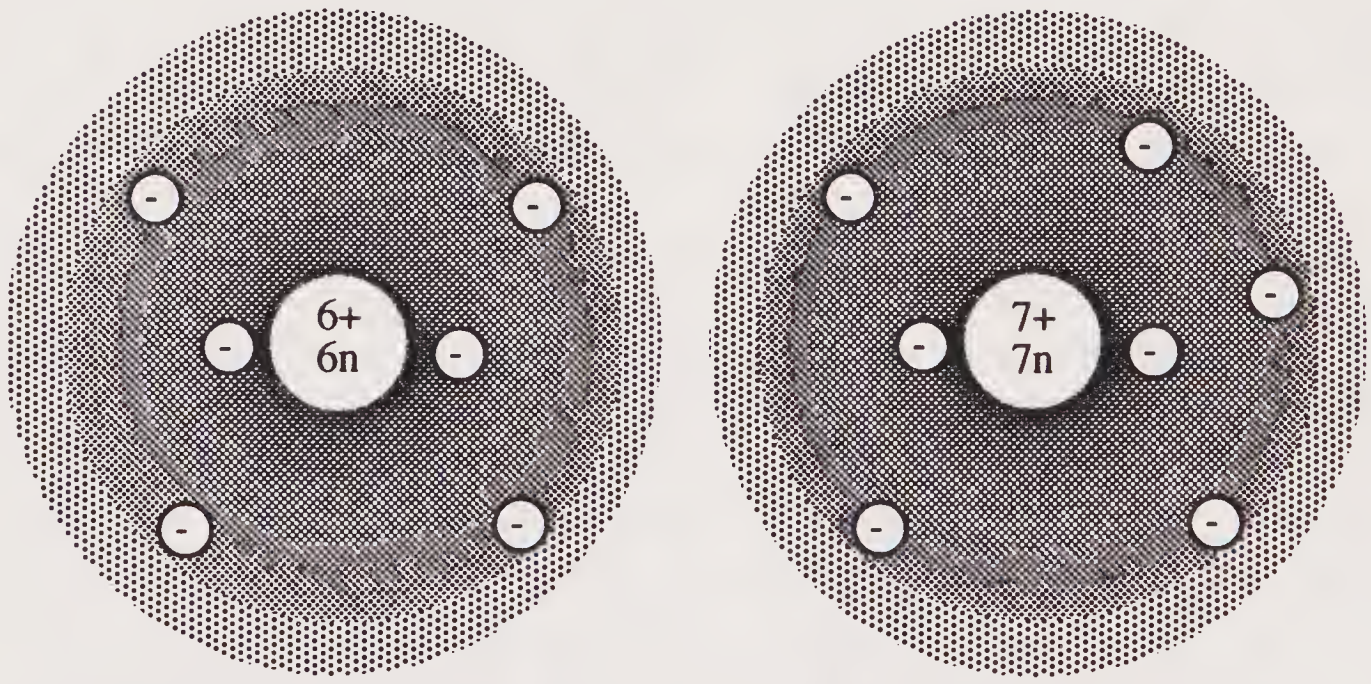
Subatomic particle	Symbol	Unit Charge	Mass number	Mass in u	Mass in grams
Proton[1]	p	+1	1	1.007277	1.6726×10^{-24}
Neutron[1]	n	0	1	1.008665	1.6749×10^{-24}
Electron[1]	e	-1	0	0.000549	9.1096×10^{-28}

[1] The mass number and charge of each of these kinds of particles may be indicated by a superscript and subscript, respectively, in the symbols ${}^{1}_{1}p$, ${}^{1}_{0}n$, and ${}^{0}_{-1}e$.

Although it is convenient to think of the proton and neutron as having the same mass, and each is assigned a mass number of 1, it is seen in Table 3.1 that their exact masses differ slightly from each other. Furthermore, the mass of an atom is not exactly equal to the sum of the masses of subatomic particles composing the atom. This is because of the energy relationships involved in holding the subatomic particles together in atoms so that the masses of the atom's constituent subatomic particles do not add up to exactly the mass of the atom.

Atom Nucleus and Electron Cloud

Protons and neutrons, which have relatively high masses compared to electrons, are contained in the positively charged **nucleus** of the atom. The nucleus has essentially all of the mass, but occupies virtually none of the volume, of the atom. An uncharged atom has the same number of electrons as protons. The electrons in an atom are contained in a cloud of negative charge around the nucleus that occupies most of the volume of the atom. These concepts are emphasized in Figure 1.2.



An atom of carbon, symbol C. Each C atom has 6 protons (+) in its nucleus, so the atomic number of C is 6. The atomic mass of C is 12.

An atom of nitrogen, symbol N. Each N atom has 7 protons (+) in its nucleus, so the atomic number of N is 7. The atomic mass of N is 14.

Figure 1.2. Atoms of carbon and nitrogen

Isotopes

Atoms with the same number of protons, but different numbers of neutrons in their nuclei are called **isotopes**. They are chemically identical atoms of the same element, but have different masses and may differ in their nuclear properties. Some isotopes are **radioactive isotopes** or **radionuclides**, which have unstable nuclei that give off charged particles and gamma rays in the form of **radioactivity**. Radioactivity may have detrimental, or even fatal, health effects; a number of hazardous substances are radioactive and they can cause major environmental problems. The most striking example of such contamination resulted from a massive explosion and fire at a power reactor in the Ukrainian city of Chernobyl in 1986.

Important Elements

An abbreviated list of a few of the most important elements that the reader should learn at this point is given in Table 1.2. A complete list of the 108 known elements is

Table 1.2. List of Some of the More Important Common Elements

Element	Symbol	Atomic Number	Atomic Mass	Significance
Aluminum	Al	13	26.9815	Abundant in Earth's crust
Argon	Ar	18	39.948	Noble gas
Arsenic	As	33	74.9216	Toxic metalloid
Bromine	Br	35	79.904	Toxic halogen
Cadmium	Cd	48	112.40	Toxic heavy metal
Calcium	Ca	20	40.08	Abundant essential element
Carbon	C	6	12.011	"Life element"
Chlorine	Cl	17	35.453	Halogen
Copper	Cu	29	63.54	Useful metal
Fluorine	F	9	18.998	Halogen
Helium	He	2	4.00260	Lightest noble gas
Hydrogen	H	1	1.008	Lightest element
Iodine	I	53	126.904	Halogen
Iron	Fe	26	55.847	Important metal
Lead	Pb	82	207.19	Toxic heavy metal
Magnesium	Mg	12	24.305	Light metal
Mercury	Hg	80	200.59	Toxic heavy metal
Neon	Ne	10	20.179	Noble gas
Nitrogen	N	7	14.0067	Important nonmetal
Oxygen	O	8	15.9994	Abundant, essential nonmetal
Phosphorus	P	15	30.9738	Essential nonmetal
Potassium	K	19	39.0983	Alkali metal
Silicon	Si	14	28.0855	Abundant metalloid
Silver	Ag	47	107.87	Valuable, reaction-resistant metal
Sulfur	S	16	32.064	Essential element, occurs in air pollutant SO_2
Sodium	Na	11	22.9898	Essential, abundant alkali metal
Tin	Sn	50	118.69	Useful metal
Uranium	U	92	238.03	Fissionable metal used for nuclear fuel
Zinc	Zn	30	65.37	Useful metal

given on the inside back cover of the book. Fortunately, most of the chemistry covered in this book requires familiarity only with the shorter list of elements in Table 1.2.

The Periodic Table

When elements are considered in order of increasing atomic number, it is observed that their properties are repeated in a periodic manner. For example, elements with atomic numbers 2, 10, and 18 are gases that do not undergo chemical reactions and consist of individual atoms, whereas those with atomic numbers larger by 1—elements with atomic numbers 3, 11, and 19—are unstable, highly reactive metals. An arrangement of the elements in a manner that reflects this recurring behavior is known as the **periodic table** (Figure 1.3). The periodic table is extremely useful in understanding chemistry and predicting chemical behavior. As shown in Figure 1.3, the entry for each element in the periodic table gives the element's atomic number, name, symbol, and atomic mass. More detailed versions of the table include other information as well.

Features of the Periodic Table

Groups of elements having similar chemical behavior are contained in vertica columns in the periodic table. **Main group** elements may be designated as A groups (1A and 2A on the left, 3A through 8A on the right). **Transition elements** are those between main groups 2A and 3A. **Noble gases** (group 8A), a group of gaseous elements that are virtually chemically unreactive, are in the far right column. The chemical similarities of elements in the same group are especially pronounced for groups 1A, 2A, 7A, and 8A.

Horizontal rows of elements in the periodic table are called **periods**, the first of which consists of only hydrogen (H) and helium (He). The second period begins with atomic number 3 (lithium) and terminates with atomic number 10 (neon), whereas the third goes from atomic number 11 (sodium) through 18 (argon). The fourth period includes the first row of transition elements, whereas lanthanides and actinides are listed separately at the bottom of the table.

Electrons in Atoms

Although a detailed discussion of the placement of electrons in atoms determines how the atoms behave chemically and, therefore, the chemical properties of each element, it is beyond the scope of this book to discuss electronic structure in detail. Several key points pertaining to this subject are mentioned here.

Electrons in atoms occupy **orbitals** in which electrons have different energies, orientations in space, and average distances from the nucleus. Each orbital may contain a maximum of 2 electrons. The placement of electrons in its orbitals determines the chemical behavior of an atom; in this respect the outermost orbitals and the electrons contained in them are the most important. These **outer electrons** are the ones beyond those of the immediately preceding noble gas in the periodic table. They are of particular importance because they become involved in the sharing and transfer of electrons through which chemical bonding occurs that results in the formation of huge numbers of different substances from only a few elements.

PERIODIC TABLE OF THE ELEMENTS

New notation →
Previous IUPAC form →
CAS version →

1 Group IA	2 IIA	3 IIIA IIIB	4 IVA IVB	5 VA VB	6 VIA VIB	7 VIIA VIIB	8	9 VIIIA VIII	10	11 IB	12 IIB	13 IIIB IIIA	14 IVB IVA	15 VB VA	16 VIB VIA	17 VIIB VIIA	18 VIIIA	Shell
1 +1 H −1 1.00794 1																	2 0 He 4.0020602 2	K
3 +1 Li 6.941 2-1	4 +2 Be 9.012182 2-2											5 +3 B 10.811 2-3	6 +2 +4 −4 C 12.011 2-4	7 +1 +2 +3 +4 +5 −1 N 14.00674 −2 −3 2-5	8 −2 O 15.9994 2-6	9 −1 F 18.9984032 2-7	10 0 Ne 20.1797 2-8	K-L
11 +1 Na 22.989768 2-8-1	12 +2 Mg 24.3050 2-8-2											13 +3 Al 26.981539 2-8-3	14 +2 +4 −4 Si 28.0855 2-8-4	15 +3 +5 −3 P 30.97362 2-8-5	16 +4 +6 −2 S 32.066 2-8-6	17 +1 +5 +7 −1 Cl 35.4527 2-8-7	18 0 Ar 39.948 2-8-8	K-L-M
19 +1 K 39.0983 -8-8-1	20 +2 Ca 40.078 -8-8-2	21 +3 Sc 44.955910 -8-9-2	22 +2 +3 +4 Ti 47.88 -8-10-2	23 +2 +3 +4 +5 V 50.9415 -8-11-2	24 +2 +3 +6 Cr 51.9961 -8-13-1	25 +2 +3 +4 +7 Mn 54.93085 -8-13-2	26 +2 +3 Fe 55.847 -8-14-2	27 +2 +3 Co 58.93320 -8-15-2	28 +2 +3 Ni 58.6934 -8-16-2	29 +1 +2 Cu 63.546 -8-18-1	30 +2 Zn 65.39 -8-18-2	31 +3 Ga 69.723 -8-18-3	32 +2 +4 Ge 72.61 -8-18-4	33 +3 +5 −3 As 74.92159 -8-18-5	34 +4 +6 −2 Se 78.96 -8-18-6	35 +1 +5 −1 Br 79.904 -8-18-7	36 0 Kr 83.80 -8-18-8	-L-M-N
37 +1 Rb 85.4678 -18-8-1	38 +2 Sr 87.62 -18-8-2	39 +3 Y 88.90585 -18-9-2	40 +4 Zr 91.224 -18-10-2	41 +3 +5 Nb 92.90638 -18-12-1	42 +6 Mo 95.94 -18-13-1	43 +4 +6 +7 Tc (98) -18-13-2	44 +3 Ru 101.07 -18-15-1	45 +3 Rh 102.90550 -18-16-1	46 +2 +4 Pd 106.42 -18-18-0	47 +1 Ag 107.8682 -18-18-1	48 +2 Cd 112.411 -18-18-2	49 +3 In 114.82 -18-18-3	50 +2 +4 Sn 118.710 -18-18-4	51 +3 +5 −3 Sb 121.757 -18-18-5	52 +4 +6 −2 Te 127.60 -18-18-6	53 +1 +5 +7 −1 I 126.90447 -18-18-7	54 0 Xe 131.29 -18-18-8	-M-N-O
55 +1 Cs 132.90543 -18-8-1	56 +2 Ba 137.327 -18-8-2	57* +3 La 138.9055 -18-9-2	72 +4 Hf 178.49 -32-10-2	73 +5 Ta 180.9479 -32-11-2	74 +6 W 183.85 -32-12-2	75 +4 +6 +7 Re 186.207 -32-13-2	76 +3 +4 Os 190.2 -32-14-2	77 +3 +4 Ir 192.22 -32-15-2	78 +2 +4 Pt 195.08 -32-16-2	79 +1 +3 Au 196.96654 -32-18-1	80 +1 +2 Hg 200.59 -32-18-2	81 +1 +3 Tl 204.3833 -32-18-3	82 +2 +4 Pb 207.2 -32-18-4	83 +3 +5 Bi 208.98037 -32-18-5	84 +2 +4 Po (209) -32-18-6	85 At (210) -32-18-7	86 0 Rn (222) -32-18-8	-N-O-P
87 +1 Fr (223) -18-8-1	88 +2 Ra 226.025 -18-8-2	89** +3 Ac 227.028 -18-9-2	104 Unq +4 (261) -32-10-2	105 Unp (262) -32-11-2	106 Unh (263) -32-12-2	107 Uns (262) -32-13-2												O P Q

*Lanthanides	58 +3 +4 Ce 140.115 -20-8-2	59 +3 Pr 140.90765 -21-8-2	60 +3 Nd 144.24 -22-8-2	61 +3 Pm (145) -23-8-2	62 +2 +3 Sm 150.36 -24-8-2	63 +2 +3 Eu 151.965 -25-8-2	64 +3 Gd 157.25 -25-9-2	65 +3 Tb 158.92534 -27-8-2	66 +3 Dy 162.50 -28-8-2	67 +3 Ho 164.93032 -29-8-2	68 +3 Er 167.26 -30-8-2	69 +3 Tm 168.93421 -31-8-2	70 +2 +3 Yb 173.04 -32-8-2	71 +3 Lu 174.967 -32-9-2		N O P
**Actinides	90 +4 Th 232.0381 -18-10-2	91 +5 +4 Pa 231.03588 -20-9-2	92 +3 +4 +5 +6 U 238.0289 -21-9-2	93 +3 +4 +5 +6 Np 237.048 -22-9-2	94 +3 +4 +5 +6 Pu (244) -24-8-2	95 +3 +4 +5 +6 Am (243) -25-8-2	96 +3 Cm (247) -25-9-2	97 +3 +4 Bk (247) -27-8-2	98 +3 Cf (251) -28-8-2	99 +3 Es (252) -29-8-2	100 +3 Fm (257) -30-8-2	101 +2 +3 Md (258) -31-8-2	102 +2 +3 No (259) -32-8-2	103 +3 Lr (260) -32-9-2		O P Q

KEY TO CHART

Atomic Number → 50 +2 ← Oxidation States
Symbol → Sn +4
1989 Atomic Weight → 118.71
18 18 4 ← Electron Configuration

The new IUPAC format numbers the groups from 1 to 18. The previous IUPAC numbering system and the system used by Chemical Abstracts Service (CAS) are also shown. For radioactive elements that do not occur in nature, the mass number of the most stable isotope is given in parentheses.

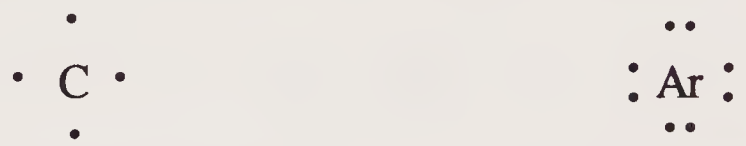
REFERENCES

1. G. J. Leigh, Editor, *Nomenclature of Inorganic Chemistry,* Blackwells Scientific Publications, Oxford, 1990.
2. *Chemical and Engineering News,* 63(5), 27, 1985.

Figure 1.3. The periodic table of the elements.

Lewis Symbols of Atoms

Outer electrons are called **valence electrons** and are represented by dots in **Lewis symbols**, as shown for carbon and argon in Figure 1.4, below:



Lewis symbol of carbon Lewis symbol of argon

Figure 1.4. Lewis symbols of carbon and argon.

The four electrons shown for the carbon atom are those added beyond the electrons possessed by the noble gas that immediately precedes carbon in the periodic table (helium, atomic number 2). Eight electrons are shown around the symbol of argon. This is an especially stable electron configuration for noble gases known as an **octet**. (Helium is the exception among noble gases in that it has a stable shell of only two electrons.) When atoms interact through the sharing, loss, or gain of electrons to form molecules and chemical compounds (see Section 1.3) many attain an octet of outer shell electrons. This tendency is the basis of the **octet rule** of chemical bonding. (Two or three of the lightest elements, most notably hydrogen, attain stable helium-like electron configurations containing two electrons when they become chemically bonded.)

Metals, Nonmetals, and Metalloids

Elements are divided between metals and nonmetals; a few elements with intermediate character are called metalloids. **Metals** are elements that are generally solid, shiny in appearance, electrically conducting, and malleable—that is, they can be pounded into flat sheets without disintegrating. They tend to have only 1–3 outer electrons, which they may lose in forming chemical compounds. Examples of metals are iron, copper, and silver. Most metallic objects that are commonly encountered are not composed of just one kind of elemental metal, but are alloys consisting of homogeneous mixtures of two or more metals. **Nonmetals** often have a dull appearance, are not at all malleable, and frequently occur as gases or liquids. Colorless oxygen gas, green chlorine gas (transported and stored as a liquid under pressure), and brown bromine liquid are common nonmetals. Nonmetals tend to have close to a full octet of outer-shell electrons, and in forming chemical compounds they gain or share electrons. **Metalloids**, such as silicon or arsenic, may have properties of both, or either metals or nonmetals.

1.3. CHEMICAL BONDING

Only a few elements, particularly the noble gases, exist as individual atoms; most atoms are joined by chemical bonds to other atoms. This can be illustrated very simply by elemental hydrogen, which exists as **molecules**, each consisting of 2 H atoms linked by a **chemical bond** as shown in Figure 1.5. Because hydrogen molecules contain 2 H atoms, they are said to be diatomic and are denoted by the **chemical formula**, H_2. The H atoms in the H_2 molecule are held together by a **covalent bond** made up of 2 electrons, each contributed by one of the H atoms, and shared between the atoms. (Bonds formed by transferring electrons between atoms are described later

in this section.) The shared electrons in the covalent bonds holding the H_2 molecule together are represented by two dots between the H atoms in Figure 1.5. By analogy with Lewis symbols defined in the preceding section, such a representation of molecules showing outer-shell and bonding electrons as dots is called a **Lewis formula**.

Figure 1.5. Molecule and Lewis formula of H_2.

Chemical Compounds

Most substances consist of two or more elements joined by chemical bonds. As an example consider the chemical combination of the elements hydrogen and oxygen shown in Figure 1.6. Oxygen, chemical symbol O, has an atomic number of 8 and an atomic mass of 16.00 and it exists in the elemental form as diatomic molecules of O_2. Hydrogen atoms combine with oxygen atoms to form molecules in which 2 H atoms are bonded to 1 O atom in a substance with a chemical formula of H_2O (water). A substance such as H_2O that consists of a chemically bonded combination of two or more elements is called a **chemical compound**. In the chemical formula for water the letters H and O are the chemical symbols of the two elements in the compound and the subscript 2 indicates that there are 2 H atoms per O atom. (The absence of a subscript after the O denotes the presence of just 1 O atom in the molecule.).

As shown in Figure 1.6, each of the hydrogen atoms in the water molecule is connected to the oxygen atom by a chemical bond composed of two electrons shared between the hydrogen and oxygen atoms. For each bond one electron is contributed by the hydrogen and one by oxygen.The two dots located between each H and O in the

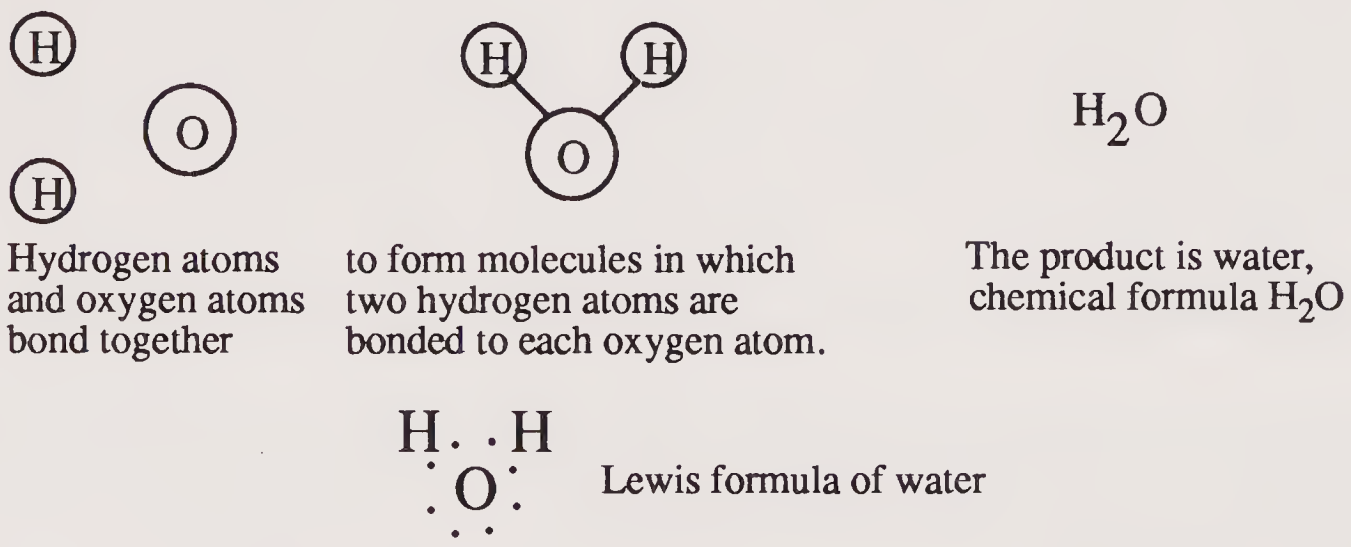


Figure 1.6. Formation and Lewis formula of a chemical compound, water.

Lewis formula of H_2O represent the two electrons in the covalent bond joining these atoms. Four of the electrons in the octet of electrons surrounding O are involved in H-

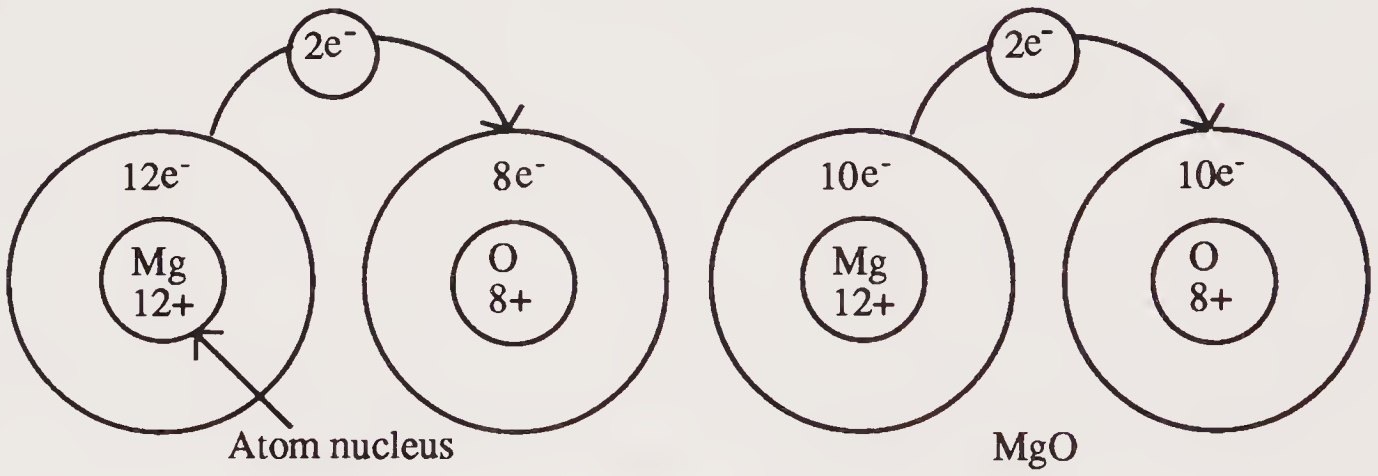
O bonds and are called bonding electrons. The other four electrons shown around the oxygen that are not shared with H are nonbonding outer electrons.

Molecular Structure

As implied by the representations of the water molecule in Figure 1.6, the atoms and bonds in H_2O form an angle somewhat greater than 90 degrees. The shapes of molecules are referred to as their **molecular geometry**, which is crucial in determining the chemical and toxicological activity of a compound and structure-activity relationships.

Ionic Bonds

As shown in Figure 1.7, the transfer of electrons from one atom to another produces charged species called **ions**. Positively charged ions are called **cations** and negatively charged ions are called **anions**. Ions that make up a solid compound are held together by **ionic bonds** in a **crystalline lattice** consisting of an ordered arrangement of the ions in which each cation is largely surrounded by anions and each anion by cations. The attracting forces of the oppositely charged ions in the crystalline lattice constitute ionic bonds in the compound.



The transfer of an electron from a magnesium atom to an oxygen atom

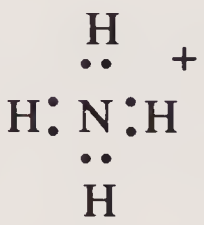
yields Mg^{2+} and O^{2-} ions that are bonded together by ionic bonds in the compound MgO.

Formation of ionic MgO as shown by Lewis formulas and symbols. In MgO, Mg has lost 2 electrons and is in the +2 oxidation state {Mg(II)} and O has gained 2 electrons and is in the -2 oxidation state.

Figure 1.7. Ionic bonds are formed by the transfer of electrons and the mutual attraction of oppositely charged ions in a crystalline lattice.

The formation of magnesium oxide is shown in Figure 1.7. In naming this compound, the cation is simply given the name of the element from which it was formed, magnesium. However, the ending of the name of the anion, ox*ide*, is different from that of the element from which it was formed, ox*ygen*.

Rather than individual atoms that have lost or gained electrons, many ions are groups of atoms bonded together covalently and having a net charge. A common example of such an ion is the ammonium ion, NH_4^+,


Lewis formula of the ammonium ion

which consists of 4 hydrogen atoms covalently bonded to a single nitrogen (N) atom and having a net electrical charge of +1 for the whole cation, as shown by its Lewis formula above.

Summary of Chemical Compounds and the Ionic Bond

The preceding several pages have just covered some material on chemical compounds and bonds that are essential to understand chemistry. To summarize, these are the following:

- Atoms of two or more different elements can form *chemical bonds* with each other to yield a product that is entirely different from the elements.
- Such a substance is called a *chemical compound.*
- The *formula* of a chemical compound gives the symbols of the elements and uses subscripts to show the relative numbers of atoms of each element in the compound.
- *Molecules* of some compounds are held together by *covalent bonds* consisting of shared electrons.
- Another kind of compound consists of *ions* consisting of electrically charged atoms or groups of atoms held together by *ionic bonds* that exist because of the mutual attraction of oppositely charged ions.

Molecular Mass

The average mass of all molecules of a compound is its **molecular mass** (formerly called molecular weight). The molecular mass of a compound is calculated by multiplying the atomic mass of each element by the relative number of atoms of the element, then adding all the values obtained for each element in the compound. For example, the molecular mass of NH_3 is 14.0 + 3 x 1.0 = 17.0. As another example consider the following calculation of the molecular mass of ethylene, C_2H_4.

1. The chemical formula of the compound is C_2H_4.
2. Each molecule of C_2H_4 consists of 2 C atoms and 4 H atoms.
3. From the periodic table or Table 1.2, the atomic mass of C is 12.0 and that of H is 1.0.
4. Therefore, the molecular mass of C_2H_4 is

$$\underbrace{12.0 + 12.0}_{\text{From 2 C atoms}} + \underbrace{1.0 + 1.0 + 1.0 + 1.0}_{\text{From 4 H atoms}} = 28.0.$$

Oxidation State

The loss of two electrons from the magnesium atom as shown in Figure 1.7 is an example of **oxidation**, and the Mg^{2+} ion product is said to be in the +2 **oxidation state**. (A positive oxidation state or oxidation number is conventionally denoted by a Roman numeral in parentheses following the name or symbol of an element as in magnesium(II) and Mg(II)). In gaining 2 negatively charged electrons in the reaction that produces magnesium oxide, the oxygen atom is **reduced** and is in the -2 oxidation state. (Unlike positive oxidation numbers, negative ones are not conventionally shown by Roman numerals in paretheses.) In chemical terms an **oxidizer** is a species that takes electrons from a reducing agent in a chemical reaction. Many hazardous waste substances are oxidizers or strong reducers and oxidation/reduction is the driving force behind many dangerous chemical reactions. For example, the reducing tendencies of the carbon and hydrogen atoms in propane cause it to burn violently or explode in the presence of oxidizing oxygen in air. The oxidizing ability of concentrated nitric acid, HNO_3, enables it to react destructively with organic matter, such as cellulose or skin.

Covalently bonded atoms that have not actually lost or gained electrons to produce ions are also assigned oxidation states. This can be done because in covalent compounds electrons are not shared equally. Therefore, an atom of an element with a greater tendency to attract electrons is assigned a negative oxidation state compared to the positive oxidation state assigned to an element with a lesser tendency to attract electrons. For example, Cl atoms attract electrons more strongly than do H atoms so that in hydrogen chloride gas, HCl, the Cl atom is in the -1 oxidation state and the H atoms are in the +1 oxidation state. **Electronegativity** values are assigned to elements on the basis of their tendencies to attract electrons.

The oxidation state (oxidation number) of an element in a compound may have a strong influence on the hazards posed by the compound. For example, chromium from which each atom has lost 3 electrons to form a chemical compound, designated as chromium(III) or Cr(III), is not toxic, whereas chromium in the +6 oxidation state (Cr(VI), chromate) is regarded as a cancer-causing chemical when inhaled.

1.4. CHEMICAL REACTIONS AND EQUATIONS

Chemical reactions occur when substances are changed to other substances through the breaking and formation of chemical bonds. For example, water is produced by the chemical reaction of hydrogen and oxygen:

Hydrogen plus oxygen yields water

Chemical reactions are written as **chemical equations**. The chemical reaction between hydrogen and water is written as the **balanced chemical equation**

$$2H_2 + O_2 \rightarrow 2H_2O \qquad (1.4.1)$$

in which the arrow is read as "yields" and separates the hydrogen and oxygen **reactants** from the water **product**. Note that because elemental hydrogen and elemental oxygen occur as *diatomic molecules* of H_2 and O_2, respectively, it is necessary to write the equation in a way that reflects these correct chemical formulas; of the elemental form. All correctly written chemical equations are **balanced**, in that

the same number of each kind of atom must be shown on both sides of the equation. The equation above is balanced because of the following:

On the left

- There are 2 H_2 *molecules* each containing 2 H *atoms* for a total of 4 H atoms on the left.
- There is 1 O_2 *molecule* containing 2 O *atoms* for a total of 2 O atoms on the left.

On the right

- There are 2 H_2O *molecules* each containing 2 H *atoms* and 1 O atom for a total of 4 H atoms and 2 O atoms on the right.

Reaction Rates

Most chemical reactions give off heat and are classified as exothermic reactions. The rate of a reaction may be calculated by the Arrhenius equation, which contains absolute temperature (K = °C + 273) in an exponential term. As a general rule the speed of a reaction doubles for each 10°C increase in temperature. Reaction rate factors are important factors in fires or explosions involving hazardous chemicals.

1.5. SOLUTIONS

A **solution** is formed when a substance in contact with a liquid becomes dispersed homogeneously throughout the liquid in a molecular form. The substance, called a **solute**, is said to **dissolve**. The liquid is called a **solvent**. There may be no readily visible evidence that a solute is present in the solvent; for example, a deadly poisonous solution of sodium cyanide in water looks like pure water! The solution may have a strong color, as is the case for intensely purple solutions of potassium permanganate, $KMnO_4$. It may have a strong odor, such as that of ammonia, NH_3, dissolved in water. Solutions may consist of solids, liquids, or gases dissolved in a solvent. Technically, it is even possible to have solutions in which a solid is a solvent, although such solutions are not discussed in this book.

Solution Concentration

The quantity of solute relative to that of solvent or solution is called the **solution concentration**. Concentrations are expressed in numerous ways. Very high concentrations are often given as percent by weight. For example, commercial concentrated hydrochloric acid is 36% HCl, meaning that 36% of the weight has come from dissolved HCl and 64% from water solvent. Concentrations of very dilute solutions, such as those of hazardous waste leachate containing low levels of contaminants, are expressed as weight of solute per unit volume of solution. Common units are milligrams per liter (mg/L) or micrograms per liter (µg/L). Since a liter of water weighs essentially 1,000 grams, a concentration of 1 mg/L is equal to 1 part per million (ppm) and a concentration of 1 µg/L is equal to 1 part per billion (ppb).

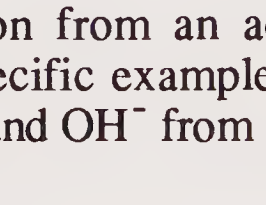
Chemists often express concentrations in moles per liter, or **molarity**, M. Molarity is given by the relationship

$$M = \frac{\text{Number of moles of solute}}{\text{Number of liters of solution}} \qquad (1.5.1)$$

The number of moles of a substance is its mass in grams divided by its molar mass. For example, the molecular mass of ammonia, NH_3, is 14 + 1 + 1 +1, so a mole of ammonia has a mass of 17 g. Therefore, 17 g of NH_3 in 1 L of solution has a value of M equal to 1 mole/L.

Water as a Solvent

Most liquid wastes are solutions or suspensions of waste materials in water. Water has some unique properties as a solvent which arise from its molecular structure as represented by the Lewis formula of water below:



The H atoms are not on opposite sides of the O atom and the two H–O bonds form an angle of 105°. Furthermore, the O atom (-2 oxidation state) is able to attract electrons more strongly than the 2 H atoms (each in the +1 oxidation state) so that the molecule is **polar**, with the O atom having a partial negative charge and the end of the molecule with the 2 H atoms having a partial positive charge. This means that water molecules can cluster around ions with the positive ends of the water molecules attracted to negatively charged anions and the negative end to positively charged cations. This kind of interaction is part of the general phenomenon of **solvation**. It is specifically called **hydration** when water is the solvent and is partially responsible for water's excellent ability to dissolve ionic compounds, including acids, bases, and salts.

Water molecules form a special kind of bond called a **hydrogen bond** with each other and with solute molecules that contain O, N, or S atoms. As its name implies, a hydrogen bond involves a hydrogen atom held between two other atoms of O, N, or S. Hydrogen bonding is partly responsible for water's ability to solvate and dissolve chemical compounds capable of hydrogen bonding.

As noted above, the water molecule is a polar species, which affects its ability to act as a solvent. Solutes may likewise have polar character. In general, solutes with polar molecules are more soluble in water than nonpolar ones. The polarity of an impurity solute in wastewater is a factor in determining how it may be removed from water. Nonpolar organic solutes are easier to take out of water by an adsorbent species such as activated carbon than are more polar solutes.

Solutions of Acids and Bases

Acid-Base Reactions

The reaction between H^+ ion from an acid and OH^- ion from a base is a **neutralization** reaction. As a specific example consider the reaction of H^+ from a solution of sulfuric acid, H_2SO_4, and OH^- from a solution of calcium hydroxide:

(1.5.2)

In addition to water, which is always the product of a neutralization reaction, the other product is calcium sulfate, $CaSO_4$. This compound is a **salt** composed of Ca^{2+} ions and SO_4^{2-} ions held together by ionic bonds. A salt, consisting of a cation other than H^+ and an anion other than OH^-, is the other product in addition to water produced when an acid and base react. Some salts are hazardous substances and environmental pollutants because of their dangerous or harmful properties. Some examples are the following:

- Ammonium perchlorate, NH_4ClO_4, (reactive oxidant)
- Barium cyanide, $Ba(CN)_2$(toxic)
- Lead acetate, $Pb(C_2H_3O_2)_2$ (toxic)
- Thallium(I) carbonate, Tl_2CO_3 (toxic)

Concentration of H^+ Ion and pH

Acids such as HCl, above, and sulfuric acid (H_2SO_4) produce H^+ ion, whereas bases, such as sodium hydroxide and calcium hydroxide (NaOH and $Ca(OH)_2$, respectively), produce hydroxide ion, OH^-. Molar concentrations of hydrogen ion, $[H^+]$, range over many orders of magnitude and are conveniently expressed by pH defined as

$$pH = -\log[H^+] \tag{1.5.3}$$

In absolutely pure water the value of $[H^+]$ is exactly 1×10^{-7} mole/L, the pH is 7.00, and the solution is **neutral** (neither acidic nor basic). **Acidic** solutions have pH values of less than 7 and **basic** solutions have pH values of greater than 7.

Strong acids and strong bases are **corrosive** substances that exhibit extremes of pH. They are destructive to materials and flesh. Strong acids can react with cyanide and sulfide compounds to release highly toxic hydrogen cyanide (HCN) or hydrogen sulfide (H_2S) gases, respectively. Bases liberate noxious ammonia gas (NH_3) from solid ammonium compounds.

Metal Ions Dissolved in Water

Metal ions dissolved in water have some unique characteristics that influence their properties as natural water constituents and heavy metal pollutants and in biological systems. The formulas of metal ions are usually represented by the symbol for the ion followed by its charge. For example, iron(II) ion (from a compound such as iron(II) sulfate, $FeSO_4$) dissolved in water is represented as Fe^{2+}. Actually, in water solution each iron(II) ion is strongly solvated and bonded to water molecules, so that the formula is more correctly shown as $Fe(H_2O)_6^{2+}$. Many metal ions have a tendency to lose hydrogen ions from the solvating water molecules as shown by the following:

$$Fe(H_2O)_6^{2+} \rightarrow Fe(OH)(H_2O)_5^{2+} + H^+ \tag{1.5.4}$$

Ions of the next higher oxidation state, iron(III), have such a tendency to lose H^+ ion in aqueous solution that, except in rather highly acidic solutions, they precipitate out as solid hydroxides, such as iron(III) hydroxide, $Fe(OH)_3$:

$$Fe(H_2O)_6^{3+} \rightarrow Fe(OH)_3(s) + 3H_2O + 3\,H^+ \qquad (1.5.5)$$

Complex Ions Dissolved in Water

It was noted above that metal ions are solvated (hydrated) by binding to water molecules in aqueous solution. Some species in solution have a stronger tendency than water to bond to metal ions. An example of such a species is cyanide ion, CN^-, which displaces water molecules from some metal ions in solution as shown below:

$$Ni(H_2O)_4^{2+} + 4CN^- \leftarrow\rightarrow Ni(CN)_4^{2-} + 4H_2O \qquad (1.5.6)$$

The species that bonds to the metal ion, cyanide in this case, is called a **ligand** and the product of the reaction is a **complex ion** or metal complex. The overall process is called **complexation**.

Colloidal Suspensions

Very small particles on the order of 1 micrometer or less in size, called **colloidal particles**, may stay suspended in a liquid for an indefinite period of time. Such a mixture is a **colloidal suspension** and it behaves in many respects like a solution. Colloidal suspensions are used in many industrial applications. Many waste materials are colloidal and are often emulsions consisting of colloidal liquid droplets suspended in another liquid, usually wastewater. One of the challenges in dealing with colloidal wastes is to remove a relatively small quantity of colloidal material from a large quantity of water by precipitating the colloid. This process is called **coagulation** or **flocculation** and is often brought about by the addition of chemical agents.

1.6. ORGANIC CHEMISTRY

Most carbon-containing compounds are **organic chemicals** and are addressed by the subject of **organic chemistry**. Organic chemistry is a vast, diverse, discipline because of the enormous number of organic compounds that exist as a consequence of the versatile bonding capabilities of carbon.[2] Such diversity is due to the ability of carbon atoms to bond to each other through single (2 shared electrons) bonds, double (4 shared electrons) bonds, and triple (6 shared electrons) bonds, in a limitless variety of straight chains, branched chains, and rings.

Among organic chemicals are included the majority of important industrial compounds, synthetic polymers, agricultural chemicals, biological materials, and most substances that are of concern because of their toxicities and other hazards. Pollution of the water, air, and soil environments by organic chemicals is an area of significant concern.

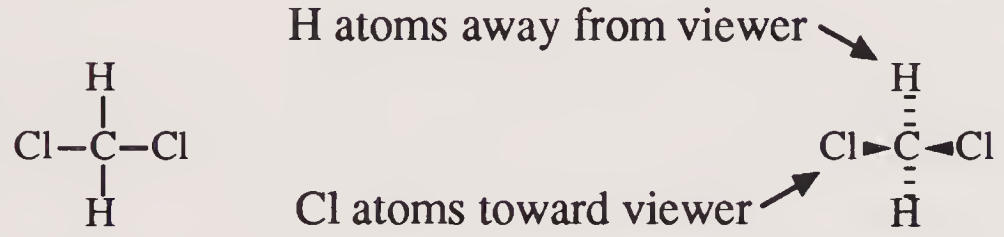
Chemically, most organic compounds can be divided among hydrocarbons, oxygen-containing compounds, nitrogen-containing compounds, sulfur-containing compounds, organohalides, phosphorus-containing compounds, or combinations of these kinds of compounds. Each of these classes of organic compounds is discussed briefly here.

All organic compounds of course contain carbon. Virtually all also contain hydrogen and have at least one C–H bond. The simplest organic compounds, and those easiest to understand, are those that contain only hydrogen and carbon. These

compounds are called **hydrocarbons** and are addressed first among the organic compounds discussed in this chapter. Hydrocarbons are used here to illustrate some of the most fundamental points of organic chemistry, including organic formulas, structures, and names.

Molecular Geometry in Organic Chemistry

The three-dimensional shape of a molecule, that is, its molecular geometry, is particularly important in organic chemistry. This is because its molecular geometry determines in part the properties of an organic molecule, particularly its interactions with biological systems. Shapes of molecules are represented in drawings by lines of normal, uniform thickness for bonds in the plane of the paper, broken lines for bonds extending away from the viewer, and heavy lines for bonds extending toward the viewer. These conventions are shown by the example of dichloromethane, CH_2Cl_2, an important organochloride solvent and extractant, illustrated in Figure 1.8.



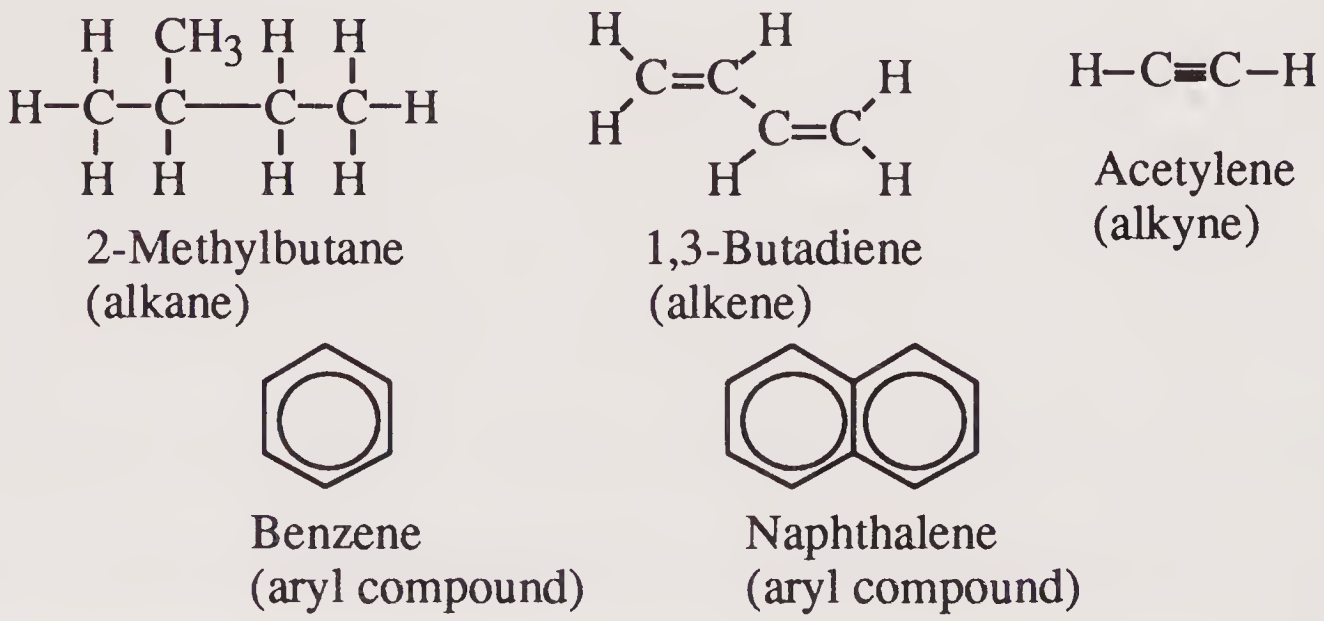
Structural formula of dichloromethane in two dimensions

Structural formula of dichloromethane represented in three dimensions

Figure 1.8. Structural formulas of dichloromethane, CH_2Cl_2; the formula on the right provides a three-dimensional representation.

1.7. HYDROCARBONS

As noted above, hydrocarbon compounds contain only carbon and hydrogen. The major types of hydrocarbons are alkanes, alkenes, alkynes, and aryl compounds. Examples of each are shown in Figure 1.9.



2-Methylbutane (alkane)

1,3-Butadiene (alkene)

Acetylene (alkyne)

Benzene (aryl compound)

Naphthalene (aryl compound)

Figure 1.9. Examples of major types of hydrocarbons.

Alkanes

Alkanes, also called **paraffins** or **aliphatic hydrocarbons**, are hydrocarbons in which the C atoms are joined by single covalent bonds (sigma bonds) consisting of two shared electrons (see Section 1.3). Some examples of alkanes are shown in Figure 1.9. As with other organic compounds, the carbon atoms in alkanes may form straight chains or branched chains. These three kinds of alkanes are, respectively, **straight-chain alkanes**, **branched-chain alkanes**, and **cycloalkanes**. As shown in Figure 1.10, a typical branched chain alkane is 2-methylbutane, a volatile, highly flammable liquid. It is a component of gasoline, which may explain why it is commonly found as an air pollutant in urban air. The general molecular formula for straight- and branched-chain alkanes is C_nH_{2n+2}, and that of cyclic alkanes is C_nH_{2n}. The four hydrocarbon molecules in Figure 1.10 contain 8 carbon atoms each. In one of the molecules, all of the carbon atoms are in a straight chain and in two they are in branched chains, whereas in a fourth 6 of the carbon atoms are in a ring.

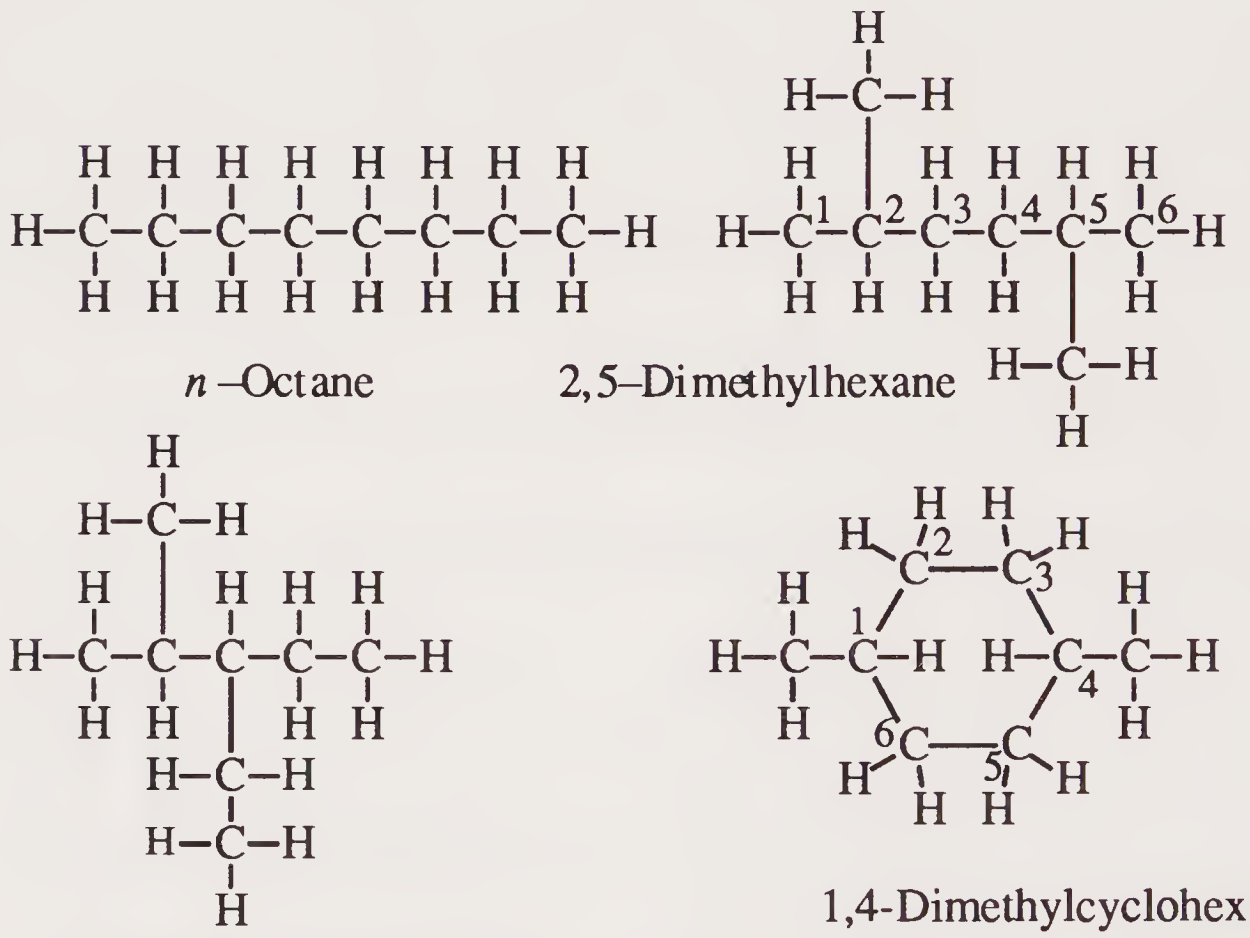


Figure 1.10. Structural formulas of four hydrocarbons, each containing 8 carbon atoms, that illustrate the structural diversity possible with organic compounds. Numbers used to denote locations of atoms for purposes of naming are shown on two of the compounds.

Formulas of Alkanes

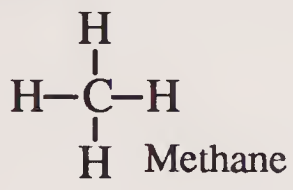
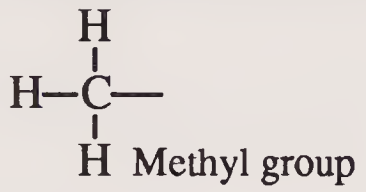
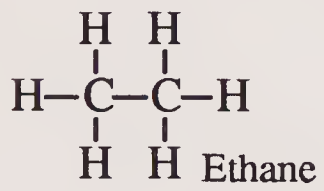
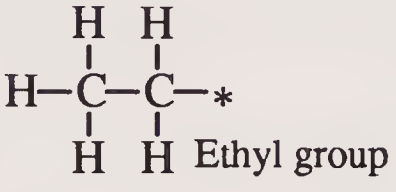
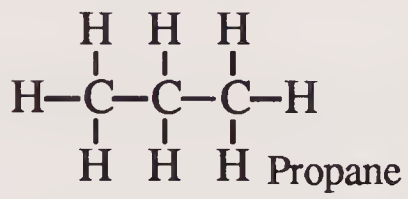
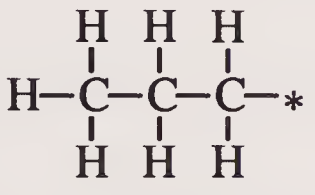
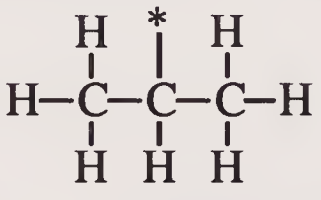
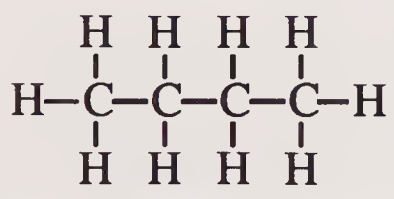
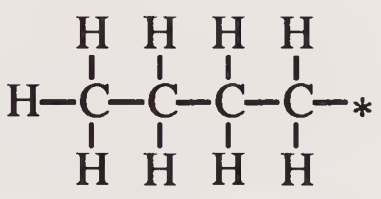
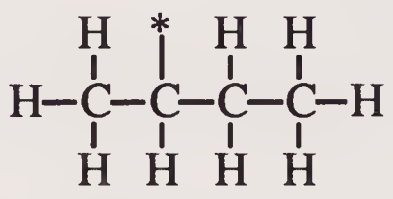
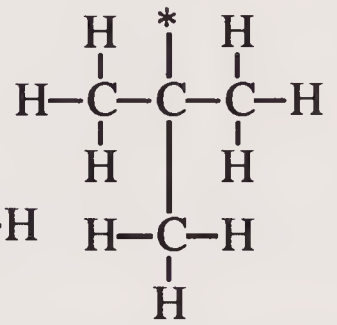
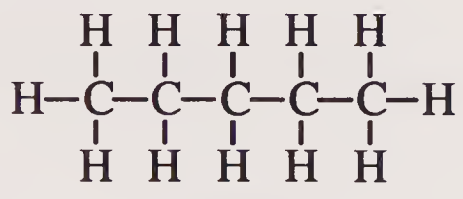
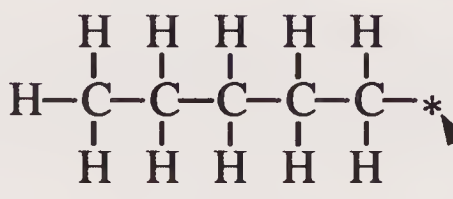
Formulas of organic compounds present information at several different levels of sophistication. **Molecular formulas**, such as that of octane (C_8H_{18}), give the number of each kind of atom in a molecule of a compound. As shown in Figure 1.10, however, the molecular formula of C_8H_{18} may apply to several alkanes, each one of which has unique chemical, physical, and toxicological properties. These different compounds are designated by **structural formulas** showing the order in which the atoms in a molecule are arranged. Compounds that have the same molecular, but different structural, formulas are called **structural isomers**. Of the compounds shown in Figure 1.10, *n*-octane, 2,5-dimethylhexane, and 2-methyl-3-ethylpentane are structural

isomers, all having the formula C_8H_{18}, whereas 1,4-dimethylcyclohexane is not a structural isomer of the other three compounds because its molecular formula is C_8H_{16}.

Alkanes and Alkyl Groups

Most organic compounds can be derived from alkanes. In addition, many important parts of organic molecules contain one or more alkane groups minus a hydrogen atom bonded as substituents onto the basic organic molecule. As a consequence of these factors the names of many organic compounds are based upon alkanes and it is useful to know the names of some of the more common alkanes and substituent groups derived from them as shown in Table 1.3.

Table 1.3. Some Alkanes and Substituent Groups Derived from Them.

Alkane	Substituent groups derived from alkane
 Methane	 Methyl group
 Ethane	 Ethyl group
 Propane	 *n*- Propyl group
 Isopropyl group	
 *n*- Butane	 *n*- Butyl group
 *sec*- Butyl group	 *tert*- Butyl group
 *n*- Pentane	 *n*- Pentyl group
Asterisk denotes point of attachment to another molecule	

Names of Alkanes and Organic Nomenclature

Systematic names, from which the structures of organic molecules can be deduced, have been assigned to all known organic compounds. The more common organic compounds, including many toxic and hazardous organic sustances, likewise have **common names** that have no structural implications. Although it is not possible

to cover organic nomenclature in any detail in this chapter, the basic approach to nomenclature is presented in the chapter along with some pertinent examples. The simplest approach is to begin with names of alkanes.

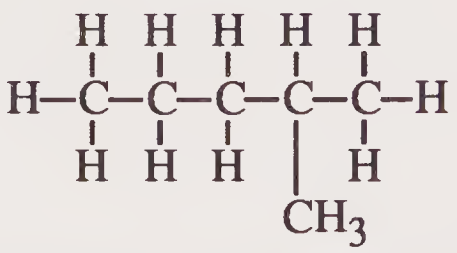
Consider the alkanes shown in Figure 1.10. The fact that *n*-octane has no side chains is denoted by "*n*", that it has 8 carbon atoms is denoted by "oct," and that it is an alkane is indicated by "ane." The names of compounds with branched chains or atoms other than H or C attached make use of numbers that stand for positions on the longest continuous chain of carbon atoms in the molecule. This convention is illustrated by the second compound in Figure 1.9. It gets the hexane part of the name from the fact that it is an alkane with 6 carbon atoms in its longest continuous chain ("hex" stands for 6). However, it has a methyl group (CH_3) attached on the second carbon atom of the chain and another on the fifth. Hence the full systematic name of the compound is 2,5-dimethylhexane, where "di" indicates two methyl groups. In the case of 2-methyl-3-ethylpentane, the longest continuous chain of carbon atoms contains 5 carbon atoms, denoted by pentane, a methyl group is attached to the second carbon atom, and an ethyl group, C_2H_5, on the third carbon atom The last compound shown in the figure has 6 carbon atoms in a ring, indicated by the prefix "cyclo," so it is a cyclohexane compound. Furthermore, the carbon in the ring to which one of the methyl groups is attached is designated by "1" and another methyl group is attached to the fourth carbon atom around the ring. Therefore, the full name of the compound is 1,4-dimethylcyclohexane.

Summary of Organic Nomenclature as Applied to Alkanes

Naming relatively simple alkanes is a straightforward process. The basic rules to be followed are the following:

1. The name of the compound is based upon the longest continuous chain of carbon atoms. (The structural formula may be drawn such that this chain is not immediately obvious.)

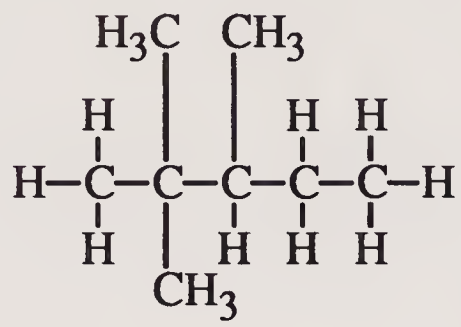
2. The carbon atoms in the longest continous chain are numbered sequentially from one end. The end of the chain from which the numbering is started is chosen to give the lower numbers for substituent groups in the final name. For example, the compound,



could be named 4-methylpentane (numbering the 5-carbon chain from the left), but should be named 2-methylpentane (numbering the 5-carbon chain from the right).

3. All groups attached to the longest continuous chain are designated by the number of the carbon atom to which they are attached and by the name of the substituent group ("2-methyl" in the example cited in Step 2, above).

4. A prefix is used to denote multiple substitutions by the same kind of group. This is illustrated by 2,2,3-trimethylpentane



in which the prefix *tri* is used to show that three methyl groups are attached to the pentane chain.

5. The complete name is assigned such that it denotes the longest continuous chain of carbon atoms and the name and location on this chain of each substituent group.

Reactions of Alkanes

Alkanes contain only C-C and C-H bonds, both of which are relatively strong. For that reason they have little tendency to undergo many kinds of reactions common to some other organic chemicals, such as acid-base reactions or low-temperature oxidation-reduction reactions. However, at elevated temperatures alkanes readily undergo oxidation, more specifically combustion, with molecular oxygen in air as shown by the following reaction of propane:

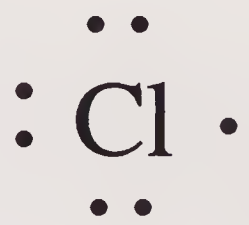
$$C_3H_8 + 5O_2 \rightarrow 3CO_2 + 4H_2O + \text{heat} \qquad (1.7.1)$$

Common alkanes are highly flammable and the more volatile lower molecular mass alkanes form explosive mixtures with air. Furthermore, combustion of alkanes in an oxygen-deficient atmosphere or in an automobile engine produces significant quantities of carbon monoxide, CO, the toxic properties of which are discussed in Section 12.3.

In addition to combustion, alkanes undergo **substitution reactions** in which one or more H atoms on an alkane are replaced by atoms of another element. The most common such reaction is the replacement of H by chlorine, to yield **organochlorine** compounds. For example, methane reacts with chlorine to give chloromethane. This reaction begins with the dissociation of molecular chlorine, usually initiated by ultraviolet electromagnetic radiation:

$$Cl_2 + \text{UV energy} \rightarrow Cl\cdot + Cl\cdot \qquad (1.7.2)$$

The Cl· product is a **free radical** species in which the chlorine atom has only 7 outer shell electrons as shown by the Lewis symbol,



instead of the favored octet of 8 outer-shell electrons. In gaining the octet required for chemical stability, the chlorine atom is very reactive. It abstracts a hydrogen from methane,

$$Cl\cdot + CH_4 \rightarrow HCl + CH_3\cdot \qquad (1.7.3)$$

to yield HCl gas and another reactive species with an unpaired electron, $CH_3\cdot$, called methyl radical. The methyl radical attacks molecular chlorine,

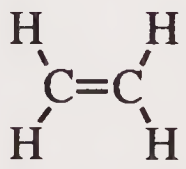
$$CH_3\cdot + Cl_2 \rightarrow CH_3Cl + Cl\cdot \qquad (1.7.4)$$

to give the chloromethane (CH_3Cl) product and regenerate $Cl\cdot$, which can attack additional methane as shown in Reaction 1.7.3. The reactive $Cl\cdot$ and $CH_3\cdot$ species continue to cycle through the two preceding reactions.

The reaction sequence shown above illustrates three important aspects of chemistry that will be shown to be very important in the discussion of atmospheric chemistry in Section 2.11. The first of these is that a reaction may be initiated by a **photochemical process** in which a photon of "light" (electromagnetic radiation) energy produces a reactive species, in this case the $Cl\cdot$ atom. The second point illustrated is the high chemical reactivity of **free radical species** with unpaired electrons and incomplete octets of valence electrons. The third point illustrated is that of **chain reactions**, which can multiply many fold the effects of a single reaction-initiating event, such as the photochemical dissociation of Cl_2.

Alkenes and Alkynes

Alkenes or **olefins** are hydrocarbons that have double bonds consisting of 4 shared electrons. The simplest and most widely manufactured alkene is ethylene,



Ethylene (ethene)

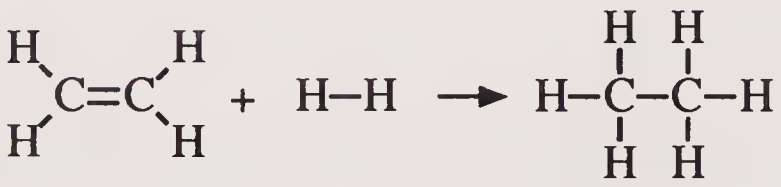
used for the production of polyethylene polymer. Another example of an important alkene is 1,3-butadiene (Figure 1.9), widely used in the manufacture of polymers, particularly synthetic rubber. The lighter alkenes, including ethylene and 1,3-butadiene, are highly flammable and form explosive mixtures with air. This was illustrated tragically by a massive explosion and fire involving leaking ethylene that destroyed a 20 billion pound per year Phillips Petroleum Company plastics plant in Pasadena, Texas, on October 23, 1989, killing more than 20 workers.

Acetylene (Figure 1.9) is an **alkyne**, a class of hydrocarbons characterized by carbon-carbon triple bonds consisting of 6 shared electrons. Highly flammable acetylene is used in large quantities as a chemical raw material and fuel for oxyacetylene torches. It forms dangerously explosive mixtures with air.

Addition Reactions

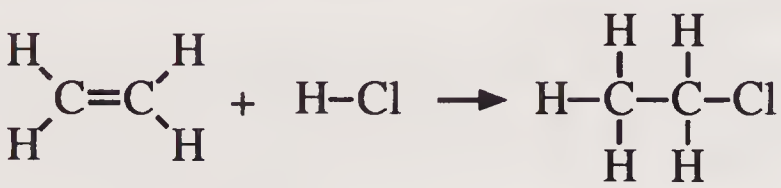
The double and triple bonds in alkenes and alkynes have "extra" electrons capable of forming additional bonds. Therefore, the carbon atoms attached to these bonds can add atoms without losing any atoms already bonded to them and the multiple

bonds are said to be **unsaturated**. Therefore, alkenes and alkynes both undergo **addition reactions** in which pairs of atoms are added across unsaturated bonds as shown in the reaction of ethylene with hydrogen to give ethane:



(1.7.5)

This is an example of a **hydrogenation reaction**, a very common reaction in organic synthesis, food processing (manufacture of hydrogenated oils), and petroleum refining. Another example of an addition reaction is that of HCl gas with acetylene to give vinyl chloride:

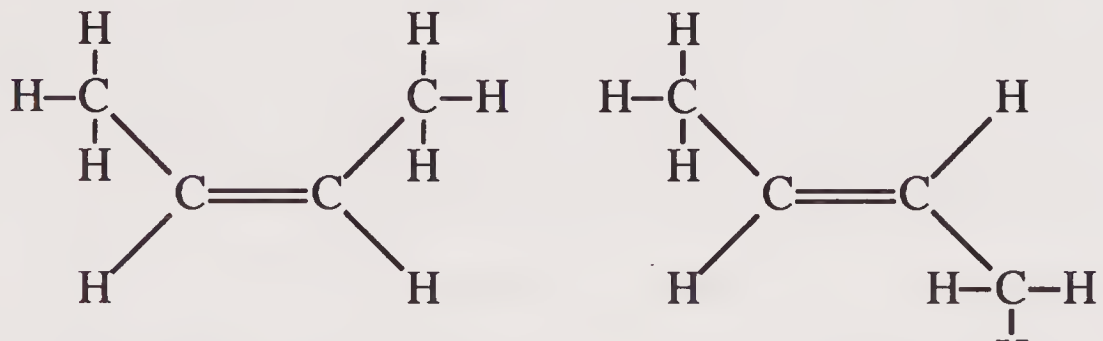


(1.7.6)

This kind of reaction, which is not possible with alkanes, adds to the chemical and metabolic versatility of compounds containing unsaturated bonds and is a factor contributing to their generally higher toxicities. It makes unsaturated compounds much more chemically reactive, more hazardous to handle in industrial processes, and more active in atmospheric chemical processes, such as smog formation (see Section 2.12).

Alkenes and *Cis-trans* Isomerism

As shown by the two simple compounds in Figure 1.11, the two carbon atoms connected by a double bond in alkenes cannot rotate relative to each other. For this reason, another kind of isomerism, called ***cis-trans***, isomerism, is possible for alkenes. *Cis-trans* isomers have different parts of the molecule oriented differently in space, although these parts occur in the same order. Both alkenes illustrated in Figure 1.11 have a molecular formula of C_4H_8. In the case of *cis*-2-butene, the two CH_3 (methyl) groups attached to the C=C carbon atoms are on the same side of the molecule, whereas in *trans*-2-butene they are on opposite sides.



Cis -2-butene

Trans -2-butene

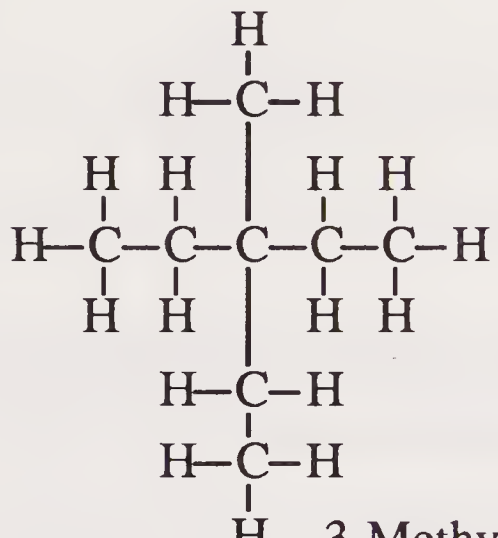
Figure 1.11. *Cis* and *trans* isomers of the alkene, 2-butene.

Condensed Structural Formulas

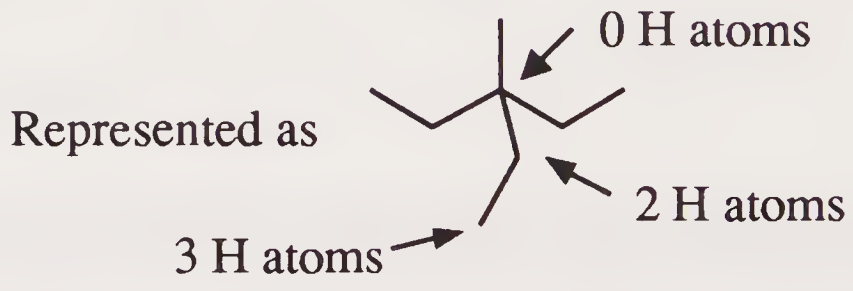
To save space, structural formulas are conveniently abbreviated as **condensed structural formulas**. The condensed structural formula of 2-methyl-3-ethylpentane is $CH_3CH(CH_3)CH(C_2H_5)CH_2CH_3$ where the CH_3 (methyl) and C_2H_5 (ethyl) groups are placed in parentheses to show that they are branches attached to the longest

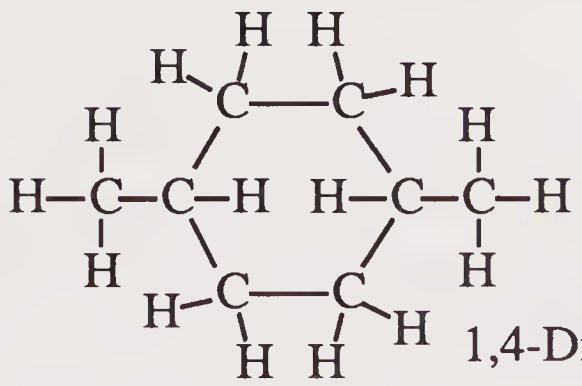
continuous chain of carbon atoms, which contains 5 carbon atoms. It is understood that each of the methyl and ethyl groups is attached to the carbon immediately preceding it in the condensed structural formula (methyl attached to the second carbon atom, ethyl to the third).

As illustrated by the examples in Figure 1.12, the structural formulas of organic molecules may be represented in a very compact form by lines and by figures such as hexagons. The ends and intersections of straight line segments in these formulas indicate the locations of carbon atoms. Carbon atoms at the terminal ends of lines are understood to have three H atoms attached, C atoms at the intersections of two lines are understood to have *two* H atoms attached to each, *one* H atom is attached to a carbon represented by the intersection of three lines, and *no* hydrogen atoms are bonded to C atoms where four lines intersect. Other atoms or groups of atoms, such as the Cl atom or OH group, that are substituted for H atoms are shown by their symbols attached to a C atom with a line.

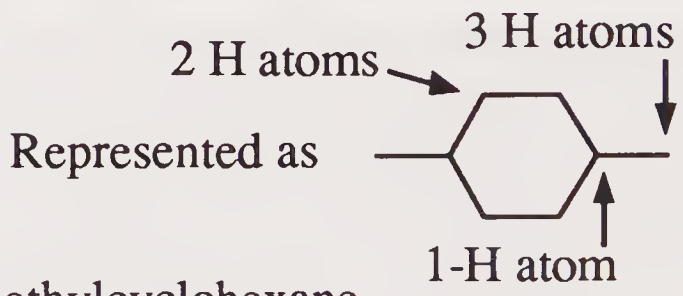


3-Methyl-3-ethylpentane





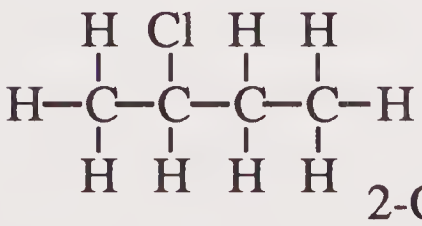
1,4-Dimethylcyclohexane



Cis -2-butene

Trans -2-butene

(See structural formulas of these compounds in the preceding figure)



2-Chlorobutane

Represented by

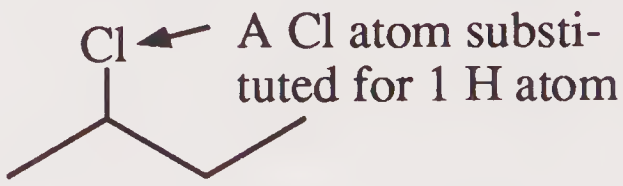


Figure 1.12. Representation of structural formulas with lines. A carbon atom is understood to be at each corner and at the end of each line. The numbers of hydrogen atoms attached to carbons at several specific locations are shown with arrows.

Aromatic Hydrocarbons

Benzene (Figure 1.13) is the simplest of a large class of **aromatic** or **aryl** hydrocarbons. Many important aryl compounds have substituent groups containing atoms of elements other than hydrogen and carbon and are called **aryl compounds** or **aromatic compounds**. Most aromatic compounds discussed in this book contain 6-carbon-atom benzene rings as shown for benzene, C_6H_6, in Figure 1.13. Aromatic compounds have ring structures and are held together in part by particularly stable bonds that contain delocalized clouds of so-called π (pi, pronounced "pie") electrons. In an oversimplified sense the structure of benzene can be visualized as resonating between the two equivalent structures shown on the left in Figure 1.13 by the shifting of electrons in chemical bonds. This structure can be shown more simply and accurately by a hexagon with a circle in it.

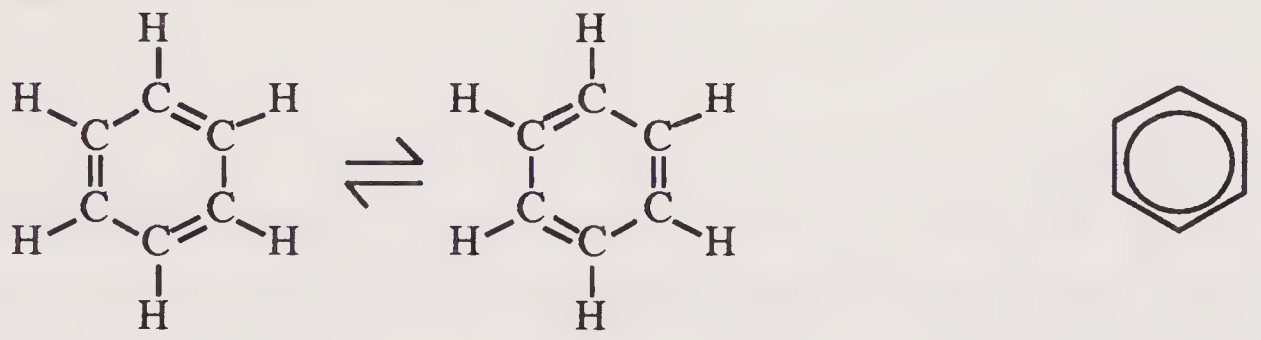


Figure 1.13. Representation of the aromatic benzene molecule with two resonance structures (left) and, more accurately, as a hexagon with a circle in it (right). Unless shown by symbols of other atoms, it is understood that a C atom is at each corner and that one H atom is bonded to each C atom.

Aryl compounds have special characteristics of **aromaticity**, which include a low hydrogen:carbon atomic ratio; C–C bonds that are quite strong and of intermediate length between such bonds in alkanes and those in alkenes; tendency to undergo substitution reactions rather than the addition reactions characteristic of alkenes; and delocalization of π electrons over several carbon atoms. The last phenomenon adds substantial stability to aromatic compounds and is known as **resonance stabilization**.

Many toxic substances, environmental pollutants, and hazardous waste compounds, such as benzene, toluene, naphthalene, and chlorinated phenols, are arenes (see Figure 1.14). As shown in Figure 1.14, some arenes, such as naphthalene and the polycyclic aromatic compound, benzo(a)pyrene, contain fused rings.

Benzene and Naphthalene

Benzene is a volatile, colorless, highly flammable liquid that is consumed as a raw material for the manufacture of phenolic and polyester resins, polystyrene plastics, alkylbenzene surfactants, chlorobenzenes, insecticides, and dyes. It is hazardous both for its ignitability and toxicity (exposure to benzene causes blood abnormalities that may develop into leukemia). Naphthalene is the simplest member of a large number of multicyclic aromatic hydrocarbons having two or more fused rings. It is a volatile white crystalline solid with a characteristic odor and has been used to make mothballs. The most important of the many chemical derivatives made from naphthalene is phthalic anhydride, from which phthalate ester plasticizers are synthesized.

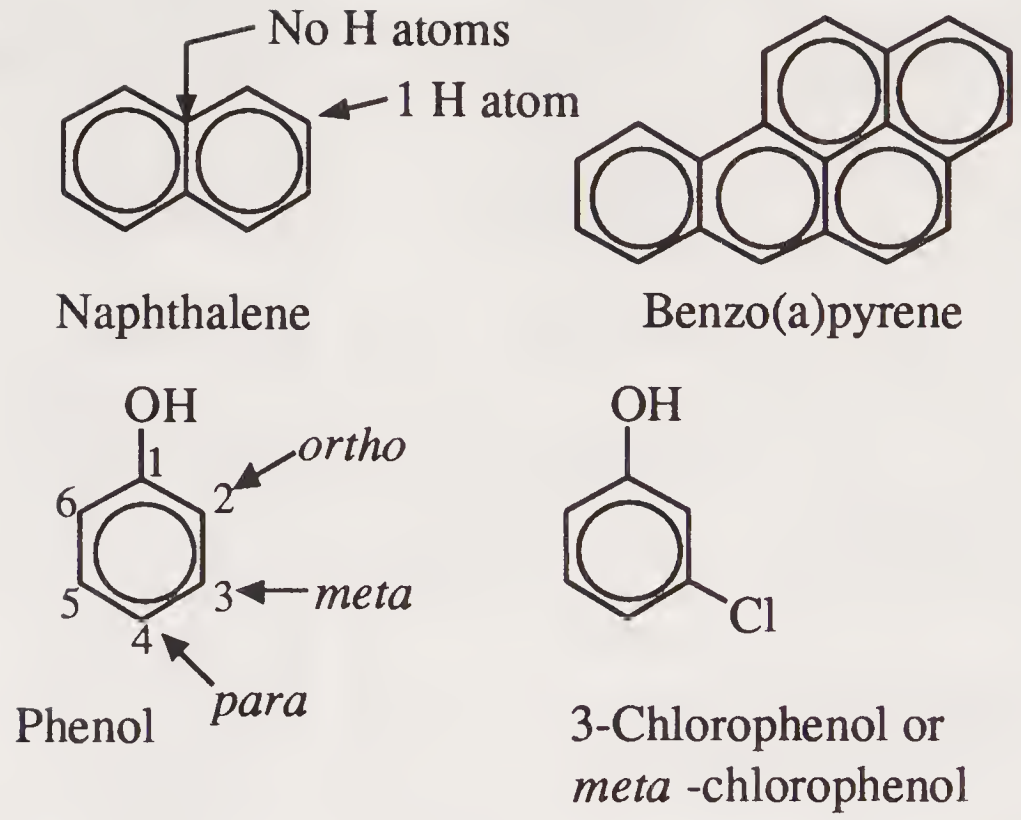


Figure 1.14. Aromatic compounds containing fused rings (top) and showing the numbering of carbon atoms for purposes of nomenclature.

Polycyclic Aromatic Hydrocarbons

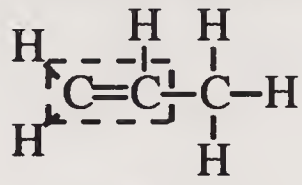
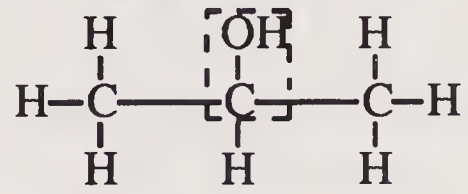
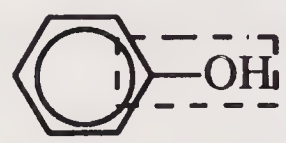
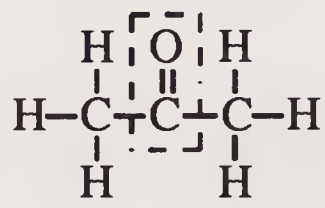
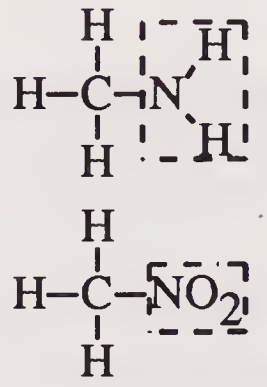
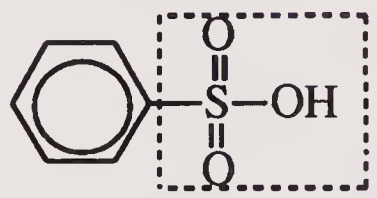
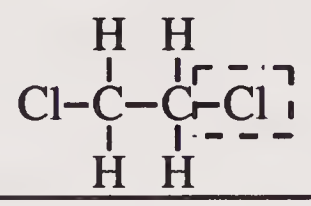
Benzo(a)pyrene (Figure 1.14) is the most studied of the polycyclic aromatic hydrocarbons (PAHs), which are characterized by condensed ring systems ("chicken wire" structures). These compounds are formed by the incomplete combustion of other hydrocarbons, a process that consumes hydrogen in preference to carbon. The carbon residue is left in the thermodynamically favored condensed aromatic ring system of the PAH compounds.

Because there are so many partial combustion and pyrolysis processes that favor production of PAHs, these compounds are encountered abundantly in the atmosphere, soil, and elsewhere in the environment from sources that include engine exhausts, wood stove smoke, cigarette smoke, and char-broiled food. Coal tars and petroleum residues such as road and roofing asphalt have high levels of PAHs. Some PAH compounds, including benzo(a)pyrene, are of toxicological concern because they are precursors to cancer-causing metabolites.

1.9. ORGANIC FUNCTIONAL GROUPS AND CLASSES OF ORGANIC COMPOUNDS

The discussion of organic chemistry so far in this chapter has emphasized hydrocarbon compounds, those that contain only hydrogen and carbon. It has been shown that hydrocarbons may exist as alkanes, alkenes, and arenes, depending upon the kinds of bonds between carbon atoms. The presence of elements other than hydrogen and carbon in organic molecules greatly increases the diversity of their chemical behavior. **Functional groups** consist of specific bonding configurations of atoms in organic molecules. Most functional groups contain at least one element other than carbon or hydrogen, although two carbon atoms joined by a double bond (alkenes) or triple bond (alkynes) are likewise considered to be functional groups. Table 1.3 shows some of the major functional groups that determine the nature of organic compounds.

Table 1.4. Examples of Some Important Functional Groups

Type of functional group	Example compound	Structural formula of functional group[1]
Alkene (olefin)	Propene (propylene)	
Alkyne	Acetylene	
Alcohol (-OH attached to alkyl group)	2-Propanol	
Phenol (-OH attached to aryl group)	Phenol	
Ketone (When  group is on end carbon, compound is an aldehyde)	Acetone	
Amine	Methylamine	
Nitro compounds	Nitromethane	
Sulfonic acids	Benzenesulfonic acid	
Organohalides	1,1–Dichloro-ethane	

[1] Functional group outlined by dashed line

Organooxygen Compounds

The most common types of compounds with oxygen-containing functional groups are epoxides, alcohols, phenols, ethers, aldehydes, ketones, and carboxylic acids. The functional groups characteristic of these compounds are illustrated by the examples of oxygen-containing compounds shown in Figure 1.15.

Ethylene oxide is a moderately to highly toxic sweet-smelling, colorless, flammable, explosive gas used as a chemical intermediate, sterilant, and fumigant. It is a mutagen and a carcinogen to experimental animals. It is classified as hazardous for both its toxicity and ignitability. **Methanol** is a clear, volatile, flammable liquid alcohol used for chemical synthesis, as a solvent, and as a fuel. It is being advocated strongly in some quarters as an alternative to gasoline that would result in significantly

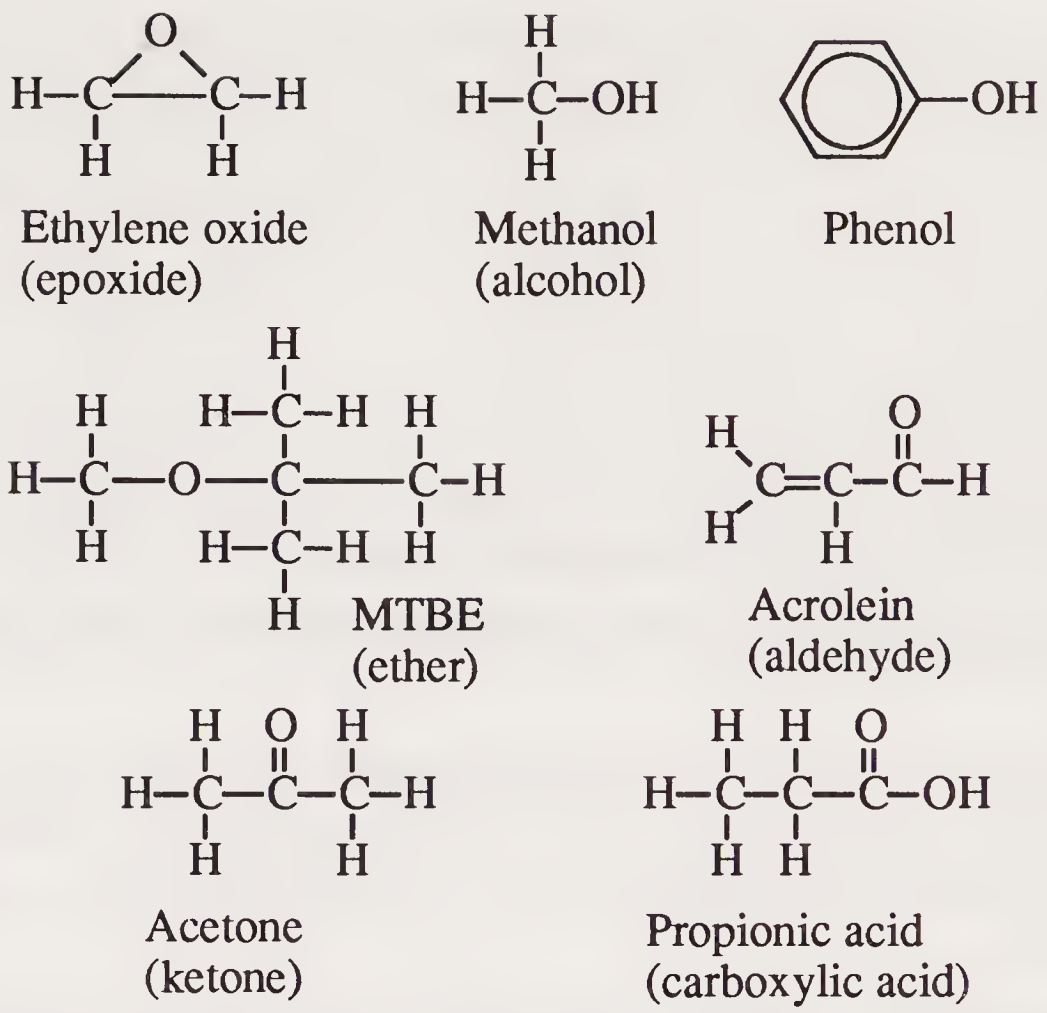
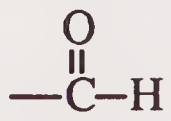


Figure 1.15. Examples of oxygen-containing organic compounds that may be significant as wastes, toxic substances, or environmental pollutants.

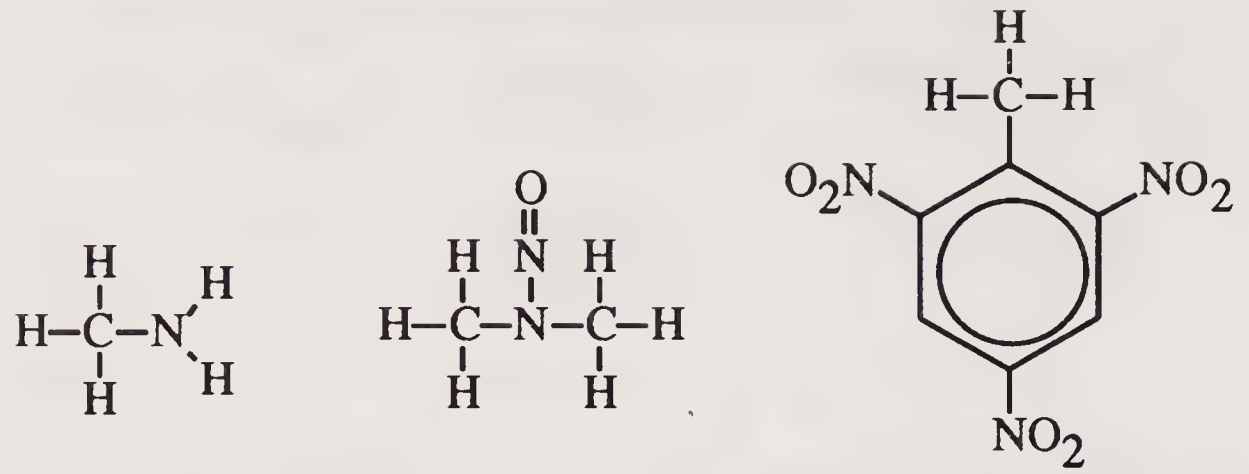
less photochemical smog formation than currently used gasoline formulations. Ingestion of methanol can be fatal and blindness can result from sublethal doses. **Phenol** is a dangerously toxic aryl alcohol widely used for chemical synthesis and polymer manufacture. **Methyltertiarybutyl ether,** MTBE, is an ether that has become the octane booster of choice to replace tetraethyllead in gasoline. **Acrolein** is an alkenic aldehyde and a volatile, flammable, highly reactive chemical. It forms explosive peroxides upon prolonged contact with O_2. An extreme lachrimator and strong irritant, acrolein is quite toxic by all routes of exposure. **Acetone** is the lightest of the ketones. Like all ketones, acetone has a carbonyl (C=O) group that is bonded to two carbon atoms (that is, it is somewhere in the middle of a carbon atom chain). Acetone is a good solvent and is chemically less reactive than the aldehydes which all have the functional group,



in which binding of the C=O to H makes the molecule significantly more reactive. **Propionic acid** is a typical organic carboxylic acid. The $-CO_2H$ group characteristic of carboxylic acids may be viewed as the most oxidized functional group on an oxygenated organic compound, and carboxylic acids may be synthesized by oxidizing aldehydes and alcohols that have an -OH group or C=O group on an end carbon atom.

Organonitrogen Compounds

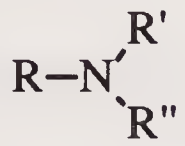
Figure 1.16 shows examples of three classes of the many kinds of compounds that contain N (amines, nitrosamines, and nitro compounds). Nitrogen occurs in many functional groups in organic compounds, some of which contain nitrogen in ring structures, or along with oxygen.



Methylamine | Dimethylnitrosamine (N-nitrosodimethylamine) | Trinitrotoluene (TNT)

Figure 1.16. Examples of organonitrogen that may be significant as wastes, toxic substances, or environmental pollutants.

Methylamine is a colorless, highly flammable gas with a strong odor. It is a severe irritant affecting eyes, skin, and mucous membranes. Methylamine is the simplest of the **amine** compounds, which have the general formula,

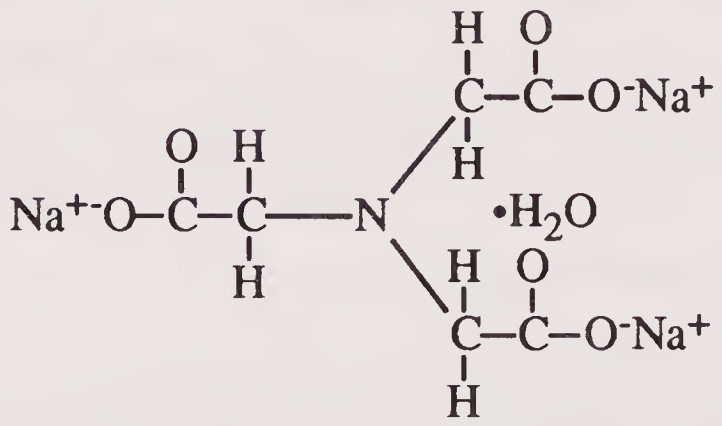


where the R's are hydrogen or hydrocarbon groups, at least one of which is the latter.

Dimethylnitrosamine is an N-nitroso compound, all of which contain the N-N=O functional group. It was once widely used as an industrial solvent, but was observed to cause liver damage and jaundice in exposed workers. Subsequently numerous other N-nitroso compounds, many produced as byproducts of industrial operations and food and alcoholic beverage processing, were found to be carcinogenic.

Solid **trinitrotoluene** (TNT) has been widely used as a military explosive. TNT is moderately to very toxic and has caused toxic hepatitis or aplastic anemia in exposed individuals, a few of whom have died from its toxic effects. It belongs to the general class of nitro compounds characterized by the presence of $-NO_2$ groups bonded to a hydrocarbon structure.

Some organonitrogen compounds are chelating agents that bind strongly to metal ions and play a role in the solubilization and transport of heavy metal wastes. Prominent among these are salts of the aminocarboxylic acids which, in the acid form, have $-CH_2CO_2H$ groups bonded to nitrogen atoms. An important example of such a compound is the monohydrate of trisodium nitrilotriacetate (NTA):



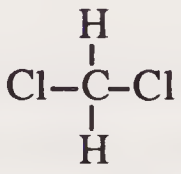
This compound is widely used in Canada as a substitute for detergent phosphates to bind to calcium ion and make the detergent solution basic. NTA is used in metal plat-

ing formulations. It is highly water soluble and quickly eliminated with urine when ingested. It has a low acute toxicity and no chronic effects have been shown for plausible doses. However, concern does exist over its interaction with heavy metals in waste treatment processes and in the environment.

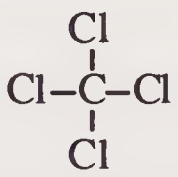
Organohalide Compounds

Organohalides (Figure 1.17) exhibit a wide range of physical and chemical properties. These compounds consist of halogen-substituted hydrocarbon molecules, each of which contains at least one atom of F, Cl, Br, or I. They may be saturated (**alkyl halides**), unsaturated (**alkenyl halides**), or aromatic (**aryl halides**). The most widely manufactured organohalide compounds are chlorinated hydrocarbons, many of which are regarded as environmental pollutants or as hazardous wastes.

Alkyl halides

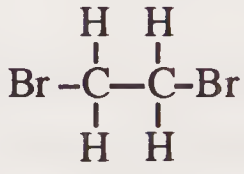


Dichloromethane (b.p. -97°C)



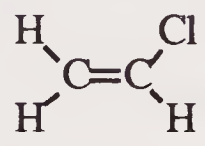
Carbon tetrachloride (bp 77°C)

Dichlorodifluoromethane (bp -29°C)

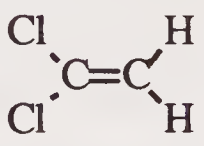


1,2-Dibromoethane (bp 131°C°

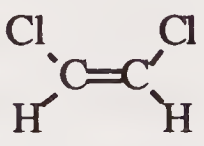
Alkenyl halides



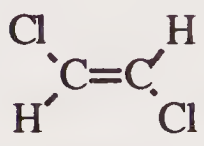
Monochloroethylene (vinyl chloride



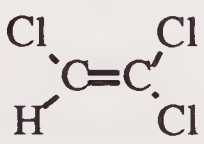
1,1,-Dichloroethylene (vinylidene chloride)



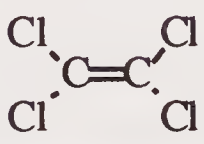
Cis -1,2-dichloroethylene



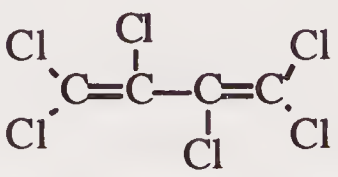
Trans -1,2-dichloroethylene



Trichloroethylene (TCE)

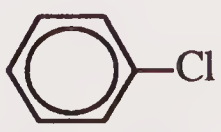


Tetrachloroethylene (perchloroethylene)

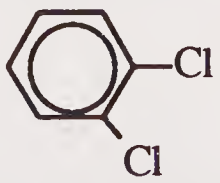


Hexachlorobutadiene

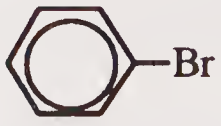
Aryl halides



Monochlorobenzene



1,2-dichlorobenzene



Bromobenzene

Figure 1.17. Some example organohalide compounds.

Alkyl Halides

Substitution of halogen atoms for one or more hydrogen atoms on alkanes gives **alkyl halides**, example structural formulas of which are given in Figure 1.17. Most of the commercially important alkyl halides are derivatives of alkanes of low molecular mass. A brief discussion of the uses of the compounds listed in Figure 1.17 is given here to provide an idea of the versatility of the alkyl halides.

Dichloromethane is a volatile liquid with excellent solvent properties for nonpolar organic solutes. It has been used as a solvent for the decaffeination of coffee, in paint strippers, as a blowing agent in urethane polymer manufacture, and to depress vapor pressure in aerosol formulations. Once commonly sold as a solvent and stain remover, highly toxic **carbon tetrachloride** is now largely restricted to uses as a chemical intermediate under controlled conditions, primarily to manufacture chlorofluorocarbon refrigerant fluid compounds, which are also discussed in this section. Insecticidal **1,2-dibromoethane** has been consumed in large quantities as a lead scavenger in leaded gasoline and to fumigate soil, grain, and fruit (Fumigation with this compound has been discontinued because of toxicological concerns). An effective solvent for resins, gums, and waxes, it serves as a chemical intermediate in the syntheses of some pharmaceutical compounds and dyes.

Alkenyl Halides

Viewed as hydrocarbon-substituted derivatives of alkenes, the **alkenyl** or **olefinic organohalides** contain at least one halogen atom and at least one carbon–carbon double bond. The most significant of these are the lighter chlorinated compounds, such as those illustrated in Figure 1.17.

Vinyl chloride is consumed in large quantities as a raw material to manufacture pipe, hose, wrapping, and other products fabricated from polyvinylchloride plastic. This highly flammable, volatile, sweet-smelling gas is a known human carcinogen.

As shown in Figure 1.17, there are three possible dichloroethylene compounds, all clear, colorless liquids. Vinylidene chloride forms a copolymer with vinyl chloride used in some kinds of coating materials. The geometrically isomeric 1,2-dichloroethylenes are used as organic synthesis intermediates and as solvents. **Trichloroethylene** is a clear, colorless, nonflammable, volatile liquid. It is an excellent degreasing and drycleaning solvent and has been used as a household solvent and for food extraction (for example, in decaffeination of coffee). Colorless, non-flammable liquid **tetrachloroethylene** has properties and uses similar to those of trichloroethylene. **Hexachlorobutadiene**, a colorless liquid with an odor somewhat like that of turpentine, is used as a solvent for higher hydrocarbons and elastomers, as a hydraulic fluid, in transformers, and for heat transfer.

Aryl Halides

Aryl halide derivatives of benzene and toluene have many uses in chemical synthesis, as pesticides and raw materials for pesticides manufacture, as solvents, and a diverse variety of other applications. These widespread uses over many decades have resulted in substantial human exposure and environmental contamination. Three example aryl halides are shown in Figure 1.17. Monochlorobenzene is a flammable liquid boiling at 132°C. It is used as a solvent, heat transfer fluid, and synthetic reagent. Used as a solvent, 1,2-dichlorobenzene is employed for degreasing hides and wool. It also serves as a synthetic reagent for dye manufacture. Bromobenzene is a liquid boiling at 156°C that is used as a solvent, motor oil additive, and intermediate for organic synthesis.

Halogenated Naphthalene and Biphenyl

Two major classes of halogenated aryl compounds containing two benzene rings are made by the chlorination of naphthalene and biphenyl and have been sold as mixtures with varying degrees of chlorine content. Examples of chlorinated naphthalenes, and polychlorinated biphenyls (PCBs discussed later), are shown in Figure 1.18. The less highly chlorinated of these compounds are liquids and those with higher chlorine contents are solids. Because of their physical and chemical stabilities and other desirable qualities, these compounds have had many uses, including heat transfer fluids, hydraulic fluids, and dielectrics. Polybrominated biphenyls (PBBs) have served as flame retardants. However, because chlorinated naphthalenes, PCBs, and PBBs are environmentally extremely persistent, their uses have been severely curtailed.

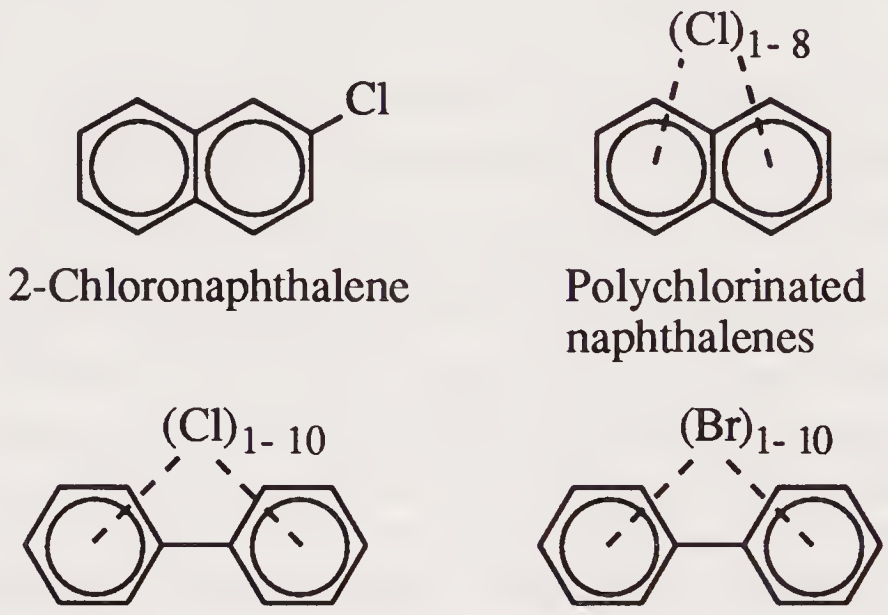


Figure 1.18. Halogenated naphthalenes and biphenyls.

Chlorofluorocarbons, Halons, and Hydrogen-Containing Chlorofluorocarbons

Chlorofluorocarbons (**CFC**s) are volatile 1- and 2-carbon compounds that contain Cl and F bonded to carbon. These compounds are notably stable and nontoxic. They have been widely used in recent decades in the fabrication of flexible and rigid foams and as fluids for refrigeration and air conditioning. The most widely manufactured of these compounds are CCl_3F (CFC-11), CCl_2F_2 (CFC-12), $C_2Cl_3F_3$ (CFC-113), $C_2Cl_2F_4$ (CFC-114), and C_2ClF_5 (CFC-115).

Halons are related compounds that contain bromine and are used in fire extinguisher systems. The most commonly produced commercial halons are $CBrClF_2$ (Halon-1211), $CBrF_3$ (Halon-1301), and $C_2Br_2F_4$ (Halon-2402), where the sequence of numbers denotes the number of carbon, fluorine, chlorine, and bromine atoms, respectively, per molecule. Halons are particularly effective fire extinguishing agents because of the way in which they stop combustion. Some fire supressants, such as carbon dioxide, act by depriving the flame of oxygen by a smothering effect, whereas water cools a burning substance to a temperature below which combustion is supported. Halons act by chain reactions (discussed previously in this section) that destroy hydrogen atoms which sustain combustion. The basic sequence of reactions involved is outlined in the following reactions:

$$CBrClF_2 + H\cdot \longrightarrow CClF_2\cdot + HBr \qquad (1.8.1)$$

$$HBr + H\cdot \longrightarrow Br\cdot + H_2 \qquad (1.8.2)$$

Chain reaction

$$Br\cdot + H\cdot \longrightarrow HBr \qquad (1.8.3)$$

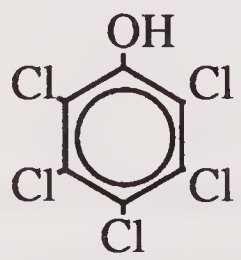
Halons are used in automatic fire extinguishing systems, such as those located in flammable solvent storage areas, and in specialty fire extinguishers, such as those on aircraft. As of 1989, there were no substitutes available for halons that had their same excellent performance characteristics.

All of the chlorofluorocarbons and halons discussed above have been implicated in the halogen atom-catalyzed destruction of atmospheric ozone. As a result of U. S. Environmental Protection Agency regulations imposed in accordance with the 1986 Montreal Protocol on Substances that Deplete the Ozone Layer, production of CFCs and halocarbons in the U. S. was curtailed starting in 1989. The most likely substitutes for these halocarbons are hydrogen-containing chlorofluorocarbons (HCFCs) and hydrogen-containing fluorocarbons (HFCs). The substitute compounds to be produced commercially first are CH_2FCF_3 (HFC-134a, a substitute for CFC-12 in automobile air conditioners and refrigeration equipment), $CHCl_2CF_3$ (HCFC-123, substitute for CFC-11 in plastic foam-blowing), CH_3CCl_2F (HCFC-141b, substitute for CFC-11 in plastic foam-blowing), $CHClF_2$ (HCFC-22, air conditioners and manufacture of plastic foam food containers). Because of the more readily broken H-C bonds that they contain, these compounds are more easily destroyed by atmospheric chemical reactions (particularly with hydroxyl radical, see Section 2.12) before they reach the stratosphere. Relative to a value of 1.0 for CFC-11, the ozone-depletion potentials of these substitutes are HFC-134a, 0; HCFC-123, 0.016; HCFC-141b, 0.081; and HCFC-22, 0.053. Concern has been expressed over toxicities and fire hazards of HCFCs, which are more reactive both chemically and biochemically than the CFCs and halons that they are designed to replace. The four leading substitutes, like the CFCs that they are designed to replace, have shown no evidence of causing skin or eye irritation, birth defects, or other short term toxic effects.[3]

The Du Pont Company, which introduced chlorofluorocarbons in the 1930s and is the largest manufacturer of them, has announced that it intends to cease production shortly after the year 2000. The company began to manufacture HFC substitutes in 1991.

Chlorinated Phenols

The chlorinated phenols, particularly **pentachlorophenol**,



Pentachlorophenol

and the trichlorophenol isomers are significant hazardous wastes. These compounds are biocides that are used to treat wood to prevent rot by fungi and to prevent termite infestation. They are toxic, causing liver malfunction and dermatitis; contaminant

polychlorinated dibenzodioxins may be responsible for some of the observed effects. Wood preservative chemicals such as pentachlorophenol are encountered at many hazardous waste sites in wastewaters and sludges.

Organosulfur Compounds

Sulfur is chemically similar to, but more diverse than oxygen. Whereas, with the exception of peroxides, most chemically combined organic oxygen is in the -2 oxidation state, sulfur occurs in the -2, +4, and +6 oxidation states. Many organosulfur compounds are noted for their foul, "rotten egg" or garlic odors. A number of example organosulfur compounds are shown in Figure 1.19.

Thiols and Thioethers

Substitution of alkyl or aryl hydrocarbon groups such as phenyl and methyl for H on hydrogen sulfide, H_2S, leads to a number of different organosulfur **thiols** (mercaptans, R–SH) and **sulfides**, also called thioethers (R–S–R). Structural formulas of examples of these compounds are shown in Figure 1.19.

Methanethiol and other lighter alkyl thiols are fairly common air pollutants that have "ultragarlic" odors; both 1- and 2-butanethiol are associated with skunk odor. Gaseous methanethiol is used as an odorant leak-detecting additive for natural gas, propane, and butane; it is also employed as an intermediate in pesticide synthesis. A toxic, irritating volatile liquid with a strong garlic odor, 2-propene-1-thiol (allyl mercaptan) is a typical alkenyl mercaptan. Benzenethiol (phenyl mercaptan), is the simplest of the aryl thiols. It is a toxic liquid with a severely "repulsive" odor.

Alkyl sulfides or thioethers contain the C-S-C functional group. The lightest of these compounds is dimethyl sulfide, a volatile liquid (bp 38°C) that is moderately toxic by ingestion. Cyclic sulfides contain the C-S-C group in a ring structure. The most common of these compounds is thiophene, a heat-stable liquid (bp 84°C) with a solvent action much like that of benzene, that is used in the manufacture of pharmaceuticals, dyes, and resins. Its saturated analog is tetrahydrothiophene, or thiophane.

Nitrogen-Containing Organosulfur Compounds

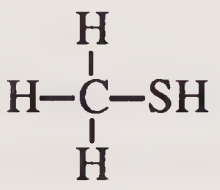
Many important organosulfur compounds also contain nitrogen. One such compound is **thiourea**, the sulfur analog of urea. Its structural formula is shown in Figure 1.19. Thiourea and **phenylthiourea** have been used as rodenticides. Commonly called ANTU, **1-naphthylthiourea** is an excellent rodenticide that is virtually tasteless and has a very high rodent:human toxicity ratio.

Sulfoxides and Sulfones

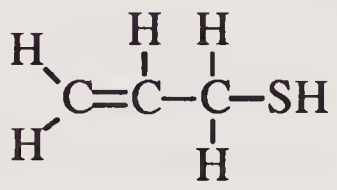
Sulfoxides and **sulfones** (Figure 1.19) contain both sulfur and oxygen. **Dimethylsulfoxide** (DMSO) is a liquid with numerous uses and some very interesting properties. It is used to remove paint and varnish, as a hydraulic fluid, mixed with water as an antifreeze solution, and in pharmaceutical applications as an anti-inflammatory and bacteriostatic agent. A polar aprotic (no ionizable H) solvent with a relatively high dielectric constant, **sulfolane** dissolves both organic and inorganic

solutes. It is the most widely produced sulfone because of its use in an industrial process called BTX processing in which it selectively extracts benzene, toluene, and xylene from aliphatic hydrocarbons; as the solvent in the Sulfinol process by which thiols and acidic compounds are removed from natural gas; as a solvent for polymerization reactions; and as a polymer plasticizer.

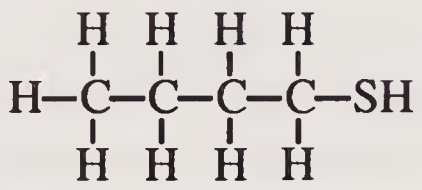
Thiols



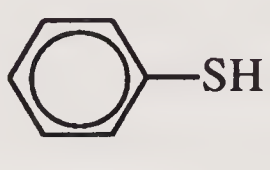
Methanethiol



2-propene-1-thiol

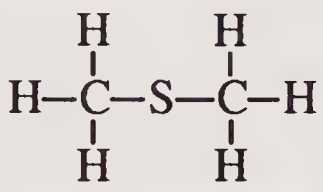


1-Butanethiol

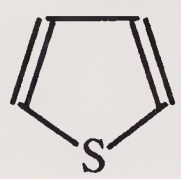


Benzenethiol

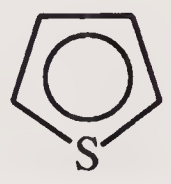
Sulfides and cyclic sulfides



Dimethyl sulfide

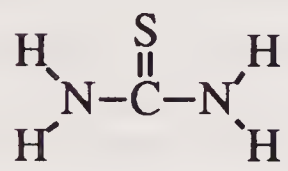


Thiophene (an unsaturated unsaturated sulfide)

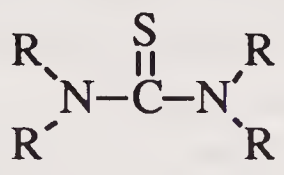


Thiophane

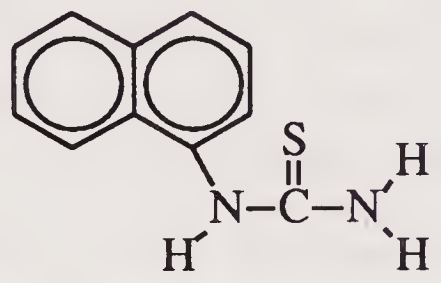
Thiourea compounds



Thiourea

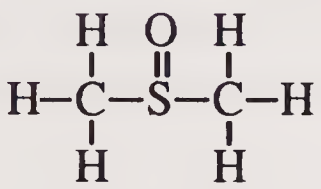


Organic derivatives of thiourea (R represents hydrocarbon substituents)



1–Naphthylthiourea (ANTU)

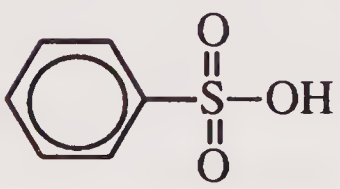
Sulfoxides and Sulfones



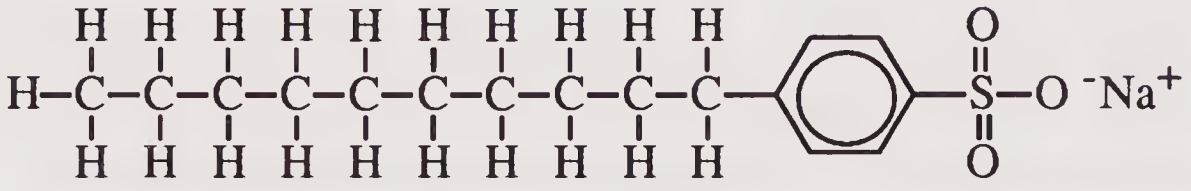
Dimethylsulfoxide (DMSO)

Sulfolane

Sulfonic acids and salts

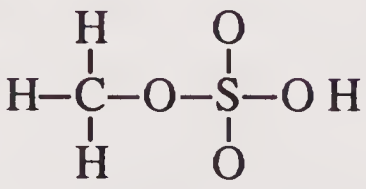


Benzenesulfonic acid

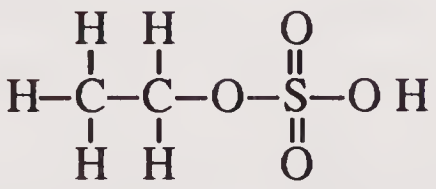


Sodium 1–(*p* –sulfophenyl)decane

Organosulfate esters



Methylsulfuric acid



Ethylsulfuric acid

Figure 1.19. Examples of organosulfur compounds.

Sulfonic Acids, Salts, and Esters

Sulfonic acids and sulfonate salts contain the $-SO_3H$ and $-SO_3^-$ groups, respectively, attached to a hydrocarbon moiety. The structural formula of benzenesulfonic acids and of sodium 1-(*p*-sulfophenyl)decane, a biodegradable detergent surfactant, are shown in Figure 1.19. The common sulfonic acids are water-soluble strong acids that lose virtually all ionizable H^+ in aqueous solution. They are used commercially to hydrolyze fat and oil esters to fatty acids and glycerol.

Organic Esters of Sulfuric Acid

Replacement of 1 H on sulfuric acid, H_2SO_4, yields an acid ester and replacement of both yields an ester. Examples of these esters are shown in Figure 1.19. Sulfuric acid esters are used as alkylating agents, which act to attach alkyl groups (such as methyl) to organic molecules, in the manufacture of agricultural chemicals, dyes, and drugs. **Methylsulfuric acid** and **ethylsulfuric acid** are oily water-soluble liquids that are strong irritants to skin, eyes, and mucous tissue.

Organophosphorus Compounds

Alkyl and Aryl Phosphines

The first two examples in Figure 1.20, illustrate that the structural formulas of alkyl and aryl phosphine compounds may be derived by substituting organic groups for the H atoms in phosphine (PH_3), the hydride of phosphorus discussed as a toxic inorganic compound in Section 12.10. **Methylphosphine** is a colorless, reactive gas. Crystalline, solid **triphenylphosphine** has a low reactivity and moderate toxicity when inhaled or ingested.

As shown by the reaction,

$$4C_3H_9P + 26O_2 \rightarrow 12O_2 + 18H_2O + P_4O_{10} \qquad (1.8.4)$$

combustion of aryl and alkyl phosphines produces P_4O_{10}, a corrosive irritant toxic substance that reacts with moisture in the air to produce droplets of corrosive orthophosphoric acid, H_3PO_4.

Organophosphate Esters

The structural formulas of three esters of orthophosphoric acid (H_3PO_4) and an ester of pyrophosphoric acid ($H_4P_2O_6$) are shown in Figure 1.20. Although **trimethylphosphate** is considered to be only moderately toxic, **tri-*o*-cresyl-phosphate, TOCP,** has a notorious record of poisonings. **Tetraethylpyrophosphate, TEPP,** was developed in Germany during World War II as a substitute for insecticidal nicotiné. Although it is a very effective insecticide, its use in that application was of very short duration because it kills almost everything else, too.

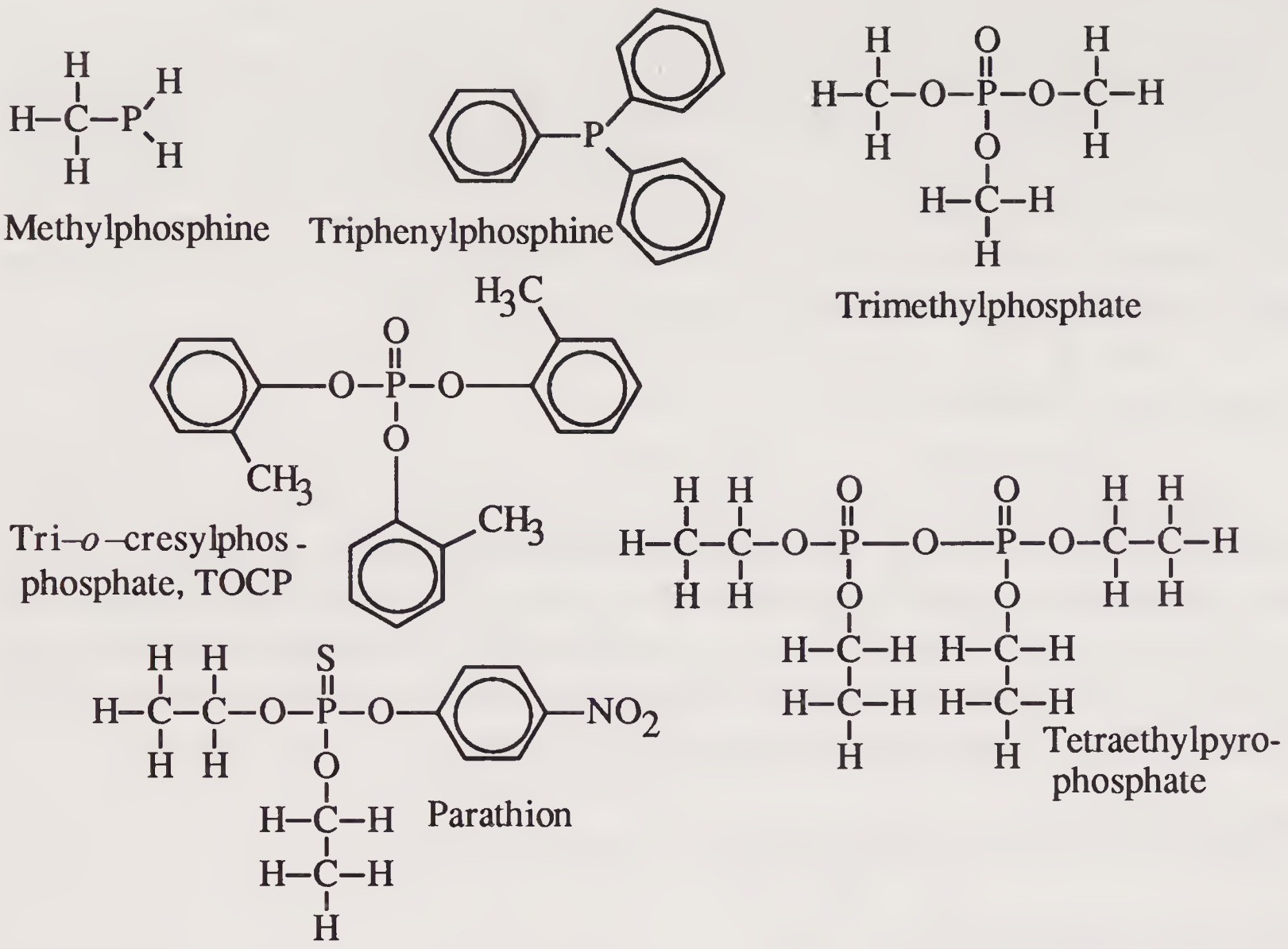


Figure 1.20. Some representative organophosphorus compounds.

Phosphorothionate Esters

Parathion, shown in Figure 1.20, is an example of **phosphorothionate** esters. These compounds are used as insecticidal acetylcholinesterase inhibitors. They contain the P=S (thiono) group, which increases their insect:mammal toxicity ratios. Since the first organophosphate insecticides were developed in Germany during the 1930s and 1940s, many insecticidal organophosphate compounds have been synthesized. One of the earliest and most successful of these is **parathion**, *O,O*-diethyl-*O*-*p*-nitrophenylphosphorothionate (banned from use in the U.S. in 1991 because of its acute toxicity to humans). From a long-term environmental standpoint organophosphate insecticides are superior to the organohalide insecticides that they largely displaced because the organophosphates readily undergo biodegradation and do not bioaccumulate.

1.9. SYNTHETIC POLYMERS

A large fraction of the chemical industry worldwide is devoted to polymer manufacture, which is very important in the area of hazardous wastes, as a source of environmental pollutants, and in the manufacture of materials used to alleviate environmental and waste problems. Synthetic **polymers** are produced when small molecules called **monomers** bond together to form a much smaller number of very large molecules. Many natural products are polymers; for example, cellulose in wood, paper, and many other materials is a polymer of the sugar glucose. Synthetic polymers form the basis of many industries, such as rubber, plastics, and textiles manufacture.

An important example of a polymer is that of polyvinylchloride, shown in Figure 1.21. This polymer is synthesized in large quantities for the manufacture of water and sewer pipe, water-repellant liners, and other plastic materials. Other major polymers include polyethylene (plastic bags, milk cartons), polypropylene, (impact-resistant plastics, indoor-outdoor carpeting), polyacrylonitrile (Orlon, carpets), polystyrene (foam insulation), and polytetrafluoroethylene (Teflon coatings, bearings); the monomers from which these substances are made are shown in Figure 1.22.

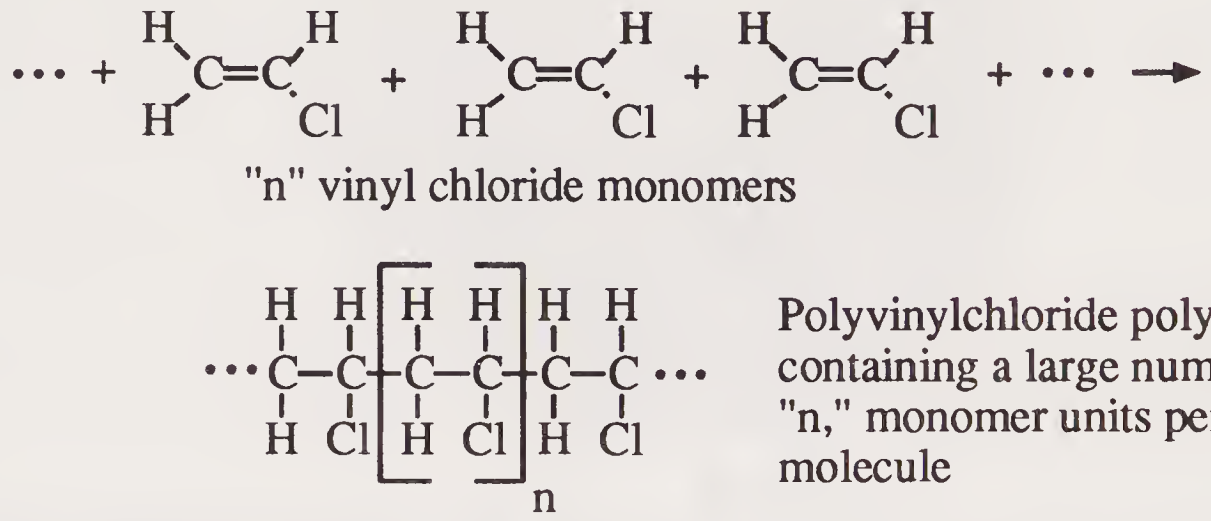


Figure 1.21. Polyvinylchloride polymer.

Many of the hazards from the polymer industry arise from the monomers used as raw materials. Many monomers are reactive and flammable, with a tendency to form explosive vapor mixtures with air. All have a certain degree of toxicity; vinyl chloride is a known human carcinogen. The combustion of many polymers may result in the evolution of toxic gases, such as hydrogen cyanide (HCN) from polyacrylonitrile or hydrogen chloride (HCl) from polyvinylchloride. Another hazard presented by plastics results from the presence of **plasticizers** added to provide essential properties, such as flexibility. The most widely used plasticizers are phthalates (see Chapter 14), which are environmentally persistent, resistant to treatment processes, and prone to undergo bioaccumulation.

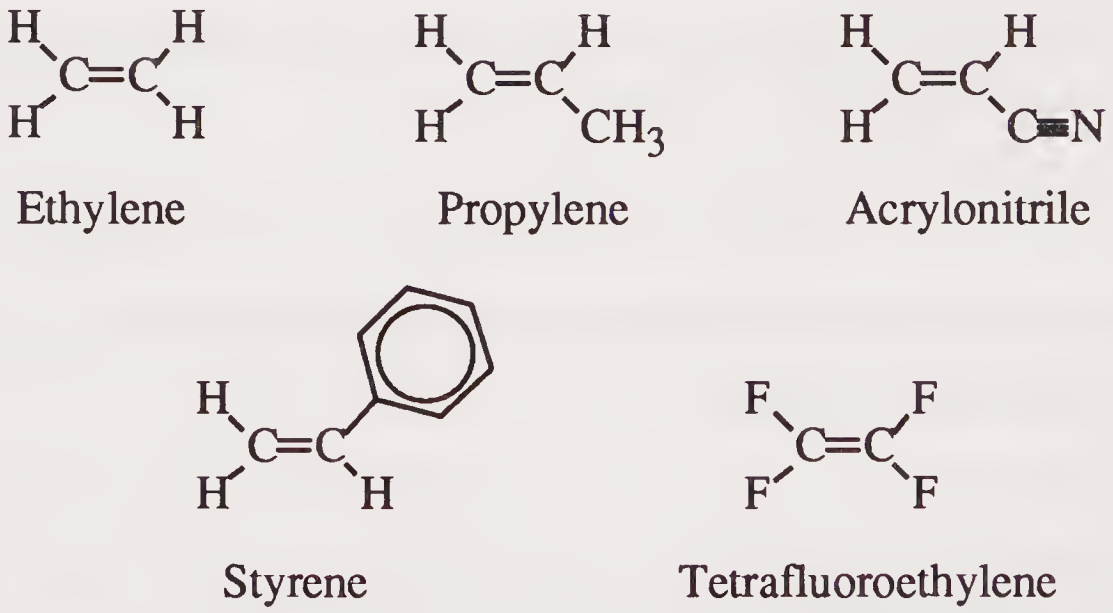


Figure 1.22. Monomers from which commonly used polymers are synthesized.

Polymers have a number of applications in waste treatment and disposal. Waste disposal landfill liners are made from synthetic polymers as are the fiber filters which remove particulate pollutants from flue gas in baghouses. Membranes used for ultrafiltration and reverse osmosis treatment of water are composed of very thin sheets of synthetic polymers. Organic solutes can be removed from water by sorption onto hydrophobic (water-repelling) organophilic beads of Amberlite XAD resin. Heavy

metal pollutants are removed from wastewater by cation exchange resins made of polymers with anionic functional groups. Typically, these resins exchange harmless sodium ion, Na^+, on the solid resin for toxic heavy metal ions in water. Figure 1.24 shows a segment of the polymeric structure of a cation exchange resin in the sodium form.

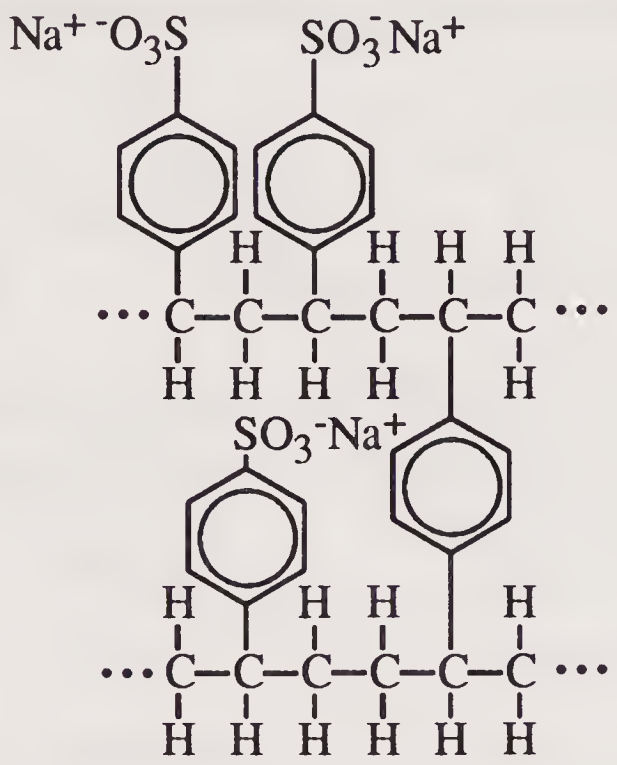


Figure 1.24. Polymeric cation exchanger in the sodium form.

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2. "Organic Chemistry," Chapter 26 in *Chemistry, The Central Science*, Theodore L. Brown, H. Eugene LeMay, Jr., and Bruce E. Bursten, Prentice Hall, Englewood Cliffs, NJ, 1991, pp. 938-969.
3. Zurer, Pamela S., "CFC Substitutes: Candidates Pass Early Toxicity Tests," *Chemical and Engineering News*, October 9, 1989, p. 4.

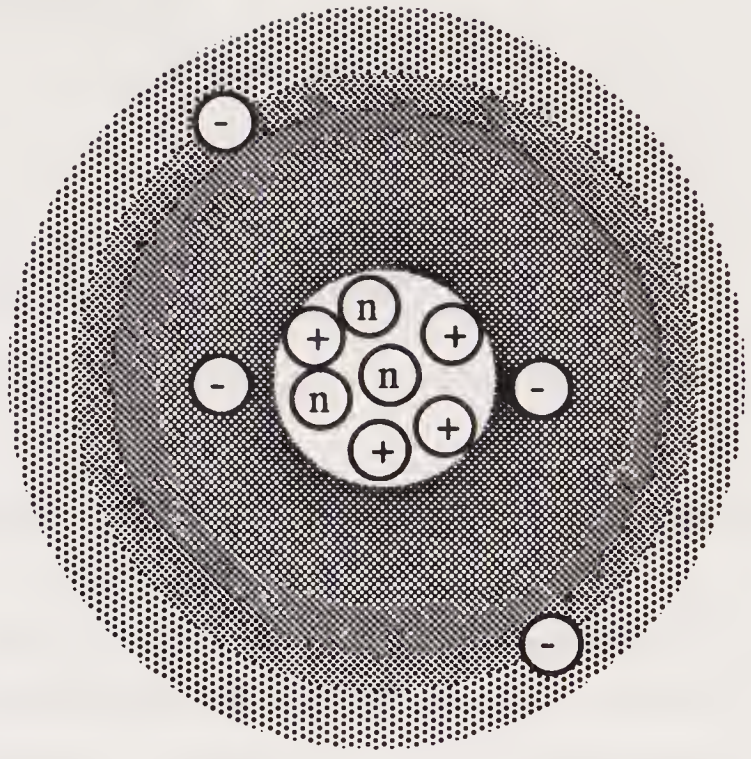
QUESTIONS

1. What distinguishes a radioactive isotope from a "normal" stable isotope?
2. Why is the periodic table so named?
3. Match the following:

1. O_2	(a) Element consisting of individual atoms
2. NH_3	(b) Element consisting of chemically bonded atoms
3. Ar	(c) Ionic compound
4. NaCl	(d) Covalently bound compound

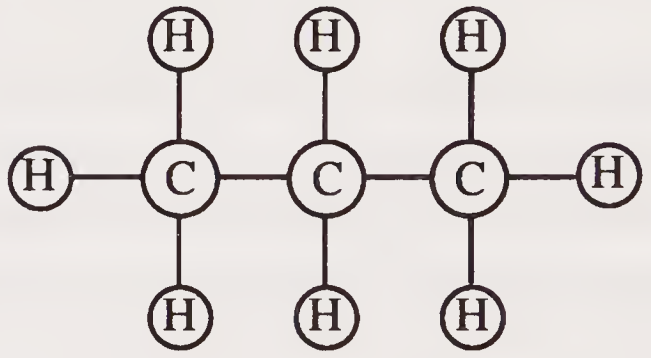
4. After examining Figure 1.7, consider what might happen when an atom of sodium (Na), atomic number 11, loses an electron to an atom of fluorine, (F), atomic number 9. What kinds of particles are formed by this transfer of a negatively charged electron? Is a chemical compound formed? What is it called?

5. Consider the following atom:



How many electrons, protons, and neutrons does it have? What is its atomic number? Give the name and chemical symbol of the element of which it is composed.

6. Give the chemical formula and molecular mass of the molecule represented below:



7. Calculate the molecular masses of (a) C_2H_2, (b) N_2H_4, (c) Na_2O, (d) O_3 (ozone), (e) PH_3, (f) CO_2.

8. Is the equation, $H_2 + O_2 \rightarrow H_2O$, a balanced chemical equation? Explain. Point out the reactants and products in the equation.

9. Define and distinguish the differences between environmental chemistry, environmental biochemistry, and toxicological chemistry.

10. An uncharged atom has the same number of _____ as protons. The electrons in an atom are contained in a cloud of _____ around the nucleus that occupies most of the _____ of the atom.

11. Match:

( ) Argon	1. A halogen
( ) Hydrogen	2. Fissionable element
( ) Uranium	3. Noble gas
( ) Chlorine	4. Has an isotope with a mass number of 2
( ) Mercury	5. Toxic heavy metal

12. The entry for each element in the periodic table gives the element's _____ _____ and the periodic table is arranged horizontally in _____ and vertically in _____.

13. Electrons in atoms occupy _____ in which electrons have different _____ _____. Each orbital may contain a maximum of _____electrons.

14. The Lewis symbol of carbon is _____ in which each dot represents a _____.

15. Elements that are generally solid, shiny in appearance, electrically conducting, and malleable are _____ whereas elements that tend to have a dull appearance, are not at all malleable, and frequently occur as gases or liquids are _____. Elements with intermediate properties are _____.

16. In the Lewis formula,

H : H

the two dots represent _____.

17. Explain why H_2 is not a chemical compound whereas H_2O is a chemical compound.

18. Using examples, distinguish between covalent and ionic bonds.

19. In terms of c, h, o, and the appropriate atomic masses, write a formula for the molecular mass of a compound with a general formula of $C_cH_hO_o$.

20. Considering oxidation/reduction phenomena, when Al reacts with O_2 to produce Al_2O_3, which contains the Al^{3+}ion, the Al is said to have been _____ and is in the _____ oxidation state.

21. Calculate the concentration in moles per liter of (a) a solution that is 27.0% H_2SO_4 by mass and that has a density of 1,198 g/L, and (b) of a solution that is 1 mg/L H_2SO_4 having a density of 1,000 g/L.

22. Calculate the pH of the second solution described in the preceding problem, keeping in mind that each H_2SO_4 molecule yields two H^+ ions.

23. Write a balanced neutralization reaction between NaOH and H_2SO_4.

24. Distinguish between solutions and colloidal suspensions.

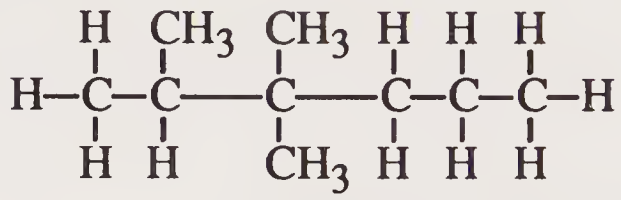
25. What is the nature of the Fe^{3+} ion? Why are solutions containing this ion acidic?

26. What kind of species is $Ni(CN)_4^{2-}$?

27. Explain the bonding properties of carbon that makes organic chemistry so diverse.

28. Distinguish among alkanes, alkenes, alkynes, and aryl compounds. To which general class of organic compounds do all belong?

29. In what sense are alkanes saturated? Why are alkenes more reactive than alkanes?
30. Name the compound below:



31. What is indicated by "*n*" in a hydrocarbon name?
32. Discuss the chemical reactivity of alkanes. Why are they chemically reactive or unreactive?
33. Discuss the chemical reactivity of alkenes. Why are they chemically reactive or unreactive?
34. What are the characteristics of aromaticity? What are the chemical reactivity characteristics of aromatic compounds?
35. Describe chain reactions, discussing what is meant by free radicals, and photochemical processes.
36. Define, with examples, what is meant by isomerism.
37. Describe how the two forms of 1,2-dichloroethylene can be used to illustrate *cis-trans* isomerism.
38. Give the structural formula corresponding to the condensed structural formula of $CH_3CH(C_2H_5)CH(C_2H_5)CH_2CH_3$.
39. Discuss how organic functional groups are used to define classes of organic compounds.
40. Give the functional groups corresponding to (a) alcohols, (b) aldehydes, (c) carboxylic acids, (d) ketones, (e) amines, (f) thiol compounds, and (g) nitro compounds.
41. Give an example compound of each of the following: epoxides, alcohols, phenols, ethers, aldehydes, ketones, and carboxylic acids.
42. Which functional group is characteristic of N-nitroso compounds, and why are these compounds toxicologically significant?
43. Give an example of each of the following: Alkyl halides, alkenyl halides, aryl halides.
44. Give an example compound of a chlorinated naphthalene and of a PCB.
45. What explains the tremendous chemical stability of CFCs? What kinds of compounds are replacing CFCs? Why?
46. How does a thio differ from a thioether?
47. How does a sulfoxides differ from a sulfones?
48. Which inorganic compound is regarded as the parent compound of alkyl and aryl phosphines? Give an example of each of these.
49. What are organophosphate esters and what is their toxicological significance?
50. Define what is meant by a polymer and give an example of one.

2

Environmental Chemistry

2.1. WHAT IS ENVIRONMENTAL CHEMISTRY?

Environmental chemistry has been defined as *the study of the sources, reactions, transport, effects, and fates of chemical species in the water, air, terrestrial and living environments.*[1] This definition is illustrated for the interactions and interchange of pollutants among the "spheres" of the environment as shown in Figure 2.1. To illustrate the definition of environmental chemistry, consider SO_2 in the environment. It may be produced and released in burning coal, which enters the

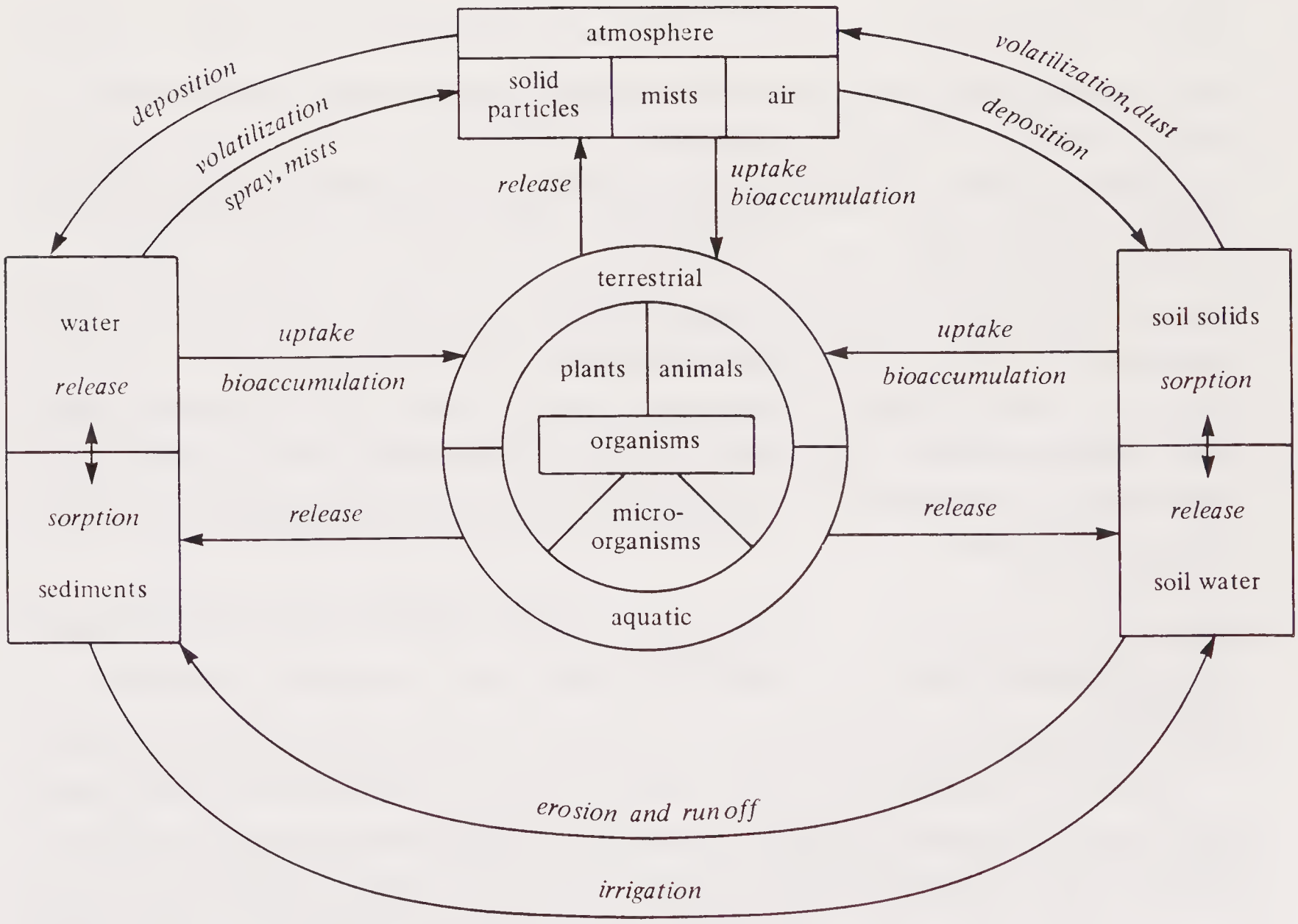


Figure 2.1. Interchange of pollutant species among the atmosphere, hydrosphere, geosphere, and biosphere.

atmosphere through the power plant stack, is transported through the atmosphere, and is oxidized to H_2SO_4 in the atmosphere. The sulfuric acid is washed from the atmosphere as "acid rain," which may harm plants, fish, or structures. Eventually the sulfuric acid reaches a semipermanent resting place, such as a body of water or the soil, known as a **sink**. As its definition implies, environmental chemistry can be divided into the four broad areas of aquatic chemistry, atmospheric chemistry, "terrestrial chemistry" (including geochemistry and soil chemistry), and environmental biochemistry, including toxicological chemistry.

2.2. AQUATIC CHEMISTRY

Water has a number of unique properties that are essential to life, many of which are due to water's ability to form hydrogen bonds. These characteristics are summarized in Table 2.1.

Table 2.1. Important Properties of Water

Property	Effects and Significance
Excellent solvent	Transport of nutrients and waste products, making biological processes possible in an aqueous medium
Highest dielectric constant of any common liquid	High solubility of ionic substances and their ionization in solution
Higher surface tension than any other liquid	Controlling factor in physiology; governs drop and surface phenomena
Transparent to visible and longer-wavelength fraction of ultraviolet light	Colorless, allowing light required for photosynthesis to reach considerable depths in bodies of water
Maximum density as a liquid at 4°C	Ice floats; vertical circulation restricted in stratified bodies of water
Higher heat of evaporation than any other material	Determines transfer of heat and water molecules between the atmosphere and bodies of water
Higher latent heat of fusion than any other liquid except ammonia	Temperature stabilized at the freezing point of water
Higher heat capacity than any other liquid except ammonia	Stabilization of temperatures of organisms and geographical regions

Figure 2.2 summarizes the more important aspects of **aquatic chemistry** as it applies to environmental chemistry. As shown in this figure, a number of chemical phenomena occur in water. Many aquatic chemical processes are influenced by the action of algae and bacteria in water. For example, it is shown that algal photosynthesis fixes inorganic carbon from HCO_3^- ion in the form of biomass (represented as $\{CH_2O\}$), in a process that also produces carbonate ion, CO_3^{2-}. Carbonate undergoes an acid-base reaction to produce OH^- ion and raise the pH, or it reacts with Ca^{2+} ion to precipitate solid $CaCO_3$. Most of the many oxidation-reduction reactions that occur in water are mediated (catalyzed) by bacteria. For example, bacteria convert inorganic nitrogen largely to ammonium ion, NH_4^+, in the oxygen-deficient

(anaerobic) lower layers of a body of water. Near the surface, where O_2 is available, bacteria convert inorganic nitrogen to nitrate ion, NO_3^-. Metals in water may be bound to organic chelating agents, such as pollutant nitrilotriacetic acid (NTA) or naturally occurring fulvic acids. Gases are exchanged with the atmosphere, and various solutes are exchanged between water and sediments in bodies of water.

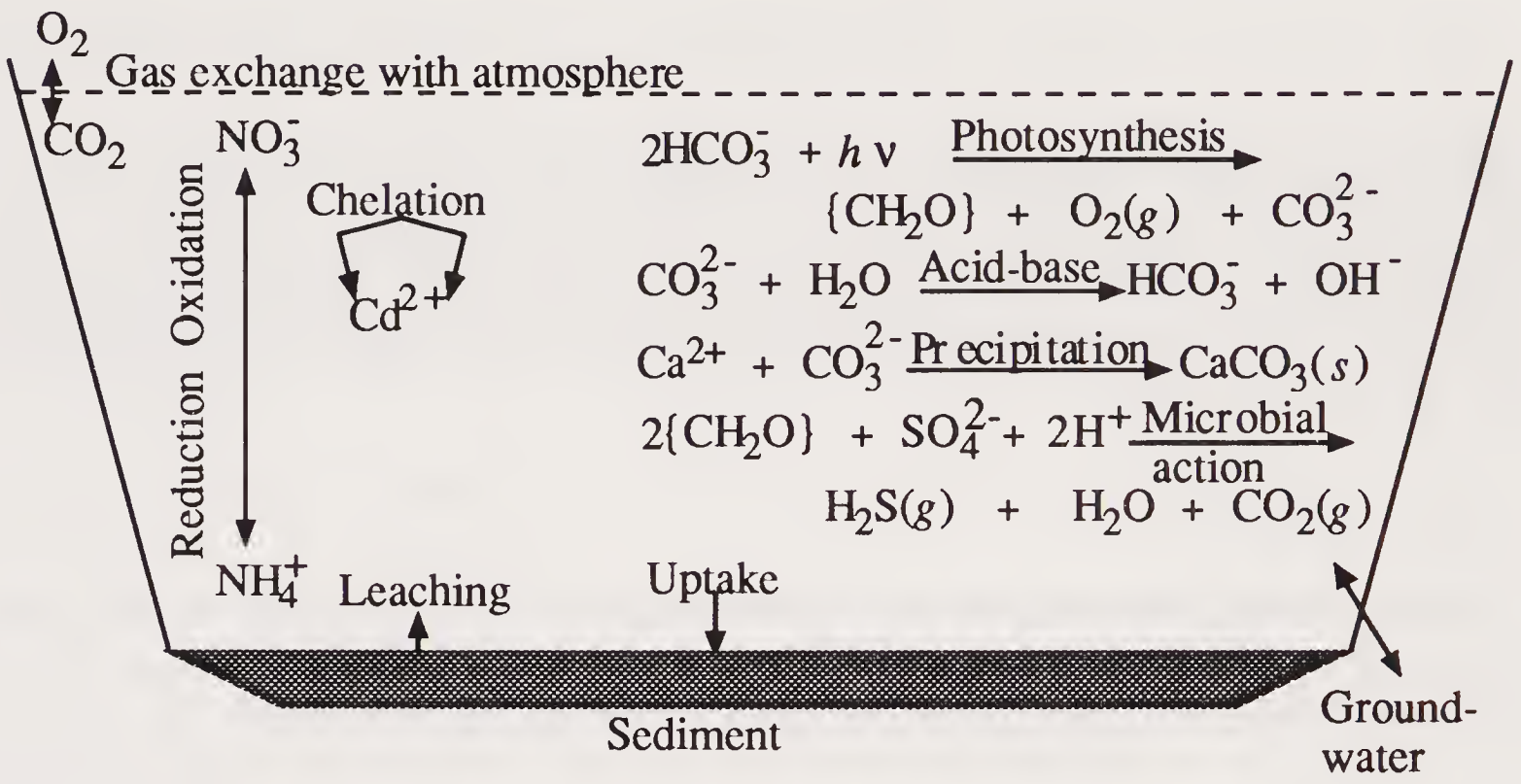


Figure 2.2. Major aquatic chemical processes.

Groundwater is of the utmost importance and concern as a water resource. This is because of groundwater's susceptibility to contamination by pollutants and improperly disposed wastes, such as those in poorly constructed landfills. Once a groundwater source is contaminated, it is extremely difficult to restore to its original condition.

Several important characteristics of unpolluted water should be noted. One of these is **gas solubility**. Since it is required to support aquatic life and maintain water quality, oxygen is the most important dissolved gas in water. Water in equilibrium with air at 25°C contains 8.3 milligrams per liter (mg/L) of dissolved O_2. Water **alkalinity** is defined as the ability of solutes in water to neutralize added strong acid. Alkalinity is generally due to bicarbonate ion, HCO_3^-, with contributions from OH^- and CO_3^{2-} at higher pH values. Water **hardness** is due to the presence of calcium ion, Ca^{2+}, and, to a lesser extent, magnesium ion, Mg^{2+}.

2.3. OXIDATION-REDUCTION

Oxidation-reduction (redox) reactions in water involve the transfer of electrons between chemical species. In natural water, wastewater, and soil, most significant oxidation-reduction reactions are carried out by bacteria, which are considered in Section 2.6.

The relative oxidation-reduction tendencies of a chemical system depend upon the activity of the electron e^-. When the electron activity is relatively high, chemical species (even including water) tend to accept electrons,

$$2H_2O + 2e^- \leftarrow\rightarrow H_2(g) + 2OH^- \qquad (2.3.1)$$

and are said to be reduced. When the electron activity is relatively low, the medium is **oxidizing**, and chemical species such as H_2O may be **oxidized** by the loss of electrons:

$$2H_2O \leftarrow\rightarrow O_2(g) + 4H^+ + 4e^- \qquad (2.3.2)$$

The relative tendency toward oxidation or reduction is based upon the electrode potential, E, which is relatively more positive in an oxidizing medium and negative in a reducing medium. It is defined in terms of the half reaction,

$$2H^+ + 2e^- \leftarrow\rightarrow H_2(g) \qquad (2.3.3)$$

for which E is defined as exactly zero when the activity of H^+ is exactly 1 (concentration approximately 1 mole per liter) and the pressure of H_2 gas is exactly 1 atmosphere. Because electron activity in water varies over many orders of magnitude, environmental chemists find it convenient to discuss oxidizing and reducing tendencies in terms of pE, a parameter analogous to pH and defined conceptually as the negative log of the electron activity:

$$pE = -\log(a_{e^-}) \qquad (2.3.4)$$

$$pH = -\log(a_{H^+}) \qquad (2.3.5)$$

The value of pE is calculated from E by the relationship,

$$pE = \frac{E}{\frac{2.303RT}{F}} \qquad (2.3.6)$$

$$\text{At } 25^\circ\text{C for E in volts, } pE = \frac{E}{0.0591}$$

where R is the gas constant, T is the absolute temperature, and F is the Faraday.

The nature of chemical species in water is usually a function of both pE and pH. A good example of this is shown by a simplified pE-pH diagram for iron in water, assuming that iron is in one of the four forms of Fe^{2+} ion, Fe^{3+} ion, solid $Fe(OH)_3$,or solid $Fe(OH)_2$ as shown in Figure 2.3. Water in which the pE is higher than that shown by the upper dashed line is thermodynamically unstable toward oxidation (Reaction 2.3.2), and below the lower dashed line water is thermodynamically unstable

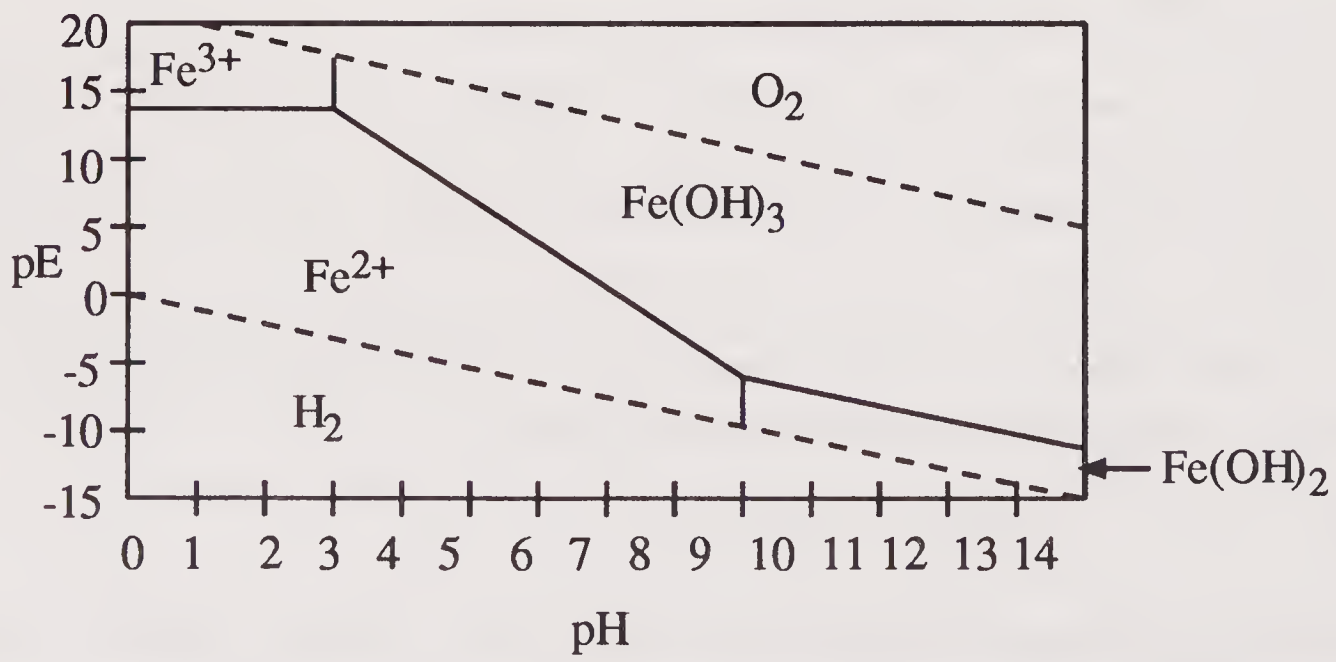


Figure 2.3. A simplified pE-pH diagram for iron in water (maximum total soluble iron concentration 1.0×10^{-5} M).

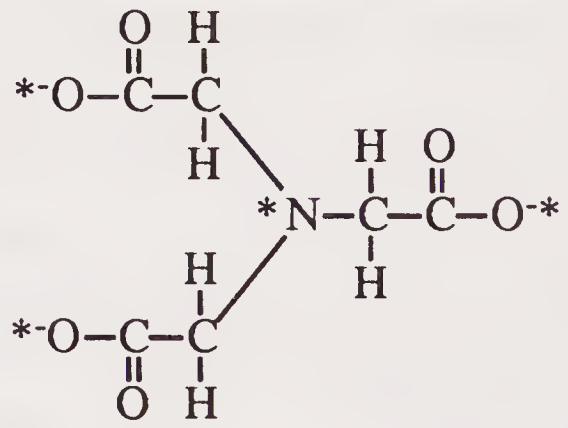
toward reduction (Reaction 2.3.1). It is seen that Fe^{3+} ion is stable only in a very oxidizing, acidic medium such as that encountered in acid mine water, whereas Fe^{2+} ion is stable over a relatively large region as reflected by the common occurrence of soluble iron(II) in oxygen-deficient groundwaters. Highly insoluble $Fe(OH)_3$ is the predominant iron species over a very wide pE-pH range.

2.4. COMPLEXATION AND CHELATION

Metal ions in water are always bonded to water molecules in the form of hydrated ions represented by the general formula, $M(H_2O)_x^{n+}$, from which the H_2O is often omitted for simplicity. Other species may be present that bond to the metal ion more strongly than does water. For example, cadmium ion, Cd^{2+}, reacts with cyanide ion, CN^-, as follows:

$$Cd^{2+} + CN^- \rightarrow CdCN^+ \qquad (2.4.1)$$

The product of the reaction is called a **complex** (or complex ion) and the cyanide ion is called a **ligand**. Some (particularly organic) ligands can bond with a metal ion in two or more places, forming particularly stable complexes. One such ligand is the nitrilotriacetate (NTA) ligand, which has the following formula:



This ion has four binding sites, each marked with an asterisk in the preceding illustration, which may simultaneously bond to a metal ion, forming a structure with three rings. Such a species is known as a **chelate**, and NTA is a **chelating** agent.

Complexation and chelation have strong effects upon metals in the environment and hazardous wastes. For example, complexation with negatively charged ligands may convert a soluble metal species from a cation, which is readily bound and immobilized by ion exchange processes in soil, to an anion, such as $Ni(CN)_4^{2-}$, that is not strongly held by soil. Thus, codisposal of metal salts and chelating agents can result in increased hazards from heavy metals. On the other hand, some chelating agents are used for the treatment of heavy metal poisoning and insoluble chelating agents, such as chelating resins, can be used to remove metals from waste streams. Metal ions chelated by hazardous waste chelating agents, such as NTA from metal plating bath solutions, may be especially mobile in water. Naturally occurring humic substance chelating agents may either increase or decrease the solubility of heavy metal wastes. [2]

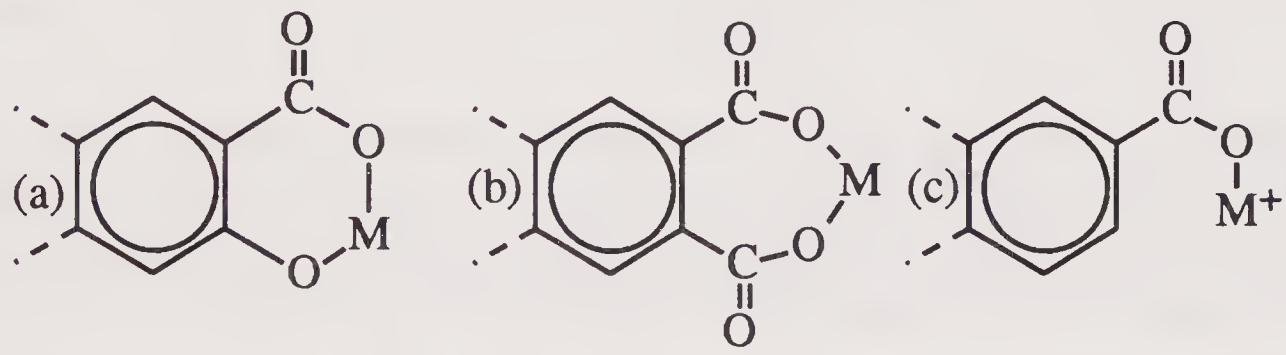
Complexation may affect the fates of environmental and hazardous waste ligand species. For example, whereas cyanide ion is very toxic, but easily oxidized in water, complexed cyanide ion is relatively less toxic, but more difficult to destroy.

Humic Substances

The most important class of complexing agents that occur naturally are the **humic substances.** These are degradation-resistant materials formed during the decomposition of vegetation that occur as deposits in soil, marsh sediments, peat, coal, lignite, or in almost any location where large quantities of vegetation have decayed. They are commonly classified on the basis of solubility. If a material containing humic substances is extracted with strong base, and the resulting solution is acidified, the products are (a) a nonextractable plant residue called **humin**; (b) a material that precipitates from the acidified extract, called **humic acid**; and (c) an organic material that remains in the acidified solution, called **fulvic acid**. (These terms do not refer to single compounds but to a wide range of compounds of generally similar origin, with many properties in common.) Because of their acid-base, sorptive, and complexing properties, both the soluble and insoluble humic substances have a strong effect upon the properties of water. In general, fulvic acid dissolves in water and exerts its effects as the soluble species. Humin and humic acid remain insoluble and affect water quality through exchange of species, such as cations or organic materials, with water.

Humic substances are polyelectrolytic macromolecules with molecular masses that range from a few hundred for fulvic acid to tens of thousands for the humic acid and humin fractions. These substances contain a carbon skeleton with a high degree of aromatic character and with a large percentage of the molecular mass incorporated in functional groups, most of which contain oxygen. The elementary composition of most humic substances is within the following ranges: C, 45-55%; O, 30-45%, H, 3-6%; N, 1-5%; and S, 0-1%.

The binding of metal ions by humic substances is one of the most important environmental qualities of humic substances. This binding can occur as chelation between a carboxyl group and a phenolic hydroxyl group, as chelation between two carboxyl groups, or as complexation with a carboxyl group (see below).



Binding of a metal ion, M^{2+}, by humic substances (a) by chelation between carboxyl and phenolic hydroxyl, (b) by chelation between two carboxyl groups, and (c) by complexation with a carboxyl group.

Organometallic Compounds

Another major type of metal species important in hazardous wastes consists of **organometallic compounds**, which differ from complexes and chelates in that the organic portion is bonded to the metal by a carbon-metal bond and the organic ligand is frequently not capable of existing as a stable separate species. Typical examples of organometallic compound species are monomethylmercury ion and dimethylmercury:

Hg^{2+}	$HgCH_3^+$	$Hg(CH_3)_2$
Mercury(II) ion	Monomethylmercury ion	Dimethylmercury

Organometallic compounds may enter the environment directly as pollutant industrial chemicals and some, including organometallic mercury, tin, selenium, and arsenic compounds, are synthesized biologically by bacteria. Some of these compounds are particularly toxic because of their mobilities in living systems and abilities to cross cell membranes.

2.5. WATER INTERACTIONS WITH OTHER PHASES

Most of the important chemical phenomena associated with water do not occur in solution, but rather through interaction of solutes in water with other phases. For example, the oxidation-reduction reactions catalyzed by bacteria occur in bacterial cells. Many organic hazardous wastes are carried through water as emulsions of very small particles suspended in water. Some hazardous wastes are deposited in sediments in bodies of water, from which they may later enter the water through chemical or physical processes and cause severe pollution effects.

Figure 2.4 summarizes some of the most significant types of interactions between water and other phases, including solids, immisicible liquids, and gases. Films of organic compounds, such as hydrocarbon liquids, may be present on the surface of water. Exposed to sunlight, these compounds are subject to photochemical reactions (see Section 2.10). Gases such as O_2 , CO_2, CH_4, and H_2S are exchanged with the atmosphere. Photosynthesis occurs in suspended cells of algae, and other biological processes, such as biodegradation of organic wastes, occur in bacterial cells. Particles contributing to the turbidity of water may be introduced by physical processes, including the erosion of streams or sloughing of water impoundment banks. Chemical processes, such as the formation of solid $CaCO_3$ illustrated in Figure 2.2, may also form particles in water.

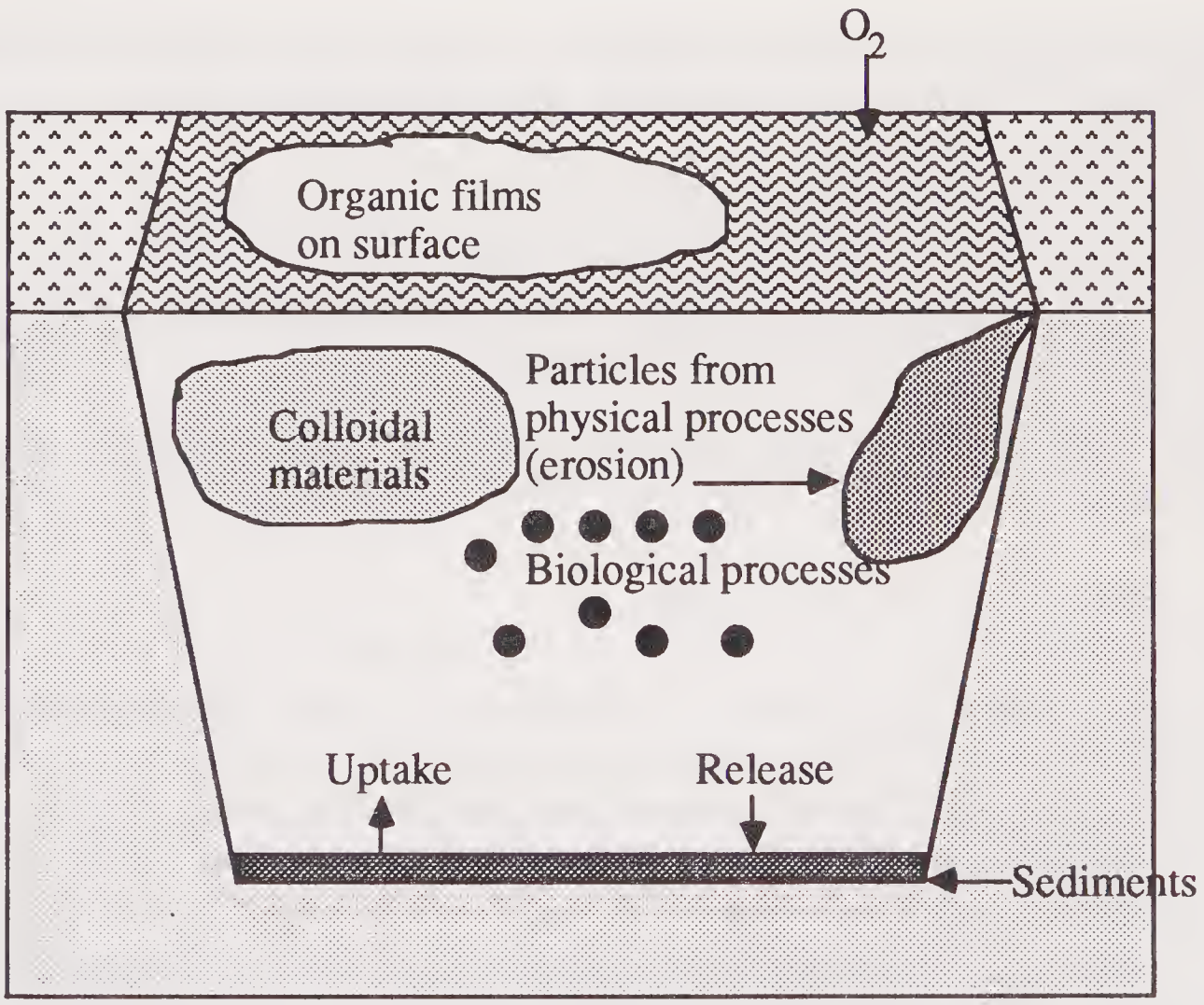


Figure 2.4. Aquatic chemical processes at interfaces between water and gases, solids, or other liquids.

Sediments are repositories of a wide variety of chemical species and the site of many chemical and biochemical processes. Anaerobic fermentation of organic matter by bacteria (see Table 2.1) produces methane gas that is evolved from sediments. Similar bacteria produce mobile $HgCH_3^+$ and $Hg(CH_3)_2$ from insoluble, relatively harmless inorganic mercury compounds. Sediments are sinks for many hazardous organic compounds and heavy metal salts that have gotten into water.

Colloidal particles are very small particles ranging from 0.001 micrometer (μm) to 1 μm in diameter. Colloids have a strong influence on aquatic chemistry. Because of their extremely small size, these particles have a very high surface-to-volume ratio. They are small enough to remain suspended in water, enabling maximum exposure to the water and solutes dissolved in it. Toxic substances in colloidal form are much more available to organisms in water than are such substances in bulk form. Special measures are required to remove colloidal particles from water. Usually, chemical treatment measures are applied to cause colloidal particles to aggregate together (processes called **coagulation** or **flocculation**), and the solids are removed by filtration.

The ability to undergo **ion exchange** processes is an important characteristic of some solids in contact with water. These processes are usually cation exchange reactions represented by

$$\{Solid\}^-M^+ + Z \leftarrow\rightarrow \{Solid\}^-Z^+ + M^+ \qquad (2.5.1)$$

where M^+ and Z^+ represent two different cations. Cationic water pollutants and hazardous wastes may be retained on sediments by cation exchange.

2.6. BACTERIA IN AQUATIC CHEMISTRY

Bacteria in water use oxidation-reduction reactions to extract the energy that they need for their own growth and reproduction. In so doing, bacteria have a very strong influence on aquatic chemistry.

Bacteria, which are discussed in considerable detail in Chapters 5-7, are single-celled organisms roughly a micrometer in size. Some bacteria require elemental molecular O_2 for their metabolic needs and are called **aerobic bacteria**. Other **anaerobic bacteria** extract their oxygen from sources such as NO_3^-, SO_4^{2-}, and organic matter, represented as $\{CH_2O\}$.

The most common bacterially mediated reaction in water and soil is the oxidation of organic matter,

$$O_2 + \{CH_2O\} \rightarrow CO_2(g) + H_2O \qquad (2.6.1)$$

a process called **aerobic respiration**. It provides the means for degrading organic wastes, such as sewage. If this reaction occurs at a rate faster than processes for replenishing oxygen in a body of water, the level of dissolved oxygen may become so diminished that the water no longer supports fish life. Some other important oxidation-reduction reactions carried out by bacteria in water are given in Table 2.1.

Bacteria interact with environmental chemicals, such as hazardous wastes, in a number of ways. Some very toxic materials impede, or even totally stop, bacterial action. A number of organic wastes are partially or completely degraded by bacteria. In a few cases, the organic products are even more toxic than the original pollutants. Bacteria can modify the soil and water medium in which pollutants or hazardous

wastes are contained, thus affecting the mobility of the wastes. Prominently, bacterial degradation of organic matter (Reaction 2.6.1) can consume all the oxygen in soil or sediment and greatly lower the pE. Under these conditions, anaerobic bacteria can reduce insoluble iron(III) oxide (hydroxide or hydrated oxide) to soluble Fe^{2+} ion. This process may release heavy metal ions incorporated with the insoluble iron(III) oxide or soluble iron(II) salts can be mobilized and seep from the site, resulting in iron contamination of the surrounding area.

Table 2.2. Some Oxidation-Reduction Reactions Mediated by Bacteria

Reaction	Significance
$2\{CH_2O\} \rightarrow CH_4 + CO_2$	Fermentation reaction, atmospheric methane source
$SO_4^{2-} + 2\{CH_2O\} + 2H^+ \rightarrow H_2S(g) + 2CO_2 + 2H_2O$	Sulfate reduction, source of atmospheric H_2S
$2FeS_2 + 2H_2O + 7O_2 \rightarrow 4H^+ + 4SO_4^{2-} + 2Fe^{2+}$	Production of acid mine water
$4NO_3^- + 5\{CH_2O\} + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$	Denitrification (conversion of fixed nitrogen back to atmospheric N_2)

2.7. WATER POLLUTANTS

Natural waters are afflicted with a wide variety of inorganic, organic, and biological pollutants, a significant fraction of which come from the improper disposal of hazardous wastes. In some cases, such as that of highly toxic cadmium, a pollutant is directly toxic at a relatively low level. In other cases the pollutant itself is not toxic, but its presence results in conditions detrimental to water quality. For example, biodegradable organic matter in water is often not toxic, but the consumption of oxygen during its degradation prevents the water from supporting fish life. Some contaminants, such as NaCl, are normal constituents of water at low levels, but harmful pollutants at higher levels. Table 2.3 (page 52) summarizes the major water pollutants and their significance.

2.8. WATER TREATMENT

The treatment of water can be considered under the two major categories of (1) treatment before use and (2) treatment of contaminated water after it has passed through a municipal water system or industrial process. In both cases, consideration must be given to contamination by toxic pollutants and hazardous wastes. In many areas, contamination by such wastes is threatening sources of municipal water supply and putting extra demands upon water treatment processes. A major objective of many hazardous waste treatment processes is the removal of water that can be safely discharged to municipal waste treatment processes (publicly owned treatment works, POTW).

Table 2.3. General Categories of Water Pollutants

Pollutant category	Examples and significance
Asbestos	Fibrous minerals known to cause lung cancer when inhaled. Effects in water are unknown.
Alkalinity, acidity, salinity	HCO_3^-, H_2SO_4, and NaCl, respectively. Harmful pollutants if in excess.
Biochemical oxygen demand, BOD	Organic matter that consumes oxygen when degraded. Affects water quality, reduces dissolved O_2 levels.
Carcinogens	Aflatoxins, nitrosamines, polycyclic aromatic hydrocarbons (PAHs), others. May cause cancer.
Detergents	Surface active agents and their builders. Harm wildlife, affect esthetics.
Fertilizers (algal nutrients)	Phosphates, K^+, NO_3^-. Cause excessive algal growth. Subsequent decay of algal biomass consumes oxygen and causes eutrophication of water.
Inorganic pollutants	CN^-, NH_3, SO_2. Toxicity, esthetics, general detrimental effects on water quality.
Metal species bound with organic entities	Heavy metal chelates, organometallic compounds. Toxicity, metal transport.
Pathogens	Disease-causing bacteria and viruses. Cause illness. Impose constraints on water reuse and increase difficulty of water treatment.
Pesticides	Parathion, carbaryl. Toxic to humans and wildlife.
Petroleum wastes, "oil spills"	Petroleum and petroleum products. Harmful to wildlife, esthetics.
Radionuclides	Radium, plutonium. Allowable only at very low levels in drinking water.
Sediments	Detrimental to wildlife and esthetics. Fill streams and and impoundments.
Sewage	Sanitary and other wastes discharged into sewer systems. Toxic, contains pathogens, consumes oxygen during biodegradation.
Trace elements and heavy metals	Cadmium, lead, arsenic. Directly toxic to wildlife and humans.
Trace organics	Aromatics, organohalides. Toxic, poorly biodegradable.

Several operations may be employed to treat water prior to use. Aeration is employed to drive off odorous gases, such as H_2S, and to oxidize soluble Fe^{2+} and Mn^{2+} ions to insoluble forms. Lime is added to remove dissolved calcium (water hardness) as solid $CaCO_3$. Aluminum sulfate coagulant may be added to form a sticky precipitate of $Al(OH)_3$, which pulls very fine particles from suspension. Various

filtration and settling processes are employed. Chlorine, Cl_2, is added to kill bacteria. However, chlorine may react with humic substances in the water to produce toxic halogenated byproducts. These can be removed by activated carbon filtration, although it is preferable to eliminate the organic materials from which the halogenated byproducts are formed prior to chlorination.

Municipal wastewater may be subjected to primary, secondary, or advanced water treatment. **Primary** water treatment consists of settling and skimming operations that remove grit, grease, and physical objects from water. **Secondary** water treatment is designed to take out biochemical oxygen demand, BOD. This is normally accomplished by introducing air and microorganisms such that biomass $\{CH_2O\}$ undergoes aerobic respiration (see Section 2.6) to remove biodegradable biomass:

$$\{CH_2O\} + O_2 \rightarrow CO_2 + H_2O \qquad (2.8.1)$$

This process is most commonly carried out in an activated sludge unit, as shown in Figure 2.5, in which biodegradation occurs in a tank through which water is pumped. Water from this tank is transferred to a settling basin where the microorganisms responsible for the degradation settle out as a sludge. The sludge is then pumped back to the tank to provide a high concentration of bacteria for the degradation process. Excess sewage sludge tends to collect hazardous waste substances, particularly heavy metals and insoluble organic species, suggesting caution in its disposal or use as fertilizer.

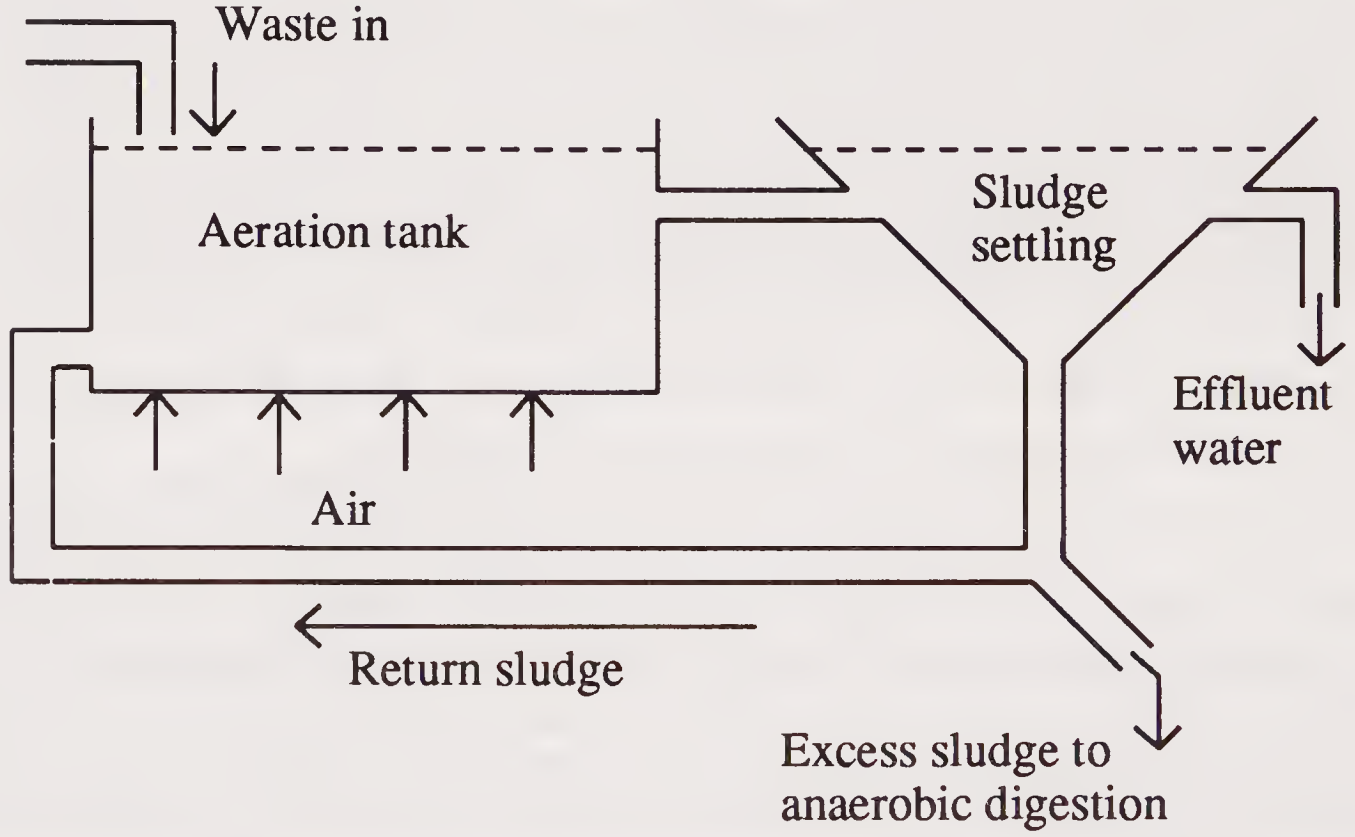


Figure 2.5. Activated sludge waste treatment process.

Advanced waste treatment is used when it is necessary to upgrade secondary sewage effluent for release to receiving waters or for further use. Special filtration techniques can be employed to remove small particles left from secondary wastewater treatment. Filtration over activated carbon removes particulate matter and even some dissolved organic substances. Special measures are employed to take out nitrate and phosphate "fertilizers," which tend to cause eutrophication in receiving waters (see Table 2.3). Excess salts can be removed by several processes, the most successful of which is reverse osmosis, in which water is forced under high pressure through a membrane that allows passage of H_2O molecules, but not dissolved salts.

2.9. THE GEOSPHERE AND SOIL

The **geosphere**, or solid Earth, is that part of the Earth upon which humans live and from which they extract most of their food, minerals, and fuels. Once thought to have an almost unlimited buffering capacity against the perturbations of humankind, the geosphere is now known to be rather fragile and subject to harm by human activities. For example, some billions of tons of Earth material are mined or otherwise disturbed each year in the extraction of minerals and coal. Two atmospheric pollutant phenomena — excess carbon dioxide and acid rain — have the potential to cause major changes in the geosphere. Too much carbon dioxide in the atmosphere may cause global heating ("greenhouse effect"), which could significantly alter rainfall patterns and turn currently productive areas of the Earth into desert regions. The low pH characteristic of acid rain can bring about drastic changes in the solubilities and oxidation-reduction rates of minerals. Erosion caused by intensive cultivation of land is washing away vast quantities of topsoil from fertile farmlands each year. In some areas of industrialized countries, the geosphere has been the dumping ground for toxic chemicals. It may be readily seen that the preservation of the geosphere in a form suitable for human habitation is one of the greatest challenges facing humankind.

Soil

Soil consists of a large variety of material composing the uppermost layer of the earth's crust upon which plants grow. Typically, soil consists of about 95% mineral matter and 5% organic material, although it may vary from almost pure mineral matter to almost pure organic material. The bulk of finely divided mineral matter in soil consists of weathered products of bedrock.

Soils are formed by the weathering (physical and chemical disintegration) of parent rocks as the result of interactive geological, hydrological, and biological processes. Soils are porous and are vertically stratified into horizons through the action of downward-percolating water and biological processes, including the production and decay of biomass. Soils are open systems that undergo continual exchange of matter and energy with the atmosphere, hydrosphere, and biosphere.

Some of the main environmental chemical aspects of soil are illustrated in Figure 2.6. The most active and important part of soil is **topsoil**, the layer in which plants are rooted and in which most biological activity occurs. In addition to finely divided mineral matter, topsoil contains water and air. During the growing season substantial amounts of water are lost to the atmosphere by sorption through plant roots followed by evaporation from leaves, a process called **transpiration**. Soil is a very active medium for biochemical processes, including bacterial fixation of atmospheric nitrogen, biodegradation of degradable organics, and oxidation-reduction transitions of inorganic substances, such as the bacterially mediated oxidation of fertilizer ammonia to nitrate, which can be utilized by plants:

$$NH_3 + 2O_2 \text{ (bacteria)} \rightarrow H^+ + NO_3^- + H_2O \qquad (2.9.1)$$

The Soil Solution

The **soil solution** is the aqueous portion of soil that contains dissolved matter from soil chemical and biochemical processes and from exchange with the hydrosphere and biosphere. This medium transports chemical species to and from soil

particles and provides intimate contact between the solutes and the soil particles. In addition to providing water for plant growth, it is an essential pathway for the exchange of plant nutrients between roots and solid soil.

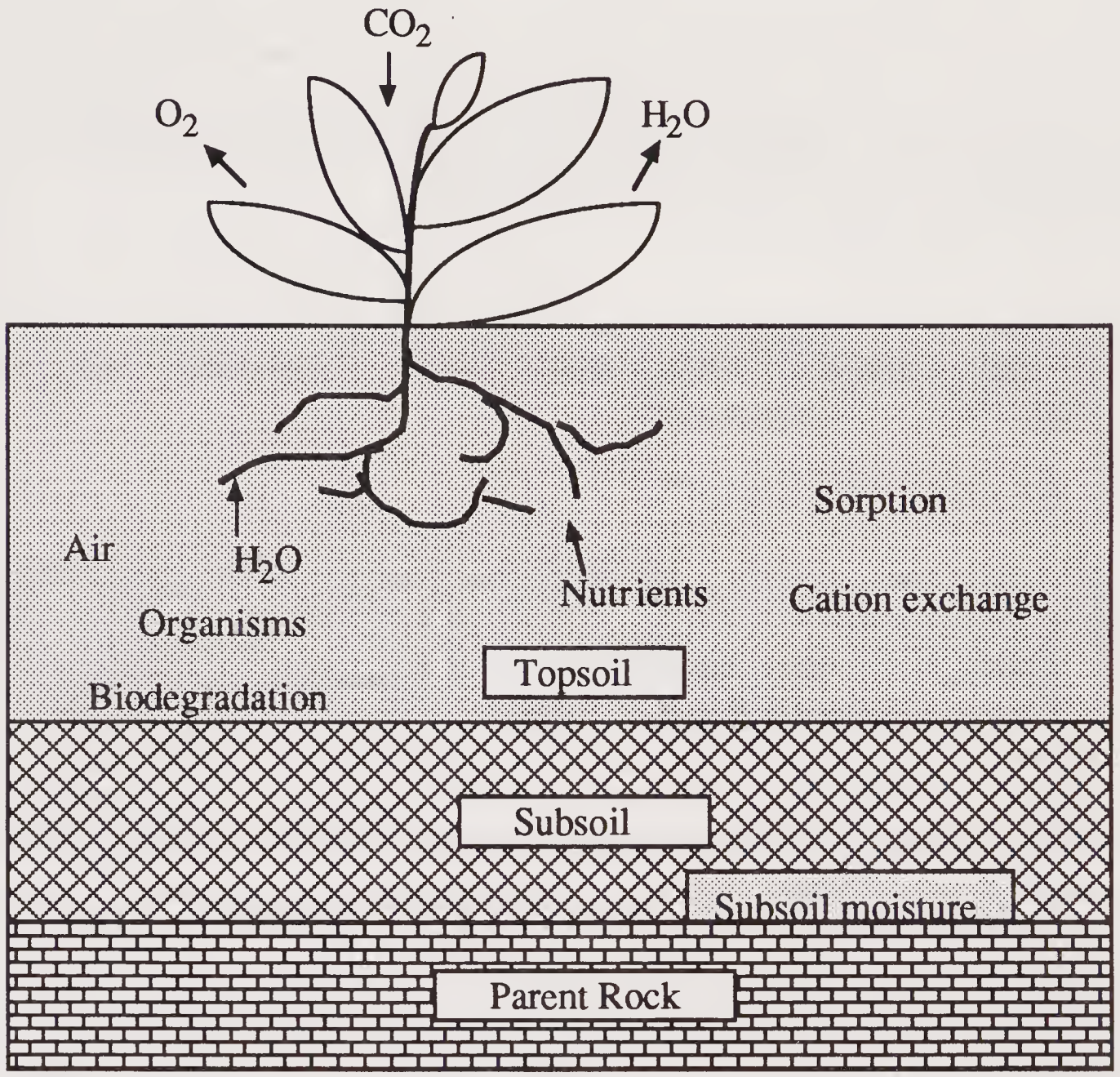


Figure 2.6. Layers of soil.

2.10. THE ATMOSPHERE

The **atmosphere** consists of the thin layer of mixed gases covering the earth's surface. Exclusive of water, atmospheric air is 78.1% (by volume) nitrogen, 21.0% oxygen, 0.9% argon, and 0.03% carbon dioxide. Normally, air contains 1-3% water vapor by volume. In addition, air contains a large variety of trace level gases at levels below 0.002%, including neon, helium, methane, krypton, nitrous oxide, hydrogen, xenon, sulfur dioxide, ozone, nitrogen dioxide, ammonia, and carbon monoxide.

The atmosphere is divided into several layers on the basis of temperature. Of these, the most significant are the troposphere extending in altitude from the earth's surface to approximately 11 kilometers (km) and the stratosphere from about 11 km to approximately 50 km. The temperature of the troposphere ranges from an average of 15°C at sea level to an average of -56°C at its upper boundary. The average temperature of the stratosphere increases from -56°C at its boundary with the troposphere to -2°C at its upper boundary. The reason for this increase is absorption of solar ultraviolet energy by ozone (O_3) in the stratosphere.

The most significant feature of the environmental chemistry of the atmosphere is the occurrence of **photochemical reactions** resulting from the absorption by

molecules of light photons, designated $h\nu$.[3] (The energy, E, of a photon of a visible or ultraviolet light photon is given by the equation, $E = h\nu$, where h is Planck's constant and ν is the frequency of light, which is inversely proportional to its wavelength. Ultraviolet radiation has a higher frequency than visible light and is, therefore, more energetic and more likely to break chemical bonds in molecules that absorb it.) One of the most significant photochemical reactions is the one responsible for the presence of ozone in the troposphere (see above), which is initiated when O_2 absorbs highly energetic ultraviolet radiation in the wavelength ranges of 135-176 nanometers (nm) and 240-260 nm in the stratosphere:

$$O_2 + h\nu \rightarrow O + O \qquad (2.10.1)$$

The oxygen atoms produced by the photochemical dissociation of O_2 react with oxygen molecules to produce ozone, O_3 ,

$$O + O_2 + M \rightarrow O_3 + M \qquad (2.10.2)$$

where M is a third body, such as a molecule of N_2 , which absorbs excess energy from the reaction. The ozone that is formed is very effective in absorbing ultraviolet radiation in the 220-330 nm wavelength range in the stratosphere, which causes the temperature increase observed in the stratosphere. The ozone serves as a very valuable filter to remove ultraviolet radiation from the sun's rays. If this radiation reached the earth's surface, it would cause skin cancer and other damage to living organisms.

Physical phenomena in the atmosphere, which are studied under the science of **meteorology**, have a strong influence on atmospheric chemistry. **Temperature inversions**, in which a layer of cold air is trapped beneath hot air, are particularly influental because they limit vertical circulation air and trap bodies of air for long periods of time. In the presence of pollutant nitrogen oxides and hydrocarbons and through the action of sunlight, which initiates photochemical reactions, photochemical smog (see Section 9.5) develops in air masses held stationary by temperature inversions.

2.11. OXIDES IN THE ATMOSPHERE

Oxides of carbon, sulfur, and nitrogen are important constituents of the atmosphere and are pollutants at higher levels. Of these, the most abundant is carbon dioxide, O_2, which is required for plant growth. As of now, carbon dioxide is present at a level of about 340 parts per million (ppm) by volume in the global atmosphere and is increasing at a rate of about 1 ppm per year. This gas reabsorbs some of the infrared radiation by which the earth's surface re-radiates energy absorbed from the sun. Increasing CO_2 levels resulting from accelerated use of fossil fuels and destruction of CO_2-fixing forests may cause an increase in the earth's temperature (greenhouse effect) with disastrous effects on climate.

Carbon monoxide, CO, is a serious air pollutant in areas where automobile exhaust emissions result in excessive levels. Carbon monoxide combines with the blood's hemoglobin (see Section 7.3) preventing it from transporting oxygen to body tissues. Illness and death can result.

Several oxides of nitrogen are significant air constituents and pollutants. Nitrous oxide, N_2O, is a non toxic atmospheric gas formed by bacterial reduction of nitrate. Although it participates in atmospheric photochemical reactions, N_2O is not generally

regarded as a pollutant. Nitric oxide, NO, and nitrogen dioxide, NO_2, are collectively denoted as "NO_x." Both are regarded as pollutants in the atmosphere. Most pollutant sources of NO_x produce predominantly NO. Internal combustion engines produce this gas by the high-temperature, high-pressure reaction of N_2 and O_2:

$$N_2 + O_2 \rightarrow 2NO \qquad (2.11.1)$$

Much of the chemically bound nitrogen in coal and residual fuel oils is converted to NO_x during combustion. Photochemical processes in the atmosphere tend to convert NO to NO_2. Further reactions can result in the formation of corrosive nitrate salts or nitric acid, HNO_3. Nitric acid is a constituent of **acid precipitation**, consisting of rainwater, snow, or fog having a very low pH.

Nitrogen dioxide is particularly significant in atmospheric chemistry because of its photochemical dissociation by light with a wavelength less than 430 nm,

$$NO_2 + h\nu \rightarrow NO + O \qquad (2.11.2)$$

to produce highly reactive O atoms. This is the first step in the formation of photochemical smog, discussed in the next section.

Sulfur dioxide, SO_2, is a reaction product of the combustion of sulfur-containing fuels, such as high-sulfur coal. Part of this sulfur dioxide is converted by the following overall atmospheric chemical process to sulfuric acid, H_2SO_4:

$$2SO_2 + O_2 + 2H_2O \rightarrow 2H_2SO_4 \qquad (2.11.3)$$

In most localities, sulfuric acid is the predominant contributor to acid precipitation.

2.12. HYDROCARBONS AND PHOTOCHEMICAL SMOG

The most abundant hydrocarbon in the atmosphere is methane, CH_4, released from underground sources as natural gas and produced by the fermentation of organic matter as shown in Table 2.2. Methane is one of the least reactive atmospheric hydrocarbons and is produced by diffuse sources, so that its participation in the formation of pollutant photochemical reaction products is minimal. The most significant atmospheric pollutant hydrocarbons are the reactive ones produced as automobile exhaust emissions. In the presence of NO, under conditions of temperature inversion (see Section 2.10), low humidity, and sunlight, these hydrocarbons produce undesirable **photochemical smog** manifested by the presence of visibility-obscuring particulate matter, oxidants such as ozone, and noxious organic species such as aldehydes. Figure 2.7 shows the variation through the day of the main types of chemical species involved in photochemical smog. Examination of the figure shows that shortly after dawn, hydrocarbons and NO increase as a result of direct emissions from automobiles. This is followed by an abrupt drop in NO and a sharp increase in NO_2 as the intensity of sunlight increases. During the afternoon, levels of oxidants and aldehydes, which give smog its irritating qualities, reach peak values.

The smog-forming process is initiated by the photodissociation of nitrogen dioxide as shown by the following reaction:

$$NO_2 + h\nu \rightarrow NO + O \qquad (2.12.1)$$

The oxygen atoms undergo abstraction reactions to remove H atoms from hydrocarbons, designated as R-H,

$$R\text{-}H + O \rightarrow R\cdot + HO\cdot \qquad (2.12.2)$$

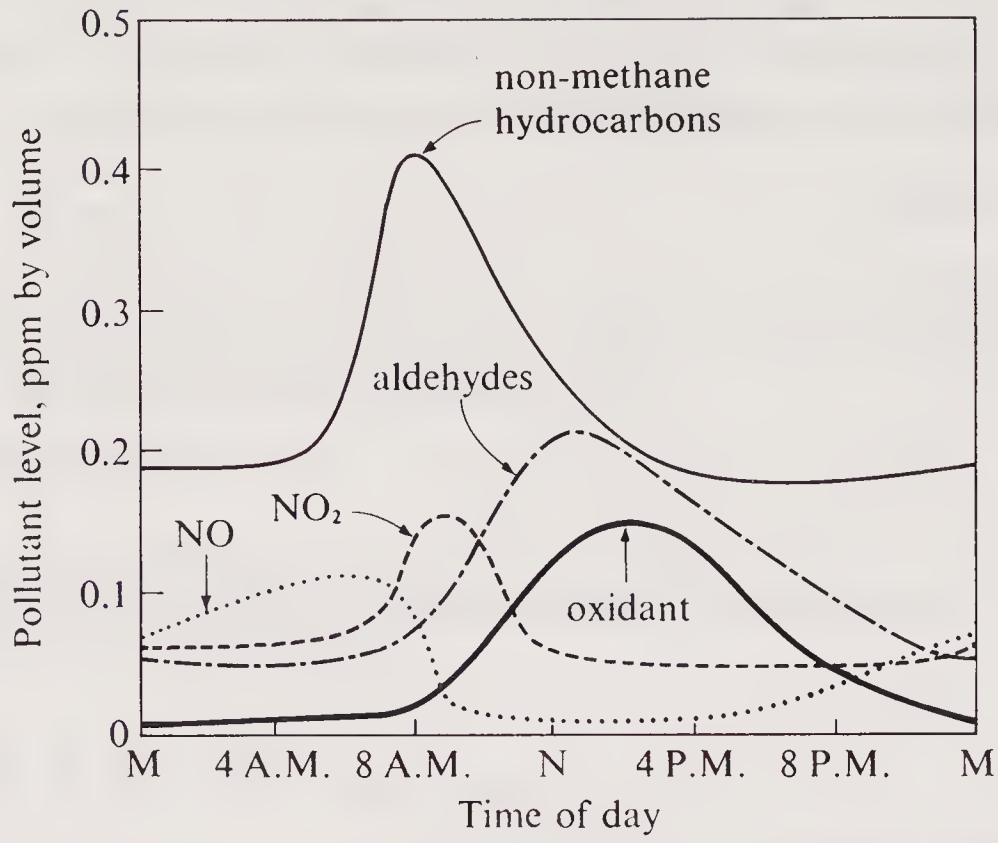


Figure 2.7. Trends in concentrations of major atmospheric species associated with photochemical smog formation.

to produce hydrocarbon radicals. (For example, if R-H were methane, CH_4, R· would be the methyl radical, H_3C·) In keeping with the representation of electrons in Lewis formulas by dots (see Section 1.2), the dots, ·, in the preceding reaction represent electrons. Single (unpaired) electrons are characteristic of usually highly reactive and unstable species called **free radicals**. The hydroxyl radical, HO·, is the most important intermediate in the smog-forming process, in that it reacts with a number of relatively stable molecules, sustaining chain reactions in which it is generated. (Hydroxyl radical is a key participant in many atmospheric chemical processes, including those in which methane and carbon monoxide are eliminated from the atmosphere by oxidation to CO_2.) The hydrocarbon free radicals, R·, react with molecules of oxygen,

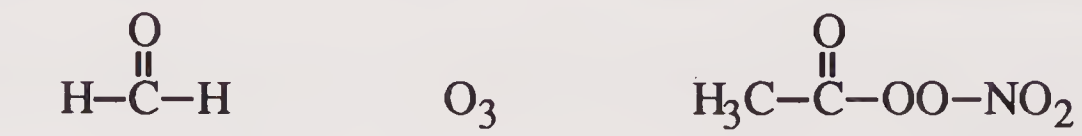
$$R\cdot + O_2 \rightarrow ROO\cdot \qquad (2.12.3)$$

to produce organic peroxyl radicals. These, in turn, undergo a number of additional reactions, of which one of the most significant is,

$$NO + ROO\cdot \rightarrow NO_2 + RO\cdot \qquad (2.12.4)$$

which is responsible for the paradoxical increase in NO_2 concentration under conditions such that this molecule is undergoing photodissociation back to NO.

Many of the irritating qualities of smog are due to the production of aldehydes and oxidants. Of the latter, the two main types are ozone and organic oxidants, such as peroxyacetyl nitrate, PAN (see below):



These compounds cause eye and respiratory tract irritation. The oxidants are quite toxic to animals and plants and cause damage to rubber and other materials.

Although the automobile is the major culprit contributing to atmospheric hydrocarbon pollution leading to photochemical smog formation, other sources of hydrocarbons are likewise important. Even pine and citrus trees emit highly reactive terpene hydrocarbons to the atmosphere, which contribute to smog formation. Volatile hydrocarbons emitted from improperly disposed hydrocarbon hazardous wastes can also add to photochemical smog.

2.13. PARTICULATE MATTER

Particles ranging from aggregates of a few molecules to pieces of dust readily visible to the naked eye are commonly found in the atmosphere. Some of these particles, such as sea salt formed by the evaporation of water from droplets of sea spray, are natural and even beneficial atmospheric constituents. Very small particles called **condensation nuclei** serve as bodies for atmospheric water vapor to condense upon and are essential for the formation of precipitation.

Colloidal-sized particles in the atmosphere are called **aerosols**. Those formed by grinding up bulk matter are known as **dispersion aerosols**, of which the latter tend to be smaller. Smaller particles are in general the most harmful because they have a greater tendency to scatter light and are the most respirable (tendency to be inhaled into the lungs).

Inorganic Particles

Figure 2.8 illustrates the basic factors responsible for the composition of inorganic particulate matter. In general, the proportions of elements in atmospheric particulate matter reflect relative abundances of elements in the parent material. The source of particulate matter is reflected in its elemental compositon, taking into consideration chemical reactions that may change the composition.

The chemical composition of atmospheric particulate matter is quite diverse. Among the constituents of inorganic particulate matter found in polluted atmospheres are salts, oxides, nitrogen compounds, sulfur compounds, various metals, and radionuclides. In coastal areas sodium and chlorine get into atmospheric particles as sodium chloride from sea spray. The major trace elements that typically occur at levels above 1 $\mu g/m^3$ in particulate matter are aluminum, calcium, carbon, iron, potassium, sodium, and silicon; note that most of these tend to originate from terrestrial sources. Lesser quantities of copper, lead, titanium, and zinc and even lower levels of antimony, beryllium, bismuth, cadmium, cobalt, chromium, cesium, lithium, manganese, nickel, rubidium, selenium, strontium, and vanadium are commonly observed. The likely sources of some of these elements are Al, Fe, Ca, and Si from soil erosion, rock dust, and coal combustion; C from incomplete combustion of carbonaceous fuels; Na and Cl from marine aerosols, chloride from incineration of organohalide polymer wastes; highly volatile Sb and Se from the combustion of oil, coal, and refuse; vanadium from combustion of residual petroleum (present at very high levels in residues from Venezuelan crude oil); zinc (which tends to occur in small particles) from combustion; and lead from combustion of leaded fuels and wastes containing lead.

Particulate carbon as soot, carbon black, coke, and graphite originates from auto and truck exhausts, heating furnaces, incinerators, power plants, and steel and foundry operations and composes one of the more visible and troublesome particulate air

pollutants. Because of its good adsorbent properties, carbon can be a carrier of gaseous and other particulate pollutants. Particulate carbon surfaces may catalyze some heterogeneous atmospheric reactions, including the important conversion of SO_2 to sulfate.

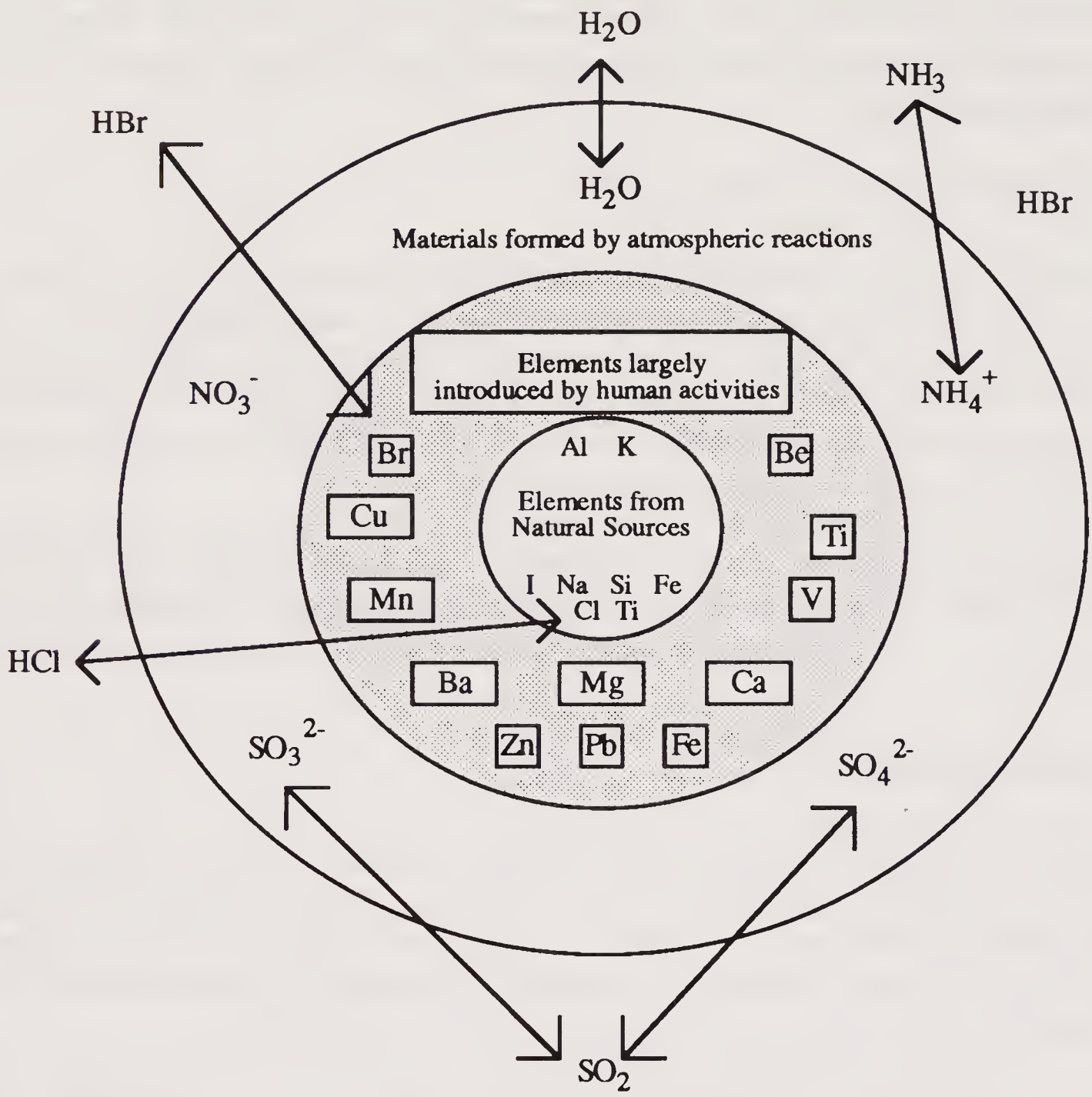


Figure 2.8. Some of the components of inorganic particulate matter and their origins.

Fly Ash

Much of the mineral particulate matter in a polluted atmosphere is in the form of oxides and other compounds produced during the combustion of high-ash fossil fuel. Much of the mineral matter in fossil fuels such as coal or lignite is converted during combustion to a fused, glassy bottom ash, which presents no air pollution problems. Smaller particles of **fly ash** enter furnace flues and are efficiently collected in a properly equipped stack system. However, some fly ash escapes through the stack and enters the atmosphere. Unfortunately, the fly ash thus released tends to consist of smaller particles that do the most damage to human health, plants, and visibility.

The composition of fly ash varies widely, depending upon the source of fuel. The predominant constituents are oxides of aluminum, calcium, iron, and silicon. Other elements that occur in fly ash are magnesium, sulfur, titanium, phosphorus, potassium, and sodium. Elemental carbon (soot, carbon black) is a significant fly ash constituent.

The size of fly ash particles is a very important factor in determining their removal from stack gas and their ability to enter the body through the respiratory tract. Although only a very small percentage of fly ash particles are in the very small range of around 0.1 μm, these particles include the majority of the total number of fly ash particles and particle surface area. Submicrometer particles probably result from a volatilization-condensation process during combustion as reflected in a higher concentration of more volatile elements, such as As, Sb, Hg, and Zn. Furthermore, the very small particles are the most difficult to remove by particulate air pollution control devices, such as electrostatic precipitators and bag houses.

Atmospheric Particle Processes

Particles undergo a number of processes in the atmosphere as summarized in Figure 2.9. The aggregation of small particles into larger ones is called **coagulation** and often precedes removal of particles from the atmosphere. Particles come out of the atmosphere by **sedimentation**, scavenging by precipitation, and **dry deposition** in which they stick upon the surface of soil or plant leaves. Particles are required for the condensation of water vapor from the atmosphere, so they are involved in rainfall. Particles serve as reaction sites for atmospheric chemical processes. These heterogeneous reactions may occur on the surfaces of particulate oxides or carbon or inside water droplets.

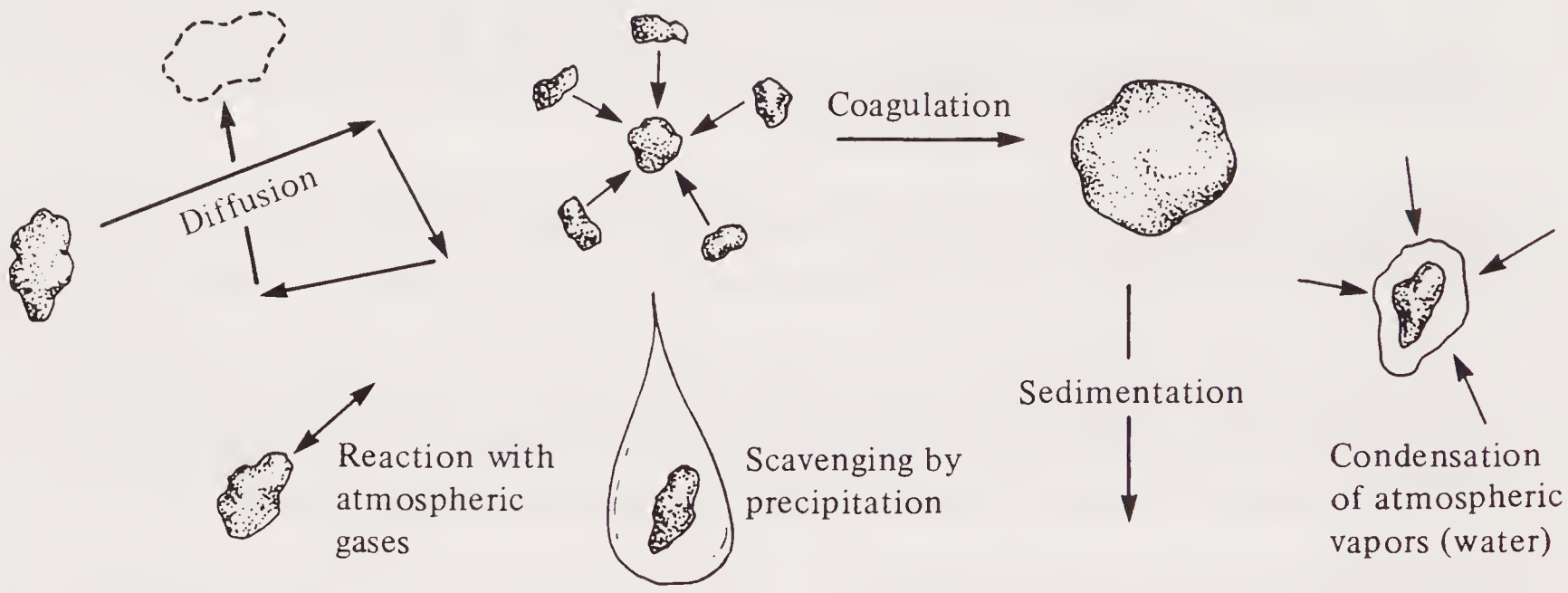


Figure 2.9. Major processes that particles undergo in the atmosphere.

2.14. INDICATOR CHEMICALS IN THE ENVIRONMENT

The release of chemical species into the environment is one of the most common and troublesome aspects of the hazardous waste problem. A large number of chemical species may be released from waste sites, and it is difficult to monitor them all. Instead, **indicator chemicals** are chosen for monitoring on the basis of their toxicities, mobilities, persistence, quantities present, and amenability to analysis.[4] Indicator chemicals are selected for a site on the basis of their **indicator score**, IS_i, a unitless number calculated by the formula,

$$IS_i = \Sigma (C_{ij} T_{ij}) \qquad (2.14.1)$$

where C_{ij} is the concentration of species i in medium j and T_{ij} is a toxicity value for the same chemical in the medium under consideration. The units of C_{ij} are in mg/L, mg/kg, and mg/m^3 for water, soil, and air, respectively, and units of T_{ij} are reciprocals of the units of C_{ij}. Values of IS_i may also be adjusted for frequencies at which chemicals are detected, and time trends for specific chemicals at the site.

The species most often selected as indicator chemicals are organic compounds and heavy metals. As rated by the frequency at which they are encountered at hazardous waste sites, the most common indicator chemicals are the following: Trichloroethylene, benzene > polychlorinated biphenyls (PCBs), tetrachloroethylene > lead > vinyl chloride, arsenic > cadmium, chromium > 1,1-dichloroethylene > xylene, 1,1,2-trichloroethylene,1,2-dichloroethane, polynuclear aromatic hydrocarbons (PAH) > 1,1,2,2-tetrachloroethane, chloroform, ethylbenzene, toluene, zinc > dichloromethane, benzo-(a)pyrene (a PAH compound) > copper, nickel, mercury, 2,3,7,8-TCDD (commonly called dioxin), phenol, pentachlorophenol, N-nitrosodiphenylamine. The chemical and toxicological properties of these species are discussed in Chapters 6-9.

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3. Wayne, Richard P., *Principles and Applications of Photochemistry*, Oxford University Press, New York, 1988.

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QUESTIONS

1. Define (a) environmental chemistry and (b) toxicological chemistry.

2. Water in equilibrium with air at 25°C contains _____ mg/L) of dissolved O_2, water alkalinity is due primarily to dissolved _____, and hardness is due primarily to _____.

3. Explain how the following reactions related to electron activity:

$$2H_2O + 2e^- \leftarrow\rightarrow H_2(g) + 2OH^-$$

$$2H_2O \leftarrow\rightarrow O_2(g) + 4H^+ + 4e^-$$

How is pE related to electron activity?

4. Based on Figure 2.3, explain what might be observed when low pE groundwater at pH 7 is exposed to the atmosphere where it is in equilibrium with atmospheric O_2.

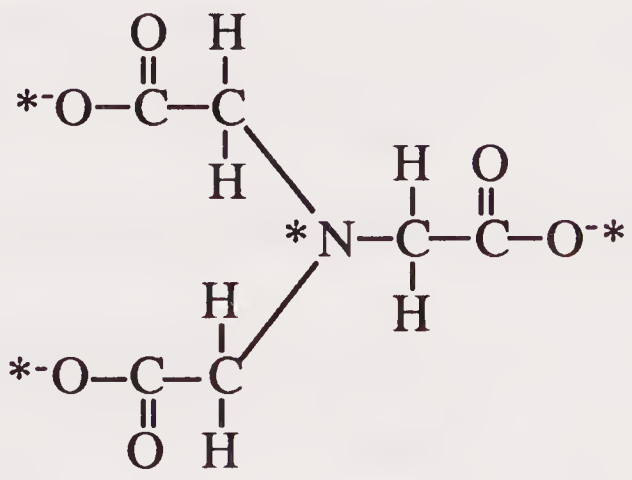
5. Match the water property on the left below with its effect or significance on the right.

(a) Higher heat capacity than any common liquid	1. High solubility of ionic substances and their ionization in solution
(b) Maximum density as a liquid at 4°C	2. Ice floats; vertical circulation restricted in stratified bodies of water
(c) Highest dielectric constant of any common liquid	3. Determines transfer of heat and water molecules between the atmosphere and bodies of water
(d) Higher heat of vaporization than any other material	4. Temperature stabilized at the freezing point of water
(e) Higher latent heat of fusion than any other common liquid	5. Stabilization of temperatures of organisms and geographical regions

6. Why is it not totally correct to regard metal ions dissolved in water as "bare ions," M^{n+}? What is a more correct way of showing such ions? How can such ions act as acids?

7. What kind of species is $CdCN^+$? What are its two components? Why does it have a +1 charge?

8. What kind of species is the following?



What do the asterisks denote?

9. What are the three classes of the most common kind of naturally-occurring chelating agents? What is the source of this material?

10. How do organometallic compounds differ from metal complexes? What is an example of an organometallic compound?

11. Which four elements are most likely to have methylated forms synthesized by bacteria?

12. List some of the properties of sediments insofar as they influence aquatic chemistry.

13. What are colloidal particles in water? What is their approximate size range?

14. What kind of process is represented by the following reaction?

$$\{Solid\}^-M^+ + Z \leftarrow\rightarrow \{Solid\}^-Z^+ + M^+$$

15. What are bacteria? What is the difference between aerobic and anaerobic bacteria?

16. What is the significance of the following bacterially-mediated reaction? What is it called?

17. Match the following:

(a) $SO_4^{2-} + 2\{CH_2O\} + 2H^+ \rightarrow H_2S(g) + 2CO_2 + 2H_2O$

(b) $2FeS_2 + 2H_2O + 7O_2 \rightarrow 4H^+ + 4SO_4^{2-} + 2Fe^{2+}$

(c) $2\{CH_2O\} \rightarrow CH_4 + CO_2$

(d) $4NO_3^- + 5\{CH_2O\} + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$

1. Production of acid mine water
2. Denitrification (conversion of fixed nitrogen back to atmospheric N_2)
3. Fermentation reaction, atmospheric methane source
4. Sulfate reduction, source of atmospheric H_2S

18. Match the following:

(a) Pathogens

(b) Carcinogens

(c) BOD

(d) Trace elements and heavy metals

(e) Alkalinity, acidity, salinity

(f) Fertilizers (algal nutrients)

1. HCO_3^-, H_2SO_4, and NaCl, respectively. Harmful pollutants if in excess
2. Organic matter that consumes oxygen when degraded.
3. Toxic metals such as lead, cadmium, and arsenic
4. Aflatoxins, nitrosamines, polycyclic aromatic hydrocarbons (PAHs), others that may cause cancer
5. Phosphates, K^+, NO_3^-. Cause excessive algal growth resulting in eutrophication
6. Impose constraints on water reuse and increase difficulty of water treatment.

19. What does aluminum sulfate form that is used in water treatment?

20. How is the following reaction used in water treatment? What does it remove from polluted water?

$$\{CH_2O\} + O_2 \rightarrow CO_2 + H_2O$$

21. How might excessive atmospheric carbon dioxide and acid rain affect the geosphere?

22. How is weathering related to soil?

23. What is the soil solution? What functions does it serve in soil?

24. Considering normal air, list two major components, three minor components (one of which may vary over a range of about 1-3% in air), and several trace-level components.

25. What are the two lowest layers of the atmosphere, and what causes the atmosphere to be divided into these two layers?

26. Using O_2 as an example, define and illustrate photochemical reactions.

27. Define and give an example of a free racical in the atmosphere.

28. Define and illustrate what is meant by chain reactions.

29. What special role is played by temperature inversions in air pollution phenomena?

30. What is the most serious effect of atmospheric CO?

31. Why is the following reaction particularly significant in atmospheric chemistry?

$$NO_2 + h\nu \rightarrow NO + O$$

32. What air pollution problem is illustrated by the following reaction?

$$2SO_2 + O_2 + 2H_2O \rightarrow 2H_2SO_4$$

33. Explain how each of the following reactions relates to a significant air pollution problem:

$$NO_2 + h\nu \rightarrow NO + O$$

$$R\text{-}H + O \rightarrow R\cdot + HO\cdot$$

$$R\cdot + O_2 \rightarrow ROO\cdot$$

$$NO + ROO\cdot \rightarrow NO_2 + RO\cdot$$

34. List and discuss the major processes that particles undergo in the atmosphere.

35. Define condensation nuclei, aerosols, and dispersion aerosols.

36. List some of the kinds of particulate inorganic matter in the atmosphere.

37. Define and explain the significance of fly ash.

38. What is meant by an indicator chemical in the environment?

3

Biochemistry

3.1. BIOCHEMISTRY

Most people have had the experience of looking through a microscope at a single cell. It may have been an ameba, alive and oozing about like a blob of jelly on the microscope slide. It may have been a cell of bacteria, stained with a dye to make it show up more plainly. Or, it may have been a beautiful cell of algae colored with bright green chlorophyll. Even the simplest of these cells is capable of carrying out a thousand or more chemical reactions. These life processes fall under the heading of **biochemistry**, that branch of chemistry that deals with the chemical properties, composition, and biologically-mediated processes of complex substances in living systems.

Biochemical phenomena that occur in living organisms are extremely sophisticated. In the human body complex metabolic processes break down a variety of food materials to simpler chemicals, yielding energy and the raw materials to build body constituents, such as muscle, blood, and brain tissue. Impressive as this may be, consider a humble microscopic cell of photosynthetic cyanobacteria only about a micrometer in size, which requires just a few simple inorganic chemicals and sunlight for its existence. This cell uses sunlight energy to convert carbon from CO_2, hydrogen and oxygen from H_2O, nitrogen from NO_3^-, sulfur from SO_4^{2-}, and phosphorus from inorganic phosphate into all the proteins, nucleic acids, carbohydrates, and other materials that it requires to exist and reproduce. Such a simple cell accomplishes what could not be accomplished by human endeavors in a vast chemical complex costing billions of dollars.

Environmental Biochemistry

Ultimately, most environmental pollutants and hazardous substances are of concern because of their effects upon living organisms. The study of the adverse effects of substances on life processes requires some basic knowledge of biochemistry Biochemistry is discussed in this chapter. It emphasizes those aspects that are especially pertinent to environmentally hazardous and toxic substances, including cell membranes, DNA, and enzymes.

Biochemical processes not only are profoundly influenced by chemical species in the environment, they largely determine the nature of these species, their degradation, and even their syntheses, particularly in the aquatic and soil environments. The study of such phenomena forms the basis of **environmental biochemistry**.

Biomolecules

The biomolecules that constitute matter in living organisms are often polymers with molecular masses of the order of a million or even larger.[1] As discussed later in this chapter, these biomolecules may be divided into the categories of carbohydrates, proteins, lipids, and nucleic acids. Proteins and nucleic acids consist of macromolecules, lipids are usually relatively small molecules, carbohydrates range from relatively small sugar molecules to high molecular mass macromolecules such as those in cellulose.

The behavior of a substance in a biological system depends to a large extent upon whether the substance is hydrophilic ("water-loving") or hydrophobic ("water-hating"). Some important toxic substances are hydrophobic, a characteristic that enables them to traverse cell membranes readily. Part of the detoxification process carried on by living organisms is to render such molecules hydrophilic, therefore water-soluble and readily eliminated from the body.

3.2. BIOCHEMISTRY AND THE CELL

The focal point of biochemistry and biochemical aspects of toxicants is the **cell**, the basic building block of living systems where most life processes are carried Bacteria, yeasts, and some algae consist of single cells. However, most living things are made up of many cells. In a more complicated organism the cells have different functions. Liver cells, muscle cells, brain cells, and skin cells in the human body are quite different from each other and do different things. Cells are divided into two major categories depending upon whether or not they have a nucleus: **eukaryotic** cells have a nucleus and **prokaryotic** cells do not. Prokaryotic cells are found predominately in single-celled organisms, particularly bacteria, and are discussed with microorganisms in Chapter 5. Eukaryotic cells occur in multicelled plants and animals — higher life forms.

Major Cell Features

Figure 3.1 shows the major features of the **eukaryotic cell**, which is the basic structure in which biochemical processes occur in multicelled organisms. These features are the following:

- **Cell membrane**, which encloses the cell and regulates the passage of ions, nutrients, lipid-soluble ("fat-soluble") substances, metabolic products, toxicants, and toxicant metabolites into and out of the cell interior because of its varying **permeability** for different substances. The cell membrane protects the contents of the cell from undesirable outside influences. Cell membranes are composed in part of phospholipids that are arranged with their hydrophilic ("water-seeking") heads on the cell membrane surfaces and their hydrophobic ("water-repelling") tails inside the membrane. Cell membranes contain bodies of proteins that are involved in the transport of some substances through the membrane. One reason the cell membrane is very important in toxicology and environmental biochemistry is because it regulates the passage of toxicants and their products into and out of the cell interior. Furthermore, when its membrane is damaged by toxic sustances, a cell may not function properly and the organism may be harmed.

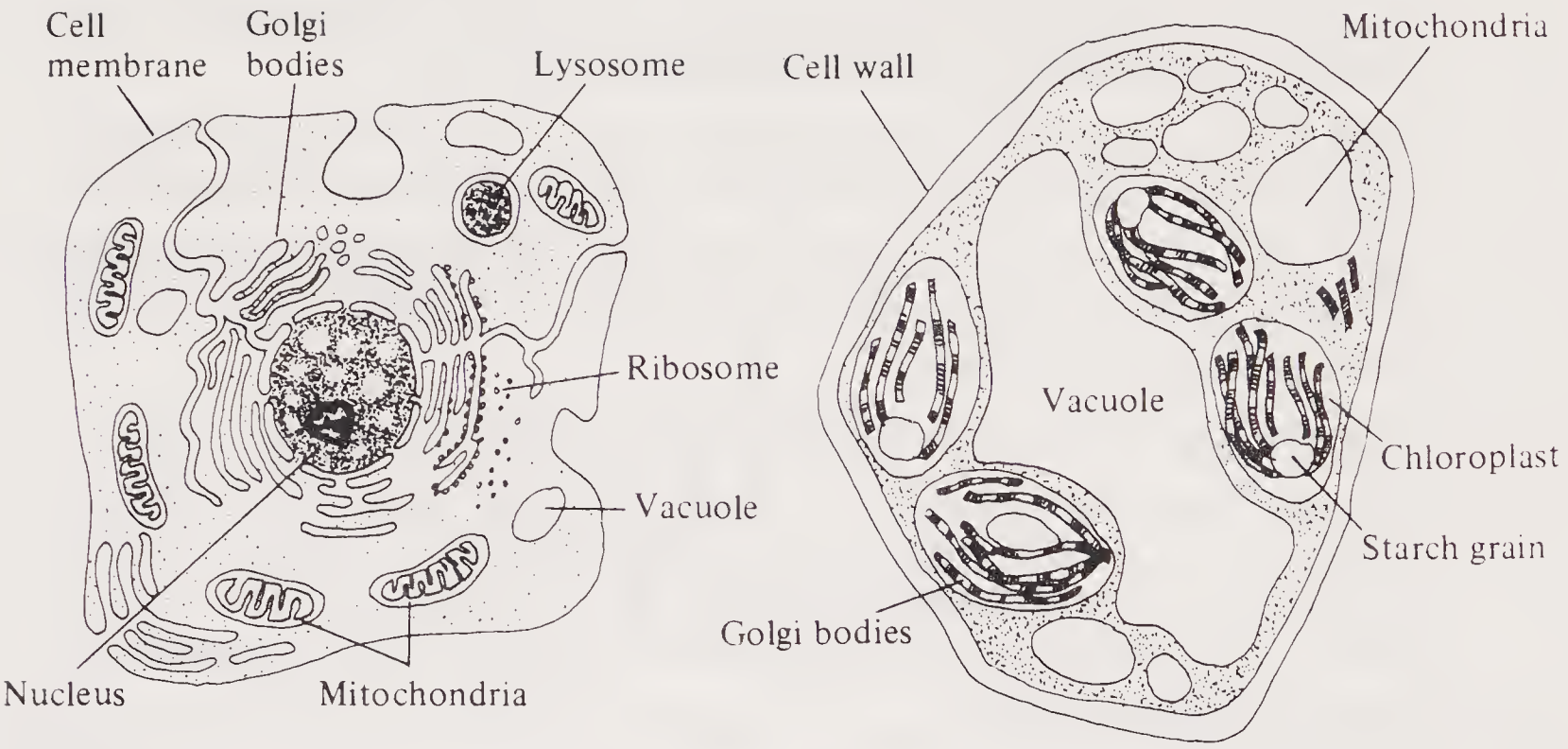


Figure 3.1. The predominant features of the eukaryotic cell in animals (left) and plants (right).

- **Cell nucleus**, which acts as a sort of "control center" of the cell. It contains the genetic directions the cell needs to reproduce itself. The key substance in the nucleus is **deoxyribonucleic** acid (**DNA**). **Chromosomes** in the cell nucleus are made up of combinations of DNA and proteins. Each chromosome stores a separate quantity of genetic information. Human cells contain 46 chromosomes. When DNA in the nucleus is damaged by foreign substances, various toxic effects, including mutations, cancer, birth defects, and defective immune system function may occur.

- **Cytoplasm**, which fills the interior of the cell not occupied by the nucleus. Cytoplasm is further divided into a water-soluble proteinaceous filler called **cytosol** and bodies suspended in it called **cellular organelles**, such as mitochondria or, in photosynthetic organisms, chloroplasts.

- **Mitochondria**, "powerhouses" which mediate energy conversion and utilization in the cell. Mitochondria are sites in which food materials — carbohydrates, proteins, and fats — are broken down to yield carbon dioxide, water, and energy, which is then used by the cell. The best example of this is the oxidation of the sugar glucose, $C_6H_{12}O_6$:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

  This kind of process is called **cellular respiration.**

- **Ribosomes**, which participate in protein synthesis.

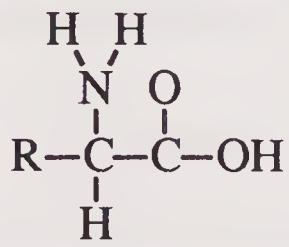
- **Endoplasmic reticulum**, which is involved in the metabolism of some toxicants by enzymatic processes.

- **Lysosome**, a type of organelle that contains potent substances capable of digesting liquid food material. Such material enters the cell through a "dent" in the cell wall, which eventually becomes surrounded by cell material. This surrounded material is called a **food vacuole**. The vacuole merges with a lysosome, and the substances in the lysosome bring about digestion of the food material. The digestion process consists largely of **hydrolysis reactions** in which large, complicated food molecules are broken down into smaller units by the addition of water.
- **Golgi bodies**, that occur in some types of cells. These are flattened bodies of material that serve to hold and release substances produced by the cells.
- **Cell walls** of plant cells. These are strong structures that provide stiffness and strength. Cell walls are composed mostly of cellulose, which will be discussed later in this chapter.
- **Vacuoles** inside plant cells that often contain materials dissolved in water.
- **Chloroplasts** in plant cells that are involved in photosynthesis (the chemical process which uses energy from sunlight to convert carbon dioxide and water to organic matter). Photosynthesis occurs in these bodies. Food produced by photosynthesis is stored in the chloroplasts in the form of **starch grains**.

3.3. PROTEINS

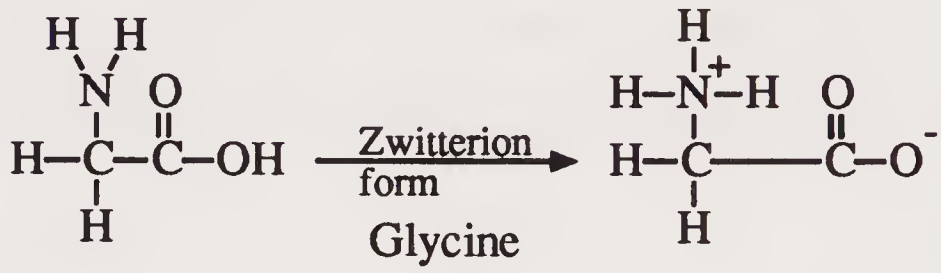
Proteins are nitrogen-containing organic compounds which are the basic units of life systems. **Simple proteins** contain only amino acids whereas **conjugated proteins** also contain groups other than amino acids, such as carbohydrates or lipids. Cytoplasm, the jelly-like liquid filling the interior of cells is made up largely of protein. Enzymes, which act as catalysts of life reactions, are specialized proteins; they are discussed later in the chapter. Proteins are made up of **amino acids** joined together in huge chains. Amino acids are organic compounds which contain the carboxylic acid group, $-CO_2H$, and the amino group, $-NH_2$. They are sort of a hybrid of carboxylic acids and amines (Section 1.9). Proteins are polymers or **macromolecules** of amino acids containing from approximately forty to several thousand amino acid groups joined by peptide linkages (discussed later in this section and illustrated in Figure 3.2). Smaller molecule amino acid polymers, containing only about ten to about forty amino acids per molecule, are called **polypeptides**. A portion of the amino acid left after the elimination of H_2O during polymerization is called a **residue**. The amino acid sequence of these residues is designated by a series of three-letter abbreviations for the amino acid.

Natural amino acids may all be represented by the general formula,

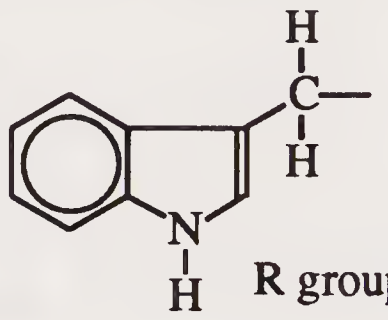


shown here with uncharged $-NH_2$ and $-CO_2H$ groups, which have a strong tendency to exchange H^+ ion and produce the **zwitterion** form as shown for glycine, below. In the general structure for amino acids above the $-NH_2$ group is always bonded to the carbon next to the $-CO_2H$ group. This C atom is in the "alpha" location, so natural

amino acids are alpha amino acids. Other groups, designated as "R," are attached to the basic alpha amino acid structure. The R groups may be as simple as an atom of H found in glycine,



or, they may be as complicated as the structure,



R group in tryptophan

found in tryptophan. As shown in Table 3.1, there are 20 common amino acids in proteins.

Table 3.1. Amino Acids That Occur in Protein

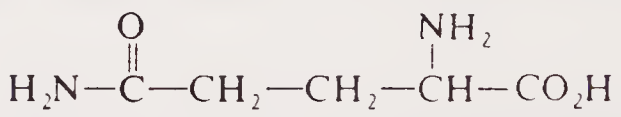
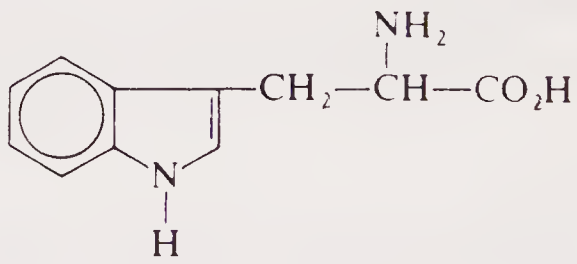
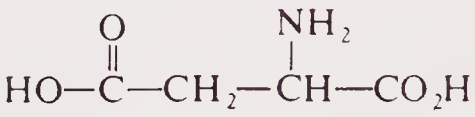
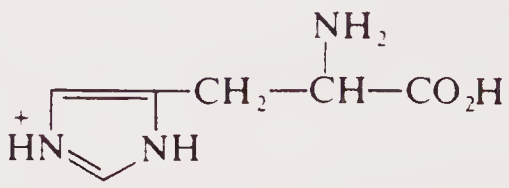
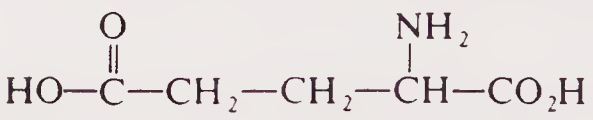
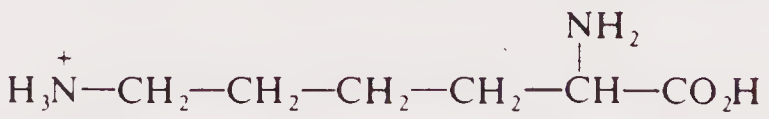
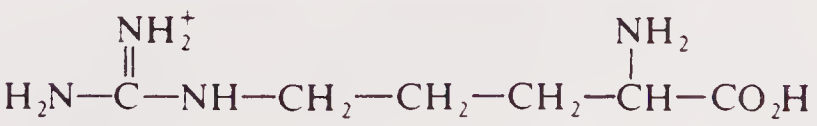
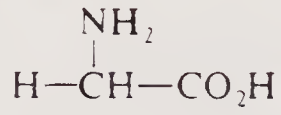
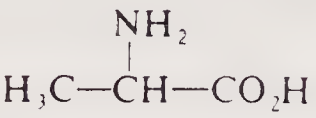
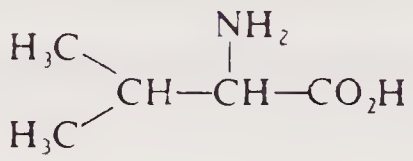
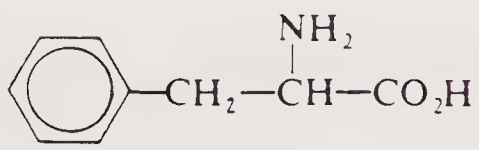
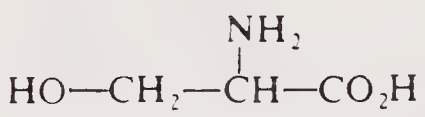
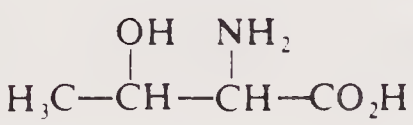
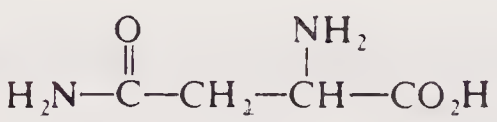
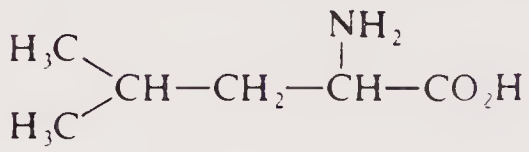
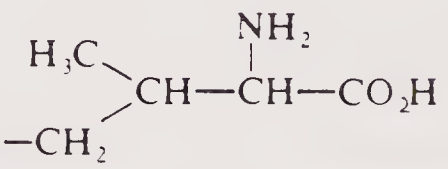
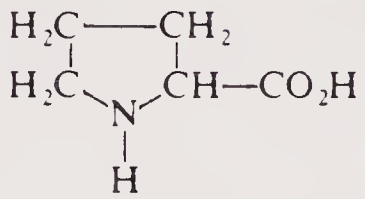
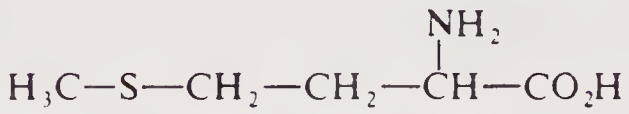
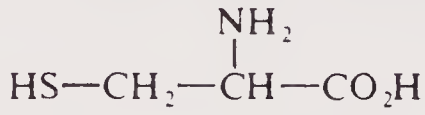
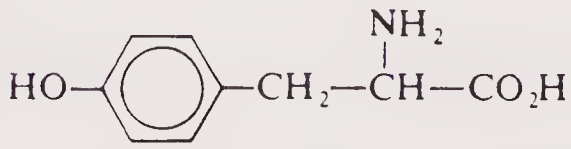
Name	Structural Formula
Glutamine (gin)	
Tryptophan (try)*	
Aspartic acid (asp)	
Histidine (his)	
Glutamic acid (glu)	
Lysine (lys)*	
Arginine (arg)	

Table 3.1 (continued from page 72). Amino Acids That Occur in Protein

Name	Structural Formula
Glycine (gly)	
Alanine (ala)	
Valine (val)*	
Phenylalanine (phe)*	
Serine (ser)	
Threonine (thr)*	
Asparagine (asn)	
Leucine (leu)*	
Isoleucine (ile)*	
Proline (pro)	
Methionine (met)*	
Cysteine (cys)	
Tyrosine (tyr)	

* Amino acids designated with an asterisk are those that are essential for humans. Unlike the other amino acids, these cannot be synthesized by the human body, and must be present in the diet. The average human requires about 70 grams of "first class" protein, contained in foods such as meat, fish, cheese, or eggs, to supply the essential amino acids.

Amino acids in proteins are joined together in a specific way. These bonds are called the **peptide linkage**. The formation of peptide linkages is a condensation process involving the loss of water. Consider as an example the condensation of alanine, leucine, and tyrosine shown in Figure 3.2. When these three amino acids join together, two water molecules are eliminated. The product is a *tri*peptide since there are three amino acids involved. The amino acids in proteins are linked as shown for this tripeptide, except that many more monomeric amino acid groups are involved.

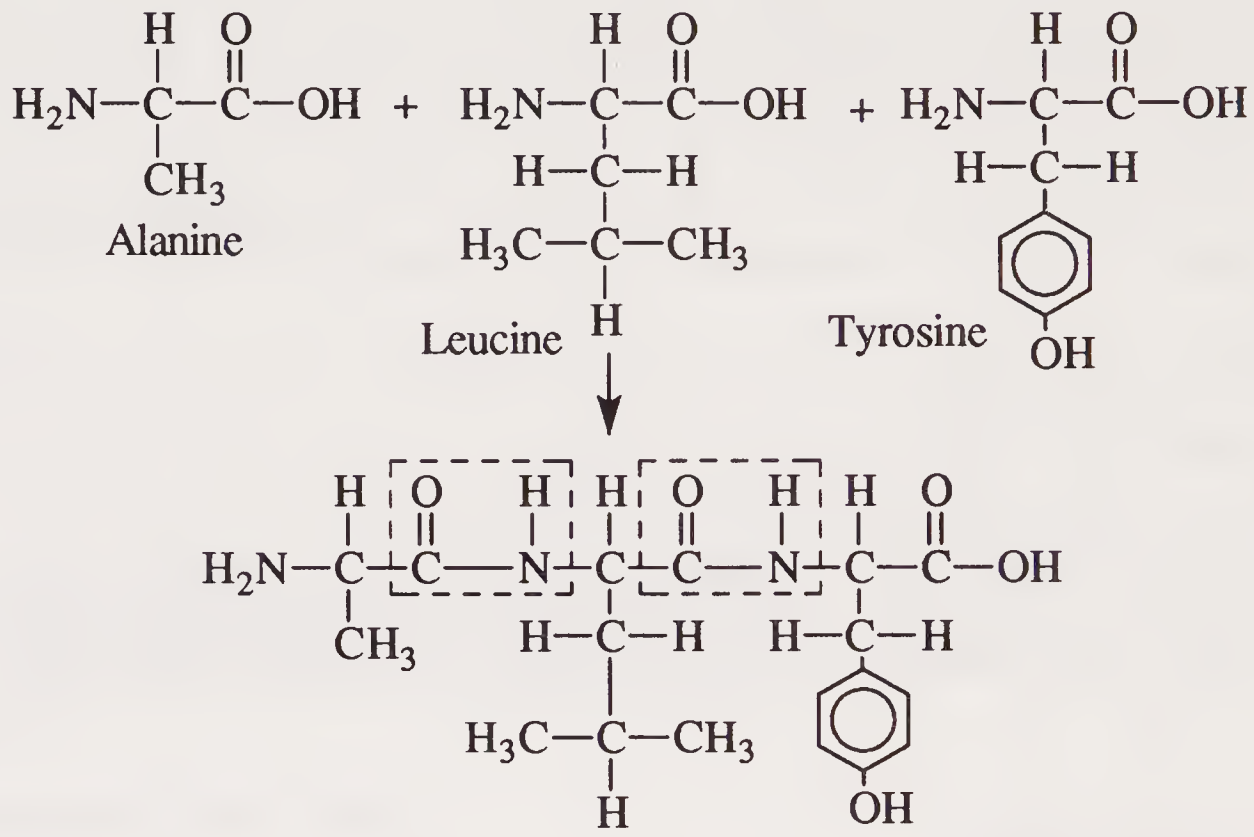


Figure 3.2. Condensation of alanine, leucine, and tyrosine to form a tripeptide consisting of three amino acids joined by peptide linkages (outlined by dashed lines).

Proteins may be divided into several types that have widely varying functions. These are given in Table 3.3.

Table 3.3. Major Types of Proteins

Type of protein	Example	Function and characteristics
Nutrient	Casein (milk protein)	Food
Storage	Ferritin	Storage of iron in animal tissues
Structural	Collagen (tendons) keratin (hair)	Structural and protective components in organisms
Contractile	Actin, myosin in muscle tissue	Strong, fibrous proteins that can contract and cause movement to occur
Transport	Hemoglobin	Transport inorganic and organic species in cell membranes and blood, between organs
Defense	- - -	Antibodies produced by the immune system that act against foreign agents, such as viruses
Regulatory	Insulin, human growth hormone	Regulate biochemical processes such as sugar metabolism or growth by binding to sites inside cells or on cell membranes
Enzymes	Acetylcholinesterase	Catalysts of biochemical reactions (see Section 3.6.)

Protein Structure

The order of amino acids in protein molecules, and the resulting three-dimensional structures that form, provide an enormous variety of possibilites for **protein structure**. This is what makes life so diverse. Proteins have primary, secondary, tertiary, and quaternary structures. The structures of protein molecules determine the behavior of proteins in crucial areas such as the processes by which the body's immune system recognizes substances that are foreign to the body. Proteinaceous enzymes depend upon their structures for the very specific functions of the enzymes.

The order of amino acids in the protein molecule determines its **primary structure**. **Secondary protein structures** result from the folding of polypeptide protein chains to produce a maximum number of hydrogen bonds between peptide linkages:

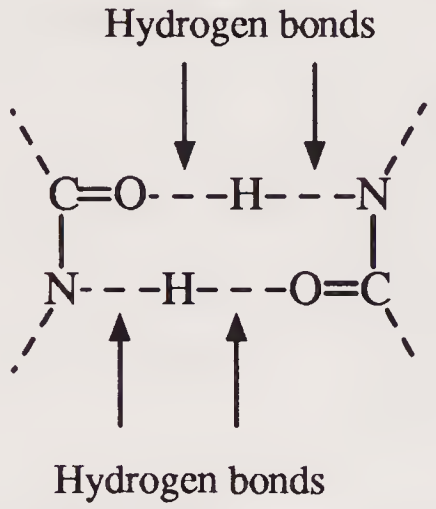


Illustration of hydrogen bonds between N and O atoms in peptide linkages, which constitute protein secondary structures

Secondary structure is influenced by amino acid side chains. Small R groups enable protein molecules to be hydrogen-bonded together in a parallel arrangement. With larger R groups the molecules tend to take a spiral form. Such a spiral is known as an **alpha-helix**.

Tertiary structures are formed by the twisting of alpha-helices into specific shapes. They are produced and held in place by the interactions of amino side chains on the amino acid residues constituting the protein macromolecules. Tertiary protein structure is very important in the processes by which enzymes identify specific proteins and other molecules upon which they act. It is also involved with the action of antibodies in blood which recognize foreign proteins by their shape and react to them. This is basically the mechanism by which immunity to a disease is developed so that antibodies in blood recognize specific proteins from viruses or bacteria and reject them.

Two or more protein molecules consisting of separate polypeptide chains may be further attracted to each other to produce a **quaternary structure**.

Fibrous Proteins

Some proteins are **fibrous proteins**, which occur in skin, hair, wool, feathers, silk, and tendons. The molecules in these proteins are long and threadlike and are laid out parallel in bundles. Fibrous proteins are quite tough and they do not dissolve in water.

An interesting fibrous protein is keratin, which is found in hair. The cross-linking bonds between protein molecules in keratin are –S–S– bonds formed from two HS— groups in two molecules of the amino acid, cysteine. These bonds largely hold hair in

place, thus keeping it curly or straight. Administration of a "permanent" to hair in a beauty salon consists of breaking the –S–S– bonds chemically, setting the hair as desired, then reforming the cross-links to hold the desired shape.

Globular Proteins

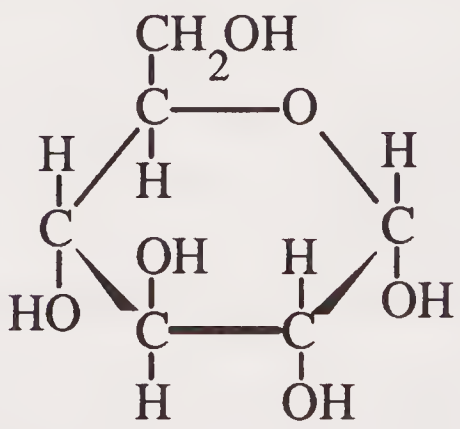
Aside from fibrous protein, the other major type of protein form is the **globular protein**. These proteins are in the shape of balls and oblongs. Globular proteins are relatively soluble in water. A typical globular protein is hemoglobin, the oxygen-carrying protein in red blood cells. Enzymes are generally globular proteins.

Denaturation of Proteins

Secondary, tertiary, and quaternary protein structures are easily changed by a process called **denaturation**. These changes can be quite damaging. Heating, exposure to acids or bases, and even violent physical action can cause denaturation to occur. The albumin protein in egg white is denatured by heating so that it forms a semisolid mass. Almost the same thing is accomplished by the violent physical action of an egg beater in the preparation of meringue. Heavy metal poisons such as lead and cadmium change the structures of proteins by binding to functional groups on the protein surface.

3.4. CARBOHYDRATES

Carbohydrates have the approximate simple formula CH_2O and include a diverse range of substances composed of simple sugars such as glucose:



Glucose molecule

High-molecular-mass **polysaccharides**, such as starch and glycogen ("animal starch"), are biopolymers of simple sugars.

When photosynthesis occurs in a plant cell, the energy from sunlight is converted to chemical energy in a carbohydrate, $C_6H_{12}O_6$. This carbohydrate may be transferred to some other part of the plant for use as an energy source. It may be converted to a water-insoluble carbohydrate for storage until it is needed for energy. Or it may be transformed to cell wall material and become part of the structure of the plant. If the plant is eaten by an animal, the carbohydrate is used for energy by the animal.

The simplest carbohydrates are the **monosaccharides**. These are also called **simple sugars**; or, because they have 6 carbon atoms, *hex*oses. Glucose (structural formula shown above) is the most common simple sugar involved in cell processes. Other simple sugars with the same formula but somewhat different structures are fructose, mannose, and galactose. These must be changed to glucose before they can

be used in a cell. Because of its use for energy in body processes, glucose is found in the blood. Normal levels are from 65 to 110 mg glucose per 100 ml of blood. Higher levels may indicate diabetes.

Units of two monosaccharides make up several very important sugars known as **disaccharides**. When two molecules of monosaccharides join together to form a disaccharide,

$$C_6H_{12}O_6 + C_6H_{12}O_6 \rightarrow C_{12}H_{22}O_{11} + H_2O \qquad (3.4.1)$$

a molecule of water is lost. Recall that proteins are also formed from smaller amino acid molecules by condensation reactions involving the loss of water molecules. Disaccharides include sucrose (cane sugar used as a sweetener), lactose (milk sugar), and maltose (a product of the breakdown of starch).

Polysaccharides consist of many simple sugar units hooked together. One of the most important polysaccharides is **starch**, which is produced by plants for food storage. Animals produce a related material called **glycogen**. The chemical formula of starch is $(C_6H_{10}O_5)_n$, where n may represent a number as high as several hundreds. What this means is that the very large starch molecule consists of many units of $C_6H_{10}O_5$ joined together. For example, if n is 100, there are 6 times 100 carbon atoms, 10 times 100 hydrogen atoms, and 5 times 100 oxygen atoms in the molecule. Its chemical formula is $C_{600}H_{1000}O_{500}$.The atoms in a starch molecule are actually present as linked rings represented by the structure shown in Figure 3.3. Starch occurs in many foods, such as bread and cereals. It is readily digested by animals, including humans.

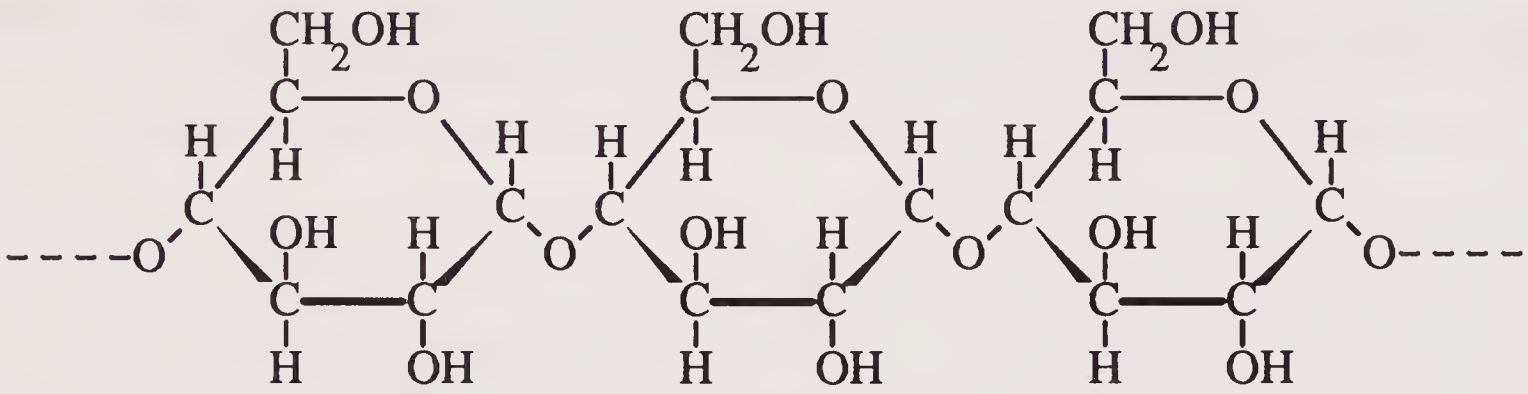


Figure 3.3. Part of a starch molecule showing units of $C_6H_{10}O_5$ condensed together.

Cellulose is a polysaccharide which is also made up of $C_6H_{10}O_5$ units. Molecules of cellulose are huge, with molecular masses of around 400,000. The cellulose structure (Figure 3.4) is similar to that of starch. Cellulose is produced by plants and forms the structural material of plant cell walls. Wood is about 60% cellulose, and cotton contains over 90% of this material. Fibers of cellulose are extracted from wood and pressed together to make paper.

Humans and most other animals cannot digest cellulose. Ruminant animals (cattle, sheep, goats, moose) have bacteria in their stomachs that break down cellulose into products which can be used by the animal. Chemical processes are available to convert cellulose to simple sugars by the reaction

$$\underset{\text{cellulose}}{(C_6H_{10}O_5)n} + nH_2O \rightarrow \underset{\text{glucose}}{nC_6H_{12}O_6} \qquad (3.4.2)$$

where n may be 2000-3000. This involves a hydrolysis reaction in which the linkages between units of $C_6H_{10}O_5$ are broken and a molecule of H_2O is added at each linkage.

Large amounts of cellulose from wood, sugar cane, and agricultural products go to waste each year. The hydrolysis of cellulose enables these products to be converted to sugars, which can be fed to animals.

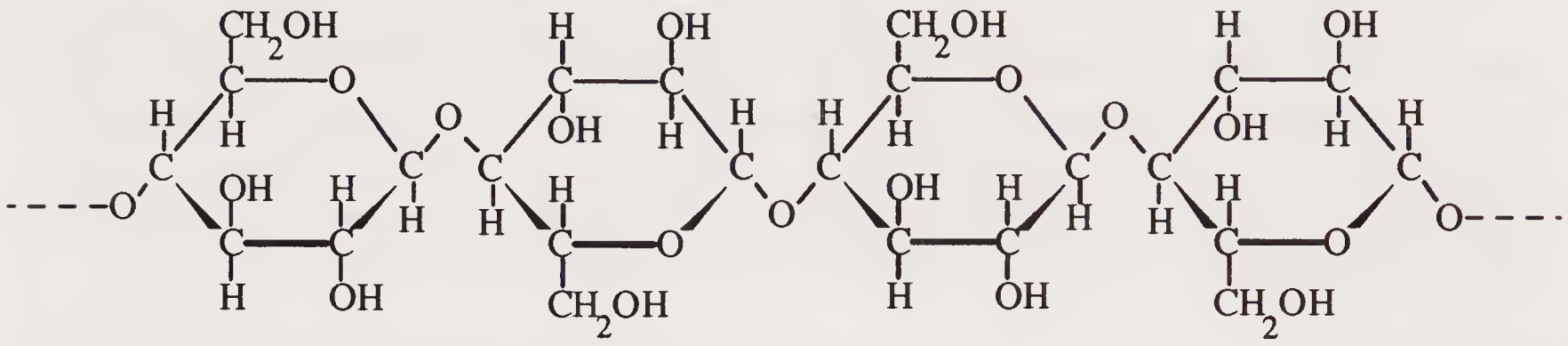


Figure 3.4. Part of the structure of cellulose.

Carbohydrate groups are attached to protein molecules in a special class of materials called **glycoproteins**. Collagen is a crucial glycoprotein that provides structural integrity to body parts. It is a major constituent of skin, bones, tendons, and cartilage.

3.5. LIPIDS

Whereas carbohydrates and proteins are characterized predominately by the monomers (monosaccharides and amino acids) from which they are composed, **lipids** are defined essentially by their physical characteristic of organophilicity. The most common lipids are fats and oils composed of **triglycerides** formed from the alcohol glycerol, $CH_2(OH)CH(OH)CH_2(OH)$, and a long-chain fatty acid such as stearic acid, $CH_3(CH_2)_{16}C(O)OH$ (Figure 3.5). Numerous other biological materials, including

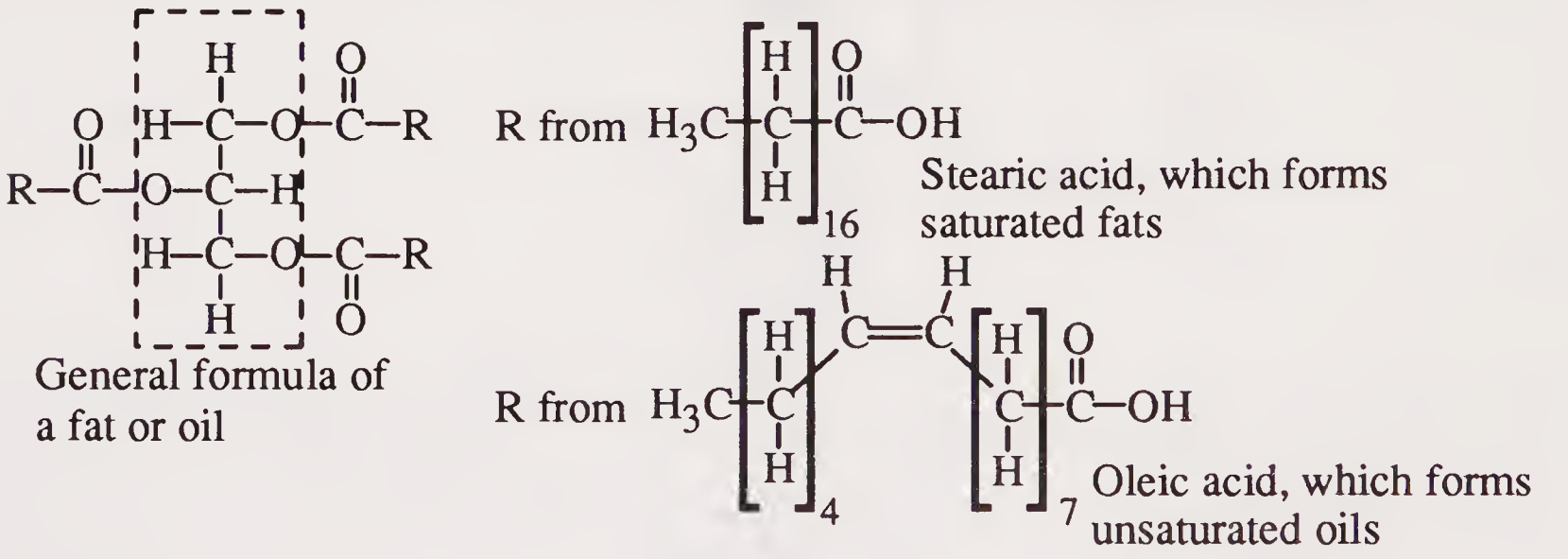


Figure 3.5. General formula of triglycerides, which make up fats and oils. The glycerol alcohol group is outlined by the dashed line and the R group is from a fatty acid and is a hydrocarbon chain, such as $-(CH_2)_{16}CH_3$.

waxes, cholesterol, and some vitamins and hormones, are classified as lipids. Common foods, such as butter and salad oils are lipids. The longer chain fatty acids, such as stearic acid, are also organic-soluble and are classified as lipids. Lipids, therefore, constitute a diverse group of biomolecules and are defined as substances that can be extracted from plant or animal matter by organic solvents, such as chloroform, diethyl ether, or toluene (Figure 3.6).

Lipids are toxicologically important for several reasons. Some toxic substances interfere with lipid metabolism, leading to detrimental accumulation of lipids. Many toxic organic compounds are poorly soluble in water, but are lipid-soluble, so that bodies of lipids in organisms serve to dissolve and store toxicants.

An important class of lipids consists of **phosphoglycerides** (glycerophosphatides). These compounds may be regarded as triglyderides in which one of the acids bonded to glycerol is orthophosphoric acid. These lipids are especially important because they are essential constituents of cell membranes. These membranes consist of bilayers in which the hydrophilic phosphate ends of the molecules are on the outside of the membrane and the hydrophobic "tails" of the molecules are on the inside.

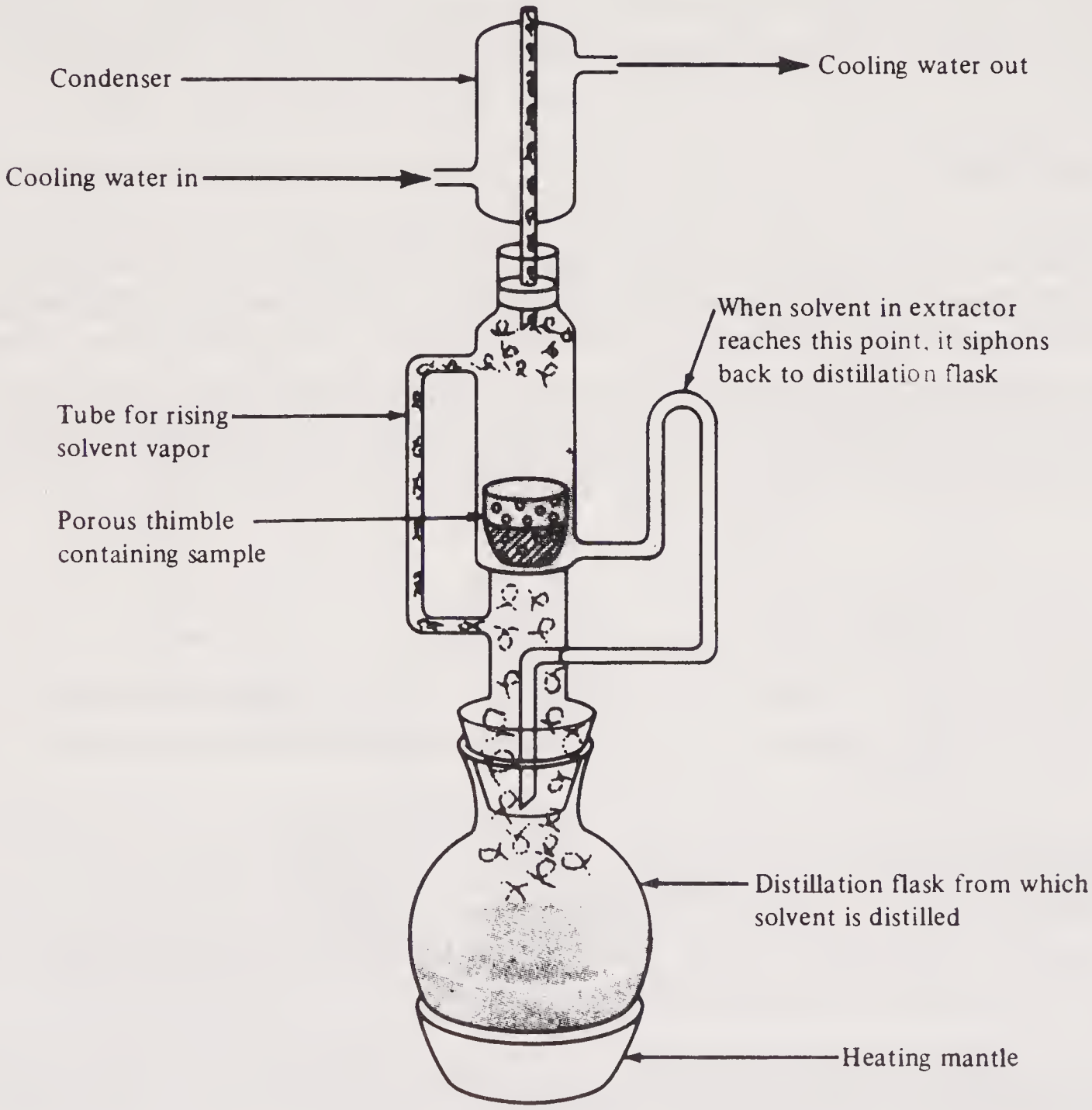
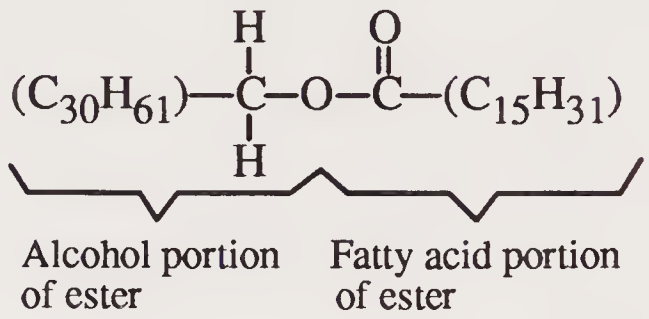
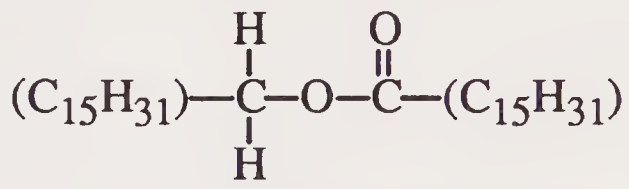


Figure 3.6. Lipids are extracted from some biological materials with a Soxhlet extractor (above). The solvent is vaporized in the distillation flask by the heating mantle, rises through one of the exterior tubes to the condenser, and is cooled to form a liquid. The liquid drops onto the porous thimble containing the sample. Siphon action periodically drains the solvent back into the distillation flask. The extracted lipid collects as a solution in the solvent in the flask.

Waxes are also esters of fatty acids. However, the alcohol in a wax is not glycerol. The alcohol in waxes is often a very long chain alcohol. For example, one of the main compounds in beeswax is myricyl palmitate,



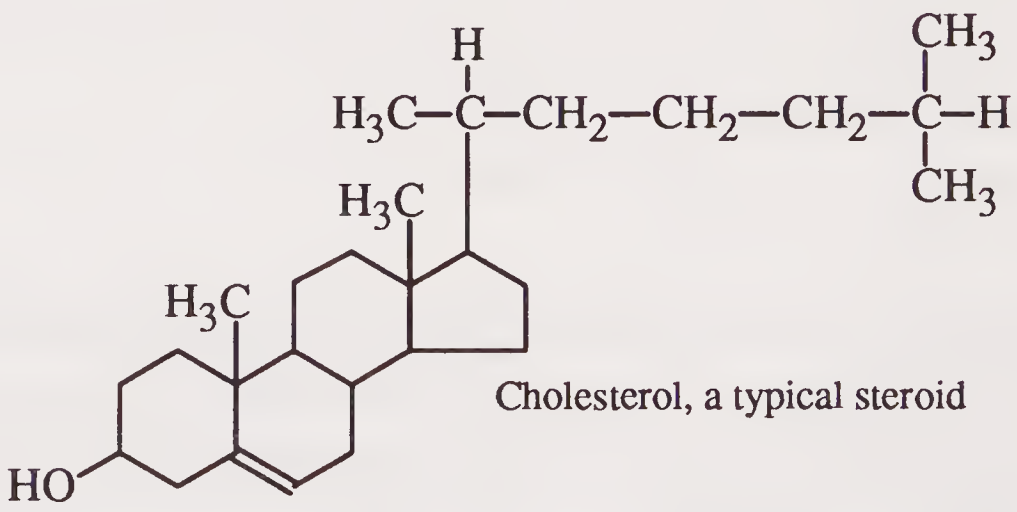
in which the alcohol portion of the ester has a very long hydrocarbon chain. Waxes are produced by both plants and animals, largely as protective coatings. Waxes are found in a number of common products. Lanolin is one of these. It is the "grease" in sheep's wool. When mixed with oils and water, it forms stable colloidal emulsions consisting of extremely small oil droplets suspended in water. This makes lanolin useful for skin creams and pharmaceutical ointments. Carnauba wax occurs as a coating on the leaves of some Brazilian palm trees. Spermaceti wax is composed largely of cetyl palmitate,



Cetyl palmitate

extracted from the blubber of the sperm whale. It is very useful in some cosmetics and pharmaceutical preparations.

Steroids are lipids found in living systems which all have the ring system shown in Figure 3.7 for cholesterol. Steroids occur in **bile salts**, which are produced by the



Cholesterol, a typical steroid

Figure 3.7. Steroids are characterized by the ring structure shown above for cholesterol.

liver and then secreted into the intestines. Their breakdown products give feces its characteristic color. Bile salts act upon fats in the intestine. They suspend very tiny fat droplets in the form of colloidal emulsions. This enables the fats to be broken down chemically and digested (see the discussion of fat digestion in Section 4.2).

Some steroids are **hormones**. Hormones act as "messengers" from one part of the body to another. As such, they start and stop a number of body functions. Male and female sex hormones are examples of steroid hormones. Hormones are given off by glands in the body called **endocrine glands**. The locations of the important endocrine glands are shown in Figure 3.8.

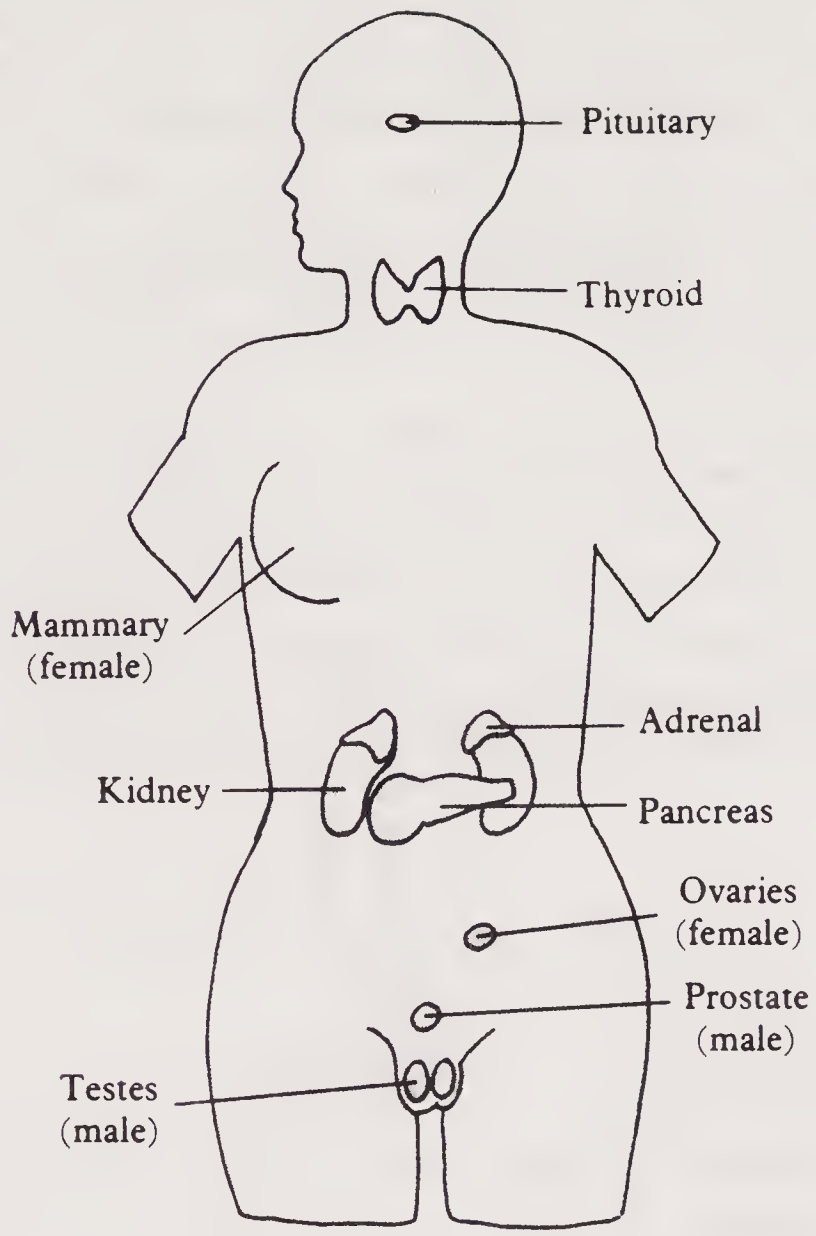


Figure 3.8. Locations of important endocrine glands.

3.6. ENZYMES

Catalysts are substances that speed up a chemical reaction without themselves being consumed in the reaction. The most sophisticated catalysts of all are those found in living systems. They bring about reactions that could not be performed at all, or only with great difficulty, outside a living organism. These catalysts are called **enzymes**. In addition to speeding up reactions by as much as ten- to a hundred-million-fold, enzymes are extremely selective in the reactions which they promote.

Nature and Action of Enzymes

Enzymes are proteinaceous substances with highly specific structures that interact with particular substances or classes of substances called **substrates**.[2] Enzymes act as catalysts to enable biochemical reactions to occur, after which they are regenerated intact to take part in additional reactions. The extremely high specificity with which enzymes interact with substrates results from their "lock and key" action based upon the unique shapes of enzymes as illustrated in Figure 3.9.

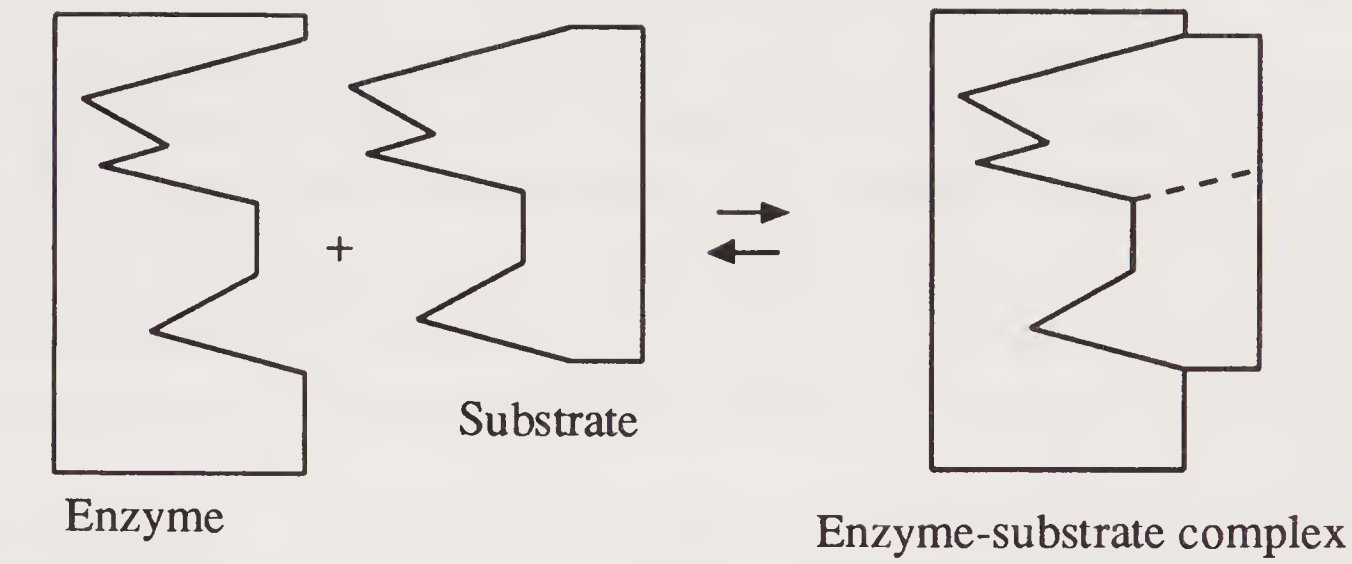


Figure 3.9. Representation of the "lock-and-key" mode of enzyme action which enables the very high specificity of enzyme-catalyzed reactions.

This illustration shows that an enzyme "recognizes" a particular substrate by its molecular structure and binds to it to produce an **enzyme-substrate complex**. This complex then breaks apart to form one or more products different from the original enzyme, regenerating the unchanged enzyme, which is then available to catalyze additional reactions. The basic process for an enzyme reaction is, therefore,

$$\text{enzyme} + \text{substrate} \leftarrow\!\!\rightarrow \text{enzyme-substrate complex} \leftarrow\!\!\rightarrow \text{enzyme} + \text{product} \qquad (3.6.1)$$

Several important things should be noted about this reaction. As shown in Figure 3.9, an enzyme acts on a specific substrate to form an enzyme-substrate complex because of the fit between their structures. As a result, something happens to the substrate molecule. For example, it might be split in two at a particular location. Then the enzyme-substrate complex comes apart, yielding the enzyme and products. The enzyme is not changed in the reaction and is now free to react again. Note that the arrows in the formula for enzyme reaction point both ways. This means that the reaction is **reversible**. An enzyme-substrate complex can simply revert back to the enzyme and the substrate. The products of an enzymatic reaction can react with the enzyme to form the enzyme-substrate complex again. It, in turn, may again form the enzyme and the substrate. Therefore, the same enzyme may act to cause a reaction to go either way.

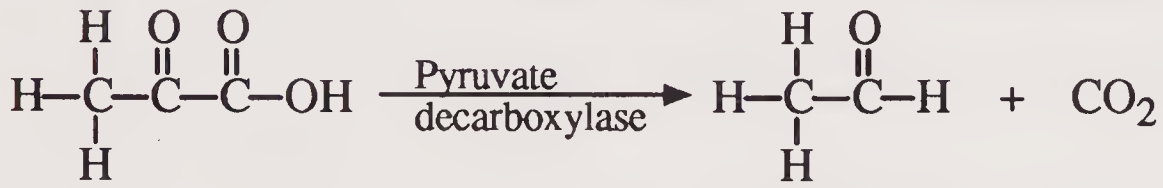
Some enzymes cannot function by themselves. In order to work, they must first be attached to **coenzymes**. Coenzymes normally are not protein materials. Some of the vitamins are important coenzymes.

Types and Names of Enzymes

Enzymes are named for what they do. For example, the enzyme given off by the stomach, which splits proteins as part of the digestion process, is called *gastric proteinase*. The "gastric" part of the name refers to the enzyme's origin in the stomach. The "proteinase" denotes that it splits up protein molecules. The common name for this enzyme is pepsin. Similarly, the enzyme produced by the pancreas that breaks down fats (lipids) is called *pancreatic lipase*. Its common name is steapsin. In general, lipase enzymes cause lipid triglycerides to dissociate and form glycerol and fatty acids.

The enzymes mentioned above are **hydrolyzing enzymes**, which bring about the breakdown of high molecular weight biological compounds by the addition of water. This is one of the most important types of the reactions involved in digestion. The three main classes of energy-yielding foods that animals eat are carbohydrates, proteins, and fats. Recall that the higher carbohydrates humans eat are largely disaccharides (sucrose, or table sugar) and polysaccharides (starch) and that these are formed by the joining together of units of simple sugars, $C_6H_{12}O_6$, with the elimination of an H_2O molecule at the linkage where they join. Proteins are formed by the condensation of amino acids, again with the elimination of a water molecule at each linkage. Fats are esters which are produced when glycerol and fatty acids link together. A water molecule is lost for each of these linkages when a protein, fat, or carbohydrate are synthesized. In order for these substances to be used as a food source, the reverse process must occur to break down large, complicated molecules of protein, fat, or carbohydrate to simple, soluble substances which can penetrate a cell membrane and take part in chemical processes in the cell. This reverse process is accomplished by hydrolyzing enzymes.

Biological compounds with long chains of carbon atoms are broken down into molecules with shorter chains by the breaking of carbon-carbon bonds. This commonly occurs by the elimination of $—CO_2H$ groups from carboxylic acids. For example, *pyruvic decarboxylase* enzyme acts upon pyruvic acid,



Pyruvic acid — Acetaldehyde (3.6.2)

to split off CO_2 and produce a compound with one less carbon. It is by such one- or two-carbon reactions that long chain compounds are eventually degraded to CO_2 in the body, or that long-chain hydrocarbons undergo biodegradation by the action of microorganims in the water and soil environments.

Oxidation and reduction are the predominant reactions for the exchange of energy in living systems. Cellular respiration discussed in connection with mitochondria in Section 3.2 is an oxidation reaction in which a carbohydrate, $C_6H_{12}O_6$, is broken down to carbon dioxide and water with the release of energy.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy} \qquad (3.6.3)$$

Actually, such an overall reaction occurs in living systems by a complicated series of individual steps. Some of these steps involve oxidation. The enzymes that bring about

oxidation in the presence of free O_2 are called **oxidases**. In general, biological oxidation-reduction reactions are catalyzed by **oxidoreductase enzymes**.

In addition to the types of enzymes discussed above, there are many enzymes that perform miscellaneous duties in living systems. Typical of these are **isomerases**, which form isomers of particular compounds. For example, there are several simple sugars with the formula $C_6H_{12}O_6$. However, only glucose can be used directly for cell processes. The other isomers are converted to glucose by the action of isomerases. **Transferase enzymes** move chemical groups from one molecule to another, **lyase enzymes** remove chemical groups without hydrolysis and participate in the formation of C=C bonds or addition of species to such bonds, and **ligase enzymes** work in conjunction with ATP (adenosine triphosphate, a high-energy molecule that plays a crucial role in energy-yielding, glucose-oxidizing metabolic processes) to link molecules together with the formation of bonds such as carbon-carbon or carbon-sulfur bonds.

Enzyme action may be affected by many different things. Enzymes require a certain hydrogen ion concentration to function best. For example, gastric proteinase requires the acid environment of the stomach to work well. When it passes into the much less acidic intestines, it stops working. This prevents damage to the intestine walls, which would occur if the enzyme tried to digest them. Temperature is critical. Not surprisingly, the enzymes in the human body work best at around 98.6°F (37°C), which is the normal body temperature. Heating these enzymes to around 140°F permanently destroys them. Some bacteria that thrive in hot springs have enzymes which work best at relatively high temperatures. Other "cold-seeking" bacteria have enzymes adapted to near the freezing point of water.

One of the greatest concerns regarding the effects of surroundings upon enzymes is the influence of toxic substances. A common mechanism of toxicity is the alteration or destruction of enzymes by toxic agents — as examples, cyanide, heavy metals, or organic compounds, such as insecticidal parathion. An enzyme that has been destroyed obviously cannot perform its designated function, whereas one that has been altered may either not function at all or may act improperly. Toxicants can affect enzymes in several ways. Parathion, for example, bonds covalently to the nerve enzyme acetylcholinesterase, which can then no longer serve to stop nerve impulses. Heavy metals tend to bind to sulfur atoms in enzymes (such as sulfur from the amino acid cysteine shown in Table 3.2) thereby altering the shape and function of the enzyme. Enzymes are denatured by some poisons causing them to "unravel" so that the enzyme no longer has its crucial specific shape.

3.7. NUCLEIC ACIDS

The "essence of life" is contained in **deoxyribonucleic acid** (**DNA**, a biopolymer with a molecular mass of 6–16 million, which stays in the cell nucleus) and **ribonucleic acid** (**RNA**, a biopolymer with a molecular mass of 20–40 thousand, which functions in the cell cytoplasm). These substances, which are known collectively as **nucleic acids**, store and pass on essential genetic information that controls reproduction and protein synthesis.

The structural formulas of the monomeric constituents of nucleic acids are given in Figure 3.10. These are pyrimidine or purine nitrogen-containing bases, two sugars, and phosphate. DNA molecules are made up of the nitrogen-containing bases adenine, guanine, cytosine, and thymine; phosphoric acid (H_3PO_4), and the simple sugar 2-deoxy-β-D-ribofuranose (commonly called deoxyribose). RNA molecules are composed of the nitrogen-containing bases adenine, guanine, uracil, and thymine; phosphoric acid (H_3PO_4), and the simple sugar β-D-ribofuranose (commonly called ribose).

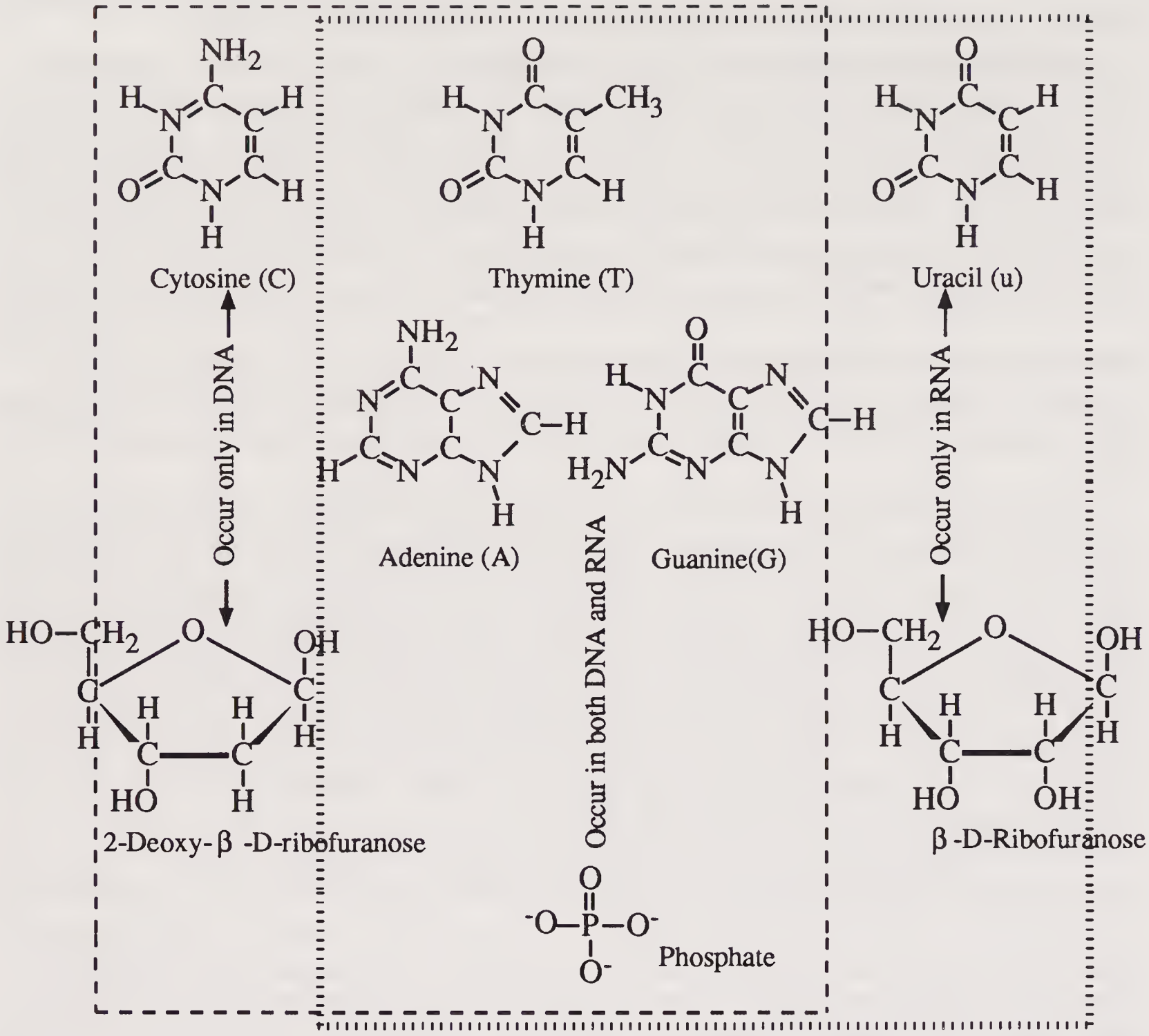
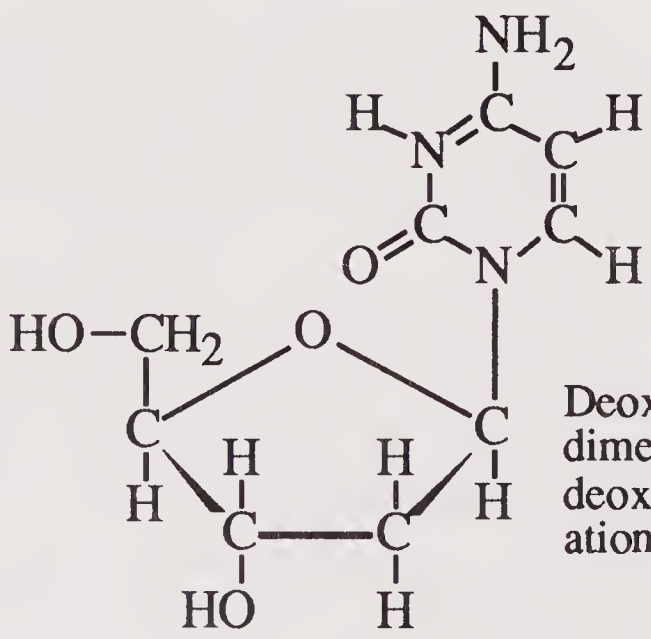


Figure 3.10. Constituents of DNA (enclosed by ----) and of RNA (enclosed by ⋯⋯).

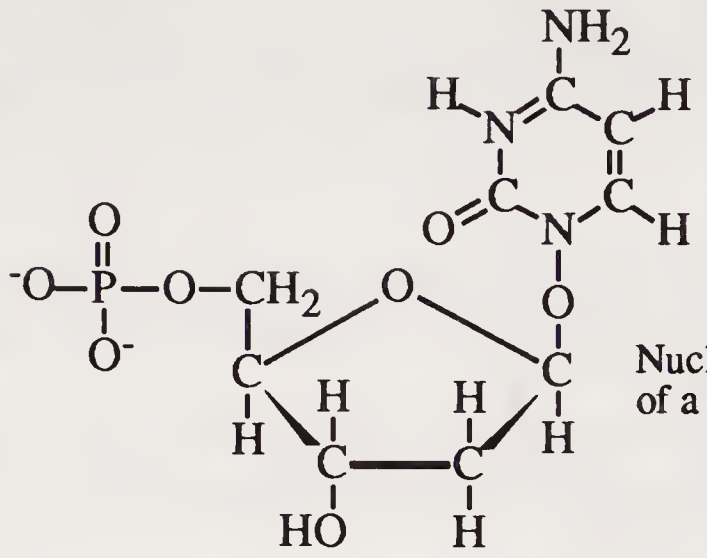
The formation of nucleic acid polymers from their monomeric constituents may be viewed as the following steps.

- Monosaccharide (simple sugar) + cyclic nitrogenous base yields **nucleoside**:



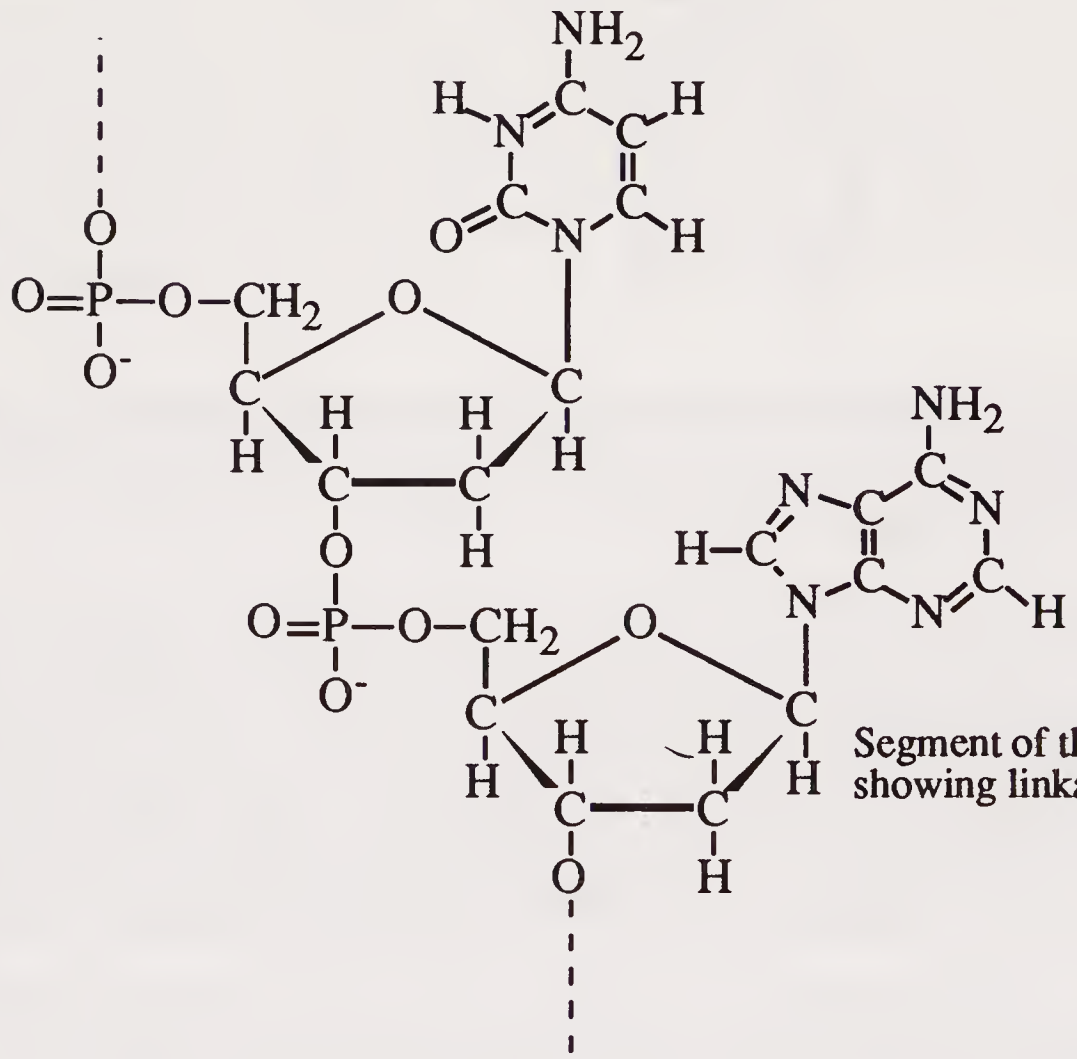
Deoxyctidine formed by the dimerization of cytosine and deoxyribose with the elimination of a molecule of H_2O.

- Nucleoside + phosphate yields **phosphate ester nucleotide**.



Nucleotide formed by the bonding of a phosphate group to deoxyctidine

- Polymerized nucleotide yields **nucleic acid**. In the nucleic acid the phosphate negative charges are neutralized by metal cations (such as Mg^{2+}) or positively charged proteins (histones).



Segment of the DNA polymer showing linkage of two nucleotides

Both DNA and RNA are high-molecular-mass biomolecules. The structure of DNA is that of the famed "double helix." It was figured out in 1953 by an American scientist, James D. Watson, and Francis Crick, a British scientist. They received the Nobel prize for this scientific milestone in 1962. This model visualizes DNA as a so-called double α-helix structure of oppositely-wound polymeric strands held together by hydrogen bonds between opposing pyrimidine and purine groups. As a result, DNA has both a primary and a secondary structure; the former is due to the sequence of nuceotides in the individual strands of DNA and the latter results from the α-helix interaction of the two strands. In the secondary structure of DNA, only cytosine can be opposite guanine and only thymine can be opposite adenine and *vice versa*. Basically, the structure of DNA is that of two spiral ribbons "counter-wound" around each other.

The two strands of DNA are **complementary** (Figure 3.11). This means that a particular portion of one strand fits like a key in a lock with the corresponding portion of another strand. If the two strands are pulled apart, each manufactures a new complementary strand, so that two copies of the original double helix result. This occurs during cell reproduction.

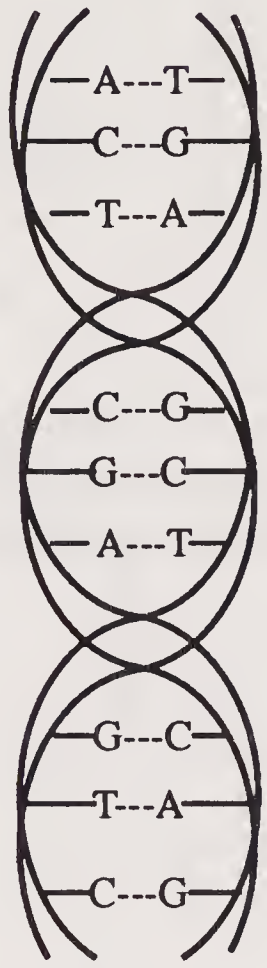


Figure 3.11. Representation of the double helix structure of DNA showing the allowed base pairs held together by hydrogen bonding between the phosphate/sugar polymer "backbones" of the two strands of DNA. The letters stand for adenine (A), cytosine (C), guanine (G), and thymine (T). The dashed lines, ---, represent hydrogen bonds.

The molecule of DNA acts as a coded message. This message consists of the genetic information contained in, and transmitted by nucleic acids. It is written by the sequence of bases from which the nucleic acids are composed. The information transmitted by DNA is somewhat like one sent by telegraph, which consists only of dots, dashes, and spaces in between. The key aspect of DNA structure that enables storage and replication of this information is the famed double helix structure of DNA mentioned above.

Portions of the DNA double helix may unravel, and one of the strands of DNA may produce a strand of RNA. This substance then goes from the cell nucleus out into the cell and regulates the synthesis of new protein. In this way, DNA regulates the function of the cell and acts to control life processes.

Nucleic Acids in Protein Synthesis

Whenever a new cell is formed, the DNA in its nucleus must be accurately reproduced from the parent cell. Life processes are absolutely dependent upon accurate protein synthesis as regulated by cell DNA. The DNA in a single cell must be capable of directing the synthesis of up to 3000 or even more different proteins. The directions for the synthesis of a single protein are contained in a segment of DNA called a **gene**. The process of transmitting information from DNA to a newly synthesized protein involves the following steps:

- The DNA undergoes **replication**. This process involves separation of a segment of the double helix into separate single strands which then replicate such that guanine is opposite cytosine (and *vice versa*) and adenine is opposite thymine (and *vice versa*). This process continues until a complete copy of the DNA molecule has been produced.

- The newly replicated DNA produces **messenger RNA (m-RNA)**, a complement of the single strand of DNA, by a process called **transcription**.

- A new protein is synthesized using m-RNA as a template to determine the order of amino acids in a process called **translation**.

Modified DNA

DNA molecules may be modified by the unintentional addition or deletion of nucleotides or by substituting one nucleotide for another. The result is a **mutation** that is transmittable to offspring. Mutations can be induced by chemical substances. This is a concern from a toxicological viewpoint because of the detrimental effects of many mutations and because substances that cause mutations often cause cancer as well. DNA malfunction may result in birth defects. The failure to control cell reproduction results in cancer. Radiation from X rays and radioactivity also disrupts DNA and may cause mutation.

3.8. RECOMBINANT DNA AND GENETIC ENGINEERING

As noted above, segments of DNA contain information for the specific syntheses of particular proteins. Within the last two decades it has become possible to transfer this information between organisms by means of **recombinant DNA technology**, which has resulted in a new industry based on **genetic engineering**. Most often the recipient organisms are bacteria, which can be reproduced (cloned) over many orders of magnitude from a cell that has acquired the desired qualities. Therefore, to synthesize a particular substance, such as human insulin or growth hormone, the required genetic information can be transferred from a human source to bacterial cells, which then produce the substance as part of their metabolic processes.

The first step in recombinant DNA gene manipulation is to lyze, or "open up" a cell that has the genetic material needed and to remove this material from the cell. Through enzyme action the sought-after genes are cut from the donor DNA chain. These are next spliced into small DNA molecules. These molecules, called **cloning vehicles**, are capable of penetrating the host cell and becoming incorporated into its genetic material. The modified host cell is then reproduced many times and carries out the desired biosynthesis.

Early concerns about the potential of genetic engineering to produce "monster organisms" or new and horrible diseases have been largely allayed, although caution is still required with this technology. In the environmental area genetic engineering offers some hope for the production of bacteria engineered to safely destroy troublesome wastes and to produce biological substitutes for environmentally damaging synthetic pesticides.

3.9. METABOLIC PROCESSES

Biochemical processes that involve the alteration of biomolecules fall under the category of **metabolism**. Metabolic processes, which are discussed in detail in Chapter 4, may be divided into the two major categories of **anabolism** (synthesis) and **catabolism** (degradation of substances). An organism may use metabolic processes to yield energy or to modify the constituents of biomolecules.

Energy-Yielding Processes

Organisms can gain energy by the following three processes:

- **Respiration** in which organic compounds undergo catabolism that requires molecular oxygen (**aerobic respiration**) or that occurs in the absence of molecular oxygen (**anaerobic repiration**). Aerobic respiration uses the **Krebs cycle** to obtain energy from the following reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

  About half of the energy released is converted to short-term stored chemical energy, particularly through the synthesis of **adenosine triphosphate** (**ATP**) nucleoside. For longer-term energy storage, glycogen or starch polysaccharides are synthesized, and for still longer-term energy storage lipids (fats) are generated and retained by the organism.

- **Fermentation**, which differs from respiration in not having an electron transport chain. Yeasts produce ethanol from sugars by respiration:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

- **Photosynthesis** in which light energy captured by plant and algal chloroplasts is used to synthesize sugars from carbon dioxide and water:

$$6CO_2 + 6H_2O + h\nu \rightarrow C_6H_{12}O_6 + 6O_2$$

Plants cannot always get the energy that they need from sunlight. During the dark they must use stored food. Plant cells, like animal cells, contain mitochondria in which stored food is converted to energy by cellular respiration.

Plant cells, which use sunlight as a source of energy and CO_2 as a source of carbon, are said to be **autotrophic**. In contrast, animal cells must depend upon organic material manufactured by plants for their food. These are called **heterotrophic** cells. They act as "middlemen" in the chemical reaction between oxygen and food material using the energy from the reaction to carry out their life processes.

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QUESTIONS AND PROBLEMS

1. What is the toxicological importance of lipids? How do lipids related to hydrophobic ("water-disliking") pollutants and toxicants?

2. What is the function of a hydrolase enzyme?

3. Match the cell structure on the left with its function on the right, below:

1. Mitochondria	(a) Toxicant metabolism
2. Endoplasmic reticulum	(b) Fills the cell
3. Cell membrane	(c) Deoxyribonucleic acid
4. Cytoplasm	(d) Mediate energy conversion and utilization
5. Cell nucleus	(e) Encloses the cell and regulates the passage of materials into and out of the cell interior

4. The formula of simple sugars is $C_6H_{12}O_6$. The simple formula of higher carbohydrates is $C_6H_{10}O_5$. Of course, many of these units are required to make a molecule of starch or cellulose. If higher carbohydrates are formed by joining together molecules of simple sugars, why is there a difference in the ratios of C, H, and O atoms in the higher carbohydrates as compared to the simple sugars?

5. Why does wood contain so much cellulose?

6. What would be the chemical formula of a *tri*saccharide made by the bonding together of three simple sugar molecules?

7. The general formula of cellulose may be represented as $(C_6H_{10}O_5)_x$ If the molecular weight of a molecule of cellulose is 400,000, what is the estimated value of x?

8. During one month a factory for the production of simple sugars, $C_6H_{12}O_6$, by the hydrolysis of cellulose processes one million pounds of cellulose. The percentage of cellulose that undergoes the hydrolysis reaction is 40%. How many pounds of water are consumed in the hydrolysis of cellulose each month?

9. What is the structure of the largest group of atoms common to all amino acid molecules?

10. Glycine and phenylalanine can join together to form two different dipeptides. What are the structures of these two dipeptides?

11. One of the ways in which two parallel protein chains are joined together, or cross linked, is by way of an —S—S— link. What amino acid to you think might be most likely to be involved in such a link? Explain your choice.

12. Fungi, which break down wood, straw, and other plant material, have what are called "exoenzymes." Fungi have no teeth and cannot break up plant material physically by force. Knowing this, what do you suppose an exoenzyme is? Explain how an enzyme might operate in the process by which fungi break down something as tough as wood.

13. Many fatty acids of lower molecular weight have a bad odor. Speculate as to the reasons that rancid butter has a bad odor. What chemical compound in produced that has a bad odor? What sort of chemical reaction is involved in its production?

14. The long-chain alcohol with 10 carbons is called decanol. What do you think would be the formula of decyl stearate? To what class of compounds would it belong?

15. Write an equation for the chemical reaction between sodium hydroxide and cetyl stearate. What are the products?

16. What are two endocrine glands that are found only in females? What are two of these glands found only in males?

17. The action of bile salts is a little like that of soap. What function do bile salts perform in the intestine? Look up the action of soaps, and explain how bile salts may function somewhat like soap.

18. If the structure of an enzyme is illustrated as,

how should the structure of its substrate be represented?

19. Look up the structures of ribose and deoxyribose. Explain where the "deoxy" came from in the name, deoxyribose.

20. In what respect is an enzyme and its substrate like two opposite strands of DNA?

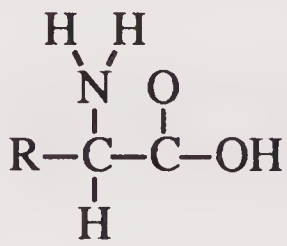
21. For what discovery are Watson and Crick noted?

22. Why does an enzyme no longer work if it is denatured?

23. What does the hydrophilic *vs.* hydrophobic character of toxic substances have to do with their toxicities.

24. Distinguish between eukaryotic cells and prokaryotic cells.

25. Explain what is meant by the following general formula and the "R" group in it:



What is a zwitterion? What is the difference between a simple and conjugated protein?

26. Match the following:

(a) Cell membrane	1. Mediate energy conversion and utilization in the cell
(b) Cell nucleus	2. Contains cytosol and cellular organelles
(c) Cytoplasm	3. Contains the genetic directions the cell needs to reproduce itself
(d) Mitochondria	4. Use energy from sunlight to convert carbon dioxide and water to organic matter
(e) Chloroplasts	5. Encloses the cell and regulates the passage of substances into and out of the cell interior

27. Describe the significant differences between the structures of structural and globular proteins.

28. Distinguish among simple sugars, disaccharides and polysaccharides. List two examples of each.

29. Define lipids, triglycerides, fatty acids, waxes, and phosphoglycerides.

30. Define bile salts and suggest how they might be tied to enzyme action.

31. Suggest how enzymes may be related chemically to proteins, how they may be involved in the synthesis of proteins, and how they may be involved in the breakdown (digestion) of proteins.

32. Discuss the meaning of the following general reaction:

$$\text{enzyme} + \text{substrate} \leftrightarrows \text{enzyme-substrate complex} \leftrightarrows \text{enzyme} + \text{product}$$

33. Why may it be reasonable that the molecular mass of RNA is much smaller than that of DNA given the function of both.

34. Explain the significance of "complementarity" in DNA structure.

35. What are the ways in which DNA may be modified, almost always with adverse consequences for the organism?

36. Define metabollic processes and the two major categories into which they may be divided.

37. Distinguish among the three metabolic processes of respiration, fermentation, and photosynthesis.

4

Metabolic Processes

METABOLISM IN ENVIRONMENTAL BIOCHEMISTRY

Metabolism, as the term is used in this book, describes the biochemical changes that substances undergo in a living organism. In a more restricted sense, the term refers to the processes by which chemical species are broken down in an organism, largely by enzymatic action, to produce energy and components for the synthesis of biomolecules required for life processes. Metabolism is an essential process for any organism because it provides the two things essential for life — energy and raw materials.

Metabolism is especially important in toxicological chemistry for two reasons: (1) Interference with metabolism is a major mode of toxic action and (2) toxic substances are transformed by metabolic processes to other materials that are usually, though not invariably, less toxic and more readily eliminated from the organism. This chapter introduces the topic of metabolism in general and addresses specifically the metabolic fates of xenobiotic compounds in living systems.

Pathways of Substances and their Metabolites in the Body

In considering metabolic processes, it is important to keep in mind the pathways of nutrients and xenobiotics in organisms. This is shown for humans and other vertebrate animals in Figure 4.1.

Regarding the uptake of materials by the body, the first component that comes to mind is the **gastrointestinal tract** beginning with the mouth, pharynx (throat) and esophagus. Some digestive and absorptive processes occur in these organs. The stomach is a major site of digestion and absorption. Beyond the stomach are the small and large intestines, rectum, and anus. The gastrointestinal tract is where essentially all ingestion of food, water, and associated contaminants occurs. Environmental and occupational exposure to toxicants, however, may occur through the respiratory tract and through skin.

4.2. DIGESTION

For most food substances and for a very limited number of toxicants, **digestion** is necessary for sorption into the body. Digestion is a hydrolysis process by which polymeric macromolecules are broken down into units that can be absorbed from the gastrointestinal tract into the circulatory system; material that cannot be absorbed is excreted as waste, usually after it has been subjected to the action of intestinal

bacteria. Digestion may begin in the mouth (starch is hydrolyzed by enzymes excreted from the salivary glands), occurs to a higher degree in the stomach, and takes place to the greatest extent in the small intestine.

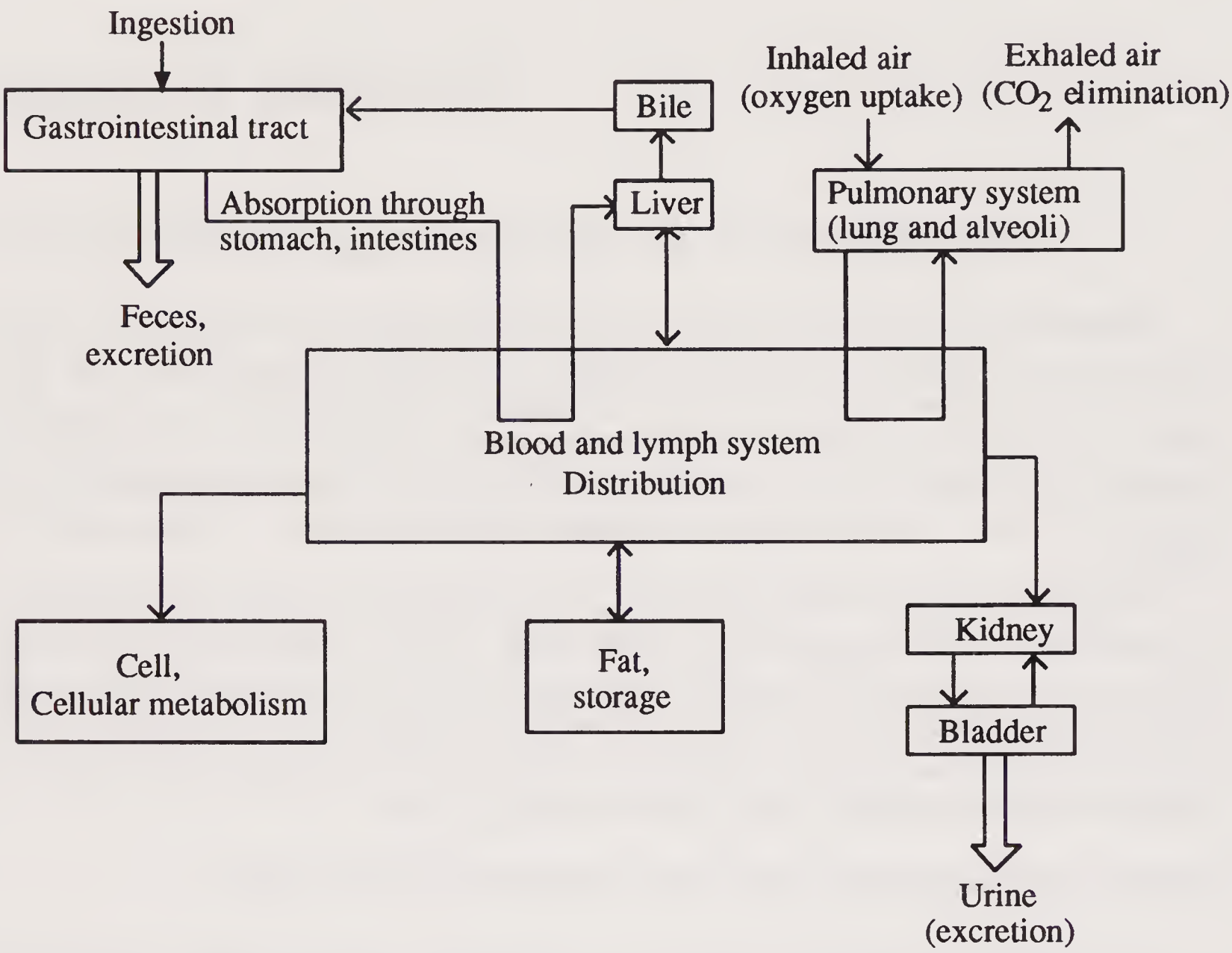
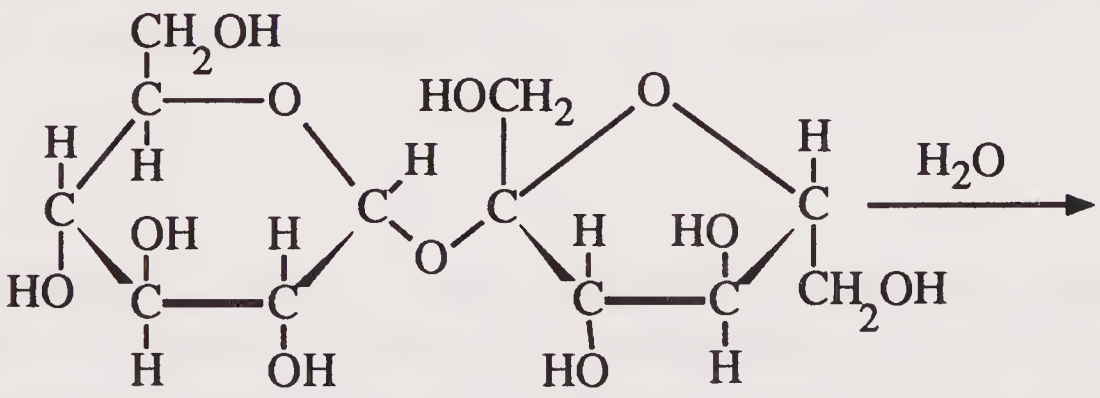


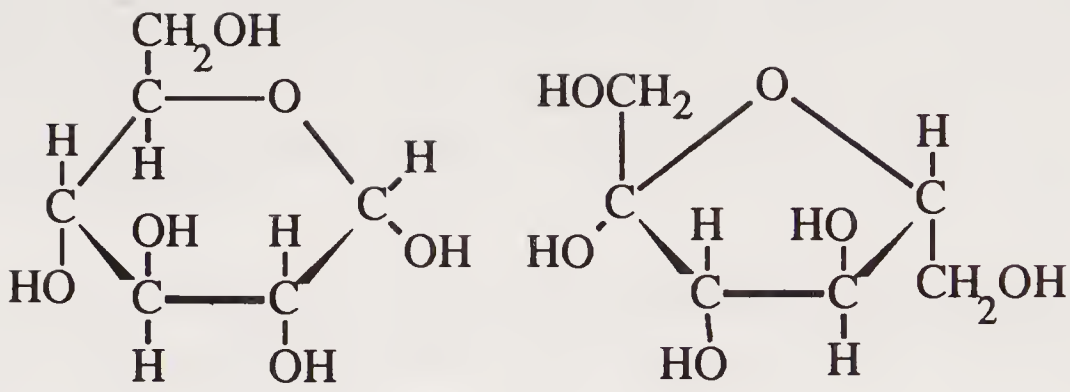
Figure 4.1. Major routes and sites of absorption, metabolism, binding, and excretion of substances in the body.

Carbohydrate Digestion

A very simple example of a digestion process is the hydrolysis of sucrose (common table sugar),



Sucrose



Glucose Fructose (4.2.1)

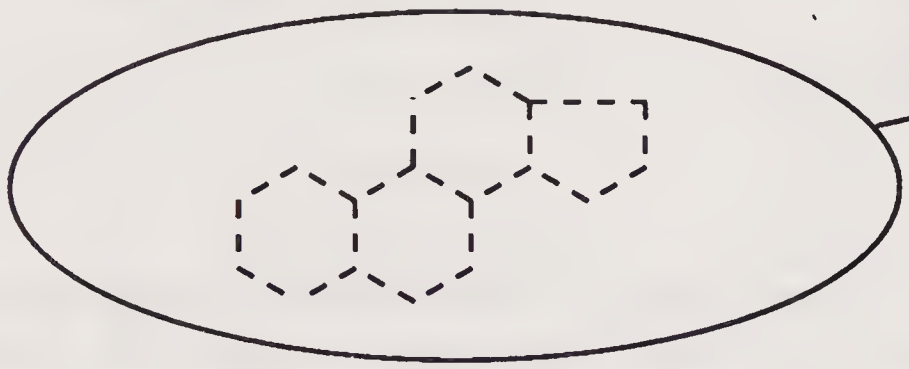
to produce glucose and fructose monosaccharides that can be absorbed through intestine walls to undergo metabolism in the body. Each digestive hydrolysis reaction has its own enzyme, with separate ones for sucrose, starch, etc. The lack of a digestive enzyme for cellulose in humans and virtually all other animals means that these animals cannot metabolize cellulose because it cannot enter the circulatory system.

Digestion of Fats

Fats and oils are the most common lipids that are digested. Digestion breaks fats down from triglycerides to di- and monoglycerides, fatty acids and their salts (soaps) and glycerol, which pass through the intestine wall, where they are resynthesized to triglycerides and transported to the blood through the lymphatic system (see Figure 4.2).

Figure 4.2. Illustration of digestion of fats (triglycerides).

A special consideration in the digestion of fats is that they are not water-soluble and cannot be placed in aqueous solution along with the water-soluble **lipase digestive enzymes**. However, intimate contact is obtained by emulsification of fats through the action of **bile salts** from glycocholic and taurocholic acids produced from cholesterol in the liver:



Representation of a bile salt showing steroid skeleton from cholesterol

Digestion of Proteins

Digestion of proteins occurs by enzymatic hydrolysis in the small intestine (Figure 4.3). The digestion of protein produces single amino acids. These can enter the bloodstream through the small intestine walls. The amino acids circulate in the bloodstream until further metabolized or used for protein synthesis; there is not a "storage depot" as such for amino acids as there is for lipids, which are stored in "fat depots" in adipose tissue. However, the body does break down protein tissue (muscle) to provide amino acids in the bloodstream.

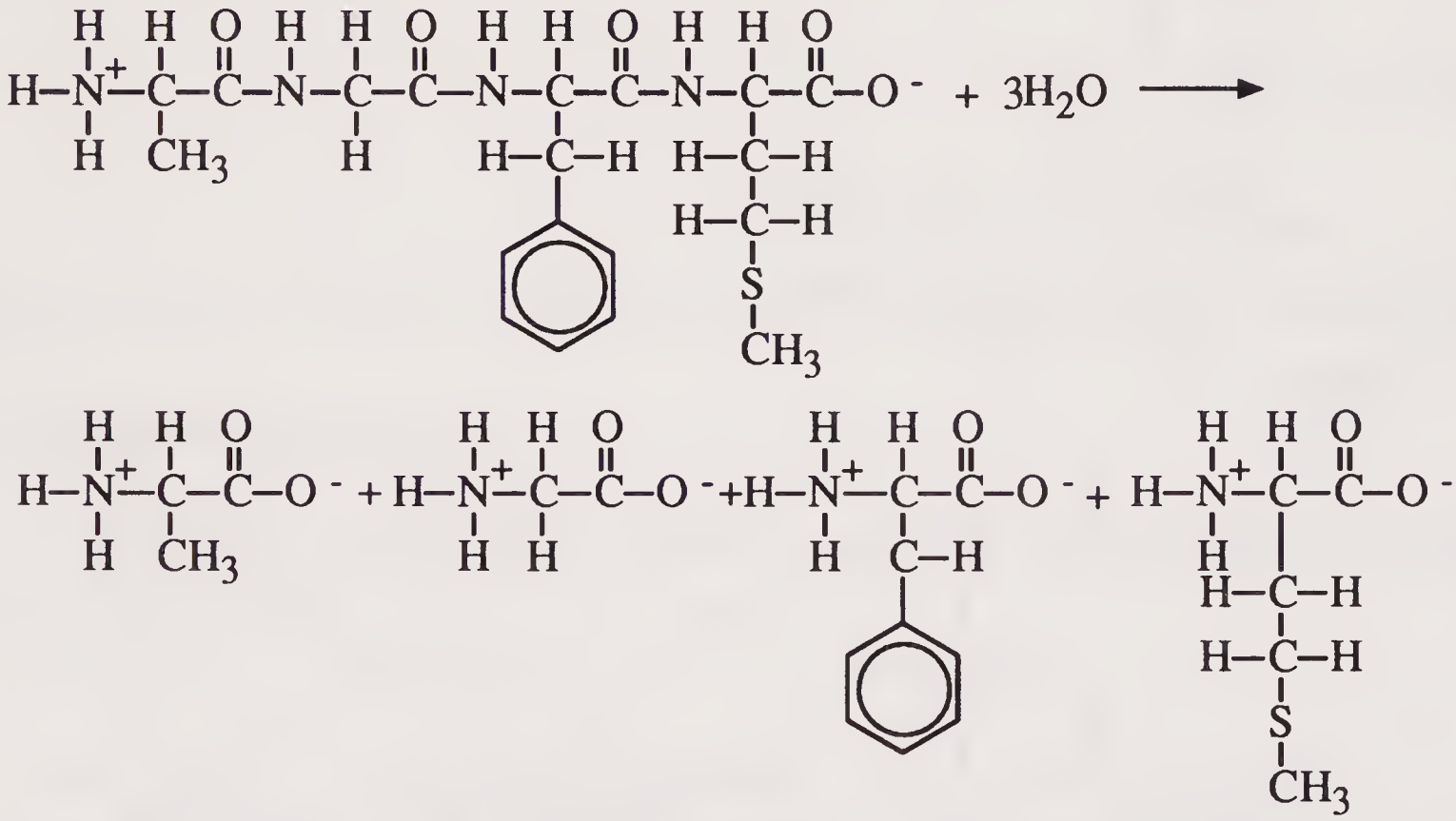


Figure 4.3. Illustration of the enzymatic hydrolysis of a tetrapeptide such as occurs in the digestion of protein.

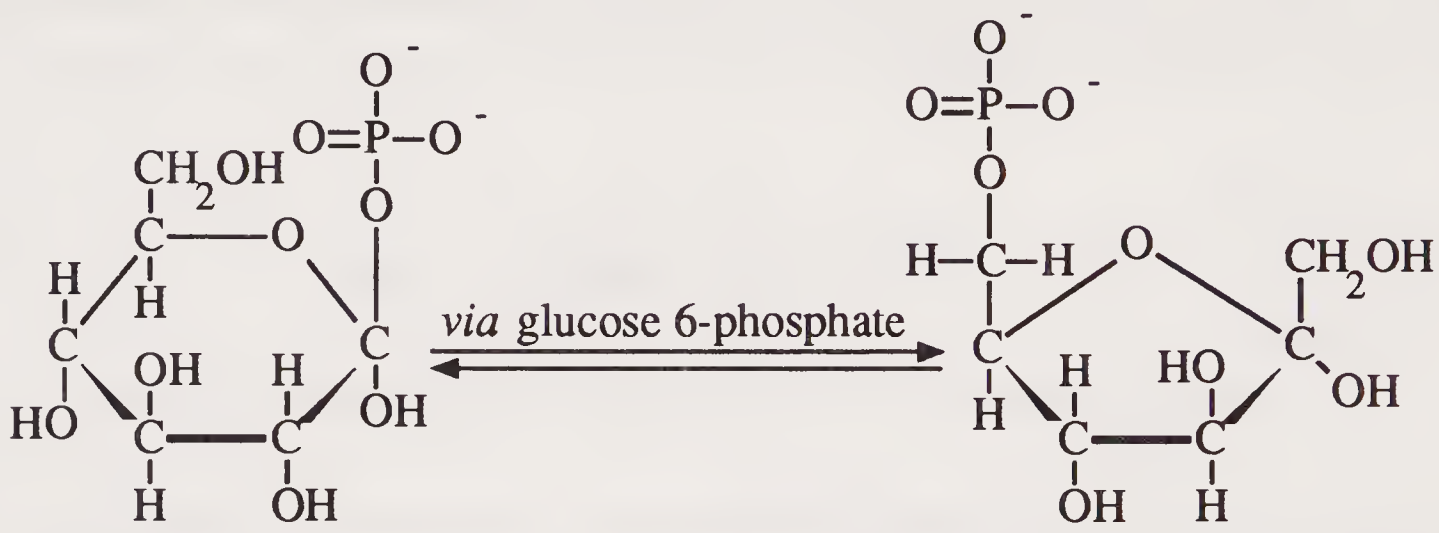
4.3. METABOLISM OF CARBOHYDRATES, FATS, AND PROTEINS

In the preceding section the digestion of carbohydrates, fats, and proteins by the enzymatic hydrolysis of their molecules was discussed. Digestion enables these materials to enter the bloodstream as relatively small molecules. Once in the bloodstream, these small molecules undergo further metabolic reactions to enable their use for

energy production and tissue synthesis. These metabolic processes are all rather complex and beyond the scope of this chapter to discuss in detail. However, the main points are covered below.

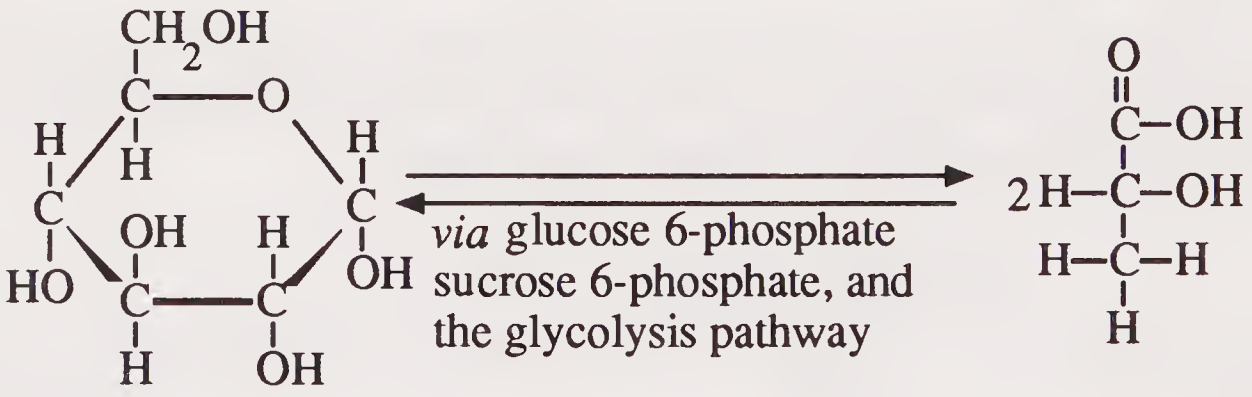
Carbohydrate Metabolism

The first major step in the metabolism of the simple sugar digestion products of carbohydrates is the conversion of glucose and fructose to their 6-phosphate conjugates, which are interconvertible:



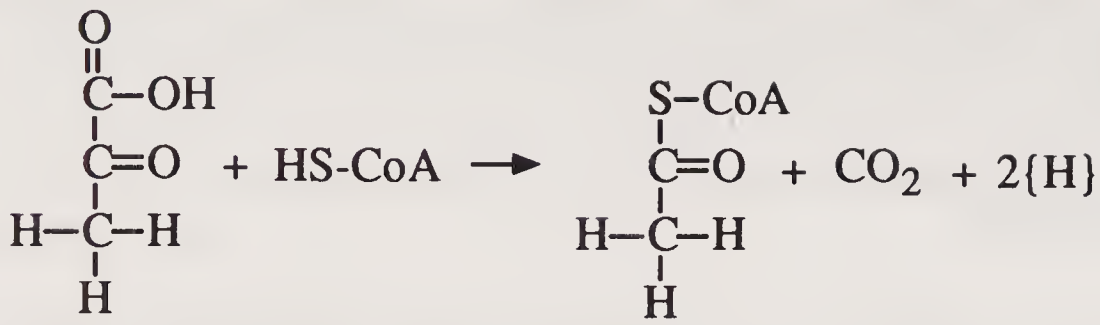
Glucose 1-phosphate Fructose 6-phosphate

From the glucose 1-phosphate form, glucose may be incorporated into macromolecular (polymeric) glycogen for storage in the animal's body. In order to obtain energy from a simple sugar, its molecule must be broken down. This can occur in a complex series of anaerobic (without O_2) enzymatically catalyzed reactions involving the high energy compounds adenosine diphosphate (ADP) and adenosine triphosphate (ATP), the oxidizing agent NAD^+, and the reducing agent NADH. The overall process can be represented as,



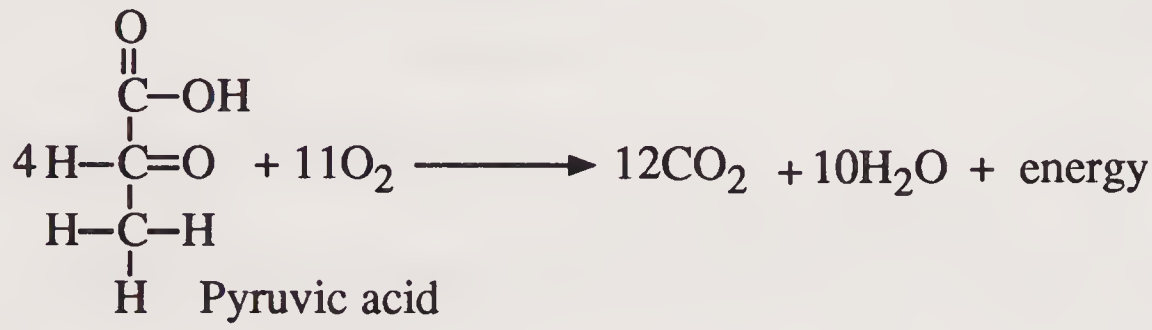
Glucose Lactic acid (4.3.1)

which releases energy and produces lactic acid, $C_3H_6O_3$. (Lactic acid that accumulates in muscle tissue faster than it can be removed during vigorous exercise causes feelings of muscle fatigue.) In this anaerobic glycolysis reaction, pyruvic acid is a precursor to lactic acid. Alternatively pyruvic acid can be broken down in the aerobic **Krebs cycle**, which does use molecular O_2. Pyruvic acid enters the Krebs cycle by attachment of its carboxylic acid group ($-CO_2H$) to the $-SH$ functional group of **coenzyme A** (CoA) to produce the following species:



(4.3.2)

At this point the two-carbon fragments attached to CoA can be assembled in chains to produce fatty acids for incorporation into fat, which is the basic mechanism by which fats are produced from carbohydrates. For energy production, the complex Krebs cycle mediates the overall reaction,



(4.3.3)

in which pyruvic acid is oxidized with molecular oxygen to carbon dioxide and water, releasing energy in the process.

Metabolism of Fats

Fats are stored and circulated through the body as triglycerides, which must undergo hydrolysis to glycerol and fatty acids before they are further metabolized. Glycerol is broken down *via* the glycolysis pathway discussed above for carbohydrate metabolism. The fatty acids are broken down in the **fatty acid cycle** in which a long-chain fatty acid goes through a number of sequential steps in which it is shortened by two-carbon fragments, producing CO_2, H_2O, and energy.

Metabolism of Proteins

A central feature of protein metabolism is the **amino acid pool** consisting of amino acids in the bloodstream. Figure 4.4 illustrates the metabolic relationship of the amino acid pool to protein breakdown, synthesis and storage.

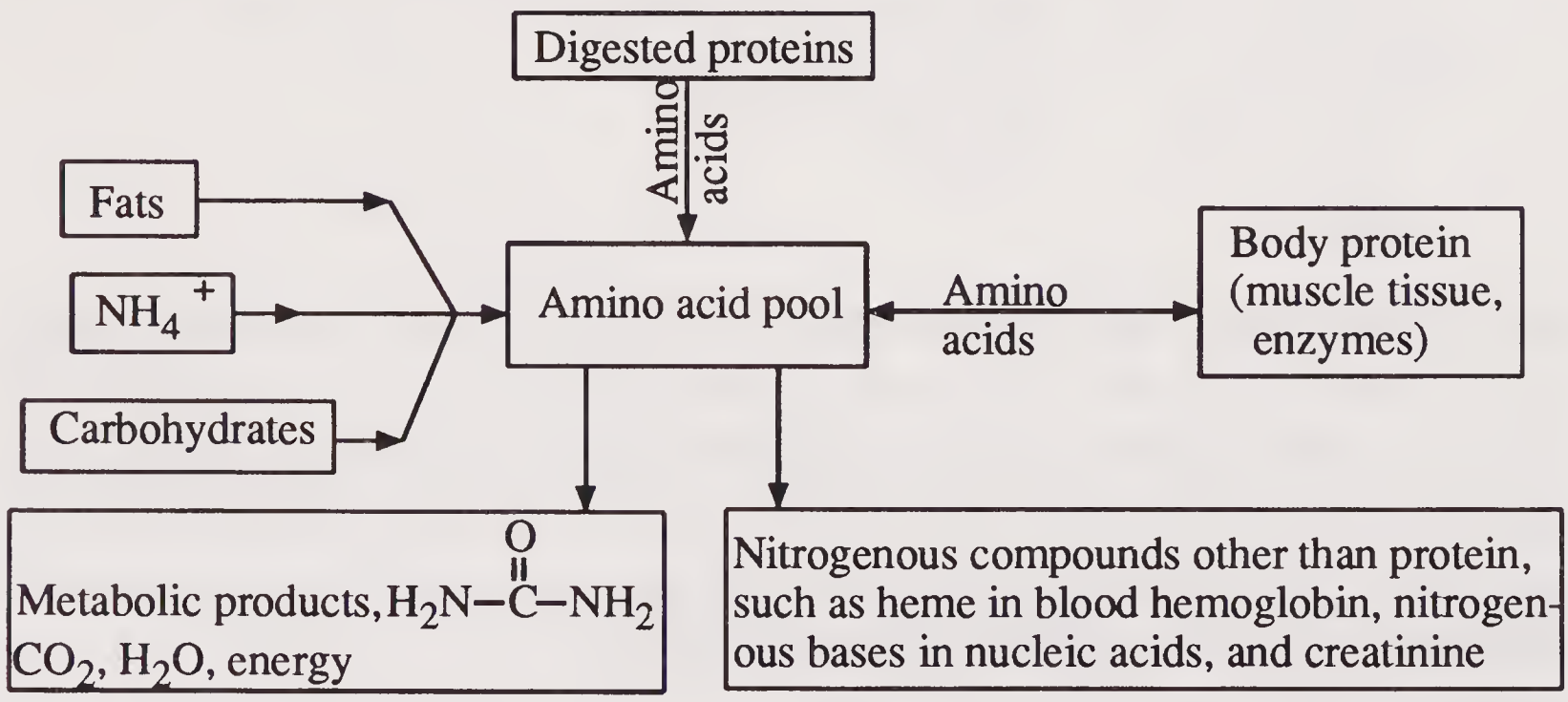
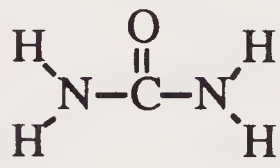


Figure 4.4. Main features of protein metabolism.

Proteins are synthesized from amino acids in the amino acid pool as discussed in Section 3.3. This occurs through the joining of H_3N^+- and $-CO_2^-$ groups at peptide bonds with the elimination of H_2O for each peptide bond formed. The body can make the majority of amino acids required. However, eight of them, the **essential amino acids**, cannot be synthesized in the human body and must be included in the diet.

The first step in the metabolic breakdown of amino acids is often the replacement of the $-NH_2$ group with a C=O group by the action of α-ketoglutaric acid in a process called **transamination**. **Oxidative deamination** then regenerates the α-ketoglutaric acid from the glutamic acid product of transamination. These processes are illustrated in Figure 4.5. As a net result of transamination N(-III) is removed from amino acids and eliminated from the body. For this to occur, nitrogen is first converted to urea,



which is eliminated from the body *via* the kidneys and bladder in urine.

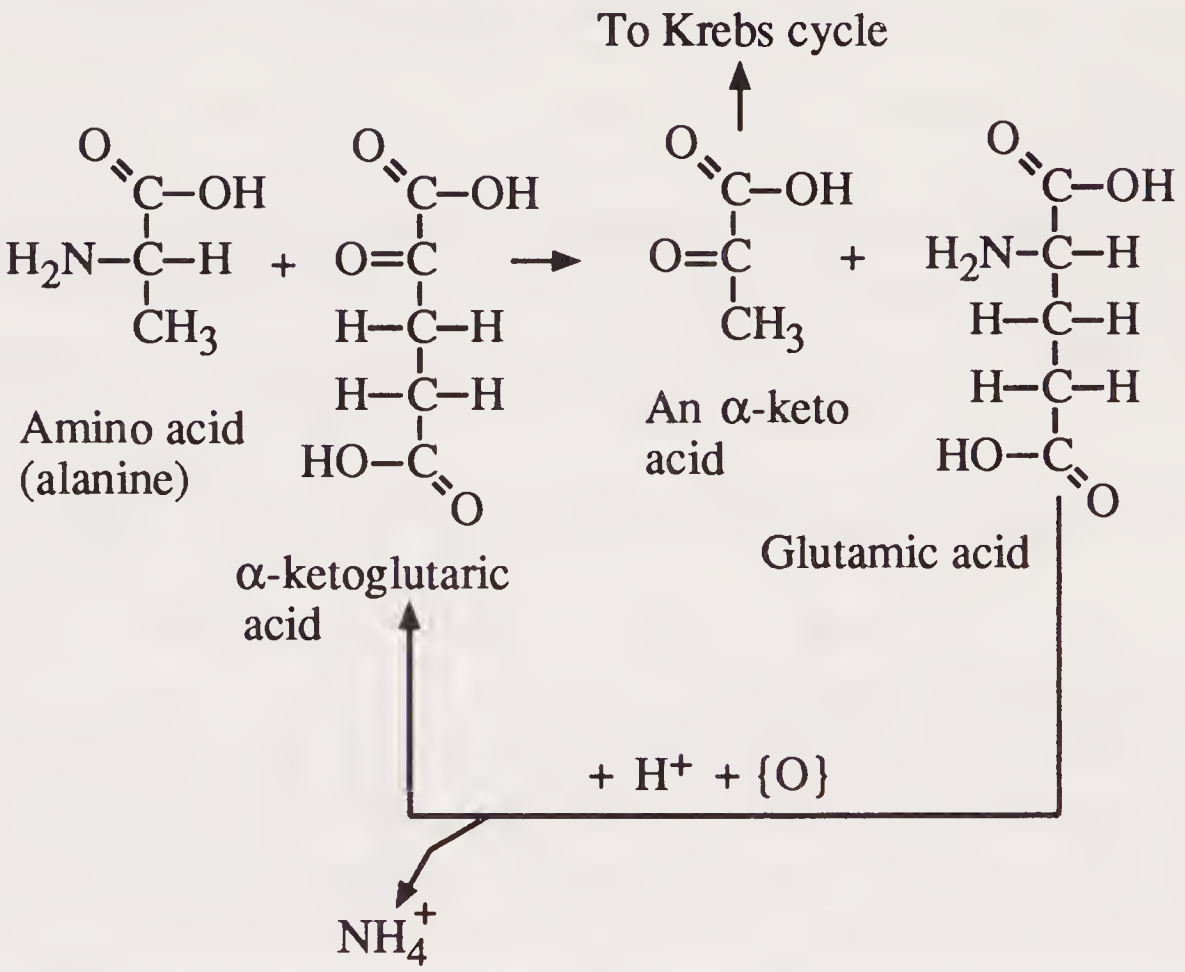


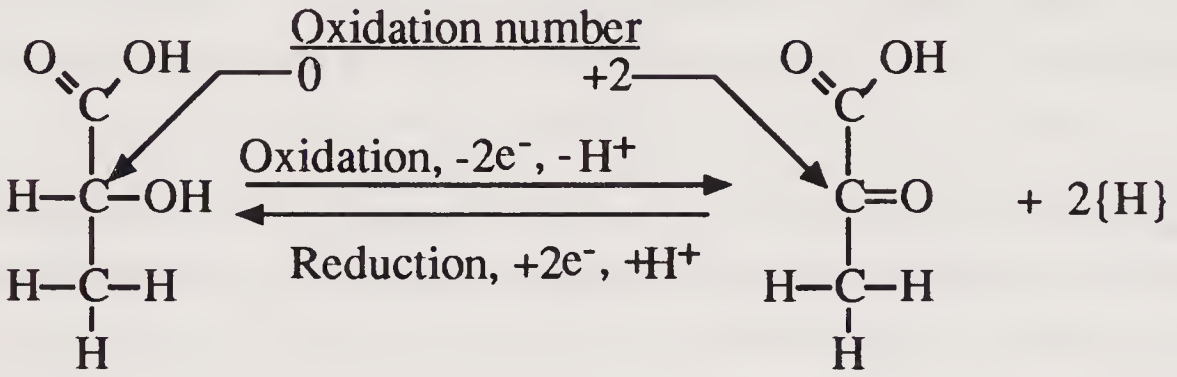
Figure 4.5. Transamination of an amino acid and regeneration of a-ketoglutaric acid by oxidative deamination.

The α-keto acids formed by transamination of amino acids are further broken down in the Krebs cycle. This process yields energy and the body's energy needs can be met with protein if insufficient carbohydrates or fats are not available.

Energy from Oxidation-Reduction Processes

Energy production by carbohydrate metabolism was mentioned earlier in this section. Energy needed by organisms is provided by enzymatically mediated oxidation-reduction reactions. Oxidation in a biological system, as in any chemical system, is the loss of electrons and reduction is the gain of electrons. A species that is oxidized by losing a negatively charged electron may maintain electrical neutrality by

losing H^+ ion; the loss of both e^- and H^+ is equivalent to the loss of a hydrogen atom, H. A biologically important example of this is the interconversion of lactic acid and pyruvic acid:

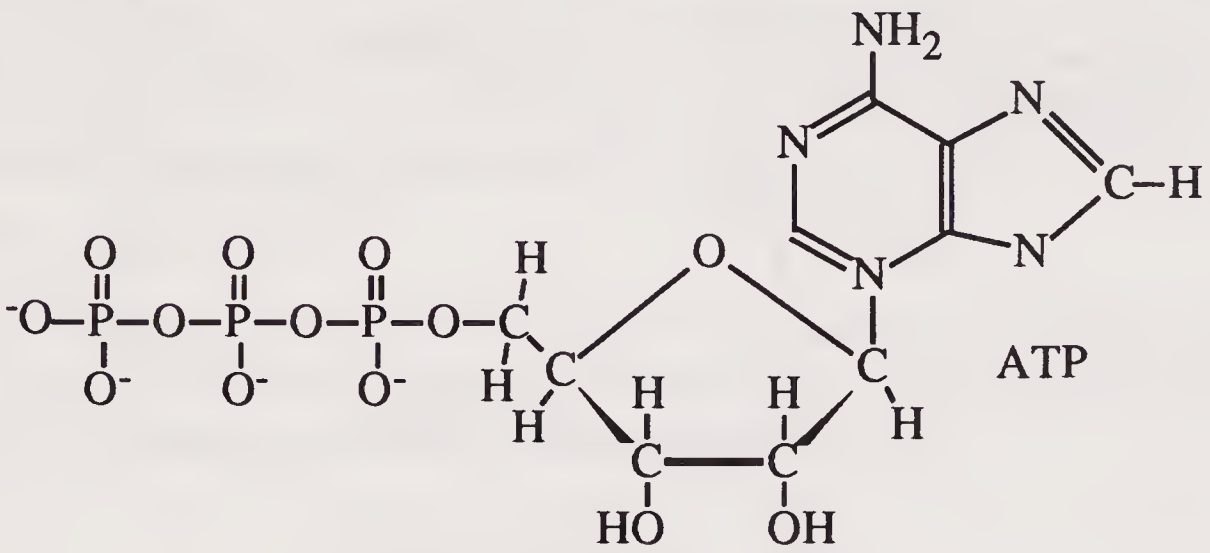


(4.3.4)

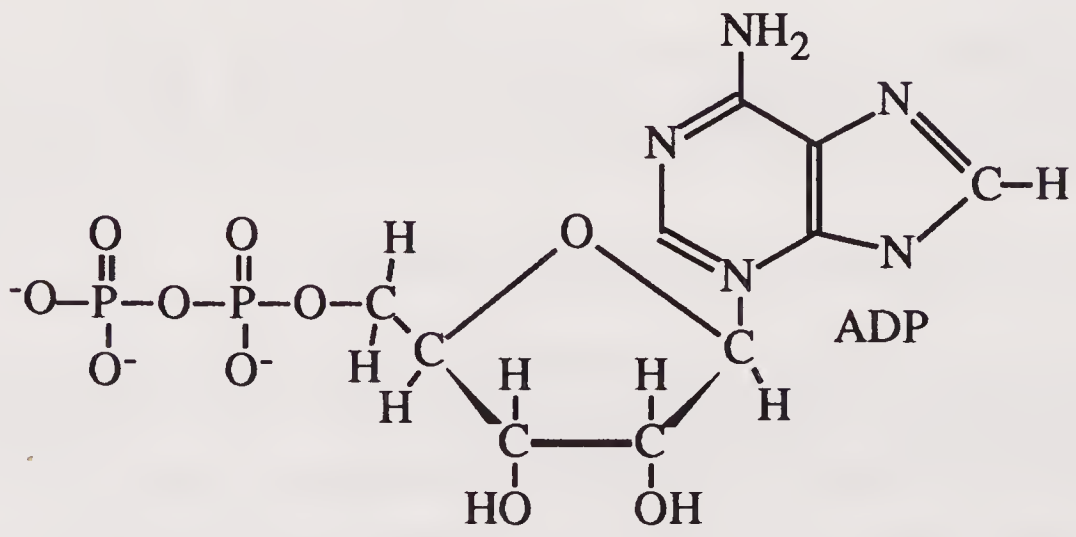
In this reaction the oxidation number of the middle carbon may be viewed as changing from 0 to +2 reflecting the loss of 2 electrons. An important aspect of enzymatic oxidation-reduction reactions involves the transfer of hydrogen atoms. This transfer is mediated by **nicotinamide adenine dinucleotide (NAD)** and **nicotinamide adenine dinucleotide phosphate (NADP)**. These two species pick up H atoms to produce $NADH_2$ and $NADPH_2$, respectively, both of which can function as hydrogen atom donors. Another pair of species involved in oxidation-reduction processes by hydrogen atom transfer consists of **flavin adenine triphosphate (FAD)** and its hydrogenated form **$FADH_2$**.

Energy Carriers

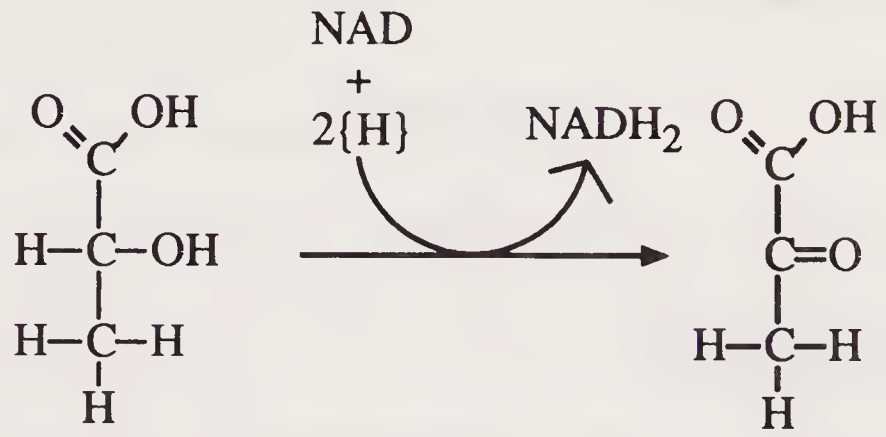
Energy generated by enzyme-mediated oxidation-reduction reactions is transferred by species that have high-energy bonds. Foremost among these is **adenosine triphosphate, ATP**:



which is generated from **adenosine diphosphate, ADP**:



A pair of species similar in function to ATP and ADP are **guanine triphosphate (GTP)** and **guanine diphosphate (GDP)**. The roles of energy-transferring and H-transferring species in enzymatic reactions are conventionally shown in reactions with curved arrows as illustrated by the following example showing the oxidation of lactic acid to pyruvic acid:



Lactic acid Pyruvic acid

(4.3.5)

Pathways of Energy Transport and Utilization

A large number of steps within several major cycles are involved in energy conversion, transport, and utilization in organisms. It is beyond the scope of this book to discuss all of these mechanisms in detail. However, it is useful to be aware of the following in relation to biochemical processes in which chemical or photochemical energy is utilized by organisms.

- **Glycolysis**, a multistepped process in which glucose is broken down in the absence of O_2 to produce lactic acid and energy.
- **Krebs cycle**, in which pyruvic acid, $C_3H_4O_3$, which enters the cycle bound to acetyl coenzyme A, undergoes the overall reaction

  $$5O_2 + 2C_3H_4O_3 \rightarrow 6CO_2 + 4H_2O + \text{energy}$$

  Molecular O_2 enters the cycle at several points, CO_2 is evolved, H is removed by $NADH_2$ and $FADH_2$, and useable energy is carried away by GTP (equivalent to ATP). Several 4-, 5-, and 6-carbon organic acids are generated as intermediates in the Krebs cycle, including citric, aconitic, isocitric, ketoglutaric, succinic, fumaric, malic, and oxaloacetic acids.
- **Transfer of electrons** from glycolysis and Krebs cycle intermediates to the electron transport chain *via* $NAD/NADH_2$ and $FAD/FADH_2$
- Release of energy to be carried by ATP by a series of oxidation-reduction processes in the **electron transport chain**.

4.4. METABOLISM OF XENOBIOTIC COMPOUNDS — PHASE I AND PHASE II REACTIONS

Toxicants or their metabolic precursors (**protoxicants**) may undergo absorption, metabolism, temporary storage, distribution, or excretion as illustrated in Figure 4.6. The remaining sections of this chapter, as well as Chapter 9, discuss the metabolic processes that toxicants undergo. Emphasis is placed on xenobiotic compounds, on chemical aspects, and on processes that lead to products that can be eliminated from the organism. Of particular importance is **intermediary xenobiotic metabolism**

which results in the formation of somewhat transient species that are different from both those ingested and the ultimate product that is excreted.[1] These species may have significant toxicological effects. Xenobiotic compounds in general are acted upon by enzymes that function on a material that is in the body naturally — an **endogenous substrate**. For example, flavin-containing monooxygenase enzyme acts upon endogenous cysteamine to convert it to cystamine, but also functions to oxidize endogenous nitrogen and sulfur compounds.

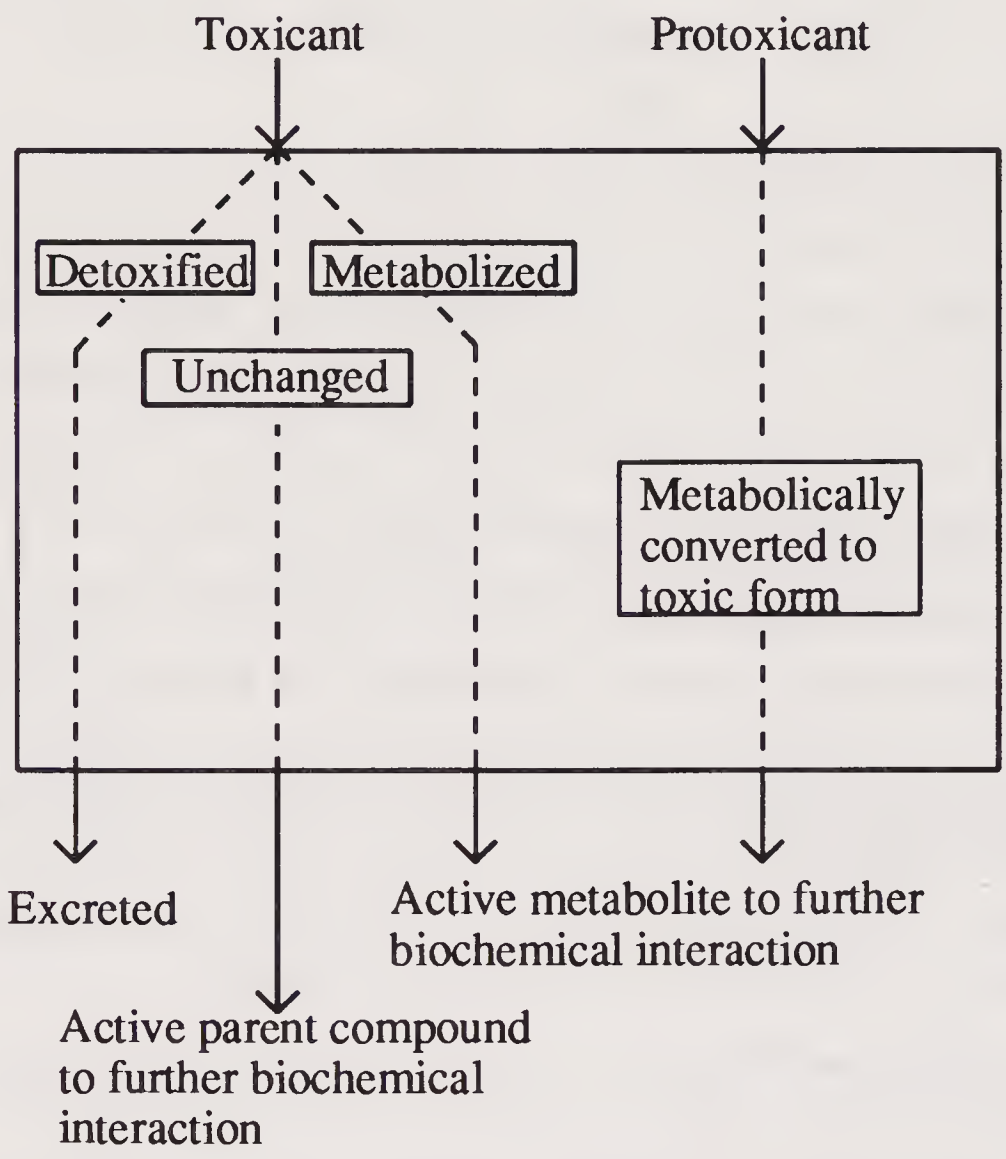


Figure 4.6. Pathways of xenobiotic species prior to their undergoing any biochemical interactions that could lead to toxic effects.

Biotransformation refers to changes in xenobiotic compounds as a result of enzyme action. Reactions that are not mediated by enzymes may also be important in some cases. As examples of nonenzymatic transformations, some xenobiotic compounds bond with endogenous biochemical species without an enzyme catalyst, undergo hydrolysis in body fluid media, or undergo oxidation/reduction processes. The metabolic phase I and phase II reactions of xenobiotics discussed here are enzymatic, however.

The likelihood that a xenobiotic species will undergo enzymatic metabolism in the body depends upon the chemical nature of the species. Compounds with a high degree of polarity, such as relatively ionizable carboxylic acids, are less likely to enter the body system and, when they do, tend to be quickly excreted. Therefore, such compounds are unavailable, or only available for a short time, for enzymatic metabolism. Volatile compounds, such as dichloromethane or diethylether, are expelled so quickly from the lungs that enzymatic metabolism is less likely. This leaves as the most likely candidates for enzymatic metabolic reactions **nonpolar lipophilic compounds**, those that are relatively less soluble in aqueous biological fluids and more attracted to lipid species. Of these, the ones that are resistant to enzymatic attack (PCBs, for example) tend to bioaccumlate in lipid tissue.

Xenobiotic species may be metabolized in a wide variety of body tissues and organs. As part of the body's defense against the entry of xenobiotic species, the most prominent sites of xenobiotic metabolism are those associated with entry into the body (see Figure 4.1). The skin is one such organ, as is the lung. The gut wall through which xenobiotic species enter the body from the gastrointestinal tract is also a site of significant xenobiotic compound metabolism. The liver is of particular significance because materials entering systemic circulation from the gastrointestinal tract must first traverse the liver.

Phase I and Phase II Reactions

The processes that most xenobiotics undergo in the body can be divided into the two categories of phase I reactions and phase II reactions.[2] A **phase I reaction** introduces reactive, polar functional groups (see Table 1.3) onto lipophilic ("fat-seeking") toxicant molecules. In their unmodified forms, such toxicant molecules tend to pass through lipid-containing cell membranes and may be bound to lipoproteins in which form they are transported through the body. Because of the functional group attached, the product of a phase I reaction is usually more water-soluble than the parent xenobiotic species, and more importantly, possesses a "chemical handle" to which a substrate material in the body may become attached so that the toxicant can be eliminated from the body. The binding of such a substrate is a **phase II reaction**, and it produces a **conjugation product** that is amenable to excretion from the body.

In general, the changes in structure and properties of a compound that result from a phase I reaction are relatively mild. Phase II processes, however, usually produce species that are much different from the parent compounds. It should be emphasized that not all xenobiotic compounds undergo both Phase I and Phase II reactions. Such a compound may undergo only a phase I reaction and be excreted directly from the body. Or a compound that already possesses an appropriate functional group capable of conjugation may undergo a phase II reaction without a preceding phase II reaction.

4.5. PHASE I REACTIONS

Figure 4.7 shows the overall processes involved in a phase I reaction. Normally a phase I reaction adds a functional group to a hydrocarbon chain or ring or modifies

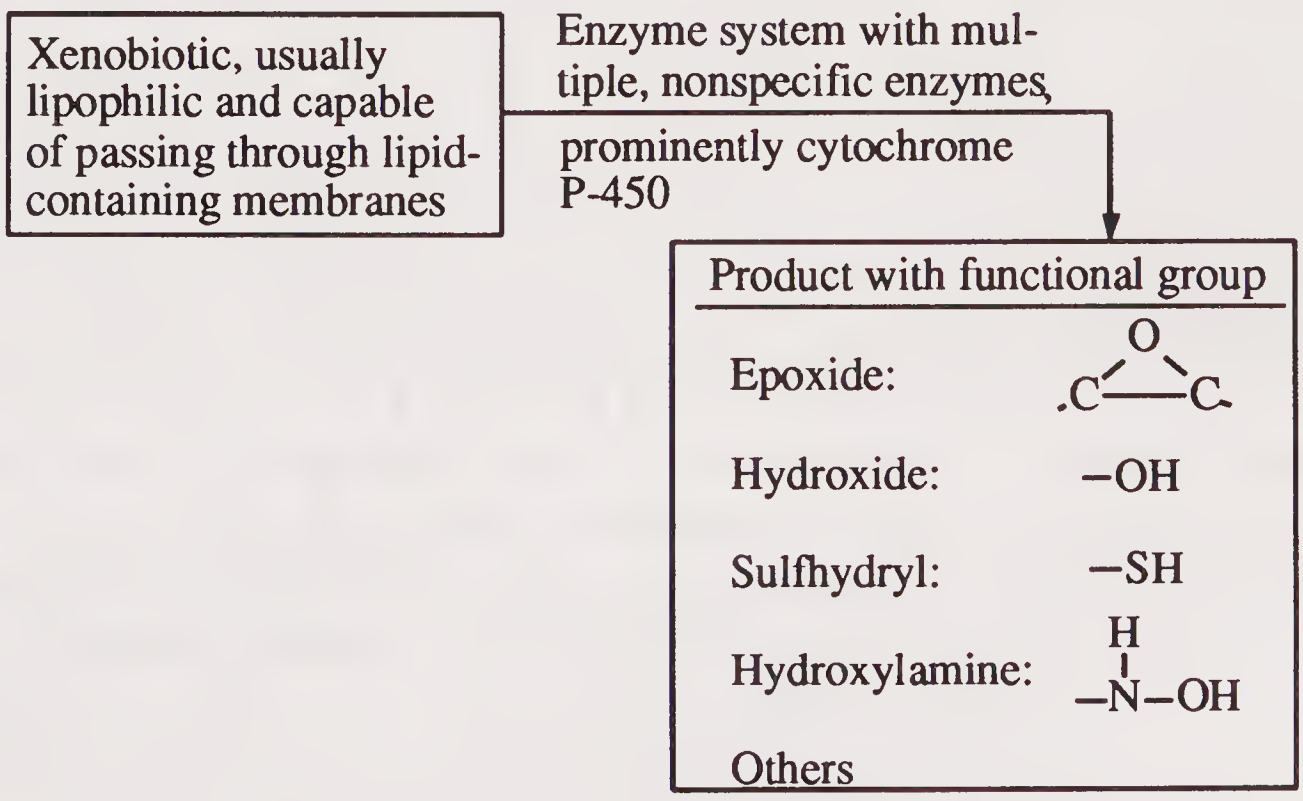
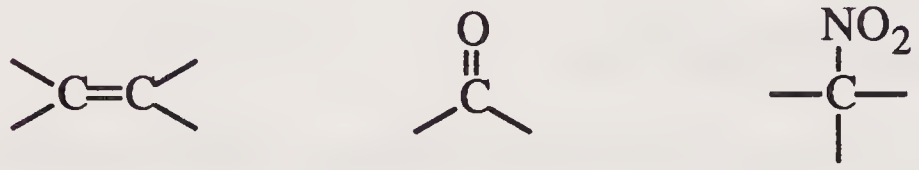


Figure 4.7. Overall process of phase I reactions.

one that is already present. The product is a chemical species that readily undergoes conjugation with some other species naturally present in the body to form a substance that can be readily excreted. Phase I reactions are of several types of which oxidation of C, N, S, and P is most important. Reduction may occur on reducible functionalities particularly those shown in Figure 4.8. Phase I reactions may also consist of hydrolysis processes, which require that the xenobiotic compound have a hydrolyzable group.



Alkenyl Carbonyl Nitro

Figure 4.8. Functionalities likely to be reduced in phase I reactions.

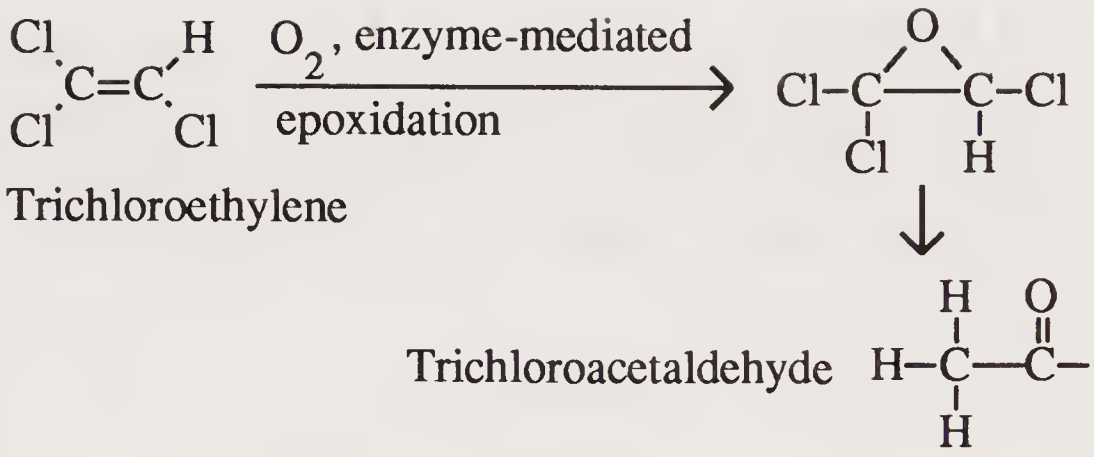
Oxidation Reactions

The most important phase I reactions are oxidation reactions, particularly those classified as microsomal mixed-function oxidase reactions. Microsomes refer to a fraction collected from the centrifugation at about 100,000 x *g* of cell homogenates and consisting of pellets. These pellets contain rough and smooth **endoplasmic reticulum** (extensive networks of membranes in cells) and Golgi bodies, which store newly synthesized molecules. **Mixed-function oxidations** occur with O_2 as the oxidizing agent, one atom of which is incorporated into the substrate, and the other going to form water:

$$\text{Substrate} + O_2 \xrightarrow[\text{oxidation}]{\text{Mixed-function}} \begin{cases} \text{Product—OH} \\ H_2O \end{cases} \qquad (4.5.1)$$

The key enzymes of the system are the cytochrome P-450 enzymes, the active sites of which contain an iron atom that can change between the +2 and +3 oxidation states.[3] These enzymes can bind to the substrate and molecular O_2 as part of the process by which the substrate is oxidized. Cytochrome P-450 is found most abundantly in the livers of vertebrates, reflecting the liver's role as the body's primary defender against systemic poisons. Cytochrome P-450 occurs in many other parts of the body, such as the kidney, ovaries, testes, and blood. The presence of this enzyme in the lung, skin, and gastrointestinal tract may reflect their defensive roles against toxicants.

Epoxidation consists of adding an oxygen atom between two C atoms in an unsaturated system as shown in Reactions 4.5.2 and 4.5.3. It is a particularly important means of metabolic attack upon aromatic rings that abound in many xenobiotic compounds. Cytochrome P-450 is involved in epoxidation reactions. Both of the epoxidation reactions shown above have the effect of increasing the toxicities of the parent compounds, a process called **intoxication.** Some epoxides are unstable, tending to undergo further reactions, usually hydroxylation (see below). A well-known example of the formation of a stable epoxide is the conversion to aldrin of the insecticide dieldrin, discussed in Chapter 16.



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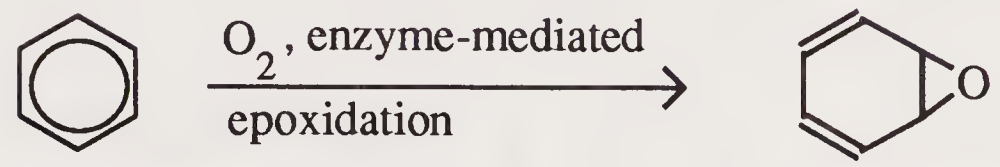
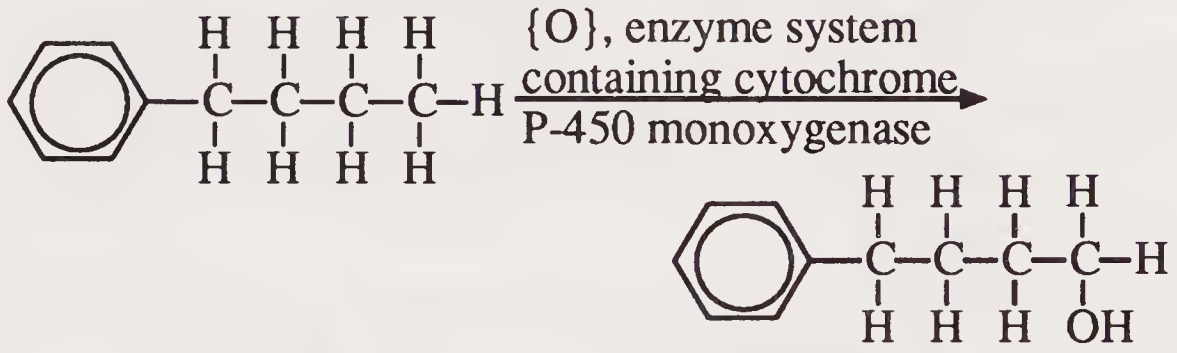


(4.5.3)

Hydroxylation

Hydroxylation is the attachment of –OH groups to hydrocarbon chains or rings. **Aliphatic hydroxylation** of alkane chains can occur on the terminal carbon atom ($-CH_3$ group or ω-carbon) or on the C atom next to the last one (ω-1-carbon) by the insertion of an O atom between C and H as shown below for the hydroxylation of the side-chain on a substituted aromatic compound:



(4.5.4)

Hydroxylation can follow epoxidation as shown by the following rearrangement reaction for benzene epoxide:

(4.5.5)

Hydroxylation can consist of the addition of more than one epoxide group. Hydroxylation and epoxidation are responsible for making several xenobiotic compounds toxic through metabolic processes. A prominent example of this phenomenon is the metabolic production of the carcinogenic 7,8-diol-9,10-epoxide of benzo(a)pyrene as illustrated by the overall reaction shown in Figure 4.9. Benzo-(a)pyrene is classified as a procarcinogen, or precarcinogen, in that metabolic action is required to convert it to a species that is carcinogenic as such (see Chapter 9).

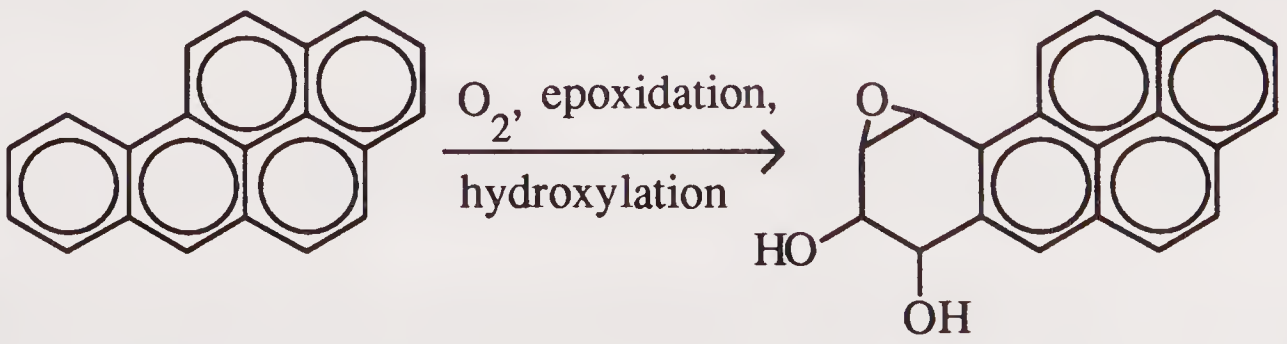


Figure 4.9 Epoxidation/hydroxylation of benzo(a)pyrene (left) to form carcinogenic 7,8-diol-9,10-benzo(a)pyrene epoxide (right).

Oxidation of Noncarbon Elements

As summarized in Figure 4.10, the oxidation of nitrogen, sulfur, and phosphorus is an important type of metabolic reaction in xenobiotic compounds. It can be an important intoxication mechanism by which compounds are made more toxic.

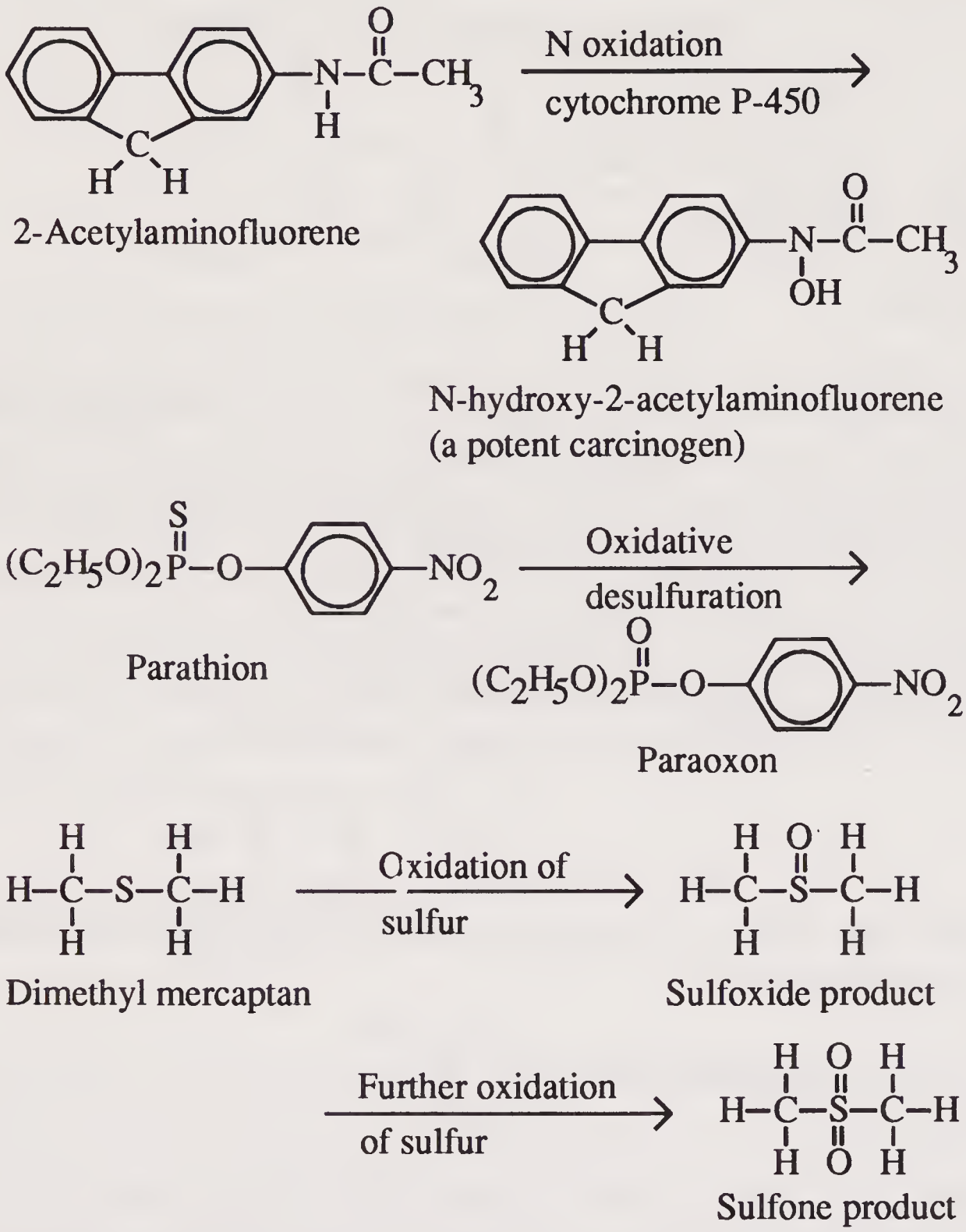


Figure 4.10. Metabolic oxidation of nitrogen, phosphorus and sulfur in xenobiotic compounds.

For example, the oxidation of nitrogen in 2-acetylaminofluorene yields potently carcinogenic N-hydroxy-2-acetylaminofluorene. Two major steps in the metabolism of the plant systemic insecticide aldicarb (Figure 4.11) are oxidation to the sulfoxide followed by oxidation to the sulfone (see discussion of sulfur compounds in Chapter

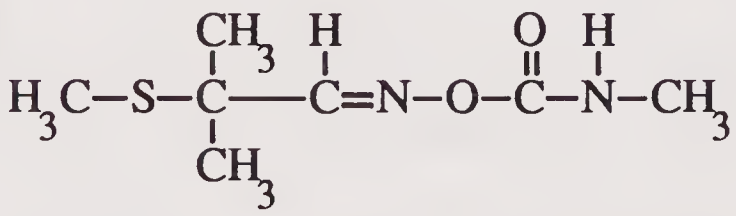


Figure 4.11. Structure of the plant systemic insecticide temik (aldicarb). The sulfur is metabolically oxidizable.

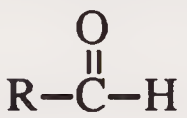
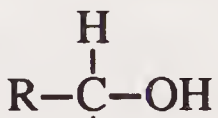
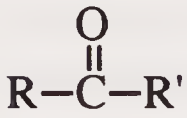
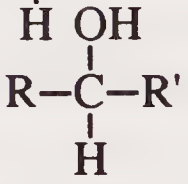
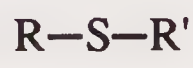
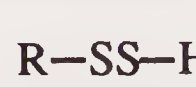
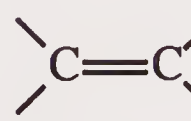
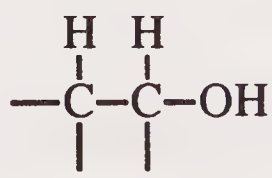
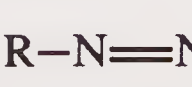
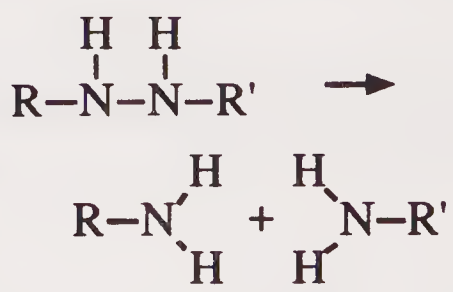
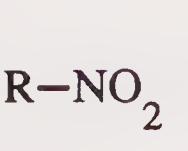
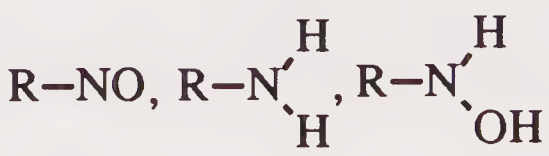
17). The oxidation of phosphorus in parathion (replacement of S by O, oxidative desulfurization) yields insecticidal paraoxon, which is much more effective than the parent compound in inhibiting acetylcholinesterase enzyme (see Section 8.8).

In addition to cytochrome P-450 enzymes, another enzyme that mediates phase I oxidations is **flavin-containing monooxygenase** (FMO), likewise contained in the endoplasmic reticulum. It is especially effective in oxidizing primary, secondary, and tertiary amines. In addition it catalyzes oxidation of other nitrogen-containing xenobiotic compounds, as well as those that contain sulfur and phosphorus, but does not bring about hydroxylation of carbon atoms.[4]

Metabolic Reductions

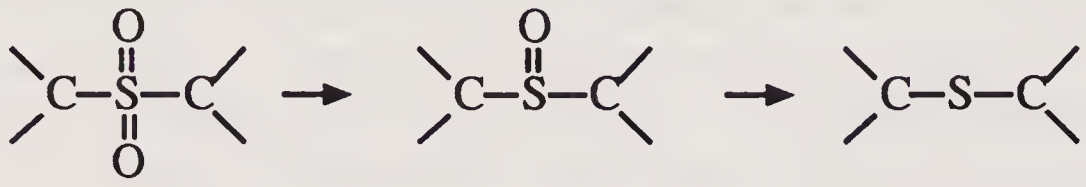
Table 4.1 summarizes the functional groups in xenobiotics that are most likely to be reduced metabolically. Reductions are carried out by **reductase enzymes**; for example, nitroreductase enzyme catalyzes the reduction of the nitro group. Reductase enzymes are found largely in the liver and to a certain extent in other organs, such as the kidney and lung.

Table 4.1. Functional Groups that Undergo Metabolic Reduction

Functional group	Process	Product
	Aldehyde reduction	
	Ketone reduction	
	Sulfoxide reduction	
	Disulfide reduction	
	Alkene reduction	
	Azo reduction	
	Nitro reduction	
As(V)	Arsenic reduction	As(III)

Most reductions of xenobiotic compounds are mediated by bacteria in the intestines, the **gut flora**. The contents of lower bowel may contain as many as 10^{10} anaerobic bacteria per gram.[5] The compounds reduced by these bacteria may enter the lower bowel either by oral ingestion (without having been absorbed through the intestinal

wall) or by secretion with bile. In the latter case the compounds may be parent materials or metabolic products of substances absorbed in upper regions of the gastrointestinal tract. Intestinal flora are known to mediate the reduction of organic xenobiotic sulfones and sulfoxides to sulfides:



(4.5.6)

Sulfone Sulfoxide Sulfide

Metabolic Dealkylation

Many xenobiotics contain alkyl groups, such as the methyl ($-CH_3$) group, attached to atoms of O, N, and S. An important step in the metabolism of many of these compounds is replacement of alkyl groups by H as shown in Figure 4.12. These reactions are carried out by mixed-function oxidase enzyme systems. Examples of these kinds of reactions with xenobiotics include O-dealkylation of methoxychlor insecticides, N-dealkylation of carbaryl insecticide, and S-dealkylation of dimethyl mercaptan.

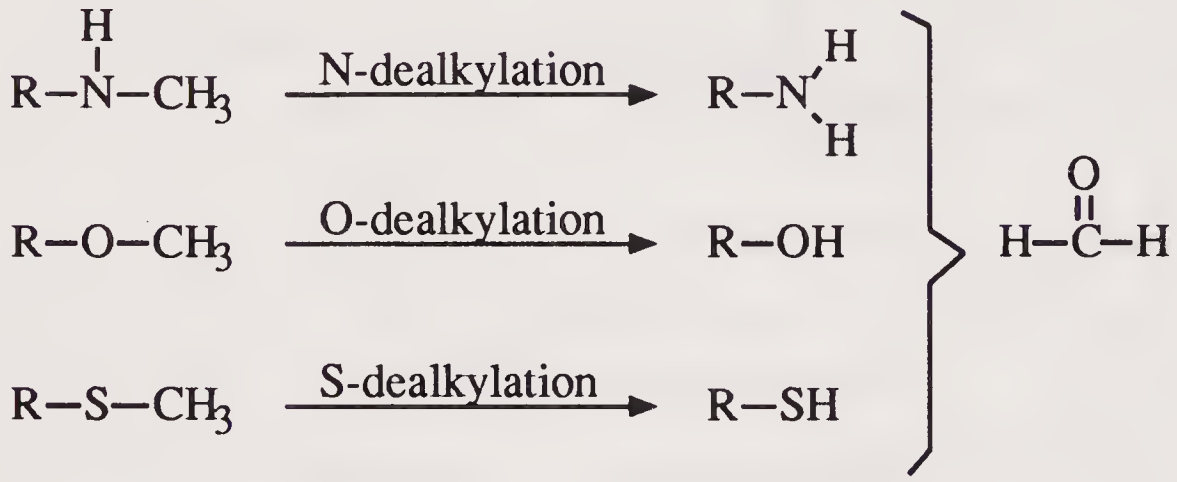


Figure 4.12. Metabolic dealkylation reactions shown for the removal of CH_3 from N, O and S atoms in organic compounds.

Metabolic Hydrolysis Reactions

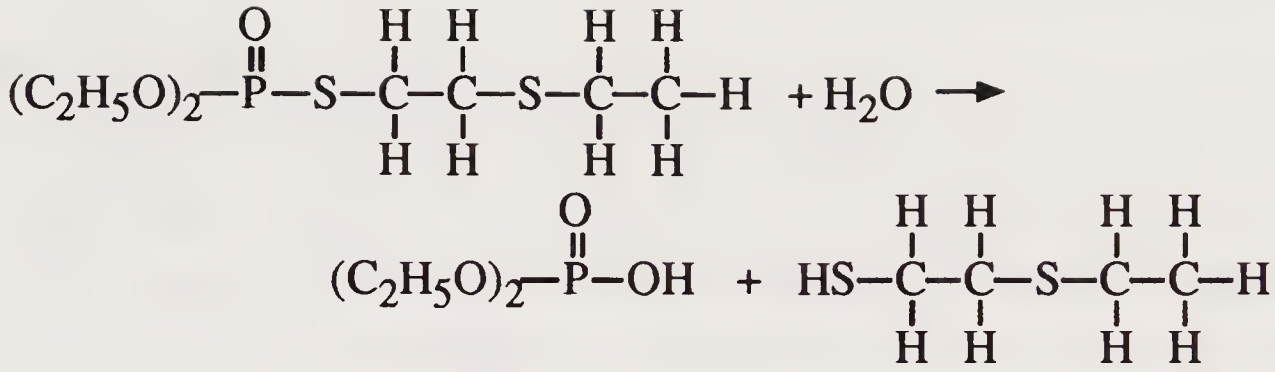
Hydrolysis involves the addition of H_2O to a molecule accompanied by cleavage of the molecule into two species. The two most common types of compounds that undergo hydrolysis are esters

(4.5.7)

and amides,

(4.5.8)

Organophosphate esters (see Chapter 18) also undergo hydrolysis as shown below for the plant systemic insecticide demeton:

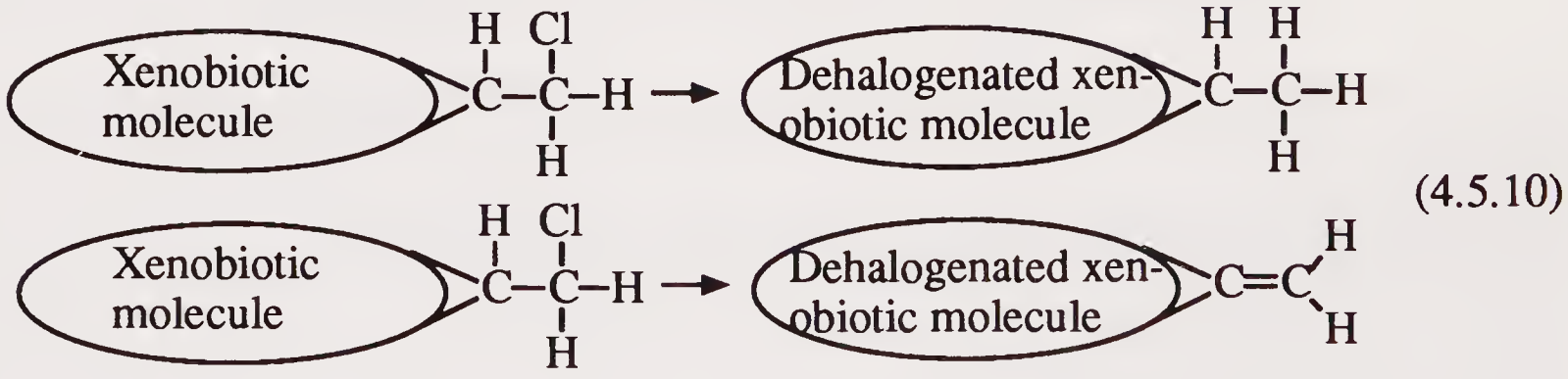


(4.5.9)

Many xenobiotic compounds, such as pesticides, are esters, amides, or organophosphate esters, and hydrolysis is a very important aspect of their metabolic fates. The types of enzymes that bring about hydrolysis are **hydrolase enzymes**. Like most enzymes involved in the metabolism of xenobiotic compounds, hydrolase enzymes occur prominently in the liver. They also occur in tissue lining the intestines, nervous tissue, blood plasma, the kidney, and muscle tissue. Enzymes that enable the hydrolysis of esters are called **esterases**, and those that hydrolyze amides are **amidases**. Aromatic esters are hydrolyzed by the action of aryl esterases and alkyl esters by aliphatic esterases. Hydrolysis products of xenobiotic compounds may be either more or less toxic than the parent compounds.

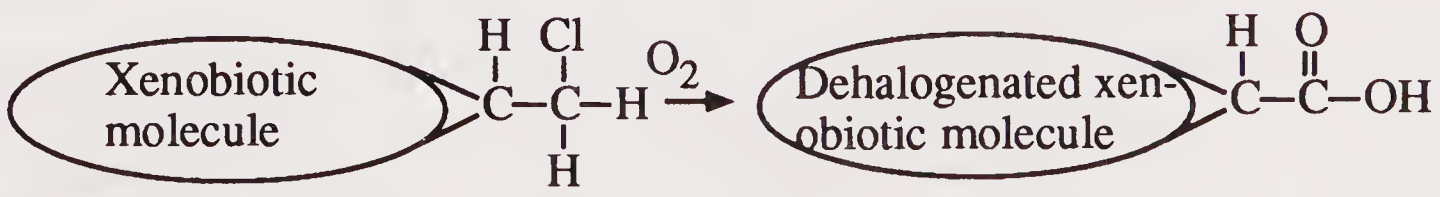
Removal of Halogen

An important step in the metabolism of the many xenobiotic compounds that contain covalently bound halogens (F, Cl, Br, I) is the removal of halogen atoms, a process called **dehalogenation**. This may occur by **reductive dehalogenation** in which the halogen atom is replaced by hydrogen, or two atoms are lost from adjacent carbon atoms, leaving a carbon-carbon double bond. These processes are illustrated by the following:



(4.5.10)

Oxidative dehalogenation occurs when oxygen is added in place of a halogen atom as shown below:



(4.5.11)

4.6. PHASE II REACTIONS OF TOXICANTS

Phase II reactions are also known as **conjugation reactions** because they involve the joining together of a substrate compound with another species that occurs normally in (is endogenous to) the body. This can occur with unmodified xenobiotic compounds, xenobiotic compounds that have undergone phase I reactions, and compounds that are not xenobiotic species. The substance that binds to these species is called an **endogenous** (present in, and produced by, the body) **conjugating agent**. Activation of the conjugating agent usually provides the energy needed for conjugation, although conjugation by glutathione or amino acids is provided by activation of the species undergoing conjugation preceding the reaction. The overall process for the conjugation of a xenobiotic compound is shown in Figure 4.13. Such a compound contains functional groups, often added as the consequence of a phase I reaction, that serve as "chemical handles" for the attachment of the conjugating agent. The product is usually less lipid-soluble, more soluble in water, less toxic, and more easily eliminated than the parent compound.

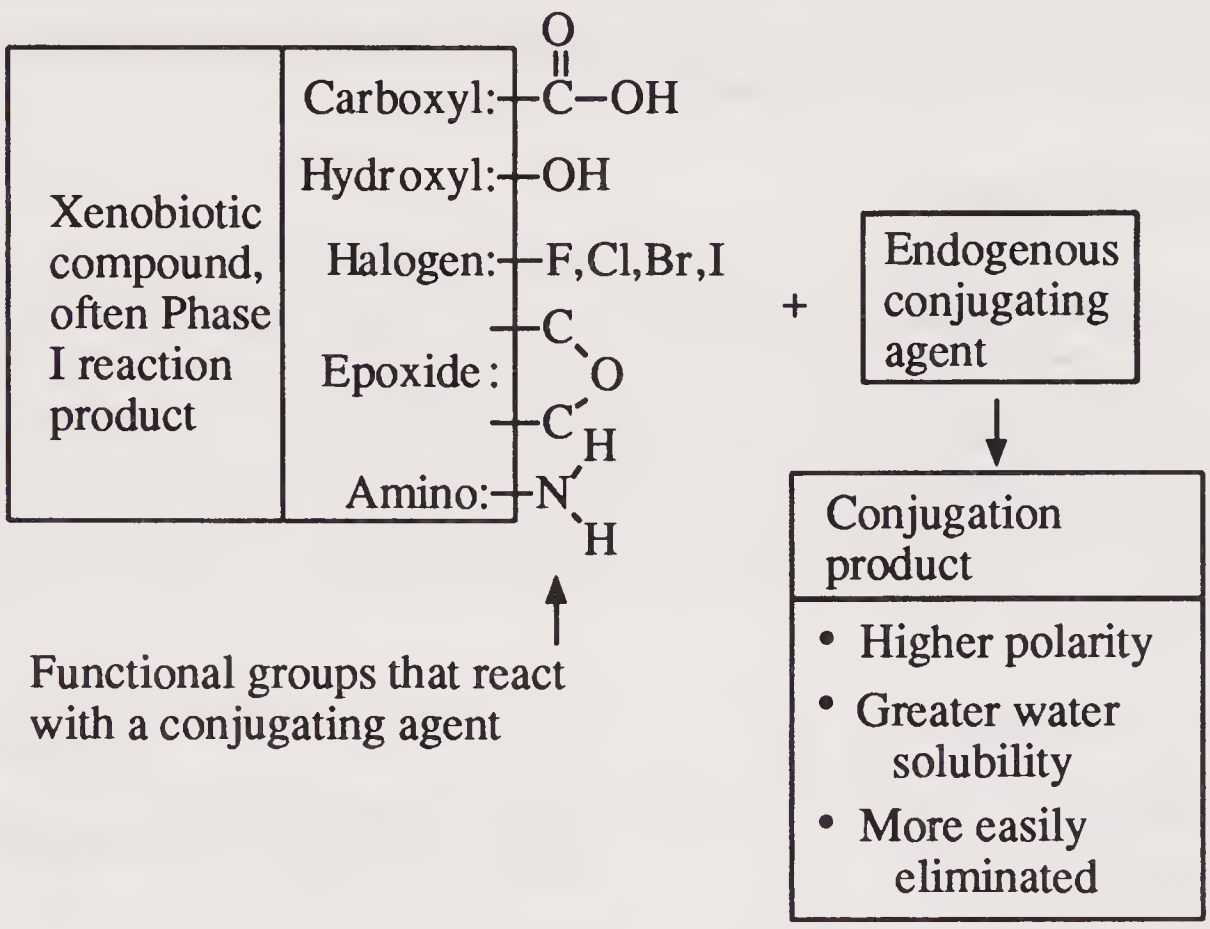
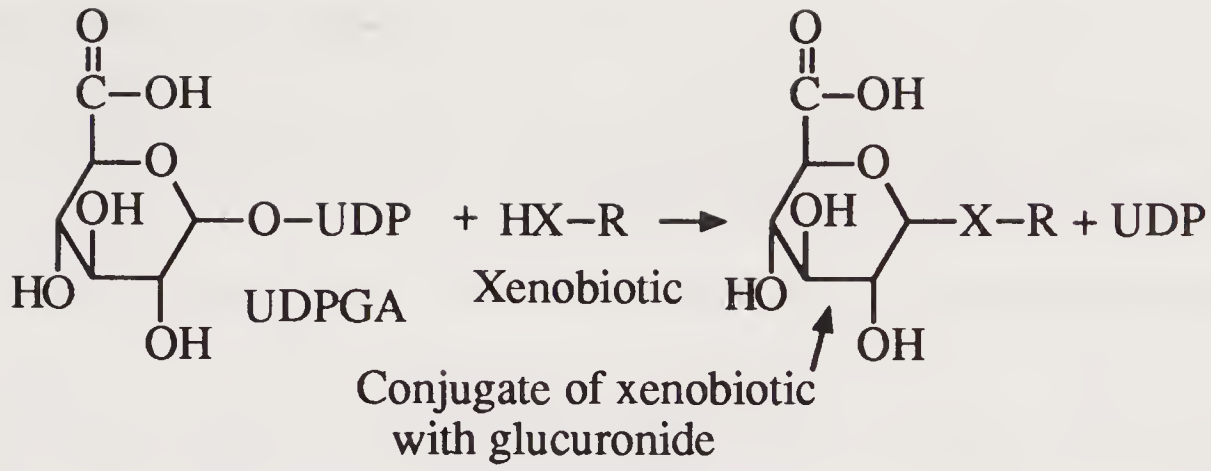


Figure 4.13. Overall process of conjugation that occurs in phase II reactions.

Conjugation by Glucuronides

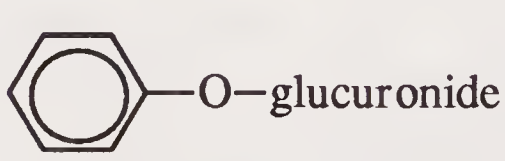
Glucuronides are the most common endogenous conjugating agents in the body. They react with xenobiotics through the action of uridine diphosphate glucuronic acid (UDPGA). This transfer is mediated by glucuronyl transferase enzymes. These enzymes occur in the endoplasmic reticulum where hydroxylated phase I metabolites of lipophilic xenobiotic compounds are produced. As a result, the lifetime of the phase I metabolites is often quite brief. A generalized conjugation reaction of UDPGA with a xenobiotic compound can be represented as the following (the uridinediphosphate (UDP) portion of these structures is moderately complicated and is not given here):



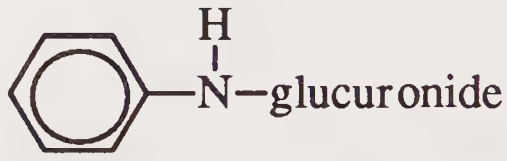
(4.6.1)

In this reaction HX-R represents the xenobiotic species in which HX is a functional group (such as –OH) and R is an organic moiety, such as the phenyl group (benzene ring less a hydrogen atom). The kind of enzyme that mediates this type of reaction is UDP glucuronyltransferase.

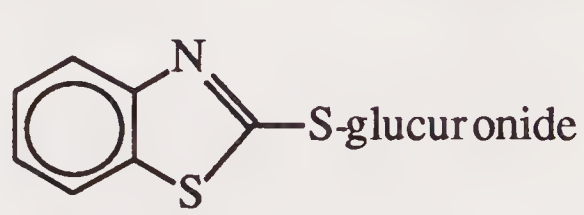
Glucuronide conjugation products may be classified according to the element to which the glucuronide is bound. These elements are oxygen, nitrogen, and sulfur, and example glucuronides involving O, N, and S atoms are shown in Figure 4.14. When the



Phenylglucuronide, an
O-glucuronide



Aniline glucuronide,
an N-glucuronide



2-Mercaptothiazole-S-glucuronide,
an S-glucuronide

Figure 4.14. Examples of O-, N-, and S-glucuronides.

functional group through which conjugation occurs is a hydroxyl group, –OH (HX– in Reaction 4.6.1) an ether glucuronide is formed. A carboxylic acid group for HX gives an ester glucuronide. Glucuronides may be attached directly to N as the linking atom, as is the case with aniline glucuronide in Figure 4.14, or through an intermediate O atom. An example of the latter is N-hydroxyacetylaminoglucuronide, for which the structure is shown in Figure 4.15. This species is of interest because it is a stronger carcinogen than its parent xenobiotic compound, N-hydroxyacetylaminofluorene, contrary to the decrease in toxicity that usually results from glucuronide conjugation.

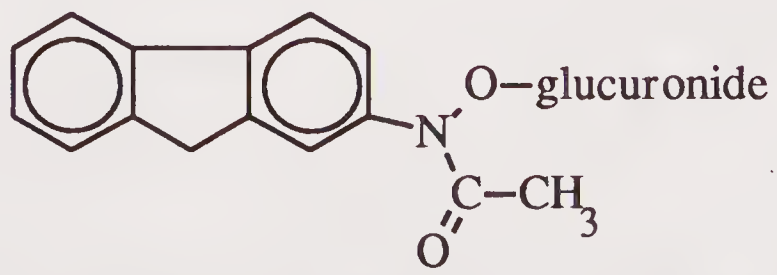


Figure 4.15. N-hydroxyacetylaminofluorene glucuronide, a more potent carcinogen than its parent compound, N-hydroxyacetylaminofluorene.

Enterohepatic circulation provides a mechanism by which the metabolic effects of some glucuronide conjugates are amplified. This phenomenon is essentially a recycling process in which a glucuronide conjugate released to the intestine with bile becomes deconjugated and re-absorbed in the intestine.

Conjugation by Glutathione

Glutathione (commonly abbreviated GSH) is a crucial conjugating agent in the body. This compound is a tripeptide, meaning that it is composed of three amino acids linked together. These amino acids and their abbreviations are glutamic acid (Glu), cysteine (Cys), and glycine (Gly, see structures in Table 3.1). The formula of glutathione may be represented as illustrated in Figure 4.16, where the SH is shown specifically because of its crucial role in forming the covalent link to a xenobiotic compound. Glutathione conjugate may be excreted directly, although this is rare. More commonly the GSH conjugate undergoes further biochemical reactions that produce mercapturic acids (compounds with N-acetylcystein attached) or other species. The overall process for the production of mercapturic acids as applied to a generic xenobiotic species, HX-R (see previous discussion), is illustrated in Figure 4.16.

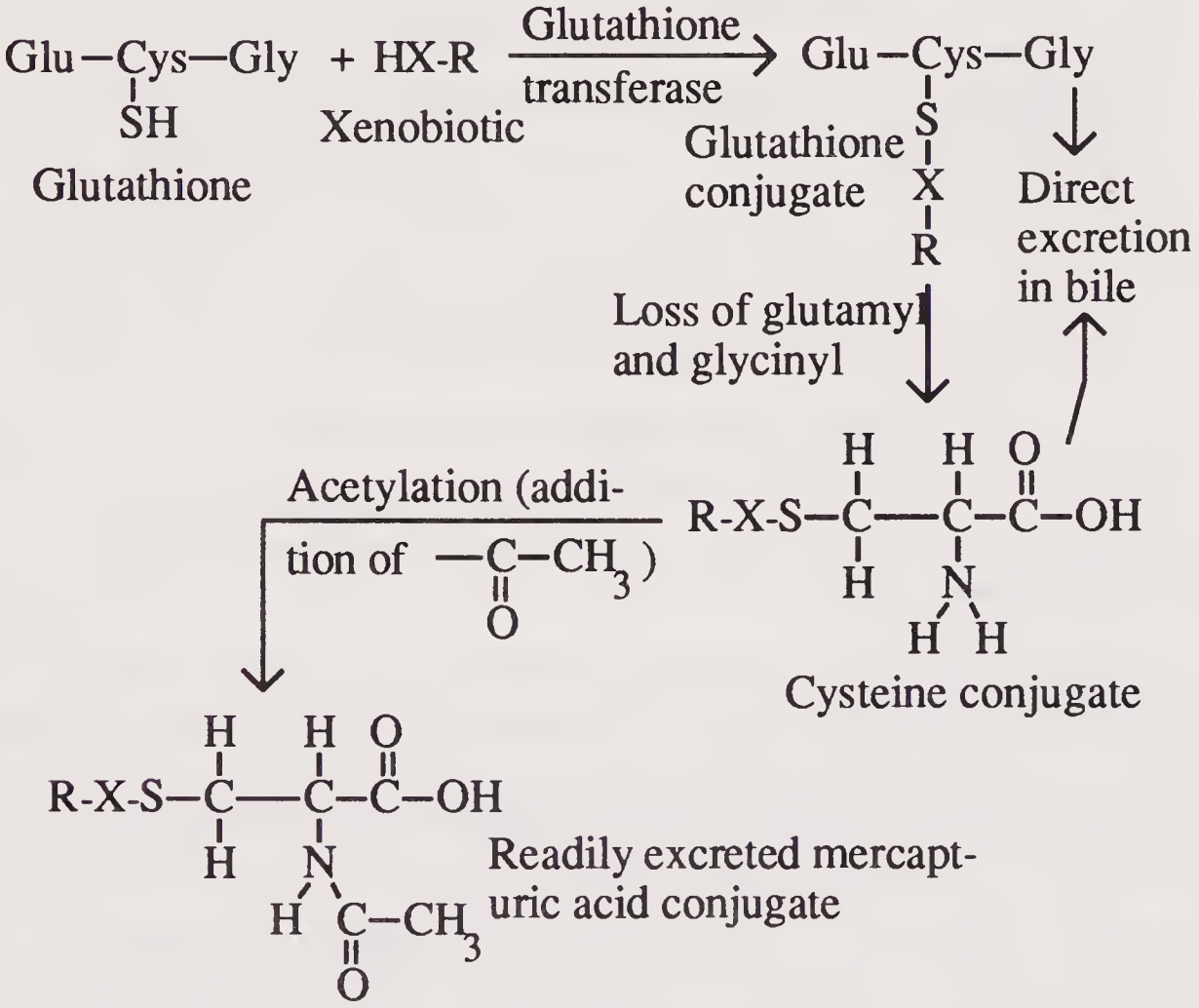
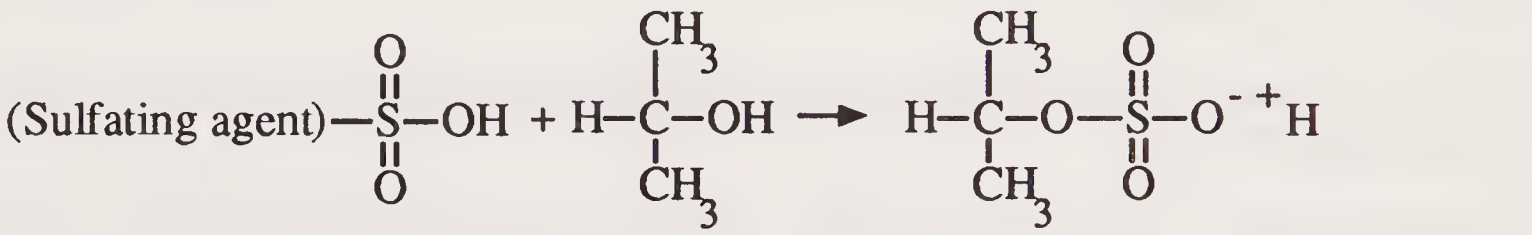


Figure 4.16. Glutathione conjugate of a xenobiotic species (HX-R) followed by formation of glutathione and cysteine conjugate intermediates (which may be excreted in bile) and acetylation to form readily excreted mercapturic acid conjugate.

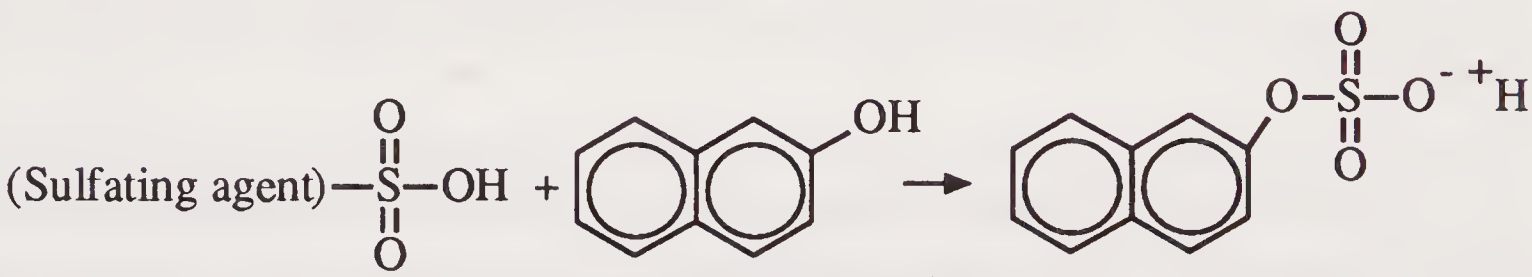
There are numerous variations on the general mechanism outlined in Figure 4.16. Glutathione forms conjugates with a wide variety of xenobiotic species including alkenes, alkyl epoxides (1,2-epoxyethylbenzene), arylepoxides (1,2-epoxynaphthalene), aromatic hydrocarbons, aryl halides, alkyl halides (methyl iodide), and aromatic nitro compounds. The glutathione transferase enzymes required for the initial conjugation are widespread in the body.

Conjugation by Sulfate

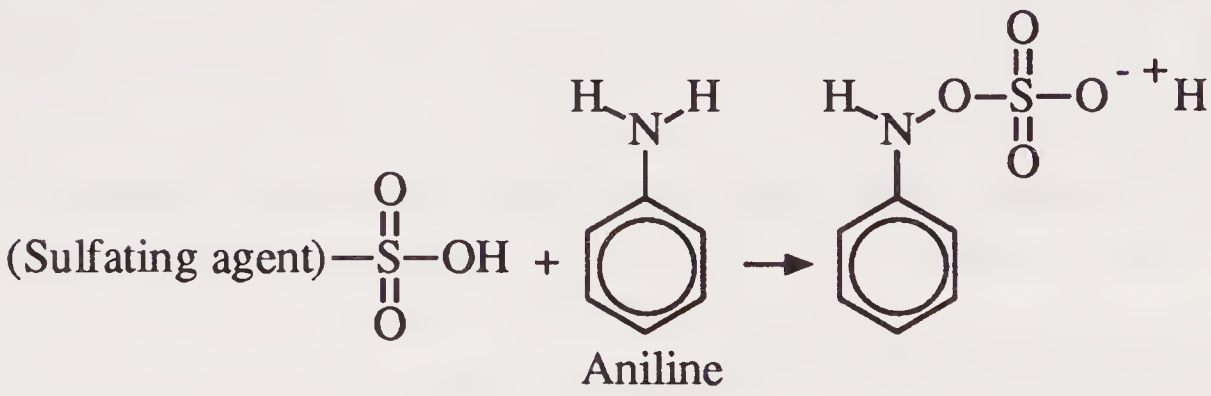
Although conjugation by sulfate requires the input of substantial amounts of energy, it is very efficient in eliminating xenobiotic species through urine because the sulfate conjugates are completely ionized and therefore highly water-soluble. The types of species that form sulfate conjugates are alcohols, phenols, and arylamines as shown by the examples in Figure 4.17. The enzymes that catalyze these reactions are sulfotransferase enzymes. The sulfating agent is a rather complex biomolecule called adenosine 3'-phosphate-5'-phosphosulfate, commonly abbreviated PAPS.



2-Propanol



2-Naphthol, a phenolic compound

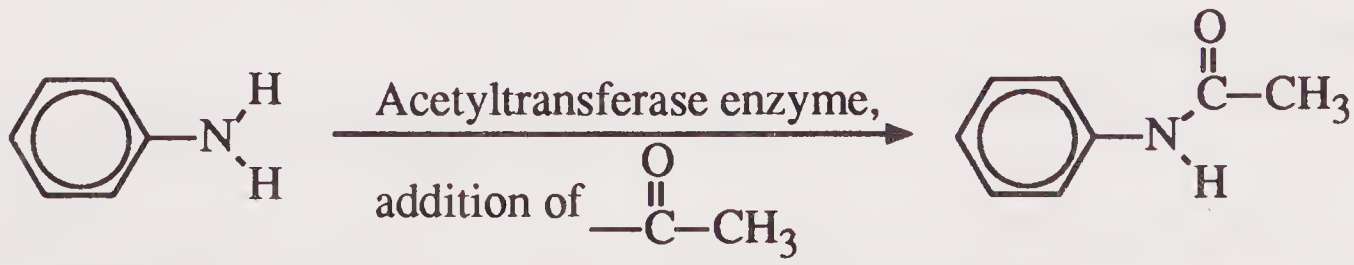


Aniline

Figure 4.17. Formation of sulfate conjugates of some xenobiotic compounds.

Miscellaneous Phase II Reactions

In addition to the important phase II reactions covered in the preceding sections, several other reactions of this type should be mentioned here. **Acetylation reactions** catalyzed by acetyltransferase enzymes involve the attachment of the acetyl moiety, shown as a final step in glutathione conjugation and the production of a mercapturic acid conjugate in Figure 4.16. Acetyl transferase enzyme acts to acetylate arylamines:[6]



(4.6.1)

Amino acids, particularly glycine, form conjugates with a number of xenobiotic compounds to give **peptide conjugates** that can be excreted from the body. **Methylation** of xenobiotics occurs with attachment of the $-CH_3$ group.

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2. Lu, Frank C., *Basic Toxicology*, 2nd ed., Hemisphere Publishing Co., New York, 1991, pp. 27-41.

QUESTIONS AND PROBLEMS

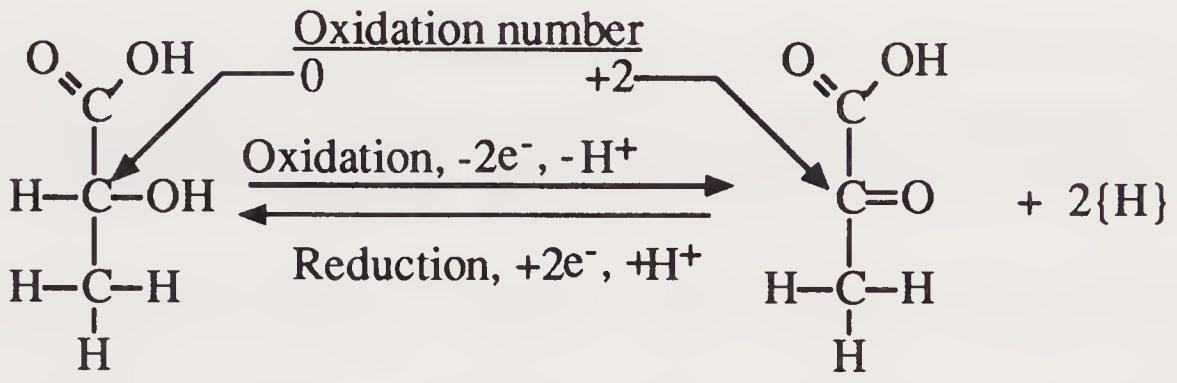
1. Define metabolism and its relationship to toxic substances.

2. Distinguish between digestion and metabolism. Why is digestion relatively unimportant in regard to toxic substances, most of which are relatively small molecules?

3. What is the fundamental difference between the digestion of fats and that of complex carbohydrates (starches) and proteins? What role is played by bile salts in the digestion of fats?

4. What are the functions of ADP, ATP, NAD^+, and NADH in metabolism?

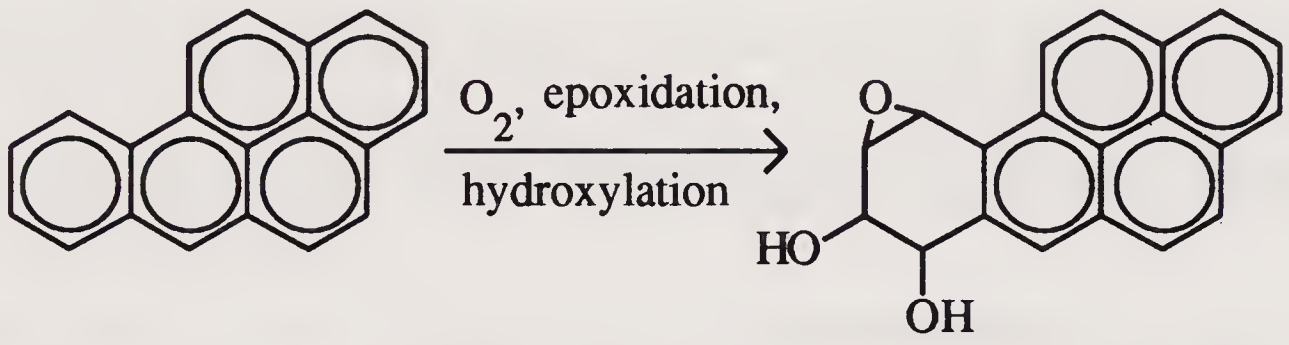
5. What is the overall reaction mediated by the Krebs cycle? What does it produce that the body needs?
6. What is the amino acid pool? What purposes does it serve?
7. What is meant by an essential amino acid?
8. What is transamination? What product of amino acid synthesis is eliminated from the body by the kidneys?
9. Discuss the meaning of the reaction below and the purpose that it serves in the body:



10. Give the definition and function of an energy carrier species in metabolism.
11. Show sites or pathways of possible toxicant absorption, metabolism, temporary storage, distribution, or excretion as illustrated in Figure 4.6.
12. What are intermediary xenobiotic metabolites? Why are they often important in toxicology?
13. Why is enzyme action relatively less likely for toxicants with a high degree of polarity and for volatile compounds? What is the nature of toxicants most likely to be subjected to enzymatic action? Of these toxicants, what is the likely fate of those that are resistant to enzymatic attack?
14. Distinguish between phase I reactions and phase II reactions of toxicants.
15. What does a phase I reaction do to a toxicant? How is a "chemical handle" involved?
16. Explain and justify the statement that in general, the changes in structure and properties of a compound that result from a phase I reaction are relatively mild whereas phase II processes usually produce species that are much different from the parent compounds. Is a phase I reaction always necessary prior to a phase II reaction?
17. The most important phase I reactions are _____, particularly those classified as _____.
18. Illustrate with examples the oxidation of nitrogen, sulfur, and phosphorus in the metabolism of xenobiotic compounds.
19. What functional groups are most likely to be reduced. Why are xenobiotics reduced in the body by enzymes that are not the body's own?
20. As applied to xenobiotic compounds, define dealkylation, hydrolysis, reductive dehalogenation and oxidative dehalogenation.

21. In what sense are phase II reactions conjugation reactions? What is an endogenous conjugating agent?

22. Define and illustrate with examples epoxidation and hydroxylation of xenobiotic substances. How do these two phenomena apply to the reaction illustrated below and what is its toxicological significance?



23. Name and briefly describe the action of the most common endogenous conjugating agent involved in phase II reactions. On what basis are its products classified?

24. Describe how enterohepatic circulation can amplify the effects of glucuronide conjugates.

25. What is the formula and nature of glutathione? From which kinds of species is it composed?

26. Other than the two conjugating agents mentioned in the preceding questions, list and describe other kinds of conjugating agents that may be involved in phase II reactions. Which one of these forms a species most likely to be eliminated through urine? Why is this species so eliminated?

5

Microbial Processes

5.1. MICROORGANISMS

Microorganisms comprise a diverse group of organisms generally capable of existing as single cells that can be seen only under a microscope. From the viewpoint of environmental chemistry, the small size of microorganisms is particularly significant because it gives them a very high surface/volume ratio enabling very rapid exchange of nutrients and metabolic products with their surroundings, resulting in exceptionally high rates of metabolic reactions. This, combined with the spectacularly fast geometric increase in population of single-celled microorganisms by fission during the log phase of growth (see Figure 5.4) enables microganisms to multiply very rapidly on environmental chemical substrates, such as biodegradable organic matter.

Microorganisms function as living catalysts that enable a vast number of chemical processes to occur in water and soil. A majority of the important chemical reactions that take place in water and soil, particularly those involving organic matter and oxidation-reduction processes, occur through bacterial intermediaries. Algae are the primary producers of biological organic matter (biomass) in water. Microorganisms are responsible for the formation of many sediment and mineral deposits; they also play the dominant role in secondary waste treatment. Pathogenic microorganisms must be eliminated from water purified for domestic use.

Microorganisms are divided into the two broad categories of **prokaryotes** and **eukaryotes**; the latter have well defined cell nuclei enclosed by a nuclear membrane, whereas the former lack a nuclear membrane and the nuclear genetic material is more diffuse in the cell. In addition, differences between these two classes of organisms occur in other categories, including location of cell respiration, means of photosynthesis, means of motility, and reproductive processes.

Microorganisms important in environmental biochemistry may be divided among three categories: bacteria, fungi, and algae. Fungi and bacteria (with the exception of photosynthetic bacteria) are classified as **reducers**, which break down chemical compounds to more simple species and thereby extract the energy needed for their growth and metabolism. Algae are classified as **producers**, because they utilize light energy and store it as chemical energy. In the absence of sunlight, however, algae utilize chemical energy for their metabolic needs. In a sense, therefore, bacteria and fungi may be looked upon as environmental catalysts, whereas algae function as aquatic solar fuel cells.

All microorganisms can be put into one of the four following classifications: Chemoheterotrophs, chemoautotrophs, photoheterotrophs, and photoautotrophs. These classifications are based upon (1) the energy source and (2) the carbon source utilized by the organism. **Chemotrophs** use chemical energy derived from oxidation-reduction reactions for their energy needs. **Autotrophs** utilize light energy from pho-

tosynthesis. **Heterotrophs** obtain their carbon from other organisms; **autotrophs** use carbon dioxide and ionic carbonates for the C that they require. Figure 5.1 summarizes the classifications into which microorganisms may be placed with these definitions.

Energy source / Carbon sources	Chemical	Photochemical (light)
Organic matter	**Chemoheterotrophs** All fungi and protozoans, most bacteria. Chemoheterotrophs use organic sources for both energy and carbon.	**Photoheterotrophs** A few specialized bacteria that use photoenergy, but are dependent on organic matter for a carbon source
Inorganic carbon (CO_2, HCO_3^-)	**Chemoautotrophs** Use CO_2 for biomass and oxidize substances such as H_2 (*Pseudomonas*), NH_4^+ (*Nitrosomonas*), S (*Thiobacillus*) for energy	**Photoautotrophs** Algae, cyanobacteria ("blue-green algae"), photosynthetic bacteria that use light energy to convert CO_2 (HCO_3^-) to biomass by photosynthesis

Figure 5.1. Classification of microorganisms among chemoheterotrophs, chemoautotrophs, photoheterotrophs, and photoautotrophs.

5.2. ALGAE

For the purposes of discussion here, **algae** may be considered as generally microscopic organisms that subsist on inorganic nutrients and produce organic matter from carbon dioxide by photosynthesis. (The term algae is somewhat difficult to define; some algae, particularly the marine kelps, are huge multicellular organisms.) The study of algae is called **phycology**.

The four main classes of unicellular algae of importance in environmental chemistry are the following:

- **Chrysophyta**, which contain pigments that give these organisms a yellow-green or golden-brown color. Chrysophyta are found in both freshwater and marine systems. They store food as carbohydrate or oil. The most well known of these algae are **diatoms**, characterized by silica-containing cell walls. Diatoms are the most prolific primary producers in sea water and are also significant as primary producers in freshwater. Diatoms are sensitive to environmental conditions of alkalinity, hardness, pH, pollutants, and temperature, so they are used to assess water quality. They produce vast mineral deposits of diatomaceous earth.

- **Chlorophyta**, commonly known as green algae, thrive near the surface of water as well as on solid surfaces of ice, snow, wood, rock, and soil. Chlorophyta are found in both freshwater and marine systems. Single-celled chlorophyta comprise most of the phytoplankton (free-floating photosynthetic organisms) in freshwater and are responsible for most of the primary

productivity in freshwaters. The predominant food storage material of these algae is starch, and their cell walls are composed of cellulose.

- **Pyrrophyta**, commonly known as dinoflagellates, resemble animals in being motile. They occur in both marine and freshwater environments. "Blooms" of *Gymnodinium* and *Gonyaulax* species release toxins that can kill millions of fish at a time. Toxins produced during incidents of this so-called "red tide" can bioaccumulate in shellfish which, eaten by humans, can cause fatal paralytic shellfish poisoning.

- **Euglenophyta** likewise exhibit characteristics of both plants and animals. Though capable of photosynthesis, these algae are not exclusively photoautotrophic (see Figure 5.1), they utilize biomass from other sources for at least part of their carbon needs, and they require external sources of some vitamins. Because of this characteristic they tend to grow in organic-rich freshwaters.

Morphologically, algae may take on numerous forms. In addition to single cells, other forms include filaments, sheets, and colonies. Algal cell walls occur in several forms including mannose polymer, cellulose covered with pectin polysaccharide, and mineral-impregnated protein incorporating SiO_2. Some algae do not have cell walls as such, but have cytoplasmic membranes thickened and strengthened by additional cellulose, protein, and minerals, including SiO_2, $CaCO_3$, and iron. Some algal cells are coated with gelatinous mucilage excretions, which in some cases form cysts that enable algal cell survival under adverse conditions.

The general nutrient requirements of algae are carbon (obtained from CO_2 or HCO_3^-), nitrogen (generally as NO_3^-), phosphorus (as some form of orthophosphate), sulfur (as SO_4^{2-}), and trace elements including sodium, potassium, calcium, magnesium, iron, cobalt, and molybdenum.

In a highly simplified form, the production of organic matter by algal photosynthesis is described by the reaction

$$CO_2 + H_2O \xrightarrow{h\nu} \{CH_2O\} + O_2(g) \qquad (5.2.1)$$

where $\{CH_2O\}$ represents a unit of carbohydrate and $h\nu$ stands for the energy of a quantum of light. Fogg[1] has represented the overall formula of the algae *Chlorella* as $C_{5.7}H_{9.8}O_{2.3}NP_{0.06}$. Using Fogg's formula for algal biomass exclusive of the phosphorus, the overall reaction for photosynthesis is:

$$5.7CO_2 + 3.4H_2O + NH_3 \xrightarrow{h\nu} C_{5.7}H_{9.8}O_{2.3}N + 6.25O_2(g) \qquad (5.2.2)$$

In the absence of light, algae metabolize organic matter in the same manner as do nonphotosynthetic organisms. Thus, algae may satisfy their metabolic demands by utilizing chemical energy from the degradation of stored starches or oils, or from the consumption of algal protoplasm itself. In the absence of photosynthesis, the metabolic process consumes oxygen, so during the hours of darkness an aquatic system with a heavy growth of algae may become depleted in oxygen.

Although algae are generally regarded as photoautotrophic, some kinds of algae require organic nutrients produced by other organisms. Some algae even act like protozoa by ingesting other unicellular organisms as food sources. Chloroplasts are

lost from some *Euglena* that are grown in the dark so that the algae become completely heterotrophic.

In addition to those discussed above, there are several other characteristics of algae that are significant to their existence. Algae can reproduce both sexually and asexually. Motility is an important characteristic of some algae. It is usually by means of flagella attached to the algal cells that propel the algal cells by means of their spiraling action.

Symbiotic relationships with other organisms are common. There are even reports of unicellular green algae growing inside hairs on polar bears, which are hollow for purposes of insulation; the sight of a green polar bear is alleged to have driven more than one arctic explorer to the brink of madness. The most common symbiotic relationship involving algae is that of **lichen** in which algae coexist with fungi; both kinds of organisms are woven into the same thallus (tubular vegetative unit). The fungus provides moisture and nutrients required by the algae, which generates food photosynthetically. Lichen are involved in weathering processes of rocks.

5.3. FUNGI

Fungi are nonphotosynthetic organisms. Most frequently they possess a filamentous structure. The morphology (structure) of fungi covers a wide range. Some fungi are as simple as the microscopic unicellular yeasts, whereas other fungi form large, intricate toadstools. The microscopic filamentous structures of fungi generally are much larger than bacteria and usually are 5-10 μm in width. Fungi are aerobic (oxygen-requiring) organisms and generally can thrive in more acidic media than can bacteria. They are also more tolerant of higher concentrations of heavy metal ions than are bacteria.

Perhaps the most important function of fungi in the environment is the breakdown of cellulose in wood and other plant materials. To accomplish this, fungal cells secrete an extracellular enzyme (exoenzyme), *cellulase*, that breaks insoluble cellulose down to soluble carbohydrates that can be absorbed by the fungal cell.

Although fungi do not grow well in water, they play an important role in determining the composition of natural waters and wastewaters because of the large amount of their decomposition products that enter water. An example of such a product is humic material, which interacts with hydrogen ions and metals (see Section 3.4).

5.4. PROTOZOA

Protozoa are microscopic animals consisting of single eukaryotic cells. They occur in a wide variety of shapes and their movement in the field of a microscope is especially fascinating to watch. Different kinds of protozoa move by means of flagella, cilia, gliding, and in the manner of amebas. Some protozoa contain chloroplasts and are photosynthetic.

Protozoa play a relatively small role in environmental biochemical processes, but are nevertheless significant in the aquatic and soil environment for the following reasons:

- Several devastating human diseases, including malaria, sleeping sickness, and some kinds of dysentery are caused by protozoa that are parasitic to the human body.

- Parasitic protozoa can cause debilitating, even fatal, diseases in livestock and wildlife.
- Vast limestone ($CaCO_3$) deposits have been formed by the deposition of shells from the *foramifera* group of protozoa.
- Protozoa are active in the oxidation of degradable biomass, particularly in sewage treatment.
- Protozoa may affect bacteria active in degrading biodegradable substances by "grazing" on bacterial cells.

Numerous kinds of protozoa are classified on the bases of morphology (physical structure), means of locomotion (flagella, cilia, pseudopodia), presence or absence of chloroplasts, presence or absence of shells, ability to form cysts (consisting of a reduced-size cell encapsulated in a relatively thick skin that can be carried in the air or by animals in the absence of water), and ability to form spores.

Though single-celled, protozoa have a fascinating variety of structures that enable them to function. The protozoal cell membrane is protected and supported by a relatively thick pellicle or by a mineral shell that may act as an exoskeleton. Food is ingested through a structure called a cytosome from which it is concentrated in a cytopharynx or oral groove, then digested by enzymatic action in a food vacuole. Residue from food digestion is expelled through a cytopyge and soluble metabolic products, such as urea or ammonia are eliminated by a contractile vacuole, which also expells water from the cell interior.

5.5. BACTERIA

Bacteria are single-celled prokaryotic microorganisms that may be shaped as rods (**bacillus**), spheres (**coccus**), or spirals (**vibrios, spirilla, spirochetes**). Bacteria cells may occur individually or grow as groups ranging from two to millions of individual cells. Most bacteria fall into the size range of 0.5-3.0 micrometers. However, considering all species, a size range of 0.3-50 μm is observed. Characteristics of most bacteria include a semirigid cell wall, motility with flagella for those capable of movement, unicellular nature (although clusters of cloned bacterial cells are common), and multiplication by binary fission in which each of two daughter cells is genetically identical to the parent cell. Like fungi and algae, bacteria produce spores, metabolically inactive bodies that form and survive under adverse conditions in a "resting" state until conditions favorable for growth occur.

The metabolic activity of bacteria is greatly influenced by their small size. Their surface-to-volume ratio is extremely large, so that the inside of a bacterial cell is highly accessible to a chemical substance in the surrounding medium. Thus, for the same reason that a finely divided catalyst is more efficient than a more coarsely divided one, bacteria may bring about very rapid chemical reactions compared to those mediated by larger organisms. Bacteria excrete exoenzymes that break down solid food material to soluble components which can penetrate bacterial cell walls, where the digestion process is completed.

Although individual bacteria cells cannot be seen by the naked eye, bacterial colonies arising from individual cells are readily visible. A common method of counting individual bacterial cells in water consists of spreading a measured volume of an appropriately diluted water sample on a plate of agar gel containing bacterial nutrients. Wherever a viable bacterial cell adheres to the plate, a bacterial colony consisting of

many cells will grow. These visible colonies are counted and related to the number of cells present initially. Because bacteria cells may already be present in groups and because individual cells may not live to form colonies, plate counts tend to grossly underestimate number of viable bacteria.

Autotrophic and Heterotrophic Bacteria

Bacteria may be divided into two main categories, autotrophic and heterotrophic. **Autotrophic bacteria** are not dependent upon organic matter for growth and thrive in a completely inorganic medium; they use carbon dioxide or other carbonate species as a carbon source. A number of sources of energy may be used, depending upon the species of bacteria; however, a biologically mediated chemical reaction always supplies the energy.

An example of autotrophic bacteria is *Gallionella*. In the presence of oxygen, these bacteria are grown in a medium consisting of NH_4Cl, phosphates, mineral salts, CO_2 (as a carbon source), and solid FeS (as an energy source). It is believed that the following is the energy-yielding reaction for this species:

$$4FeS(s) + 9O_2 + 10H_2O \rightarrow 4Fe(OH)_3(s) + 4SO_4^{2-} + 8H^+ \qquad (5.5.1)$$

Starting with the simplest inorganic materials, autotrophic bacteria must synthesize all of the complicated proteins, enzymes, and other materials needed for life processes. It follows, therefore, that the biochemistry of autotrophic bacteria is quite complicated. Because of their consumption and production of a wide range of minerals, autotrophic bacteria are involved in many geochemical transformations.

Heterotrophic bacteria depend upon organic compounds, both for their energy and for the carbon required to build their biomass. They are much more common in occurrence than autotrophic bacteria. Heterotrophic bacteria are the microorganisms primarily responsible for the breakdown of pollutant organic matter in waters and of organic wastes in biological waste-treatment processes.

Aerobic and Anaerobic Bacteria

Another classification system for bacteria depends upon their requirement for molecular oxygen. **Aerobic bacteria** require oxygen as an electron receptor:

$$O_2 + 4H^+ + 4e^- \rightarrow H_2O \qquad (5.5.2)$$

Anaerobic bacteria function only in the complete absence of molecular oxygen. Frequently, molecular oxygen is quite toxic to anaerobic bacteria.

A third class of bacteria, **facultative bacteria**, utilize free oxygen when it is available and use other substances as electron receptors (oxidants) when molecular oxygen is not available. Common oxygen substitutes in water are nitrate ion (see Section 7.12) and sulfate ion (see Section 7.13).

5.6. THE PROKARYOTIC BACTERIAL CELL

The eukaryotic cell was discussed in Chapter 3 and illustrated in Figure 3.1. It is the type of cell found in most organisms, including animals, plants, algae, protozoa, and fungi. Bacteria and cyanobacteria, however, consist of prokaryotic cells. The occurrence of prokaryotic cells is confined to unicellular organisms.

Figure 5.2 illustrates a generic prokaryotic bacterial cell. Several structural and functional features distinguish prokaryotic cells, the most obvious of which is the lack of membranes enclosing the nucleus and organelles. Two more subtle differences are the presence of a unique type of polymer, peptidoglycan, in cell walls in prokaryotic cells and the absence of histone proteins associated with DNA in prokaryotic cells.

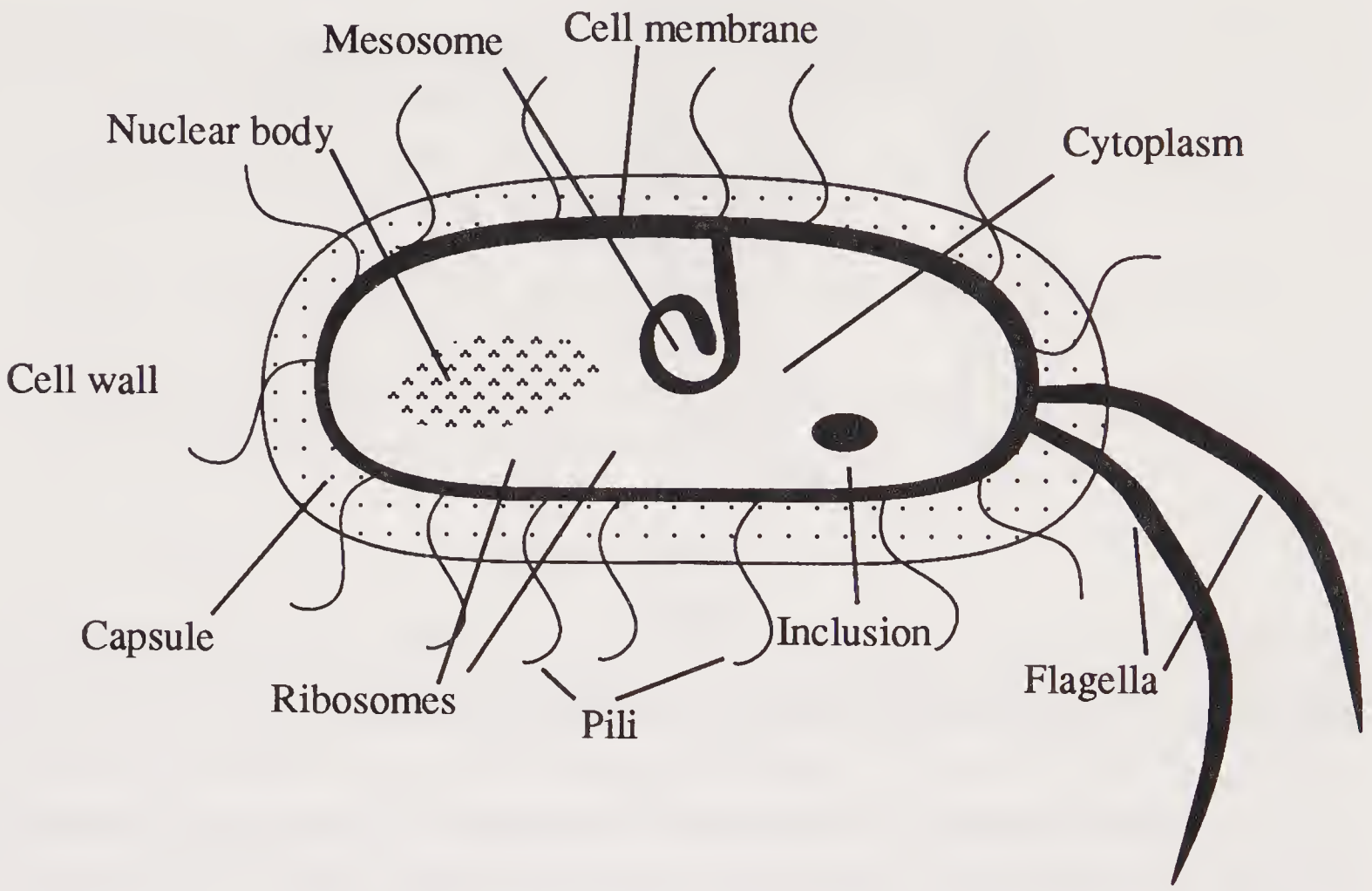


Figure 5.2. Generic prokaryotic bacterial cell illustrating major cell features.

Bacterial cells are enclosed in a **cell wall**, of cross-linked polymeric material called *peptidoglycan* and composed of two kinds of sugars (N-acetylglucosamine and N-acetylmuramic acid) and amino acids. Peptide cross bridges in the polymer consisting of chains of one to five amino acids give the cell wall rigidity. The cell wall holds the contents of the bacterial cell and determines the shape of the cell. Cell walls are subject to damage by antibiotics and by *lysozyme*, an enzyme produced by eukaryotic cells as a defense mechanism against bacteria. Secreted in mucus, tears, saliva, and other body secretions, this enzyme hydrolyzes the sugar chains in the peptidoglycan causing it to break down. The cell wall in many bacteria is frequently surrounded by a **slime layer** (capsule). This layer protects the bacteria from the action of defensive white blood cells in the body and helps the bacterial cells to adhere to surfaces.

The **cell membrane** or **cytoplasmic membrane** composed of somewhat more than half protein and somewhat less than half phospholipid occurs as a thin layer only about 7 nanometers in thickness on the inner surface of the cell wall enclosing the cellular cytoplasm. The cytoplasmic membrane is of crucial importance to cell function in that it controls the nature and quantity of materials transported into and out of the cell. It is also very susceptible to damage from some toxic substances. Antibiotics of the polymyxin class and disinfectant quaternary ammonium compounds and alcohols kill bacteria by destroying the cytoplasmic membrane.

Figure 5.3 illustrates the basic structure of the cytoplasmic membrane. It is seen to be a bilayer in which water-soluble ionic phosphate functional groups ("heads") are

on the surface and lipophilic hydrocarbon "tails" are inside the membrane. Bodies of proteins, many of which penetrate the entire bilayer, are also part of the membrane. These structural features largely determine transport of materials into and out of the cell interior.

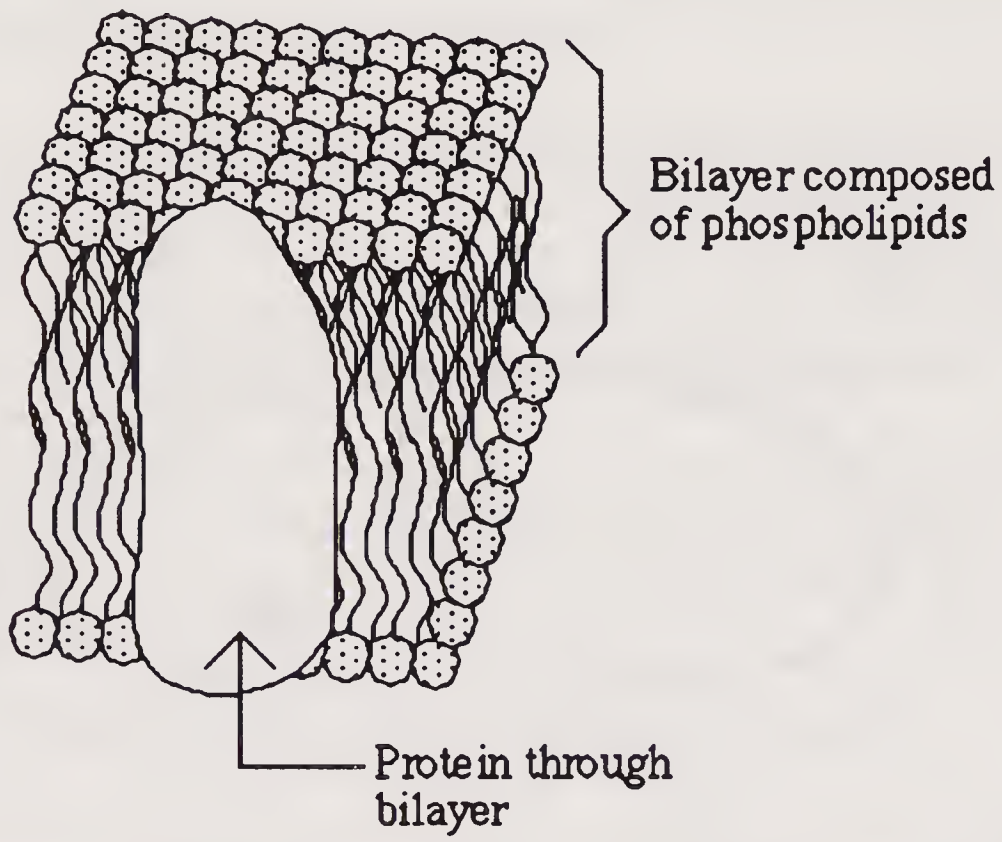


Figure 5.3. Representation of the cross-section of a bacterial cell cytoplasmic membrane showing the phospholipid bilayer and protein bodies that penetrate the membrane.

A crucial characteristic of cell plasma membranes is their **selective permeability** (**semipermeability**) that allows for the passage of some kinds of substances, while restricting others. In general, substances that readily traverse the membrane are water, uncharged small molecules with molecular masses less than several hundred (monosaccharides, amino acids), and lipid-soluble substances (because of the phospholipid content of the membrane). Ions do not go through the membranes readily because of their charges, nor do macromolecules because of their large sizes. To break down the latter into components that can enter the cell, cytoplasmic membranes produce exoenzymes that act outside the cell.

Folds in the cytoplasmic membrane called **mesosomes** serve several functions. One of these is to increase the surface area of the membrane to enhance transport of materials through it. Another function is to act as a site for division of the cell during reproduction. Bacterial DNA is separated at the mesosome during cell division.

Hairlike **pili** on the surface of a bacterial cell enable the cell to stick to surfaces. Specialized **sex pili** enable nucleic acid transfer between bacterial cells during an exchange of genetic material. Somewhat similar to pili — but larger, more complex, and fewer in number — are **flagella**, moveable appendages that cause bacterial cells to move by their whipping action. Bacteria with flagella are termed **motile**.

Bacterial cells are filled with an aqueous solution and suspension containing proteins, lipids, carbohydrates, nucleic acids, ions, and other materials. Collectively, these materials are referred to as **cytoplasm**, the medium in which the cell's metabolic processes are carried out. The major consituents of cytoplasm are the following:

- **Nuclear body**, not considered to be a true nucleus because of its lack of a nuclear membrane. The nuclear body consists of a single DNA macromolecule and controls metabolic processes and reproduction.

- **Inclusions** of reserve food material, consisting of fats, carbohydrates, and even elemental sulfur.
- **Ribosomes**, sites of protein synthesis containing protein and RNA.

Material Transport across the Cytoplasmic Membrane

The transport of nutrients into cells and the movement of waste products out of cells is of critical importance for bacterial metabolism. This movement is regulated by the cytoplasmic membrane. The two driving forces behind this transport are:

- **Concentration gradient** in which a substance moves from an area of higher to one of lower concentration.
- **Active transport** in which cellular energy is expended to move material counter to a concentration gradient.
- **Group translocation** in which the form of the species inside the cell is different from the form outside the cell.

Passive Transport

Movement of material along a concentration gradient is classified as a **passive process**, in that materials simply move from a region of higher to one of lower concentration. In cases where the selectivity of the membrane is not involved in the transport process, movement is regarded as **simple diffusion**. Oxygen required for cellular respiration moves into a cell, and carbon dioxide produced by respiration moves out of a cell, by simple diffusion. **Osmosis** occurs when the cell membrane is selectively permeable to solvent (water) molecules, but does not allow for the movement of solute molecules, such as dissolved sugars. Therefore, water molecules move in a direction to equalize the effective solute concentrations on either side of a membrane, that is, from a relatively dilute solution to a more concentrated one. In an enclosed membrane this occurs until the **osmotic pressure** on the more dilute side is sufficient to establish an equilibrium between the molecules moving in and out. Most bacterial cells are in contact with an outside environment that is an aqueous solution with a lower concentration than the cellular contents; such a solution is called a **hypotonic solution** and the cells are swollen because of the osmotic pressure of the cell contents. Bacterial cells placed in a **hypertonic solution** of higher concentration than the cell contents tend to lose water to the outside and to become shriveled. Solutions with the same effective concentrations are termed **isotonic solutions** and no osmotic pressure differential is established between such solutions.

Carrier proteins or **permeases** facilitate the movement of some substances through the cytoplasmic membrane from a region of higher to one of lower concentration. This process is selective for specific kinds of some substances and is called **facilitated diffusion**. Exoenzymes act outside the cell to break down macromolecules and produce small molecules that may enter a cell *via* facilitated diffusion.

Active Transport

Simple diffusion and facilitated diffusion do not work to move substances from regions of lower to regions of higher concentration. Nevertheless, this is exactly what is required in many cases, such as the movement of nutrients from a dilute medium

into a bacterial cell interior. However, substances can move through a cytoplasmic membrane against a concentration gradient using energy from ATP (an energy-carrier compound discussed in Section 4.3), a process called **active transport**. Active transport makes use of carrier proteins that are specific for each species moved.

Group Translocation

Group translocation usually involves formation of a phosphate ester. The most common example of this kind of phenomenon in bacteria is the transport of sugar molecules into the cells as phosphate esters. The sugar becomes bound with a phosphorylated enzyme in the cell cytoplasmic membrane and released to the cytoplasm as a phosphate ester.[2]

5.7. KINETICS OF BACTERIAL GROWTH

The population size of bacteria and unicellular algae as a function of time in a growth culture is illustrated by Figure 5.4, which shows a **population curve** for a bacterial culture. Such a culture is started by inoculating a rich nutrient medium with

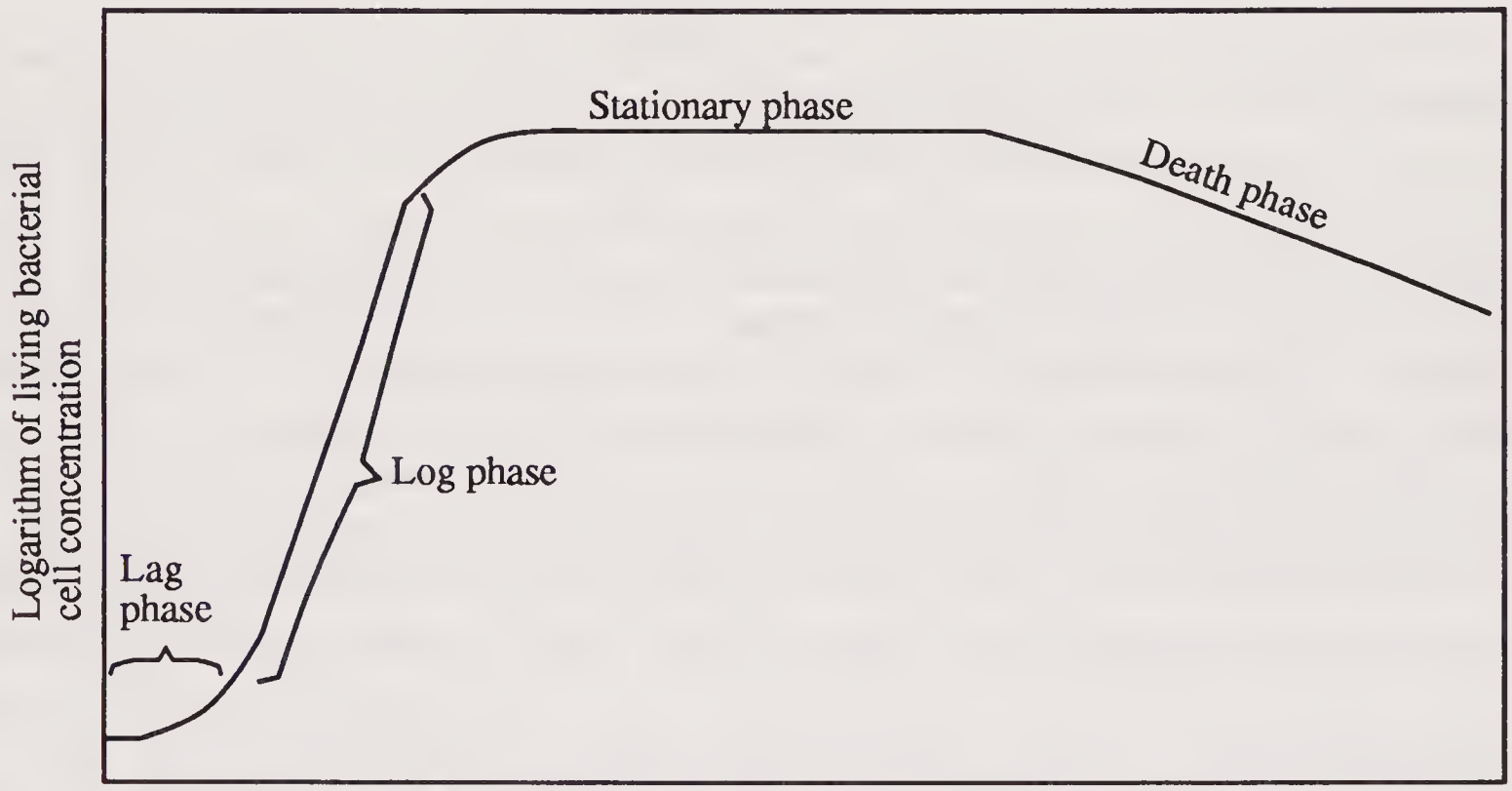


Figure 5.4. Population curve for a bacterial culture.

a small number of bacterial cells. The population curve consists of four regions. The first region is characterized by little bacterial reproduction and is called the **lag phase**. The lag phase occurs because the bacteria must become acclimated to the new medium. Following the lag phase comes a period of very rapid bacterial growth. This is the **log phase**, or exponential phase, during which the population doubles over a regular time interval called the **generation time**. This behavior can be described by a mathematical model in which growth rate is proportional to the number of individuals present and there are no limiting factors such as death or lack of food:

$$\frac{dn}{dt} = kN \qquad (5.7.1)$$

This equation can be integrated to give

$$\ln \frac{N}{N_0} = kt \qquad \text{or } N = N_0 e^{kt} \tag{5.7.2}$$

where N is the population at time t and N_0 is the population at time t = 0. Thus, another way of describing population growth during the log phase is to say that the logarithm of bacterial population increases linearly with time. The generation time, or doubling time, is $(\ln 2)/k$, analogous to the half-life of radioactive decay. Fast growth during the log phase can cause very rapid microbial transformations of chemical species in water.

The log phase terminates and the **stationary phase** begins when a limiting factor is encountered. Typical factors limiting growth are depletion of an essential nutrient, build-up of toxic material, and exhaustion of oxygen. During the stationary phase, the number of viable cells remains virtually constant. Depending upon the bacterial species and other circumstances, the stationary phase may be either very long or very short in duration. After the stationary phase, the bacteria begin to die faster than they reproduce, and the population enters the **death phase**.

5.8. BACTERIAL METABOLISM

Bacteria obtain the energy and raw materials needed for their metabolic processes and reproduction by mediating chemical reactions. Nature provides a large number of such reactions, and bacterial species have evolved that utilize many of these. As a consequence of their participation in such reactions, bacteria are involved in many biogeochemical processes in water and soil. Bacteria are essential participants in many important elemental cycles in nature, including those of nitrogen, carbon, and sulfur. They are responsible for the formation of many mineral deposits, including some of iron and manganese. On a smaller scale, some of these deposits form through bacterial action in natural water systems and even in pipes used to transport water.

Bacterial metabolism addresses the biochemical processes by which chemical species are modified in bacterial cells. It is basically a means of deriving energy and cellular material from nutrient substances. Figure 5.5 summarizes the essential features of bacterial metabolism. The two major divisions of bacterial metabolism are

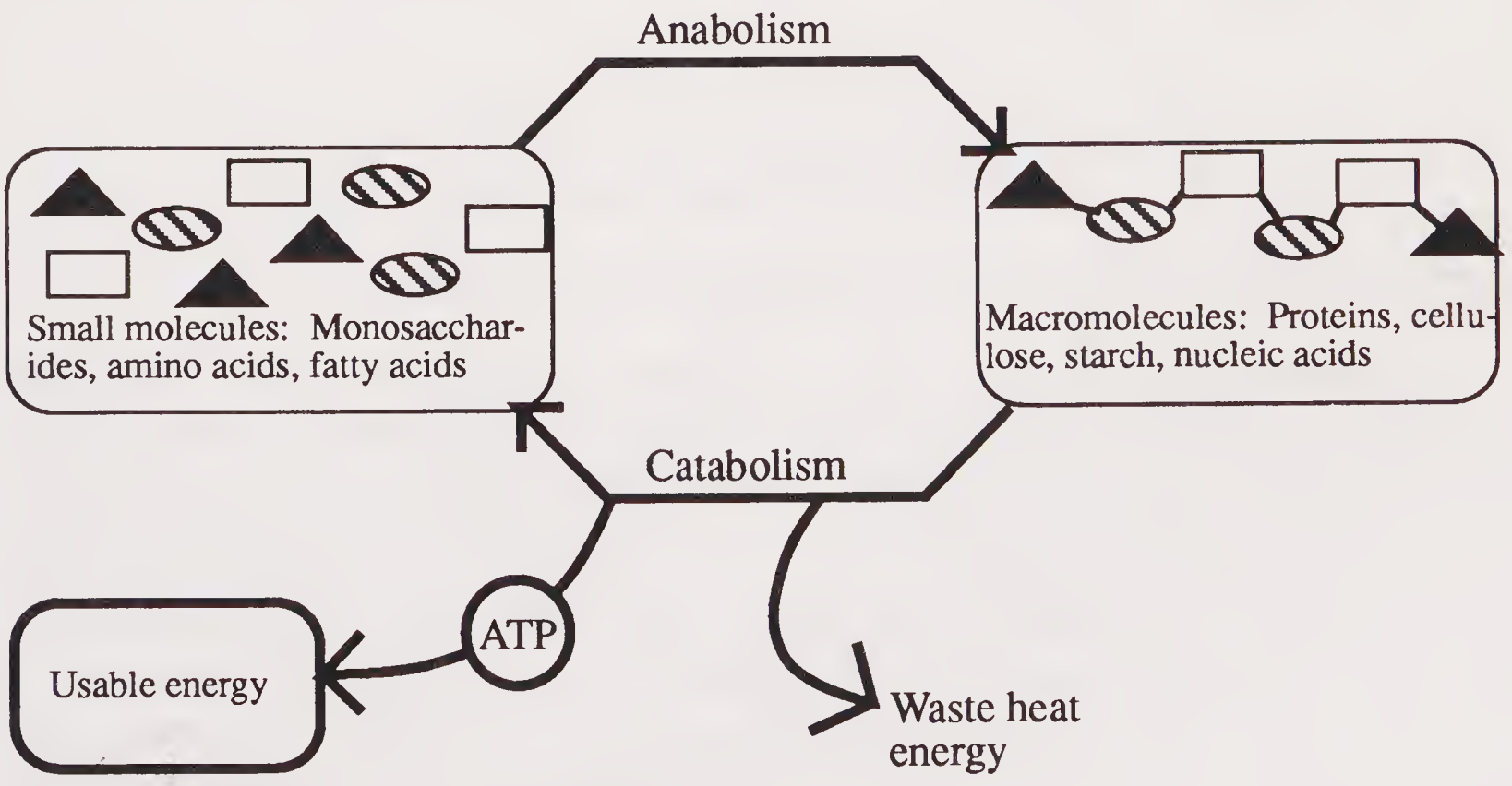


Figure 5.5. Bacterial metabolism and energy production.

catabolism, degradative metabolism which breaks macromolecules down to their small monomeric constituents, and **anabolism**, synthetic metabolism in which small molecules are assembled into large ones. The former releases energy, part of which is expended as waste heat and the remainder of which is retained as energy that can be used by cellular processes. Useable energy released by catabolism and carried by molecules with high-energy bonds is utilized by energy-consuming anabolic processes to build macromolecules required for cell function and reproduction.

Enzymes in Bacterial Metabolism

Bacterial metabolic reactions are mediated by enzymes, biochemical catalysts endogenous to living organisms that were covered in detail in Chapter 3. Enzymatic processes in bacteria are essentially the same as those in other organisms. At this point, however, it is useful to review several factors that influence bacterial enzyme activity and, therefore, bacterial growth.

Figure 5.6 illustrates the effect of **substrate concentration** on enzyme activity. It is seen that enzyme activity increases in a linear fashion up to a value that represents saturation of the enzyme activity. Beyond this concentration, increasing substrate levels do not result in increased enzyme activity. This kind of behavior is reflected in bacterial activity which increases with available nutrients up to a saturation value. Superimposed on this plot in a bacterial system is increased bacterial population, which, in effect, increases the amount of available enzyme.

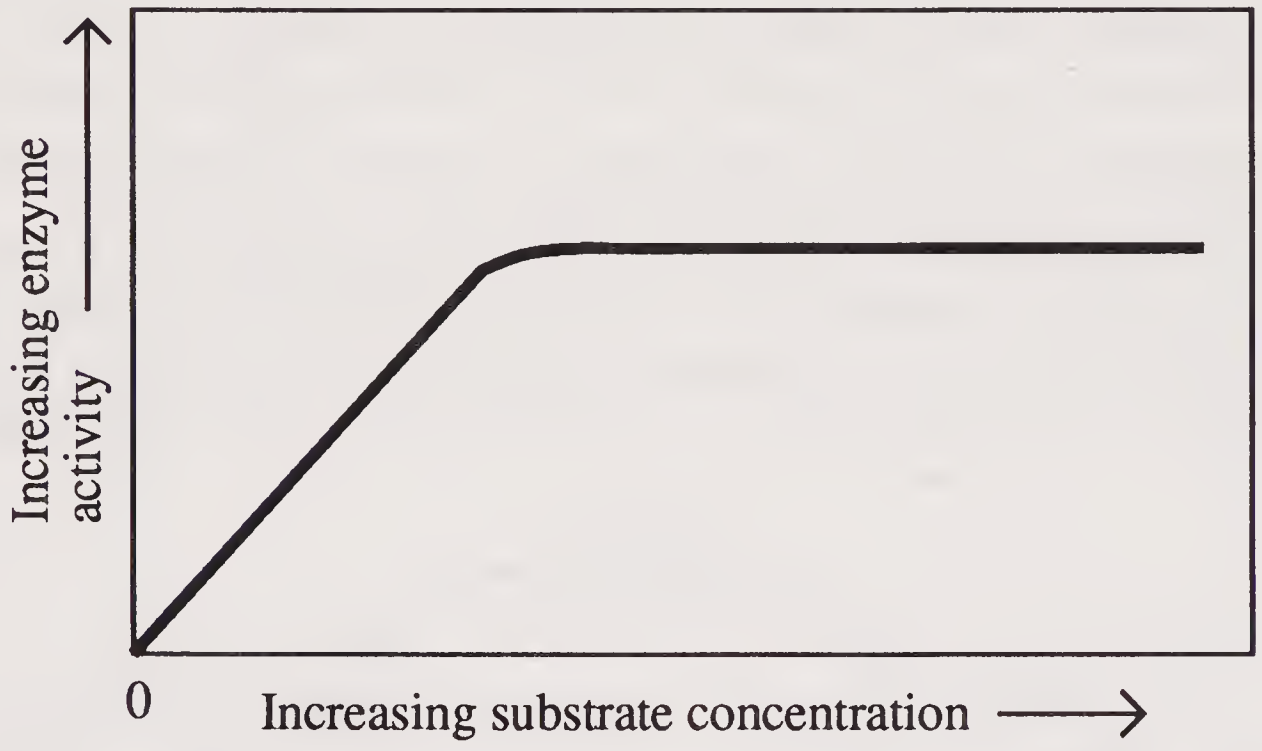


Figure 5.6. Effect of increasing substrate concentration on enzyme activity.

Temperature has a strong effect upon enzyme activity and therefore on bacterial growth and metabolism. Figure 5.7 shows the effect of temperature on enzyme activity. It is seen that over a relatively short range of temperature, a plot of enzyme activity as a function of the reciprocal of the absolute temperature, $1/K$, is linear. Students of physical chemistry will recognize such a relationship as an Arrhenius plot, which relates a kinetic rate constant to temperature. Such curves show a maximum growth rate with an optimum temperature that is skewed toward the high temperature end of the curve and exhibit an abrupt dropoff beyond the temperature maximum. This occurs because enzymes are destroyed by being denatured at temperatures not far above the optimum.

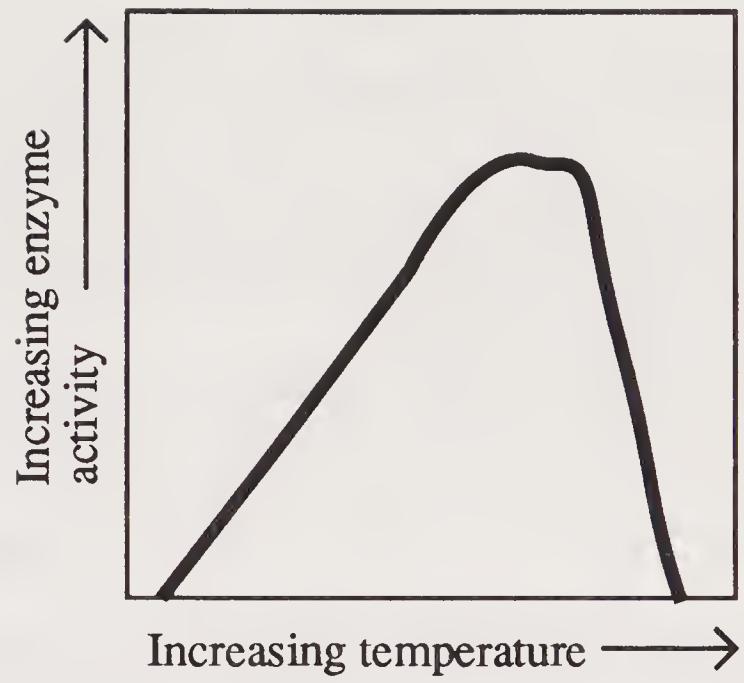


Figure 5.7. Enzyme activity as a function of temperature. A plot of bacterial growth *vs.* temperature has the same shape.

Bacterial metabolic activity as a function of temperature shows exactly the same kind of plot as that illustrated in Figure 5.7. However, a given temperature does not affect different kinds of bacteria in the same way, since they have different optimum temperatures for growth. **Psychrophilic bacteria** are bacteria having temperature optima below approximately 20°C. The temperature optima of **mesophilic bacteria** lie between 20°C and 45°C. Bacteria having temperature optima above 45°C are called **thermophilic bacteria**. The temperature range for optimum growth of bacteria is remarkably wide, with some bacteria being able to grow at 0°C, and some thermophilic bacteria existing at temperatures as high as 80°C.

Figure 5.8 is a plot of pH *vs.* enzyme activity. Although the optimum pH will vary somewhat, enzymes typically have a pH optimum around neutrality. Enzymes tend to become denatured at pH extremes. This behavior likewise is reflected in plots of bacterial metabolism as a function of pH. For some bacteria, such as those that generate sulfuric acid by the oxidation of sulfide or organic acids by fermentation processes, the pH optimum may be quite acidic.

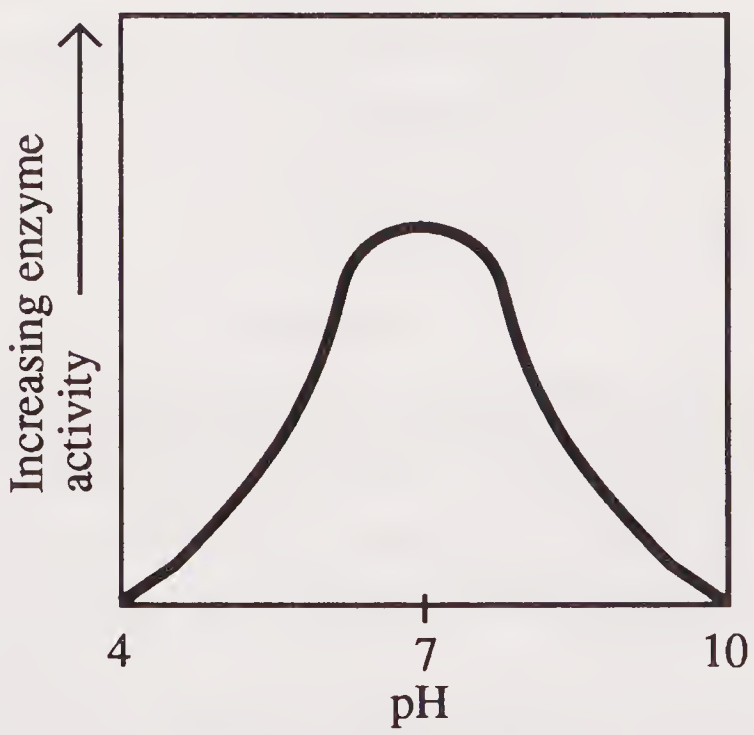


Figure 5.8. Enzyme activity as a function of pH.

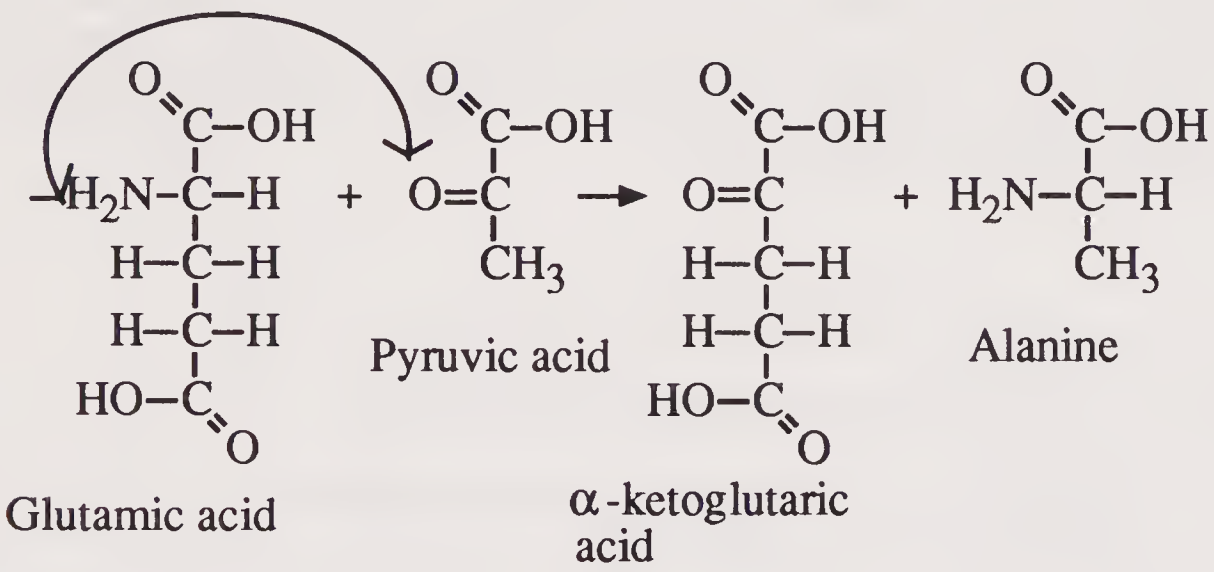
5.9. ENERGY UTILIZATION BY BACTERIA

Energy derived by bacteria from metabolic processes is utilized for bacterial functions, such as movement, and for production of biomass. Heterotrophic bacteria obtain their energy from catabolism as they break down complex nutrient substances. In general, bacteria obtain energy by mediating oxidation-reduction reactions. These processes must occur continually because only a relatively small portion of the energy released is available to the bacterial cells.

The most common use of the energy obtained by bacteria through catabolism is for reproduction and growth. This requires continual synthesis of new proteins, polysaccarides, and lipids. The processes involved in synthesizing these crucial biomolecules are in general those described in some detail in Chapter 4 and are reviewed briefly here.

A key distinction among bacteria has to do with the terminal electron acceptor in the electron transport chain involved in the process by which bacteria gain energy by oxidizing food materials (see Section 4.3). If the terminal electron acceptor is molecular O_2, the process is **aerobic respiration**. If it is another reducible species, commonly including SO_4^{2-}, NO_3^-, HCO_3^-, or iron(III), the process is called **anaerobic respiration**. As examples, *Desulfovibrio* bacteria convert SO_4^{2-} to H_2S, *Methanobacterium* reduce HCO_3^- to CH_4, and assorted bacteria reduce NO_3^- to NO_2^-, N_2O, N_2, or NH_4^+.

Bacteria must have amino acids in order to make their required proteins. Amino acids can be obtained from the breakdown of nutrient proteins. More versatile bacteria can produce all their required amino acids by biosynthesis from inorganic substances and organic (carboxylic) acid intermediates in the Krebs cycle. In order to convert carboxylic acids to nitrogen-containing amino acids, an $-NH_2$ group must be attached to the carboxylic acid. This is a process called **amination**. Amination that occurs as a transfer of $-NH_2$ to an organic acid from an amino acid is called **transamination** as shown by the following example for the biosynthesis of the amino acid alanine:



(5.9.1)

The final step in protein synthesis is the assembly of amino acids into protein macromolecules. Preceding that, however, it is necessary for bacteria to obtain or synthesize the required amino acids, as discussed above. In addition to forming proteins, amino acids synthesized by bacterial cells can be utilized to make the purine and pyrimidine base constituents of DNA and RNA. The amino acids glycine, aspartic acid, and glutamine are so utilized.

Microorganisms can obtain simple sugars from the hydrolysis of complex carbohydrates or from biosynthesis from simple inorganic materials. An important example of the latter is the biosynthesis of glucose by algae using photochemical energy, for which the overall reaction is the following:

$$6CO_2 + 6H_2O + h\nu \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (5.9.2)$$

The most prominent polysaccharide synthesized by bacteria is glycogen, for which adenosine diphosphoglucose is the raw material. The glycogen thus synthesized can be stored as inclusions in bacterial cells.

Bacteria must synthesize lipids as essential components of bacterial cytoplasmic membranes. In a sense, bacterial synthesis of fats is much the reverse of fat catabolism. The glycerol backbone of the fat molecule is made from a glycolysis intermediate, dihydroxyacetone phosphate. The fatty acid chains bound to the glycerol are made by successive two-carbon additions of acetyl groups carried by acetylcoenzyme A.

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1. Boyd, Robert F., *General Microbiology*, Times Mirror/Mosby, St. Louis, MO, 1984.

2. Tortora, Gerard J, Berdell R. Funke, and Christine L. Case, *Microbiology: An Introduction*, The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California, 1982.

QUESTIONS AND PROBLEMS

1. What is the main distinguishing feature between prokaryotes and eukaryotes. Classify the major types of microorganisms between these two classes of organisms.

2. Match:

(a) Chemotrophs	1. Obtain their carbon from other organisms
(b) Heterotrophs	2. Utilize light energy from photosynthesis
(c) Autotrophs	3. Use chemical energy derived from oxidation/reduction reactions for their energy needs
(d) Chemautotrophs	4. Use carbon dioxide and ionic carbonates for their carbon needs

3. Describe the nature and significance of the most common symbiotic relationship involving algae. What are the contributions of each of the parties to this relationship?

4. Define fungi. What is the most important function of fungi in the environment? How do they accomplish this function?

5. What are protozoa? How do they resemble animals? How do some of their members resemble algae? Describe their environmental significance, role in disease, in geochemical processes.

6. Discuss the transport of nutrients into cells and the movement of waste products out of cells considering concentration gradient, active transport, and group translocation. In connection with the discussion, consider hypotonic, hypertonic, and isotonic solutions, carrier proteins, and facilitated diffusion.

7. Match:

(a) Chrysophyta	1. Resemble animals in being motile
(b) Chlorophyta	2. Comprise most of the phytoplankton (free-floating photosynthetic organisms) in freshwater and are responsible for most of the primary productivity in freshwaters
(c) Pyrrophyta	3. Use chemical energy derived from oxidation/reduction reactions for their energy needs
(d) Euglenophyta	4. Use carbon dioxide and ionic carbonates for their carbon needs.

8. Define bacteria and describe their shapes, size range, and classifications based upon nutrient sources and oxygen sources.

9. Match the following pertaining to the prokaryotic bacterial cell:

(a) Cell wall	1. Resemble animals in being motile
(b) Cell membrane	2. Consists of a single DNA macromolecule and controls metabolic processes and reproduction
(c) Ribosomes	3. Holds the contents of the bacterial cell and determines the shape of the cell
(d) Nuclear body	4. Sites of protein synthesis containing protein and RNA

10. The following data were obtained for numbers of bacterial cells per mL of polluted water:

Time elapsed, hr	Concentration of cells, millions per mL
0	1.1
1	0.9
2	1.0
3	1.4
4	1.8
5	3.6
6	7.2
8	29

Describe what is going on in terms of the bacterial population curve.

11. What are the primary functions of bacterial metabolism?

12. Describe bacterial metabolism in terms of natural elemental cycles, and biogeochemical processes.

13. Distinguish between aerobic respiration and anaerobic respiration in respect to bacterial metabolism and give examples of each.

14. When molecular oxygen is used as the terminal electron receptor by bacteria in mediating energy-yielding oxidation-reduction reactions, the process is called aer-

obic repiration. What is it called when some other species is used as the terminal electron receptor? What are some of these species?

15. Describe each of the following curves in terms of bacterial growth and metabolism:

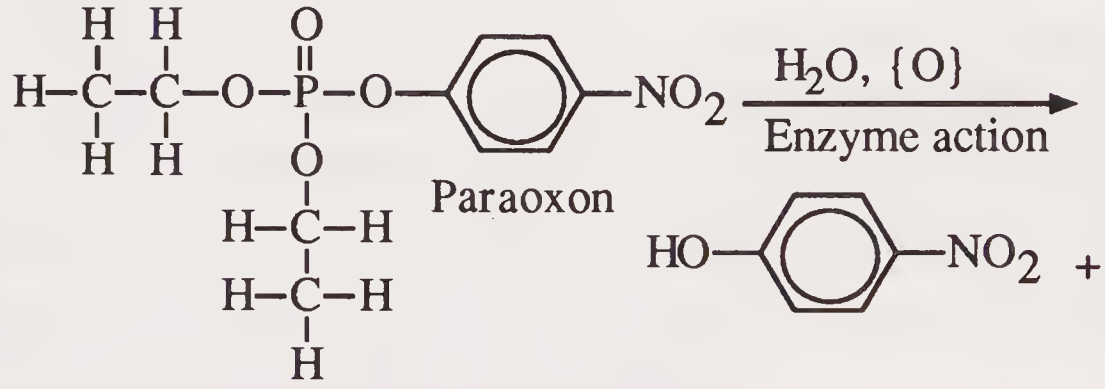
6

Biodegradation and Bioaccumulation

6.1. INTRODUCTION

The water and soil environments receive a variety of xenobiotic compounds that are foreign to living systems. Microorganisms in water and soil act upon these compounds by using them directly as substrates for energy and biomass production, through cometabolism along with primary metabolic processes, by conjugation, or by bioaccumulation. Of these processes, biodegradation, the metabolic breakdown of substances by microorganisms, is the most important.

Detoxication refers to the biological conversion of a toxic substance to a less toxic species, which may still be a relatively complex, or even more complex material. An example of detoxication is illustrated below for the enzymatic conversion of paraoxon (a highly toxic organophosphate insecticide) to *p*-nitrophenol, which has only about 1/200 the toxicity of the parent compound:



(6.1.1)

The uptake and concentration of environmental chemicals by living systems is covered under the heading of **bioaccumulation**.[1] In a general sense the term refers to substances dissolved and suspended in water or contained in sediments, soil, food, or drinking water that are taken into an organism by diffusion from aqueous solution and by ingestion. The term applies especially to aquatic organisms, particularly fish. It may be extended to whole series of organisms in food chains. Uptake of environmental chemicals through food chains can result in much higher levels of the chemicals in organisms than would be expected from simple bioaccumulation, thereby resulting in **biomagnification**. Biomagnification can occur, for example, in a succession of organisms starting with herbivores (which live on plant material), and progressing through detritovores (which feed on residues from the herbivores) and terminating with carnivores.[2]

Of all the biologically-mediated processes that may operate on environmental xenobiotic species, the uptake by organisms and related phenomena, such as biomagnification, usually changes the substance least. Therefore, biological uptake without any metabolic alteration of xenobiotic substances is addressed in this chapter first.

The process opposite to that in which organisms take up substances from water can be observed as a lowered concentration of xenobiotic in tissue when the organism is placed in an uncontaminated environment. This loss of substance back to the surroundings is called **depuration**. Depuration may occur through passive mechanisms of diffusion or desorption. It may also occur by active excretion or egestion on the part of the organism. Biotransformation that changes the substance to a different form may also occur.

The length to time corresponding to a 50 percent probability that a molecule of a substance will be eliminated from an organism is the **half-time** or **half-life** of the substance. If an organism is placed in uncontaminated water, such as a fish placed in clean water, the half-time is measured as the period required for half of the substance to be eliminated from the organism, or for the tissue concentration to reach half its initial value.

6.2. BIOCONCENTRATION

The tendency of a chemical to leave aqueous solution and enter a food chain is important in determining its environmental effects and is expressed through the concept of bioconcentration. **Bioconcentration** (Figure 6.1) may be viewed as a spec-

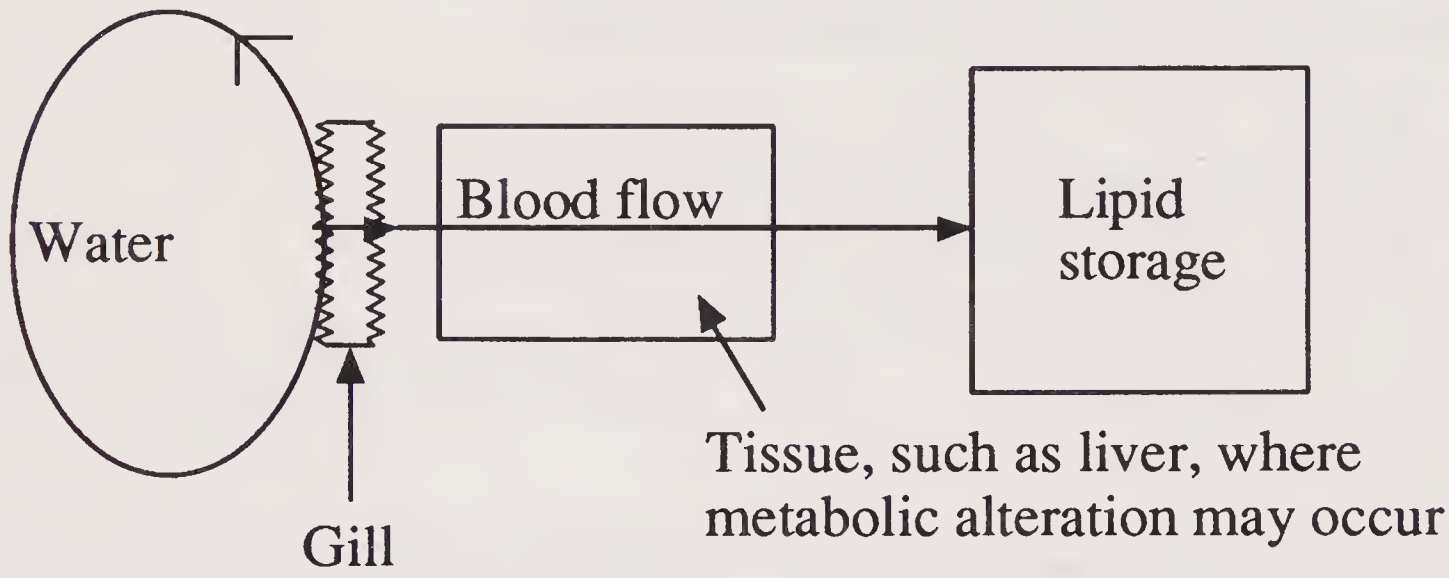


Figure 6.1. Overall pathway of bioconcentration.

ial case of bioaccumulation in which a *dissolved substance* is selectively taken up from water solution and concentrated in tissue by nondietary routes.[3] Bioconcentration applies especially to the concentration of materials from water into fish. This is because fish obviously live in water; they are an important source of human food and are, therefore, potential sources of toxicants; and they are readily used as test organisms. As illustrated in Figure 6.1, the model of bioconcentration is based upon a process by which contaminants in water traverse fish gill epithelium and are transported by the blood through highly vascularized tissues to lipid tissue, which serves as a storage sink for hydrophobic substances. Transport through the blood is affected by several factors including rate of blood flow and degree and strength of binding to blood plasma protein. Prior to reaching the lipid tissue sink, some of the compound may be metabolized to different forms. The concept of bioconcentration is most applicable under the following conditions:

- The substance is taken up and eliminated *via* passive transport processes
- The substance is metabolized slowly
- The substance has a relatively low water solubility
- The substance has a relatively high lipid solubility

Substances that undergo bioconcentration are hydrophobic and tend to undergo transfer from water media to fish lipid tissue. The simplest model of bioconcentration views the phenomenon on the basis of the physical properties of the contaminant and does not account for physiologic variables (such as variable blood flow) or metabolism of the substance. Such a simple model forms the basis of the **hydrophobicity model** of bioconcentration[4] in which bioconcentration is regarded from the viewpoint of a dynamic equilibrium between the substance dissolved in aqueous solution and the same substance dissolved in lipid tissue.

Variables in Bioconcentration

There are several important variables in estimating bioconcentration. These are discussed briefly here.

A basic requirement for uptake of a chemical species from water is **bioavailability**. Normally uptake is viewed in terms of absorption from true water solution. Biouptake may be severely curtailed for substances with extremely low water solubilities or that are bound to particulate matter. Dissolved organic matter may also bind to substances and limit their biouptake.

Although the simple bioconcentration model assumes relatively unhindered movement of a contaminant across the barriers between water and lipid tissue, such is often not the case. The uptake of an organic species can be a relatively complex process in which the chemical must traverse membranes in the gills and skin to reach a final lipid sink. A **physiological component** of the process by which a chemical species moves across membranes tends to cause bioconcentration to deviate from predictions based on hydrophobicity alone.

Some evidence suggests that the **lipid content** of the subject organism affects bioconcentration. Higher lipid contents in an organism may to a degree be associated with relatively higher BCF values. The lipid levels at the site of entry (gill in fish) may be relatively more important than those in the whole organism.

Molecular shape and size seem to play a role in bioconcentration. There are steric hindrances to the movement of large molecules across membranes compared to molecules of about the same mass but having smaller cross-sectional areas. For larger molecules this results in slower transfer and a lower BCF.

Although a simple bioconcentration model assumes rapid movement of a hydrophobic contaminant through an organism, **distribution** may be relatively slow. The predominant limiting factor in this case is the blood flow. Slow transport to lipid tissue sinks can result in lower apparent BCF values than would be the case if true equilibrium were attained.

Biotransfer From Sediments

Because of the strong attraction of hydrophobic species for insoluble materials such as humic matter, many organic pollutants in the aquatic environment are held by sediments in bodies of water.[5] Bioaccumulation of these materials must, therefore, consider transfer from sediment to water to organism as illustrated in Figure 6.2.

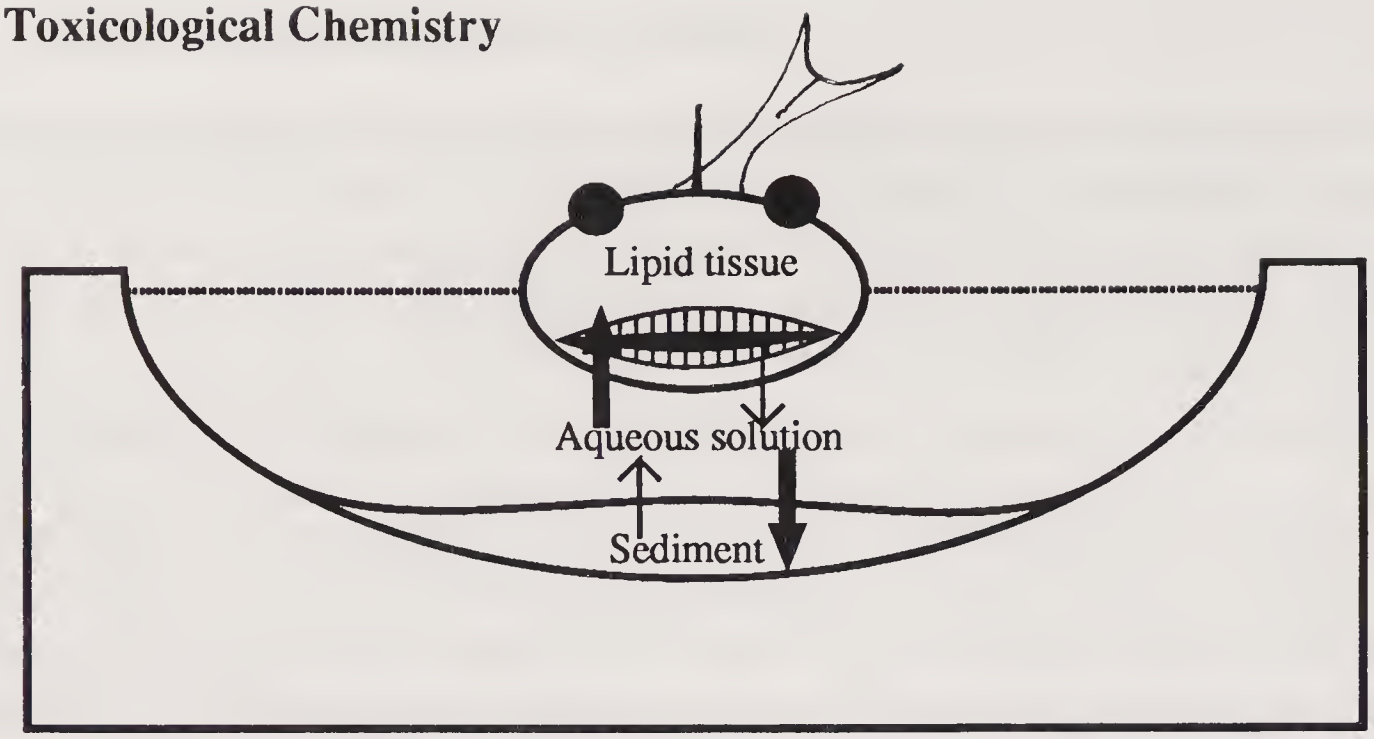


Figure 6.2. Partitioning of a hydrophobic chemical species among sediment, water, and lipid tissue. Heavier arrows denote the preference of the chemical for sediment and lipid tissue compared to aqueous solution.

6.3. BIOCONCENTRATION AND BIOTRANSFER FACTORS

Bioconcentration Factor

Quantitatively, the *hydrophobicity model* of bioconcentration is viewed in the classical thermodynamic sense as an equilibrium between the uptake and elimination of substance "X:"

$$X(aq) \leftrightarrow X(lipid) \qquad (6.3.1)$$

Using k_u as the rate constant for uptake and k_e as the rate constant for elimination leads to the following definition of **bioconcentration factor, BCF**:

$$BCF = \frac{k_u}{k_e} = \frac{[X(lipid)]}{[X(aq)]} \qquad (6.3.2)$$

When $[X(lipid)]/[X(aq)]$ = BCF, the rates of uptake and elimination are equal, the concentration of the xenobiotic substances remains constant (at constant $[X(aq)]$), and the system is in a condition of **dynamic equilibrium** or **steady state**. Some typical values of BCF are given in Table 6.1.

Table 6.1. Some Example Bioconcentration Factors[1]

Chemical	Fish Species	Temperature, °C	BCF
PCB	Sunfish	5	6.0×10^3
PCB	Sunfish	15	5.0×10^4
PCB	Trout	5	7.4×10^3
PCB	Trout	15	1.0×10^4
Hexachlorobenzene	Rainbow trout	15	5.5×10^3
Hexachlorobenzene	Fathead minnow	15	1.6×10^4

[1] From values cited in Barron, Mace G., "Bioconcentration," *Environmental Science and Technology*, **24**, 1612-1618 (1990).

Evidence for the validity of the hydrophobicity model of bioconcentration is provided by correlations of it with the **octanol–water partition coefficient**, K_{ow} using *n*-octanol as a surrogate for fish lipid tissue. The measurement of K_{ow} consists of determining the concentration of a hydrophobic contaminant in water-immiscible *n*-octanol relative to water with which it is in equilibrium. Typical K_{ow} values range from 10 to 10^7 corresponding to BCF values of 1 to 10^6. Such K_{ow}/BCF correlations have proven to be reasonably accurate when narrowly defined for a specified class of compounds, most commonly poorly metabolized organohalides. Major inconsistencies appear when attempts are made to extrapolate from one class of contaminants to another.

Biotransfer Factor

A useful measure of bioaccumulation from food and drinking water by land animals is the **biotransfer factor**, BTF, defined as,

$$\text{BTF} = \frac{\text{Concentration in tissue}}{\text{Daily intake}} \qquad (6.3.3)$$

where the concentration in tissue is usually expressed in mg/kg and daily intake in mg/d. This expression can be modified to express other parameters, such as concentration in milk. As is the case for bioconcentration factors for fish in water, BTF shows a positive correlation with K_{ow} values.[6] Representative BTF values for biotransfer to beef are given in Table 6.2.

Table 6.2 Some Example Biotransfer Factors for Beef and milk.[1]

		Biotransfer factor	
Chemical	Log K_{ow}	Beef	Milk
Arochlor 1254 (PCB)	6.47	-1.28	-1.95
Chordane	6.00	-2.13	-3.43
DDT	5.76	-1.55	-2.62
Endrin	5.16	-1.92	-2.76
Lindane	3.66	-1.78	-2.60
2,4,5-T	3.36	-4.82	-2.60
TCDD	6.15	-1.26	-1.99
Toxaphene	5.50	-2.79	-3.20

[1] For a more complete listing see, Travis, Curtis C., and Angela D. Arms, "Bioconcentration of Organics in Beef, Milk, and Vegetation," *Environmental Science and Technology*, **22**, 271-274 (1988).

Bioconcentration by Vegetation

Like fish and mammals, vegetation can absorb organic contaminants. In the case of vegetation the bioconcentration factor can be expressed relative to the mass of compound per unit mass of soil. The exact expression for vegetation is,

$$\text{BCF} = \frac{\text{Concentration in plant tissue}}{\text{Concentration in soil}} \qquad (6.3.4)$$

where the concentration in plant tissue is given in units of mg/kg dry plant tissue and the concentration in soil is in units of mg/kg dry soil. Table 6.3 gives some typical

values of BCF for plants relative to log K_{ow}. It is seen that for uptake of hydrophobic substances by plants BCF values are less than 1 and tend to decrease with increasing K_{ow}, the opposite of the trend observed in animals. This is explained by the transport of organic substances by water from soil to plant tissue, which increases with increasing water solubility of the compound and, therefore, with decreasing K_{ow}.

Table 6.3. Some Example Bioconcentration Factors[1]

Chemical	Log K_{ow}	log BCF
Arochlor 1254 (PCB)	6.47	-1.77
3,4-Dichloroaniline	2.69	-0.30
Diflubenzuron	3.82	-0.53
Heptachlor	5.44	-1.48
Lindane	3.66	-0.41
TCDD	6.15	-1.87

[1] For a more complete listing see, Travis, Curtis C., and Angela D. Arms, "Bioconcentration of Organics in Beef, Milk, and Vegetation," *Environmental Science and Technology*, **22**, 271-274 (1988).

6.4. BIODEGRADATION

Biodegradation may involve relatively small changes in the parent molecule, such as substitution or modification of a functional group.[7] In the most favorable cases, however, the compound is completely destroyed and the end result is conversion of relatively complex organic compounds to CO_2, H_2O, and inorganic salts, a process called **mineralization**. Usually the products of biodegradation are molecular forms that tend to occur in nature and that are in greater thermodynamic equilibrium with their surroundings.

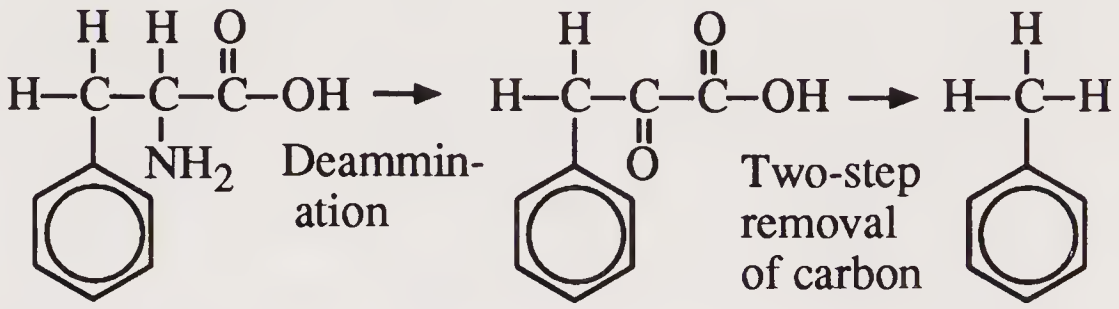
Biochemical Aspects of Biodegradation

Several terms should be reviewed in considering the biochemical aspects of biodegradation. *Biotransformation* is what happens to any substance that is *metabolized* by the biochemical processes in an organism and is altered by these processes. *Metabolism* is divided into the two general categories of *catabolism*, which is the breaking down of more complex molecules, and *anabolism*, which is the building up of life molecules from simpler materials.The substances subjected to biotransformation may be naturally occurring or *anthropogenic* (made by human activities). They may consist of *xenobiotic* molecules that are foreign to living systems.

It should be emphasized that biodegradation of an organic compound occurs in a stepwise fashion and is usually not the result of the activity of a single specific organism. Usually several strains of microorganisms, often existing synergistically, are involved. These may utilize different metabolic pathways and a variety of enzyme systems.

Although biodegradation is normally regarded as degradation to simple inorganic species such as carbon dioxide, water, sulfates, and phosphates, the possibility must always be considered of forming more complex or more hazardous chemical species. An example of the latter is the production of volatile, soluble, toxic methylated forms of arsenic and mercury from inorganic species of these elements by bacteria under

anaerobic conditions. There is some evidence to suggest that toluene, an "anthropogenic compound," can be produced metabolically from the amino acid phenyalanine by anaerobic bacteria. This would occur by successive deammination and decarboxylation as follows:



Phenylalanine Toluene

It is well known that microbial communities exposed to xenobiotic compounds develop the ability to break these compounds down metabolically. This has become particularly obvious from studies of biocidal compounds in the environment.[8] In general, such compounds are readily degraded by bacteria that have been exposed to the compounds for prolonged periods, but not by bacteria from unexposed sites. The development of microbial cultures with the ability to degrade materials to which they are exposed is described as **metabolic adaptation**. In rapidly multiplying microbial cultures metabolic adaptation can include genetic changes that favor microorganisms that can degrade a specific kind of pollutant. Metabolic adaptation may also include increased numbers of microorganisms capable of degrading the substrate in question and enzyme induction.

Cometabolism

Xenobiotic compounds are usually attacked by enzymes whose prime function is to react with other compounds, a process that provides neither carbon nor energy called **cometabolism**. Cometabolism usually involves relatively small modifications of the substance that is cometabolized (the secondary substrate) relative to the primary substrate. The enzymes that carry out cometabolism tend to be relatively non-specific. As an environmentally significant example of cometabolism, at least one strain of bacteria degrades trichloroethylene with an enzyme system that acts predominantly on phenol. The enzyme activity can be induced by exposure to phenol, after which it acts on trichloroethylene.

In pure cultures of microorganisms, the products of cometabolism tend to accumulate and often do not undergo further degradation. However, in mixed cultures, which are the norm for environmental systems, they may serve as substrates for other organisms so that complete biodegradation results. Therefore, studies of biodegradation in pure cultures are usually of limited utility in predicting what happens in the environment.

An example of cometabolism of pollutants is provided by the white rot fungus, *Phanerochaete chrysosporium*, which degrades a number of kinds of organochlorine compounds, including DDT, PCBs, and chlorodioxins, under the appropriate conditions. The enzyme system responsible for this degradation is one that the fungus uses to break down lignin in plant material under normal conditions.

General Factors in Biodegradation

The rates and efficacy of biodegradation of organic substances depend upon several obvious factors. These include the concentration of the substrate compound, nature and concentration of the final electron acceptor (most commonly O_2) presence of phosphorus and nitrogen nutrients, availability of trace element nutrients, the presence of a suitable organism, absence of toxic substances, and the presence of appropriate physical conditions (temperature, growth matrix). In addition to their biochemical properties, the physical properties of compounds, including volatility, water-solubility, organophilicity, tendency to be sorbed by solids, and charge play a role in determining the biodegradability of organic compounds.

To a large extent, xenobiotic compounds in the aquatic environment are bound with sediments and suspended solid materials, such as humic acids. This binding plays a large role in biodegradation. Indeed, the structure of the bound form of the xenobiotic, such as a humic acid complex with a synthetic organic compound, may largely determine its rate of enzymatic degradation.

Low concentrations of surfactants may affect rates of biodegradation. A study of the effects of surfactants on biodegradation[9] showed enhanced biodegradation of phenanthrene sorbed to organic-rich soils, even though the surfactant did not seem to promote desorption of either the biphenyl or phenanthrene studied.

Some studies suggest that biodegradation rates of substances at relatively higher concentrations are not extrapolatable to very low concentrations (see Figure 6.3). That could explain the persistence of very low residual levels of some biodegradable substances in water or soil.[10]

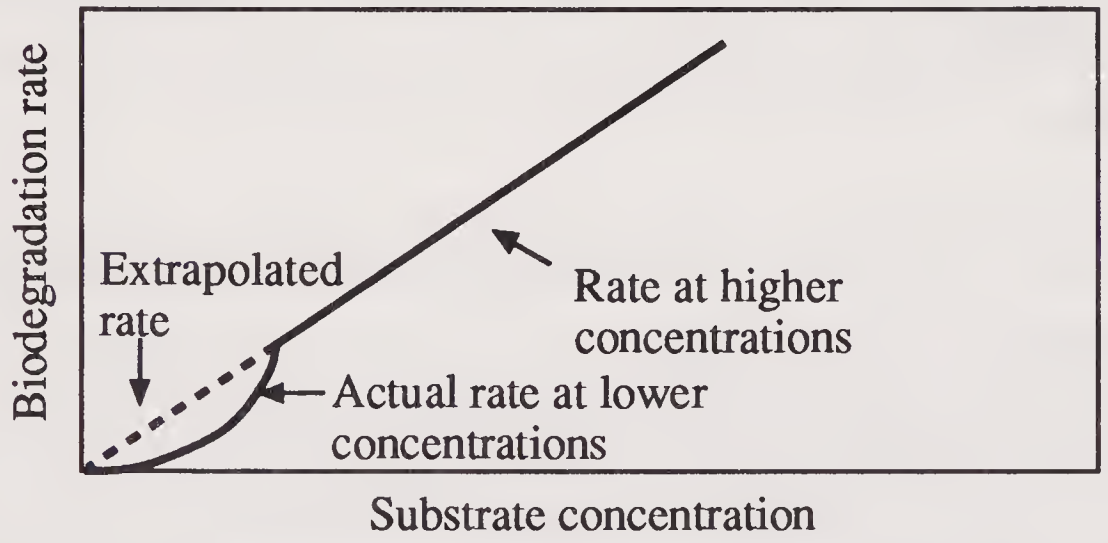


Figure 6.3. Biodegradation *vs*. substrate concentration showing that rates may not extrapolate well to lower concentrations.

An example of the effects of the presence of toxic materials is provided by the biodegradation of polycyclic aromatic hydrocarbons (PAH compounds).[11] PAH compounds from fuel and petroleum sludges spilled on soil undergo biodegradation with relative ease, whereas PAHs from creosote contamination are poorly biodegradable. This observation may be explained by the bactericidal properties of creosote components that inhibit the growth of organisms responsible for degrading PAH compounds.

Competition from other organisms may be a factor in biodegradation of pollutants. "Grazing" by protozoa may result in consumption of bacterial cells responsible for the biodegradation of particular compounds.

Trace amounts of micronutrients are needed to support biological processes and as constituents of enzymes. Important micronutrients are calcium, magnesium, potassium, sodium, chlorine, cobalt, iron, vanadium, and zinc. Sometimes sulfur, phosphorus, and micronutrients must be added to media in which microorganisms are used to degrade hazardous wastes in order for optimum growth to occur.

Biodegradability

The amenability of a compound to chemical attack by microorganisms is expressed as its **biodegradability**. The biodegradability of a compound is influenced by its physical characteristics, such as solubility in water and vapor pressure, and by its chemical properties, including molecular mass, molecular structure, and presence of various kinds of functional groups, some of which provide a "biochemical handle" for the initiation of biodegradation. With the appropriate organisms and under the right conditions, even substances that are biocidal to most microorganisms can undergo biodegradation. For example, normally bactericidal phenol is readily metabolized by the appropriate bacteria acclimated to its use as a carbon and energy source.

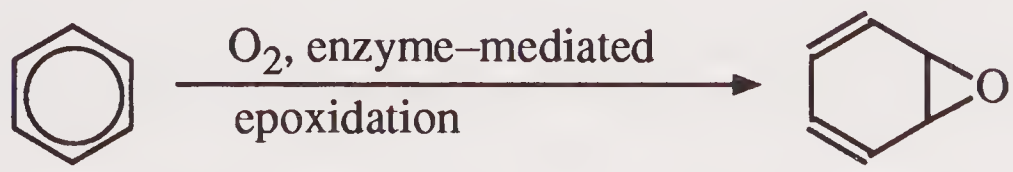
Recalcitrant or **biorefractory** substances are those that resist biodegradation and tend to persist and accumulate in the environment. Such materials are not necessarily toxic to organisms, but simply resist their metabolic attack. Even some compounds regarded as biorefractory may be degraded by microorganisms adapted to their biodegradation. Examples of such compounds and the types of microorganisms that can degrade them include endrin (*Arthrobacter*), DDT (*Hydrogenomonas*), phenylmercuric acetate (*Pseudomonas*), and raw rubber (*Actinomycetes*).

6.5. ENZYMATIC PROCESSES IN BIODEGRADATION

It should be noted that by no means all reactions involved in the breakdown of chemical species in the water and soil environments are biologically mediated enzyme-catalyzed reactions. Nonenzymatic reactions including hydrolysis, oxidation-reduction, surface-catalyzed, photolytic, and ion-exchange reactions are often significant. Overall, however, enzymatic biodegradation of organic matter by microorganisms in the aquatic and terrestrial environments is a crucial environmental process. It occurs by way of a number of stepwise, microbially catalyzed reactions. These reactions and examples of them are discussed individually in this section.

Oxidation

Oxidation occurs by the action of oxygenase enzymes. The microbially catalyzed conversion of aldrin to dieldrin is an example of epoxide formation, a major step in many oxidation mechanisms. **Epoxidation** consists of adding an oxygen atom between two C atoms in an unsaturated system as shown below:



(6.5.1)

Epoxidation is a particularly important means of metabolic attack upon aromatic rings that abound in many xenobiotic compounds.

Microbial Oxidation of Hydrocarbons

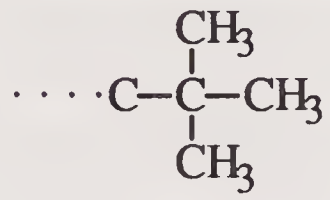
The degradation of hydrocarbons by microbial oxidation is an important environmental process because it is the primary means by which petroleum wastes are eliminated from water and soil. Bacteria capable of degrading hydrocarbons include *Micrococcus*, *Pseudomonas*, *Mycobacterium*, and *Nocardia*.

The most common initial step in the microbial oxidation of alkanes involves conversion of a terminal $-CH_3$ group to a $-CO_2$ group. More rarely, the initial enzymatic attack involves the addition of an oxygen atom to a nonterminal carbon, forming a ketone. After formation of a carboxylic acid from the alkane, further oxidation normally occurs by metabolic processes that involve removal of two-carbon atom fragments in each step. This process, illustrated by the following reaction, is called β–oxidation:

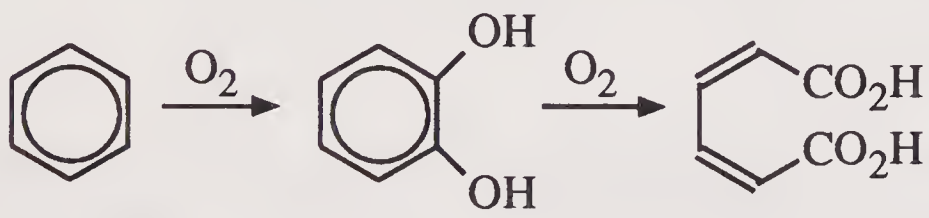
$$CH_3CH_2CH_2CH_2CO_2H + 3O_2 \rightarrow CH_3CH_2CO_2H + 2CO_2 + 2H_2O \qquad (6.5.2)$$

The oxidation of fatty acids involves oxidation of the β–carbon atom, followed by removal of two-carbon fragments. This occurs by way of a complicated cycle with a number of steps involving ATP and coenzyme A. The residue at the end of each cycle is an organic acid with two fewer carbon atoms than its precursor at the beginning of the cycle.

Hydrocarbon degradability varies with compound structure, and microorganisms show a strong preference for straight-chain hydrocarbons. A major reason for this preference is that branching inhibits β–oxidation at the site of the branch. The presence of a quaternary carbon (below) particularly inhibits alkane degradation.



Ring cleavage is a crucial step in the ultimate degradation of hydrocarbon aromatic rings. Despite their chemical stability, aromatic rings are susceptible to microbial oxidation. The overall process leading to ring cleavage is

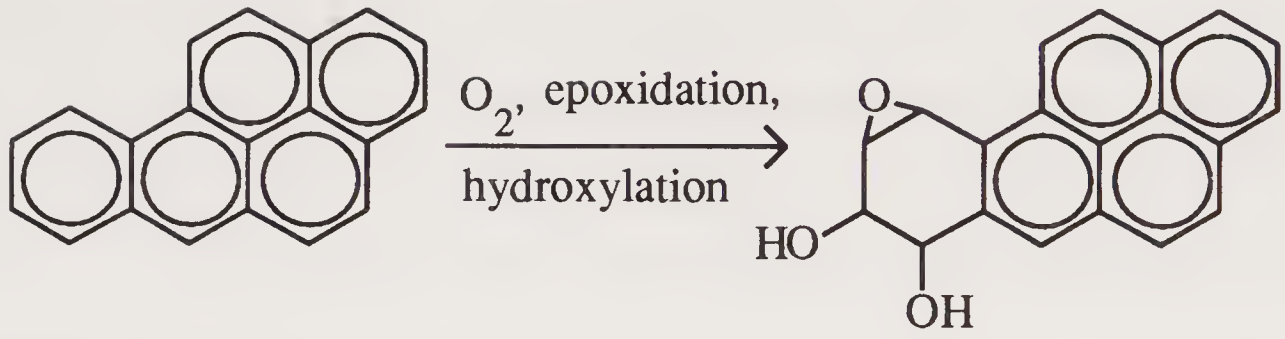


(6.5.3)

in which cleavage is preceded by addition of –OH to adjacent carbon atoms.

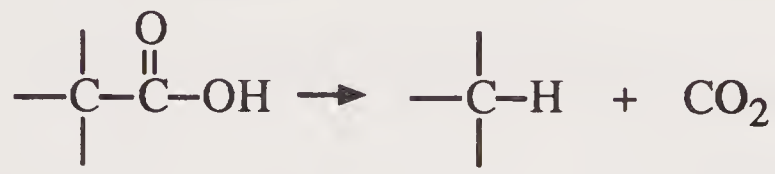
Hydroxylation often accompanies microbial oxidation of hydrocarbon structures. It is the attachment of –OH groups to hydrocarbon chains or rings. It can follow epoxidation as shown by the following rearrangement reaction for benzene epoxide:

Hydroxylation can consist of the addition of more than one epoxide group. An example of epoxidation and hydroxylation is the metabolic production of the 7,8-diol-9,10-epoxide of benzo(a)pyrene (a product that is capable of binding to cellular DNA and causing mutations and cancer) as illustrated below:



Decarboxylation

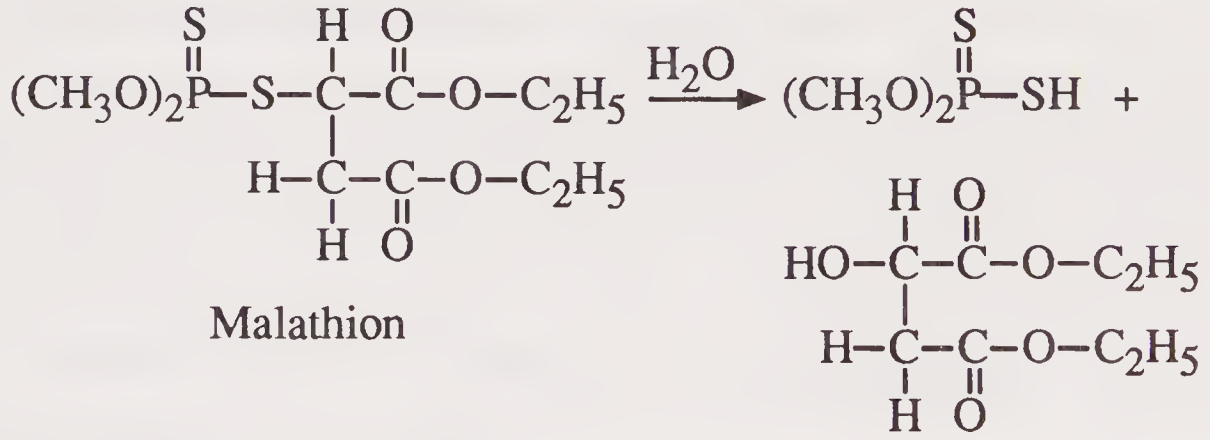
Decarboxylation is the replacement of the $-CO_2H$ with an H atom or –OH group. In the former case the reaction may be represented as follows:



(6.5.4)

Hydrolysis

Hydrolysis, which involves the addition of H_2O to a molecule accompanied by cleavage of the molecule into two species, is a major step in microbial degradation of many hydrolyzable pollutant xenobiotic compounds, especially pesticidal esters, amides, organophosphate esters, and nitriles (compounds with the –C≡N group). The types of enzymes that bring about hydrolysis are **hydrolase enzymes**, those that enable the hydrolysis of esters are called **esterases**, and those that hydrolyze amides are **amidases**. At least one species of *Pseudomonas* hydrolyzes malathion in a type of hydrolysis reaction typical of those by which pesticides are degraded:



Malathion

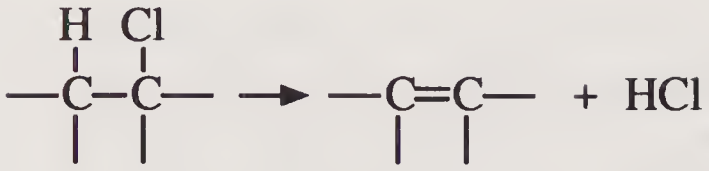
(6.5.5)

Reduction

Reductions are carried out by **reductase enzymes**; for example, nitroreductase enzyme catalyzes the reduction of the nitro group. Table 4.1 in Chapter 4 gives the major kinds of reduction reactions. An oxygen-free environment is reducing, so microbial reductions are carried out by anaerobic bacteria.

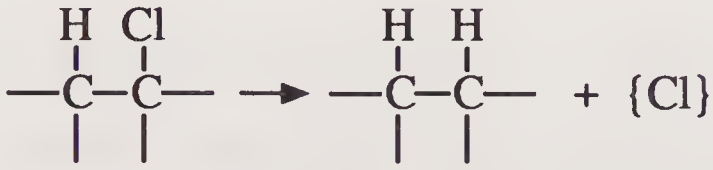
Dehalogenation

Dehalogenation reactions involve the bacterially-mediated removal or replacement of a halogen atom, usually chlorine, on a xenobiotic molecule. Microbially-mediated dehalogenation reactions are less facile than some of the other major biodegradation processes, which explains the persistence of organohalide xenobiotic compounds. The three ways in which dehalogenation may occur are (1) **dehydrohalogenation** in which H and a halogen atom are removed from adjacent carbon atoms,



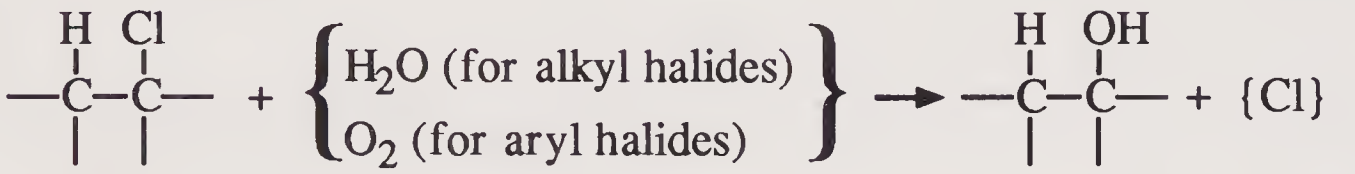
(6.5.6)

(2) **reductive dehalogenation** in which H replaces the halogen atom,



(6.5.7)

and (3) **hydrolytic dehalogenation** in which the halogen atom is replaced by –OH:



(6.5.8)

There appear to be two distinct mechanisms for the dechlorination of aromatic compounds during biodegradation. In one of these the ring is cleaved before the chlorine atom is eliminated and in the other the chlorine atom is first removed.

Demethylation

Many environmentally significant organic compounds contain alkyl groups, such as the methyl ($-CH_3$) group, attached to atoms of O, N, and, less commonly, S and C. An important step in the microbial metabolism of many of these compounds is **dealkylation**, replacement of alkyl groups by H as shown in Figure 6.4. Examples of these kinds of reactions include O-dealkylation of methoxychlor insecticides, N-dealkylation of carbaryl insecticide, and S-dealkylation of dimethyl mercaptan. Alkyl groups removed by dealkylation usually are attached to oxygen, sulfur, or nitrogen atoms; those attached to carbon are normally not removed directly by microbial processes.

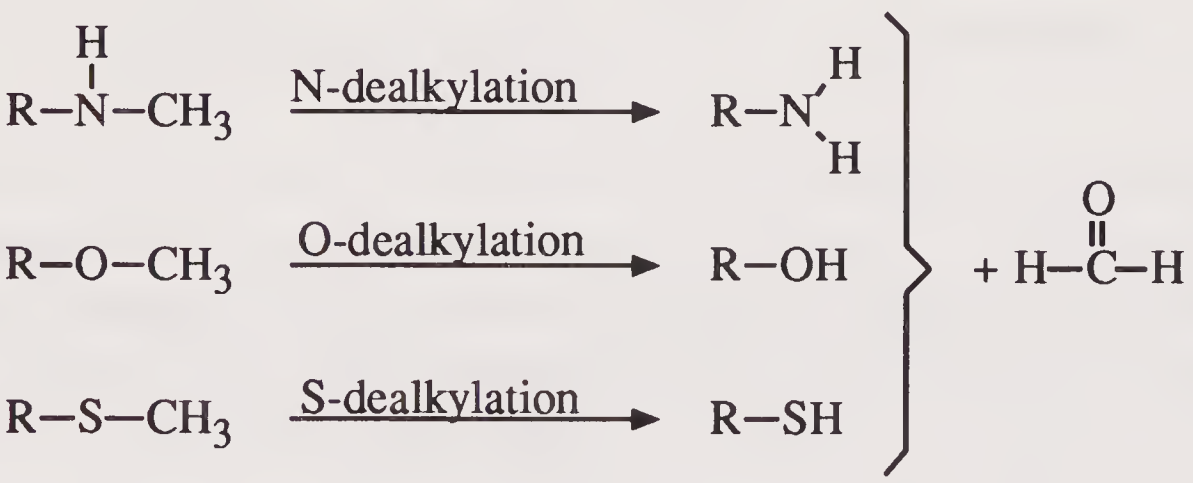


Figure 6.4. Metabolic dealkylation reactions shown for the removal of CH_3 from N, O and S atoms in organic compounds.

Conjugation Reactions

Conjugation reactions involve the attachment of a biochemical group, such as glucuronide, to a xenobiotic substance or its metabolite. As noted in Section 4.6, conjugation processes are usually carried out by higher organisms as a detoxication and elimination mechanism. Conjugation reactions are less common among

microorganisms, but can occur through attachment of groups such as amino acids or organic acids. Perhaps the most common and significant microbial process of conjugation, broadly defined, is **methylation** in which a $-CH_3$ group is attached to a metal or metalloid atom in an inorganic compound. An important example of this is the microbially mediated methylation of arsenic species as shown by the following reactions:

$$H_3AsO_4 + 2H^+ + 2e^- \rightarrow H_3AsO_3 + H_2O \qquad (6.5.9)$$

$$H_3AsO_3 \xrightarrow{\text{Methylcobalamin}} \underset{\text{Methylarsinic acid}}{CH_3AsO(OH)_2} \qquad (6.5.10)$$

$$CH_3AsO(OH)_2 \xrightarrow{\text{Methylcobalamin}} \underset{\text{(Dimethylarsinic acid)}}{(CH_3)_2AsO(OH)} \qquad (6.5.11)$$

$$(CH_3)_2AsO(OH) + 4H^+ + 4e^- \rightarrow (CH_3)_2AsH + 2H_2O \qquad (6.5.12)$$

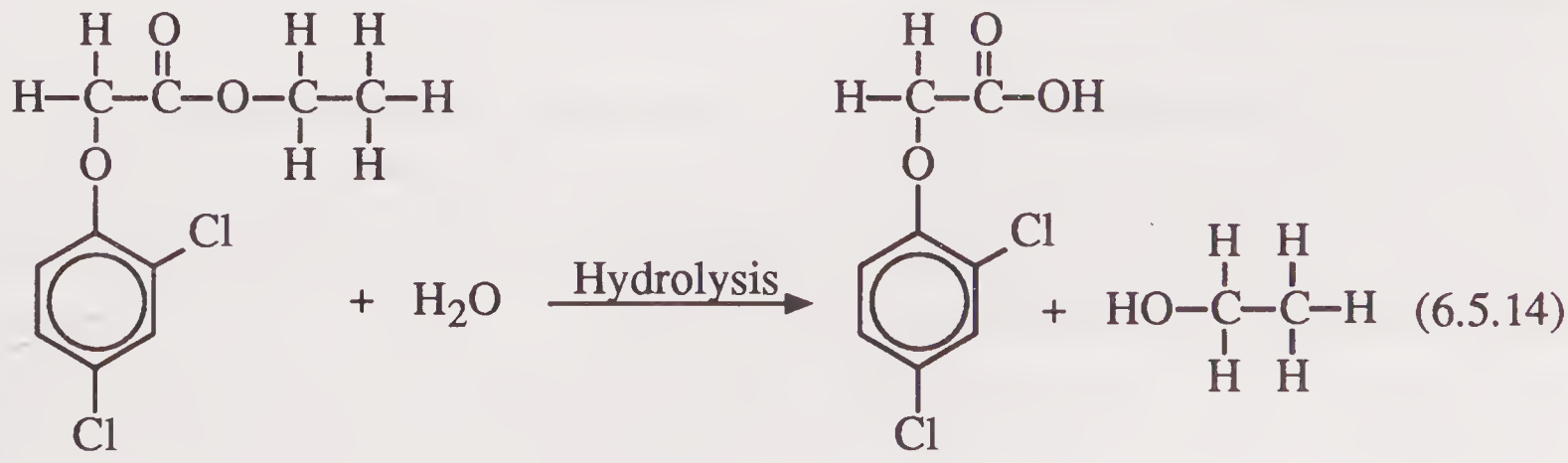
The unexpectedly high concentrations of mercury found in water and in fish tissues result from methylation of inorganic mercury, by anaerobic bacteria in sediments to produce soluble monomethylmercury ion, CH_3Hg^+, and volatile dimethylmercury, $(CH_3)_2Hg$. Mercury from these compounds becomes concentrated in fish lipid (fat) tissue and the bioconcentration factor from water to fish may exceed 10^3. As is the case with arsenic, the methylating agent by which inorganic mercury is converted to methylmercury compounds is methylcobalamin, a vitamin B_{12} analog:

$$HgCl_2 \xrightarrow{\text{Methylcobalamin}} CH_3HgCl + Cl^- \qquad (6.5.13)$$

It is believed that the bacteria that synthesize methane produce methylcobalamin as an intermediate in the synthesis. Thus, waters and sediments in which anaerobic decay is occurring provide the conditions under which methylmercury production occurs. In neutral or alkaline waters, the formation of dimethyl mercury, $(CH_3)_2Hg$, is favored. This volatile compound can escape to the atmosphere.

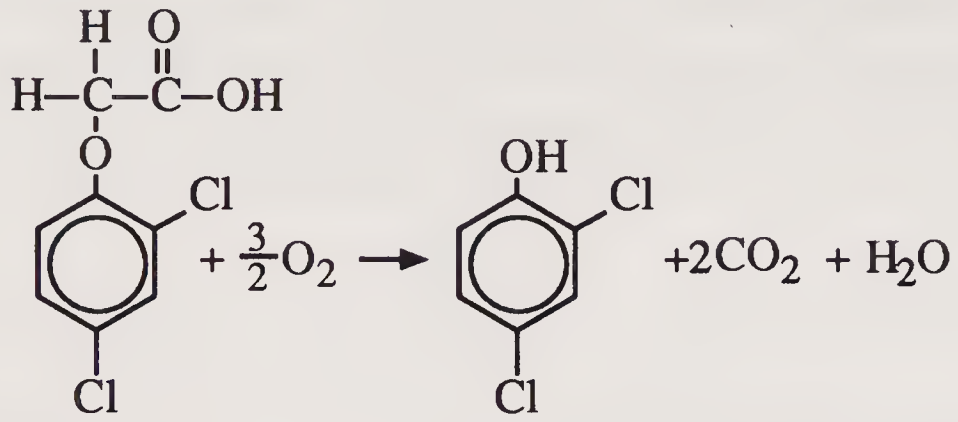
Biodegradation of 2,4-D

Herbicidal 2,4-D provides an informative example of ways in which several of the biodegradation processes discussed above are involved in the breakdown of an environmental pollutant. A 2,4-D ester undergoes hydrolysis:



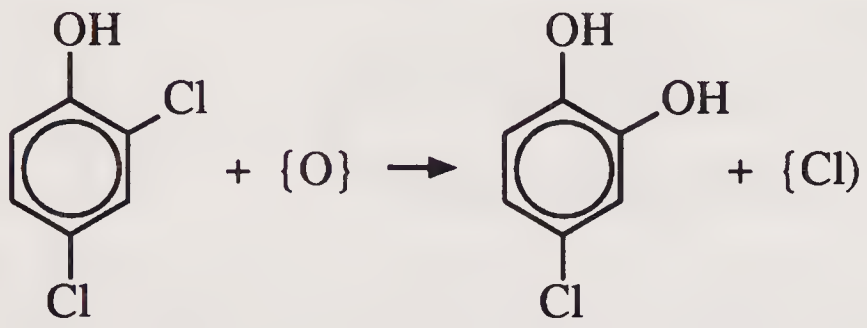
(6.5.14)

The aliphatic acid portion of the residue may be oxidized:



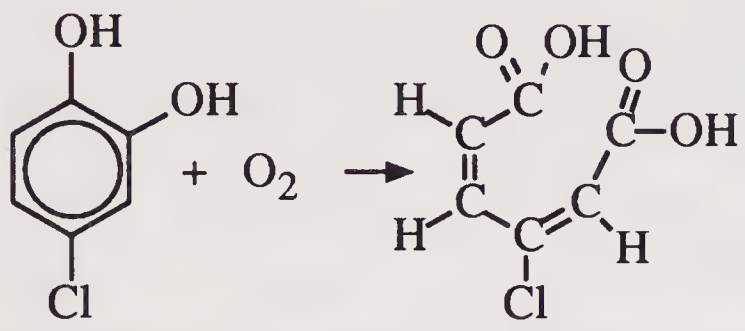
(6.5.15)

Chlorine may be removed by hydrolytic dehalogenation:



(6.5.16)

And the ring may be cleaved:



(6.5.17)

6.6. BIODEGRADATION IN MUNICIPAL AND NONCHEMICAL WASTE TREATMENT

The most common deliberate application of biodegradation is for destruction of "nonchemical" sewage, food, and agricultural wastes. Treatment processes for these wastes make use of **bioreactors** that enable contact of the wastes with a high concentration of microorganisms in a relatively compact container. Examples of bioreactors used to treat municipal sewage are discussed here; applications of these devices are readily extended to food processing, agricultural, and even some types of chemical wastes.

Biodegradation of Wastewater Constituents

Secondary wastewater treatment as it is practiced uses biodegradation in bioreactors to remove **biochemical oxygen demand** (BOD) that would otherwise consume oxygen in water receiving the wastewater. Representing biodegradable matter in wastewater as $\{CH_2O\}$, oxygen is consumed by microbially mediated aerobic respiration reactions (see also Section 7.11):

$$\{CH_2O\} + O_2 \rightarrow CO_2 + H_2O \qquad (6.6.1)$$

In addition to the products shown above, this process also yields biomass and energy. If the wastes were discharged without treatment, the same oxygen-consuming process would occur in a "natural water" lake, stream, or groundwater aquifer. However, when this occurs, the biodegradation of the wastes in receiving waters consumes oxygen needed for fish life and overall water quality.

Secondary wastewater treatment by biological processes takes advantage of the tendency for oxygen to be consumed by biodegradation of organic wastes to dispose of these wastes before they get into the aquatic environment. Microorganisms provided with added oxygen are allowed to degrade organic material in solution or in suspension until the BOD of the waste has been reduced to acceptable levels. The waste is oxidized biologically under conditions controlled for optimum bacterial growth and at a site where this growth does not influence the environment.

One of the simplest biological waste treatment processes is the **trickling filter** (Figure 6.5) in which wastewater is sprayed over rocks or other solid support material covered with microorganisms. The structure of the trickling filter is such that contact of the wastewater with air is allowed and degradation of organic matter occurs by the action of the microorganisms.

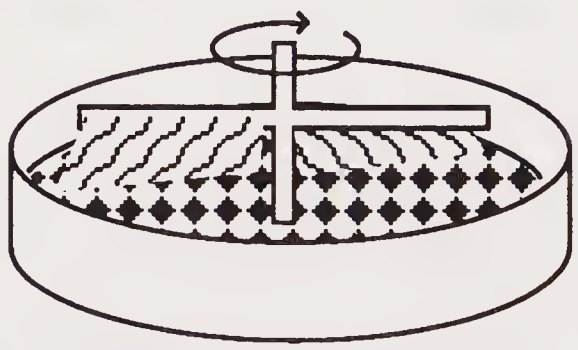


Figure 6.5. Trickling filter for secondary waste treatment.

Rotating biological reactors, another type of treatment system, consist of groups of large plastic discs mounted close together on a rotating shaft. The device is positioned such that at any particular instant half of each disc is immersed in wastewater and half exposed to air. The shaft rotates constantly, so that the submerged portion of the discs is always changing. The discs, usually made of high-density polyethylene or polystyrene, accumulate thin layers of attached biomass, which degrades organic matter in the sewage. Oxygen is absorbed by the biomass and by the layer of wastewater adhering to it during the time that the biomass is exposed to air.

Both trickling filters and rotating biological reactors are examples of **fixed-film biological** (FFB) processes. The greatest advantage of these processes is their low energy consumption. The energy consumption is minimal because it is not necessary to pump air or oxygen into the water, as is the case with the popular activated sludge process described below.

The **activated sludge process**, Figure 6.6, is probably the most versatile and effective of all biological waste treatment processes. Microorganisms in the aeration tank convert organic material in wastewater to microbial biomass and CO_2. Organic nitrogen is converted to ammonium ion or nitrate. Organic phosphorus is converted to orthophosphate. The microbial cell matter formed as part of the waste degradation processes is normally kept in the aeration tank until the microorganisms are past the log phase of growth (Chapter 5, Figure 5.4), at which point the cells flocculate relatively well to form settleable solids. These solids accumulate in the bottom of a settler and a fraction of them is discarded. Part of the solids, the return sludge, is

recycled to the head of the aeration tank and comes into contact with fresh sewage. The combination of a high concentration of "hungry" cells in the return sludge and a rich food source in the influent sewage provides optimum conditions for the rapid degradation of organic matter.

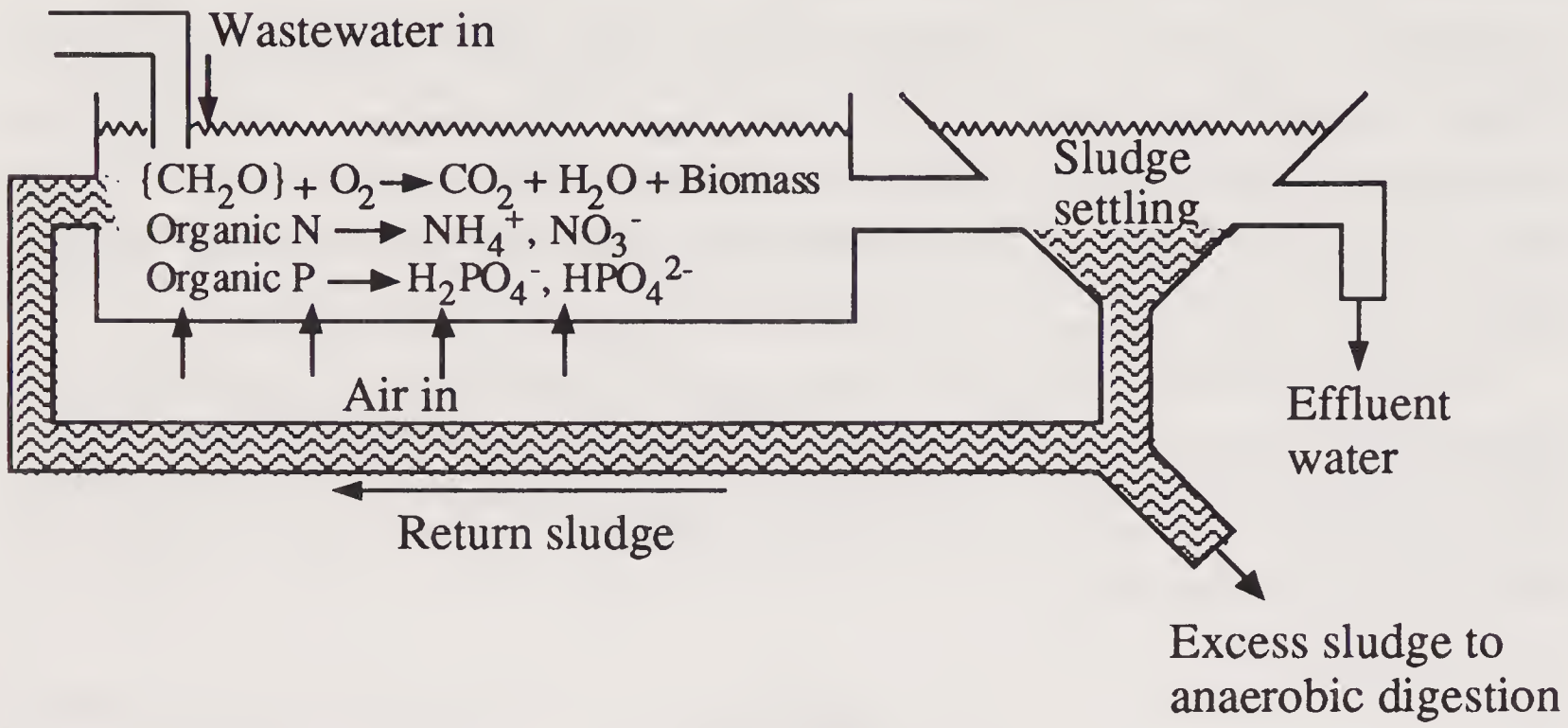


Figure 6.6. Activated sludge process.

The degradation of organic matter that occurs in an activated sludge facility also occurs in streams and other aquatic environments. However, in general, when a degradable waste is put into a stream, it encounters only a relatively small population of microorganisms capable of carrying out the degradation process. Thus, several days may be required for the build-up of a sufficient population of organisms to degrade the waste. In the activated sludge process, continual recycling of active organisms provides the optimum conditions for waste degradation, and a waste may be degraded within the very few hours that it is present in the aeration tank.

The activated sludge process provides two pathways for the removal of BOD, as illustrated schematically in Figure 6.7. BOD may be removed by (1) oxidation of organic matter to provide energy for the metabolic processes of the microorganisms, and (2) synthesis, incorporation of the organic matter into cell mass. In the first pathway, carbon is removed in the gaseous form as CO_2. The second pathway provides for

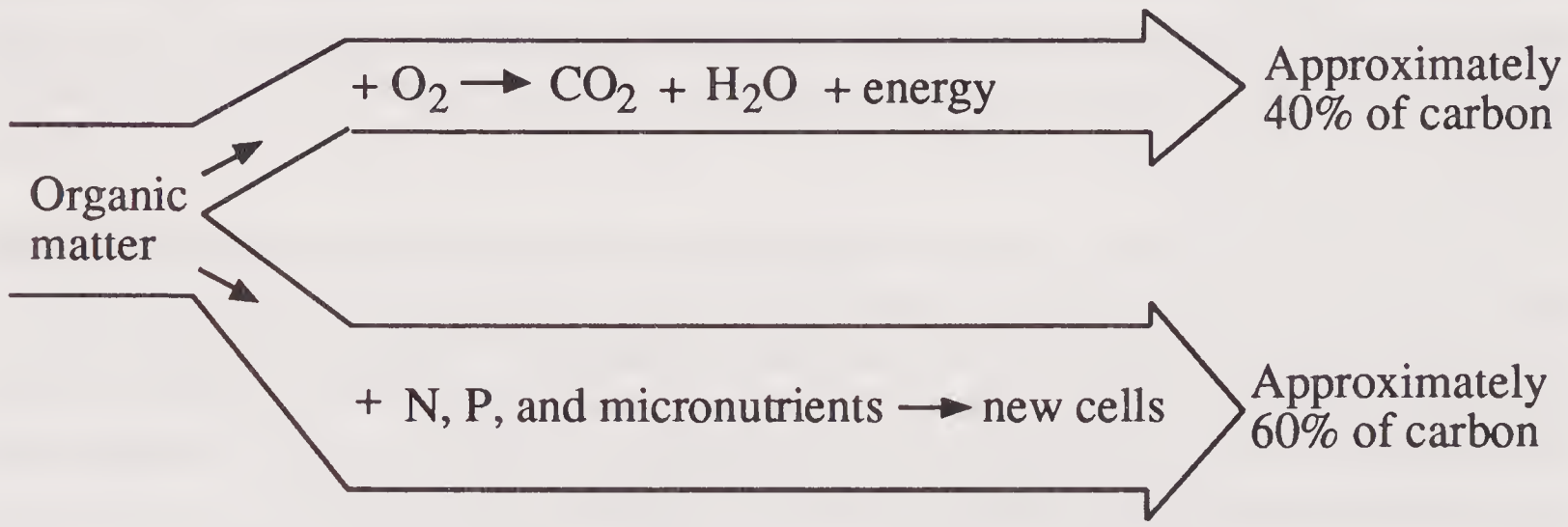


Figure 6.7. Pathways for the removal of BOD in biological wastewater treatment.

removal of carbon as a solid in biomass. That portion of the carbon converted to CO_2 is vented to the atmosphere and does not present a disposal problem. The disposal of waste sludge, however, is a problem, primarily because it is only about 1% solids and contains many undesirable components. Normally, partial water removal is accomplished by drying on sand filters, vacuum filtration, or centrifugation. The dewatered sludge may be incinerated or used as landfill. To a certain extent, sewage sludge may be digested in the absence of oxygen by methane-producing anaerobic bacteria to produce methane and carbon dioxide,

$$2\{CH_2O\} \rightarrow CH_4 + CO_2 \qquad (6.6.2)$$

a process that reduces both the volatile-matter content and the volume of the sludge by about 60%. A carefully designed plant may produce enough methane to provide for all of its power needs.

Nitrification (the microbially mediated conversion of ammonium nitrogen to nitrate; see Section 7.12), is a significant process that occurs during biological waste treatment. Ammonium ion is normally the first inorganic nitrogen species produced in the biodegradation of nitrogenous organic compounds. It is oxidized, under the appropriate conditions, first to nitrite by *Nitrosomonas* bacteria,

$$2NH_4^+ + 3O_2 \rightarrow 4H^+ + 2NO_2^- + 2H_2O \qquad (6.6.3)$$

then to nitrate by *Nitrobacter*:

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad (6.6.4)$$

These reactions occur in the aeration tank of the activated sludge plant and are favored in general by long retention times, low organic loadings, large amounts of suspended solids, and high temperatures. Nitrification can reduce sludge settling efficiency because the denitrification reaction

$$4NO_3^- + 5\{CH_2O\} + 4H^+ \rightarrow 2N_2(g) + 5CO_2(g) + 7H_2O \qquad (6.6.5)$$

occurring in the oxygen-deficient settler causes bubbles to form on the sludge floc (aggregated sludge particles), making it so buoyant that it floats to the top. This prevents settling of the sludge and increases the organic load in the receiving waters.

Under the appropriate conditions, however, advantage can be taken of the nitrification phenomenon to remove nutrient nitrogen from water by following it with denitrification. The first step is an essentially complete conversion of ammonia and organic nitrogen to nitrate under strongly aerobic conditions, achieved by more extensive than normal aeration of the sewage by the reactions discussed above. The second step is the reduction of nitrate to nitrogen gas. This reaction is also bacterially catalyzed and requires a carbon source and a reducing agent such as methanol, CH_3OH, as shown in Reaction 6.6.7. The denitrification process may be carried out either in a tank or on a carbon column.

$$6NO_3^- + 5CH_3OH + 6H^+ \text{ (Denitrifying bacteria)} \rightarrow 3N_2(g) + 5CO_2 + 13H_2O \qquad (6.6.6)$$

6.7. BIODEGRADATION IN HAZARDOUS WASTE TREATMENT

Throughout the 1980s and into the present decade, interest has accelerated in the use of microorganisms for waste treatment. This can be done in several ways, including **land treatment**, where wastes are put on soil for degradation, or by **composting**, where wastes are mixed with porous, aerated material, such as wood shavings. For more sophisticated treatment bioreactors are used. One of the more intriguing possibilities is to stimulate the growth of waste-degrading bacteria onsite and underground, a process that can be called **subsurface biorestoration**.[12]

A number of hazardous waste compounds are susceptible to destruction by biodegradation. Properties of hazardous wastes can be changed to increase biodegradability. This is especially true of wastes that consist of several constituents, one or more of which inhibit biological processes. Sometimes a waste substance that is toxic to microorganisms at a relatively high concentration is degraded well in more dilute media. Biodegradation that is inhibited by extremes of pH in the waste may occur when excess acid or base is neutralized. Toxic organic and inorganic substances, such as heavy metal ions, can be removed in some cases prior to biological treatment. Substances that are precursors to formation of solid precipitates that form during biodegradation and inhibit it can be removed. For example, soluble iron(II) needs to be removed because it oxidizes to form bacteria-inhibiting deposits of gelatinous iron(III) hydroxide during aerobic treatment of wastes:

$$O_2 + 4Fe^{2+}(aq) + 10H_2O \rightarrow 4Fe(OH)_3(s) + 8H^+ \qquad (6.7.1)$$

Biodegradability of Waste Compounds

Practically all classes of synthetic organic compounds can be at least partially degraded by various microorganisms. These classes include nonhalogenated alkanes, halogenated alkanes (trichloroethane, dichloromethane) nonhalogenated aromatic compounds (benzene, naphthalene, benzo(a)pyrene), halogenated aromatic compounds (hexachlorobenzene, pentachlorophenol), phenols (phenol, cresols), polychlorinated biphenyls, phthalate esters , and pesticides (chlordane, parathion).

For the most part, anthropogenic compounds resist biodegradation much more strongly than do naturally occurring compounds. This is generally due to the absence of enzymes that can bring about an initial attack on the compound (phase I reactions). As mentioned earlier in this chapter, a number of physical and chemical characteristics of a compound determine its amenability to biodegradation. Such characteristics include hydrophobicity, solubility, volatility, and affinity for lipids. Some organic structural groups impart particular resistance to biodegradation. These include branched carbon chains, ether linkages, meta-substituted benzene rings, chlorine, amines, methoxy groups, sulfonates, and nitro groups.

Microorganisms in Waste Treatment

Several groups of microorganisms are capable of partial or complete degradation of hazardous organic compounds. Among the aerobic bacteria, those of the *Pseudomonas* family are the most widespread and most adaptable to the degradation of synthetic compounds. These bacteria degrade biphenyl, naphthalene, DDT, and many other compounds. Anaerobic bacteria are very fastidious and difficult to study in the laboratory because they require oxygen-free (anoxic) conditions and pE values of

less than -3.4 in order to survive. These bacteria catabolize biomass through hydrolytic processes, breaking down proteins, lipids, and saccharides. They are also known to reduce nitro compounds to amines, degrade nitrosamines, promote reductive dechlorination, reduce epoxide groups to alkenes, and break down aromatic structures. **Actinomycetes** are microorganisms that are morphologically similar to both bacteria and fungi. They are involved in the degradation of a variety of organic compounds, including degradation-resistant alkanes, and lignocellulose. Other compounds attacked include pyridines, phenols, nonchlorinated aromatics, and chlorinated aromatics. Fungi are particularly noted for their ability to attack long-chain and complex hydrocarbons and are more successful than bacteria in the initial attack on PCB compounds. Phototrophic microorganisms, which include algae, photosynthetic bacteria, and cyanobacteria (see Chapter 5) tend to concentrate organophilic compounds in their lipid stores and induce photochemical degradation of the stored compounds. For example, *Oscillatoria* can initiate the biodegradation of naphthalene by the attachment of –OH groups.

Waste Treatment with Anaerobic Bacteria

In order to degrade wastes microbially, bacteria require nutrients and a *terminal electron acceptor* (see Section 5.5), usually molecular O_2, but including other species, such as SO_4^{2-}, NO_3^-, and even organic matter, itself. To a degree, anaerobic bacteria, which use terminal electron acceptors other than O_2, will perform subsurface biorestoration without human intervention (see the discussion of anaerobic degradation of PCBs in Section 6.8). A possibility that may not have been exploited yet to a significant degree is to add nutrients, but not oxygen, to underground sites to stimulate the growth of anaerobic bacteria. This should both enable anaerobic bacteria to metabolize wastes and, by consuming any residual oxygen, produce conditions especially conducive to their growth. The major steps in anaerobic waste treatment are outlined in Figure 6.8.

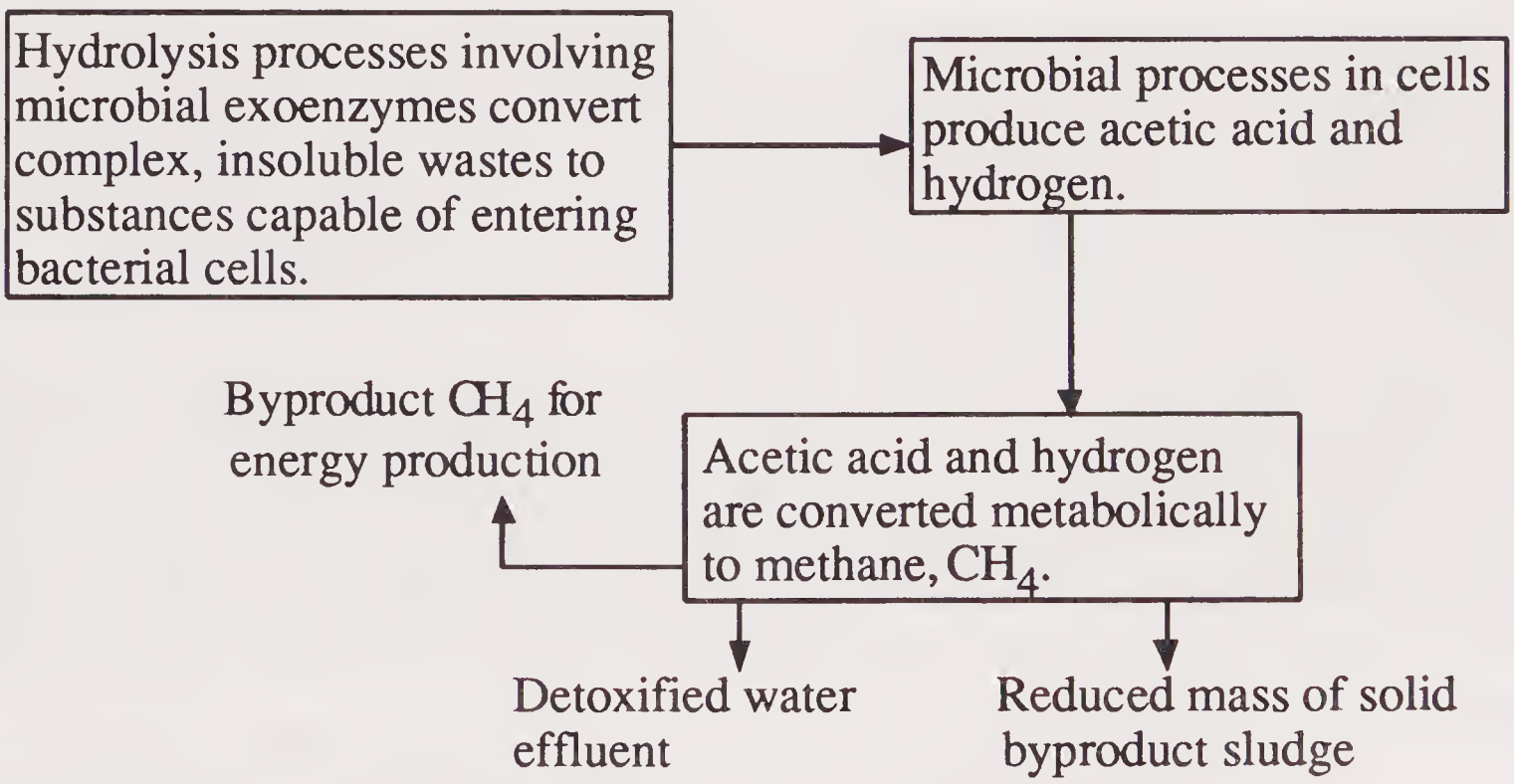


Figure 6.8. Major steps in anaerobic waste treatment.

Although not widely practiced as a means of waste treatment, the anaerobic treatment of industrial wastes offers some important advantages:[13]

- Production of byproduct methane that can be used for fuel
- Relatively low production of sludge (sludge from aerobic waste treatment may be further degraded anaerobically)
- Low nutrient and energy requirements and no requirement for oxygen
- An enclosed system that enables odor control (Not surprisingly, in the U.S. one of the leading states in anaerobic waste treatment research is Iowa, where odors from production, treatment, and disposal of hog feedlot wastes has caused rifts between pork producers and their neighbors.)

One reason that anaerobic treatment has lagged despite the advantages listed above is that it is a rather complex process requiring careful control of pertinent parameters. Probably five main groups of bacteria are involved overall. There are three major steps in anaerobic treatment of industrial wastes as outlined in Figure 6.8.

Waste Treatment with Aerobic Bacteria

For the most part, the use of biodegradation for waste treatment has concentrated on promoting the growth of aerobic bacteria in waste media. This is done by adding a source of oxygen, essential nutrients, and bacteria (which are often present as "indigenous subsurface microflora" in sufficient numbers already). The overall process is, therefore, the following:

$$\text{Wastes} + O_2 + \text{Nutrients} \xrightarrow[\text{bacteria}]{\text{Aerobic}} \text{Biomass} + CO_2 + \text{Inorganic products} \qquad (6.7.2)$$

Oxygen can be added as gaseous O_2, although the solubility of this species in water limits the maximum O_2 concentration to about 40 mg/L. Another alternative is to add hydrogen peroxide solution, which reacts as follows to produce O_2:

$$2H_2O_2 \rightarrow O_2 + 2H_2O \qquad (6.7.3)$$

At levels above approximately 100 mg/L, hydrogen peroxide is toxic to some bacteria. Up to 10 times that concentration can be used, though, by first acclimating the bacteria to the peroxide. Nitrogen and phosphorus are the nutrients most commonly added. Essential trace elements may also promote the growth of bacteria. Usually the required organisms are already present and their growth in sufficient numbers is promoted by the added nutrient materials and oxygen source.

In some cases better results are obtained by adding along with the nutrients a carbon source called an **inducer**. This can stimulate more vigorous bacterial growth and enable biodegradation by *cometabolism* in which the pollutant is degraded along with the inducer. At least one strain of phenol-metabolizing *Acinetobacter* cometabolizes trichloroethylene, $CHCl{=}CCl_2$. An important potential application of cometabolism uses methane, which enables growth of bacteria for which CH_4 is a carbon source:

$$CH_4 + 2O_2 \xrightarrow[\text{enzyme}]{\text{Monooxygenase}} CO_2 + 2H_2O + \text{biomass} + \text{energy} \qquad (6.7.4)$$

The bacteria are **methanotrophs**. In addition to metabolizing hydrocarbons, the monooxygenase enzyme in methanotrophic bacteria is capable of cometabolizing low-

molecular-mass organohalides, including chlorine-substituted methane, ethane, and ethylene compounds, such as $CHCl_3$, C_2HCl_3, and $CHCl{=}CCl_2$, respectively.

Typically, the raw materials needed for biorestoration are pumped along with water into the site underground through an injection well, and products are pumped out through a production well. The water from the production well may be discharged if it meets acceptable standards, or it may be recharged with nutrients and re-injected.

Outlook for Subsurface Biorestoration

Despite the euphoria in some quarters over subsurface biorestoration, there are many pitfalls. The site must be sufficiently permeable and uniform to permit addition of nutrients and oxygen without bypassing large volumes within the site; permeability gets to be a particular problem when bacteria must also be added. Toxic substances, such as heavy metal contaminants may prevent the growth of the kinds of bacteria that degrade the wastes. Oxygen reacting with soluble iron(II) underground,

$$O_2 + 4Fe^{2+}(aq) + 10H_2O \rightarrow 4Fe(OH)_3(s) + 8H^+ \qquad (6.7.5)$$

can precipitate gelatinous $Fe(OH)_3$, which clogs the pores in the underground medium. Essential trace element nutrients may be precipitated or be removed by ion-exchange processes, such that they are not available to promote the growth of bacteria. Many wastes are mixtures of several components, only some of which may be degraded. It may be hard to achieve and maintain physical conditions, such as temperature, required for optimum biodegradation. In favorable cases these problems either do not exist or can be circumvented.

6.8. BIODEGRADATION OF PCBs

The biodegradation of PCBs in New York's Hudson River provides an interesting example of microbial degradation of environmental chemicals.[14] During the mid-1900s polychlorinated biphenyls were legally discharged in large quantities to the Hudson River as waste products from electrical equipment manufacture. As a result, these virtually insoluble, dense, hydrophobic materials accumulated in the river's sediments, causing serious concern about their effects on water quality as a result of their bioaccumulation in fish. Methods of removal, such as dredging, were deemed prohibitively expensive and likely to cause severe contamination and disposal problems.

Although it was well known that aerobic bacteria could degrade PCBs with only one or two Cl atom constituents, most of the PCB congeners discharged to the sediments had multiple chlorine atom constituents, specifically an average of 3.5 Cl atoms per PCB molecule at the time the PCBs were discharged.[15] However, investigations during the late 1980s revealed that the PCBs in the sediments had been largely converted to mono- and dichloro substituted forms. This conversion had to be due to the action of anaerobic bacteria. Such bacteria do not use PCBs as a carbon source, but rather as electron acceptors in the overall process,

$$\{CH_2O\} + H_2O + 2\text{Cl-PCB} \rightarrow CO_2 + 2H^+ + 2Cl^- + 2\text{H-PCB} \qquad (6.8.1)$$

where Cl-PCB represents a site of chlorine substitution on a PCB molecule and H-PCB represents a site of hydrogen substitution. The net result of this process is the replacement of Cl by H on the more highly chlorine-substituted PCB molecules. The circumstances under which this took place in the sediments included anaerobic

conditions and very long residence times. These are ideal for the growth of anaerobic bacteria, which tend to carry out their metabolic processes slowly, with relatively low efficiency compared to aerobic organisms, and in complex synergistic relationships with other anaerobic bacteria.

The mono- and dichloro substituted PCBs are degraded by aerobic bacteria. Aerobic processes oxidize the molecules and cleave the aromatic rings as shown in Figure 6.9. Ultimately, the PCBs are mineralized by conversion to inorganic chloride, carbon dioxide, and water.

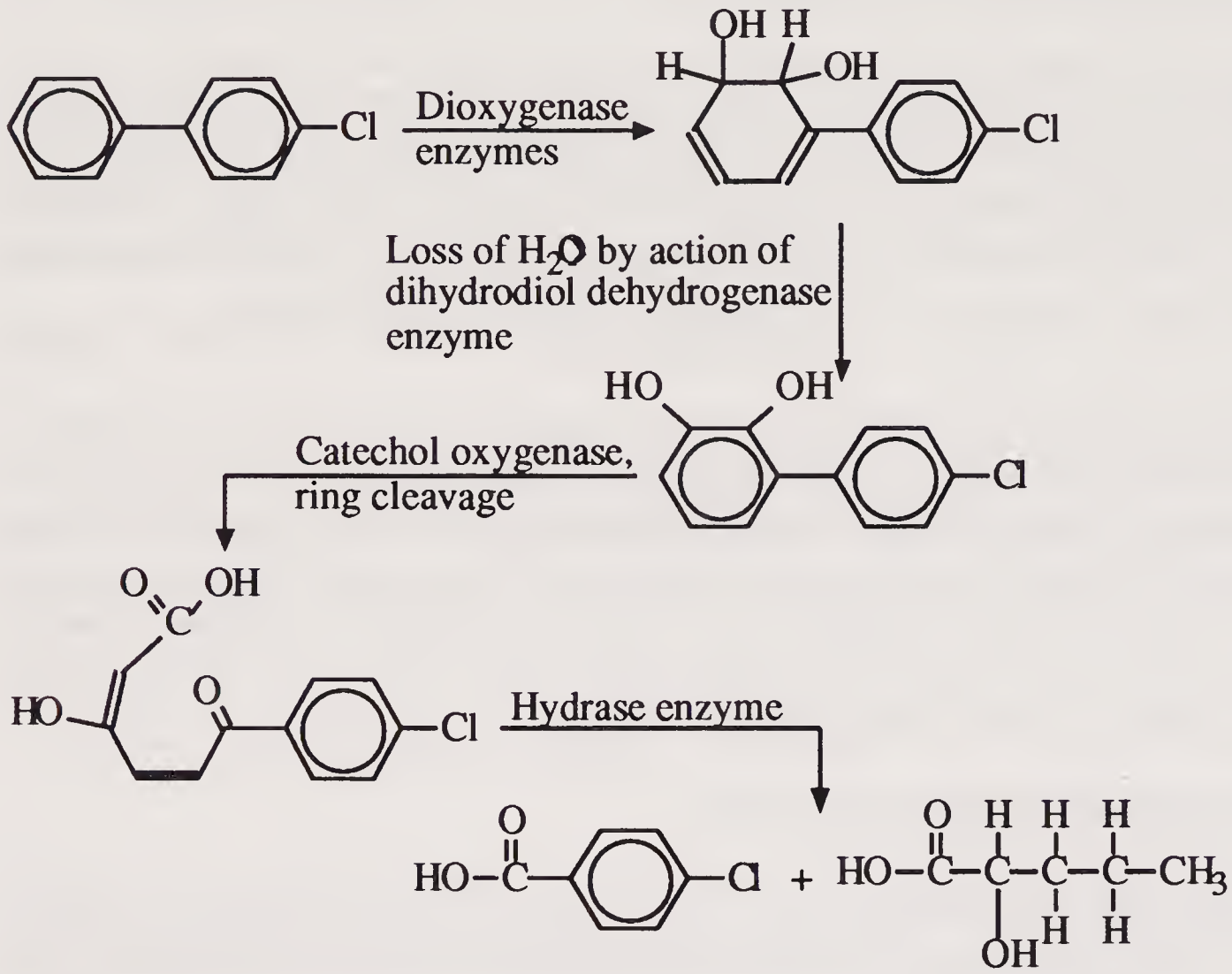


Figure 6.9. Enzymatic processes involved in the initial attack of aerobic bacteria on mono- and dichloro substituted PCBs.

Ideally, in the case of PCBs in sediments, about half of the work of remediation by biodegradation—conversion of highly substituted PCBs to molecules with one or two chlorines—is done by the slow, steady action of anaerobic bacteria without human intervention. Since the PCB products tend to stay in anaerobic surroundings, some assistance is required to finish the biodegradation. Experiments are underway on the Hudson River sediments to introduce aerobic bacteria acclimated to PCB biodegradation, along with the oxygen and nutrients required for their growth. As a means of treating PCB wastes, such degradation can be carried out *in situ*, in composting operations on land, or in bioreactors where a continuous culture works on waste soil, slurries, or sludges pumped through it. Treatment in place has advantages of low cost and minimal site disturbance, whereas bioreactors are generally much faster and more effective.

6.9. BIODEGRADATION OF PETROLEUM PRODUCTS

The biodegradation of petroleum is essential to the elimination of oil spills (about 5×10^6 metric tons per year). This oil is degraded by both marine bacteria and filamentous fungi. In some cases, the rate of degradation is limited by available nitrate and phosphate.

The physical form of crude oil makes a large difference in its degradability. Degradation in water occurs at the water-oil interface. Therefore, thick layers of crude oil prevent contact with bacterial enzymes and O_2. Apparently, bacteria synthesize an emulsifier that keeps the oil dispersed in the water as a fine colloid and therefore accessible to the bacterial cells.

Numerous kinds of organic materials are effectively treated by biodegradation. Much of what is known about this area has been learned from petroleum production, storage, transport, and processing sites where, throughout most of this century, unintentional experiments in biodegradation have been conducted as indigenous microorganisms have metabolized a variety of petroleum products spilled on soil. A significant amount of information is now available regarding the biodegradation of petroleum hydrocarbons, which are largely readily broken down by bacteria. Table 6.4 summarizes what is known about the amenability to biodegradation of petroleum constituents and organic substances commonly synthesized from petroleum.

Table 6.4. Biodegradability of Petroleum Products

Readily degraded, predominantly low-mass, naturally occurring, straight-chain or simple aromatic molecules	Less readily degraded, predominantly high-molecular-mass, synthetic, branched-chain, often highly chlorinated molecules
Light, straight-chain aliphatic hydrocarbons	Asphalt, bitumens, tars, waxes
Simple aromatic hydrocarbons, benzene, toluene, lighter PAHs[1]	Heavier PAHs
	Highly branched hydrocarbons
Oxygenated compounds, alcohols, ketones, carboxylic acids	PCBs
	Dioxins
Some lighter, less highly chlorinated organohalides, vinyl chloride	Highly chlorinated compounds without functional groups, such as hydrolyzable esters or $-CO_2H$ that are readily attacked

[1] Polycyclic aromatic hydrocarbons

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QUESTIONS

1. Define detoxication and cite an example of the phenomenon.
2. Distinguish among the following: bioaccumulation, biomagnification, and bioconcentration.
3. What is the basis of the hydrophobicity model? Under what circumstances is it most readily applicable?
4. What is the role of molecular size and shape in bioconcentration? Why may molecular mass alone be insufficient to predict bioconcentration?
5. What is the bioconcentration factor, BCF? How is it calculated mathematically? What are representative values of BCF?
6. How is the octanol-water partition coefficient employed in predicting biouptake? How is this use justified?
7. In which respect do the values and trends of the bioconcentration factors for vegetation differ from those of animals? What is the explanation?
8. Define biodegradation. Define each of the following terms related to biodegradation: mineralization, biotransformation, metabolism, catabolism.
9. How is cometabolism involved in the biodegradation of xenobiotic compounds? What is a secondary substrate in cometabolism? How does *Phanerochaete chrysosporium* illustrate secondary metabolism?
10. List the general factors involved in biodegradation. How might such factors influence biodegradation? What is the influence of binding of xenobiotic compounds to sediments in biodegradation?
11. Some studies suggest that biodegradation rates of substances at relatively higher concentrations are not extrapolatable to very low concentrations. Discuss the effects that this might have upon the long-term persistence of very low levels of pollutants.
12. Nonenzymatic reactions that may be involved in the breakdown of chemical species in the water and soil environments include hydrolysis, oxidation-reduction, surface-catalyzed, photolytic, and ion-exchange reactions. Which of these may also be are biologically mediated enzyme-catalyzed reactions?
13. Match each of the following pertaining to microbially mediated oxidation reactions in biodegradation:

(a) Oxidation	1. Adding an O atom between two C atoms
(b) Ring cleavage	2. Especially important for aromatic compounds
(c) Hydroxylation	3. Mediated by oxygenase enzymes
(d) Epoxidation	4. Frequently follows epoxidation

14. Discuss and illustrate an example of a compound that undergoes epoxidation and hydroxylation that is of particular toxicological importance.
15. Name and describe a process involving H2O that is especially important in the microbial degradation of pesticidal esters, amides, organophosphate esters, and nitriles.

16. Describe a reaction that involves both a reduction and removal of halogen from an organic compound.
17. Conjugation reactions are less common among microorganisms than they are in higher organisms. Why might this be so, given the ways in which these two general classes of organisms must handle toxic substances?
18. List some physical and chemical properties of a compound as well as some biochemical conditions that are favorable for biodegradability to occur.
19. Critique the statement that "recalcitrant or biorefractory substances are those that are toxic to microorganisms that would otherwise degrade them."
20. What is a bioreactor? How are such devices used in the treatment of municipal wastes? Give at least two examples. What do they actually eliminate in municipal wastewater?
21. Describe why the activated sludge process is particularly efficient in treating wastes in municipal wastes. What is the "sludge"?
22. What mineralization processes may occur in municipal wastewater treatment? Which elements other than carbon are mineralized? How are microorganisms involved? What is the environmental significance of these mineralization processes?
23. What may be the adverse effects of soluble iron(II) in biological treatment of hazardous waste substances? What may be the importance of "biorefractory" or "biorecalcitrant" compounds in biological treatment of such wastes?
24. What function may be served by SO_4^{2-}, NO_3^-, and similar compounds in the biodegradation of wastes in the absence of molecular O_2?
25. What purpose may be served by H_2O_2 as an additive in the treatment of wastes in place? What other materials may have to be added for such treatment?
26. Although aerobic bacteria degrade PCBs with only one or two Cl atoms, why, and how, are PCBs with more Cl substituents degraded in river sediments?
27. What do some bacteria do to change the physical form of crude oil to enhance its biodegradability?

7

Aquatic and Soil Environmental Biochemistry

7.1. MICOORGANISMS IN SOIL AND WATER

Microorganisms in the soil and aquatic environments are of the utmost importance in determining environmental and environmental chemical phenomena. Such microorganisms include bacteria, fungi, protozoa and algae. Of these, bacteria are very important in both soil and water. Fungi are relatively more important as *reducers* (which serve to break down biodegradable material) in soil and algae are relatively more important in water as primary *producers* of biomass.

This chapter addresses the important question of microbial activity in both the geosphere and the hydrosphere. In addition to degrading both biologically and chemically produced organic matter, microorganisms mediate oxidation-reduction reactions, fix atmospheric nitrogen, serve as the foundation for the aquatic food chain, release mineral nutrients (inorganic carbon dioxide, nitrates, and phosphates) required by plants, and participate in other environmentally significant processes. Of utmost importance is the role of microorganisms in elemental cycles, such as those of carbon, nitrogen, sulfur, and phosphorus. These kinds of microbial processes are addressed in this chapter.

7.2. SOIL, A MICROBIAL HABITAT

In order to consider microorganisms in soil, it is important to have an appreciation of soil as a hospitable environment for fungi, bacteria, and other single-celled organisms. **Soil**, discussed briefly in Section 2.9, is a variable mixture of minerals, organic matter, and water, capable of supporting plant life on the earth's surface.[1] It is the final product of the weathering action of physical, chemical, and biological processes on rocks. The organic portion of soil consists of plant biomass in various stages of decay. High populations of bacteria, fungi, and animals such as earthworms may be found in soil. Soil contains air spaces and generally has a loose texture (Figure 7.1).

Typical soils exhibit distinctive layers with increasing depth. These layers are called **horizons**. Horizons form as the result of complex interactions among processes that occur during weathering. Rainwater percolating through soil carries dissolved and colloidal solids to lower horizons where they are deposited. Biological processes, such as bacterial decay of residual plant biomass, produces slightly acidic CO_2, organic acids, and complexing compounds that are carried by rainwater to lower horizons where they interact with clays and other minerals, altering the properties of the

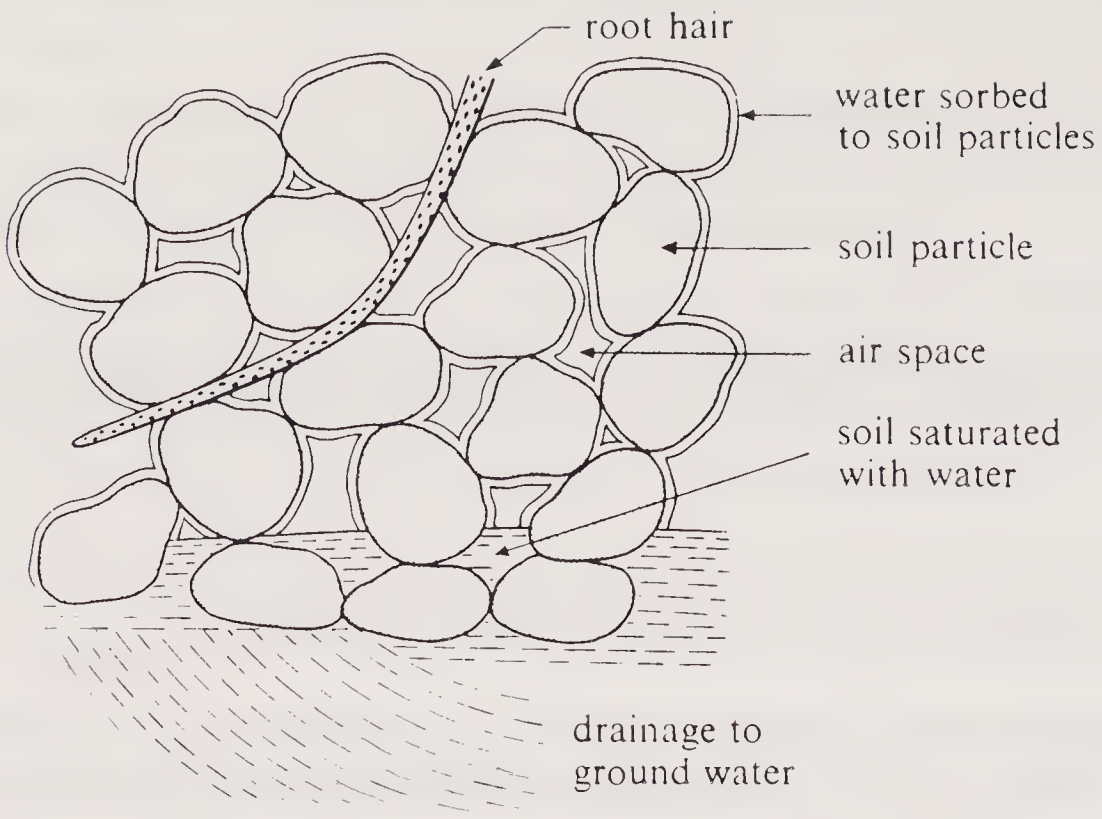


Figure 7.1. Fine structure of soil, showing solid, water, and air phases.

minerals. Of these the layer of maximum microbiological activity is the top layer known as the A horizon, or **topsoil**. Typically 10-100 cm thick, topsoil contains most of the soil organic matter.

Water and Air in Soil

Water is part of the three-phase, solid-liquid-gas system making up soil; it is essential for the growth of microorganisms. Normally, because of the small size of soil particles and the presence of small capillaries and pores in the soil, the water phase is not totally independent of soil solid matter. The availability of water to plants and microorganisms is governed by gradients arising from capillary and gravitational forces. The availability of nutrient solutes in water depends upon concentration gradients and electrical potential gradients. Water present in larger spaces in soil is relatively more available to microorganisms and plants and easily drains away. Water held in smaller pores, or between the unit layers of clay particles is bound much more strongly. Soils high in organic matter may hold appreciably more water than other soils, but it is relatively less available to organisms because of physical and chemical sorption of the water by the organic matter.

As soil becomes waterlogged (water-saturated) it undergoes drastic changes in physical, chemical, and biological properties. Oxygen in such soil is rapidly used up by the respiration of microorganisms that degrade soil organic matter. One of the most marked chemical effects of waterlogging is a reduction of pE (see Section 2.3) by the action of organic reducing agents acting through bacterial catalysts. Thus, the redox condition of the soil becomes much more reducing, and the soil pE may drop from that of water in equilibrium with air (+13.6 at pH 7) to 1 or less. One of the more significant results of this change is the mobilization of iron and manganese as soluble iron(II) and manganese(II) through bacterially mediated reduction of their insoluble higher oxides:

$$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O \qquad (7.2.1)$$

$$Fe_2O_3 + 6H^+ + 2e^- \rightarrow 2Fe^{2+} + 3H_2O \qquad (7.2.2)$$

Some soluble metal ions such as Fe^{2+} and Mn^{2+} are toxic to some microorganisms and to plants at high levels.

Roughly 35% of the volume of typical soil is composed of air-filled pores. Whereas the normal dry atmosphere at sea level contains 21% O_2 and 0.03% CO_2 by volume, these percentages may be quite different in soil air because of the decay of organic matter:

$$\{CH_2O\} + O_2 \rightarrow CO_2 + H_2O \qquad (7.2.3)$$

This process consumes oxygen and produces CO_2. As a result, the oxygen content of air in even relatively well aerated soil may be as low as 15%, and the carbon dioxide content may be several percent. Thus, the decay of organic matter in soil increases the equilibrium level of dissolved CO_2 in groundwater. This lowers the pH and contributes to weathering of carbonate minerals.

The Inorganic Component of Soil

The weathering of parent rocks and minerals to form the inorganic soil components results ultimately in the formation of inorganic colloids. These colloids are repositories of water and plant nutrients, which may be made available to plants as needed. Inorganic soil colloids often absorb toxic substances in soil, thus playing a role in detoxication of substances that otherwise would harm plants. The abundance and nature of inorganic colloidal material in soil are obviously important factors in determining soil productivity.

The most common elements in the earth's crust are oxygen, silicon, aluminum, iron, calcium, sodium, potassium, and magnesium. Therefore, minerals composed of these elements — particularly silicon and oxygen — constitute most of the mineral fraction of the soil. Common soil mineral constituents are finely divided quartz (SiO_2), orthoclase ($KAlSi_3O_8$), albite ($NaAlSi_3O_8$), epidote ($4CaO{\cdot}3(AlFe)_2O_3{\cdot}6SiO_2{\cdot}H_2O$), geothite ($FeO(OH)$), magnetite ($Fe_3O_4$), calcium and magnesium carbonates ($CaCO_3$, $CaCO_3{\cdot}MgCO_3$), and oxides of manganese and titanium.

Organic Matter in Soil

Though typically comprising less than 5% of a productive soil, organic matter largely determines soil productivity. It serves as a source of food for microorganisms; undergoes chemical reactions such as ion exchange; and influences the physical properties of soil. Some organic compounds even contribute to the weathering of mineral matter, the process by which soil is formed. For example, $C_2O_4^{2-}$, oxalate ion, produced as a soil fungi metabolite, occurs in soil as the calcium salts whewellite and weddelite. Oxalate in soil water dissolves minerals, thus speeding the weathering process and increasing the availability of nutrient ion species. This weathering process involves oxalate complexation of iron or aluminum in minerals, represented by the reaction

$$3H^+ + M(OH)_3(s) + 2CaC_2O_4(s) \rightarrow M(C_2O_4)_2^-(aq) + 2Ca^{2+}(aq) + 3H_2O \qquad (7.2.4)$$

in which M is Al or Fe. Some soil fungi produce citric acid, and other chelating organic acids, which react with silicate minerals and release potassium and other nutrient metal ions held by these minerals.

The accumulation of organic matter in soil is strongly influenced by temperature and by the availability of oxygen. Since the rate of biodegradation decreases with decreasing temperature, organic matter does not degrade rapidly in colder climates and tends to build up in soil. In water and in waterlogged soils, decaying vegetation does not have easy access to oxygen, and organic matter accumulates. The organic content may reach 90% in areas where plants grow and decay in soil saturated with water.

Soil humus is by far the most significant organic component of soil. Humus, composed of a base-soluble fraction called humic and fulvic acids and an insoluble fraction called humin, is the residue left when bacteria and fungi biodegrade plant material. The bulk of plant biomass consists of relatively degradable cellulose and degradation-resistant lignin, which is a polymeric substance with a higher carbon content than cellulose. Among lignin's prominent chemical components are aromatic rings connected by alkyl chains, methoxyl groups, and hydroxyl groups. Lignin is the precursor of most soil humus. Humic materials in soil strongly sorb many solutes in soil water and have a particular affinity for heavy polyvalent cations. Sorption of organic substances by soil humus may influence their biodegradation by microorganisms.

The Soil Rhizosphere

A particularly important part of the topsoil is the **rhizosphere**, that portion of the soil in the immediate vicinity of plant roots. As an environment for microorganisms, the rhizosphere is strongly influenced by the plant roots, which provide nutrients and influence conditions such as moisture level, oxygen level, and pH. Furthermore, some highly significant microbial species grow in close association with plant roots and root hairs (see the discussion of nitrogen-fixing *Rhizobium* bacteria in Section 7.12).

Bacteria associated with plant roots tend to exist as colonies at locations where nutrient-containing fluids are excreted from the plant roots. Plant root secretions contain a rich variety of substances that are utilized by bacteria and fungi. These substances include amino acids; sugars, such as glucose, maltose, and xylose; enzymes; growth factors, such as B vitamins; nucleic acid consituents, including the nitrogen-containing bases shown in Figure 3.10; and organic acids (acetic, oxalic, citric, fatty acids). In addition, roots may secrete substances that stimulate the growth of fungi or cause fungal spores to germinate.

Rhizospheric microorganisms provide a number of benefits to plants. One of these is the conversion of organic nitrogen, sulfur, and phosphorus in decaying biomass to inorganic forms (NO_3^-, SO_4^{2-}, HPO_4^{2-}, $H_2PO_4^-$), a process called **mineralization**. The liberation of soluble Fe^{2+} and Mn^{2+} by microorganism-mediated processes accompanied by reduction of pE was shown in Reactions 7.2.1 and 7.2.2; such reactions may be significant in making nutrient metal ions available for uptake by plant roots. Microorganisms may lower the pH by producing CO_2 and organic acids, thereby making nutrient metal ions available by acid dissolution:

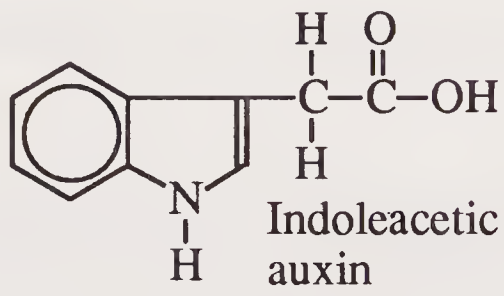
$$H^+(\text{microbially generated}) + CaCO_3(s) + H_2O \rightarrow Ca^{2+}(aq) + 2HCO_3^-(aq) \qquad (7.2.5)$$

In addition microbial synthesized chelating agents (see Section 2.4) may solubilize plant nutrient metal ions from insoluble forms.

There are several other effects of microorganisms in the rhizosphere that may be beneficial to plant growth. The biodegradation of organic matter in the rhizosphere,

$$\{CH_2O\} + O_2(g) \rightarrow CO_2 + H_2O \qquad (7.2.6)$$

generates carbon dioxide gas required by plants for photosynthesis. Under some circumstances the flux of microbially generated CO_2 from the soil surface is high enough to significantly enrich the air around the plant leaves with carbon dioxide, thereby accelerating growth. It is possible that rhizospheric microorganisms produce antibiotics that are active against bacterial and fungal plant pathogens. Finally, bacteria in the rhizosphere produce some plant growth regulators, such as indolacetic acid (below) which may have effects on plants, such as stimulating plant root growth.



Indoleacetic acid or auxin

7.3. MICROORGANISMS IN SOIL

Most healthy soils are vast microbial cultures with a large variety of microrganisms growing in the soil. Soil microorganisms include bacteria and fungi; algae grow on the surface of wet soil exposed to light. Strong relationships exist among the kinds of microorganisms in soil. Microorganisms have a strong influence on soil and the ecosystem as a whole through the following:

- Biodegradation of plant and animal biomass, releasing nutrients to the soil and forming soil humus
- Microbial processes essential to maintaining the balance and well-being of the biosphere
- Biodegradation, biotransformation, and detoxication of xenobiotic substances, particularly herbicides, applied to soil
- In some cases the presence of disease-causing microorganisms in soil
- Mediation of important oxidation-reduction reactions, such as conversion of fertilizer NH_4^+ to NO_3^-, the form of nitrogen that can be assimilated by most terrestrial plants
- In some cases formation of pollutant species in soil (for example, groundwater contaminant nitrate produced by bacterially mediated oxidation of of ammoniacal nitrogen)

7.4. SOIL FUNGI

Fungi may be quite abundant in soil. The abundance of soil fungi is appreciated by considering that a single gram of soil may contain up to 1000 meters of fungal mycelium (mass of filamentous fungal hyphae).

Soil fungi serve to degrade substances that are not readily broken down by bacteria. In terms of bulk the most important such substances are cellulose, hemicellulose, and lignin, which are the three major structural materials in plants. Largely as a consequence of their partial degradation of lignin, fungi are instrumental in forming humic substances, the most prominent organic constituents of soil. Fungal degradation of plant material enables release of ammoniacal nitrogen, which is converted to plant nutrient nitrate by bacteria. Fungi may be important in determining the physical properties of soil, largely by virtue of their binding of soil particles into matrices that facilitate transport of soil air and soil water. On the negative side, soil is the repository of some kinds of fungi that cause disease in plants and even animals.

Fungi are particularly well adapted to persisting in soil and reproducing rapidly when water, temperature, and nutrient conditions become right by virtue of their formation of resting structures, such as various kinds of spores. These bodies can remain viable under highly adverse conditions and resume fungal growth and reproduction when conditions become right.

7.5. ACTINOMYCETES

Actinomycetes are bacteria that grow in soil and resemble fungi somewhat in their structure and behavior. The resemblance is especially true of filamentous branching species of actinomycetes. Like fungi, actinomycetes are spore-forming microorganisms. Unlike fungi, but like bacteria, they consist of prokaryotic, rather than eukaryotic cells. Actinomycetes are often very abundant in soil, where they are distinguished from other kinds of bacteria by their characteristic morphology, relatively slow growth rates, and limited variety of substrates that they can use for food sources.

Soil actinomycetes produce metabolic products that give soil an "earthy" odor. Like fungi, they produce extracellular enzymes that can act outside the cell to break down biomass. One of the useful functions of soil actinomycetes is their ability to degrade biorecalcitrant compounds, including pesticides. They metabolize petroleum hydrocarbons; in the case of *Arthobacter* soil actinomycetes produce emulsifying agents that facilitate the biodegradation of water-insoluble hydrocarbons.

The *Streptomyces* bacteria are among the more important actinomycetes microorganisms. Of particular significance is their production of antibiotic secondary metabolites. These include substances such as erythromycin and streptomycin that deter the growth of other, unrelated bacteria. Some of these kinds of substances are very useful as bactericidal pharmaceutical agents.

7.6. SOIL BACTERIA

Normally greater in number of cells, but smaller in total biomass than soil fungi, bacteria in soil usually carry out the greater fraction of soil biochemical processes. This is especially true of anaerobic processes, which fungi cannot mediate. Bacteria are obviously of great importance in the environmental chemistry of soil.

There exists a vast variety of soil bacteria dominated by *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, and *Pseudomonas*. Such bacteria have members shaped as rods, coccoidal rods, and cocci; those that form spores as well as those that do not; gram-positive bacteria; and gram-negative bacteria (based upon cell response to standard staining procedures).

From the standpoint of their environmental biochemistry, however, the most important characteristic of bacteria is their variable nutritional and metabolic charac-

teristics. Soil bacteria degrade biomass, destroy xenobiotic contaminants (such as residual herbicides), and mediate oxidation/reduction processes involving carbon, nitrogen, sulfur, iron, and manganese species. Various kinds of bacteria may require nutrients, such as amino acids or vitamins that they cannot make; these may be produced by other organisms, including other bacteria.

The soil bacteria that grow first and most rapidly are those that degrade common food materials — carbohydrates, proteins, and organic acids from lipids. Numbers of such bacteria increase rapidly when readily metabolized nutrients are added to soil, and their metabolic activity diminishes abruptly when the nutrient sources are removed. Other heterotrophic bacteria use more degradation-resistant substances as nutrient sources. Such sources include petroleum products, humic material, and undegraded residues left over after more biodegradable constituents have been removed. The latter group of bacteria grows more slowly and is less responsive to fluctuations in nutrient availability.

7.7. SOIL ENVIRONMENTAL FACTORS IN BACTERIAL GROWTH

A variety of environmental factors influence the growth of soil bacteria. One of the simpler of these is simply the **physical nature** of the soil. Soil surface area is important, and colloidal-sized soil particles (those ranging downward from about 1 micrometer in size) are of particular importance because of their high surface/volume ratio, surface charge, and ability to exchange and concentrate nutrient cations. The metabolic activity of bacterial cells and of their exoenzymes may be influenced by binding to soil colloidal particles

A major factor in determining bacterial activity in soil is **soil moisture**, which must be present for bacterial growth to occur. Soil moisture is the solvent medium in the all-important soil solution (see Section 2.9), which carries nutrients to, and waste products away from bacterial cells. A high level of soil moisture reflecting a waterlogged condition prevents air movement in soil, thus making it anaerobic, inhospitable to the growth of oxygen-requiring bacteria and fungi, and conducive to the growth of anaerobic bacteria.

Soil solution **pH** strongly influences microbial growth. Many kinds of bacteria do not thrive under acidic, low pH conditions, so that acid-tolerant fungi predominate. Some bacteria, such as those involved in the oxidation of FeS_2, thrive at low pH, whereas others are strongly inhibited. An example of the latter is nitrogen-fixing *Rhizobium* bacteria that lives symbiotically attached to legume roots.

Nutrient availability is crucial to the growth of soil bacteria. The most rapid bacterial growth and reproduction occurs when readily metabolized carbohydrates, proteins, and fatty acids are available, but bacterial growth can be sustained by less readily metabolized substrates as well. The bacterial biomass formed by readily degraded substances serves in turn as a nutrient source for other bacteria, protozoa, or fungi.

Oxygen availability determines the rate of bacterial growth and the kinds of bacteria that can grow in soil. Most bacteria, including actinomycetes, require oxygen for growth, as do all fungi. In a nutrient-rich soil, aeration processes are too slow to provide needed oxygen, so the growth of aerobic bacteria is limited. In extreme cases oxygen is not available and anaerobic bacteria take over. The presence of absence of oxygen combined with the biochemical action of bacteria determines soil **pE**, explained in Section 2.3 as a measure of the relative oxidizing and reducing ability of soil.

Soil **temperature** strongly influences bacterial metabolism rates. Based upon their temperature preferences, (see Figure 5.7) bacteria can be classified among psychrophilic ("cold-loving"), mesophilic, and thermophilic ("heat-loving") bacteria. For the temperature range encountered in most soils, the metabolic rate of psychrophilic bacteria is too low for them to dominate soil environmental chemical processes and soil temperatures are rarely high enough to favor thermophilic bacteria, such as those that grow in hot springs. Therefore, most soil environmental chemical processes are carried out by mesophilic bacteria. Soil temperature has a variety of other effects that influence bacterial growth, such as its influence on aeration and water evaporation.

Several kinds of substances may act to inhibit the growth of soil bacteria or to influence the kinds of bacteria that grow. A high level of salts in the soil solution leads to the predominance of salt-tolerant halophilic bacteria. Inhibitory hydrogen sulfide, H_2S, is released by anaerobic bacteria using sulfate as an electron acceptor in the biodegradation of biomass ($\{CH_2O\}$):

$$SO_4^{2-} + 2\{CH_2O\} + 2\,H^+ \rightarrow H_2S + 2CO_2 + 2H_2O \qquad (7.7.1)$$

The acidification of the soil solution from pollutant sources, such as acid rain, or by bacterially-mediated reactions, such as

$$2O_2 + NH_4^+ \rightarrow 2H^+ + NO_3^- + H_2O \qquad (7.7.2)$$

can release inhibitory metals (Cu^{2+}, for example) by solubilization of metal salts or oxides. Some bacteria release substances that inhibit the growth of competing microorganisms as a form of "biochemical warfare."

7.8. INTERACTIONS AMONG SOIL BACTERIA

As a rich and diverse microbial ecosystem, soil shows many interactions among various kinds of bacteria and other kinds of microorganisms. These may be broadly divided between **beneficial** and **antagonistic** interactions. The two kinds of interactions are addressed briefly here.

Beneficial Relationships

Figure 7.2 illustrates the three general categories of beneficial interactions among bacteria and soil microorganisms in general. These are discussed below.

Mutualism

Mutualism implies a very strong interdependence between two specific kinds of organisms to the extent that neither can thrive without the other. Although examples of true mutualism between two kinds of bacteria have not been shown conclusively, there are cases of mutualism between two kinds of microorganism. Some bacteria exist within the cells of protozoa. The bacteria clearly derive nutrients from the protozoa. The benefit to the latter is not well known, but must exist because the protozoa do not grow without the bacteria. Another example of mutualism is that of lichens consisting of a fungus growing in association with a photosynthetic microorganism. The latter may consist of algae or cyanobacteria and provides organic carbon to the fungus. The fungus in turn provides nutrients, a moist condition, and a "physical anchor" for growth of the photosynthetic microorganism.

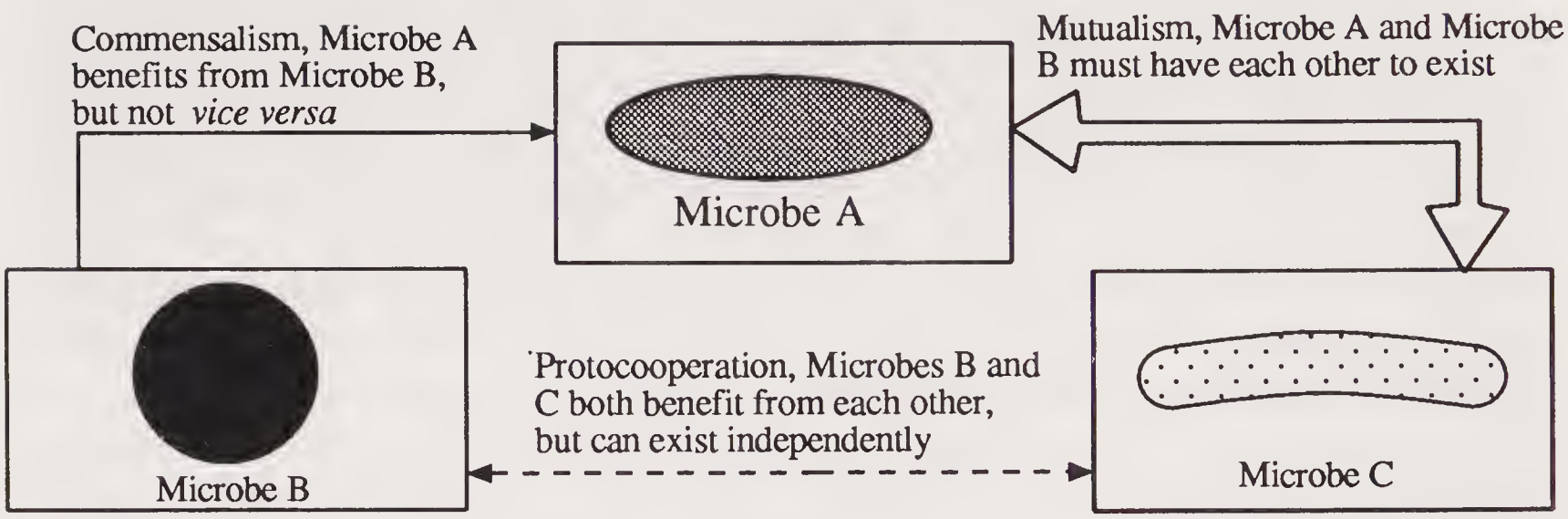


Figure 7.2. Beneficial interactions among bacteria and microorganisms.

Although one partner is not a microorganism, good examples of mutualism are provided by nitrogen-fixing bacteria growing on the roots of leguminous plants, such as soybeans, alfalfa, and clover. Leguminous plants have a symbiotic (mutually advantageous) relationship with bacteria capable of fixing atmospheric nitrogen that grow on the plant root structures. The nitrogen-fixing bacteria in legumes exist in special structures on the roots called root nodules (see Fig. 7.3). The rod-shaped bacteria that fix nitrogen are members of a special genus called *Rhizobium*. Although all species of *Rhizobium* appear to be very similar, they exhibit a great deal of specificity in their choice of host plants. Curiously, legume root nodules also contain a form of hemoglobin, which must somehow be involved in the nitrogen-fixation process.

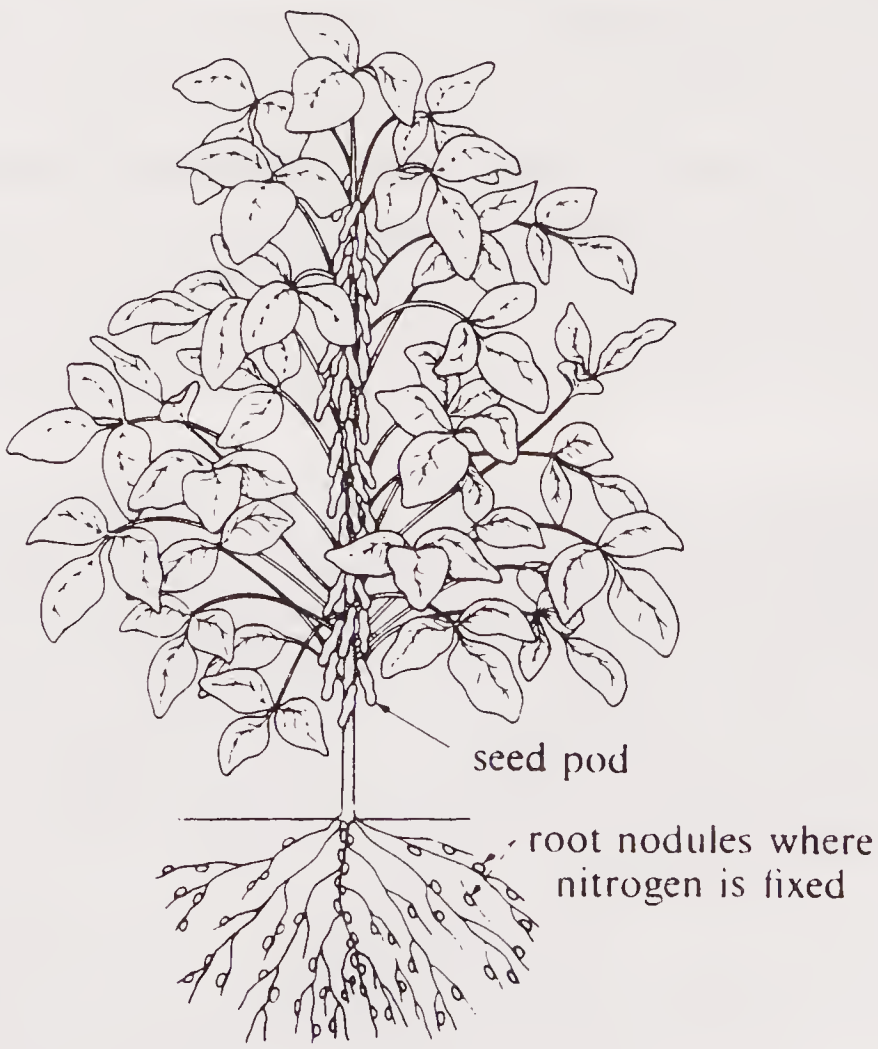
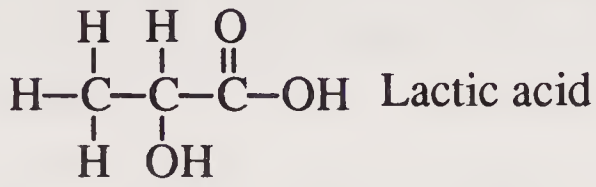


Figure 7.3. A soybean plant, showing root nodules where nitrogen is fixed.

Protocooperation

Protocooperation is similar to mutualism, but with a less rigid relationship between the organisms. The two organisms involved can exist well without each other, the organism specificity is relatively less, and the arrangement may be only temporary. One example would be where each organism produces nutrients needed by the other. Another specific example would be the removal of toxic microbially-generated H_2S by a microorganism that can oxidize hydrogen sulfide as a means of energy production. Metabolically-produced lactic acid (below) accumulates to toxic levels in cultures of *Streptococcus lactis* which produces it, but is removed and used as food by fungal *Geotrichum candidum.*


Lactic acid

Commensalism

Commensalism benefits only one of the organisms involved. Typically, fungi may partially degrade cellulose to give some products that are not utilized by the fungi. However, if these products serve as nutrients for bacteria that cannot utilize cellulose directly, the bacteria benefit from the action of the fungi, providing nothing in return. Sorption of toxic organophilic xenobiotic compounds by cell wall lipids of one species of bacteria may enable growth of another microorganism.

Antagonistic Relationships

"It is a jungle down there," as soil microorganisms compete and wage biological warfare for dominance in their ecological niches. This area of soil microbiology is addressed under the term **antagonisms**. The most basic kind of antagonism is simple **competition** between microorganisms, usually for nutrients or oxygen. Microorganisms may even compete for space in which to grow. When, as is usually the case, available food is less than that which can be utilized by the microorganisms present, competition occurs. In general, the microorganisms predominate that reproduce most rapidly, metabolize the food most quickly, and are most versatile in the kinds of nutrients that they will accept.

Predation

Numerous examples exist in which one kind of microorganism attacks another kind and utilizes its biomass as a food and nutrient source. This kind of phenomenon may be called **predation** (typically when one microorganism feeds on another) or **parasitism** (typically when a smaller microorganism invades the host cells of a larger microorganism). Protozoa commonly act as predators and feed ("graze") upon bacteria or other protozoa. *Actinomycetes* and other fungi may produce enzymes that cause lysis (rupture) of fungal cells, the contents of which serve as a food source for the predators.

Viruses, which are much smaller than their host cells, exist only as parasites; soil fungi, protozoa, and bacteria (including *Actinomycetes* and cyanobacteria) may all be afflicted with parastic viruses. Both bacteria and fungi act as parasites to algal cells growing on the surface of soil.

7.9. THE HYDROSPHERE AS A MICROBIAL ENVIRONMENT

The hydrosphere — lakes, streams, oceans, groundwater, even ice and snowpack — provides hospitable media for the growth of a variety of microorganisms. In considering aquatic microbiology it is useful to review some of the characteristics of water as discussed in Chapters 1 and 2. Of particular importance are the unique properties of water listed in Table 2.1. Some other characteristics of water and bodies of water are reviewed briefly here.

Characteristics of Bodies of Water

The physical condition of a body of water strongly influences the chemical and biological processes that occur in the water. **Surface water** occurs primarily in streams, lakes, and reservoirs. Lakes (both natural and constructed) may be classified as oligotrophic, eutrophic, or dystrophic, an order that often parallels the life of the lake. **Oligotrophic** lakes are deep, generally clear, deficient in nutrients, and without much biological activity; their population of microorganisms tends to be low. **Eutrophic** lakes have more nutrients, usually as the result of runoff from forests, grasslands, and agricultural lands. Eutrophic waters support more life, including microorganisms, and are more turbid. **Dystrophic** lakes are shallow, clogged with plant life, and normally contain colored water with a low pH. **Wetlands** are flooded areas in which the water is shallow enough to enable growth of bottom-rooted plants.

Impounding water in reservoirs may have some profound effects upon water quality. These changes result from factors such as different velocities, changed detention time, and altered surface-to-volume ratios relative to the streams that were impounded. Some resulting beneficial changes due to impoundment are a decrease in the level of organic matter, a reduction in turbidity, and a decrease in hardness (calcium and magnesium content). Some detrimental changes are lower oxygen levels due to decreased reaeration, decreased mixing, accumulation of pollutants, lack of bottom scour produced by flowing water scrubbing a stream bottom, and increased growth of algae. Algal growth may be enhanced when suspended solids settle from impounded water, causing increased exposure of the algae to sunlight. Stagnant water in the bottom of a reservoir may be of low quality. Oxygen levels frequently go to almost zero near the bottom, and odorous hydrogen sulfide is produced by the microbially mediated reduction of sulfur compounds in the low oxygen environment. Insoluble iron(III) and manganese(IV) species are reduced by bacterially-catalyzed reactions soluble iron(II) and manganese(II) ions, which must be removed prior to using the water.

Estuaries constitute another type of body of water, consisting of arms of the ocean into which streams flow. The mixing of fresh and salt water gives estuaries unique chemical and biological properties. Estuaries are the breeding grounds of much marine life, which makes their preservation very important.

Water's unique temperature-density relationship results in the formation of distinct layers within nonflowing bodies of water, as shown in Figure 7.4. During the summer a surface layer (**epilimnion**) is heated by solar radiation and, because of its lower density, floats upon the bottom layer, or **hypolimnion**. This phenomenon is called **thermal stratification.** When an appreciable temperature difference exists between the two layers, they do not mix but behave independently and have very different chemical and biological properties. Stratification of this sort is very important to microbial activity. The epilimnion, which is exposed to light, may have a heavy growth of algae. As a result of exposure to the atmosphere and (during daylight

hours) because of the photosynthetic activity of algae, the epilimnion contains relatively higher levels of dissolved oxygen and generally is aerobic. In the hypolimnion, bacterial action on biodegradable organic material may cause the water to become anaerobic. As a consequence, chemical species in a relatively reduced form tend to predominate in the hypolimnion.

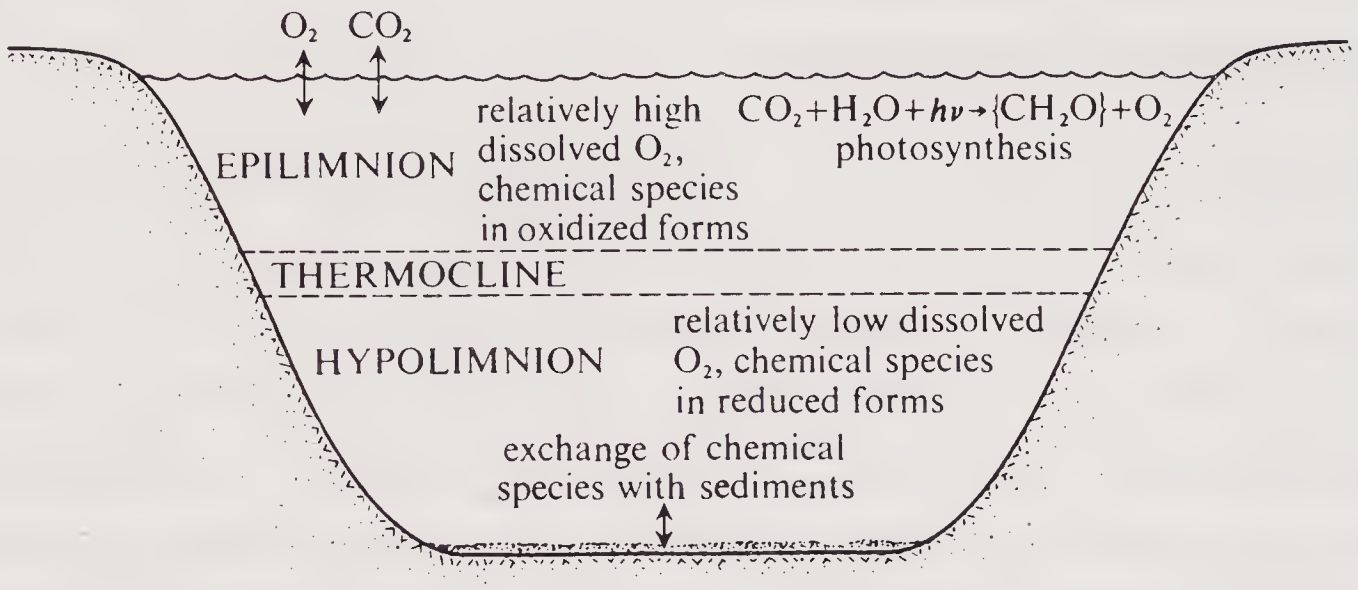


Figure 7.4. Stratification of a lake.

The shear-plane, or layer between epilimnion and hypolimnion, is called the **thermocline**. During the autumn, when the epilimnion cools, a point is reached at which the temperatures of the epilimnion and hypolimnion are equal. This disappearance of thermal stratification causes the entire body of water to behave as a hydrological unit, and the resultant mixing is known as **overturn**. An overturn also generally occurs in the spring. During the overturn, the chemical and physical characteristics of the body of water become much more uniform, and a number of chemical, physical, and biological changes may result. Overturn is especially important for both algae and bacteria because microbial activity tends to increase dramatically during and following overturn because of the mixing of nutrients.

The chemistry and biology of the Earth's vast oceans are unique because of the ocean's high salt content, great depth, and other factors. Oceanographic chemistry is a discipline in its own right. The environmental problems of the oceans have increased greatly in recent years because of ocean dumping of pollutants, oil spills, and increased utilization of natural resources from the oceans.

Aquatic Life

The living organisms (**biota**) in an aquatic ecosystem may be classified as either autotrophic or heterotrophic. **Autotrophic** biota utilize solar or chemical energy to fix elements from simple, nonliving inorganic material into complex life molecules that compose living organisms. Algae are typical autotrophic aquatic organisms. Generally, CO_2, NO_3^-, and $H_2PO_4^-/HPO_4^{2-}$ are sources of C, N, and P, respectively, for autotrophic organisms. Organisms that utilize solar energy to synthesize organic matter from inorganic materials are called **producers**.

Heterotrophic organisms utilize the organic substances produced by autotrophic organisms as energy sources and as the raw materials for the synthesis of their own

biomass. **Decomposers** (or **reducers**) are a subclass of the heterotrophic organisms and consist of chiefly bacteria and fungi, which ultimately break down material of biological origin to the simple compounds originally fixed by the autotrophic organisms.

The ability of a body of water to produce living material is known as its **productivity.** Productivity results from a combination of physical and chemical factors. Though required to maintain the food chain in a body of water, excessive productivity results in a set of conditions characterized by choking with excessive plant growth and reduction of aquatic oxygen levels to unhealthy levels. Such a condition is called **eutrophication**.

Life forms higher than algae and bacteria — fish, for example — comprise a comparatively small fraction of the biomass in most aquatic systems. The influence of these higher life forms upon aquatic chemistry is minimal, and they are not further considered here.

Turbulence is an important factor in mixing and transport processes in water. Some small organisms (**plankton**), largely microorganisms, depend upon water currents for their own mobility. Water turbulence is largely responsible for the transport of nutrients to living organisms and of waste products away from them. It plays a role in the transport of oxygen, carbon dioxide, and other gases through a body of water and in the exchange of these gases at the water-atmosphere interface. Moderate turbulence is generally beneficial to aquatic life.

Dissolved oxygen (DO) frequently is the key substance in determining the extent and kinds of life in a body of water. Oxygen deficiency is fatal to many aquatic animals such as fish. The presence of oxygen can be equally fatal to many kinds of anaerobic bacteria.

Biochemical oxygen demand, BOD, is another important water-quality parameter. It refers to the amount of oxygen utilized when the organic matter in a given volume of water is degraded biologically. A body of water with a high biochemical oxygen demand, and no means of rapidly replenishing the oxygen, obviously cannot sustain organisms that require oxygen.

Carbon dioxide is produced by respiratory processes in waters and sediments and can also enter water from the atmosphere. Carbon dioxide is required for the photosynthetic production of biomass by algae and in some cases is a limiting factor. High levels of carbon dioxide produced by the degradation of organic matter in water can cause excessive algal growth and productivity.

The levels of nutrients in water frequently determine its productivity. Aquatic plant life requires an adequate supply of carbon (CO_2), nitrogen (nitrate), phosphorus (orthophosphate), and trace elements such as iron. In many cases, phosphorus is the limiting nutrient and is generally controlled in attempts to limit excess productivity.

The salinity of water also determines the kinds of life forms present. Irrigation waters may pick up harmful levels of salt. Marine life obviously requires or tolerates salt water, whereas many freshwater organisms are intolerant of salt.

Groundwater

Groundwater (Figure 7.5) is a vital resource in its own right that plays a crucial role in geochemical processes, many of which are strongly influenced by microbial action. The nature, quality and mobility of groundwater are all strongly dependent upon the rock formations in which the water is held. Physically, an important characteristic of such formations is their **porosity**, which determines the percentage of

rock volume available to contain water. A second important physical characteristic is **permeability**, which describes the ease of flow of the water through the rock. High permeability is usually associated with high porosity. However, clays tend to have low permeability even when a large percentage of the volume is filled with water.

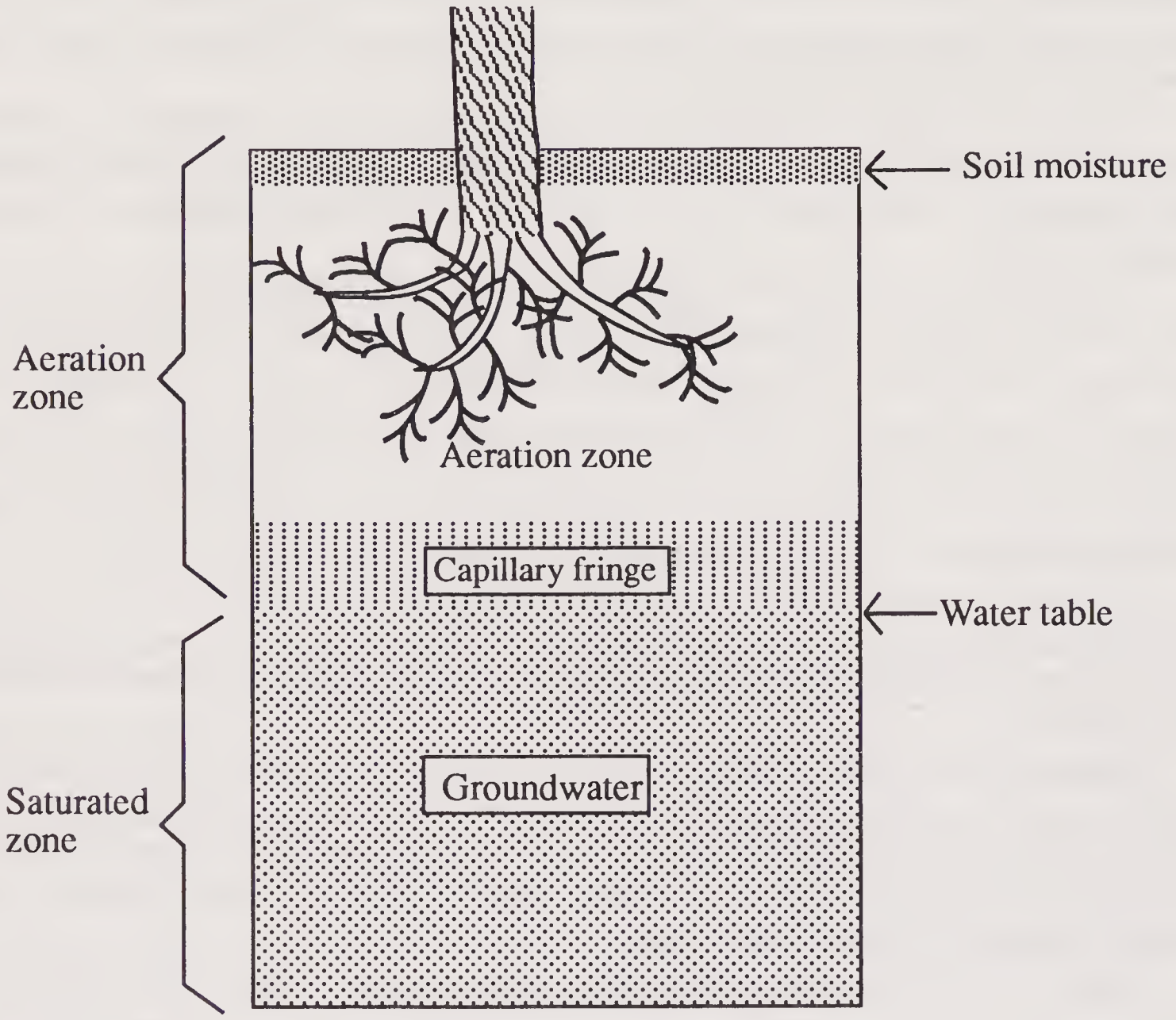


Figure 7.5. Some major features of the distribution of water underground.

The nature of water underground is illustrated by Figure 7.5. Most groundwater originates as **meteoritic** water from precipitation in the form of rain or snow. If water from this source is not lost by evaporation, transpiration, or to stream runoff, it may infiltrate into the ground. Initial amounts of water from precipitation onto dry soil are held very tightly as a film on the surfaces and in the micropores of soil particles in a **belt of soil moisture**. At intermediate levels, the soil particles are covered with films of water, but air is still present in larger voids in the soil. The region in which such water is held is called the **unsaturated zone** or **zone of aeration** and the water present in it is **vadose water**. At lower depths in the presence of adequate amounts of water, all voids are filled to produce a **zone of saturation**, the upper level of which is the **water table**. Water present in a zone of saturation is called **groundwater**. Because of its surface tension, water is drawn somewhat above the water table by capillary-sized passages in soil in a region called the **capillary fringe**.

7.10. MICOORGANISMS IN WATER

Microorganisms are of the utmost importance in water for a number of reasons as listed below:

- Through their ability to fix inorganic carbon, algae and photosynthetic bacteria are the predominant producers of the biomass that supports the rest of the food chain in bodies of water.
- As catalysts of aquatic chemical reactions, bacteria mediate most of the significant oxidation-reduction processes that occur in water.
- By breaking down biomass and mineralizing essential elements, especially nitrogen and phosphorus, aquatic microorganisms play a key role in nutrient cycling.
- Aquatic microorganisms are essential for the major biogeochemical cycles.
- Aquatic bacteria are responsible for the breakdown and detoxication of many xenobiotic pollutants that get into the hydrosphere.

The free-floating organisms, most of microscopic size, in water constitute *plankton*. Animal plankton are called **zooplankton**, free-floating algae labelled **phytoplankton**, and **bacterioplankton** consist of free-floating bacteria. The latter two kinds of plankton are the predominant food source for zooplankton, which serve as food for higher animals.

Although microscopic plankton predominate in nonflowing waters, microorganisms in streams and rivers tend to be attached to objects to keep from being swept away by the water flow. In relatively clear flowing water, for example, mats consisting of algae interspersed with bacteria are attached to bottom rocks. The sediments, or **benthos** regions of both streams and quiescent bodies of water tend to accumulate large bacterial populations. Here the bacteria feed upon plant and animal remains that deposit in the benthos.

The conditions of most natural waters are conducive to relatively small populations of microrganisms, typically around 1×10^6 cells per mL for bacteria in water. Much of the relatively small population of aquatic microorganisms is due to low nutrient concentrations. Higher levels of nutrients may even be inhibiting, and microorganisms for which this is true are called **oligotrophic**. Other water conditions that tend to contribute to such relatively low microorganism populations nonoptimum and variable temperature, pH values considerably different from neutral, and variations in light intensity.

Both aerobic and anaerobic bacteria are important in the hydrosphere. The bacteria in deep stratified bodies of water may be predominantly anaerobic. This is especially true during the winter months when neither mixing with air or photosynthetic activity is sufficient to provide oxygen required for aerobic bacteria. During the summer these two mechanisms may enable growth of aerobic bacteria in the epilimnion while anaerobic bacteria predominate in the hypolimnion.

Microbially Mediated Elemental Transitions and Cycles

The remainder of this chapter deals with microbially mediated elemental transitions and cycles, which are largely oxidation-reduction processes mediated for the most part by bacteria, although algae, fungi, and protozoa may be involved as well. Though emphasizing microbial reactions in water, consideration of elemental cycles must take account of soil microbial processes as well.

7.11. MICROBIAL TRANSFORMATIONS OF CARBON

The carbon cycle is represented in Figure 7.6. A relatively small, but highly significant, portion of global carbon is in the atmosphere as CO_2. A very large amount of carbon is present as minerals, particularly calcium and magnesium carbonates. Another fraction of carbon is fixed as petroleum and natural gas, with a much larger

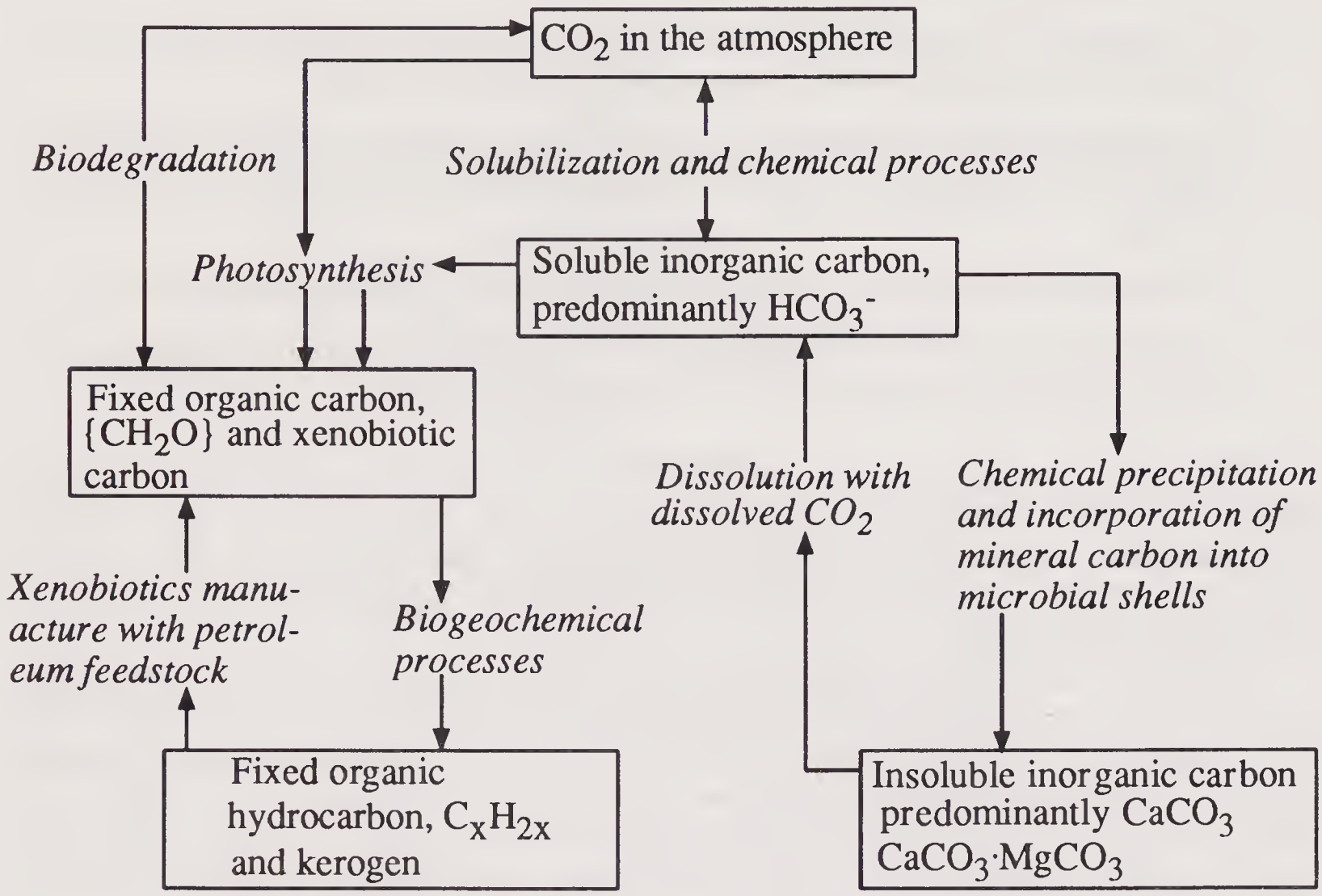


Figure 7.6. Important aspects of the biogeochemical carbon cycle.

amount as hydrocarbonaceous kerogen, coal, and lignite. Manufacturing processes are used to convert hydrocarbons to xenobiotic compounds with functional groups containing halogens, oxygen, nitrogen, phosphorus, or sulfur. Though a very small amount of total environmental carbon, these compounds are particularly significant because of their toxicological chemical effects.

Microorganisms are strongly involved in the carbon cycle, mediating crucial biochemical reactions discussed later in this section. Photosynthetic algae are the predominant carbon-fixing compounds in water; as they consume CO_2, the pH of the water is raised enabling precipitation of $CaCO_3$ and $CaCO_3 \cdot MgCO_3$. Organic carbon fixed by microorganisms is transformed by biogeochemical processes to fossil petroleum, kerogen, coal, and lignite. Microorganisms degrade organic carbon from biomass, petroleum, and xenobiotic sources, ultlimately returning it to the atmosphere as CO_2.

Photosynthesis

Carbon is an essential life element and composes a high percentage of the dry weight of microorganisms. For most microorganisms, the bulk of net energy-yielding or energy-consuming metabolic processes involve changes in the oxidation state of

carbon. These chemical transformations of carbon have important environmental implications. For example, when algae and other plants fix CO_2 as carbohydrate, represented as $\{CH_2O\}$,

$$CO_2 + H_2O \xrightarrow{h\nu} \{CH_2O\} + O_2(g) \qquad (7.11.1)$$

carbon changes from the +4 to the 0 oxidation state. Energy from sunlight is stored as chemical energy in organic compounds. However, when the algae die, bacterial decomposition results in the reverse of the biochemical process represented by the above reaction, energy is released, and oxygen is consumed.

Respiration

In the presence of oxygen, the principal energy-yielding reaction of bacteria is the oxidation of organic matter. Since it is generally more meaningful to compare reactions on the basis of the reaction of one electron-mole, the aerobic degradation of organic matter is conveniently written for the transfer of one mole of electrons,

$$-\frac{1}{4}\{CH_2O\} + -\frac{1}{4}O_2(g) \rightarrow -\frac{1}{4}CO_2 + -\frac{1}{4}H_2O \qquad (7.11.2)$$

for which the free-energy change is -29.9 kcal/electron-mole (number of kilocalories of free energy released when 1 mole, 6.02×10^{23}, electrons is transferred from carbon in CH_2O to produce carbon in CO_2). This general type of reaction is called **aerobic respiration**, and from it bacteria and other microorganisms extract the energy needed to carry out their metabolic processes; to synthesize new cell material; for reproduction; and for locomotion.

Degradation of Biomass by Soil Bacteria and Fungi

One of the most important functions of soil bacteria and fungi is the biodegradation of dead organic matter consisting predominantly of plant residues. In addition to preventing accumulation of excess waste residue, this composting process converts organic carbon, nitrogen, sulfur, and phosphorus to simple organic forms that can be utilized by plants and is a key part of the biogeochemical cycles of these elements. It also leaves a humus residue that is required for optimum physical form of soil.

Plants provide a rich source of food for microorganisms. The predominant biodegradable plant materials are cellulose and related hemicelluloses. Another polysaccharide, starch, is one of the most readily biodegraded plant products. In addition to other saccharidic substances, dead plant biomass includes biodegradable oil, fat, and wax lipids. Lignin, a relatively nonbiodegradable plant constituent that acts as sort of a "glue" to hold woody tissue together is nevertheless significant in the biodegradation of plant biomass. This is because the partial biodegradation products of lignin remain in soil and form crucial soil organic matter (humus).

Partial microbial decomposition of organic matter is a major step in the production of peat, lignite, coal, oil shale, and petroleum. Under reducing conditions, particularly below water, the oxygen content of the original plant material (approximate empirical formula, $\{CH_2O\}$) is lowered, leaving materials with relatively higher carbon contents.

Methane-Forming Bacteria

The production of methane in anoxic (oxygen-less) sediments is favored by high organic levels and low nitrate and sulfate levels. Methane production plays a key role in local and global carbon cycles as the final step in the anaerobic decomposition of organic matter. This process is the source of about 80% of the methane entering the atmosphere.

The carbon from microbially produced methane can come from either the reduction of CO_2 or the fermentation of organic matter, particularly acetate. The overall reaction for the anaerobic degradation of organic matter by methane-forming bacteria is the following, which involves a free-energy change of -5.55 kcal per electron-mole:

$$\frac{1}{4}\{CH_2O\} \rightarrow \frac{1}{8}CH_4 + \frac{1}{8}CO_2 \qquad (7.11.3)$$

This reaction, in reality a series of complicated processes, is a **fermentation reaction**, defined as an oxidation-reduction process in which both the oxidizing agent and reducing agent are organic substances. It may be seen that only about one-fifth as much free energy is obtained from one electron-mole of methane formation as from a one electron-mole reaction involving complete oxidation of one electron-mole of the organic matter, Reaction 7.11.2.

There are four main categories of methane-producing bacteria. These bacteria, differentiated largely by morphology, are *Methanobacterium*, *Methanobacillus*, *Methanococcus*, and *Methanosarcina*. The methane-forming bacteria are *obligately anaerobic*; that is, they cannot tolerate the presence of molecular oxygen. The necessity of avoiding any exposure to oxygen makes the laboratory culture of these bacteria very difficult.

Methane formation is a valuable process responsible for the degradation of large quantities of organic wastes, both in biological waste-treatment processes and in nature. Methane production is used in biological waste-treatment plants to further degrade excess sludge from the activated sludge process. In the bottom regions of natural waters, methane-forming bacteria degrade organic matter in the absence of oxygen. This eliminates organic matter which would otherwise require oxygen for its biodegradation.

Bacterial Utilization of Hydrocarbons

Methane is oxidized under aerobic conditions by a number of strains of bacteria. One of these, *Methanomonas*, is a highly specialized organism that cannot use any material other than methane as an energy source. Methanol, formaldehyde, and formic acid are intermediates in the microbial oxidation of methane to carbon dioxide. Several types of bacteria can degrade higher hydrocarbons and use them as energy and carbon sources.

Microbial Utilization of Carbon Monoxide

Carbon monoxide is removed from the atmosphere by contact with soil as a result of the activity of soil microorganisms. Fungi capable of CO metabolism include some commonly-occurring strains of the ubiquitous *Penicillium* and *Aspergillus*. It is also possible that some bacteria are involved in CO removal. Whereas some microorganisms metabolize CO, other aquatic and terrestrial organisms produce this gas.

Microbial Processes in Waste Degradation

Microbially mediated transformations of carbon are the most common means of treating municipal wastewater. Such wastes are treated in a bioreactor, of which the most widely used is the *activated sludge* process described in Section 2.8 and illustrated in Figure 2.5. In such a reactor wastes undergo *aerobic respiration*,

$$\{CH_2O\} + O_2(g) \rightarrow CO_2 + H_2O + \text{biomass} \qquad (7.11.4)$$

converting biodegradable organic carbon, $\{CH_2O\}$, to CO_2, which is evolved to the atmosphere, and to biomass, which settles out of the system. The net result is the removal of degradable potentially oxygen-consuming organic material.

The microbially active sludge in an activated sludge waste treatment process is based upon an organic matrix or **floc** held together by organic matter secreted by bacteria, particularly *Zooglea ramigera*. Organic matter in wastewater serves as a food source for the *Zooglea*, *Flavobacterium*, *Pseudomonas*, and *Vibrio*, bacteria in the floc, enabling it to increase in size. Protozoa feed on the bacteria in the floc and are an important part of the overall biodegradation process. Substantial amounts of carbon are released as CO_2 from the metabolic activities of both the bacteria and protozoa. After a significant residence time in the aeration tank of the waste treatment process the floc is allowed to settle, part is pumped back into the aeration tank to destroy additional organic matter, and part is taken to anaerobic digestion, later to be discarded as byproduct sewage sludge. Other kinds of sewage treatment processes, particularly the *trickling filter* process, make use of a fixed film of microorganisms on a support. In addition to bacteria, the microbial community in the film usually contains significant levels of algae and fungi.

7.12. MICROBIAL TRANSFORMATIONS OF NITROGEN

Some of the most important microorganism-mediated chemical reactions in aquatic and soil environments are those involving nitrogen compounds. They are summarized in the **nitrogen cycle** shown in Figure 7.7. This cycle describes the dynamic processes through which nitrogen is interchanged among the atmosphere, organic matter, and inorganic compounds. It is one of nature's most vital elemental cycles.

Among the biochemical transformations in the nitrogen cycle are *nitrogen fixation*, whereby molecular nitrogen is fixed as organic nitrogen; *nitrification*, the process of oxidizing ammonia to nitrate; *nitrate reduction*, the process by which nitrogen in nitrate ion is reduced to form compounds having nitrogen in a lower oxidation state; and *denitrification*, the reduction of nitrate and nitrite to N_2, with a resultant net loss of nitrogen gas to the atmosphere. Each of these important chemical processes will be discussed separately.

Nitrogen Fixation

The overall microbial process for the **nitrogen fixation**, the binding of atmospheric nitrogen in a chemically combined form,

$$3\{CH_2O\} + 2N_2 + 2H_2O + 4H^+ \rightarrow 3CO_2 + 4NH_4^+ \qquad (7.12.1)$$

is actually quite complicated and not completely understood. Biological nitrogen fixation is a key biochemical process in the environment and is essential for plant growth in the absence of synthetic fertilizers.

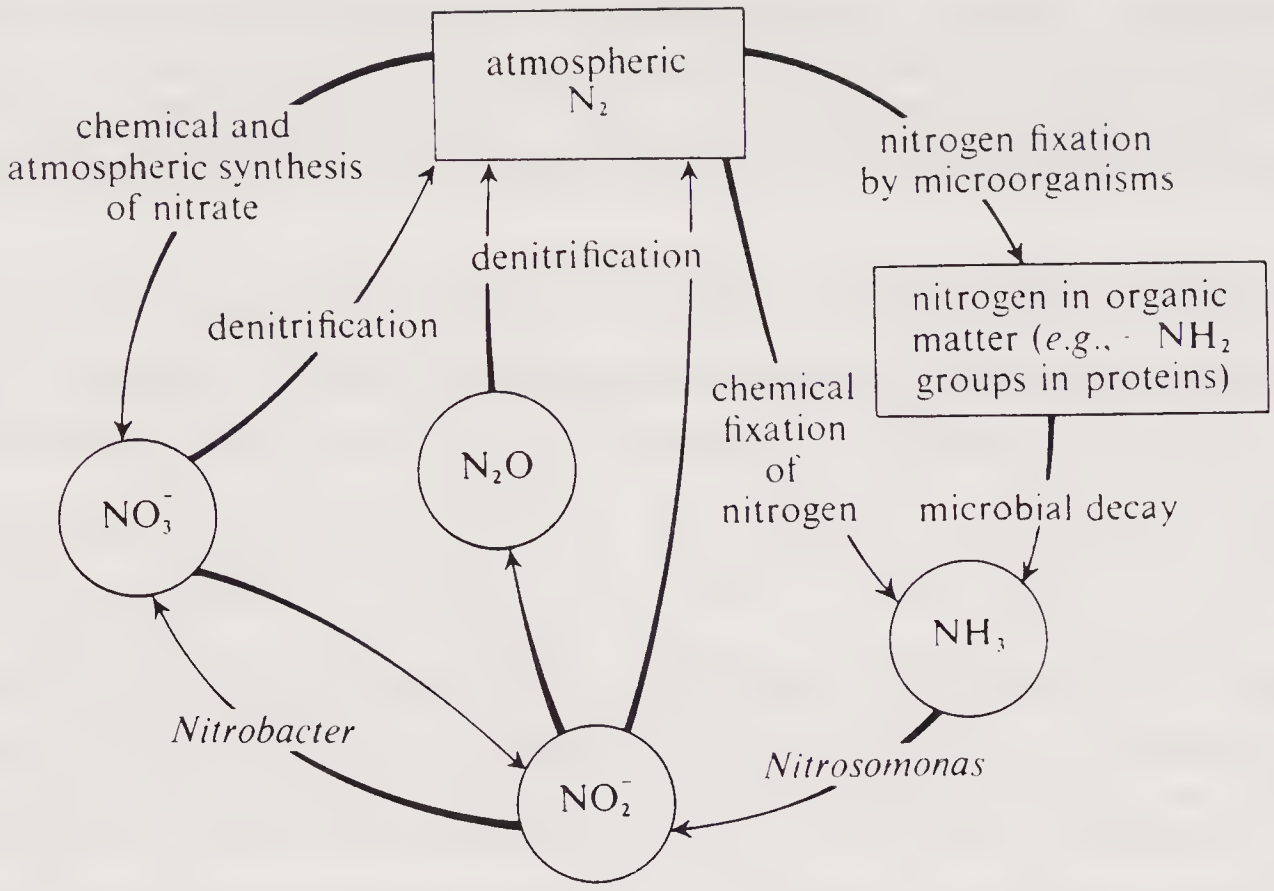


Figure 7.7. Aspects of the nitrogen cycle.

Only a few species of aquatic microorganisms have the ability to fix atmospheric nitrogen. Among the aquatic bacteria that can do so are photosynthetic bacteria, *Azotobacter*, and several species of *Clostridium*. The best-known and most important form of nitrogen-fixing bacteria is *Rhizobium*, which enjoys a symbiotic (mutually advantageous) relationship with leguminous plants such as clover or alfalfa. The *Rhizobium* bacteria are found in root nodules, special structures attached to the roots of legumes and connected directly to the vascular (circulatory) system of the plant, enabling the bacteria to derive photosynthetically produced energy directly from the plant. Thus, the plant provides the energy required to break the strong triple bonds in the dinitrogen molecule, converting the nitrogen to a reduced form which is directly assimilated by the plant. When the legumes die and decay, NH_4^+ ion is released and is converted by microorganisms to nitrate ion which is assimilable by other plants.

Some nonlegume angiosperms fix nitrogen through the action of actinomycetes bacteria contained in root nodules. Shrubs and trees in the nitrogen-fixing category are abundant in fields, forests, and wetlands throughout the world. Their rate of nitrogen fixation is comparable to that of legumes.

Nitrification

Nitrification, the conversion of N(-III) to N(V), is a very common and extremely important process in water and in soil. Aquatic nitrogen in thermodynamic equilibrium with air is in the +5 oxidation state as NO_2^-, whereas in most biological compounds, nitrogen is present as N(-III), such as $-NH_2$ in amino acids. The overall nitrification reaction, below, is highly favored from a thermodynamic viewpoint:

$$2O_2 + NH_4^+ \rightarrow NO_3^- + 2H^+ + H_2O \qquad (7.12.2)$$

Nitrification is especially important in nature because nitrogen is absorbed by plants primarily as nitrate. When fertilizers are applied in the form of ammonium salts or anhydrous ammonia, a microbial transformation to nitrate enables maximum assimilation of nitrogen by the plants.

In nature, nitrification is catalyzed by two groups of bacteria, *Nitrosomonas* and *Nitrobacter*. *Nitrosomonas* bacteria bring about the transition of ammonia to nitrite,

$$NH_3 + -\ 2O_2 \rightarrow H^+ + NO_3^- + H_2O \qquad (7.12.3)$$

whereas *Nitrobacter* mediate the oxidation of nitrite to nitrate:

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \qquad (7.12.4)$$

Both of these highly specialized types of bacteria are *obligate aerobes*; that is, they function only in the presence of molecular O_2. These bacteria are also *chemolithotrophic*, meaning that they can utilize oxidizable inorganic materials as electron donors in oxidation reactions to yield needed energy for metabolic processes.

For the aerobic conversion of one electron-mole of ammoniacal nitrogen to nitrite ion at pH 7.00,

$$\frac{1}{4}O_2 + \frac{1}{6}NH_4^+ \rightarrow \frac{1}{6}NO_2^- + \frac{1}{3}H^+ + \frac{1}{6}H_2O \qquad (7.12.5)$$

the free-energy change is -10.8 kcal. The free-energy change for the aerobic oxidation of one electron-mole of nitrite ion to nitrate ion,

$$\frac{1}{4}O_2 + \frac{1}{2}NO_2^- \rightarrow \frac{1}{2}NO_3^- \qquad (7.12.6)$$

is -9.0 kcal. Both steps of the nitrification process involve an appreciable yield of free energy.

Nitrate Formation

As a general term, **nitrate reduction** refers to microbial processes by which nitrogen in chemical compounds is reduced to lower oxidation states. In the absence of free oxygen, nitrate may be used by some bacteria as an alternate electron receptor. The most complete possible reduction of nitrogen in nitrate ion involves the acceptance of 8 electrons by the nitrogen atom, with the consequent conversion of nitrate to ammonia (+V to -III oxidation state). Nitrogen is an essential component of protein, and any organism that utilizes nitrogen from nitrate for the synthesis of protein must first reduce the nitrogen to the -III oxidation state (ammoniacal form). However, incorporation of nitrogen into protein generally is a relatively minor use of the nitrate undergoing microbially mediated reactions and is more properly termed nitrate assimilation.

Generally, when nitrate ion functions as an electron receptor, the product is NO_2^-:

$$\frac{1}{2}NO_3^- + \frac{1}{4}\{CH_2O\} \rightarrow \frac{1}{2}NO_2^- + \frac{1}{4}H_2O + \frac{1}{4}H_2O \qquad (7.12.7)$$

The free-energy yield per electron-mole is only about 2/3 of the yield when oxygen is the oxidant; however, nitrate ion is a good electron receptor in the absence of O_2. One of the factors limiting the use of nitrate ion in this function is its relatively low concentration in most waters. Furthermore, nitrite, NO_2^-, is relatively toxic and tends to inhibit the growth of many bacteria after building up to a certain level.

Denitrification

An important special case of nitrate reduction is **denitrification**, in which the reduced nitrogen product is a nitrogen-containing gas. At pH 7.00, the free-energy change per electron-mole of reaction,

$$\frac{1}{5}NO_3^- + \frac{1}{4}\{CH_2O\} + \frac{1}{5}H^+ \rightarrow \frac{1}{10}N_2 + \frac{1}{4}CO_2 + \frac{7}{20}H_2O \qquad (7.12.8)$$

is -2.84 kcal. Denitrification is an important process in nature. It is the mechanism by which fixed nitrogen is returned to the atmosphere. Denitrification is also useful in advanced water treatment for the removal of nutrient nitrogen. Because nitrogen gas is a nontoxic volatile substance that does not inhibit microbial growth, and since nitrate ion is a very efficient electron acceptor, denitrification allows the extensive growth of bacteria under anaerobic conditions.

Loss of nitrogen to the atmosphere may also occur through the formation of N_2O and NO by bacterial action on nitrate and nitrite catalyzed by the action of several types of bacteria. Production of N_2O relative to N_2 is enhanced during denitrification in soils by increased concentrations of NO_3^-, NO_2^-, and O_2.

7.13. MICROBIAL TRANSFORMATIONS OF SULFUR

Sulfur Cycle

Aspects of the sulfur cycle are shown in Figure 7.8. The major microbially mediated processes in this cycle are discussed in this section.

Sulfur Compounds

Sulfur compounds are very common in water and soil. Sulfate ion, SO_4^{2-}, is found in varying concentrations in practically all natural waters. Organic sulfur compounds, both those of natural origin and pollutant species, are very common in natural aquatic systems, and the degradation of these compounds is an important microbial process. Sometimes the degradation products, such as the odiferous and toxic H_2S, cause serious problems with water quality.

Oxidation of H_2S and Reduction of Sulfate by Bacteria

Although organic sulfur compounds often are the source of H_2S in water, H_2S commonly is produced by the microbial reduction of sulfate. The bacteria *Desulfovibrio* can reduce sulfate ion to H_2S. In so doing, they utilize sulfate as an electron acceptor in the oxidation of organic matter. The overall reaction is the following:

$$SO_4^{2-} + 2\{CH_2O\} + 2\,H^+ \rightarrow H_2S + 2CO_2 + 2H_2O \qquad (7.13.1)$$

Actually, other bacteria besides *Desulfovibrio* are required to oxidize organic matter completely to CO_2 using a sulfate electron acceptor. Both *Desulfuromonas* and *Desulfotomaculum* are involved in sulfate reduction to sulfide. Because of the high concentration of sulfate ion in sea water, bacterially mediated formation of H_2S causes pollution problems in some coastal areas and is a major source of atmospheric sulfur. In waters where sulfide formation occurs, the sediment is often black in color due to the formation of FeS.

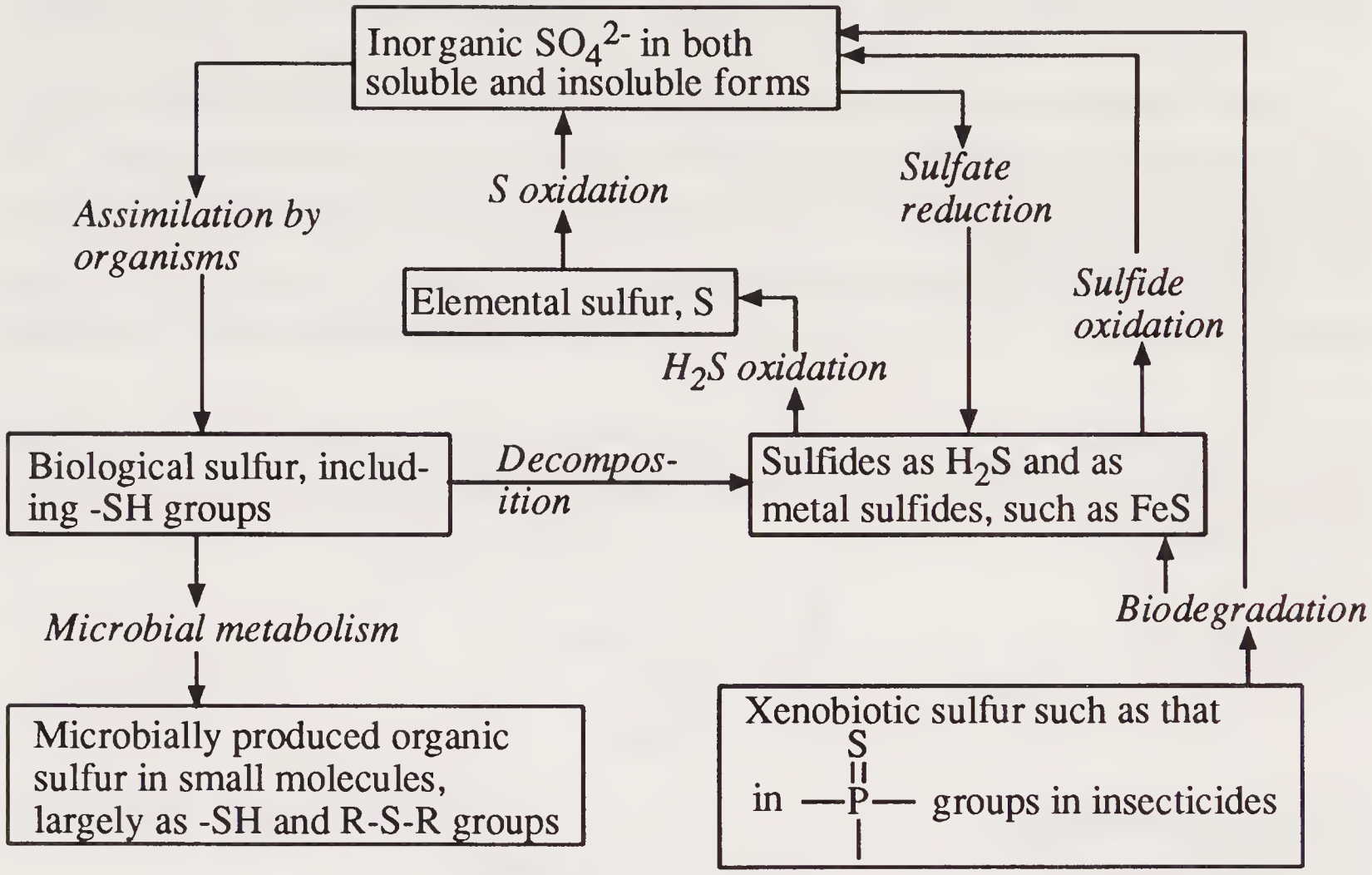


Figure 7.8. Important aspects of the sulfur cycle.

Whereas some bacteria can reduce sulfate ion to H_2S, others can oxidize hydrogen sulfide to higher oxidation states. Both photosynthetic bacteria (*Chromatium*, *Chlorobium*, *Thiocystis*, *Thiospirillum*) and nonphotosynthetic bacteria (*Beggiatoa*, *Sulfolobus*, *Thiobacillus*, *Thiomicrosporia*) oxidize H_2S. The purple sulfur bacteria and green sulfur bacteria derive energy for their metabolic processes through the oxidation of H_2S. These bacteria utilize CO_2 as a carbon source and are strictly anaerobic. The aerobic colorless sulfur bacteria may use molecular oxygen to oxidize H_2S,

$$2H_2S + O_2 \rightarrow 2S + 2H_2O \qquad (7.13.2)$$

elemental sulfur,

$$2S + 2H_2O + 3O_2 \rightarrow 4H^+ + 2SO_4^{2-} \qquad (7.13.3)$$

or thiosulfate ion:

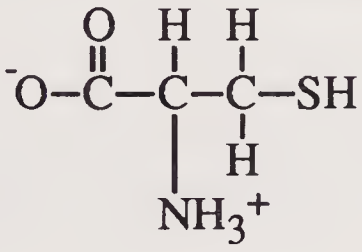
$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2H^+ + 2SO_4^{2-} \qquad (7.13.4)$$

Oxidation of sulfur in a low oxidation state to sulfate ion produces sulfuric acid, a strong acid. One of the colorless sulfur bacteria, *Thiobacillus thiooxidans* is tolerant of 1 normal acid solutions, a remarkable acid tolerance. This degree of acid tolerance enables bacteria such as *Thiobacillus thiooxidans* to produce, and thrive in, acidic waters that can cause severe environmental problems. The most significant of these is acid mine water produced by the microbially mediated oxidation of pyritic sulfur and discussed in Section 7.16.

Microorganism-Mediated Degradation of Organic Sulfur Compounds

Sulfur occurs in many types of biological compounds. As a consequence, organic sulfur compounds of natural and pollutant origin are very common in water. The degradation of these compounds is an important microbial process having a strong effect upon water quality.

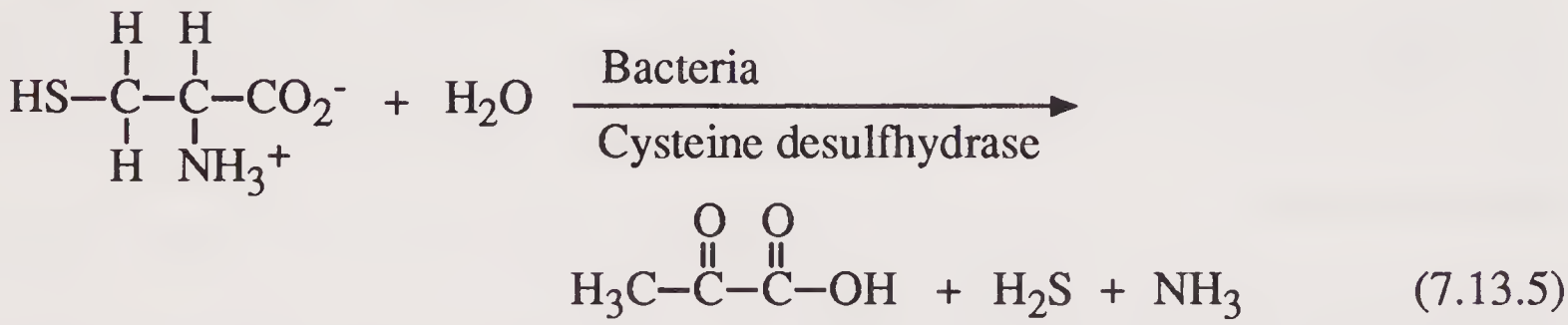
Among some of the common sulfur-containing functional groups found in aquatic organic compounds are hydrosulfide (–SH), disulfide (–SS–), sulfide (–S–), sulfoxide (), sulfonic acid ($-SO_2OH$), thioketone (), and thiazole (a hetero cyclic sulfur group). Protein contains some amino acids with sulfur functional groups — cysteine,



cystine, and methionine — whose breakdown is important in natural waters. The amino acids are readily metabolized by bacteria and fungi.

The biodegradation of sulfur-containing amino acids can result in production of volatile organic sulfur compounds such as methyl thiol, CH_3SH, and dimethyl disulfide, CH_3SSCH_3. These compounds have strong, unpleasant odors. Their formation, in addition to that of H_2S, accounts for much of the odor associated with the biodegradation of sulfur-containing organic compounds.

Hydrogen sulfide is formed from a large variety of organic compounds through the action of a number of different kinds of microorganisms. A typical sulfur-cleavage reaction producing H_2S is the conversion of cysteine to pyruvic acid through the action of cysteine desulfhydrase enzyme in bacteria:



(7.13.5)

Because of the numerous forms in which organic sulfur may exist, a variety of sulfur products and biochemical reaction paths must be associated with the biodegradation of organic sulfur compounds.

7.14. MICROBIAL TRANSFORMATIONS OF PHOSPHORUS

Phosphorus Compounds

Figure 7.9 illustrates important aspects of the phosphorus cycle. It involves natural and pollutant sources of phosphorus including biological, organic, and inorganic phosphorus. Soil and aquatic microbial processes are very important in the phosphorus cycle. Of particular importance is the fact that phosphorus is the most

common limiting nutrient in water, particularly for the growth of algae. Bacteria are even more effective than algae in taking up phosphate from water, accumulating it as excess cellular phosphorus that can be released to support additional bacterial growth if the supply of phosphorus becomes limiting. Microorganisms that die release phosphorus that can support additional organisms. Some bacteria are even "phosphorus-dissolving" in that they produce acidic substances that bring about the release of inorganic phosphorus from poorly soluble phosphorus minerals. Such bacteria are called **phosphoclastic** bacteria.

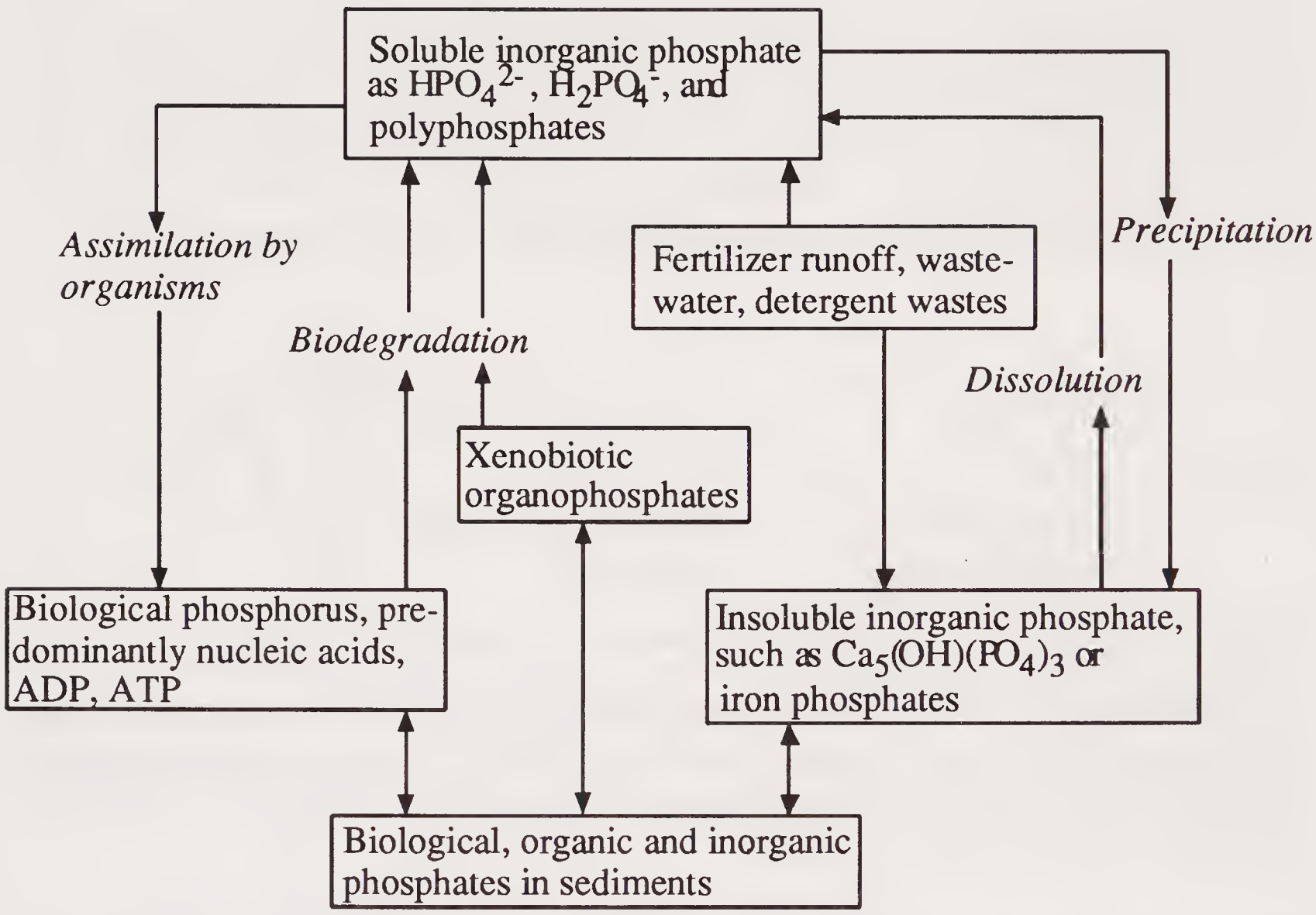
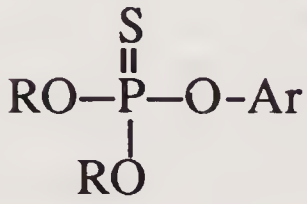


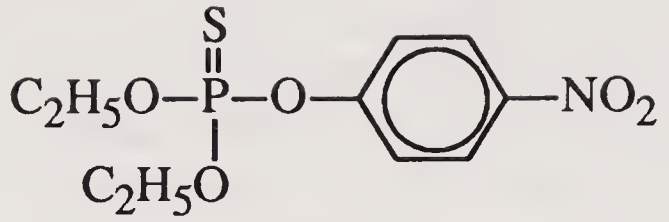
Figure 7.9. Important aspects of the phosphorus cycle.

Biodegradation of phosphorus compounds is important in the environment for two reasons. The first of these is that it is a *mineralization* process that releases inorganic phosphorus from the organic form thereby providing a source of algal nutrient orthophosphate and, secondly, biodegradation deactivates highly toxic organophosphate compounds, such as the organophosphate insecticides.

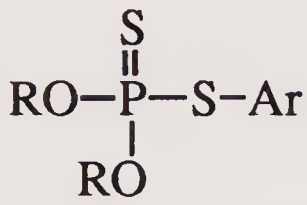
The organophosphorus compounds of greatest environmental concern tend to be sulfur-containing **phosphorothionate** and **phosphorodithioate** ester insecticides with the general formulas illustrated in Figure 7.10, where R represents a hydrocarbon or substituted hydrocarbon moiety. These are used because they exhibit higher ratios of insect:mammal toxicity than do their nonsulfur analogs. The metabolic conversion of P=S to P=O (oxidative desulfuration, such as in the conversion of parathion to paraoxon) in organisms is responsible for the insecticidal activity and mammalian toxicity of phosphorothionate and phosphorodithioate insecticides. The biodegradation of these compounds is an important environmental chemical process. Fortunately, unlike the organohalide insecticides that they largely displaced, the organophosphates readily undergo biodegradation and do not bioaccumulate.



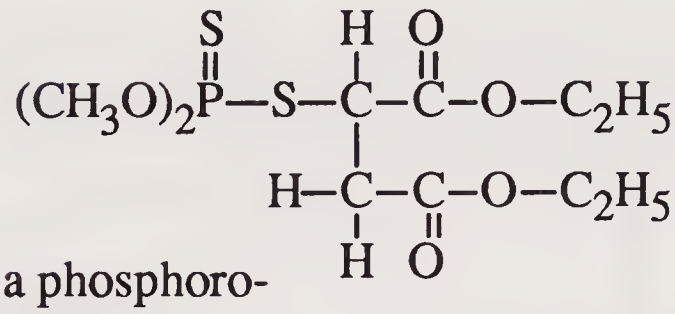
General formula of phosphorothionates



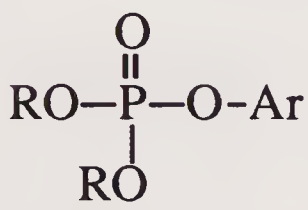
Parathion



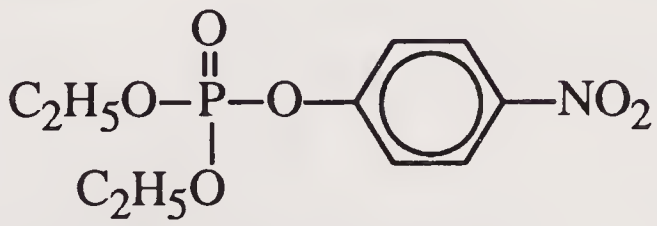
General formula of phosphorodithioates



Malathion, a phosphorodithioate insecticide



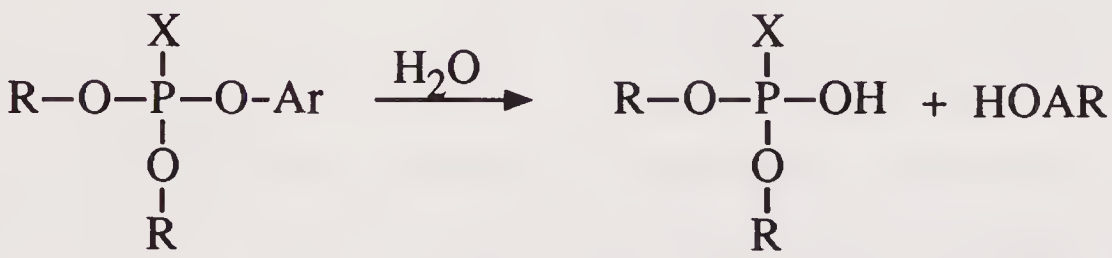
General formula of phosphate esters



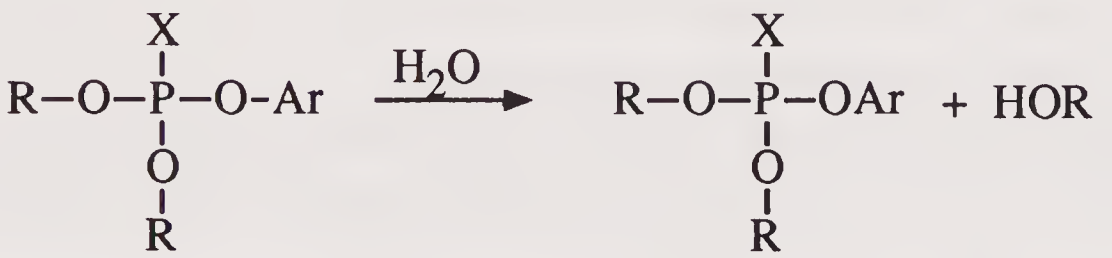
Paraoxon, a phosphate ester insecticide

Figure 7.10. Phosphorothionate, phosphorodithioate, and phosphate ester insecticides.

Hydrolysis is an important step in the biodegradation of phosphorothionate, phosphorodithioate, and phosphate ester insecticides as shown by the following general reactions where R is an alkyl group, Ar is a substituent group that is frequently aromatic, and X is either S or O:



(7.14.1)



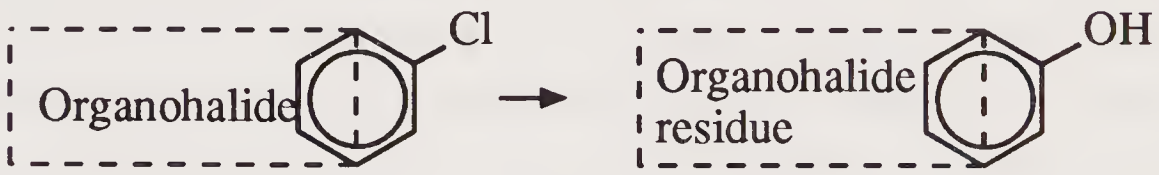
(7.14.2)

7.15. MICROBIAL TRANSFORMATIONS OF HALOGENS AND ORGANOHALIDES

Among the more important microbial processes that operate on pollutant xenobiotic compounds in soil and water are those involving the degradation of organohalide compounds. Such compounds, particularly the organochloride compounds, are among the more abundant air and water pollutants and hazardous waste constituents. Some

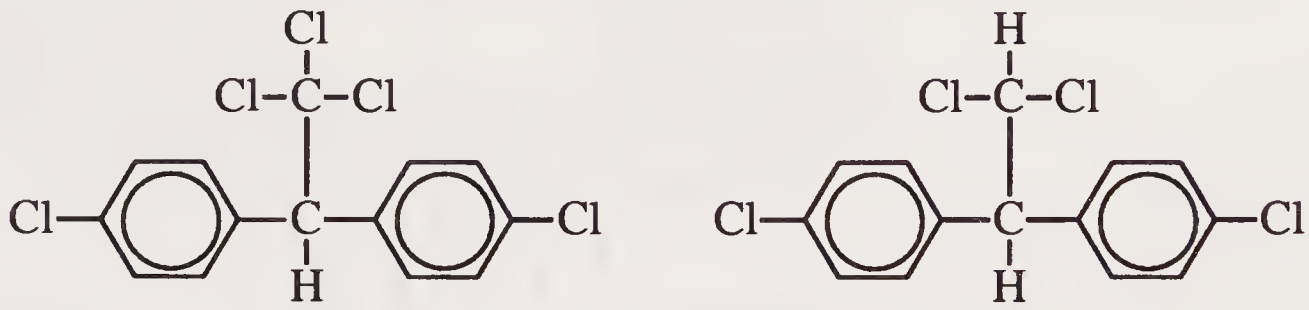
are relatively toxic and even carcinogenic, and they tend to accumulate in lipid tissues.

The key step in biodegradation of organohalide compounds is **dehalogenation** reactions, which involve the replacement of a halogen atom:



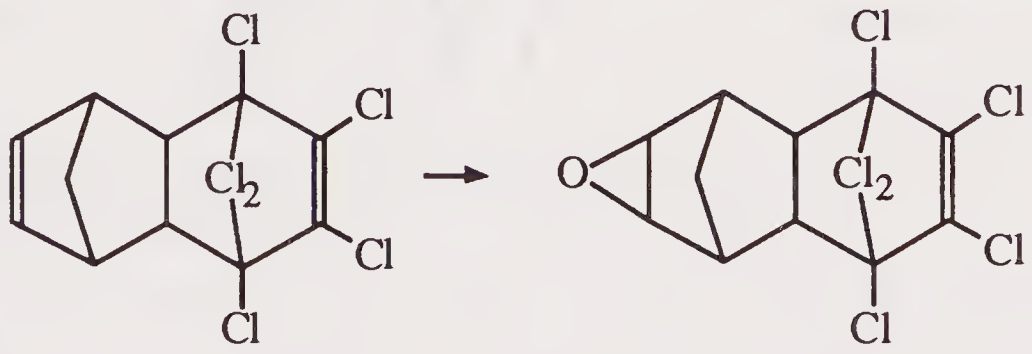
Microorganisms need not utilize a particular organohalide compound as a sole carbon source in order to affect its degradation. This is due to the phenomenon of **cometabolism**, which results from a lack of specificity in the microbial degradation processes. Thus, bacterial degradation of small amounts of an organohalide compound may occur while the microorganism involved is metabolizing much larger quantities of another substance.

Bioconversion of DDT to replace Cl with H yields DDD:



(7.15.1)

The latter compound is more toxic to some insects than DDT and is even manufactured as a pesticide. The same situation applies to microbially mediated conversion of aldrin to dieldrin:



(7.15..2)

7.16. MICROBIAL TRANSFORMATIONS OF METALS AND METALLOIDS

Some bacteria, including *Ferrobacillus*, *Gallionella*, and some forms of *Sphaerotilus*, utilize iron compounds in obtaining energy for their metabolic needs. These bacteria catalyze the oxidation of iron(II) to iron(III) by molecular oxygen:

$$2Fe(II) + 4H^+ + O_2 \rightarrow 4Fe(III) + 2H_2O \qquad (7.16.1)$$

The carbon source for some of these bacteria is CO_2. Since they do not require organic matter for carbon, and because they derive energy from the oxidation of inorganic matter, these bacteria may thrive in environments where organic matter is absent.

The microorganism-mediated oxidation of iron(II) is not a particularly efficient means of obtaining energy for metabolic processes. For the reaction

$$FeCO_3(s) + -\frac{1}{4}O_2 + -\frac{3}{2}H_2O \rightarrow 4Fe(OH)_3(s) + CO_2 \qquad (7.16.2)$$

the change in free energy is approximately 10 kcal/electron-mole. Approximately 220 g of iron(II) must be oxidized to produce 1.0 g of cell carbon. The calculation assumes CO_2 as a carbon source and a biological efficiency of 5%. The production of only 1.0 g of cell carbon would produce approximately 430 g of solid $Fe(OH)_3$, so that large deposits of hydrated iron(III) oxide form in areas where iron-oxidizing bacteria thrive.

Some of the iron bacteria, notably *Gallionella*, secrete large quantities of hydrated iron(III) oxide in the form of intricately branched structures. The bacterial cell grows at the end of a twisted stalk of the iron oxide. Individual cells of *Gallionella*, photographed through an electron microscope, have shown that the stalks consist of a number of strands of iron oxide secreted from one side of the cell (Figure 7.11).

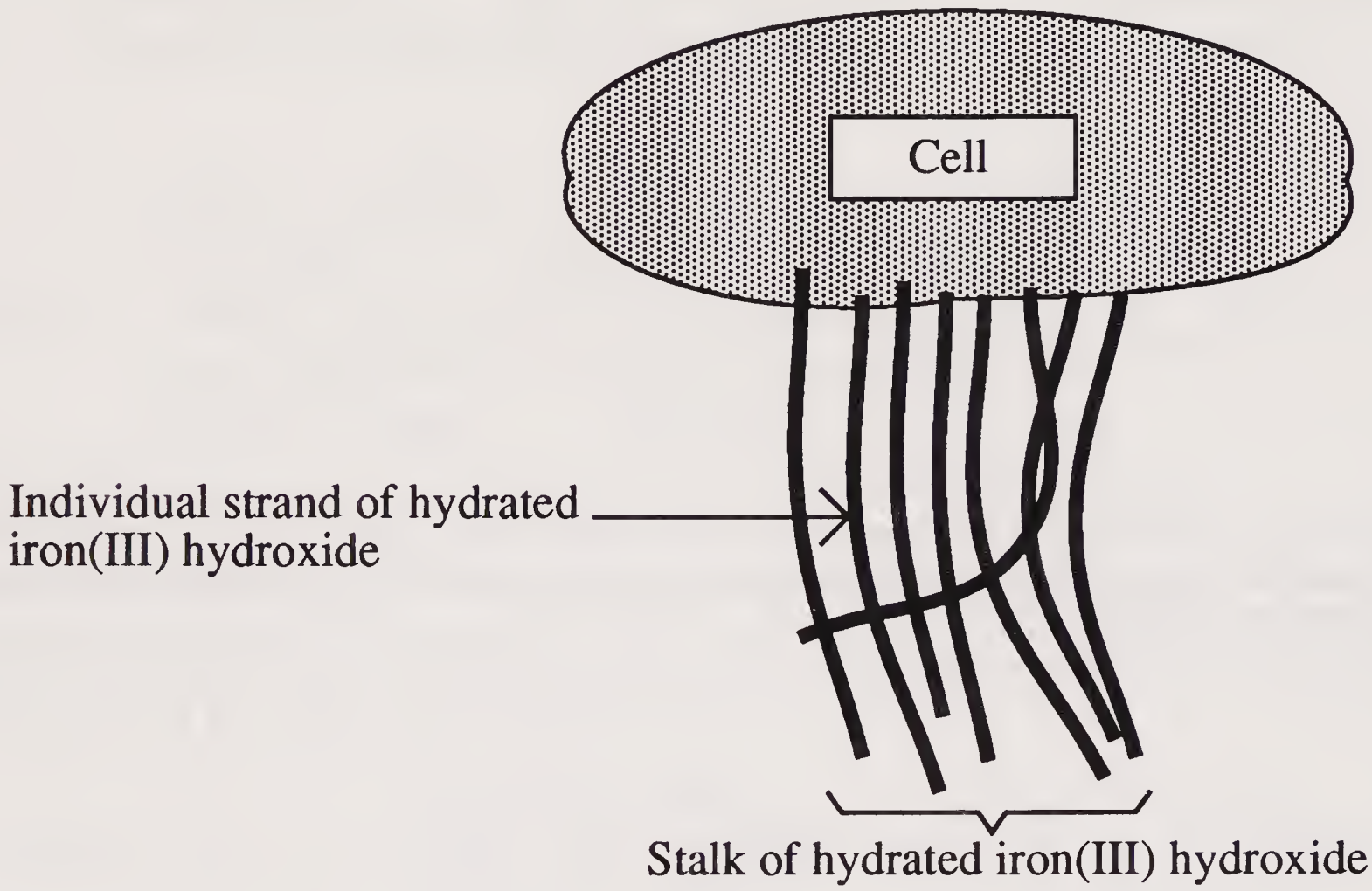


Figure 7.11. Sketch of a cell of *Gallionella* showing iron(III) oxide secretion.

At nearly neutral pH values, bacteria deriving energy by mediating the air oxidation of iron(II) must compete with direct chemical oxidation of iron(II) by O_2. The latter process is relatively rapid at pH 7. As a consequence, these bacteria tend to grow in a narrow layer in the region between the oxygen source and the source of iron(II). Therefore, iron bacteria are sometimes called *gradient organisms*, and they grow at intermediate pE values.

Acid Mine Waters

One consequence of bacterial action on metal compounds is acid mine drainage, one of the most common and damaging problems in the aquatic environment. Many waters flowing from coal mines and draining from the "gob piles" left over from coal processing and washing are practically sterile due to high acidity.

Acid mine water results from the presence of sulfuric acid produced by the oxidation of pyrite, FeS_2. Microorganisms are closely involved in the overall process, which consists of several reactions. The first of these reactions is the oxidation of pyrite:

$$2FeS_2(s) + 2H_2O + 7O_2 \rightarrow 4H^+ + 4SO_4^{2-} + 2Fe^{2+} \qquad (7.16.3)$$

The next step is the oxidation of ferrous ion to ferric ion,

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \qquad (7.16.4)$$

a process that occurs very slowly at the low pH values found in acid mine waters. Below pH 3.5, the iron oxidation is catalyzed by the iron bacterium *Thiobacillus* ferrooxidans, and in the pH range 3.5-4.5 it may be catalyzed by a variety of *Metallogenium*, a filamentous iron bacterium. Other bacteria that may be involved in acid mine water formation are *Thiobacillus thiooxidans* and *Ferrobacillus ferrooxidans*. The ferric ion further dissolves pyrite by chemical interaction,

$$2FeS_2(s) + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad (7.16.5)$$

which in conjunction with Reaction 5.11.4 constitutes a cycle for the dissolution of pyrite. $Fe(H_2O)_6^{3+}$ is an acidic ion and at pH values much above 3, the iron(III) precipitates as unsightly amorphous, semigelatinous hydrated iron(III) oxide:

$$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3(s) + 3H^+ \qquad (7.16.6)$$

Microbial Transitions of Selenium

Directly below sulfur in the periodic table, the metalloid selenium is subject to bacterial oxidation and reduction. These transitions are important because selenium is a crucial element in nutrition, particularly of livestock. Diseases related to either selenium excesses or deficiency have been reported in at least half of the states of the U.S. and in 20 other countries, including the major livestock-producing countries. Livestock in New Zealand, in particular, suffer from selenium deficiency.

Microorganisms are closely involved with the selenium cycle, and microbial reduction of oxidized forms of selenium has been known for some time. A soil-dwelling strain of *Bacillus megaterium* has been found to be capable of oxidizing elemental selenium to selenite, SeO_3^{2-} .

Microbial Transitions of Mercury

An interesting and significant aspect of mercury metabolism by bacteria that are resistant to the toxic effects of mercury compounds including inorganic $HgCl_2$, mercury chloride compounds(CH_3HgCl, C_2H_5HgCl, and C_3H_7HgCl), and phenylmercury(II) acetate ($C_6H_5HgOC(O)CH_3$) is the ability of these organisms to convert toxic mercury forms to volatile mercury(0).[2] Bacteria of *Bacillus*, *Pseudomonas*, and *Moraxella* genera have been found to have mercury(0)-volatization capabilities. Along with methylmercury formation by methylating bacteria, the bacterial production of volatile mercury(0) may play a significant role in the environmental mercury cycle.

LITERATURE CITED

1. "Soil Chemistry," Chapter 16 in *Environmental Chemistry*, Stanley E. Manahan, Lewis Publishers/CRC Press, Boca Raton, Florida, 1991, pp. 381-400.

2. Nakamura, Kunihiko, Taizo Sakata, and Hideomi, Nakahara, "Volatilization of Methylmercury–Volatizing Bacteria in Minimata Bay Sediment," *Bull. Environ. Contam. Toxicol.* **41**, 651-656 (1988).

QUESTIONS AND PROBLEMS

1. What are the characteristic layers of soil called? How might they be formed? Of these layers, which is the most microbiologically active?

2. What happens microbially when soil becomes waterlogged? How is this manifested chemically? Why do Fe^{2+} and Mn^{2+} become mobilized as a result?

3. What are some of the functions and effects of organic matter in soil? How might it contribute to weathering? Which is the most important of the organic components in soil?

4. What is the rhizosphere in soil? What does it have to do with bacteria? What benefits do rhizospheric bacteria have for plants through their interactions with plant roots?

5. List the effects of soil microorganisms on biodegradation of biomass, transorrmations of xenobiotic substances, mediation of oxidation-reduction reactions in soil.

6. In what sense are soil fungi complementary to soil bacteria in biodegradation processes?

7. Discuss any special significance of soil actinomycetes in the soil. What are their special properties?

8. From the standpoint of their environmental biochemistry, what is the most important characteristic of bacteria?

9. Compare the metabolic activities of soil bacteria that degrade common food materials to those that utilize petroleum products, humic material, and undegraded residues left over after more biodegradable constituents have been removed.

10. What kinds of soil microorganisms tend to predominate under acidic, low pH conditions?

11. Distinguish among psychrophilic, mesophilic, and thermophilic bacteria. Which of these tends to predominate? Why?

12. On what basis are the two major catergories of interactions between microorganisms divided? In this context define mutualism, protocooperation, competition, predation, and parasitism.

13. How does stratification of a body of water into an epilimnion and a hypolimnion influence microbial activity in the water?

14. Define each of the following and briefly list the role of each in aquatic environmental systems: Autotrophic, heterotrophic, productivity, eutrophication, bichemical oxygen demand.

15. What are some of the special environmental chemical and microbiological characteristics of groundwater?

16. In what respects are microorganisms "of the utmost importance" in water?

17. List two ways in which microbial activity has been involved in the formation of $CaCO_3$ deposits.

18. How are methane-producing bacteria utilized in wastewater treatment? Are they used for the direct treatment of wastewater or indirectly?

19. What particular role is played by *Zooglea ramigera* for wastewater treatment using the activated sludge process?

20. List the various kinds of microbially mediated processes, the reactants, and the products in the nitrogen cycle.

21. What important natural process is illustrated by the following reaction:

$$3\{CH_2O\} + 2N_2 + 2H_2O + 4H^+ \rightarrow 3\,CO_2 + 4NH_4^+$$

22. Give a reason for writing a microbially mediated reaction in the following form:

$$\frac{1}{4}O_2 + \frac{1}{6}NH_4^+ \rightarrow \frac{1}{6}NO_2^- + \frac{1}{3}H^+ + \frac{1}{6}H_2O$$

23. Explain how might the reaction below might be involved in pollutant production:

$$SO_4^{2-} + 2\{CH_2O\} + 2\,H^+ \rightarrow H_2S + 2CO_2 + 2H_2O$$

24. Give a "remarkable characteristic" of *Thiobacillus thiooxidans*.

25. What do phosphoclastic bacteria do?

26. What is microbial dehalogenation? Why is it important in the environment? How does it involve cometabolism?

27. Give the reactions and list the ways in which bacteria are involved in the production of acid mine waters.

8

Toxicology

8.1. INTRODUCTION

Poisons and Toxicology

A **poison**, or **toxicant**, is a substance that is harmful to living organisms because of its detrimental effects on tissues, organs, or biological processes. **Toxicology** is the science of poisons. These definitions are subject to a number of qualifications. Whether a substance is poisonous depends upon the type of organism exposed, the amount of the substance, and the route of exposure. In the case of human exposure, the degree of harm done by a poison can depend strongly upon whether the exposure is to the skin, by inhalation, or through ingestion. For example, a few parts per million of copper in drinking water can be tolerated by humans. However, at that level it is deadly to algae in their aquatic environment, whereas at a concentration of a few parts per *billion* copper is a required nutrient for the growth of algae. Subtle differences like this occur with a number of different kinds of substances.

History of Toxicology

The origins of modern toxicology can be traced to M. J. B. Orfila (1787-1853), a Spaniard born on the island of Minorca. In 1815 Orfila published a classic book,[1] the first ever devoted to the harmful effects of chemicals on organisms. This work discussed many aspects of toxicology recognized as valid today. Included are the relationships between the demonstrated presence of a chemical in the body and observed symptoms of poisoning, mechanisms by which chemicals are eliminated from the body, and treatment of poisoning with antidotes.

Since Orfila's time, the science of toxicology has developed at an increasing pace with advances in the basic biological, chemical, and biochemical sciences. Prominent among these advances are modern instruments and techniques for chemical analysis that provide the means for measuring chemical poisons and their metabolites at very low levels and with remarkable sensitivity, thereby greatly extending the capabilities of modern toxicology.

This chapter deals with toxicology in general, including the routes of exposure and clinically observable effects of toxic substances. The information is presented primarily from the viewpoint of human exposure and readily observed detrimental effects of toxic substances on humans. To a somewhat lesser extent this material applies to other mammals, especially those used as test organisms. It should be kept in mind that many of the same general principles discussed apply also to other of the more complex organisms, such as fish and even plants.

Although LD_{50} (as discussed in Section 8.2, the dose required to kill half of test subjects) is often the first parameter to come to mind in discussing degrees of toxicity, mortality is usually not a good parameter for toxicity measurement. Much more widespread than fatal poisoning, and certainly more subtle, are various manifestations of morbidity ("unhealthiness"). As discussed in this chapter, there are many ways in which morbidity is manifested. Some of these, such as effects on vital signs, are obvious. Others, such as some kinds of immune system impairment, can be observed only with sophisticated tests. Various factors must be considered, such as minimum dose or the latency period (often measured in years for humans) for an observable response to be observed. Furthermore, it is important to distinguish **acute toxicity**, which has an effect soon after exposure, and **chronic toxicity**, which has a long latency period.

Toxicological Chemistry

Toxicological chemistry relates chemistry to toxicology. It deals with the chemical nature of toxic substances, how they are changed biochemically, and how xenobiotic substances and their metabolites react biochemically in an organism to exert a toxic effect. Chapter 9 is devoted to defining and explaining toxicological chemistry and the last 10 chapters of the book cover the toxicological chemistry of various kinds of toxic substances.

Toxicity-Influencing Factors

Classification of Factors

It is useful to categorize the factors that influence toxicity within the three following classifications: (1) the toxic substance and its matrix, (2) circumstances of exposure and (3) the subject and its environment (see Figure 8.1). These are considered in the following sections.

Form of the Toxic Substance and its Matrix

Toxicants to which subjects are exposed in the environment or occupationally, particularly through inhalation, may be in several different physical forms. **Gases** are substances such as carbon monoxide in air that are normally in the gaseous state under ambient conditions of temperature and pressure. **Vapors** are gas-phase materials that can evaporate or sublime from liquids or solids. Benzene or naphthalene can exist in the vapor form. **Dusts** are respirable solid particles produced by grinding bulk solids, whereas **fumes** are solid particles from the condensation of vapors, often metals or metal oxides. **Mists** are liquid droplets.

Generally a toxic substance is in solution or mixed with other substances. A substance with which the toxicant is associated (the solvent in which it is dissolved or the solid medium in which it is dispersed) is called the **matrix**. The matrix may have a strong effect upon the toxicity of the toxicant.

Numerous factors may be involved with the toxic substance itself. If the substance is a toxic heavy metal cation, the nature of the anion with which it is associated can be crucial. For example, barium ion, Ba^{2+}, in the form of insoluble barium sulfate, $BaSO_4$, is routinely used as an X-ray-opaque agent in the gastrointestinal tract for diagnostic purposes (barium enema X-ray). This is a safe procedure; however, *soluble* barium salts such as $BaCl_2$ are deadly poisons when ntroduced into the gastrointestinal tract.

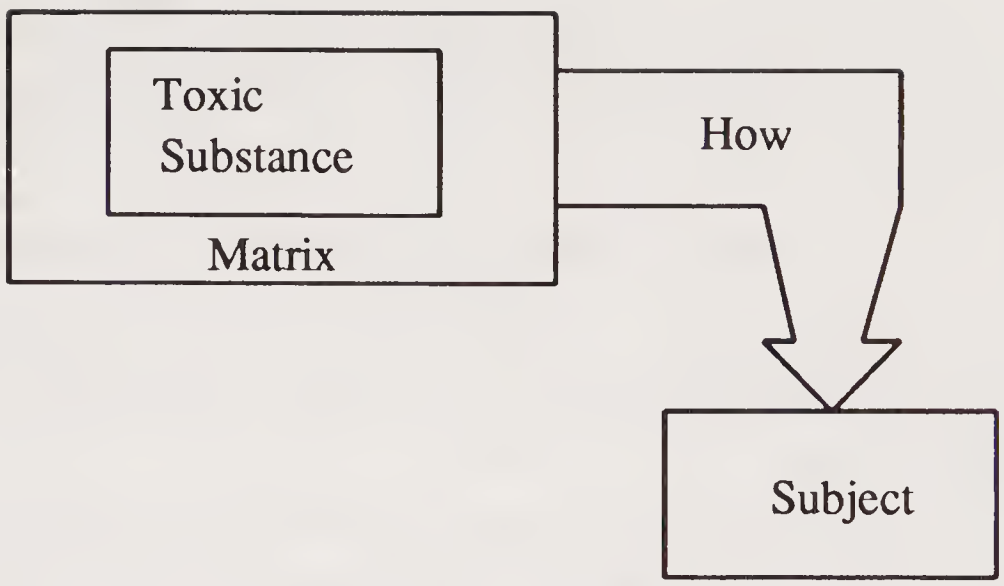


Figure 8.1. Toxicity is influenced by the nature of the toxic substances and its matrix, the subject exposed, and the conditions of exposure.

The pH of the toxic substance can greatly influence its absorption and, therefore, its toxicity. An example of this phenomenon is provided by aspirin, one of the most common causes of poisoning in humans. The chemical name of aspirin is sodium acetylsalicylate, the acidic form of which is acetylsalicylic acid (HAsc), a weak acid that ionizes as follows:

$$HAsc \rightleftharpoons H^+ + Asc^- \quad K_a = \frac{[H^+][Asc^-]}{[HAsc]} = 6 \times 10^{-4} \qquad (8.1.1)$$

The pK_a of HAsc is 3.2 and at a pH substantially below 3.2 most of this acid is in the neutral HAsc form. This neutral form is easily absorbed by the body, especially in the stomach, where the contents have a low pH of about 1. Many other toxic substances exhibit acid-base behavior and pH is a factor in their uptake

Solubility is an obvious factor in determining the toxicity of systemic poisons. These must be soluble in body fluids or converted to a soluble form in the organ or system through which they are introduced into the body. Some insoluble substances that are ingested pass through the gastrointestinal tract without doing harm, whereas they would be quite toxic if they could dissolve in body fluids (see the example of barium sulfate cited above).

As noted at the beginning of this section, the degree of toxicity of a substance may depend on its matrix. The solvent or suspending medium is called the **vehicle**. For laboratory studies of toxicity, several vehicles are commonly used. Among the most common of these are water and aqueous saline solution. Lipid-soluble substances may be dissolved in vegetable oils. Various organic liquids are used as vehicles. Dimethylsulfoxide is a solvent that has some remarkable abilities to carry a solute dissolved in it into the body. The two major classes of vehicles for insoluble substances are the natural gums and synthetic colloidal materials. Examples of the former are tragacanth and acacia, whereas methyl cellulose and carboxymethylcellulose are examples of the latter.

Some drug formulations contain **excipients** that have been added to give a desired consistency or form. In some combinations excipients have a marked influence upon toxicity. **Adjuvants** are excipients that may increase the effect of a toxic substance or enhance the pharmacologic action of a drug. For example, dithiocarbamate fungicides may have their activities increased by the addition of 2-mercaptothiazole.

A variety of materials other than those discussed above may be present in formulations of toxic substances. **Dilutents** increase bulk and mass. Common examples of these are salts, such as calcium carbonate and dicalcium phosphate; carbohydrates, including sucrose and starch; the clay kaolin; and milk solids. Among the **preservatives** used are sodium benzoate, phenylmercuric nitrate and butylated hydroxyanisole (an antioxidant). "Slick" substances such as cornstarch, calcium stearate, and talc act as **lubricants**. Various gums and waxes, starch, gelatin and sucrose are used as **binders**. Gelatin, carnauba wax and shellac are applied as **coating agents**. Cellulose derivatives and starch may be present as **disintegrators** in formulations containing toxicants.

Decomposition may affect the action of a toxic substance. Therefore the stability and storage characteristics of formulations containing toxicants should be considered. A toxic substance may be contaminated with other materials that affect toxicity. Some contaminants may result from decomposition.

Circumstances of Exposure

There are numerous variables related to the ways in which organisms are exposed to toxic substances. One of the most crucial of these, **dose,** is discussed in Section 8.3. Another important factor is the **toxicant concentration**, which may range from the pure substance (100%) down to a very dilute solution of a highly potent poison. Both the **duration** of exposure per exposure incident and the **frequency** of exposure are important. The **rate** of exposure, inversely related to the duration per exposure, and the total time period over which the organism is exposed are both important situational variables. The exposure **site** and **route** also affect toxicity.

It is possible to classify exposures on the basis of acute *vs.* chronic and local *vs.* systemic exposure, giving four general categories. **Acute local** exposure occurs at a specific location over a time period of a few seconds to a few hours and may affect the exposure site, particularly the skin, eyes or mucous membranes. The same parts of the body can be affected by **chronic local** exposure, but the time span may be as long as several years. **Acute systemic** exposure is a brief exposure or exposure to a single dose and occurs with toxicants that can enter the body, such as by inhalation or ingestion, and affect organs such as the liver that are remote from the entry site. **Chronic systemic** exposure differs in that the exposure occurs over a prolonged time period.

The Subject

The first of two major classes of factors in toxicity pertaining to the subject and its environment consists of **factors inherent to the subject**. The most obvious of these is the **taxonomic classification** of the subject, that is, the species and strain. With test animals it is important to consider the **genetic status** of the subjects, including whether they are littermates, half-siblings (different fathers), or the products of inbreeding. Body mass, sex, age, and degree of maturity are all factors in toxicity. **Immunological status** is important. Another area involves the general well-being of the subject. It includes disease and injury, diet, state of hydration, and the subject's "psychological state" as affected by the presence of other species and/or members of the opposite sex, crowding, handling, rest, and activity.

The other of the two major classes of factors related to the subject and its environment consists of **environmental factors**. Among these are ambient atmosphere conditions of temperature, pressure, and humidity, as well as composition

of the atmosphere, including the presence of atmospheric pollutants, such as ozone or carbon monoxide. Light and noise and the patterns in which they occur are important. Social and housing (caging) conditions may also influence response of subjects to a toxicant.

8.2. EXPOSURE TO TOXIC SUBSTANCES

Perhaps the first consideration in toxicology is **exposure** of an organism to a toxic substance. In discussing exposure sites for toxicants it is useful to consider the major routes and sites of exposure, distribution, and elimination of toxicants in the body as shown in Figure 8.2. The major routes of accidental or intentional exposure to toxicants by humans and other animals are the skin (percutaneous route), the lungs (inhalation, respiration, pulmonary route), and the mouth (oral route); minor routes of exposure are rectal, vaginal, and parenteral (intravenous or intramuscular, a common means for the administration of drugs or toxic substances in test subjects). The way that a toxic substance is introduced into the complex system of an organism is strongly dependent upon the physical and chemical properties of the substance. The pulmonary system is most likely to take in toxic gases or very fine, respirable solid or liquid particles. In other than a respirable form, a solid usually enters the body orally. Absorption through the skin is most likely for liquids, solutes in solution, and semisolids, such as sludges.

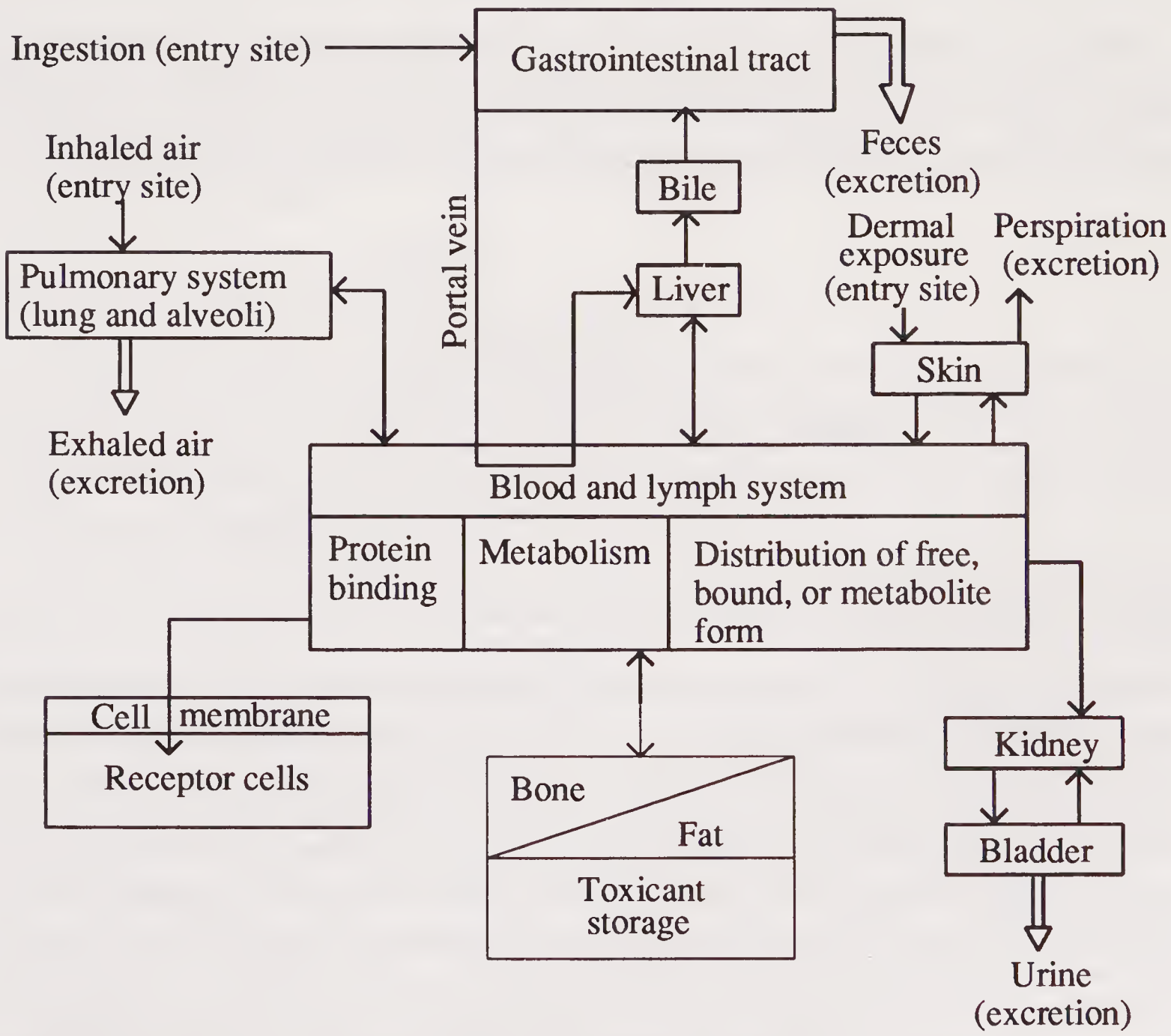


Figure 8.1. Major sites of exposure, metabolism, and storage, routes of distribution and elimination of toxic substances in the body.

The defensive barriers that a toxicant may encounter vary with the route of exposure. For example, elemental mercury is readily absorbed, often with devastating effect, through the alveoli in the lungs much more readily than through the skin or gastrointestinal tract. Most test exposures to animals are through ingestion or gavage (introduction into the stomach through a tube). Pulmonary exposure is often favored with subjects that may exhibit refractory behavior when noxious chemicals are administered by means requiring a degree of cooperation from the subject. Intravenous injection may be chosen for deliberate exposure when it is necessary to know the concentration and effect of a xenobiotic substance in the blood. However, pathways used experimentally that are almost certain not to be significant in accidental exposures can give misleading results when they avoid the body's natural defense mechanisms.

Percutaneous Exposure

Toxicants can enter the skin through epidermal cells, sebaceous gland cells, or hair follicles. By far the greatest area of the skin is composed of the epidermal cell layer, and most toxicants absorbed through the skin do so through epidermal cells. Despite their much smaller total areas, however, the cells in the follicular walls and in sebaceous glands are much more permeable than epidermal cells.

Skin Permeability

Figure 8.3 illustrates the absorption of a toxic substance through the skin and its entry into the circulatory system, where it may be distributed through the body. Often the skin suffers little or no harm at the site of entry of systemic poisons, which may act with devastating effects upon receptors far from the location of absorption.

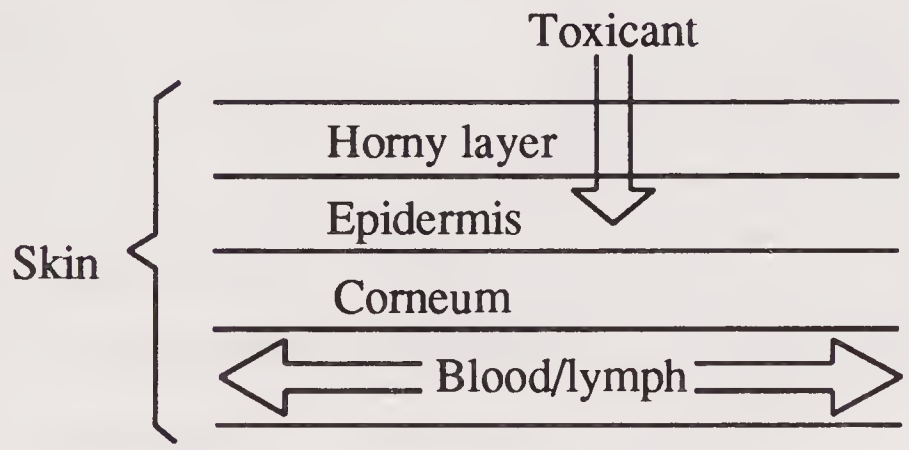


Figure 8.3. Absorption of a toxic substance through the skin.

The permeability of the skin to a toxic substance is a function of both the substance and the skin. The permeability of the skin varies with both the location and the species that penetrates it. In order to penetrate the skin significantly, a substance must be a liquid or gas or significantly soluble in water or organic solvents. In general, nonpolar, lipid-soluble substances traverse skin more readily than do ionic species. Substances that penetrate skin easily include lipid-soluble endogenous substances (hormones, vitamins D and K) as well as a number of xenobiotic compounds. Common examples of these are phenol, nicotine, and strychnine. Some military poisons, such as the "nerve gas" Sarin (see Section 18.8), permeate the skin very readily, which greatly adds to their hazards. In addition to the rate of transport through the skin, an additional factor that influences toxicity via the percutaneous route is the blood flow at the site of exposure.

Barriers to Skin Absorption

The major barrier to dermal absorption of toxicants is the **stratum corneum** or horny layer (see Figure 8.3). The permeability of skin is inversely proportional to the thickness of this layer, which varies by location on the body in the order soles and palms > abdomen, back, legs, arms > genital (perineal) area. Evidence of the susceptibility of the genital area to absorption of toxic substances is to be found in accounts of the high incidence of cancer of the scrotum among chimney sweeps in London described by Sir Percival Pott, Surgeon General of Britain during the reign of King George III. The cancer-causing agent was coal tar condensed in chimneys. This material was more readily absorbed through the skin in the genital areas than elsewhere leading to a high incidence of scrotal cancer. (The chimney sweeps' conditions were aggravated by their lack of appreciation of basic hygienic practices, such as bathing and regular changes of underclothing.) Breaks in epidermis due to laceration, abrasion, or irritation increase the permeability, as do inflammation and higher degrees of skin hydration.

Measurement of Dermal Toxicant Uptake

There are two principal methods for determining the susceptibility of skin to penetration by toxicants. The first of these is measurement of the dose of the substance received by the organism using chemical analysis, radiochemical analysis of radioisotope-labelled substances, or observation of clinical symptoms. Secondly, the amount of substance remaining at the site of administration may be measured. The latter approach requires control of nonabsorptive losses of the substance, such as those that occur by evaporation.

Pulmonary Exposure

The pulmonary system is the site of entry for numerous toxicants. Examples of toxic substances inhaled by human lungs include fly ash and ozone from polluted atmospheres, vapors of volatile chemicals used in the workplace, tobacco smoke, radioactive radon gas, and vapors from paints, varnishes, and synthetic materials used for building construction.

The major function of the lungs is to exchange gases between the bloodstream and the air in the lungs. This includes especially the absorption of oxygen by the blood and the loss of carbon dioxide. Gas exchange occurs in a vast number of alveoli in the lungs where a tissue the thickness of only one cell separates blood from air. The thin, fragile nature of this tissue makes the lungs especially susceptible to absorption of toxicants and to direct damage from toxic substances. Furthermore, the respiratory route enables toxicants entering the body to bypass organs that have a screening effect (the liver is the major "screening organ" in the body and it acts to detoxify numerous toxic substances). These toxicants can enter the bloodstream directly and be transported quickly to receptor sites with minimum intervention by the body's defense mechanisms.

As illustrated in Figure 8.4, there are several parts of the pulmonary system that can be affected by toxic substances. The upper respiratory tract consisting of the nose, throat, trachea, and bronchi retains larger particles that are inhaled. The retained particles may cause upper respiratory tract irritation. Cilia, which are small hair-like

appendages in the upper respiratory tract, move with a sweeping motion to remove captured particles. These substances are transported to the throat from which they may enter the gastrointestinal tract and be absorbed by the body. Gases such as ammonia (NH_3) and hydrogen chloride (HCl) that are very soluble in water are also removed from air predominantly in the upper respiratory tract and may be very irritating to tissue in that region.

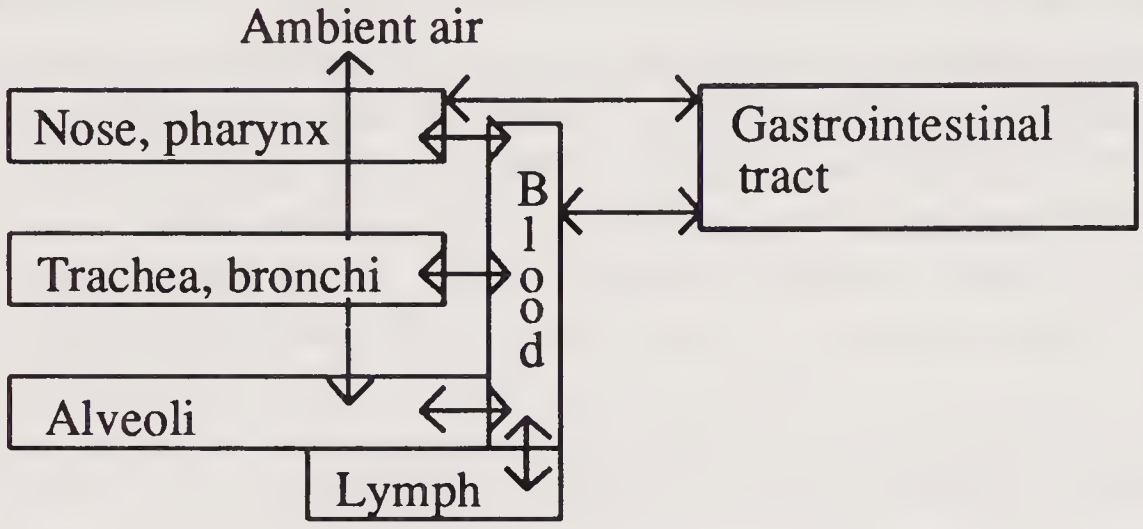


Figure 8.4. Pathways of toxicants in the respiratory system.

Gastrointestinal Tract

The gastrointestinal tract may be regarded as a tube through the body from the mouth to the anus, the contents of which are external to the rest of the organism system. Therefore, any systemic effect of a toxicant requires its absorption through the mucosal cells that line the inside of the gastrointestinal tract. Caustic chemicals can destroy or damage the internal surface of the tract and are viewed as nonkinetic poisons which act mainly at the site of exposure.

Mouth, Esophagus, and Stomach

Most substances are not readily absorbed in the mouth or esophagus; one of several exceptions is nitroglycerin administered for certain heart disfunctions and absorbed if left in contact with oral tissue. The stomach is the first part of the gastrointestinal tract where substantial absorption and translocation to other parts of the body may take place. The stomach is unique because of its high content of HCl and consequent low pH (about 1.0). Therefore, some substances that are ionic at pH near 7 and above are neutral in the stomach so that they readily traverse the stomach walls. In some cases absorption is affected by stomach contents other than HCl. These include food particles, gastric mucin, gastric lipase, and pepsin.

Intestines

The small intestine is effective in the absorption and translocation of toxicants. The pH of the contents of the small intestine is close to neutral, so that weak bases that are charged (HB^+) in the acidic environment of the stomach are uncharged (B) and absorbable in the intestine. The small intestine has a large surface area favoring absorption. Intestinal contents are moved through the intestinal tract by peristalsis. This has a mixing action on the contents and enables absorption to occur the length of the intestine. Some toxicants slow down or stop peristalsis (paralytic ileus), thereby slowing the absorption of the toxicant itself.

The Intestinal Tract and the Liver

The intestine/blood/liver/bile loop constitutes the **enterohepatic circulation** system (see Figure 8.5). A substance absorbed through the intestines goes either directly to the lymphatic system or to the **portal circulatory system**. The latter carries

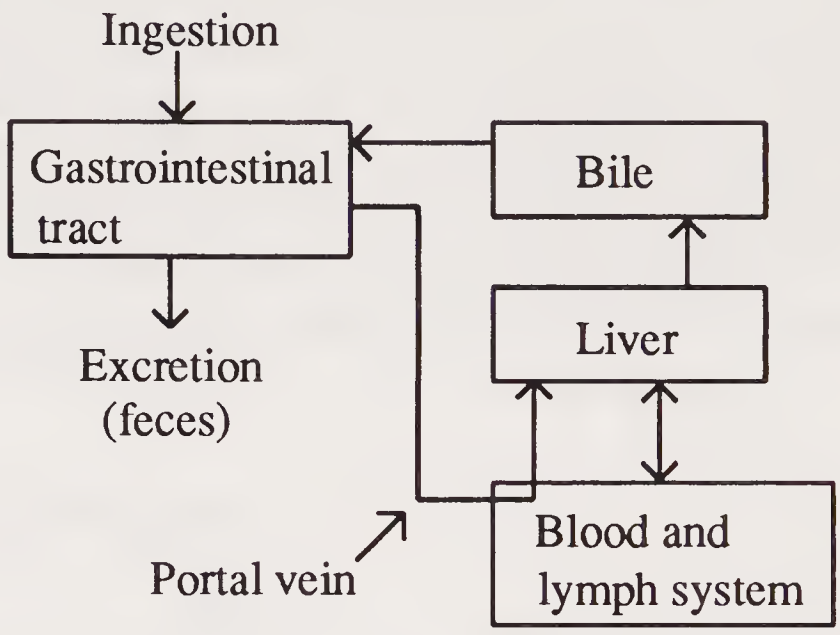


Figure 8.5. Representation of enterohepatic circulation.

blood to the portal vein that goes directly to the liver. The liver serves as a screening organ for xenobiotics, subjecting them to metabolic processes that usually reduce their toxicities, and secretes these substances or a metabolic product of them back to the intestines. For some substances there are mechanisms of active excretion into the bile in which the substances are concentrated by 1–3 orders of magnitude over levels in the blood. Other substances enter the bile from blood simply by diffusion.

8.3. DOSE-RESPONSE RELATIONSHIPS

Toxicants have widely varying effects upon organisms. Quantitatively, these variations include minimum levels at which the onset of an effect is observed, the sensitivity of the organism to small increments of toxicant, and levels at which the ultimate effect (particularly death) occurs in most exposed organisms. Some essential substances, such as nutrient minerals, have optimum ranges above and below which detrimental effects are observed.

Factors such as those just outlined are taken into account by the **dose-response** relationship, which is one of the key concepts of toxicology.[2] **Dose** is the amount, usually per unit body mass, of a toxicant to which an organism is exposed. **Response** is the effect upon an organism resulting from exposure to a toxicant. In order to define a dose-response relationship, it is necessary to specify a particular response, such as death of the organism, as well as the conditions under which the response is obtained, such as the length of time from administration of the dose. Consider a specific response for a population of the same kinds of organisms. At relatively low doses, none of the organisms exhibits the response (for example, all live) whereas at higher doses, all of the organisms exhibit the response (for example, all die). In between, there is a range of doses over which some of the organisms respond in the specified manner and others do not, thereby defining a dose-response curve. Dose-response relationships differ among different kinds and strains of organisms, types of tissues, and populations of cells.

Figure 8.6 shows a generalized dose-response curve. Such a plot may be obtained, for example, by administering different doses of a poison in a uniform manner to a homogeneous population of test animals and plotting the cumulative percentage of deaths as a function of the log of the dose. The result is normally an S-shaped curve as shown in Figure 8.6. The dose corresponding to the mid-point (inflection point) of such a curve is the statistical estimate of the dose that would cause death in 50 percent of the subjects and is designated as LD_{50}. The estimated doses at which 5 percent (LD_5) and 95 percent (LD_{95}) of the test subjects die are obtained from the graph by reading the dose levels for 5 percent and 95 percent fatalities, respectively. A relatively small difference between LD_5 and LD_{95} is reflected by a steeper S-shaped curve and vice versa. Statistically, 68 percent of all values on a dose-response curve fall within ± 1 standard deviation of the mean at LD_{50} and encompass the range from LD_{16} to LD_{84}.

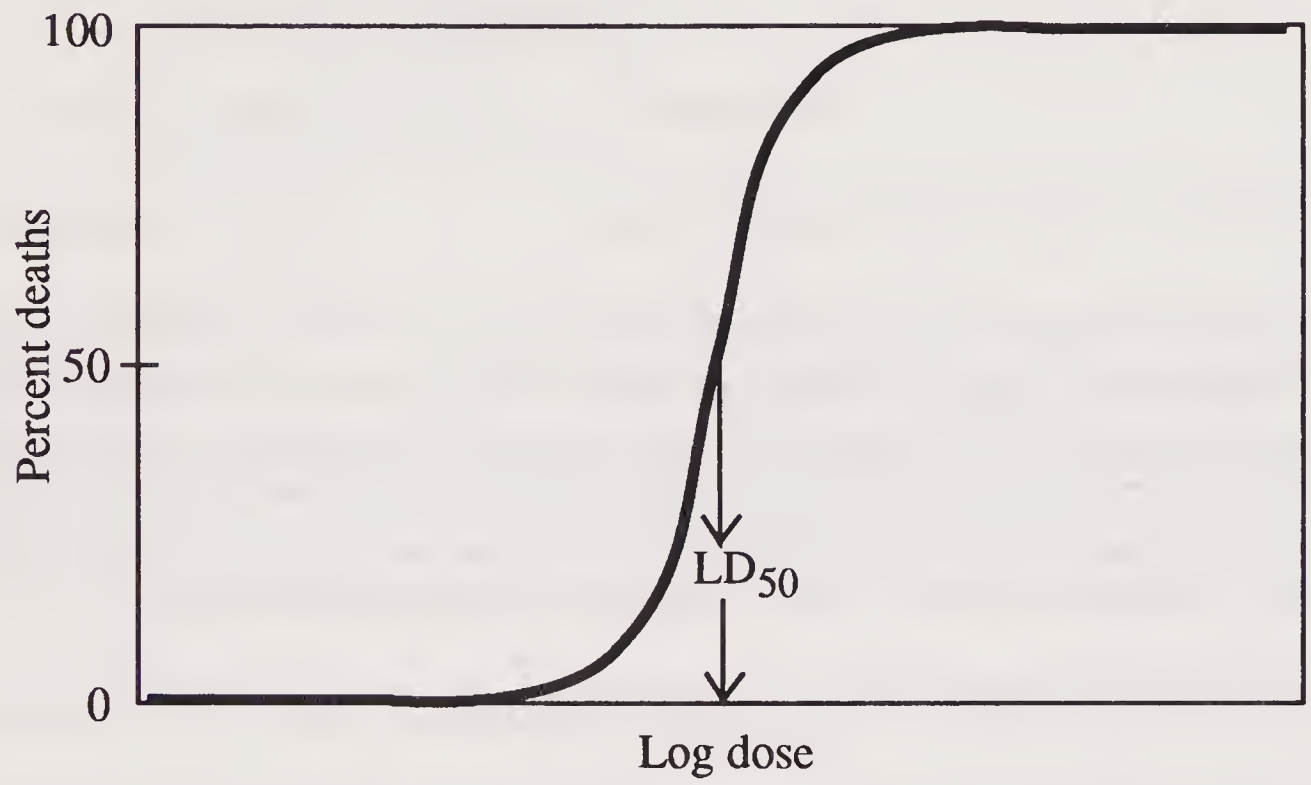


Figure 8.6. Illustration of a dose-response curve in which the response is the death of the organism. The cumulative percentage of deaths of organisms is plotted on the Y axis.

8.4. RELATIVE TOXICITIES

Table 8.1 illustrates standard **toxicity ratings** that are used to describe estimated toxicities of various substances to humans. Reference is made to them in this book to denote toxicities of substances. Their values range from 1 (practically nontoxic) to 6 (supertoxic). In terms of fatal doses to an adult human of average size, a "taste" of a supertoxic substances (just a few drops or less) is fatal. A teaspoonful of a very toxic substance could have the same effect. However, as much as a quart of a slightly toxic substance might be required to kill an adult human.

When there is a substantial difference between LD_{50} values of two different substances, the one with the lower value is said to be the more **potent**. Such a comparison must assume that the dose-response curves for the two substances being compared have similar slopes (see Figure 8.6). If this is not the case, the substance for which the dose-response curve has the lesser slope may be toxic at a low dose where the other substance is not toxic at all. Put another way, the relative LD_5s of the substances may be reversed from the relative LD_{50}s.

Table 8.1. Toxicity Scale with Example Substances.*

Substance	Approximate LD_{50}	Toxicity rating
	-10^5	1. Practically nontoxic > 1.5 x 10^4 mg/kg
DEHP[1] →	–	
Ethanol →	-10^4	2. Slightly toxic, 5 x 10^3 – 1.5 x 10^4 mg/kg
Sodium chloride →	–	
Malathion →	-10^3	3. Moderately toxic, 500 – 5000 mg/kg
Chlordane →	–	
Heptachlor →	-10^2	4. Very toxic, 50 – 500 mg/kg
	–	
Parathion →	– 10	5. Extremely toxic, 5 – 50 mg/kg
	–	
TEPP[2] →	– 1	
	–	
Tetrodotoxin[3] →	– 10^{-1}	
	–	
	– 10^{-2}	6. Supertoxic, <5 mg/kg
	–	
TCDD[4] →	– 10^{-3}	
	–	
	– 10^{-4}	
	–	
Botulinus toxin →	– 10^{-5}	

[1] Bis(2-ethylhexyl)phthalate; 2 Tetraethylpyrophosphate; 3 toxin from pufferfish; 4 TCDD represents 2,3,7,8,-tetrachlorodibenzodioxin, commonly called "dioxin."

* Doses are in units of mg of toxicant per kg of body mass. Toxicity ratings on the right are given as numbers ranging from 1 (practically nontoxic) through 6 (supertoxic) along with estimated lethal oral doses for humans in mg/kg. Estimated LD50 values for substances on the left have been measured in test animals, usually rats, and apply to oral doses.

Nonlethal Effects

So far, toxicities have been described primarily in terms of the ultimate effect, that is, deaths of organisms, or lethality This is obviously an irreversible consequence of exposure. In many, and perhaps most, cases, **sublethal** and **reversible** effects are of greater importance. This is obviously true of drugs, where death from exposure to a registered therapeutic agent is rare, but other effects, both detrimental and beneficial, are usually observed. By their very nature, drugs alter biologic processes; therefore, the potential for harm is almost always present. The major consideration in establishing drug dose is to find a dose that has an adequate therapeutic effect without undesirable side effects. A dose-response curve can be established for a drug that progresses from noneffective levels through effective, harmful, and even lethal levels. A low slope for this curve indicates a wide range of effective dose and a wide **margin of safety** (see Figure 8.7). This term applies to other substances, such as pesticides, for which it is desirable to have a large difference between the dose that kills a target species and that which harms a desirable species.

8.5. REVERSIBILITY AND SENSITIVITY

Sublethal doses of most toxic substances are eventually eliminated from an organism's system. If there is no lasting effect from the exposure, it is said to be **reversible**. However, if the effect is permanent, it is termed **irreversible**. Irreversible effects of exposure remain after the toxic substance is eliminated from the organism. Figure 8.7 illustrates these two kinds of effects. For various chemicals and different subjects, toxic effects may range from the totally reversible to the totally irreversible.

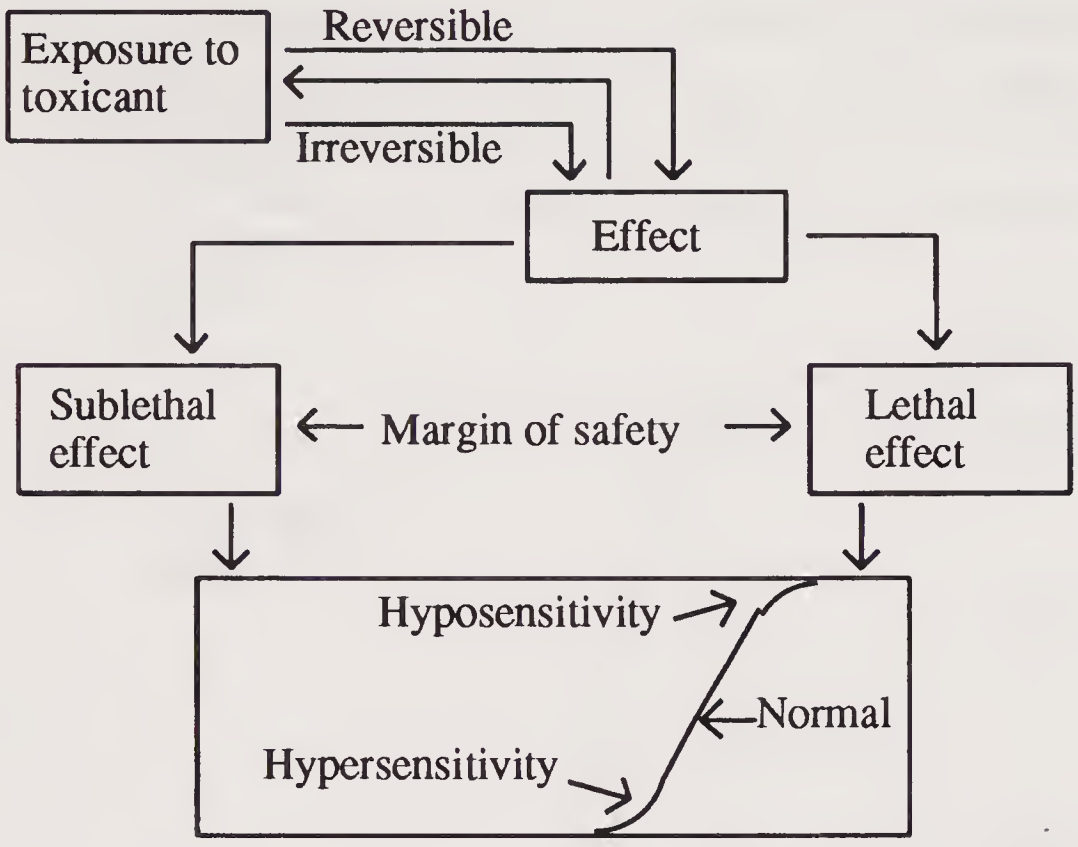


Figure 8.7. Effects of and responses to toxic substances.

Hypersensitivity and Hyposensitivity

Examination of the dose-response curve shown in Figure 8.6 reveals that some subjects are very sensitive to a particular poison (for example, those killed at a dose corresponding to LD_5), whereas others are very resistant to the same substance (for example, those surviving a dose corresponding to LD_{95}). These two kinds of responses illustrate **hypersensitivity** and **hyposensitivity**, respectively; subjects in the mid-range of the dose-response curve are termed **normals**. These variations in response tend to complicate toxicology in that there is not a specific dose guaranteed to yield a particular response, even in a homogeneous population.

In some cases hypersensitivity is induced. After one or more doses of a chemical, a subject may develop an extreme reaction to it. This occurs with penicillin, for example, in cases where people develop such a severe allergic response to the antibiotic that exposure results in death if countermeasures are not taken.

8.6. XENOBIOTIC AND ENDOGENOUS SUBSTANCES

Xenobiotic substances are those that are foreign to a living system, whereas those that occur naturally in a biologic system are termed **endogenous**. Endogenous substances are usually required within a particular concentration range in order for metabolic processes to occur normally. Levels below a normal range may result in a toxic response or even death, and the same effects may occur above the normal range. This kind of response is illustrated in Figure 8.8.

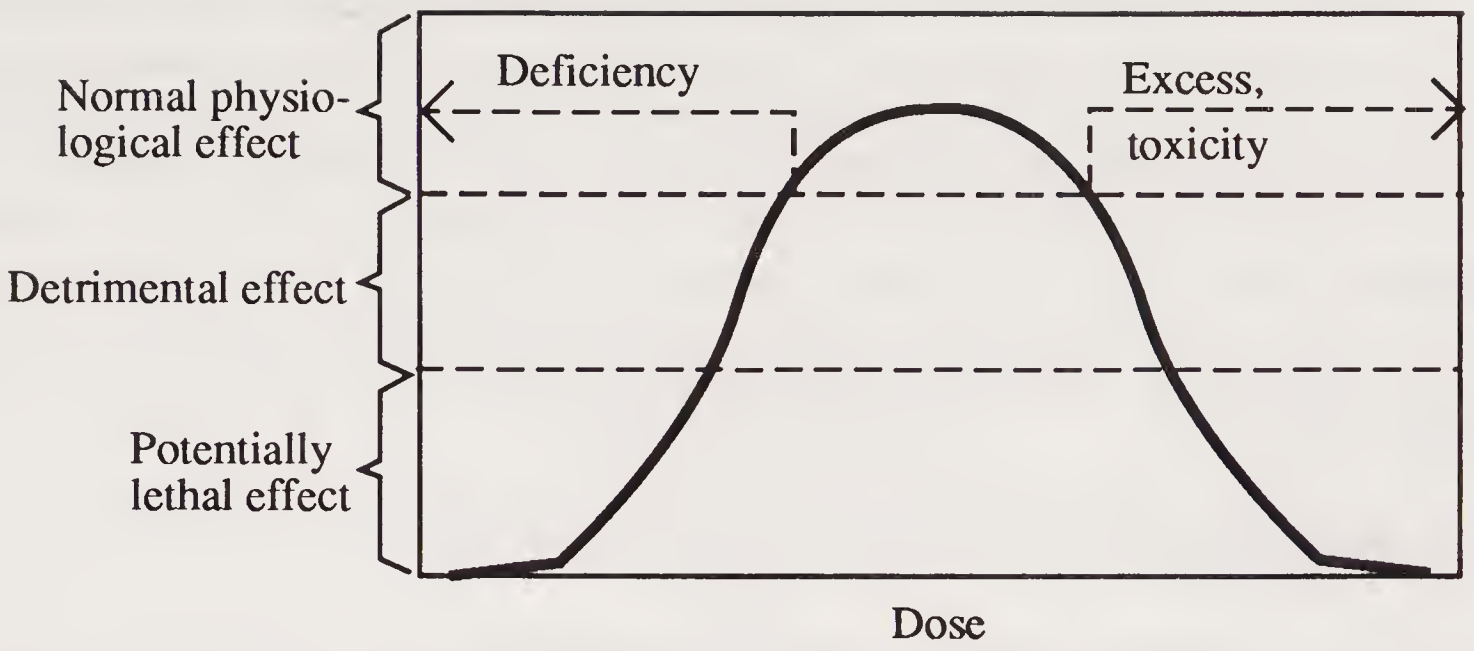


Figure 8.8. Biologic effect of an endogenous substance in an organism showing optimum level, deficiency, and excess.

Examples of Endogenous Substances

Examples of endogenous substances in organisms include various hormones, glucose (blood sugar), and some essential metal ions, including Ca^{2+}, K^{+}, and Na^{+}. The optimum level of calcium in human blood serum occurs over a rather narrow range of 9 – 9.5 milligrams per deciliter (mg/dL). Below these values a toxic response known as hypocalcemia occurs, manifested by muscle cramping. At serum levels above about 10.5 mg/dL hypercalcemia occurs, the major effect of which is kidney malfunction.

8.7. KINETIC AND NONKINETIC TOXICOLOGY

Nonkinetic toxicology deals with generalized harmful effects of chemicals that occur at an exposure site; a typical example is the destruction of skin tissue by contact with concentrated nitric acid, HNO_3. Nonkinetic toxicology applies to those poisons that are not metabolized or transported in the body or subject to elimination processes that remove them from the body. The severity of a nonkinetic insult depends upon both the characteristic of the chemical and the exposure site. Injury increases with increasing area and duration of the exposure, with the concentration of the toxicant in its matrix (for example, the concentration of HNO_3 in solution) and with the susceptibility of the exposure site to damage. The toxic action of the substance ceases when its chemical reaction with tissue is complete or when it is removed from the exposure site. Nonkinetic toxicology is also called **nonmetabolic** or **nonpharmacologic** toxicology.

Kinetic Toxicology

Kinetic toxicology, also known as **metabolic** or **pharmacologic** toxicology, involves toxicants that are transported and metabolized in the body. Such substances are called **systemic poisons** and they are studied under the discipline of **systemic toxicology**. Systemic poisons may cross cell membranes (see Chapter 3) and act upon **receptors** such as cell membranes, bodies in the cells, and specific enzyme systems. The effect is dose-responsive and it is terminated by processes that may include metabolic conversion of the toxicant to a metabolic product, chemical binding, storage and excretion from the organism.

In an animal a xenobiotic substance may be bound reversibly to a plasma protein in an inactivated form. A polar xenobiotic substance, or a polar metabolic product, may be excreted from the body in solution in urine. Nonpolar substances delivered to the intestinal tract in bile are eliminated with feces. Volatile nonpolar substances such as carbon monoxide tend to leave the body via the pulmonary system. The ingestion, biotransformation, action on receptor sites, and excretion of a toxic substance may involve complex interactions of biochemical and physiological parameters. The study of these parameters within a framework of metabolism and kinetics is called **toxicometrics**.

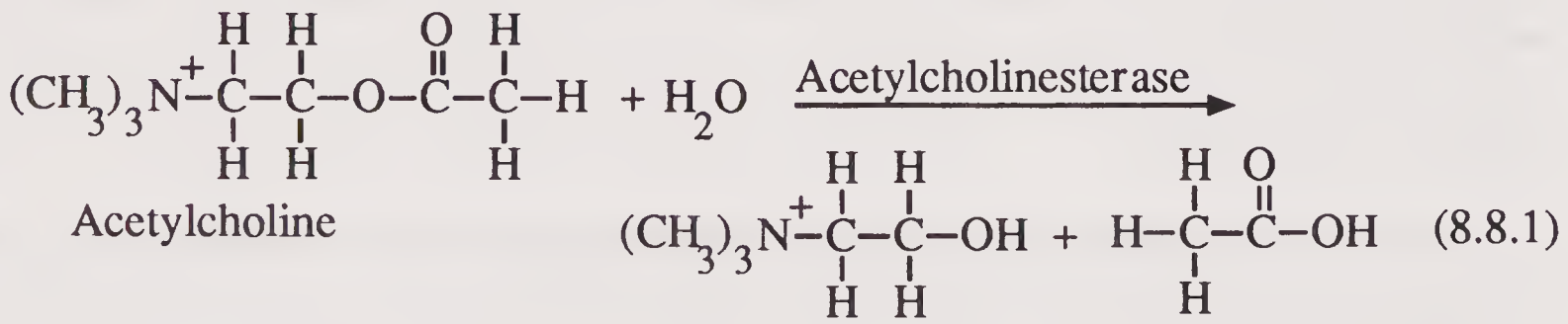
8.8. RECEPTORS AND TOXIC SUBSTANCES

A toxic substance that enters the body through any of the possible entry sites (ingestion, inhalation, skin) undergoes biochemical transformations that can increase or decrease its toxicity, affect its ability to traverse cell membranes, or enable its elimination from the body. A substance involved in a kinetic toxicological process (see Section 8.7) generally enters the blood and lymph system before it has any effect. Plasma proteins may inactivate the toxic substance by binding reversibly to it. The substance often undergoes biotransformation, which most commonly occurs in the liver, but may also take place in other types of tissue as well. These reactions are catalyzed by enzymes, most frequently mixed function oxidases. Toxicants can either stimulate or inhibit enzyme action. It is obvious that biochemical actions and transformations of toxicants are varied and complex. They are discussed in greater detail in Chapter 9.

Receptors

As noted in the preceding section, there are various *receptors* upon which xenobiotic substances or their metabolites act. In order to bind to a receptor, the substance has to have the proper structure or, more precisely, the right **stereochemical molecular configuration** (see Chapters 1 and 3). Receptors are almost always proteinaceous materials, normally enzymes. Nonenzyme receptors include opiate (nerve) receptors, gonads, or the uterus.

An example of a toxicant acting upon a receptor will be cited here; the topic is discussed in greater detail in Chapter 9. One of the most commonly cited examples of an enzyme receptor that is adversely affected by toxicants is that of **acetylcholinesterase**. It acts upon **acetylcholine** as shown by the reaction



Acetylcholine (8.8.1)

Acetylcholine is classified as a neurotransmitter. As such, it is a key substance involved with transmission of nerve impulses in the brain, skeletal muscles, and other areas where nerve impulses occur. An essential step in the proper function of any nerve impulse is its cessation (see Figure 8.9) which requires hydrolysis of acetylcholine by Reaction 8.7.1. Some xenobiotics, such as organophosphate compounds

(see Chapter 18) and carbamates (see Chapter 15) inhibit acetylcholinesterase, with the result that acetylcholine accumulates and nerves are overstimulated. Adverse effects may occur in the central nervous system, autonomic nervous system, and at neuromuscular junctions. Convulsions, paralysis, and finally, death may result.

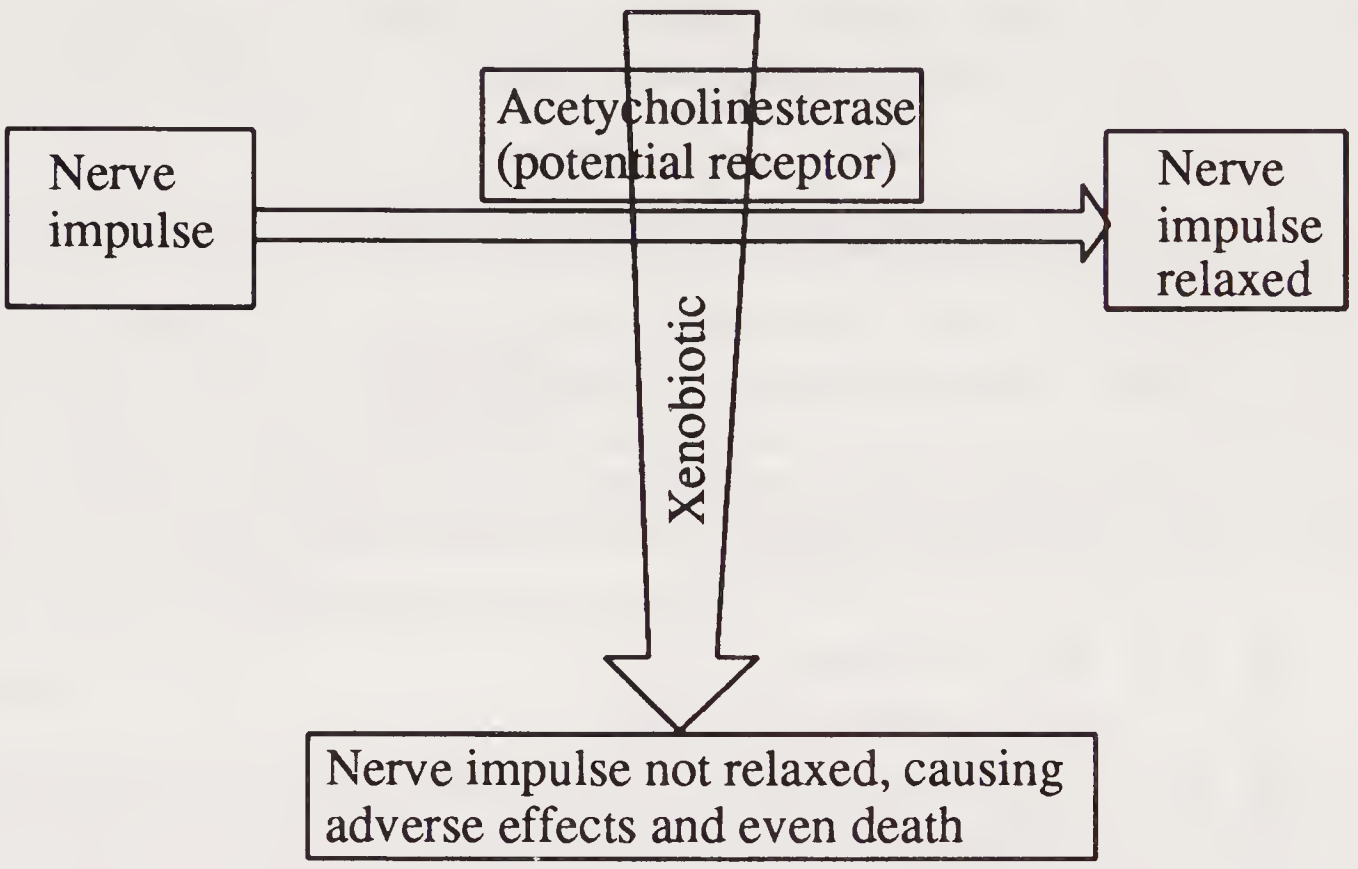


Figure 8.9. Example of a toxicant acting on a receptor to cause an adverse effect.

8.9. PHASES OF TOXICITY

Having examined the routes by which toxicants enter the body, it is now appropriate to consider what happens to them in the body and what their effects are. The action of a toxic substance can be divided into two major phases as illustrated in Figure 8.10. The **kinetic phase** involves absorption, metabolism, temporary storage, distribution, and, to a certain extent, excretion of the toxicant or its precursor compound called the **protoxicant**. In the most favorable scenario for an organism, a toxicant is absorbed, detoxified by metabolic processes, and excreted with no harm resulting. In the least favorable case, a protoxicant that is not itself toxic is absorbed and converted to a toxic metabolic product which is transported to a location where it has a detrimental effect. The **dynamic phase** is divided as follows: (1) the toxicant reacts with a receptor or target organ in the **primary reaction** step, (2) there is a biochemical response and (3) physiological and/or behavioral manifestations of the effect of the toxicant occur.

8.10. TOXIFICATION AND DETOXIFICATION

As shown for the kinetic phase in Figure 8.10, a xenobiotic substance may be (1) detoxified by metabolic processes and eliminated from the body, (2) made more toxic (toxified) by metabolic processes and distributed to receptors, or (3) passed on to receptors as a metabolically unmodified toxicant. A metabolically unmodified toxicant is called an **active parent compound** and a substance modified by metabolic processes is an **active metabolite**. Both types of species may be involved in the dynamic phase.

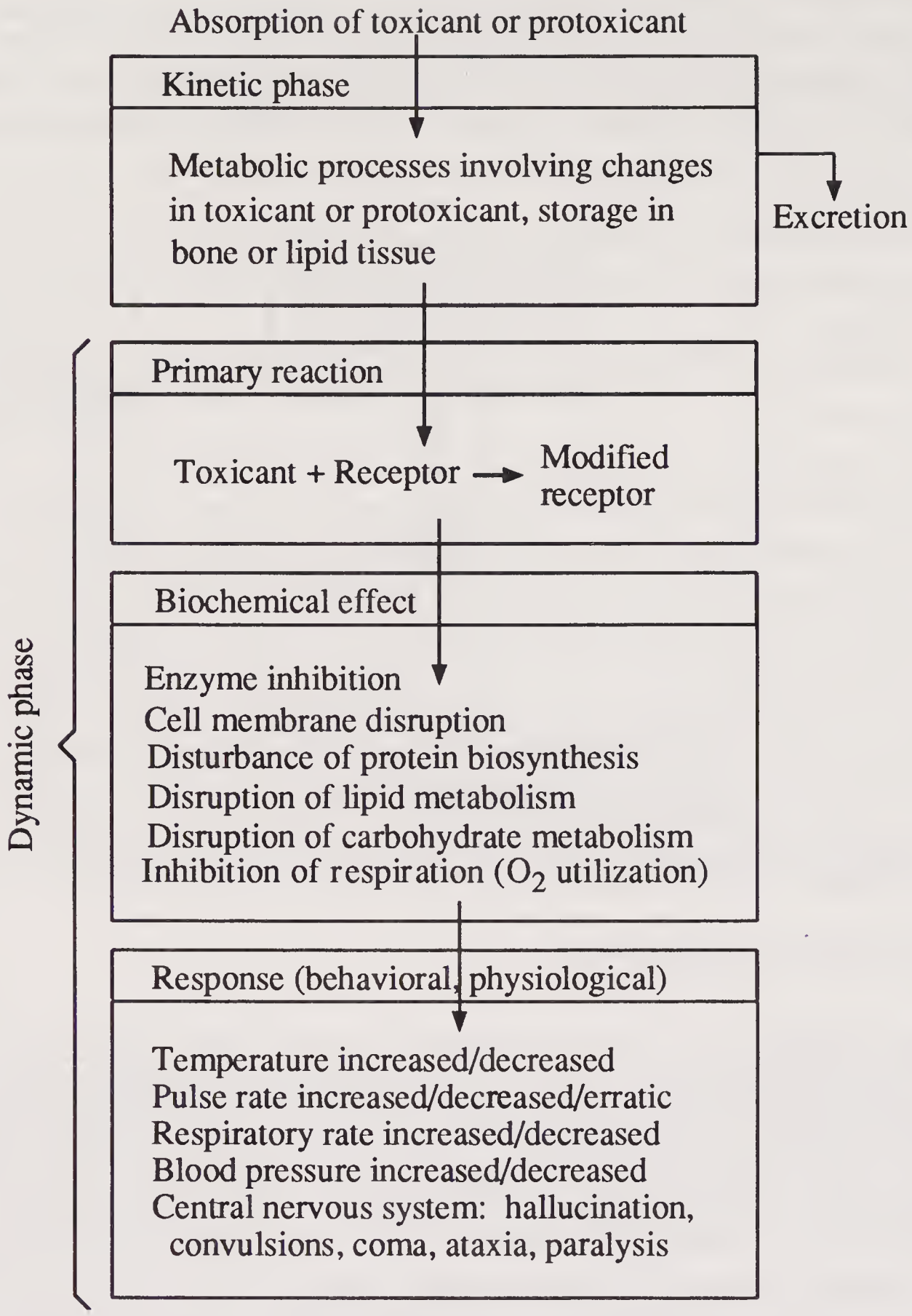


Figure 8.10. Different major steps in the overall process leading to a toxic response.

During the kinetic phase an active parent compound can be present in blood, liver, or **extrahepatic tissue** (nonliver tissue), and in the latter two it may be converted to an inactive metabolite or metabolites. An inactive parent metabolite may produce a toxic metabolite or metabolites in the liver or in extrahepatic tissue; in both these locations a toxic metabolite may be changed to an inactive form. Therefore, the kinetic phase involves a number of pathways by which a xenobiotic substance is converted to a toxicant that can act on a receptor or to a substance that is eliminated from the organism.

Synergism, Potentiation, and Antagonism

The biological effects of two or more toxic substances can be different in kind and degree from those of one of the substances alone. One of the ways in which this can occur is when one substance affects the way in which another undergoes any of

the steps in the kinetic phase as shown in Figure 8.10. Chemical interaction between substances may affect their toxicities. Both substances may act upon the same physiologic function or two substances may compete for binding to the same receptor. When both substances have the same physiologic function, their effects may be simply **additive** or they may be **synergistic** (the total effect is greater than the sum of the effects of each separately). **Potentiation** occurs when an inactive substance enhances the action of an active one and **antagonism** when an active substance decreases the effect of another active one.

8.11. BEHAVIORAL AND PHYSIOLOGICAL RESPONSES

The final part of the overall toxicological process outlined in Figure 8.10 consists of **behavioral** and **physiological responses**, which are observable symptoms of poisoning. These are discussed here, primarily in terms of responses seen in humans and other animals. Nonanimal species exhibit other kinds of symptoms from poisoning; for example, plants suffer from leaf mottling, pine needle loss, and stunted growth as a result of exposure to some toxicants.

Vital Signs

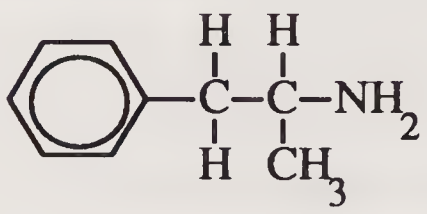
Human subjects suffering from acute poisoning usually show alterations in the **vital signs**, which consist of **temperature**, **pulse rate**, **respiratory rate**, and **blood pressure**. These are discussed here in connection with their uses as indicators of toxicant exposure.

Some toxicants that affect body temperature are shown in Figure 8.11 (page 210). Among those that increase body temperature are benzadrine, cocaine, sodium fluoroacetate, tricyclic antidepressants, hexachlorobenzene, and salicylates (aspirin). In addition to phenobarbital and ethanol, toxicants that decrease body temperature include phenothiazine, clonidine, glutethimide, and haloperidol.

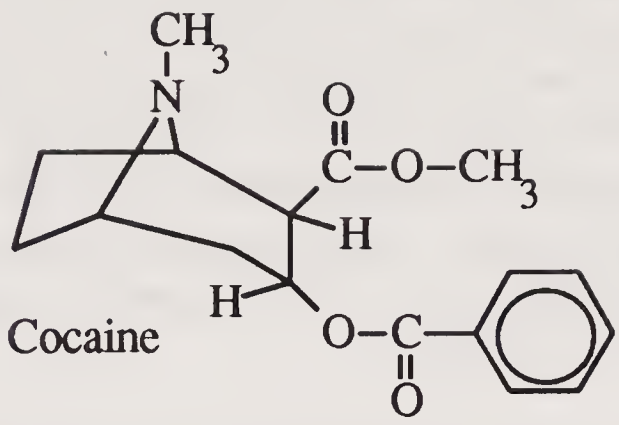
Toxicants may have three effects on pulse rate. These are **bradycardia** (decreased rate), **tachycardia** (increased rate), and **arrhythmia** (irregular pulse). Alcohols may cause either bradycardia or tachycardia. Amphetamines, belladonna alkaloids, cocaine, and tricyclic antidepressants (see imiprimine hydrochloride in Figure 8.12 on page 210) may cause either tachycardia or arrhythmia. Toxic doses of digitalis may result in bradycardia or arrhythmia. The pulse rate is decreased by toxic exposure to carbamates, organophosphates, local anesthetics, barbiturates, clonidine, muscaric mushroom toxins, and opiates. In addition to the substances mentioned above, those that cause arrhythmia are arsenic, caffeine, belladonna alkaloids, phenothizine, theophylline, and some kinds of solvents.

Among the toxicants that increase respiratory rate are cocaine, amphetamines, and fluoroacetate (all of which were shown in Figure 8.11), nitrites (compounds containing the NO_2^- ion), methanol (CH_3OH), salicylates, and hexachlorobenzene. Cyanide and carbon monoxide may either increase or decrease respiratory rate. Alcohols other than methanol, analgesics, narcotics, sedatives, phenothiazines, and opiates in toxic doses decrease respiratory rate. The structural formulas of some compounds that affect respiratory rate are shown in Figure 8.13 on page 211.

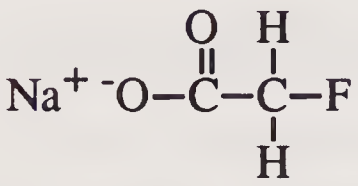
Amphetamines and cocaine (Figure 8.11) tricyclic antidepressants (see imiprimine hydrochloride in Figure 8.12), phenylcyclidines, and belladonna alkaloids at toxic levels increase blood pressure. Overdoses of antihypertensive agents decrease blood pressure, as do toxic doses of opiates, barbiturates, iron, nitrite, cyanide, and mushroom toxins.



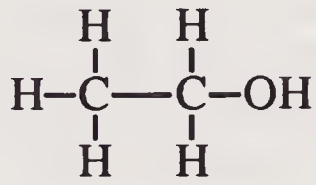
Amphetamine
(benzadrine)



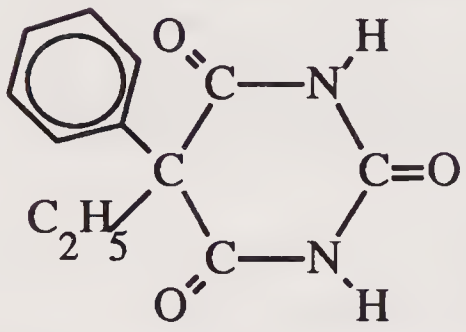
Cocaine



Sodium fluoroacetate
(fluoroacetate ion)

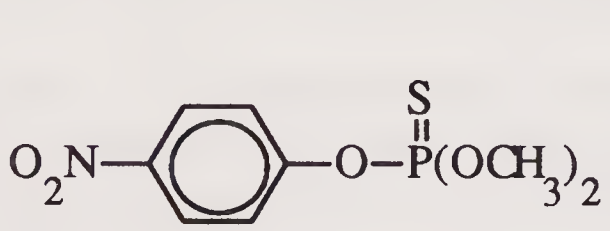


Ethanol

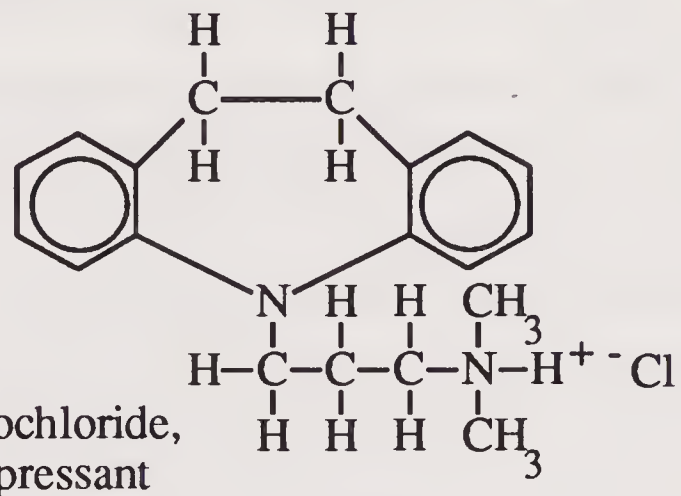


Phenobarbital
(a barbiturate)

Figure 8.11. Examples of toxicants that affect body temperature. Amphetamine, cocaine, and fluoroacetate increase body temperature; phenobarbital and ethanol decrease it.



Methyl parathion



Imiprimine hydrochloride,
a tricyclic antidepressant

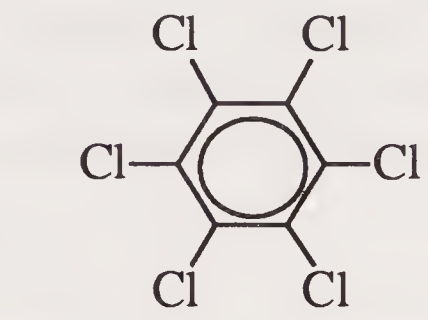
Figure 8.12. Structures of toxicants that can affect pulse rate. Methyl parathion, a commonly used plant insecticide, can cause bradycardia. Imiprimine hydrochloride, a tricyclic antidepressant, can cause either tachycardia or arrhythmia.

Skin Symptoms

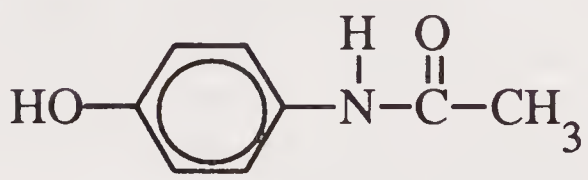
In many cases the skin exhibits evidence of exposure to toxic substances. The two main skin characteristics observed as evidence of poisoning are skin color and degree of skin moisture. Excessively dry skin tends to accompany poisoning by tricyclic antidepressants, antihistamines, and belladonna alkaloids. Among the toxic substances for which moist skin is a symptom of poisoning are mercury, arsenic, thallium, carbamates, and organophosphates. The skin appears flushed when the subject has been exposed to toxic doses of carbon monoxide, nitrites, amphetamines, monsodium glutamate, and tricyclic antidepressants. Higher doses of cyanide, carbon monoxide, and nitrites give the skin a **cyanotic** appearance (blue color due to oxygen deficiency in the blood). Skin may appear **jaundiced** (yellow because of the presence of bile pigments in the blood) when the subject is poisoned by a number of toxicants including arsenic, arsine gas (AsH_3), iron, aniline dyes, and carbon tetrachloride.

Odors

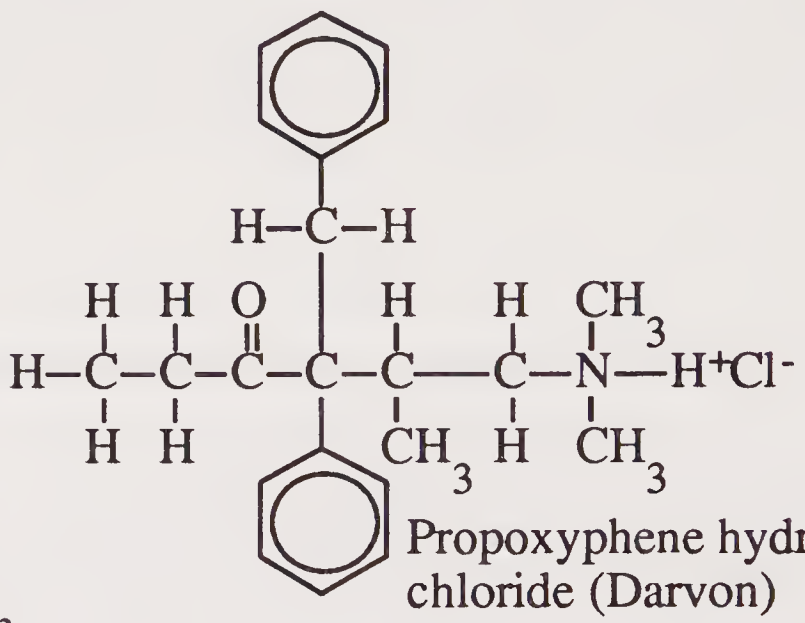
Toxic levels of some materials cause the body to have unnatural **odors** because of parent compound toxicants or their metabolites secreted through the skin, exhaled through the lungs, or present in tissue samples. Some examples of odorous species are shown in Figure 8.14. In addition to the odors noted in the figure, others symptomatic of poisoning include aromatic odors from hydrocarbons and the odor of violets arising from the ingestion of turpentine. Alert pathologists have uncovered evidence of poisoning murders by noting the bitter almond odor of HCN in tissues of victims of criminal cyanide poisoning. A characteristic rotten egg odor is evidence of hydrogen sulfide (H_2S) poisoning. The same odor has been reported at autopsies of carbon disulfide poisoning victims. Even very slight exposures to some selenium compounds cause an extremely potent garlic breath odor.



Hexachlorobenzene

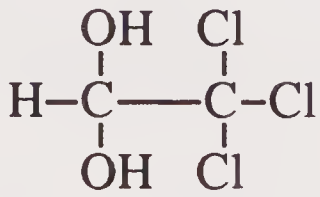


Acetaminophen

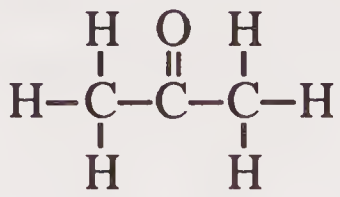


Propoxyphene hydrochloride (Darvon)

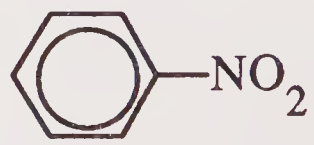
Figure 8.13. Some compounds that affect respiratory rate. Acetaminophen is one of the simple analgesics, which in therapeutic doses relieve pain without any effect upon an individual's consciousness. Propoxyphene hydrochloride is a narcotic analgesic, a class of substances which can cause biochemical changes in the body leading to chemical dependency.



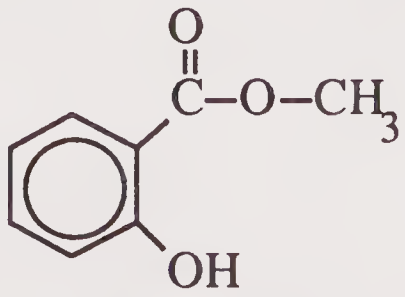
Chloral hydrate
(pear odor)



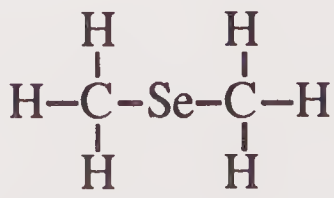
Acetone
(acetone odor)



Nitrobenzene
(shoe polish)



Methyl salicylate
(wintergreen)



Dimethyl selenide
(garlic)

Figure 8.14. Some toxicants and the odors they produce in exposed subjects.

Eyes

Careful examination of the eyes can reveal evidence of poisoning. The response, both in size and reactivity, of the pupils to light can provide useful evidence of a response to toxicants. Both voluntary and involuntary movement of the eyes can be significant. The appearance of eye structures, including optic disc, conjunctiva, and blood vessels, can be significant. Eye **miosis**, defined as excessive or prolonged contraction of the eye pupil, is a toxic response to a number of substances, including alcohols, carbamates, organophosphates, and phenycyclidine. The opposite response **mydriasis** (excessive pupil dilation) is caused by amphetamines, belladonna alkaloids, glutethimide, and tricyclic antidepressants, among others. **Conjunctivitis** is a condition marked by inflammation of the conjunctiva, the mucus membrane that covers the front part of the eyeball and the inner lining of the eyelids. Corrosive acids and bases (alkalies) cause conjunctivitis, as do exposures to nitrogen dioxide, hydrogen sulfide, methanol, and formaldehyde. **Nystagmus**, the involuntary movement of the eyeballs, usually in a side-to-side motion, is observed in poisonings by some toxicants, including barbiturates, phenycyclidine, phentoin, and ethychlorovynol.

Mouth

Examination of the mouth provides evidence of exposure to some toxicants. Caustic acids and bases cause a moist condition of the mouth. Other toxicants that cause the mouth to be more moist than normal include mercury, arsenic, thallium, carbamates, and organophosphates. A dry mouth is symptomatic of poisoning by tricyclic antidepressants, amphetamines, antihistamines, and glutethimide.

Gastrointestinal Tract

The gastrointestinal tract responds to a number of toxic substances, usually by pain, vomiting, or paralytic ileus (see "intestines" in Section 8.2). Severe gastrointestinal pain is symptomatic of poisoning by arsenic or iron. Both of these substances can cause vomiting, as can acids, bases, fluorides, salicylates, and theophyllin. Paralytic ileus can result from ingestion of narcotic analgesics, tricyclic antidepressants, and clonidine.

Central Nervous System

The central nervous system responds to poisoning by exhibiting symptoms such as **convulsions**, **paralysis**, **hallucinations**, and **ataxia** (lack of coordination of voluntary movements of the body). Other behavioral symptoms of poisoning include agitation, hyperactivity, disorientation, and delirium.

Among the many toxicants that cause convulsions are chlorinated hydrocarbons, amphetamines, lead, organophosphates, and strychnine. There are several levels of **coma**, the term used to describe a lowered level of consciousness. At level 0 the subject may be awakened and will respond to questions. At level 1 withdrawal from painful stimuli is observed and all reflexes function. A subject at level 2 does not withdraw from painful stimuli, although most reflexes still function. Levels 3 and 4 are characterized by the absence of reflexes; at level 4 respiratory action is depressed and the cardiovascular system fails. Among the many toxicants that cause coma are

narcotic analgesics, alcohols, organophosphates, carbamates, lead, hydrocarbons, hydrogen sulfide, benzodiazepines, tricyclic antidepressants, isoniazid, phenothiazines, and opiates.

8.12. IMMUNE SYSTEM RESPONSE

The **immune system** is the body's defense against biological systems that would harm it. The most obvious of these consist of **infectious agents**, such as viruses or bacteria. Also included are **neoplastic cells**, which give rise to cancerous tissue. The immune system produces **immunoglobin**, a substance consisting of proteins bound to carbohydrates. This material functions as **antibodies** against **immunogen** or **antigen** macromolecules of polysaccharides, nucleic acids, or proteins characteristic of invasive foreign virus, bacteria, or other biological materials. The cells that the immune system uses to provide protection are called **leukocytes**.

Toxicants can adversely affect the immune system in several ways.[3] **Immunosuppression** occurs when the body's natural defense mechanisms are impaired by agents such as toxicants. Radiation and drugs such as chemotherapeutic agents, anticonvulsants, and corticosteroids can have immunosuppressive effects. Immunosuppressants are deliberately used to prevent rejection of transplanted organs. In some cases toxicants adversely alter the mechanisms by which the immune system defends the body against pathogens and neoplastic cells. Another effect of toxicants on the immune system occurs through the loss of its ability to control proliferation of cells, resulting in leukemia or lymphoma.

Foreign agents can cause the immune system to overreact with an extreme, self-destructive response called **allergy** or **hypersensitivity**. This reaction probably occurs after the foreign agents or their metabolites become associated with large molecules endogenous to the body. Among the many substances that cause allergy are metals (beryllium, chromium, nickel), penicillin, formaldehyde, pesticides, food additives, resins, and plasticizers.

Uncontrolled proliferation is another immune system disfunction that can occur. It may be manifested by lymphoma, leukemia, or related conditions.

In some cases xenobiotic compounds adversely alter **host defense mechanisms**. This may reduce the body's ability to resist pathogenic bacteria or viruses or to combat neoplasia.

Autoimmunity develops as a condition in which the body develops an allergic response to its own biomolecules. What essentially occurs is that to a degree it loses the ability to distinguish foreign antigens from its own antigens. Several important diseases are caused by autoimmune response, including rheumatoid arthritis and systemic lupus erythematosus. Binding of xenobiotic molecules to body proteins can induce autoimmunity. Chemicals that have been so implicated include heavy metals, hydrazine, epoxy resins, and chlorinated ethylene compounds.

Effects upon the immune system are gaining increasing recognition as factors in toxicology and in evaluating the toxicity of various substances. There are numerous ways of evaluating potential effects upon the immune system. The most modern methods are reproducible and sensitive and have been standardized and validated.[4] One is a "two-tier method" that makes use of numerous tests made directly on the test organism and on samples taken from it. The first tier consists of relatively simple tests such as measurements of body mass, blood count, examination of tissue (histology), and ability to form antibodies. The second tier consists of more sophisticated tests, such as the *Streptococcus* challenge, that measure host resistance. Bone marrow evaluations are also employed as second-tier tests.

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QUESTIONS AND PROBLEMS

1. Distinguish between acute toxicity and chronic toxicity.
2. Distinguish among acute local exposure, chronic local exposure, acute systemic exposure and chronic systemic exposure to toxicants.
3. List and discuss the major routes and sites of exposure, distribution, and elimination of toxicants in the body.
4. What function is served by the stratum corneum in exposure of the body to toxic substances?
5. Explain why the lungs are regarded as the place where substances external to the body have the most intimate contact with body fluids. In what sense does pulmonary intake of a toxicant evade important "screening organs."
6. Why are ammonia (NH_3) and hydrogen chloride (HCl) removed from air predominantly in the upper respiratory tract?
7. In what sense is the gastrointestinal tract "external" to the body?
8. How do the different regions of the gastrointestinal tract influence the uptake of toxicants, such as weak acids, that have different acid-base behavior?
9. What are the major components of the enterohepatic circulation system? What is the portal circulatory system?
10. Describe the nature and significance of the dose-response curve. What is the significance of its inflection point (mid-point). Define dose and response.
11. How do toxicity ratings relate to the potency of a toxicant?

12. Define sublethal effects, reversible effects, and margin of safety. What is an irreversible toxic effect?
13. What are hypersensitivity and hyposensitivity? Can these phenomena be related in any respect to the immune system?
14. What is the distinction between a xenobiotic substance and an endogenous substance? What are some examples of endogenous substances?
15. Define nonkinetic toxicology and how it relates to corrosive substances. What is kinetic toxicology and how does it relate to systemic poisons?
16. What is a receptor? In what way may acetylcholinesterase act as a receptor? What happens when this enzyme becomes bound to a toxic substance?
17. What is a protoxicant? What may happen to a protoxicant in the kinetic phase?
18. What are the three major divisions of the dynamic phase? In which of these is a receptor acted upon by a toxicant?
19. Distinguish between an active parent compound and an active metabolite in toxicology.
20. Differentiate among synergism, potentiation, and antagonism. What is an additive effect?
21. Define bradycardia, tachycardia, and arrhythmia. What are some of the toxicants that may cause each?
22. Distinguish between a cyanotic appearance of skin and a jaundiced appearance. Which kinds of toxicants may cause each?
23. List the major biological agents against which the body's immune system defends. How are leukocytes involved in this defense?
24. Define and give the significance of immunosuppression, hypersensitivity, uncontrolled proliferation, and autoimmunity.

9

Toxicological Chemistry

9.1. INTRODUCTION

As defined in Section 1.1, **toxicological chemistry** is defined as the chemistry of toxic substances with emphasis upon their interactions with biologic tissue and living systems. This chapter expands upon this definition to define toxicological chemistry in more detail. Earlier chapters of the book have outlined the essential background required to understand toxicological chemistry. In order to comprehend this topic, it is first necessary to have an appreciation of the chemical nature of inorganic and organic chemicals, the topic of Chapter 1. An understanding of biochemistry, covered in Chapter 3, is required to comprehend the ways in which xenobiotic substances in the body undergo biochemical processes and, in turn, affect these processes. Additional perspective is provided by the discussion of metabolic processes in Chapter 4. The actual toxicities and biologically manifested effects of toxicants are covered in Chapter 8. Finally, an understanding of the environmental biochemistry of toxicants requires an appreciation of environmental chemistry, which is outlined in Chapter 2.

Chemical Nature of Toxicants

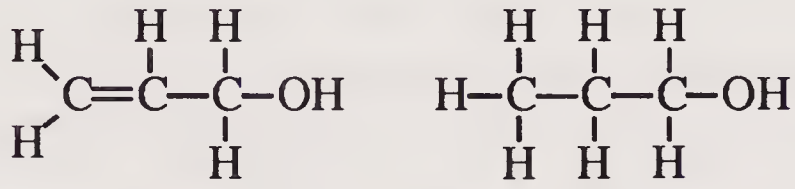
It is not possible to exactly define a set of chemical characteristics that make a chemical species toxic. This is because of the large variety of ways in which a substance can interact with substances, tissues, and organs to cause a toxic response. Because of subtle differences in their chemistry and biochemistry, similar substances may vary enormously in the degrees to which they cause a toxic response. For example, consider the toxic effects of carbon tetrachloride, CCl_4, and a chemically closely-related chlorofluorocarbon, dichlorodifluoromethane, CCl_2F_2. Both of these compounds are completely halogenated derivatives of methane possessing very strong carbon-halogen bonds. As discussed in Section 16.2, carbon tetrachloride is considered to be dangerous enough to have been banned from consumer products in 1970. It causes a large variety of toxic effects in humans, with chronic liver injury being the most prominent. Dichlorodifluoromethane, a Freon compound, is regarded as nontoxic, except for its action as a simple asphyxiant and lung irritant at high concentrations.

An increasingly useful branch of toxicological chemistry is the one dealing with **structure-activity relationships**. By relating the chemical structure and physical characteristics of various compounds to their toxic effects, it is possible to predict the toxicological effects of other compounds and classes of compounds.

With the qualification that there are exceptions to the scheme, it is possible to place toxic substances into several main categories. These are listed below:

- Substances that exhibit **extremes of acidity, basicity, dehydrating ability**, or **oxidizing power**. Examples include concentrated sulfuric acid (a strong acid with a tendency to dehydrate tissue), strongly basic sodium hydroxide, and oxidant elemental fluorine, F_2. Such species tend to be nonkinetic poisons (see Section 8.7) and corrosive substances that destroy tissue by massively damaging it at the site of exposure.

- **Reactive substances** that contain bonds or functional groups that are particularly prone to react with biomolecules in a damaging way. One reason that diethyl ether, (C_2H_5)-O-(C_2H_5), is relatively nontoxic is because of its lack of reactivity resulting from the very strong C-H bonds in the ethyl groups and the very stable C-O-C ether linkage. A comparison of allyl alcohol with 1-propanol (structural formulas below),



Allyl alcohol Propyl alcohol

shows that the former is a relatively toxic irritant to the skin, eyes, and respiratory tract that also damages liver and kidneys, whereas 1-propanol is one of the less toxic organic chemicals with an LD_{50} about 100 times that of allyl alcohol. As shown by the structures, allyl alcohol differs from 1-propanol in having the relatively reactive alkenyl group, C=C.

- **Heavy metals**, broadly defined, contain a number of members that are toxic by virtue of their interaction with enzymes, tendency to bond strongly with sulfhydryl (-SH) groups on proteins, and other effects.

- **Binding species** are those that bond to biomolecules, altering their function in a detrimental way. This binding may be reversible, as is the case with the binding of carbon monoxide with hemoglobin (see Chapter 12), which deprives hemoglobin of its ability to attach molecular O_2 and carry it from the lungs to body tissues. The binding may be irreversible. An example is that which occurs when an electron-deficient carbonium ion, such as H_3C^+ (an electrophile), binds to a nucleophile, such as an N atom on guanine attached to DNA.

- **Lipid-soluble compounds** are frequently toxic because of their ability to traverse cell membranes and similar barriers in the body. Lipid-soluble species frequently accumulate to toxic levels through biouptake and biomagnification processes (see Chapter 6).

- Chemical species that induce a toxic response based largely on their **chemical structures**. Such toxicants often produce an allergic reaction as the body's immune system recognizes the foreign agent, causing an immune system response. Lower molecular mass substances that act in this way usually must become bound to endogenous proteins to form a large enough species to induce an allergic response.

9.2. BIOCHEMICAL TRANSFORMATIONS

The toxicological chemistry of toxicants is strongly tied to their metabolic reactions and fates in the body. The metabolism of xenobiotic compounds was discussed in Section 4.4. The toxicological chemistry of biochemical transformations of xenobiotics is reviewed here.

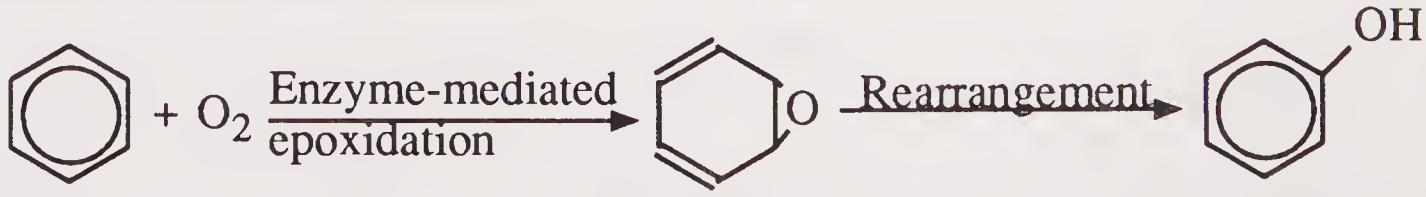
As summarized in Chapter 8, Figure 8.10, systemic poisons in the body undergo (1) biochemical reactions through which they have a toxic effect and (2) biochemical processes that increase or reduce their toxicities or which change toxicants to forms that are readily eliminated from the body. This section reviews the metabolic processes that toxicants undergo with emphasis on toxicological chemical aspects.

In dealing with xenobiotic compounds, the body metabolizes them in ways that usually reduce toxicity and facilitate removal of the substance from the body.[1] The initial attack on a xenobiotic compound (which are typically lipophilic with a tendency to pass through lipid-containing cell membranes and to bind to blood lipoproteins) usually involves hydrolysis or oxidation and, less commonly, reduction. As defined in Section 4.5, this is a **phase I reaction**, which usually introduces reactive polar functional groups onto toxicant molecules, making them more water-soluble than the parent xenobiotic species and providing access to a functional group that can undergo subsequent reactions. The parent xenobiotic compound or its phase I metabolic product may undergo a conjugation reaction with a conjugating agent endogenous to the body. This process is called a **phase II reaction**, and it produces a **conjugation product** that normally (but not always) is less toxic than the parent xenobiotic compound or its phase I metabolite and more readily excreted from the body.

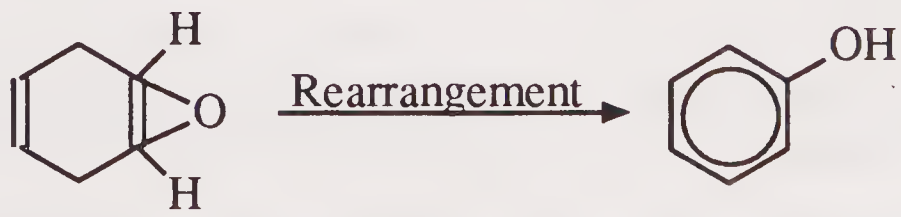
Phase I Reactions

The most common phase I reactions are oxidations. As discussed in Section 4.5, these usually occur in cell microsomes. A large variety of microsomal oxidation reactions may occur. These are summarized below:

- **Oxidation of carbon atoms**. One such reaction is oxidation of alkyl carbon ($\cdots CH_2CH_2CH_3$), such as carbon in an alkyl compound or an alkyl side-chain group on an aromatic compound. Another broad category of this kind of reaction is aryl oxidation of aromatic groups. It usually occurs by introduction of an epoxide group followed by hydroxylation as shown by the following:



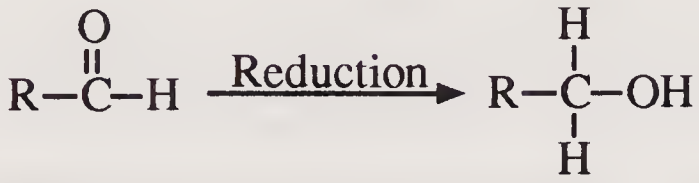
- **Hydroxylation** consisting of the attachment of –OH groups to hydrocarbon chains or rings. It can follow epoxidation as shown by the following rearrangement reaction for benzene epoxide:

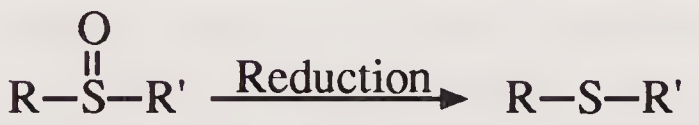


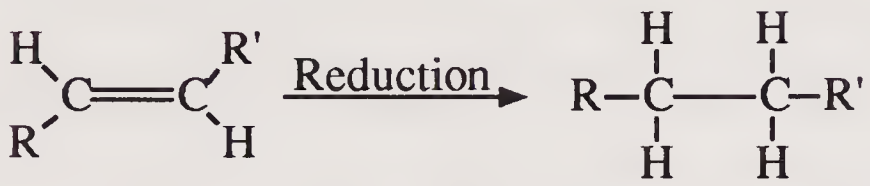
- **Oxidation of noncarbon elements**, particularly nitrogen, sulfur, and phosphorus

Several phase I reactions other than oxidation are important. The most important of these are reduction, dealkylation, hydrolysis and removal of halogen as listed below:

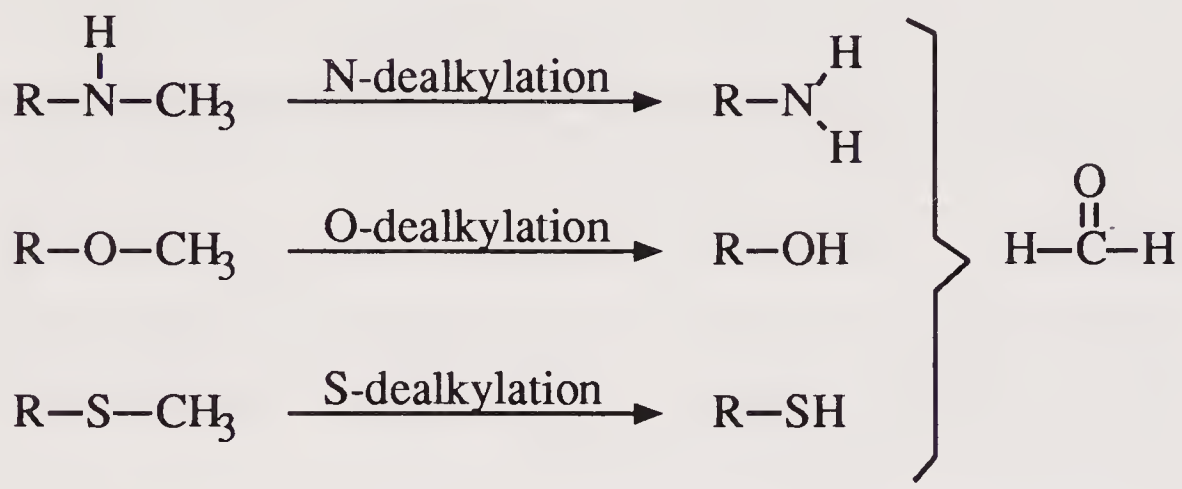
- **Reduction**, such as the following:







- **Dealkylation**, particularly the removal of the methyl ($-CH_3$) group, attached to atoms of O, N, and S:



- **Hydrolysis**, splitting a molecule with insertion of H_2O

- **Removal of halogen**, usually Cl from an organochlorine compound

Phase II Reactions

As discussed in Section 4.6, phase II reactions get their name from the fact that they often follow phase I modification of a xenobiotic substance. Phase II reactions are conjugation reactions because they involve the joining together of a substrate compound with a conjugating agent endogenous to the body. The most prominent conjugations are the following:

- Conjugation by **glucuronide**, which is the most common endogenous conjugating agent in the body

- Conjugation by **glutathione**, a tripeptide consisting of glutamic acid, cysteine, and glycine

- Conjugation by **sulfate**, which requires a high input of energy, but produces a readily eliminated, highly water-soluble product

9.3. BIOCHEMICAL MECHANISMS OF TOXICITY

A critical aspect of toxicological chemistry is that which deals with the biochemical mechanisms and reactions by which xenobiotic compounds and their metabolites interact with biomolecules to cause an adverse toxicological effect. This section addresses the major aspects of biochemical mechanisms and processes of toxicity.

Interference with Enzyme Action

Enzymes are extremely important because they must function properly to enable essential metabolic processes to occur in cells. Substances that interfere with the proper action of enzymes obviously have the potential to be toxic. Many xenobiotics that adversely affect enzymes are **enzyme inhibitors** that slow down or stop enzymes from performing their normal functions as biochemical catalysts. Stimulation of the body to make enzymes that serve particular purposes, a process called **enzyme induction**, is also important in toxicology.

The body contains numerous endogenous enzyme inhibitors that serve to control enzyme-catalyzed processes. When a toxicant acts as an enzyme inhibitor, however, an adverse effect usually results. An important example of this is the action of ions of heavy metals, such as mercury (Hg^{2+}), lead (Pb^{2+}), and cadmium (Cd^{2+}), which have strong tendencies to bind to sulfur-containing functional groups, especially –SS–, –SH, and –S–CH_3. These functional groups are often present on the active sites of enzymes, which, because of their specific three-dimensional structures, bind with high selectivity to the substrate species upon which the enzymes act. Toxic metal ions may bind strongly to sulfur-containing functional groups in enzyme active sites, thereby inhibiting the action of the enzyme. Such a reaction is illustrated in Figure 9.1 for Hg^{2+} ion binding to sulfhydryl groups on an enzyme active site:

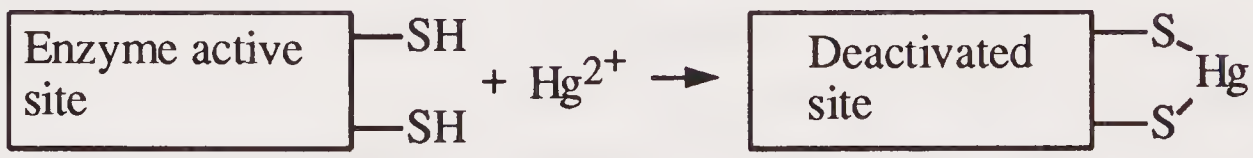


Figure 9.1. Binding of a heavy metal to an enzyme active site.

Inhibition of Metalloenzymes

Substitution of foreign metals for the metals in metalloenzymes (those that contain metals as part of their structures) is an important mode of toxic action by metals. A common mechanism for cadmium toxicity is the substitution of this metal for zinc, a metal that is present in many metalloenzymes. This substitution occurs readily because of the chemical similarities between the two metals (for example, Cd^{2+} and Zn^{2+} behave very much alike in solution). Some enzymes that are affected adversely by the substitution of cadmium for zinc are adenosine triphosphate, alcohol dehydrogenase, and carbonic anhydrase.

Inhibition by Organic Compounds

The covalent bonding of organic xenobiotic compounds to enzymes as shown in Equation 9.3.1 can cause enzyme inhibition. Such bonding occurs most commonly

through hydroxyl (–OH) groups on enzyme active sites. Covalent bonding of xenobiotic compounds is one of the major ways in which acetylcholinesterase (an enzyme crucial to the function of nerve impulses, see Section 8.8) can be inhibited. An organophosphate compound, such as the nerve gas compound diisopropyl-phosphorfluoridate (a reactant in Equation 9.3.1), may bind to acetylcholinesterase, thereby inhibiting the enzyme.

$$(C_3H_7O)_2\overset{\overset{O}{\|}}{P}\text{—}F + HO\text{—(acetylcholinesterase)} \longrightarrow HF + (C_3H_7O)_2\overset{\overset{O}{\|}}{P}\text{—}O\text{—(acetylcholinesterase)} \qquad (9.3.1)$$

Biochemistry of Mutagenesis

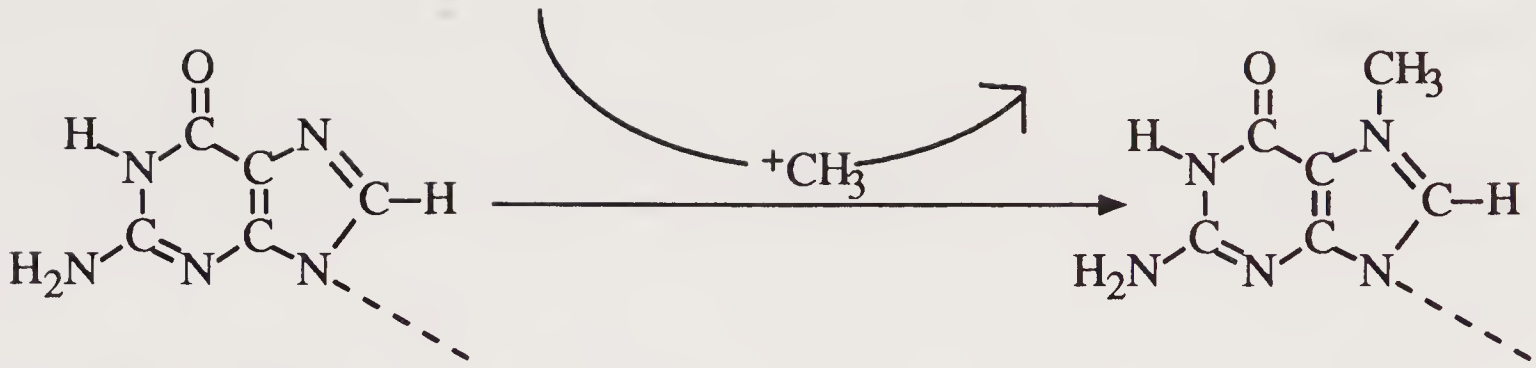
Mutagenesis is the phenomenon in which inheritable traits result from alterations of DNA. Although mutation is a normally occurring process that gives rise to diversity in species, most mutations are harmful. The toxicants that cause mutations are known as **mutagens**. These toxicants, often the same as those that cause cancer or birth defects, are a major toxicological concern.

To understand the biochemistry of mutagenesis, it is important to recall from Chapter 3 that DNA contains the nitrogenous bases adenine, guanine, cytosine, and thymine. The order in which these bases occur in DNA determines the nature and structure of newly produced RNA, a substance produced as a step in the synthesis of new proteins and enzymes in cells. Exchange, addition, or deletion of any of the nitrogenous bases in DNA alters the nature of RNA produced and can change vital life processes, such as the synthesis of an important enzyme.[2] This phenomenon, which can be caused by xenobiotic compounds, is a mutation that can be passed on to progeny, usually with detrimental results.

There are several ways in which xenobiotic species may cause mutations. It is beyond the scope of this work to discuss these mechanisms in detail. For the most part, however, mutations due to xenobiotic substances are the result of chemical alterations of DNA, such as those discussed in the two examples below.

Nitrous acid, HNO_2, is an example of a chemical mutagen that is often used to cause mutations in bacteria. To understand the mutagenic activity of nitrous acid it should be noted that three of the nitrogenous bases — adenine, guanine, and cytosine — contain the amino group, $-NH_2$. The action of nitrous acid is to replace amino groups with doubly bonded oxygen atoms, thereby placing keto groups (C=O) in the rings of the nitrogenous bases and converting them to other compounds. When this occurs, the DNA may not function in the intended manner, causing a mutation to occur.

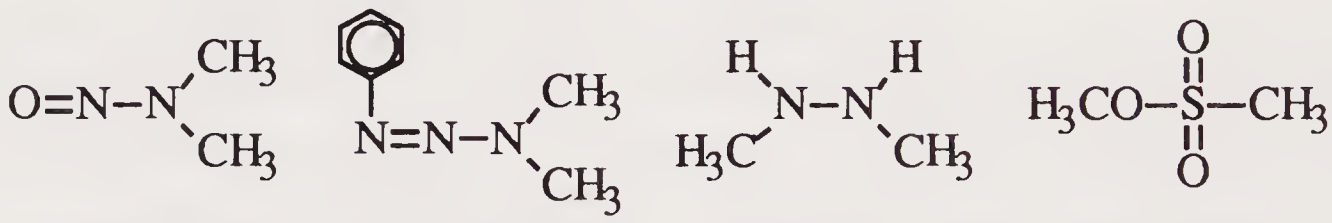
Alkylation consisting of the attachment of a small alkyl group, such as $-CH_3$ or $-C_2H_5$, to an N atom on one of the nitrogenous bases in DNA is one of the most common mechanisms leading to mutation. The methylation of "7" nitrogen in guanine in DNA to form N^7 guanine is shown in Figure 9.2. O-alkylation may also occur by attachment of a methyl or other alkyl group to the oxygen atom in guanine.



Guanine bound to DNA Methylated guanine in DNA

Figure 9.2. Alkylation of guanine in DNA.

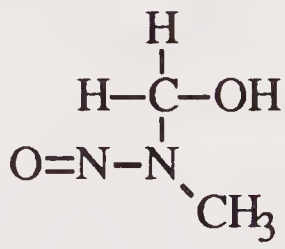
A number of mutagenic substances act as alkylating agents. Prominent among these are the compounds shown in Figure 9.3.



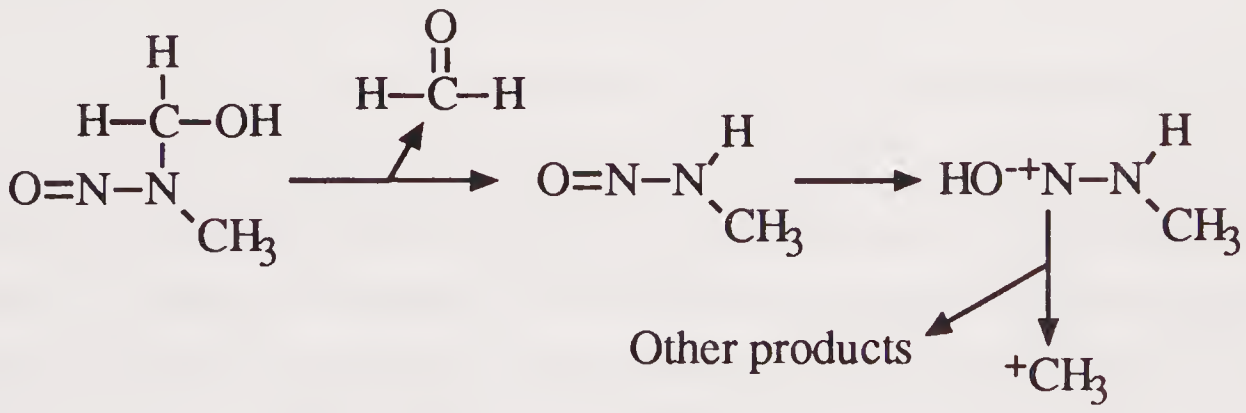
Dimethylnitrosamine 3,3-Dimethyl-1-phenyltriazine 1,2-Dimethylhydrazine Methylmethanesulfonate

Figure 9.3. Examples of simple alkylating agents capable of causing mutations.

Alkylation occurs by way of generation of positively charged electrophilic species that bond to electron-rich nitrogen or oxygen atoms on the nitrogenous bases in DNA. The generation of such species usually occurs by way of biochemical and chemical processes. For example, dimethylnitrosamine (structure in Figure 9.3) is activated by oxidation through cellular NADPH (see Section 4.3) to produce the following highly reactive intermediate:



This product undergoes several nonenzymatic transitions, losing formaldehyde and generating a carbonium ion, $^{+}CH_3$, that can methylate nitrogenous bases on DNA:



(9.3.2)

Tris

One of the more notable mutagens is tris(2,3-dibromopropyl)phosphate, commonly called "tris," that was used as a flame retardant in children's sleepwear. Tris was found to be mutagenic in experimental animals and metabolites of it were found in children wearing the treated sleepwear. This strongly suggested that tris is absorbed through the skin and its uses were discontinued.

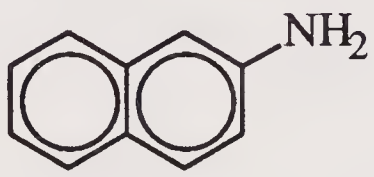
Carcinogenesis

Cancer is a condition characterized by the uncontrolled replication and growth of the body's own cells (somatic cells). **Carcinogenic agents** may be categorized as follows:[3]

- Chemical agents, such as nitrosamines and polycyclic aromatic hydrocarbons
- Biological agents, such as hepadna viruses or retro viruses
- Ionizing radiation, such as X-rays
- Genetic factors, such as selective breeding

Clearly, in some cases, cancer is the result of the action of synthetic and naturally occurring chemicals. The role of xenobiotic chemicals in causing cancer is called **chemical carcinogenesis**. It is often regarded as the single most important facet of toxicology and clearly the one that receives the most publicity.

Chemical carcinogenesis has a long history. In 1775 Sir Percivall Pott, Surgeon General serving under King George III of England, observed that chimney sweeps in London had a very high incidence of cancer of the scrotum, which he related to their exposure to soot and tar from the burning of bituminous coal. (This occupational health hazard was exacerbated by their aversion to bathing and changing underwear.) A German surgeon, Ludwig Rehn, reported elevated incidences of bladder cancer in dye workers exposed to chemicals extracted from coal tar; 2-naphthylamine



was shown to be largely responsible. Other historical examples of carcinogenesis include observations of cancer from tobacco juice (1915), oral exposure to radium from painting luminescent watch dials (1929), tobacco smoke (1939), and asbestos (1960).

Biochemistry of Carcinogenesis

Large expenditures of time and money on the subject in recent years have yielded a much better understanding of the biochemical bases of chemical carcinogenesis.[3] The overall processes for the induction of cancer may be quite complex, involving numerous steps. However, it is generally recognized that there are two major steps in carcinogenesis: an initiation stage followed by a promotional stage. These steps are further subdivided as shown in Figure 9.4.

Initiation of carcinogenesis may occur by reaction of a **DNA-reactive species** with DNA or by the action of an **epigenetic carcinogen** that does not react with DNA and is carcinogenic by some other mechanism. Most DNA-reactive species are **genotoxic carcinogens** because they are also mutagens. These substances react irreversibly with DNA. They are either electrophilic or, more commonly, metabolically activated to form electrophilic species, as is the case with electrophilic $^{+}CH_3$ gener-

ated from dimethylnitrosamine, as discussed under mutagenesis above. Cancer-causing substances that require metabolic activation are called **precarcinogens** or **procarcinogens**. The metabolic species actually responsible for carcinogenesis is termed an **ultimate carcinogen**. Some species that are intermediate metabolites between precarcinogens and ultimate carcinogens are called **proximate carcinogens**. Carcinogens that do not require biochemical activation are categorized as **primary** or **direct-acting carcinogens**. Some example procarcinogens and primary carcinogens are shown in Figure 9.5.

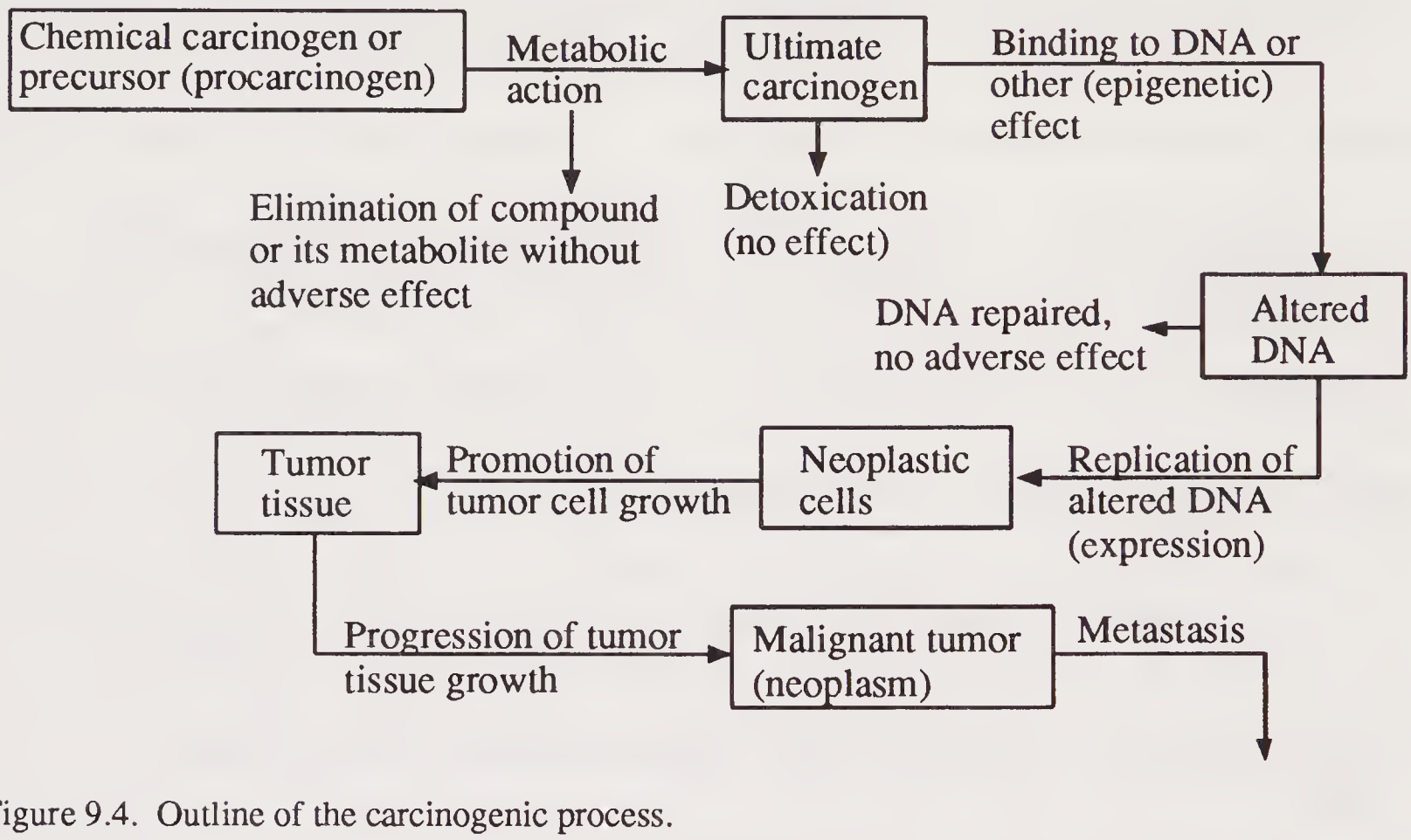
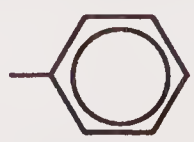


Figure 9.4. Outline of the carcinogenic process.

Most substances classified as epigenetic carcinogens are **promoters** that act after initiation. Manifestations of promotion include increased numbers of tumor cells and decreased length of time for tumors to develop (shortened latency period). Promoters do not initiate cancer, are not electrophilic, and do not bind with DNA. The classic example of a promotor is a substance known chemically as decanoyl phorbol acetate or phorbol myristate acetate, a substance extracted from croton oil.

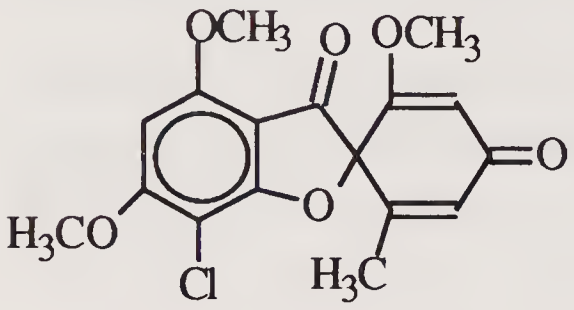
Alkylating Agents in Carcinogenesis

Chemical carcinogens usually have the ability to form covalent bonds with macromolecular life molecules.[4] Such covalent bonds can form with proteins, peptides, RNA, and DNA. Although most binding is with other kinds of molecules, which are more abundant, the DNA adducts are the significant ones in initiating cancer. Prominent among the species that bond to DNA in carcinogenesis are the alkylating agents which attach alkyl groups — such as methyl (CH_3) or ethyl (C_2H_5) — to DNA. A similar type of compound, **arylating agents**, act to attach aryl moieties, such as the phenyl group

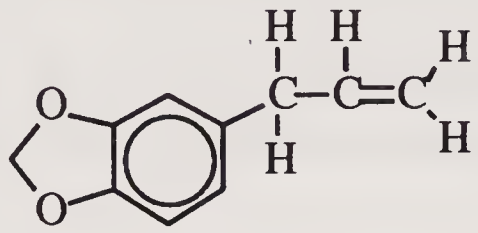

Phenyl group

to DNA. As shown by the examples in Figure 9.6, the alkyl and aryl groups become attached to N and O atoms in the nitrogenous bases that compose DNA. This alteration in the DNA can initiate the sequence of events that results in the growth and

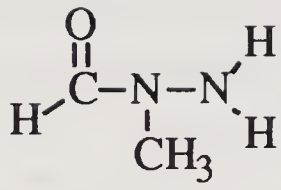
Naturally occurring carcinogens that require bioactivation



Griseofulvin (produced by *Penicillium griseofulvum*)

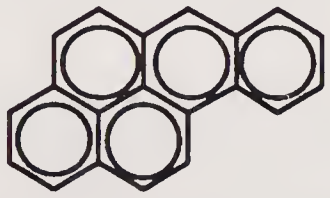


Saffrole (from sassafras)

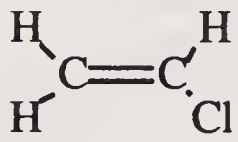


N-methyl-N-formylhydrazine (from edible false morel mushroom)

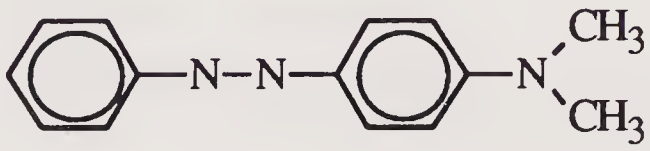
Synthetic carcinogens that require bioactivation



Benzo(a)pyrene

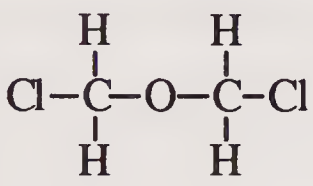


Vinyl chloride

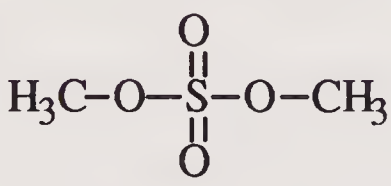


4-Dimethylaminoazobenzene

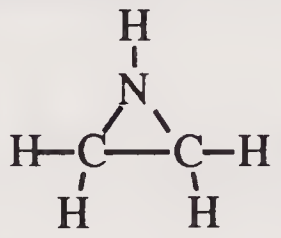
Primary carcinogens that do not require bioactivation



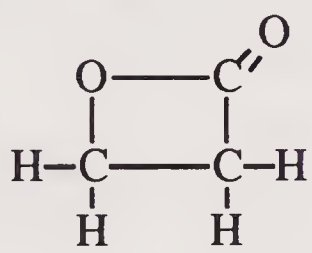
Bis(chloromethyl)-ether



Dimethyl sulfate



Ethyleneimine



β-Propiolactone

Figure 9.5. Examples of the major classes of naturally occurring and synthetic carcinogens, some of which require bioactivation and others of which act directly.

replication of neoplastic (cancerous) cells. The reactive species that donate alkyl groups in alkylation are usually formed by metabolic activation as shown for dimethylnitrosamine in the discussion of mutagenesis earlier in this section.

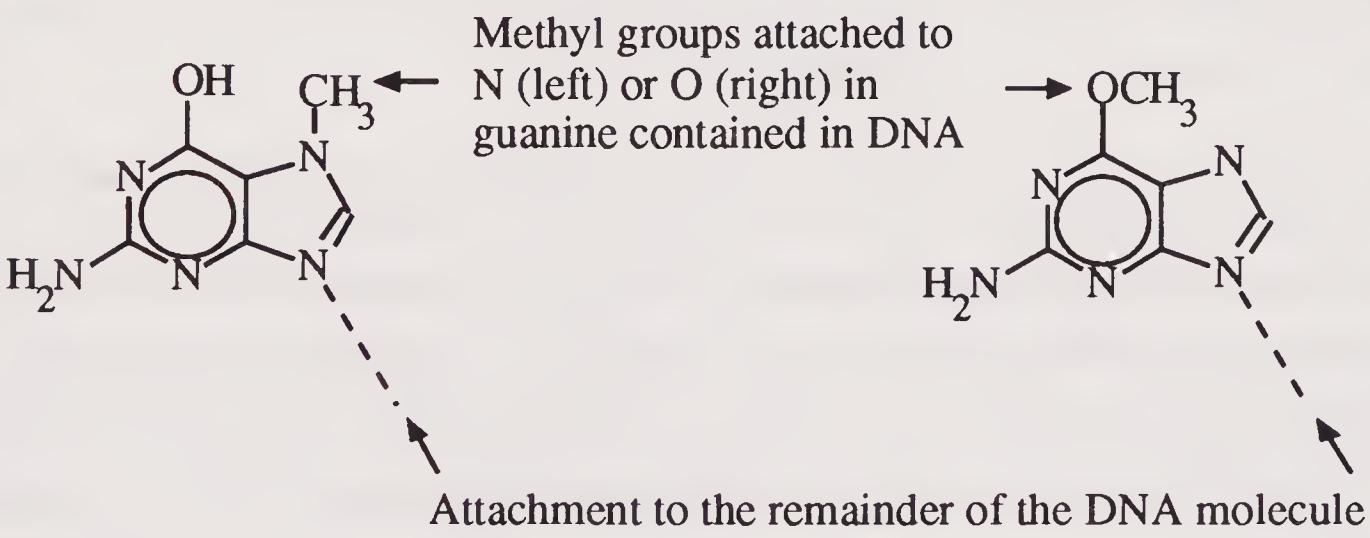


Figure 9.6. Alkylated (methylated) forms of the nitrogenous base guanine.

Testing for Carcinogens

In some cases chemicals are known to be carcinogens from epidemiological studies of exposed humans. Animals are used to test for carcinogenicity, and the results can be extrapolated with some uncertainty to humans. The most broadly

applicable test for potential carcinogens is the **Bruce Ames** procedure, which actually reveals mutagenicity. The principle of this method is the reversion of mutant histidine-requiring *Salmonella* bacteria back to a form that can synthesize their own histidine.[6] The bacteria are inoculated onto a medium that does not contain histidine, and those that mutate back to a form that can synthesize histidine establish colonies which are assayed on the growth medium, thereby providing both a qualitative and quantitative indication of mutagenicity. The test chemicals are mixed with homogenized liver tissue to simulate the body's alteration of chemicals (conversion of procarcinogens to ultimate carcinogens). Up to 90% correlation has been found between mutagenesis on this test and known carcinogenicity of test chemicals.

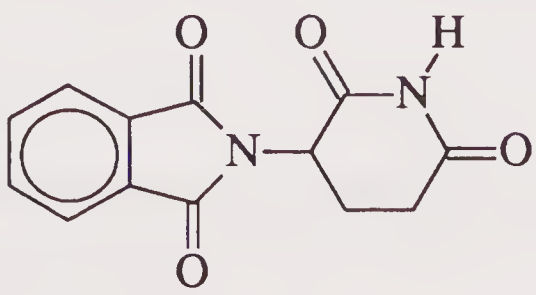
Biochemistry of Teratogenesis

Teratology is the science of birth defects caused by radiation, viruses, and chemicals, including drugs. Xenobiotic chemical species that cause birth defects are called **teratogens**. Teratogens affect developing embryos adversely, often with remarkable specificity in regard to effect and stage of embryo development when exposed. A teratogen may cause a specific effect when exposure occurs on a definite number of days after conception; if exposure occurs only a few days sooner or later, no effect, or an entirely different one, may be observed. Although mutations in germ cells (egg or sperm cells) may cause birth defects (e.g., Down's syndrome), teratology usually deals with defects arising from damage to embryonic or fetal cells.

The biochemical aspects of teratology are not particularly well understood. Several kinds of biochemical mechanisms are probably involved. One such mechanism is interference with DNA synthesis, which alters the function of nucleic acids in cell replications resulting in effects that are expressed as birth defects. Exposure to teratogenic xenobiotic substances may result in either an absence or excess of chromosomes. Enzyme inhibition (see Section 9.3) by xenobiotics can result in birth defects. Xenobiotics that deprive the fetus of essential substrates (for example, vitamins), that interfere with energy supply, or that alter the permeability of the placental membrane may all cause birth defects.

Thalidomide

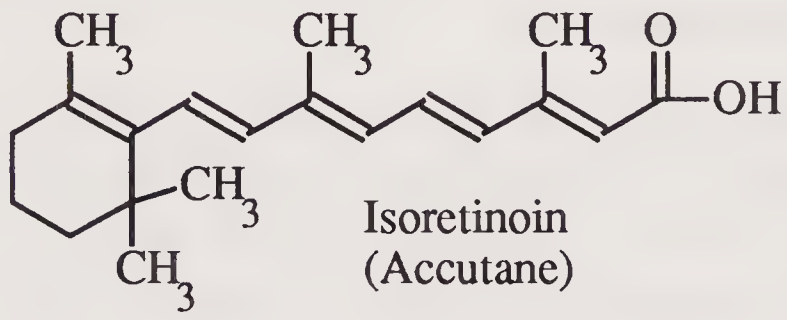
Perhaps the most notorious teratogen is thalidomide (see below), a sedative-hypnotic drug used in Europe and Japan in 1960-1961. Some infants born to women who had taken thalidomide from days 35 through 50 of their pregnancies were born suffering from amelia or phocomelia, the absence or severe shortening, respectively, of the limbs. About 10,000 children were affected. The biochemical action of thalidomide leading to teratogenesis is not well understood. Possibilities include adverse modification of DNA and interference with the metabolism of folic acid or glutamic acid.[7]



Thalidomide

Accutane

In 1988 the U.S. Food and Drug Administration estimated that **Accutane**, used as an anti-acne medication, may have been reponsible for approximately 1,000 birth defects in children born to women taking the drug during the period 1982–1986.[8] The chemical name for Accutane is isoretinoin, and it is chemically related to retinoic acid, Vitamin A, which likewise is teratogenic at excessive levels. Exposure of the fetus to the Accutane over a period of only several days can result in birth defects such as severe facial malformations, heart defects, and mental retardation.[9]



Isoretinoin
(Accutane)

Xenobiotics and the Immune System

The immune system response to toxicants, **immunogenesis**, was discussed in Section 8.12. Perhaps the most common biochemical response to a xenobiotic compound is the production of antibodies to xenobiotic compounds (antigens) bound as conjugates with proteins. In such a case the foreign body is called a **hapten**. Antibody production is the basis for the body's immunologic response to many small-molecule xenobiotics. The result of this response is an **allergic reaction**, which may be quite severe, or even fatal.

A variety of chemical and therapeutic agents are known to cause allergic reactions in susceptible individuals.[10] Several prominent examples of these are the following:

- Formaldehyde used in a large number of consumer products, resins, and wood products. This agent causes type I hypersensitivity manifested by respiratory symptoms including rhinitis, bronchial asthma, and asthmatic bronchitis.

- Trimellitic anhydride used in chemical synthesis. Type II hypersensitivity manifested by adverse effects on blood, including hemolytic anemia and bone marrow depression may be caused by exposure to trimellitic anhydride. This agent may also cause type III hypersensitivity resulting from deposition of antigen-antibody complexes in tissue and causing symptoms such as rheumatoid disease or pneumonitis.

- A variety of agents including antibiotic penicillin, beryllium, mercapto-benzothiazole, phthalic anhydride, and dichromate salts cause type IV allergic reactions, one common symptom of which is contact dermatitis.

- Immunosuppression (see Section 8.12) can result from exposure to a number of agents including ozone, benzene, asbestos, silica, nitrogen mustards and several metals.

Ionizing Radiation

Although not a chemical agent as such, ionizing radiation, such as X-rays or alpha particles from ingested alpha emitters, cause chemical reactions that have toxic, even fatal, effects. The toxicologic effects of radiation have to do with its physical and chemical interactions with matter and the biological consequences that result.[11] Ionizing radiation alters chemical species in tissue and can lead to significant and harmful alterations in the tissue and in the cells that make up the tissue. Radon and radium, two radioactive elements of particular concern for their potential to expose humans to ionizing radiation, are discussed in Chapter 10.

There is not room here to discuss the detailed mechanisms by which exposure to radiation causes adverse responses. Much of the effects of radiation result from its interaction with water to produce active species that include superoxide ($O_2 \cdot^-$), hydroxyl radical (HO·), hydroperoxyl radical (HOO·), and hydrogen peroxide (H_2O_2). These species oxidize cellular macromolecules. When DNA is so affected, mutagenesis and carcinogenesis may result. Ionizing radiation can also interact with organic substances to produce a carbonium ion, such as $^+CH_3$, that can alkylate nitrogenous bases on DNA:

9.4. Toxicological Chemistry of the Elements

This section addresses the toxicological chemistry of elemental species. These substances are covered further in Chapter 10.

Many elements are not very toxic in their chemically uncombined elemental forms. This is because a majority of elemental forms are nonvolatile solids that cannot become systemic poisons unless they react to form a chemically active compound. The elements that may have toxic effects include strongly reducing metals that form corrosive bases (sodium, potassium), strongly oxidizing nonmetals that form corrosive acids (the halogens), oxygen as ozone, white phosphorus, and mercury. Others may be included; for example, noble gases and nitrogen act as simple asphyxiants by simply depriving an organism of oxygen. Other toxic elemental forms are not discussed because exposure to them is unlikely.

Strongly Reducing Base-Forming Metals

"Strongly reducing base-forming metals" are exemplified by sodium and potassium. These are extremely reactive substances with only limited exposure to humans. Reaction with water, such as water in tissues, may be represented as

$$Na + H_2O \rightarrow NaOH + \{H\} \qquad (9.4.1)$$

where {H} stands for reductant (nascent hydrogen) produced by the reaction. The reactive {H} intermediate, if it is even produced, goes very rapidly to H_2. It is readily seen that exposure to alkali metals can cause localized base burns.

Strongly Oxidizing Acid-Forming Nonmetals

Elemental forms that can be classified as "strongly oxidizing acid-forming nonmetals" are **halogens** — fluorine, chlorine, bromine and iodine. Their properties are best exemplified by elemental chlorine (Cl_2, mp -101°C, bp -34.5°C) a highly reactive and toxic gas. It reacts with water to form both strong acid (HCl) and strongly oxdizing species (HOCl, {O}),

$$Cl_2 + H_2O \leftarrow\rightarrow HCl + HOCl \quad (9.4.2)$$

$$Cl_2 + H_2O \leftarrow\rightarrow 2HCl + \{O\} \quad (9.4.3)$$

where HOCl is oxidant hypochlorous acid and {O} stands for nascent oxygen (in a chemical sense regarded as freshly generated, highly reactive oxygen atoms). These reactions occur in moist tissue lining the respiratory tract and in eye tissue, causing severe irritation and other effects.

Fluorine, F_2, is an even stronger oxidant than chlorine. Of all the elements, fluorine is the most reactive and the most electronegative (a measure of tendency to acquire electrons as an oxidizing agent).

Bromine (Br_2, mp -7.3°C, bp 58.7°C) is a dark red liquid that is chemically similar to fluorine and chlorine. Like chlorine and fluorine, bromine is a toxic irritant to the respiratory tract and eyes because it attacks their mucous membranes. Pulmonary edema may result from severe bromine poisoning. Elemental iodine (I_2, solid, sublimes at 184°C) consists of violet-black rhombic crystals with a lustrous metallic appearance. More irritating to the lungs than bromine or chlorine, its general effects are similar to these elements. However, exposure to iodine is limited by its low vapor pressure compared to liquid bromine or gaseous chlorine or fluorine.

Oxygen

The harmful effects of molecular oxygen in the body result from its reduction products, such as hydroperoxyl radical:

$$O_2 + e^- + H^+ \rightarrow HO_2\cdot \quad (9.4.4)$$

where the dot denotes an unpaired electrons. Other reduction products of molecular oxygen are H_2O_2 (hydrogen peroxide), and HO· (hydroxyl radical) are very chemically reactive. The radicals and hydrogen peroxide vigorously attack tissue and DNA, causing what are called oxidative lesions. Oxygen appears to interfere with a number of enzymatic pathways including glycolysis, respiration, and electron transport. Enzyme and coenzyme activities requiring free -SH groups appear to be susceptible to the presence of oxygen.

Ozone, O_3, is a toxic potent oxidant. In the body ozone reacts to produce reactive free radicals. These species become involved in destructive oxidative processes, particularly reaction with -SH groups and lipid peroxidation. This is a process in which the C=C double bonds in unsaturated lipids are attacked by free radicals and undergo chain reactions in the presence of O_2, resulting in their oxidative destruction. These effects can be mitigated by radical scavengers and antioxidants. Species rich in sulfhydryl groups, such as metallothionien shown in Equation 9.4.7 act as antidotes to ozone poisoning.

White Phosphorus

White phosphorus is the most common and by far the most toxic elemental form of phosphorus. It can be absorbed into the body particularly through inhalation, as well as through the oral and dermal routes. Although poorly water soluble, it tends to be soluble in lipids. Its high vapor pressure and lipid solubility cause particular hazards from inhalation, and chronic poisoning occurs largely through the uptake of low concentrations of white phosphorus through the lungs. Little is known about the toxicological chemistry of white phosphorus.

Mercury

Elemental mercury is the only metal that is a liquid at room temperature, and its relatively high vapor pressure contributes to its toxicological hazard. Monatomic elemental mercury in the vapor state, Hg(g), is absorbed from inhaled air by the pulmonary route to the extent of about 80%, but only a small fraction so absorbed leaves the body by the same route. In part because of its lipid solubility, Hg(0) in the body is absorbed by red blood cells and nerve cells.[12]

Elemental mercury undergoes biotransformation to mercury(II),

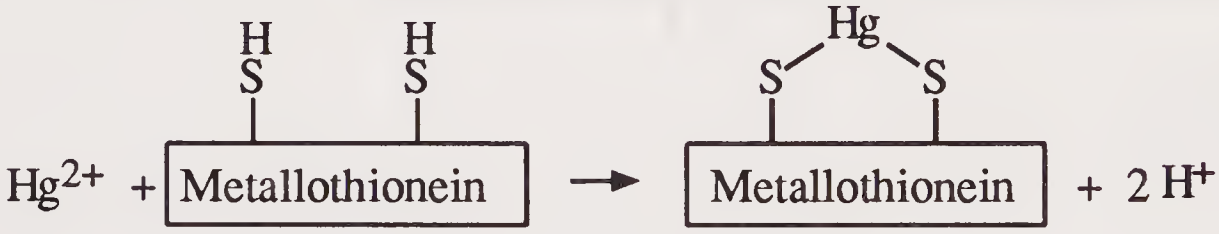
$$\text{Hg(0)} \xrightarrow[\text{oxidation}]{\text{Metabolic}} \text{Hg(II)} \qquad (9.4.5)$$

which is the toxicologically active form, largely because of its affinity for biological sulfhydryl groups:

$$\text{R-SH} + \text{Hg(II)} \rightarrow \text{R-S-Hg} \qquad (9.4.6)$$

Much cell injury results from this kind of binding to mitochondrial and microsomal enzymes. Chronic mercury poisoning is manifested primarily by effects on the central nervous system, including symptoms of excitability and tremor.

Kidney tissue of organisms exposed to mercury induce production of metallothionein, which consists of two similar sulfhydryl-rich proteins with a low molecular mass of about 6,500, discussed further in Section 10.4. Binding of mercury to metallothionein (below) may be involved in its detoxication.



(9.4.7)

9.5. TOXICOLOGICAL CHEMISTRY OF INORGANIC SPECIES

Cyanide

Highly toxic **cyanide** is encountered either in the form of gaseous **hydrogen cyanide** (HCN) or **cyanide ion** (CN^- present in cyanide salts such as KCN). A particular danger with hydrogen cyanide is its gaseous nature. Cyanide salts, such as NaCN, tend to be highly water-soluble. Acids can liberate hydrogen cyanide from cyanide ion by the following reaction:

$$H^+(aq) + CN^-(aq) \rightarrow HCN(g) \qquad (9.5.1)$$

As discussed in Section 12.2, the toxic action of cyanide results from its ability to bond chemically to iron(III) in ferricytochrome oxidase (Fe(III)-oxid), an iron-containing metalloprotein that acts as an acceptor of electrons during the oxidation of glucose. This prevents reduction of the crucial ferricytochrome oxidase enzyme to ferrouscytochrome oxidase (Fe(II)-oxid). The net result of this is that cyanide intoxication deprives the body of oxygen by acting as a **chemical asphyxiant**.

The metabolic pathway for the detoxification of cyanide involves conversion to the less toxic thiocyanate by the *rhodanase* enzyme-catalyzed reaction with thiosulfate or colloidal sulfur:

$$CN^- + S_2O_3^{2-} \xrightarrow[\text{Rhodanase}]{} SCN^- + SO_3^{2-} \qquad (9.5.2)$$

Carbon Monoxide

Carbon monoxide, CO, is a toxic gas that enters the blood stream through the lungs and reacts with hemoglobin (Hb) as follows where O_2Hb is oxyhemoglobin and COHb is carboxyhemoglobin:

$$O_2 + CO \rightarrow COHb + O_2 \qquad (9.5.3)$$

Carboxyhemoglobin is several times more stable than oxyhemoglobin and ties up the hemoglobin so that it cannot carry oxygen to body tissues.

Nitrogen Oxides

The two most common oxides of nitrogen are **nitric oxide** (NO) and **nitrogen dioxide** (NO_2), designated collectively as NO_x. Inhalation of NO_2 causes severe irritation of the innermost parts of the lungs resulting in pulmonary edema and fatal bronchiolitis fibrosa obliterans. The biochemical action of NO_2 includes disruption of some enzyme systems, such as lactic dehydrogenase. Nitrogen dioxide probably acts as an oxidizing agent similar to, though weaker than, ozone, which is discussed in Section 9.4. Included is the formation of free radicals, particularly the hydroxyl radical HO·. Like ozone, it is likely that NO_2 causes lipid peroxidation.

Hydrogen Halides

The hydrogen halides are HF, HCl, HBr, and HI. The most dangerous of these is hydrogen fluoride (mp -83.1°C, bp 19.5°C), which may be in the form of a clear, colorless liquid, a gas, or an aqueous solution of hydrofluoric acid. Hydrogen fluoride and hydrochloric acid are so reactive that they will even attack silica, SiO_2:

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O \qquad (9.5.4)$$

Both hydrogen fluoride and hydrofluoric acid, referred to collectively as HF, are extreme irritants to any part of the body that they contact.

Hydrogen chloride (HCl, mp -114°C, bp -84.8°C) is a major industrial chemical that may be encountered as a gas, pressurized liquid, or aqueous solution called **hydrochloric acid**. Hydrogen chloride is not nearly as toxic as HF. Because of its high affinity for water, HCl vapor tends to dehydrate tissue of the eyes and respiratory tract.

Hydrogen bromide (HBr, mp -87°C, bp -66.5°C) and **hydrogen iodide** (HI, mp -50.8°C, bp -35.4°C) are both pale yellow or colorless gases, although contamination by their respective elements tends to impart some color to these compounds. Both are very dense gases, 3.5 g/L for HBr and 5.7 g/L for HI at 0°C and atmospheric pressure. These compounds are used much less than HCl. Both are irritants to the skin and eyes and to the oral and respiratory mucous membranes.

Interhalogen Compounds

Halogens form compounds among themselves and with oxygen. Some of these compounds are important industrially and toxicologically. Some of the more important such compounds are mentioned here.

Interhalogen Compounds

Any of the elements in the order F>Cl>Br>I can oxidize any of the halogens following it to produce **interhalogen compounds**,[13] such as chlorine monofluoride, ClF, bromine trifluoride, BrF_3, iodine pentafluoride, IF_5,bromine monochloride, BrCl, and iodine tribromide, IBr_3. For the most part interhalogen compounds exhibit extreme reactivity. They react with water or steam to produce hydrohalic acid solutions (HF, HCl) and nascent oxygen {O}. They tend to be potent oxidizing agents for organic matter and oxidizable inorganic compounds. These chemical properties are reflected in the toxicities of the interhalogen compounds. Toxicologically, interhalogen compounds tend to be powerful corrosive irritants that acidify, oxidize, and dehydrate tissue, attacking the skin, eyes and mucous membranes of the mouth, throat, and pulmonary systems.

Halogen Oxides

The oxides of the halogens, such as fluorine monoxide, OF_2, chlorine heptaoxide, Cl_2O_7, and iodine pentoxide, I_2O_5, tend to be unstable and reactive. Commercially, the most important of the halogen oxides is chlorine dioxide, ClO_2, used as a substitute for chlorine in water disinfection. For the most part, the halogen oxides are highly reactive substances with toxicity and hazard characteristics similar to those of the interhalogen compounds.

Hypochlorous Acid and Hypochlorites

The halogens form several oxyacids and their corresponding salts. Of these, the most important is hypochlorous acid (HOCl) formed by the following reaction:

$$Cl_2 + H_2O \leftarrow\rightarrow HCl + HOCl \qquad (9.5.5)$$

Hypochlorous acid and hypochlorites are used for bleaching and disinfection. They produce active (nascent) oxygen ({O}) as shown by the reaction below:

$$HClO \rightarrow H^+ + Cl^- + \{O\} \qquad (9.5.6)$$

The resulting oxidizing action is largely responsible for the toxicity of hypochloric acid and hypochlorites. These compounds are irritants to eye, skin, and mucous membrane tissue.

Nitrogen Halides and Hydrohalides

The major nitrogen halides are gaseous nitrogen trifluoride, NF_3; liquid nitrogen trichloride, NCl_3; solid nitrogen tribromide, NBr_3; violently reactive nitrogen triiodide, NI_3; and tetrafluorohydrazine, N_2H_4. Although the nitrogen halides are

considered to be very toxic, direct exposure to them tends to be limited because of their reactivity, which may destroy the compound before exposure.

Monochloramine and Dichloramine

The substitution of Cl for H on ammonia can form monochloramine, NH_2Cl, and dichloramine, $NHCl_2$. These compounds are added to drinking water to provide residual disinfection.

Inorganic Compounds of Silicon

Silica

Silica, SiO_2, is a hard mineral substance known as quartz in the pure form and occurring in a variety of minerals such as sand, sandstone, and diatomaceous earth. Exposure to silicon in materials used for construction, sand blasting, refractories manufacture, and many other industrial applications, causes **silicosis**, a lung condition manifested by fibrosis and nodules in the lung. The observation of abnormalities in antibodies and cellular immunological functions associated with silicosis suggests that it may involve immune system response.[14]

Asbestos

Asbestos describes a group of fibrous silicate minerals, such as those of the serpentine group, approximate formula $Mg_3P(Si_2O_5)(OH)_4$. Asbestos has many properties, such as insulating abilities and heat resistance, that have given it numerous uses in structural materials, brake linings, insulation, and pipe manufacture. As described in Section 12.9 and discussed in detail in reference works on asbestos,[15] inhalation of the fibers may cause asbestosis (a pneumonia condition), mesothelioma (tumor of the mesothelial tissue lining the chest cavity adjacent to the lungs), and bronchogenic carcinoma (cancer originating with the air passages in the lungs).

Silanes, Silicon Halides, and Halohydrides

Compounds of silicon with hydrogen are called **silanes**, the simplest of which is silane, SiH_4. Inorganic silanes produced for commercial use are dichloro- and trichlorosilane, SiH_2Cl_2 and $SiHCl_3$, respectively, used as intermediates in the synthesis of organosilicon compounds and in the production of high-purity silicon for semiconductors. In general, not much is known about the toxicities of silanes. Chlorosilanes are irritants to eye, nasal, and lung tissue.

Although all four **silicon tetrahalides** with the general formula SiX_4 are known to exist, only silicon tetrachloride, $SiCl_4$, is produced in significant quantities. It is used to manufacture fumed silica (finely divided SiO_2). The **silicon halohydrides** (general formula $H_{4-x}SiX_x$) that is commercially important is trichlorosilane, $HSiCl_3$. Silicon tetrachloride and trichlorosilane, both fuming liquids with suffocating odors, react with water to give off HCl vapor.

Calcium Oxide

Calcium oxide, CaO, also known as quicklime or unslaked lime, reacts vigrously and exothermically with water to produce calcium hydroxide:

$$CaO + H_2O \rightarrow Ca(OH)_2 + \text{heat} \qquad (9.5.7)$$

The effects of this reaction can be especially harmful to eye tissue. Because of the alkaline nature of calcium hydroxide it causes alkali burns. The effect is aggravated by the heat generated. Furthermore, calcium hydroxide is not very soluble and exposure to solid CaO tends to produce moist clumps on the eye surface that are not readily removed by irrigation with water.[16]

Inorganic Phosphorus Compounds

Phosphine

Phosphine (PH_3, mp -132°C, bp -88°C) is a colorless gas that undergoes autoignition at 100°C. It is used for the synthesis of organophosphorus compounds. Phosphine gas is a very toxic, potentially fatal pulmonary tract irritant and central nervous system depressant.

Phosphorus Pentoxide

The most common phosphorus oxide is P_4O_{10}, commonly misnamed **phosphorus pentoxide**. The freshly produced oxide is a fluffy white powder that removes water from air to form syrupy orthophosphoric acid,

$$P_4O_{10} + 6H_2O \rightarrow 4H_3PO_4 \qquad (9.5.8)$$

a corrosive irritant to skin, eyes and mucous membranes that dehydrates and acidifies exposed tissue.

Phosphorus Halides

Phosphorus halides (general formulas PX_3 and PX_5) include phosphorus trifluoride (PF_3), a colorless gas (mp -152°C, bp -102°C), and phosphorus pentabromide (PBr_5,) a yellow solid that decomposes at approximately 100°C. Of these compounds the most important commercially is phosphorus pentachloride used as a catalyst in organic synthesis, as a chlorinating agent and as a raw material to make phosphorus oxychloride ($POCl_3$). The strong reaction of phosphorus halides with water (below) makes them strong irritants to eyes, skin, and mucous membranes.

$$PCl_5 + 4H_2O \rightarrow H_3PO_4 + 5HCl \qquad (9.5.9)$$

Phosphorus Oxyhalides

Of the known phosphorus oxyhalides (POF_3, $POCl_3$, $POBr_3$) the one with commercial uses is phosphorus oxychloride ($POCl_3$). Its uses are similar to those of phosphorus trichloride, acting in chemical synthesis as a chlorinating agent and for the production of organic chemical intermediates. The liquid compound evolves toxic vapors and it is a strong irritant to the eyes, skin, and mucous membranes.

Inorganic Compounds of Sulfur

Hydrogen Sulfide

Hydrogen sulfide (H_2S) is a colorless gas (mp -86°C, bp -61°C) with a foul rotten-egg odor. It is produced in large quantities as a byproduct of coal coking and petroleum refining and massive quantities are removed in the cleansing of sour natural gas. A very toxic substance, hydrogen sulfide causes death by asphyxiation as a consequence of respiratory system paralysis.

Sulfur Dioxide and Sulfites

Sulfur dioxide (SO_2) is a common air pollutant produced by the combustion of pyrite (FeS_2) in coal and organically bound sulfur in coal and fuel oil as shown by the two following reactions:

$$4FeS_2 + 11O_2 \rightarrow 2Fe_2O_3 + 8SO_2 \qquad (9.5.10)$$

$$S(\textit{organic, in fuel}) + O_2 \rightarrow SO_2 \qquad (9.5.11)$$

Sulfur dioxide is an irritant to the eyes, skin, mucous membranes, and respiratory system. As a water-soluble gas, it is largely removed in the upper respiratory tract (see Section 8.2).

Dissolved in water, sulfur dioxide produces **sulfurous acid**, H_2SO_3; **hydrogen sulfite ion**, HSO_3^-; and **sulfite ion**, SO_3^{2-}. Sodium sulfite (Na_2SO_3) has been used as a chemical food preservative, although some individuals are hypersensitive to it.

Sulfuric Acid

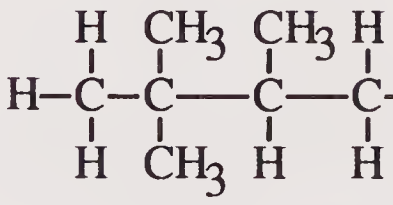
Sulfuric acid , H_2SO_4, the most widely produced synthetic organic chemical, is a severely corrosive poison. In addition to being a strong acid, it acts as a dehydrating agent in the concentrated liquid form, removing liquid from tissue and destroying it. Sulfuric acid in the concentrated form can also function as an oxidant.

9.6. ORGANIC COMPOUNDS AND HYDROCARBONS

This section addresses the toxicological chemistry of the simplest organic compounds, hydrocarbons, which contain only carbon and hydrogen. Subsequent sections discuss organic compounds that contain other elements.

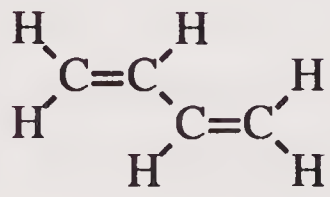
Hydrocarbons may be grouped into the categories listed below, with an example of each shown:

- **Alkanes**



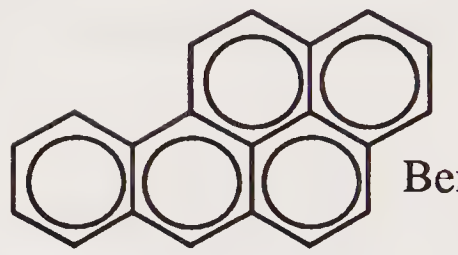
2,2,3,-Trimethylbutane

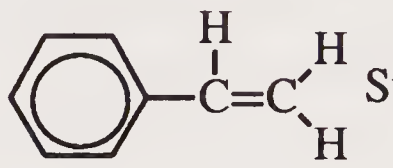
- **Unsaturated nonaromatic**



1,3-Butadiene

- **Aromatic**  Benzene

- **Polycyclic aromatic hydrocarbons**  Benzo(a)pyrene

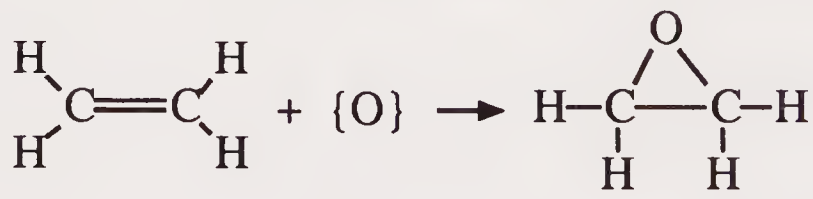
- **Mixed hydrocarbons**  Styrene

Alkanes

Alkanes are hydrocarbons in which the C atoms are joined by single covalent bonds.The two major classes of alkane reactions are **oxidation** to produce CO_2 and H_2O and **substitution**, especially with Cl_2, to produce compounds such as CH_2Cl_2 that contain elements other than carbon and hydrogen. The comparatively low chemical reactivity for alkanes is reflected in their relative lack of toxicity.

Unsaturated Nonaromatic Hydrocarbons

Unsaturated hydrocarbons, alkenes and **alkynes**, have multiple bonds. Alkenes may undergo **addition reactions** such as the following biochemically mediated epoxidation reaction:

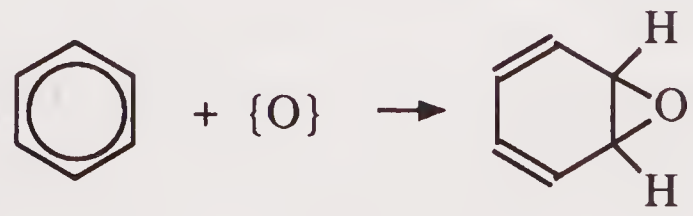


(9.6.1)

This kind of reaction, which is not possible with alkanes, adds to the chemical and metabolic, as well as toxicological, versatility of compounds containing unsaturated bonds.

Aromatic Hydrocarbons

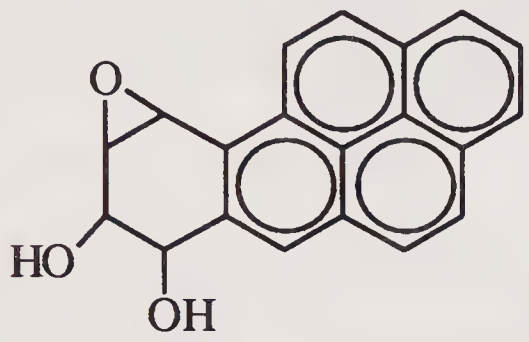
Aromatic hydrocarbons are usually those with 6-membered benzene rings. Though unsaturated, their chemical and toxicological chemical characteristics are quite different from those of alkenes. The most significant toxicological chemical reaction of an aromatic compound is the metabolic oxidation of benzene to form an epoxide that likely reacts with cell nucleophiles, damaging or destroying the cells:



(9.6.2)

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are high-carbon, low-hydrogen hydrocarbons formed by the incomplete combustion of other hydrocarbons. Benzo(a)pyrene is the most studied of the PAHs. It undergoes metabolic epoxidation and hydration to form the mutagenic and carcinogenic 7,8-diol-9,10-epoxide metabolic product:

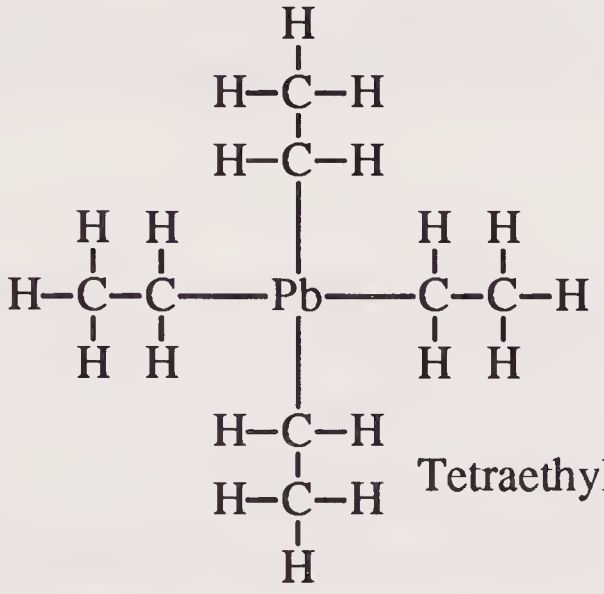


7,8–Diol–9,10–epoxide of benzo(a)pyrene

9.7. ORGANOMETALLIC AND ORGANOMETALLOID COMPOUNDS

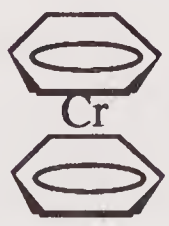
Organometallic compounds and **organometalloids** are those in which a metal or metalloid atom is bonded to at least one carbon atom in an organic group. Examples of the major types of such compounds are the following:

- Organometallic compounds with sigma-covalent metal-carbon bonds:



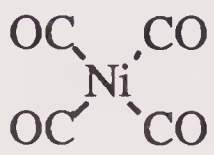
Tetraethyllead

- Metals bonded to π electron systems on organic groups:



Dibenzene chromium

- Carbonyl compounds in which a metal is bonded to CO:



Nickel carbonyl

The toxicological chemistry of organometallic compounds may be divided roughly between direct effects of the intact compound and effects of its degradation products. In the latter category are effects of the products of inorganic compounds of the metal released by hydrolysis in tissue. For example, hydrolysis of cyclopentadienylsodium,

$$C_5H_5^-Na^+ + H_2O \rightarrow C_5H_6 + Na^+OH^- \qquad (9.7.3)$$

yields highly caustic NaOH base that can be very damaging to tissue. In contrast, the toxicological action of tetraethyllead is different from that of inorganic lead. Attempts to remove lead from the body by treatment with chelating agents are ineffective in treating tetraethyllead poisoning, but reasonably helpful in alleviating effects of inorganic lead poisoning. This observation implies significantly different toxicological chemical mechanisms of distribution, metabolism, and toxicity of tetraethyllead compared to inorganic lead.

9.8. OXYGEN-CONTAINING ORGANIC COMPOUNDS

Figure 9.7 summarizes the categories of oxygen-containing compounds that are most significant for their toxicological chemistry. These are listed in generally increasing order of their degree of oxygenation.

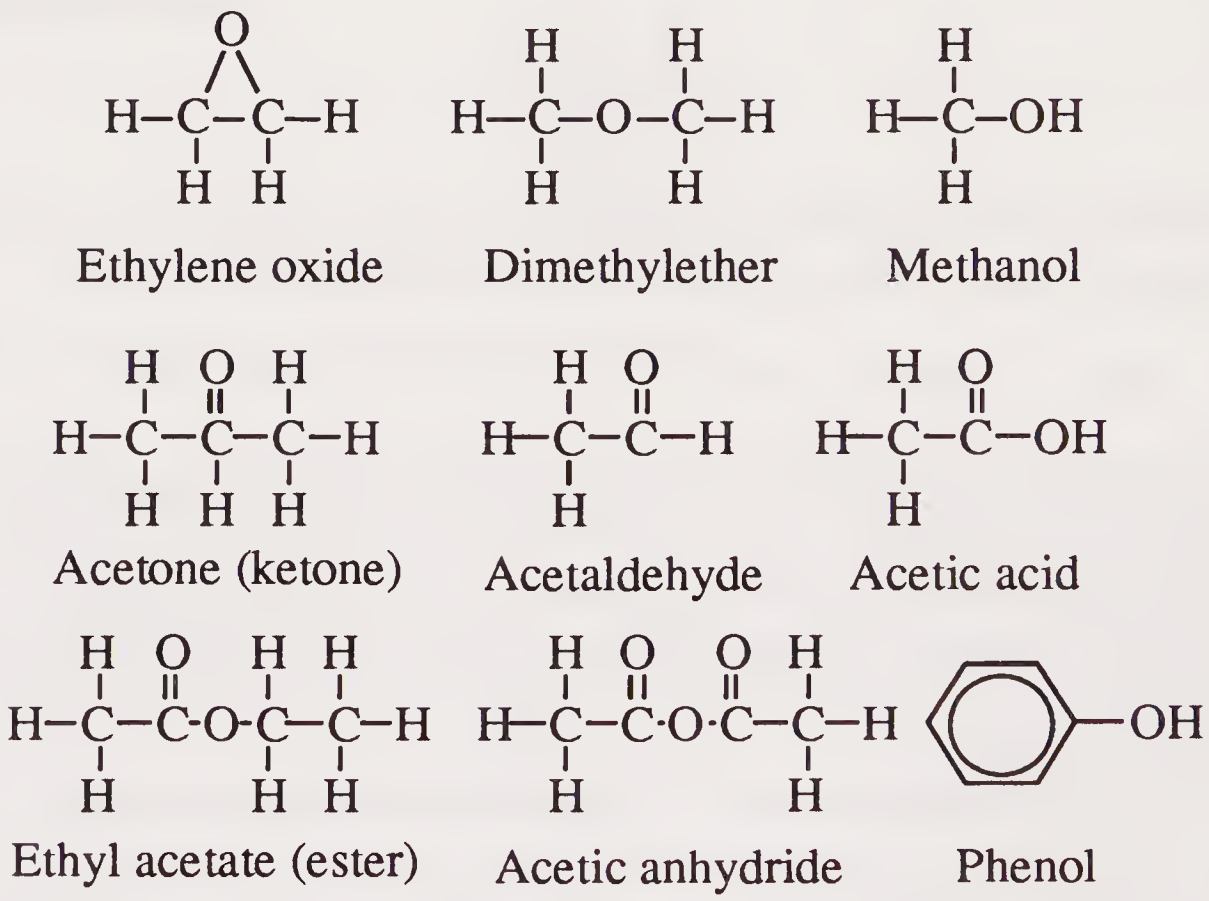
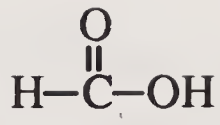
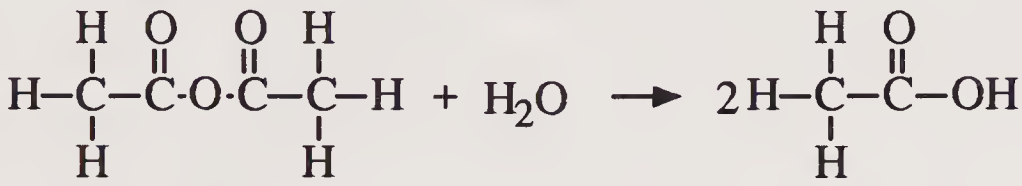


Figure 9.7. Classes of oxygen-containing organic compounds of toxicological chemical significance.

Organic **oxides** or **epoxides** have an oxygen atom bridging between two adjacent C atoms as shown for ethylene oxide in Figure 9.7. Oxides tend to be electrophilic, such that they can bind to electron-rich entities on DNA, possibly acting as mutagens or carcinogens. Because of the strength of the C–O bond, simple aliphatic **ethers** are relatively unreactive in the body, which limits their systemic toxicological effects. **Alcohols**, **aldehydes**, and **ketones** are metabolized in the body and have a variety of toxic effects. Because their carbonyl (C=O) group is on an end carbon, aldehydes tend to be more active and toxic. With the exception of formic acid,



which is atypical, **carboxylic acids** tend to have relatively low toxicities and are involved as intermediates in normal metabolic processes. Simple aliphatic **esters** tend to be widespread in nature; they are common flavor constituents that are usually readily metabolized in the body. In contrast, many **acid anhydrides**, such as acetic anhydride, exhibit a very active toxicological chemistry. Acetic anhydride reacts with water in tissue to undergo a hydrolysis reaction to form acetic acid:

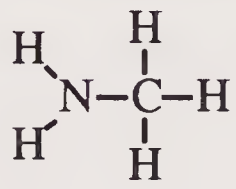


(9.8.1)

Acetic anhydride is a very toxic systemic poison that is especially corrosive to the skin, eyes, and upper respiratory tract, causing blisters and burns that heal only slowly. Several **aromatic alcohols**, the most prominent of which is phenol, are notably toxic.

9.9. ORGANONITROGEN COMPOUNDS

The simplest of the organonitrogen compounds are the amines, such as methylamine:

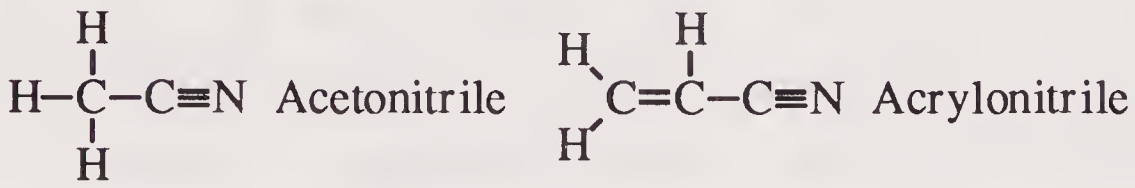


The low-molecular-mass amines are rapidly and easily taken into the body by all common exposure routes. They are basic and react with water in tissue,

$$R_3N + H_2O \rightarrow R_3N^+ + OH^- \qquad (9.9.1)$$

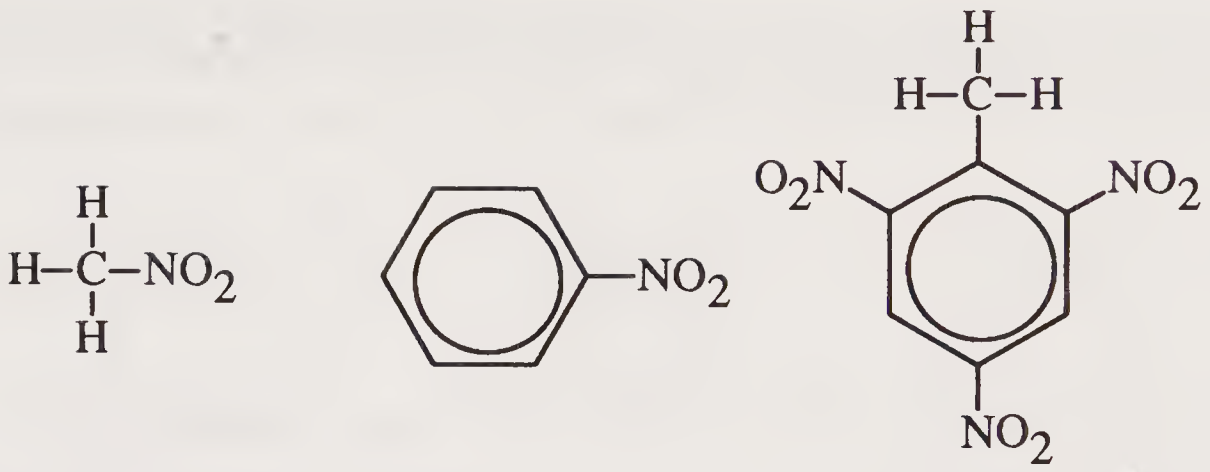
raising the pH of the tissue to harmful levels, thereby acting as corrosive poisons and causing several toxic effects.

In addition to amines, another significant class of organonitrogen compounds containing only C, H, and N consists of nitriles, such as acetonitrile and acrylonitrile,



which contain the -C≡N functional group. Acetonitrile is a relatively safe compound with a number of industrial uses. However, some nitriles, such as acrylonitrile, which contains an unsaturated hydrocarbon group, may be metabolized to release highly toxic HCN.

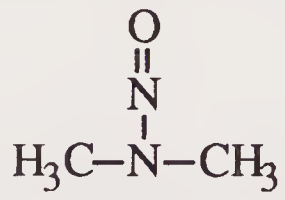
A number of organonitrogen compounds that are relatively toxic contain oxygen in addition to nitrogen. Prominent among these are the nitro compounds, such as those shown in Figure 9.8, which contain the $-NO_2$ functional group. These compounds exhibit a variety of toxicological chemical behavior. For example, nitrobenzene converts hemoglobin to methemoglobin, which cannot carry oxygen to body tissue.



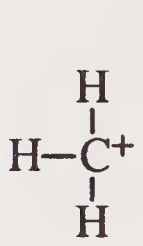
Nitromethane Nitrobenzene Trinitrotoluene (TNT)

Figure 9.8. Some major nitro compounds.

Of particular significance among organic compounds that contain both nitrogen and oxygen are **N-nitroso** compounds (**nitrosamines**), such as dimethylnitrosamine,

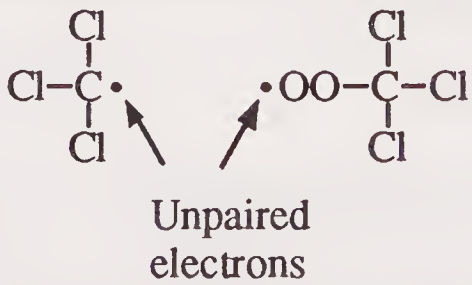


a known carcinogen once widely used as an industrial solvent. Dimethylnitrosamine and several other nitrosamines are known to be carcinogenic. As discussed in Section 9.4, this effect is due to their ability to form electrophilic carbonium ions that bond to DNA, such as the following:



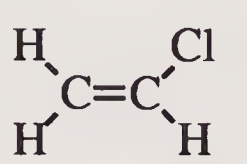
9.10. ORGANOHALIDE COMPOUNDS

Organohalide compounds exhibit a variety of toxicological chemical behavior. Of the simple alkyl chlorides, carbon tetrachloride, CCl_4, has the most notorious record of toxicity. Used for many years as a degreasing solvent, in home fire extinguishers, and for other industrial and consumer product applications, carbon tetrachloride compiled a grim record of toxic effects which led the U. S. Food and Drug Administration (FDA) to prohibit its household use in 1970. Carbon tetrachloride is a systemic poison that affects the nervous system when inhaled and the gastrointestinal tract, liver, and kidneys when ingested. The biochemical mechanism of carbon tetrachloride toxicity involves reactive radical species including those shown below:

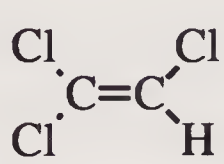


These compounds react with biomolecules, such as proteins and DNA. The most damaging such reaction is **lipid peroxidation**, which occurs in the liver and consists of the attack of free radicals on unsaturated lipid molecules, followed by oxidation of the lipids through a free radical mechanism.

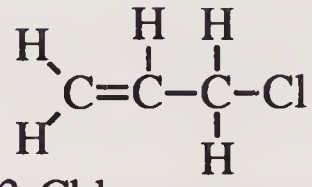
The most significant **alkenyl** or **olefinic organohalides** are the lighter chlorinated compounds, such as the following:



Monochloroethylene (vinyl chloride)



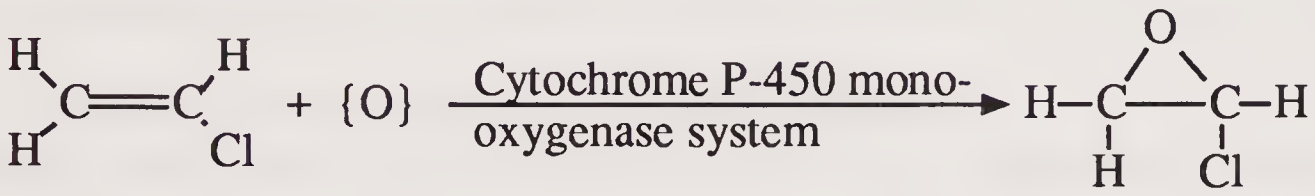
Trichloroethylene (TCE)



3-Chloropropene (allyl chloride)

Because of their widespread use and disposal in the environment, the numerous acute and chronic toxic effects of the alkenyl halides are of considerable concern.

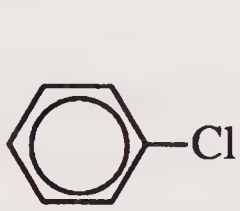
The most toxicologically significant alkenyl halide is vinyl chloride, exposure to which has been widespread because of its use in polyvinylchloride polymer manufacture. Most vinyl chloride exposure is through the pulmonary route. It affects the central nervous system, respiratory system, liver, and blood and lymph systems. In the liver vinyl chloride is oxidized,



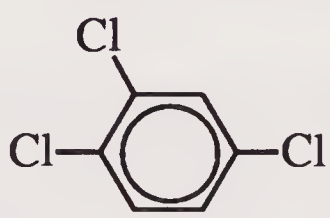
(9.10.1)

to produce an electrophilic epoxide that can cause cancer by binding with cellular DNA.[17]

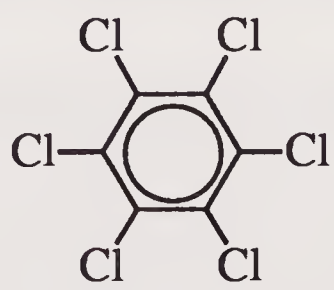
A number of significant organohalide compounds are **aryl halides**, such as the compounds shown in Figure 9.9. Of these compounds, some of the most notable for their toxicological chemistry are the **polychlorinated biphenyls, PCBs,** which have from 1 to 10 chlorine atoms bonded to the biphenyl hydrocarbon skeleton. As discussed in Section 16.4, these are lipophilic materials that tend to bioaccumulate. Although they are degradation-resistant, the PCBs can undergo biodegradation (see Section 6.8).



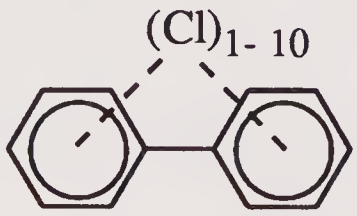
Monochlorobenzene



1,2,4-Trichlorobenzene



Hexachlorobenzene



Polychlorinated biphenyls (PCBs)

Figure 9.9. Some examples of aryl halides.

9.11. ORGANOSULFUR COMPOUNDS

The structural formulas of examples of some typical organosulfur compounds are given in Figure 9.10. Of these compounds, those that are closest to H_2S (discussed as an inorganic toxicant in Section 9.6) are the thiols. Like H_2S, the alkyl thiols are precursors to cytochrome oxidase poisons. Dimethyl sulfide, an alkyl sulfide or

thioether, is a volatile liquid that is moderately toxic by ingestion. Replacement of both of the H atoms on H_2SO_4 by a hydrocarbon substituent (for example, $-CH_3$) produces **dimethylsulfate**, a primary carcinogen.

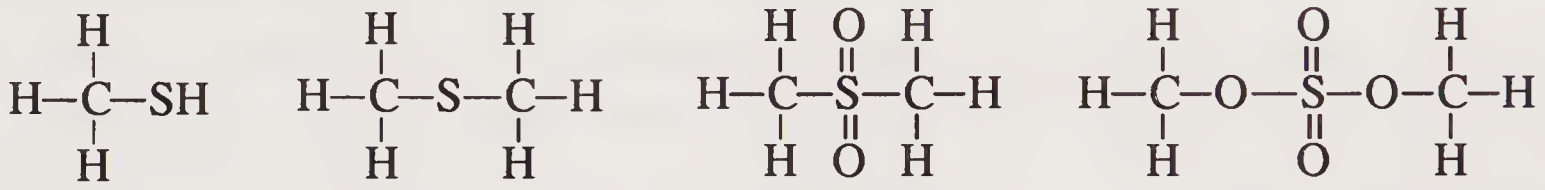


Figure 9.10. Example organosulfur compounds.

9.12. ORGANOPHOSPHORUS COMPOUNDS

Organophosphorus compounds have varying degrees of toxicity. Some of these compounds, such as the "nerve gases" produced as industrial poisons, are deadly in minute quantities. Some of the more toxicologically significant organophosphorus compounds are organophosphates, such as those shown in Figure 9.11. **Trimethylphosphate** is probably moderately toxic when ingested or absorbed through the skin, whereas moderately toxic **triethylphosphate**, $(C_2H_5O)_3PO$, damages nerves and inhibits acetylcholinesterase. Notoriously toxic **tri-*o*-cresylphosphate, TOCP,** apparently is metabolized to products that inhibit acetylcholinesterase. Exposure to TOCP causes degeneration of the neurons in the body's central and peripheral nervous

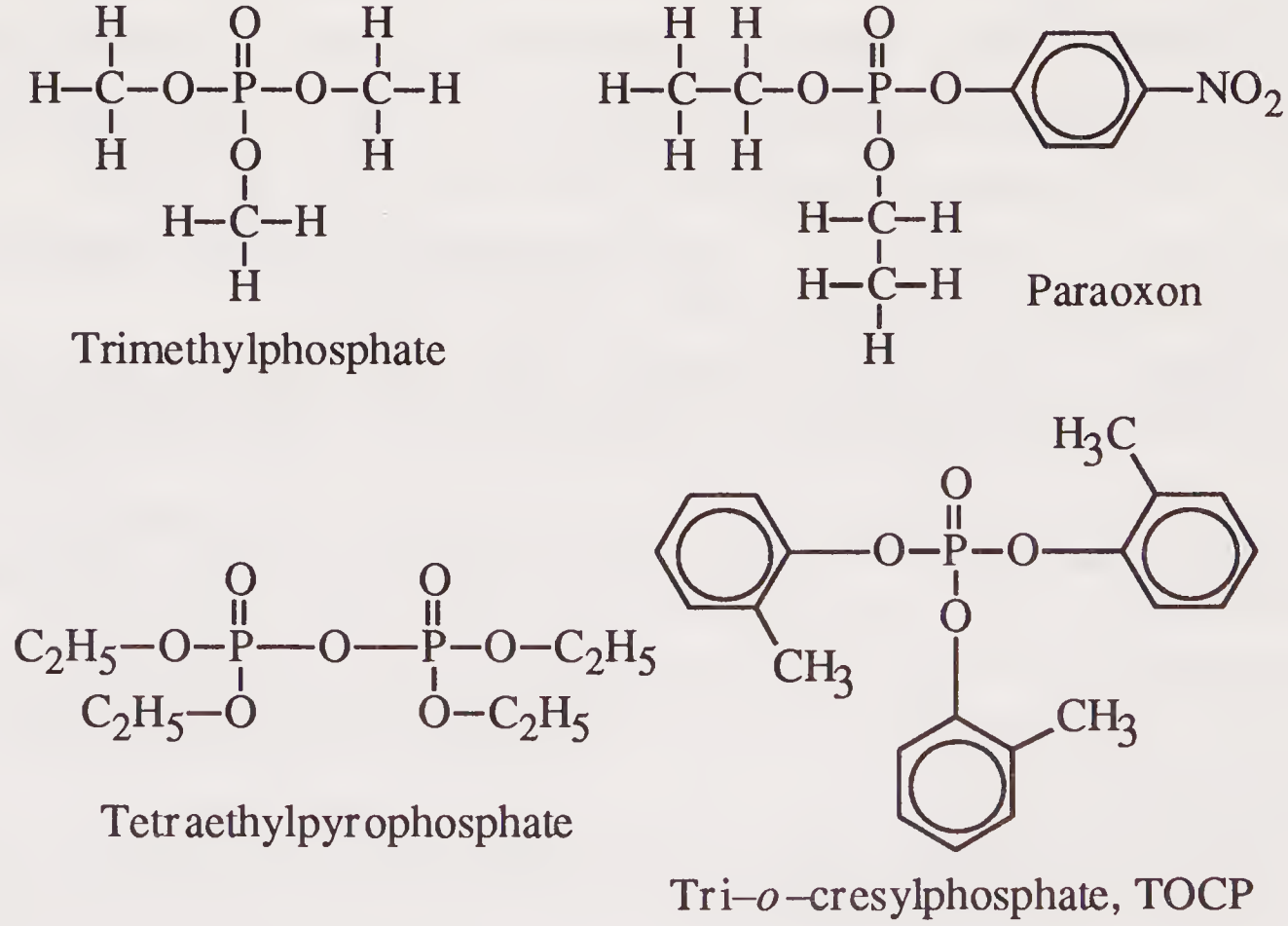


Figure 9.11. Some organophosphate esters.

systems with early symptoms of nausea, vomiting, and diarrhea accompanied by severe abdominal pain. About 1–3 weeks after these symptoms have subsided, peripheral paralysis develops manifested by "wrist drop" and "foot drop," followed by slow recovery, which may be complete or leave a permanent partial paralysis.

Briefly used in Germany as a substitute for insecticidal nicotine, **tetraethylpyrophosphate, TEPP**, is a very potent acetylcholinesterase inhibitor. With a toxicity rating of 6 (supertoxic), TEPP is deadly to humans and other mammals.

Organophosphorus Military Poisons

Powerful inhibitors of acetylcholinesterase enzyme, organophosphorus "nerve gas" military poisons such as **Sarin** and **VX** (Figure 9.12) are among the most toxic synthetic compounds ever made. A systemic poison to the central nervous system that is readily absorbed as a liquid through the skin, Sarin may be lethal at doses as low as about 0.0l mg/kg; a single drop can kill a human. Other examples of these deadly poisons are **Tabun** (O-ethyl N,N-dimethylphosphoramidocyanidate), **Soman** (*o*-pinacolyl methylphosphonofluoridate), and "**DF**" (methylphosphonyldifluoride).

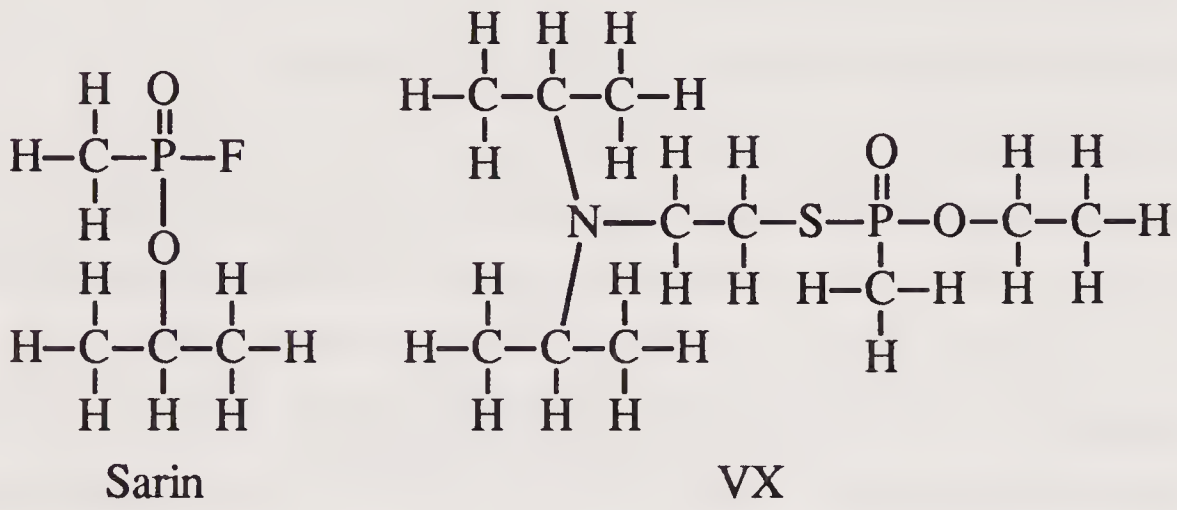


Figure 9.12. Examples of organophosphate military poisons.

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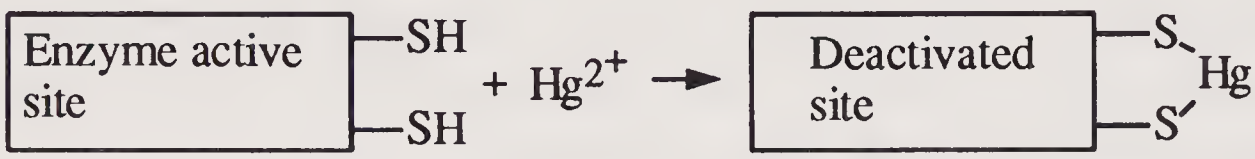
QUESTIONS AND PROBLEMS

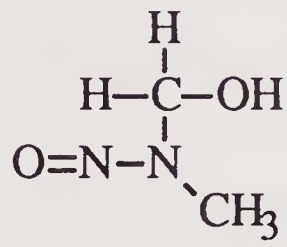
1. Define toxicological chemistry. What is the significance of structure-activity relationships in toxicological chemistry?
2. One of the main categories of toxic substances consists of those that exhibit extremes of acidity, basicity, dehydrating ability, or oxidizing power. Give an example of a substance in each of these categories.
3. Match the following:

(a) Solid P_4O_{10}	1. Lipid-soluble substance
(b) CO	2. Binding species
(c) Formaldehyde	3. Dehydrating substance
(d) PCB	4. Particularly likely to bond with -SH
(e) Pb^{2+}	5. Particularly likely to cause an allergic response

4. Define and distinguish the two main phases of biochemical transformations that toxicants can undergo in the body.
5. Define which of the following processes is **least likely** to be a phase I reaction: epoxide formation, reduction, dealkylation, bonding with sulfate, hydrolysis, or removal of halogen.
6. Define what the following process shows in terms of a biochemical mechanism of toxicity:



7. What are the roles of nitrogenous bases in mutagenesis? What often happens to these nitrogenous bases that results in a mutation?
8. What does the following species have to do with the mutagenicity of dimethylnitrosamine compound?



9. Match the following:

(a) Procarcinogen	1. An irreversible process that does not necessarily lead to cancer
(b) Genotoxic carcinogens	2. Do not require bioactivation
(c) Initiation	3. The metabolic species actually responsible for carcinogenesis
(d) Ultimate carcinogen	4. Are also mutagens
(e) Direct-acting carcinogens	5. Require metabolic activation

10. Explain what alkylating agents have to do with carcinogenesis. Which kind of biomolecule is a receptor for alkylation?
11. What are the two main types of groups to which alkylating agents may attach in nitrogenous bases?
12. What is one of the main distinctions between vinyl chloride and dimethyl sulfate as carcinogens?
13. In what sense is the Bruce Ames test not strictly a test for carcinogenicity? Why is homogenized liver tissue used in this test?
14. List some of the main ways in which teratogens may act biochemically to cause birth defects.
15. What are two therapeutic drugs known to be potent teratogens?
16. What are some of the major clinical symptoms associated with allergy?
17. List the active species produced by ionizing radiation? How are these species related to the effects of oxidants?

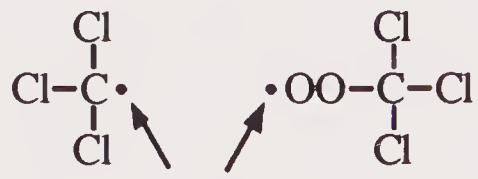
18. List two reactions, the first of which is a biotransformation, by which Hg(0) exerts a toxic effect.
19. What is the biochemical mechanism for cyanide poisoning?
20. What is the biochemical mechanism for toxicity of carbon monoxide?
21. What are some of the toxicological chemical characteristics held in common by interhalogen compounds and halogen oxides?
22. Match the following:

(a) PH_3	1. May cause asbestosis
(b) P_4O_{10}	2. A very toxic colorless gas that undergoes auto-ignition at 100°C
(c) $Mg_3P(Si_2O_5)(OH)_4$	3. A silicon hydrohalide
(d) CaO	4. Causes alkali burns to eyes
(e) $HSiCl_3$	5. Removes water from air

23. In what sense might phosphorus oxychloride be expected to have some toxicological chemical similarities to elemental chlorine?
24. Match the following:

(a) H_2SO_4	1. From SO_2 dissolving in water
(b) H_2SO_3	2. Acidic, dehydrating oxidant in concentrated form
(c) SO_2	3. Sulfite
(d) FeS_2	4. Burns to form the compound listed above it
(e) SO_3^{2-}	5. Formed from burning the compound below it

25. What are five major categories of hydrocarbons divided on the basis of their formulas, chemical characteristics, and potential for toxicological effects?
26. What are three major categories of organometallic compounds?
27. List nine categories of oxygen-containing organic compounds.
28. List two major categories of organonitrogen compounds that contain only C, H, and N. Give a major category of organonitrogen compounds that also contain oxygen, which are known for their carcinogenicity.
29. What produces the species illustrated below and what is their toxicological chemical significance?



30. What is the major toxicological effect of organophosphate compounds?
31. List nine categories of oxygen-containing organic compounds.

10

Toxic Elements

10.1. INTRODUCTION

It is somewhat difficult to define what is meant by a toxic element. Some elements, such as white phosphorus, chlorine, and mercury, are quite toxic in the elemental state. Others, such as carbon, nitrogen and oxygen, are harmless as usually encountered in their normal elemental forms. With the exception of those noble gases that are not known to combine chemically, all elements can form toxic compounds. A prime example is hydrogen cyanide. This extremely toxic compound is formed from three elements that are nontoxic in the uncombined form, which produce compounds that are essential constituents of living matter, but which bonded together in the simple HCN molecule constitute a deadly substance.

The three categories of elements considered here are the following:

- Those that are notable for the toxicities of most of their compounds
- Those that form very toxic ions
- Those that are very toxic in their elemental forms

Elements in these three classes are discussed in this chapter as **toxic elements**, with the qualification that this category is somewhat arbitrary. With the exceptions of phosphorus and chlorine, elements known to be essential to life processes in humans have not been included as toxic elements.

10.2. TOXIC ELEMENTS AND THE PERIODIC TABLE

It is most convenient to consider elements from the perspective of the periodic table, which is shown in Figure 1.3 and discussed in Section 1.2. Recall that the three main types of elements, based upon their chemical and physical properties as determined by the electron configurations of their atoms, are metals, nonmetals, and metalloids. Metalloids (B, Si, Ge, As, Sb, Te, At), show some characteristics of both metals and nonmetals. The nonmetals consist of those few elements in groups 4A–7A above and to the right of the metalloids. The noble gases, only some of which form a limited number of very unstable chemical compounds of no toxicological significance, are in group 8A. All the remaining elements, including the lanthanide and actinide series, are metals. Elements in the periodic table are broadly distinguished between representative elements in the A groups of the periodic table and transition metals constituting the B groups, the lanthanide series, and the actinide series.

10.3. ESSENTIAL ELEMENTS

Some elements are essential to the composition or function of the body. Since the body is mostly water, hydrogen and oxygen are obviously essential elements. Carbon (C) is a component of all life molecules, including proteins, lipids, and carbohydrates. Nitrogen, N, is in all proteins. The other essential nonmetals are phosphorus (P), sulfur (S), chlorine (Cl), selenium (Se), fluorine (F), and iodine (I). The latter two are among the essential trace elements that are required in only small quantities, particularly as constituents of enzymes or as cofactors (nonprotein species essential for enzyme function).[1] The metals present in macro amounts in the body are sodium (Na), potassium (K), and calcium (Ca). Essential trace elements are chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), magnesium (Mg), molybdenum (Mo), nickel (Ni), and perhaps one or more elements that have not yet been established as essential.

10.4. METALS IN AN ORGANISM

Metals are mobilized and distributed through environmental chemical processes that are strongly influenced by human activities. A striking example of this phenomenon is illustrated by the lead content of the Greenland ice pack.[2] Starting at very low levels before significant industrialization had occurred, the lead content of the ice increased in parallel with the industrial revolution, showing a strongly accelerated upward trend beginning in the 1920s with the introduction of lead into gasoline. With their curtailment of the use of leaded gasoline, some countries are now showing decreased lead levels, a trend that hopefully will extend globally within the next several decades.

Metals in the body are almost always in an oxidized or chemically combined form; mercury is a notable exception in that elemental mercury vapor readily enters the body through the pulmonary route. The simplest form of a chemically bound metal in the body is the hydrated cation, of which $Na(H_2O)_6^+$ is the most abundant example in the body. At pH values ranging upward from somewhat less than 7 (neutrality), many metal ions tend to be bound to one or more hydroxide groups; an example is iron(II) in $Fe(OH)(H_2O)_5^+$. Some metal ions have such a strong tendency to lose H^+ that, except at very low pH values, they exist as the insoluble hydroxides. A common example of this phenomenon is iron(III) which is very stable as the insoluble hydrated iron(III) oxide, $Fe_2O_3 \cdot xH_2O$, or hydroxide, $Fe(OH)_3$. Metals can bond to some anions in body fluids. For example, in the strong hydrochloric acid medium of the stomach, some iron(III) may be present as $HFeCl_4$, where the acid in the stomach prevents formation of insoluble $Fe(OH)_3$ and a high concentration of chloride ion is available to bond to iron(III). Ion pairs may exist that consist of positively charged metal cations and negatively charged anions endogenous to body fluids. These do not involve covalent bonding between cations and anions, but rather an electrostatic attraction, such as in the ion pairs, $Ca^{2+}HCO_3^-$ or $Ca^{2+}Cl^-$.

Complex Ions and Chelates

With the exception of group 1A metals and the somewhat lesser exception of group 2A metals, there is a tendency for metals to form **complexes** with **electron donor** functional groups on **ligands** consisting of anionic or neutral inorganic or

organic species. In such cases, covalent bonds are formed between the **central metal ion** and the ligands. Usually the resulting complex has a net charge and is called a complex ion; $FeCl_4^-$ is such an ion. In many cases an organic ligand has two or more electron donor functional groups that may simultaneously bond to a metal ion to form a complex with one or more rings in its structure. A ligand with this capability is called a **chelating agent**, and the complex is a **metal chelate.** Copper(II) ion forms such a chelate with the anion of the amino acid glycine as shown in Figure 10.1. This chelate is very stable.

Organometallic compounds constitute a large class of metal-containing species with properties quite different from those of the metal ions. These are compounds in which the metal is covalently bonded to carbon in an organic moiety, such as the methyl group, $-CH_3$. Unlike metal complexes, which can reversibly dissociate to the metal ions and ligands, the organic portions of organometallic compounds are not normally stable by themselves. The chemical and toxicological properties of organometallic compounds are discussed in detail in Chapter 11, so space will not be devoted to them here. However, it should be mentioned that neutral organometallic compounds tend to be lipid-soluble, a property that enables their facile movement across biologic membranes. They often remain intact during movement through biological systems and so become distributed in these systems as lipid-soluble compounds.

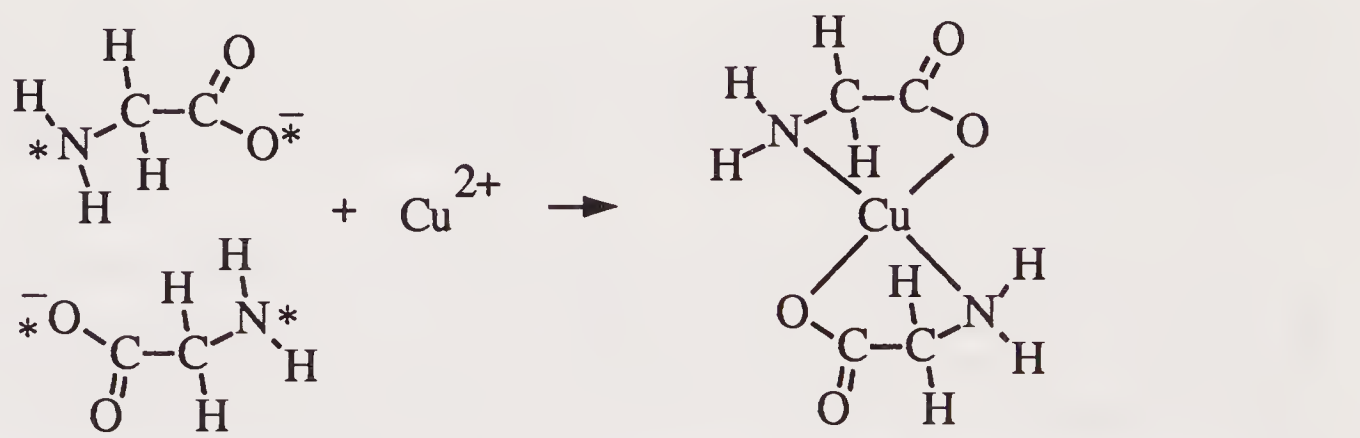


Figure 10.1. Chelation of Cu^{2+} by glycinate anion ligands to form the glycinate chelate. Each electron donor group on the glycinate anion chelating agents is designated with an asterisk. In the chelate the central copper(II) metal ion is bonded in 4 places and the chelate has two rings composed of the 5-atom sequence Cu-O-C-C-N.

A phenomenon not confined to metals, **methylation** is the attachment of a methyl group to an element and is a significant natural process responsible for much of the environmental mobility of some of the heavier elements.[3] Among the elements for which methylated forms are found in the environment are cobalt, mercury, silicon, phosphorus, sulfur, the halogens, germanium, arsenic, selenium, tin, antimony, and lead.

Metal Toxicity

Inorganic forms of most metals tend to be strongly bound by protein and other biologic tissue. Such binding increases bioaccumulation and inhibits excretion. There is a significant amount of tissue selectivity in the binding of metals. For example, toxic lead and radioactive radium are accumulated in osseous (bone) tissue, whereas the kidneys accumulate cadmium and mercury. Metal ions most commonly bond with amino acids, which may be contained in proteins (including enzymes) or polypeptides.

The electron-donor groups most available for binding to metal ions are amino and carboxyl groups (see Figure 10.2). Binding is especially strong for many metals to thiol (sulfhydryl) groups, which is particularly significant because the –SH groups are common components of the active sites of many crucial enzymes, including those that are involved in cellular energy output and oxygen transport. The amino acid that usually provides –SH groups in enzyme active sites is cysteine, as shown in Figure 10.2. The imidazole group of the amino acid histidine is a common feature of enzyme active sites with strong metal binding capabilities.

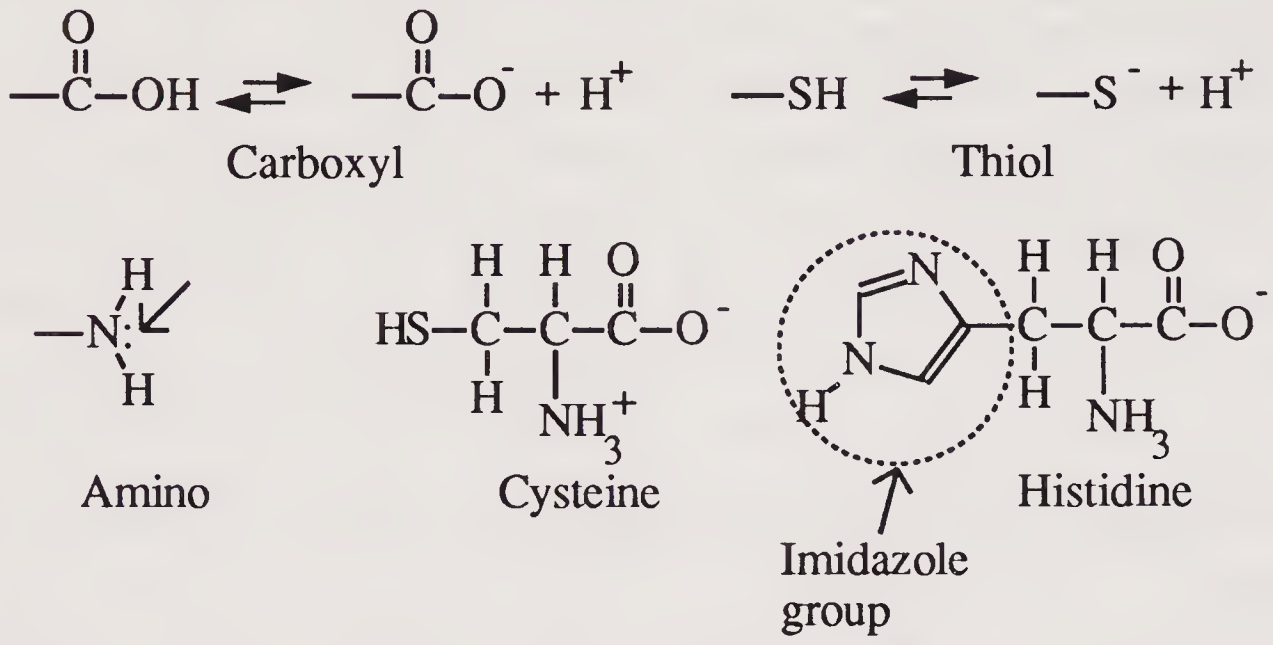


Figure 10.2. Major binding groups for metal ions in biologic tissue (carboxyl, thiol, amino) and amino acids with strong metal binding groups in enzyme active sites (cysteine, histidine). The arrow pointing to the amino group designates an unshared pair of electrons available for binding metal ions. The thiol group is a weak acid that usually remains un-ionized until the hydrogen ion is displaced by a metal ion.

The absorption of metals is to a large extent a function of their chemical form and properties. Pulmonary intake results in the most facile absorption and rapid distribution through the circulatory system. Absorption through this route is often very efficient when the metal is in the form of respirable particles less than 100 μm in size, as volatile organometallic compounds (see Chapter 11) or (in the case of mercury) as the elemental metal vapor. Absorption through the gastrointestinal tract is affected by pH, rate of movement through the tract, and presence of other materials. Particular combinations of these factors can combine to make absorption very high or very low.

Metals tend to accumulate in target organs, and a toxic response is observed when the level of the metal in the organ reaches or exceeds a threshold level. Often the organs most affected are those involved with detoxication or elimination of the metal. Therefore, the liver and kidneys are often affected by metal poisoning. The form of the metal can determine which organ is adversely affected. For example, lipid-soluble elemental or organometallic mercury damages the brain and nervous system, whereas Hg^{2+} ion may attack the kidneys.

Because of the widespread opportunity for exposure combined with especially high toxicity, some metals are particularly noted for their toxic effects. These are discussed separately in the following sections in the general order of their appearance in groups in the periodic table.

Beryllium

Beryllium (Be) is a group 2A element with the electron configuration $\{He\}2s^2$. It is the first metal in the periodic table to be notably toxic. When fluorescent lamps and neon lights were first introduced, they contained beryllium phosphor, and a

number of cases of beryllium poisoning resulted from the manufacture of these light sources and the handling of broken lamps. Modern uses of beryllium in ceramics, electronics, and alloys require special handling procedures to avoid industrial exposure.

Beryllium has a number of toxic effects. Of these, the most common involve the skin. Skin ulceration and granulomas have resulted from exposure to beryllium. Hypersensitization to beryllium can result in skin dermatitis, acute conjunctivitis, and corneal laceration.

Chronic berylliosis may occur with a long latent period of 5–20 years. The most damaging effect of chronic berylliosis is lung fibrosis and pneumonitis. In addition to coughing and chest pain, the subject suffers from fatigue, weakness, loss of weight, and dyspnea (difficult, painful breathing). The impaired lungs do not transfer oxygen well. Other organs that can be adversely affected are the liver, kidneys, heart, spleen, and striated muscles.

The chemistry of beryllium is atypical compared to that of the other group 1A and group 2A metals. Atoms of Be are the smallest of all metals, having an atomic radius of 111 pm (picometers). The beryllium ion, Be^{2+}, has an ionic radius of only 35 pm, which gives it a high polarizing ability, a tendency to form molecular compounds rather than ionic compounds, and a much greater tendency to form complex compounds than other group 1A or 2A ions.[4]

Vanadium

Vanadium (V) is a transition metal with an electron configuration of $\{Ar\}4s^2 3d^3$. In the combined form it exists in the +3, +4, and +5 oxidation states, of which the +5 is the most common. Vanadium is of concern as an environmental pollutant because of its high levels in residual fuel oils and subsequent emission as small particulate matter from the combustion of these oils in urban areas. Vanadium occurs as chelates of the porphyrin type in crude oil and it concentrates in the higher boiling fractions during the refining process. A major industrial use of vanadium is in catalysts, particularly those in which sulfur dioxide is oxidized in the production of sulfuric acid. The other major industrial uses of vanadium are for hardening steel, as a pigment ingredient, in photography, and as an ingredient of some insecticides. In addition to environmental exposure from the combustion of vanadium-containing fuels, there is some potential for industrial exposure.

Probably the vanadium compound to which people are most likely to be exposed is vanadium pentoxide, V_2O_5. Exposure normally occurs via the respiratory route, and the pulmonary system is the most likely to suffer from vanadium toxicity. Bronchitis and bronchial pneumonia are the most common pathological effects of exposure; skin and eye irritation may also occur. Severe exposure can also adversely affect the gastrointestinal tract, kidneys, and nervous system.

Chromium

Chromium (Cr) is a transition metal with an electron configuration of $\{Ar\}4s^1 3d^5$. In the chemically combined form it exists in all oxidation states from +2 through +6, of which the +3 and +6 are the more notable.

In strongly acidic aqueous solution, chromium(III) may be present as the hydrated cation $Cr(H_2O)_6^{3+}$. At pH values above approximately 4 this ion has a strong tendency to precipitate from solution as shown by the reaction:

$$Cr(H_2O)_6^{3+} \rightarrow Cr(OH)_3 + 3H^+ + 3H_2O \qquad (10.4.1)$$

The two major forms of chromium(VI) in solution are the yellow chromate, CrO_4^{2-}, and orange dichromate, $Cr_2O_7^{2-}$. The latter predominates in acidic solution as shown by the following reaction, the equilibrium of which is forced to the left by higher levels of H^+:

$$Cr_2O_7^{2-} + H_2O \leftarrow\rightarrow 2HCrO_4^- \leftarrow\rightarrow 2H^+ + 2CrO_4^{2-} \qquad (10.4.2)$$

Chromium in the +3 oxidation state is an essential trace element (see Section 10.3) required for glucose and lipid metabolism in mammals, and a deficiency of it gives symptoms of diabetes mellitus. However, chromium must also be discussed as a toxicant because of its toxicity in the +6 oxidation state, commonly called **chromate**. Exposure to chromium(VI) usually involves chromate salts, such as Na_2CrO_4. These salts tend to be water-soluble and readily absorbed into the bloodstream through the lungs. The carcinogenicity of chromate has been demonstrated by studies of exposed workers. Exposure to atmospheric chromate may cause bronchogenic carcinoma with a latent period of 10–15 years. In the body, chromium(VI) is readily reduced to chromium(III) as shown in Reaction 10.3; however, the reverse reaction does not occur in the body.

$$CrO_4^{2-} + 8H^+ + 3e^- \rightarrow Cr^{3+} + 4H_2O \qquad (10.4.3)$$

The interaction of chromium(VI) and bacteria can be important. Chromate-tolerant bacteria can be used to remove chromium(VI) from wastewater. One such study has used *Enterobacter cloacae* growing under anaerobic conditions to bring about the reduction of Cr(VI) to Cr(III) (see Reaction 10.4.3), resulting in the precipitation of insoluble chromium(III)[4]. This process differs from preceding work reported in the literature in that removal of the chromium appears to be the result of bacterially mediated reduction of chromate, and not simply sorption of chromium by the bacterial biomass.

Cadmium

Along with mercury and lead, cadmium (Cd) is one of the "big three" heavy metal poisons. Cadmium occurs as a constituent of lead and zinc ores, from which it can be extracted as a by-product. Cadmium is used to electroplate metals to prevent corrosion, as a pigment, as a constituent of alkali storage batteries, and in the manufacture of some plastics.

Cadmium is located at the end of the second row of transition elements and has the electron configuration $\{Kr\}5s^24d^{10}$. The two outer s electrons in cadmium are the only ones involved in bonding, and the +2 oxidation state of the element is the only one exhibited in its compounds. In its compounds, cadmium occurs as the Cd^{2+} ion. Cadmium is directly below zinc in the periodic table and behaves much like zinc. This may account in part for cadmium's toxicity; because zinc is an essential trace element, cadmium substituting for zinc could cause metabolic processes to go wrong.

The toxic nature of cadmium was revealed in the early 1900s as a result of workers inhaling cadmium fumes or dusts in ore processing and manufacturing operations. Welding or cutting metals plated with cadmium or containing cadmium in

alloys, or the use of cadmium rods or wires for brazing or silver-soldering, can be a particularly dangerous route to pulmonary exposure.[5] Acute pulmonary symptoms of cadmium exposure are usually caused by the inhalation of cadmium oxide dusts and fumes, which results in cadmium pneumonitis characterized by edema and pulmonary epithelium necrosis. Chronic exposure sometimes produces emphysema severe enough to be disabling. The kidney is generally regarded as the organ most sensitive to chronic cadmium poisoning. The function of renal tubules is impaired by cadmium as manifested by excretion of both high molecular mass proteins (such as albumin) and low molecular mass proteins.

The most spectacular and publicized occurrence of cadmium poisoning resulted from dietary intake of cadmium by people in the Jintsu River Valley near Fuchu, Japan. The victims were afflicted by *itai, itai* disease, which means "ouch, ouch" in Japanese. The symptoms are the result of painful osteomalacia (bone disease) combined with kidney malfunction. Cadmium poisoning in the Jintsu River Valley was attributed to irrigated rice contaminated from an upstream mine producing lead, zinc, and cadmium.

In general, cadmium is poorly absorbed through the gastrointestinal tract. A mechanism exists for its active absorption in the small intestine through the action of the low-molecular-mass calcium-binding protein CaBP. The production of this protein is stimulated by a calcium-deficient diet, which may aggravate cadmium toxicity.

Cadmium is a highly **cumulative** poison with a biologic half-life estimated at about 20-30 years in humans. About half of the body burden of cadmium is found in the liver and kidneys. The total body burden reaches a plateau in humans around age 50. Cigarette smoke is a source of cadmium, and the body burden of cadmium is about 1.5 to 2 times greater in smokers than in nonsmokers of the same age.

Cadmium in the body is known to affect several enzymes. It is believed that the renal damage that results in proteinuria from cadmium is the result of cadmium adversely affecting enzymes responsible for reabsorption of proteins in kidney tubules.[6] Cadmium also reduces the activity of delta-aminolevulinic acid synthetase (Figure 10.3), arylsulfatase, alcohol dehydrogenase, and lipoamide dehydrogenase, whereas it enhances the activity of delta-aminolevulinic acid dehydratase, pyruvate dehydrogenase, and pyruvate decarboxylase.

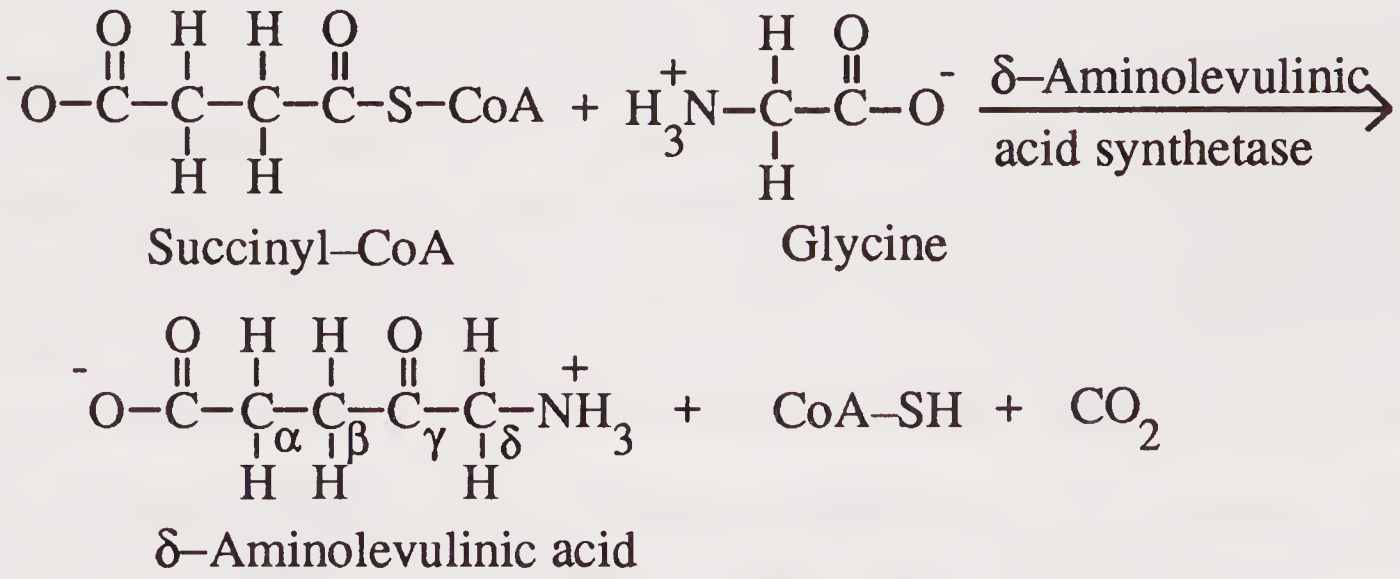


Figure 10.3. Path of synthesis of delta-aminolevulinic acid (coenzyme A abbreviated as CoA). Cadmium tends to inhibit the enzyme responsible for this process.

Cadmium is excreted from the body in both urine and feces. The mechanisms of cadmium excretion are not well known.

Mercury

Mercury is directly below cadmium in the periodic table, but has a considerably more varied and interesting chemistry than cadmium or zinc. Elemental mercury is the only metal that is a liquid at room temperature, and its relatively high vapor pressure contributes to its toxicological hazard. Mercury metal is used in electric discharge tubes (mercury lamps), gauges, pressure-sensing devices, vacuum pumps, valves, and seals. It was formerly widely used as a cathode in the chlor-alkali process for the manufacture of NaOH and Cl_2, a process that has been largely discontinued in part because of mercury pollution resulting from it.

In addition to the uses of mercury metal, mercury compounds have a number of applications.[7] Mercury(II) oxide, HgO, is commonly used as a raw material for the manufacture of other mercury compounds. Mixed with graphite, it is a constituent of the Ruben-Mallory dry cell for which the cell reaction is the following:

$$Zn + HgO \rightarrow ZnO + Hg \qquad (10.4.4)$$

Mercury(II) acetate, $Hg(C_2H_3O_2)_2$, is made by dissolving HgO in warm 20% acetic acid. This compound is soluble in a number of organic solvents. Mercury(II) chloride is quite toxic. The dangers of exposure to $HgCl_2$ are aggravated by its high water solubility and relatively high vapor pressure compared to other salts. Mercury(II) fulminate, $Hg(ONC)_2$, is used as an explosives' detonator. In addition to the +2 oxidation state, mercury can also exist in the +1 oxidation state as the dinuclear Hg_2^{2+} ion. The best known mercury(I) compound is mercury(I) chloride, Hg_2Cl_2, commonly called calomel. It is a constituent of calomel reference electrodes, such as the well-known saturated calomel electrode, SCE.

A number of organomercury compounds are known. These compounds and their toxicities are discussed further in Chapter 11.

Absorption and Transport of Elemental and Inorganic Mercury

Monatomic elemental mercury in the vapor state, Hg(g), is absorbed from inhaled air by the pulmonary route to the extent of about 80%. Inorganic mercury compounds are absorbed through the intestinal tract and in solution through the skin.

Although elemental mercury is rapidly oxidized to mercury(II) in erythrocytes (red blood cells), a large fraction of elemental mercury absorbed through the pulmonary route reaches the brain prior to oxidation and enters that organ because of the lipid solubility of mercury(0). This mercury is subsequently oxidized in the brain and remains there. Inorganic mercury(II) tends to accumulate in the kidney.

Metabolism, Biologic Effects, and Excretion

Like cadmium, mercury(II) has a strong affinity for sulfhydryl groups in proteins, enzymes, hemoglobin, and serum albumin. Because of the abundance of sulfhydryl groups in active sites of many enzymes, it is difficult to establish exactly which enzymes are affected by mercury in biological systems.

The effect upon the central nervous system following inhalation of elemental mercury is largely psychopathological. Among the most prominent symptoms are tremor (particularly of the hands) and emotional instability characterized by shyness, insomnia, depression, and irritability. These symptoms are probably the result of

damage to the blood-brain barrier. This barrier regulates the transfer of metabolites, such as amino acids, to and from the brain. Brain metabolic processes are probably disrupted by the effects of mercury.

The kidney is the primary target organ for Hg^{2+}. Chronic exposure to inorganic mercury(II) compounds causes proteinuria. In cases of mercury poisoning of any type, the kidney is the organ with the highest bioaccumulation of mercury.

Excretion of inorganic mercury occurs through the urine and feces. The mechanisms by which excretion occurs are not well understood.

Minimata Bay

The most notorious incident of widespread mercury poisoning in modern times occurred in the Minimata Bay region of Japan during the period 1953-1960. Mercury waste from a chemical plant draining into the bay contaminated seafood consumed regularly by people in the area. Overall, 111 cases of poisoning with 43 deaths and 19 congenital birth defects were documented. The seafood was found to contain 5-20 parts per million of mercury.

Lead

Lead (Pb) ranks fifth behind iron, copper, aluminum, and zinc in industrial production of metals.[8] About half of the lead used in the United States goes for the manufacture of lead storage batteries. Other uses include solders, bearings, cable covers, ammunition, plumbing, pigments, and caulking.

Metals commonly alloyed with lead are antimony (in storage batteries), calcium and tin (in maintenance-free storage batteries), silver (for solder and anodes), strontium and tin (as anodes in electrowinning processes), tellurium (pipe and sheet in chemical installations and nuclear shielding), tin (solders), and antimony and tin (sleeve bearings, printing, high-detail castings).

The electron configuration of lead is $\{Xe\}4f^{14}5d^{10}6s^26p^2$ and it has four valence electrons. Lead is one of a small group of "p-block" metals that are representative elements with partially filled *p* orbitals. Inorganic lead exists in chemical compounds in both the +2 and +4 oxidation states. Lead(II) compounds are predominantly ionic (e.g., $Pb^{2+}SO_4^{2-}$), whereas lead(IV) compounds tend to be covalent (e.g., tetraethyllead, $Pb(C_2H_5)_4$). Some lead(IV) compounds, such as PbO_2, are strong oxidants. Lead forms several basic lead salts, such as $Pb(OH)_2 \cdot 2PbCO_3$, which was once the most widely used white paint pigment and the source of considerable chronic lead poisoning to the children who ate peeling white paint. Many compounds of lead in the +2 oxidation state (lead(II)) and a few in the +4 oxidation state (lead(IV)) are useful. The two most common of these are lead dioxide and lead sulfate, which are participants in the following reversible reaction that occurs during the charge and discharge of a lead storage battery:

$$Pb + PbO_2 + 2H_2SO_4 \underset{\text{Charge}}{\overset{\text{Discharge}}{\leftarrow\!\rightarrow}} 2PbSO_4 + 2H_2O \qquad (10.4.5)$$

Lead halides have several important uses, such as that of $PbCl_2$ in asbestos brake linings and clutch disks. Lead hydroxide, $Pb(OH)_2$, is a component of sealed nickel-cadmium batteries. Basic lead sulfates ($xPbO \cdot PbSO_4$, x = 1–4) are used as paint pigments. Dibasic lead phosphite ($2PbO_2 \cdot PbHPO_3 \cdot 1/2H_2O$) is used as a stabilizer in polyvinylchloride plastic to give this polymer desired qualities of thermal stability, weathering resistance, and electrical insulation.

In addition to the inorganic compounds of lead, there are a number of organolead compounds, such as tetraethyllead. These are discussed in Chapter 11.

Exposure and Absorption of Inorganic Lead Compounds

Although industrial lead poisoning used to be very common, it is relatively rare now[9] because of previous experience with the toxic effects of lead and protective actions that have been taken. Lead is a common atmospheric pollutant, and absorption through the respiratory tract is the most common route of human exposure. The greatest danger of pulmonary exposure comes from inhalation of very small respirable particles of lead oxide (particularly from lead smelters and storage battery manufacturing) and lead carbonates, halides, phosphates, and sulfates. The other major route of lead absorption is the gastrointestinal tract. Lead(II) may have much the same transport mechanism as calcium in the gastrointestinal tract. It is known that lead absorption decreases with increased levels of calcium in the diet and vice versa.

Transport and Metabolism of Lead

A striking aspect of lead in the body is its very rapid transport to bone and storage there. Lead tends to undergo bioaccumulation in bone throughout life, and about 90% of the body burden of lead is in bone after long-term exposure. Of the soft tissues, the liver and kidney tend to have somewhat elevated lead levels.

Measurement of the concentration of lead in the blood is the standard test for recent or ongoing exposure to lead. This test is used routinely to monitor industrial exposure to lead and in screening children for lead exposure.

The most common biochemical effect of lead is inhibition of the synthesis of heme, a complex (see Section 11.4) of a substituted porphyrin and Fe^{2+} in hemoglobin and cytochromes. Lead interferes with the conversion of delta-aminolevulinic acid to porphobilinogen, as shown in Figure 10.4, with a resulting accumulation of metabolic products. Hematological damage results. Lead inhibits enzymes that have sulfhydryl groups. However, the affinity of lead for the –SH group is not as great as that of cadmium or mercury.

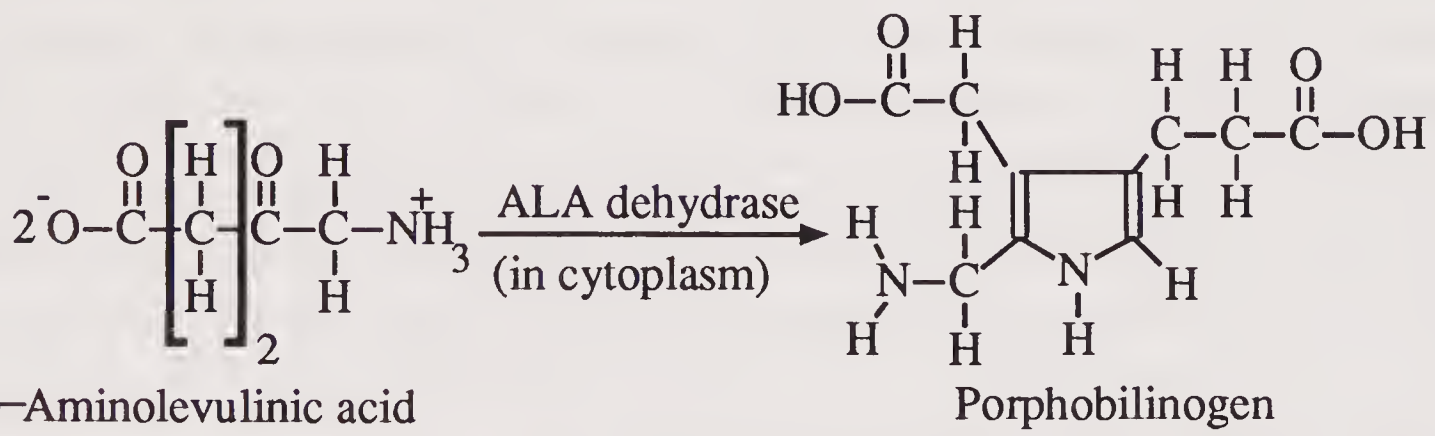


Figure 10.4. Synthesis of porphobilinogen from δ-aminolevulinic acid, a major step in the overall scheme of heme synthesis that is inhibited by lead in the body.

Manifestations of Lead Poisoning

Lead adversely affects a number of systems in the body. The inhibition of the synthesis of hemoglobin by lead has just been noted. This effect, plus a shortening of the life span of erythrocytes, results in anemia, a major manifestation of lead poisoning.

The central nervous system is adversely affected by lead. Psychopathological symptoms include restlessness, dullness, irritability, and memory loss. The subject may experience ataxia, headaches, and muscular tremor. In extreme cases, convulsions followed by coma and death may occur. Lead affects the peripheral nervous system, and lead palsy used to be a commonly observed symptom in lead industry workers and miners suffering from lead poisoning. Even in the absence of lead palsy, peripheral nerves are adversely affected by chronic lead poisoning.

Lead causes reversible damage to the kidney through its adverse effect upon proximal tubules. This impairs the processes by which the kidney absorbs glucose, phosphates, and amino acids prior to secretion of urine. A longer-term effect of lead ingestion on the kidney is general degradation of the organ (chronic nephritis) including glomular atrophy, interstitial fibrosis, and sclerosis of vessels.

Reversal of Lead Poisoning and Therapy

Some effects of lead poisoning, such as those upon proximal tubules of the kidney and inhibition of heme synthesis, are reversible upon removal of the source of lead exposure. Lead poisoning can be treated by chelation therapy in which the lead is solubilized and removed by a chelating agent (see Section 10.4). One such chelating agent is ethylenediaminetetraacetic acid (EDTA) which binds strongly to most +2 and +3 cations (Figure 10.5). It is administered for lead poisoning therapy in the form of

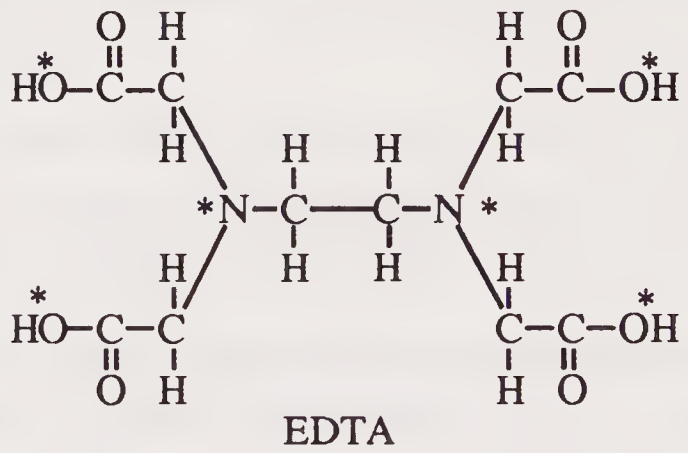


Figure 10.5. The non-ionized form of ethylenediaminetetraacetic acid, EDTA. (Asterisks denote binding sites.)

the calcium chelate. The ionized Y^{4-} form chelates metal ions by bonding at one, two, three, or all four carboxylate groups ($-CO_3^{2-}$) and one or both of the 2 N atoms (see glycinate-chelated structure in Figure 10.1). EDTA is administered as the calcium chelate for the treatment of lead poisoning to avoid any net loss of calcium by solubilization and excretion.

Another compound used to treat lead poisoning is British anti-Lewisite (BAL), originally developed to treat arsenic-containing poison gas Lewisite. As shown in Figure 10.6, BAL chelates lead through its sulfhydryl groups, and the chelate is excreted through the kidney and bile.

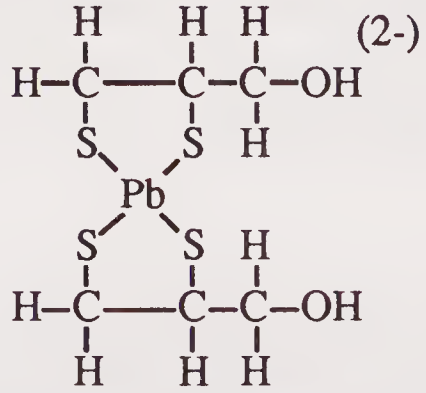
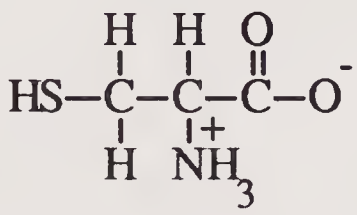


Figure 10.6. Lead chelated by the lead antidote British anti-Lewisite, BAL.

Defenses Against Heavy Metal Poisoning

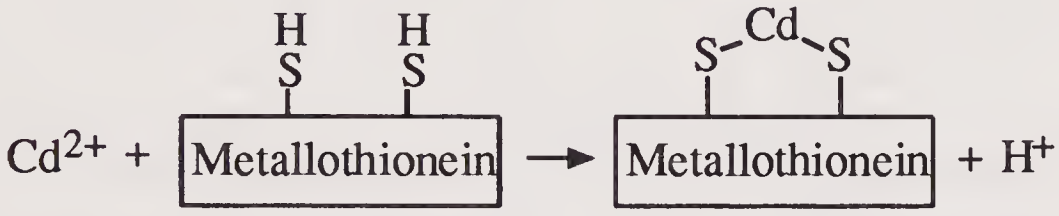
Organisms have some natural defenses against heavy metal poisoning.[10] Several factors are involved in regulating the uptake and physiological concentrations of heavy metals. For example, higher levels of calcium in water tend to lower the bioavailability of metals such as cadmium, copper, lead, mercury, and zinc by fish and the presence of chelating agents affects the uptake of such metals. Some evidence suggests that mechanisms developed to maintain optimum levels of essential metals, such as zinc and copper, are utilized to minimize the effects of chemically somewhat similar toxic heavy metals, of which cadmium, lead, and zinc are prime examples.

An interesting feature of heavy metal metabolism is the role of intracellular **metallothionein**, which consists of two similar proteins with a low molecular mass of about 6,500. As a consequence of a high content of the amino acid cysteine:



Cysteine

metallothionein contains a large number of thiol (sulfhydryl, –SH) groups. These groups bind very strongly to other heavy metals, particularly mercury, silver, zinc, and tin. The metal most investigated for its interaction with metallothionein is cadmium. The general reaction of metallothionein with cadmium ion is the following:



(10.4.6)

By binding with metallothionein, the mobility of metals by diffusion is greatly reduced and the metals are prevented from binding to enzymes or other proteins essential to normal metabolic function.

Metallothionein has been isolated from virtually all of the major mammal organs, including liver, kidney, brain, heart, intestine, lung, skin, and spleen. Nonlethal doses of cadmium, mercury, and lead induce synthesis of metallothionein. In test animals, nonlethal doses of cadmium followed by an increased level of metallothionein in the body have allowed later administration of doses of cadmium at a level fatal to nonacclimated animals, but without fatalities in the test subjects.

Endogenous substances other than metallothionein may be involved in minimizing the effects of heavy metals and excreting them from the body. Hepatic (liver) glutathione, discussed as a phase II conjugating agent in Section 4.6, plays a role in the excretion of several metals in bile. These include the essential metals copper and zinc; toxic cadmium, mercury(II), and lead(II) ions; and organometallic methymercury.[11]

10.5. METALLOIDS: ARSENIC

Sources and Uses

Arsenopyrite and loellingite are both arsenic minerals that can be smelted to produce elemental arsenic. Both elemental arsenic and arsenic trioxide (As_2O_3) are produced commercially; the latter is the raw material for the production of numerous

arsenic compounds. Elemental arsenic is used to make alloys with lead and copper.[12] Arsenic compounds have a number of uses, including applications in catalysts, bactericides, herbicides, fungicides, animal feed additives, corrosion inhibitors, pharmaceuticals, veterinary medicines, tanning agents, and wood preservatives. Arsenicals were the first drugs to be effective against syphilis and they are still used to treat amebic dysentery. Arsobal, or Mel B, an organoarsenical, is the most effective drug for the treatment of the neurological stage of African trypanosomiasis for which the infectious agents are *Trypanosoma gambiense* or *T. rhodesiense*.

Exposure and Absorption of Arsenic

Arsenic can be absorbed through both the gastrointestinal and pulmonary routes. Although the major concern with arsenic is its effect as a systemic poison, arsenic trichloride ($AsCl_3$) and the organic arsenic compound, Lewisite (used as a poison gas in World War I) can penetrate skin; both of these compounds are very damaging at the point of exposure and are strong vesicants (causes of blisters).[13] The common arsenic compound As_2O_3 is absorbed through the lungs and intestines. The degree of coarseness of the solid is a major factor in how well it is absorbed. Coarse particles of this compound tend to pass through the gastrointestinal tract and to be eliminated with the feces.

Arsenic occurs in the +3 and +5 oxidation states, and inorganic compounds in the +3 oxidation state are generally more toxic. The conversion to arsenic(V) is normally favored in the environment, which somewhat reduces the overall hazard of this element.

Arsenic is a natural constituent of most soils. It is found in a number of foods, particularly shellfish. The average adult ingests somewhat less than 1 milligram of arsenic per day through natural sources.

Metabolism, Transport, and Toxic Effects of Arsenic

Biochemically, arsenic acts to coagulate proteins, forms complexes with coenzymes, and inhibits the production of ATP (see Section 4.3). Like cadmium and mercury, arsenic is a sulfur-seeking element. Arsenic has some chemical similarities to phosphorus,[14] and it substitutes for phosphorus in some biochemical processes, with adverse metabolic effects. Figure 10.7 (p. (262) summarizes one such effect. The top reaction in the figure illustrates the enzyme-catalyzed synthesis of 1,3-diphosphoglycerate from glyceraldehyde 3-phosphate. The product undergoes additional reactions to produce adenosine triphosphate (ATP), an essential energy-yielding substance in body metabolism. When arsenite AsO_3^{3-} is present it bonds to glyceraldehyde 3-phosphate to yield a product that undergoes nonenzymatic spontaneous hydrolysis. This prevents ATP formation.

Antidotes to arsenic poisoning take advantage of the element's sulfur-seeking tendencies and contain sulfhydryl groups. One such antidote is 2,3-mercaptopropanol (BAL) discussed in the preceding section as an antidote for lead poisoning.

10.6. NONMETALS

Although molecular oxygen is essential for respiratory processes in aerobic organisms, it is reduced in the body to active species that can be harmful.[15] Successive additions of an electron (e^-) and a hydrogen ion (H^+) to a molecule of O_2

produce $HO_2\cdot$ (hydroperoxyl radical or superoxide), H_2O_2 (hydrogen peroxide), and $HO\cdot$ (hydroxyl radical, produced along with a molecule of H_2O). The dot beside the formulas $HO\cdot$ and $HO_2\cdot$ denotes that each one of these species contains an unpaired electron. Such species are called **radicals** and are very chemically reactive. These radicals and chemically reactive hydrogen peroxide attack tissue and DNA either directly or through their reaction products. The damage done is sometimes referred to as oxidative lesions. Radicals are scavenged from a living system by several enzymes, including peroxidase, superoxide dismutase, and catalase. Oxidative lesions on DNA may be repaired by DNA repair enzymes.

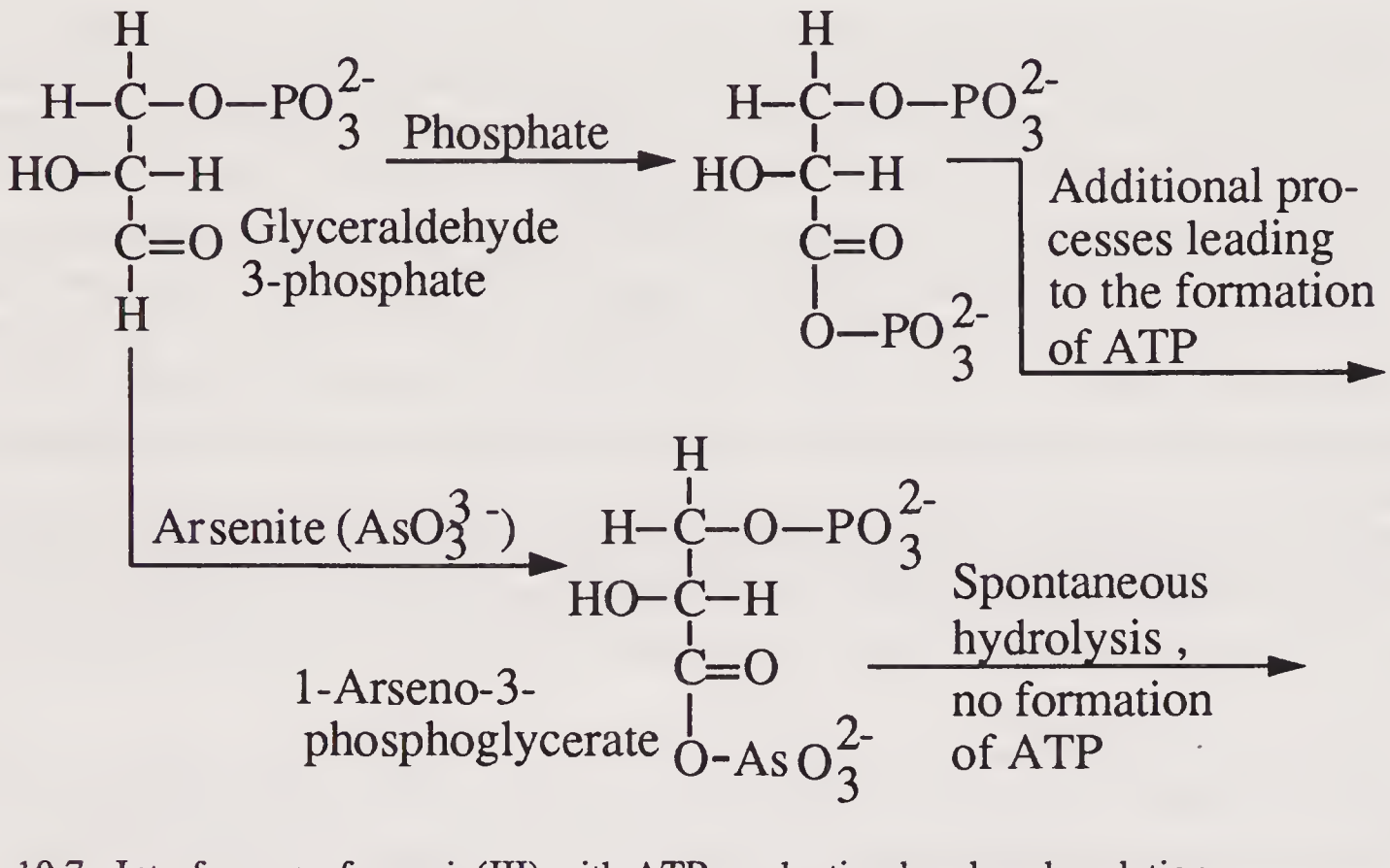


Figure 10.7. Interference of arsenic(III) with ATP production by phosphorylation.

Phosphorus

The most common elemental form of phosphorus, white phosphorus, is highly toxic. White phosphorus (mp 44°C, bp 280°C) is a colorless waxy solid, sometimes with a yellow tint. It ignites spontaneously in air to yield a dense fog of finely divided, highly deliquescent P_4O_{10}:

$$P_4 + 5O_2 \rightarrow P_4O_{10} \qquad (10.6.1)$$

White phosphorus can be absorbed into the body particularly through inhalation, as well as through the oral and dermal routes. It has a number of systemic effects, including anemia, gastrointestinal system dysfunction, and bone brittleness. Acute exposure to relatively high levels results in gastrointestinal disturbances and weakness due to biochemical effects on the liver. Chronic poisoning occurs largely through the inhalation of low concentrations of white phosphorus and causes necrosis (tissue death) of the jawbone, brittleness in other bones, and deterioration of teeth, which can result in their loss. **Phossy jaw** results from necrosis and fracture of the jawbone in exposed individuals. Severe eye damage can result from chronic exposure to elemental white phosphorus.

The Halogens

The elemental halogens — fluorine, chlorine, bromine, and iodine — are all toxic. Both fluorine and chlorine are highly corrosive gases that are very damaging to exposed tissue. All the halogens are used in their elemental forms and can properly be discussed with toxic elements. However, the toxicological properties of these elements are very similar in many cases to those of interhalogen compounds formed between the various halogens, which are covered in Chapter 12, and discussion of halogen toxicity is deferred to that chapter.

Radionuclides

Radon

In section 9.3 the toxicological effects of ionizing radiation were mentioned, and radon was cited as a source of such radiation. Some authorities believe that of all the elemental toxicants radon is the one that is most likely to eventually cause death in humans.[16] The threat of radon in the environment and in indoor air have been summarized in books on the subject.[17,18] Radon's toxicity is not the result of its chemical properties, because it is a noble gas and does not enter into any normal chemical reactions. However, it is a radioactive element (radionuclide) that emits positively charged alpha particles, the largest and — when emitted inside the body — the most damaging form of radioactivity. Furthermore, the products of the radioactive decay of radon are also alpha emitters. Alpha particles emitted from a radionuclide in the lung cause damage to cells lining the lung bronchi and other tissues, resulting in processes that can cause cancer.

Radon is a decay product of radium which in turn is produced by the radioactive decay of uranium. During its brief lifetime, radon may diffuse upward through soil and into dwellings through cracks in basement floors. Radioactive decay products of radon become attached to particles in indoor air, are inhaled, and lodge in the lungs until they undergo radioactive decay, damaging lung tissue. Synergistic effects between radon and smoking appear to be responsible for most of the cases of cancer associated with radon exposure.

Radium

A second radionuclide to which humans are likely to be exposed is **radium**, Ra. Occupational exposure to radium is known to have caused cancers in humans. The most likely route for human exposures to low doses is through drinking water.[19] Areas in the United States where significant radium contamination of water has been observed include the uranium-producing regions of the western U.S., Iowa, Illinois, Wisconsin, Missouri, Minnesota, Florida, North Carolina, Virginia, and the New England states.

The maximum contaminant level (MCL) for total radium (^{226}Ra plus ^{228}Ra) in drinking water is specified by the U.S. Environmental Protection Agency as 5 pCi/L (picoCuries per liter) where a picoCurie is 0.037 disintegrations per second. Perhaps as many as several hundred municipal water supplies in the U.S. exceed this level and require additional treatment to remove radium. Fortunately, conventional water softening processes, which are designed to take out excessive levels of calcium, are relatively efficient in removing radium from water.

Fission Products

The anthropogenic radionuclides of most concern are those produced as fission products from nuclear weapons and nuclear reactors. The most devastating release from the latter source to date resulted from the April 26, 1986, explosion, partial meltdown of the reactor core, and breach of confinement structures by a power reactor at Chernobyl in the Ukraine. This disaster released 5×10^7 curies of radionuclides from the site, which contaminated large areas of Soviet Ukraine and Byelorussia, as well as areas of Scandinavia, Italy, France, Poland, Turkey, and Greece.

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QUESTIONS

1. Why is it difficult to define what is meant by a "toxic element"? What are the major categories of toxic elements? Give an example of each.

2. Into which four main categories are elements divided in the periodic table? Why does one of these categories consist of elements of no toxicological chemical significance? What might be a toxicity characteristic of these "nontoxic" elements?

3. What has the Greenland icepack revealed about the environmental chemistry and distribution of a toxicologically significant element?

4. List and explain the forms in which metals may occur in the body.

5. What is a metal chelate? How are metal complexes related to chelates? In what sense may water be regarded as a ligand and metal ions dissolved in water regarded as complex ions?

6. What is the distinguishing feature of organometallic compounds as related to metal complexes? How is methylation related to organometallic compounds?

7. Which two kinds of functional molecules in biomolecules are most available for bonding to metal ions by complexation? Which other functional group forms especially strong bonds with some important toxic heavy metals? In what common biological compound produced as a defense against heavy metal poisoning is this functional group most abundant?

8. In what form are metals most likely to be taken in by the pulmonary route? What is one very special case of a toxic heavy metal taken in by this route?

9. What are the major toxic effects of beryllium? What may be said about the latent period for beryllium poisoning?

10. Although metal ions are generally not very soluble in hydrocarbons, vanadium occurs at high levels in some crude oil products. What is there about vanadium in crude oil that enables this to occur?

11. What are the most common oxidation states of chromium. Of these, why is chromium in the lower oxidation state generally insignificant in water?

12. In what respect does cadmium's chemical similarity to zinc possibly contribute to the toxicity of cadmium? Which organ in the body is most susceptible to cadmium poisoning?

13. What is a cumulative poison? In what sense is cadmium a cumulative poison? What might be a metabolic explanation for why a poison is cumulative?

14. Match the following:

(a) $PbSO_4$	1. Organometallic compound
(b) $Pb(C_2H_5)_4$	2. In sealed nickel/cadmium batteries
(c) PbO_2	3. Basic salt
(d) $Pb(OH)_2 \cdot 2PbCO_3$	4. Strong oxidant
(e) $Pb(OH)_2$	5. Ionic lead(II) compound

15. Match the following:

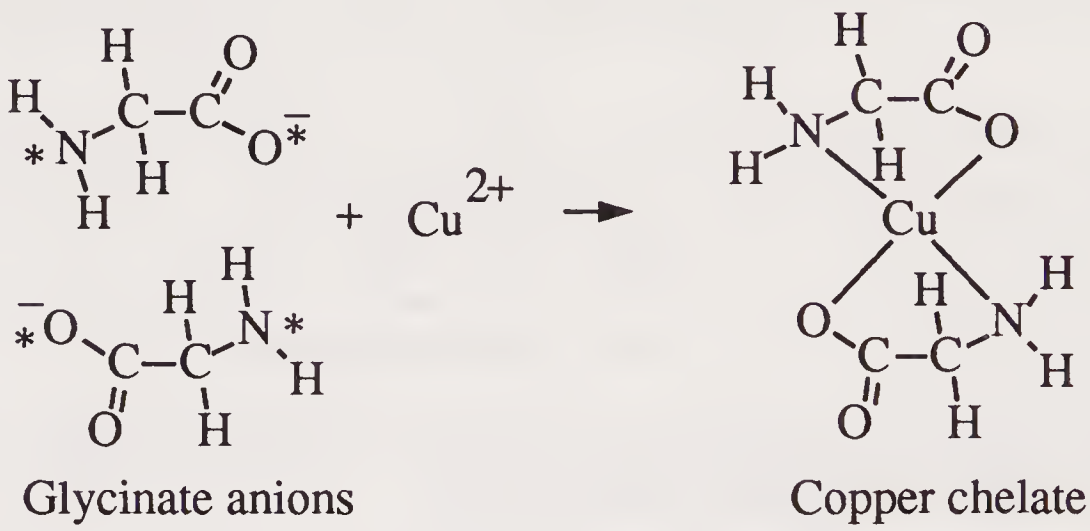
(a) Hg metal	1. In Ruben- Mallory dry cell
(b) HgO	2. Very soluble in water
(c) $Hg(C_2H_3O_2)_2$	3. Explosives' detonator
(d) $HgCl_2$	4. Used in gauges
(e) $Hg(ONC)_2$	5. Soluble in a number of organic solvents

16. What is the predominant function of the blood-brain barrier? How is it affected by mercury?

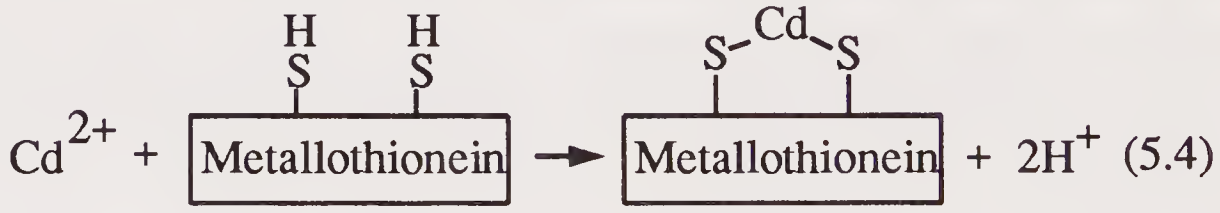
17. What is the greatest single use for lead? How might this use lead to lead exposure?

18. What is the effect of calcium on the absorption of dietary lead? How might this effect be explained?

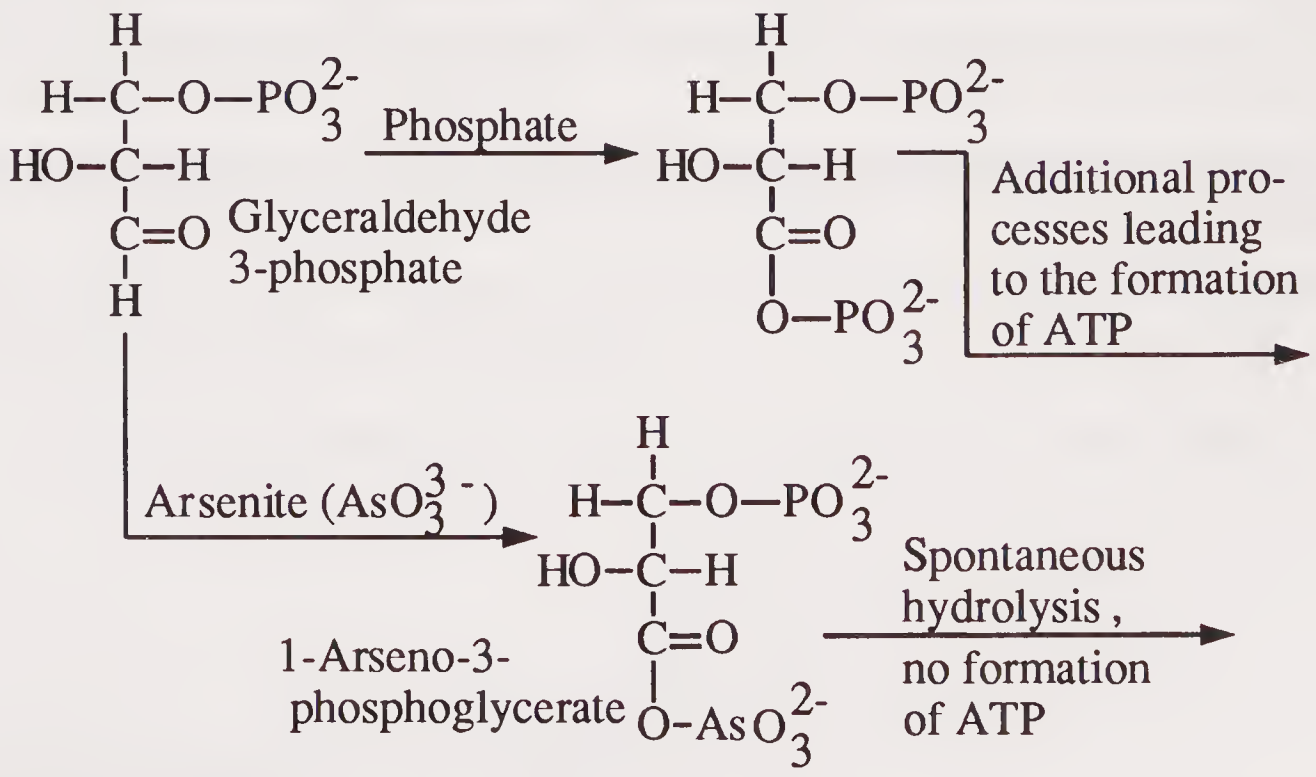
19. What is the major biochemical effect of lead, and how is this effect manifested?
20. What are the toxic effects of lead and cadmium on the kidney?
21. What is used as a therapeutic agent for lead poisoning? Why is this antidote always administered with calcium?
22. What are some of the uses of elemental arsenic and of arsenic compounds? How might these uses lead to human exposure?
23. Which of the oxidation states of arsenic is most likely to be toxic?
24. Explain what is shown by the illustration below:



25. What toxicological chemical effect is illustrated by the figure below:



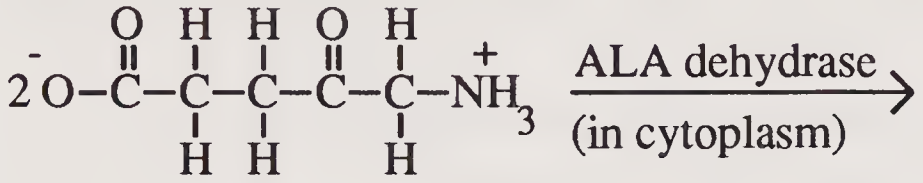
26. Explain what is shown by the following figure:



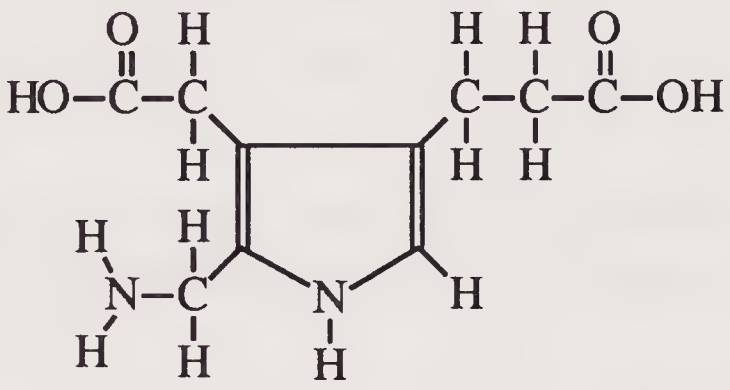
27. List the respects in which arsenic is similar to cadmium and mercury as well as phosphorus? Why is its chemical similarity to phosphorus especially damaging?

28. In what respects do antidotes to arsenic poisoning take advantage of arsenic's sulfur-seeking tendencies? What is the name and chemical formula of one such antidote?

29. Explain what the following figure shows about toxicological chemistry:



δ–Aminolevulinic acid



Porphobilinogen

30. Phosphorus and arsenic are chemically similar. Compare the toxic effects of elemental and combined phosphorus and arsenic.

31. Although noble gases are chemically unreactive, and cannot be toxic because of any chemical interactions, one such gas is particularly toxic by non-chemical mechanisms. Which noble gas is that, and why is it toxic?

32. Which metallic element, though chemically not similar to radon, operates through a similar mode of toxic action? What is the most likely route of exposure to this element?

33. Designate which of the following is **not** true of the toxicological hazard or effects of lead: (a) inhibition of the synthesis of hemoglobin, (b) particularly hazardous from inhalation of the elemental metal, (c) psychopathological symptoms including restlessness, dullness, irritability, and memory loss, (d) effects on the peripheral nervous system, (e) reversible damage to the kidney through its adverse effect upon proximal tubules.

34. Which radicals are produced by oxygen in the body? What are radicals? Why are they toxic?

11

Organometallics and Organometalloids

11.1. THE NATURE OF ORGANOMETALLIC AND ORGANOMETALLOID COMPOUNDS

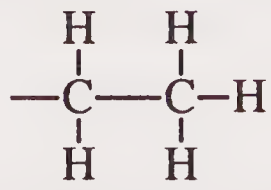
An **organometallic compound** is one in which the metal atom is bonded to at least one carbon atom in an organic group. An **organometalloid compound** is a compound in which a metalloid element is bonded to at least one carbon atom in an organic group. The metalloid elements are shown in the periodic table of the elements in Figure 1.3 and consist of boron, silicon, germanium, arsenic, antimony, tellurium, and astatine (a very rare radioactive element). In subsequent discussions, *organometallic* will be used as a term to designate both organometallic and organometalloid compounds and *metal* will refer to both metals and metalloids, unless otherwise indicated. Given the predominance of the metals among the elements, and the ability of most to form organometallic compounds, it is not surprising that there are so many organometallic compounds, and new ones are being synthesized regularly. Fortunately, only a small fraction of these compounds are produced in nature or for commercial use, which greatly simplifies the study of their toxicities.

A further clarification of the nature of organometallic compounds is based upon the **electronegativities** of the elements involved, i.e., the abilities of covalently bonded atoms to attract electrons to themselves. Electronegativity values range from 0.86 for cesium to 4.10 for fluorine. The value for carbon is 2.50, and all organometallic compounds involve bonds between carbon and an element with an electronegativity value of less than 2.50. The value of the electronegativity of phosphorus is 2.06, but it is so nonmetallic in its behavior that its organic compounds are not classified as organometallic compounds.

11.2. CLASSIFICATION OF ORGANOMETALLIC COMPOUNDS

The simplest way to classify organometallic compounds for the purpose of discussing their toxicology is the following:[1]

1. Those in which the organic group is an alkyl group such as ethyl:



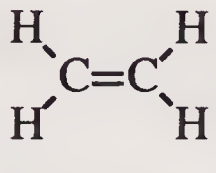
in tetraethyllead, $Pb(C_2H_5)_4$.

2. Those in which the organic group is carbon monoxide:

$$:C\equiv O:$$

(In the preceding Lewis formula of CO each dash, –, represents a pair of bonding electrons, and each pair of dots, :, represents an unshared pair of electrons.) Compounds with carbon monoxide bonded to metals, some of which are quite volatile and toxic, are called **carbonyls**.

3. Those in which the organic group is a π electron donor, such as ethylene or benzene.



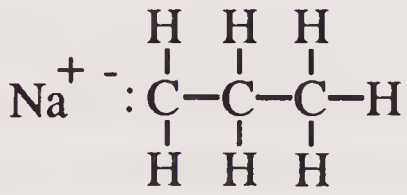
Ethylene

Benzene

Combinations exist of the three general types of compounds outlined above, the most prominent of which are arene carbonyl species in which a metal atom is bonded to both an aromatic entity such as benzene and to several carbon monoxide molecules. A more detailed discussion of the types of compounds and bonding follows.

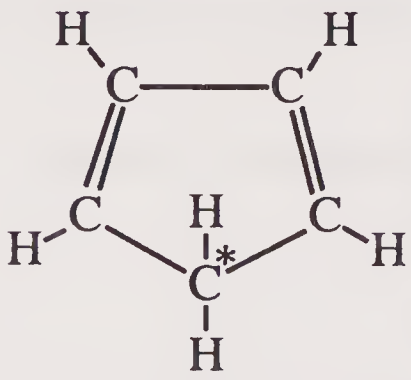
Ionically Bonded Organic Groups

Negatively charged hydrocarbon groups are called **carbanions**. These can be bonded to group IA and IIA metal cations, such as Na^+ and Mg^{2+}, by predominantly ionic bonds. In some carbanions the negative charge is localized on a single carbon atom. For species in which conjugated double bonds and aromaticity are possible, the charge may be delocalized over several atoms, thereby increasing the carbanions' stability (see Figure 11.1).



Negative charge localized on a single carbon atom in propylsodium

Negative charge delocalized in the 5-carbon ring of cyclopentadiene (see cyclopentadiene below)



Cyclopentadiene. Loss of H^+ from the carbon marked with an asterisk gives the negatively charged cyclopentadienide anion.

Figure 11.1. Carbanions showing localized and delocalized negative charges.

Ionic organic compounds involving carbanions react readily with oxygen. For example, ethylsodium, $C_2H_5^-Na^+$, self-ignites in air. Ionic organometallic compounds are extremely reactive in water, as shown by the following reaction:

$$C_2H_5^-Na^+ \xrightarrow{H_2O} \text{Organic products} + NaOH \qquad (11.2.1)$$

One of the products of such a reaction is a strong base, such as NaOH, which is very corrosive to exposed tissue.

Organic Groups Bonded with Classical Covalent Bonds

A major group of organometallic compounds has carbon-metal covalent single bonds in which both the C and metal (or metalloid) atoms contribute one electron each to be shared in the bond (in contrast to ionic bonds in which electrons are transferred between atoms). The bonds produced by this sharing arrangement are sigma- covalent bonds in which the electron density is concentrated between the two nuclei. Since in all cases the carbon atom is the more electronegative atom in this bond (see Section 11. l), the electrons in the bond tend to be more attracted to the more electronegative atom and the covalent bond has a **polar** character as denoted by the following:

$$\overset{\delta+}{M}\!\!-\!\!\overset{\delta-}{C}$$

When the electronegativity difference is extreme, such as when the metal atom is Na, K, or Ca, an ionic bond is formed. In cases of less extreme differences in electronegativity, the bond may be only partially ionic; i.e., it is intermediate between a covalent and ionic bond. Organometallic compounds with classical covalent bonds are formed with representative elements and with zinc, cadmium, and mercury, which have filled *d* orbitals. In some cases these bonds are also formed with transition metals. Organometallic compounds with this kind of bonding comprise some of the most important and toxicologically significant organometallic compounds. Examples of such compounds are shown in Figure 11.2.

The two most common reactions of sigma-covalently bonded organometallic compounds are oxidation and hydrolysis (see Chapter 1). These compounds have very high heats of combustion because of the stabilities of their oxidation products, which consist of the metal oxide, water, and carbon dioxide as shown by the following reaction for the oxidation of dimethyl zinc:

$$Zn(C_2H_5)_2 + 7O_2 \rightarrow ZnO(s) + 5H_2O(g) + 4CO_2(g) \qquad (11.2.2)$$

Industrial accidents in which the combustion of organometallic compounds generates respirable, toxic metal oxide fumes can certainly pose a hazard.

The organometallic compounds most likely to undergo hydrolysis are those with ionic bonds, compounds with relatively polar covalent bonds and those with vacant atomic orbitals (see Chapter 1) on the metal atom, which can accept more electrons. These provide sites of attack for the water molecules. For example, liquid trimethylaluminum reacts almost explosively with water or water and air:

$$Al(CH_3)_3 \xrightarrow[\{O_2\}]{H_2O} ZnO(s) + Al(OH)_3 + \text{Organic products} \qquad (11.2.3)$$

In addition to the dangers posed by the vigor of the reaction, it is possible that noxious organic products are evolved. Accidental exposure to air in the presence of moisture can result in the generation of sufficient heat to cause complete combustion of trimethylaluminum to the oxides of aluminum and carbon and to water.

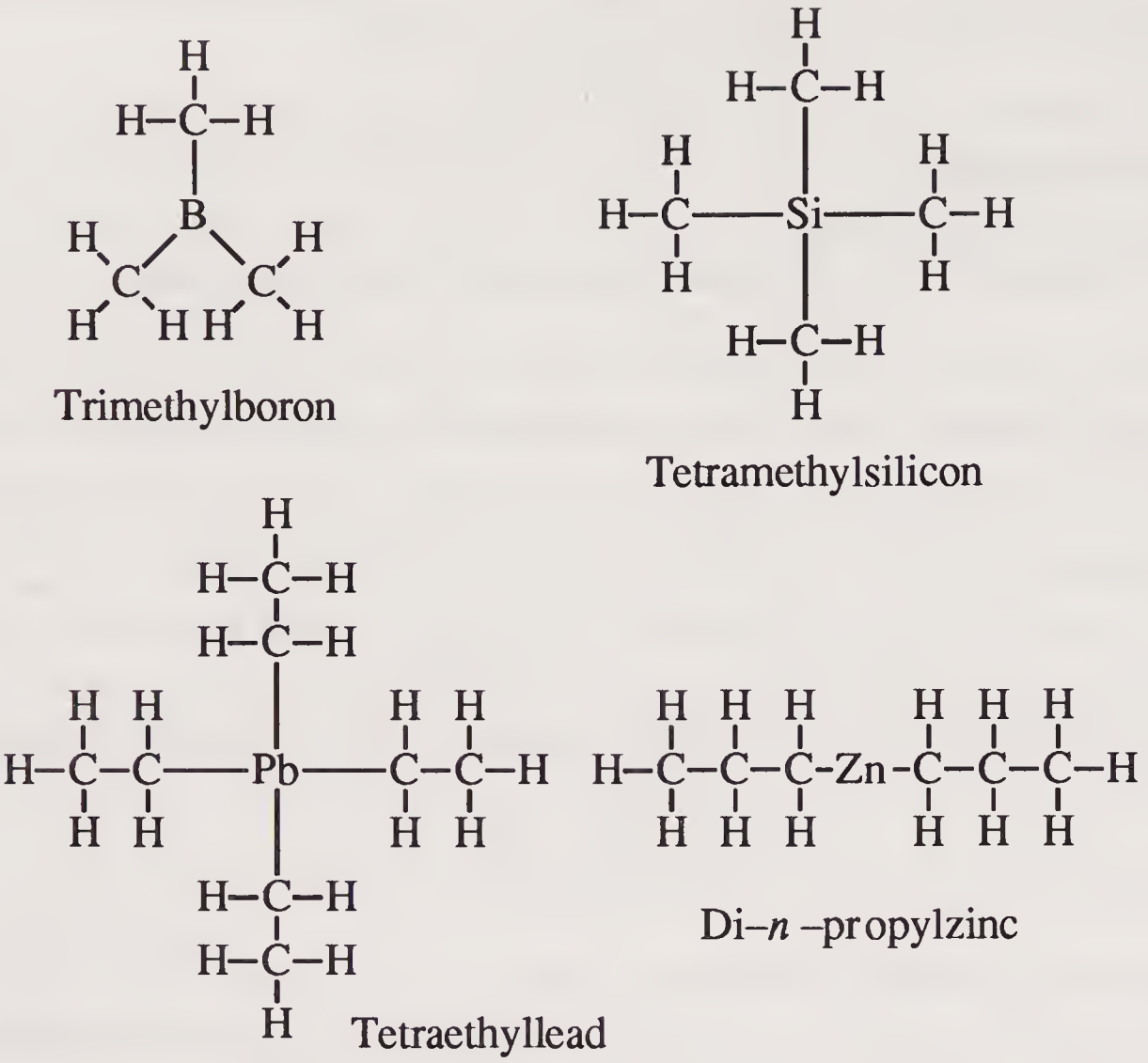


Figure 11.2. Some organometallic compounds with sigma-covalent metal-carbon bonds.

Organometallic Compounds with Dative Covalent Bonds

Dative covalent bonds, or coordinate covalent bonds, are those in which electrons are shared (as in all covalent bonds) but in which both electrons involved in each bond are contributed from the same atom. Such bonds occur in organometallic compounds of transition metals having vacant *d* orbitals. It is beyond the scope of this book to discuss such bonding in detail and the reader needing additional information is referred to works on organometallic compounds.[1,2] The most common organometallic compounds that have dative covalent bonds are **carbonyl compounds**, which are formed from a transition metal and carbon monoxide, where the metal is usually in the -1, 0, or +1 oxidation state. In these compounds the carbon atom on the carbon monoxide acts as an electron-pair donor as shown by the following:

$$M + :CO: \rightarrow \underset{\uparrow}{M{:}CO:} \qquad (11.2.4)$$

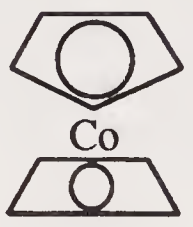
Dative bond

Most carbonyl compounds have several carbon monoxide molecules bonded to a metal.

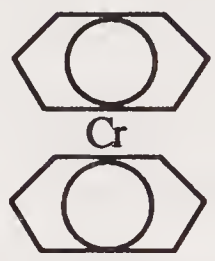
Many transition metal carbonyl compounds are known. The one of these that is the most significant toxicologically because of its widespread occurrence and extremely poisonous nature is the nickel carbonyl compound, $Ni(CO)_4$. Perhaps the next most abundant is $Fe(CO)_5$. Other examples are $V(CO)_6$ and $Cr(CO)_6$. In some cases bonding favors compounds with two metal atoms per molecule, such as $(CO)_5Mn\text{-}Mn(CO)_5$ or $(CO)_4Co\text{-}Co(CO)_4$.

Organometallic Compounds Involving π-Electron Donors

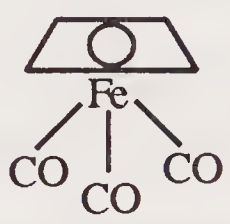
Unsaturated hydrocarbons, such as ethylene, butadiene, cyclopentadiene, and benzene contain π-electrons that occupy orbitals that are not in a direct line between the two atoms bonded together but are above and below a plane through that line. These electrons can participate in bonds to metal atoms in organometallic compounds. Furthermore, the metal atoms in a number of organometallic compounds are bonded to both a π-electron donor organic species — most commonly the cyclopentadienyl anion with a -1 charge — and one or more CO molecules. A typical compound of this class is cyclopentadienylcobalt-dicarbonyl, $C_5H_5Co(CO)_2$. Examples of these compounds and of compounds consisting of metals bonded only to organic π-electron donors are shown in Figure 11.3.



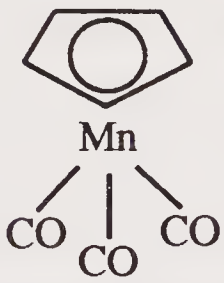
Compound of Co with cyclopentadienyl ion and cyclobutadiene



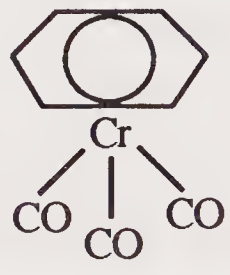
Dibenzene chromium



Cyclobutadiene-irontricarbonyl



Cyclopentadienylmanganesetricarbonyl



Benzenechromium-tricarbonyl

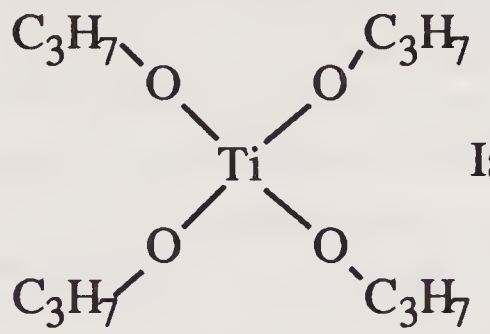
Figure 11.3. Compounds of metals with π-electron donor hydrocarbons and with carbon monoxide.

11.3. MIXED ORGANOMETALLIC COMPOUNDS

So far in this chapter the discussion has centered upon compounds in which all of the metal bonds are with carbon. A large number of compounds exist that have at least one bond between the metal and a C atom on an organic group, as well as other covalent or ionic bonds between the metal and atoms other than carbon. Because they have at least one metal-carbon bond, as well as properties, uses and toxicological effects typical of organometallic compounds, it is useful to consider such compounds along with organometallic compounds. Examples are monomethylmercury chloride, CH_3HgCl, in which the organometallic CH_3Hg^+ ion is ionically bonded to the chloride anion. Another example is phenyldichloroarsine, $C_6H_5AsCl_2$, in which a phenyl group is covalently bonded to arsenic through an As-C bond, and two Cl atoms are also covalently bonded to arsenic.

A number of compounds exist that consist of organic groups bonded to a metal atom through atoms other than carbon. Although they do not meet the strict definition thereof, such compounds can be classified as organometallics for the discussion of their toxicology and aspects of their chemistry. An example of such a compound is isopropyl titanate, $Ti(OC_3H_7)_4$, also called titanium isopropylate. This compound is a colorless liquid melting at 14.8°C and boiling at 104° C. Its behavior is more that of an organometallic compound than that of an inorganic compound, and by virtue of its

titanium content it is not properly classified as an organic compound. The term "organometal" is sometimes applied to such a compound. For toxicological considerations it may be regarded as an organometallic compound.



Isopropyl titanate

Several compounds are discussed in this chapter that have some organometallic character, but which also have formulas, structures, and properties of inorganic or organic compounds. These compounds could be called "mixed organometallics." However, so long as the differences are understood, compounds such as isopropyl titanate (see above) that do not meet all the criteria of organometallic compounds can be regarded as such for the discussion of their toxicities.

11.4. ORGANOMETALLIC COMPOUND TOXICITY

Some organometallic compounds have been known and used for decades, so that their toxicological properties are rather well known. Prominent among these are organoarsenicals used as drugs, organomercury fungicides, and tetramethyl- and tetraethyllead used as antiknock additives for gasoline. Since about 1950 there has been very substantial growth in chemical research devoted to organometallic compounds and large numbers and varieties of these compounds have been synthesized. Although the applications of organoarsenicals and organomercury compounds as human drugs and pesticides have declined sharply because of their toxicities, environmental effects, and the development of safer substitutes, a wide variety of new organometallic compounds has come into use for various purposes, such as catalysis and chemical synthesis. Toxicological experience is lacking for many of these compounds, so they should be treated with great caution until proven safe. Many are very reactive chemically, so that they are hazardous to directly exposed tissue, even if not toxic systemically.

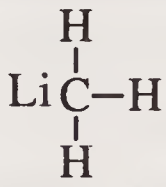
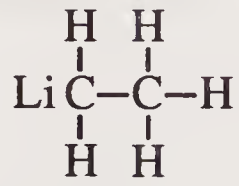
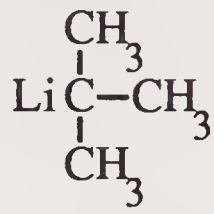
11.5. COMPOUNDS OF GROUP 1A METALS

Lithium Compounds

Table 11.1 shows some organometallic lithium compounds. It is seen from their formulas that these compounds are ionic. As discussed in Section 11.2, 1A metals have low electronegativities and form ionic compounds with hydrocarbon anions. Of these elements, lithium tends to form metal-carbon bonds with the most covalent character; therefore, lithium compounds are more stable (though generally quite reactive) compared to other organometallic compounds of Group 1A metals, most likely to exist as liquids or low-melting-point solids, and generally more soluble in organic solvents.[3] These compounds are moisture-sensitive, both in the pure state and in solution, and can undergo spontaneous ignition when exposed to air.

The most widely used organolithium compound is *n*-butyllithium (see formulas of related compounds in Table 11.1), used as an initiator for the production of elastomers by solution polymerization, predominantly of styrene-butadiene.

Table 11.1. Some Organometallic Compounds of Lithium

Name	Formula	Properties and Uses
Methyllithium		Initiator for solution polymerization of elastomers
Ethyllithium		Transparent crystals melting at 95°C, pyrophoric,[1] decomposes in water
Tert-butyllithium		Colorless crystalline solid subliming at 70-80°C, used as synthesis reagent
Phenyllithium		Colorless pyrophoric solid used in Grignard-type reactions to attach a phenyl group

[1] Pyrophoric: Spontaneously flammable in air.

Lithium forms a very unstable carbonyl, for which the toxicity is suspected of being high. The formula of this compound is LiCOCOLi, written in this manner to show that the two CO molecules form bridges between two Li atoms.

Unless otherwise known, the toxicities of lithium organometallic compounds should be regarded as those of lithium compounds and of organometallic compounds in general. The latter were discussed in Section 11.4. Lithium oxide and hydroxide are caustic bases, and they may be formed by the combustion of lithium organometallic compounds or by their reaction with water.

Lithium ion, Li^+, is a central nervous system toxicant that causes dizziness, prostration, anorexia, apathy, and nausea. It can also cause kidney damage and, in large doses, coma and death.

Compounds of Group 1A Metals Other than Lithium

As discussed in Section 11.2, group 1A metals form ionic metal-carbon bonds. Organometallic compounds of group 1A metals other than lithium have metal-carbon bonds with less of a covalent character than the corresponding bonds in lithium compounds and tend to be especially reactive. Compounds of rubidium and cesium are rarely encountered outside the laboratory, so their toxicological significance is relatively minor. Therefore, aside from lithium compounds, the toxicology of sodium and potassium compounds is of most concern.

Both sodium and potassium salts are natural constituents of body tissues and fluids as Na^+ and K^+ ions, respectively, and are not themselves toxic at normal physiological levels. The oxides and hydroxides of both these metals are very caustic, corrosive substances that damage exposed tissue. Oxides are formed by the combustion of sodium and potassium organometallics, and hydroxides are produced by the reaction of the oxides with water or by direct reaction of the organometallics with water, as shown below for cyclopentadienylsodium:

$$C_5H_5^-Na^+ + H_2O \rightarrow C_5H_6 + NaOH \qquad (11.5.1)$$

Both sodium and potassium form carbonyl compounds, NaCO and $(KCO)_6$, respectively. Both compounds are highly reactive solids prone to explode when exposed to water or air. Decomposition of the carbonyls gives off caustic oxides and hydroxides of Na and K, as well as toxic carbon monoxide.

Sodium and potassium form alkoxide compounds with the general formula $M^{+-}OR$, in which R is a hydrocarbon group. Typically, sodium reacts with methanol:

$$2CH_3OH + 2Na \rightarrow 2Na^{+-}OCH_3 + H_2 \qquad (11.5.2)$$

to yield sodium methoxide and hydrogen gas. The alkoxide compounds are highly basic and caustic, reacting with water to form the corresponding hydroxides as illustrated by the following reaction:

$$K^{+-}OCH_3 + H_2O \rightarrow KOH + CH_3OH \qquad (11.5.3)$$

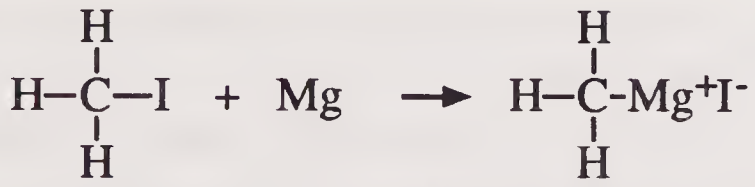
11.6. COMPOUNDS OF GROUP 2A METALS

The organometallic compound chemistry of the 2A metals is similar to that of the 1A metals and ionically-bonded compounds predominate. As is the case with lithium in Group 1A, the first 2A element, beryllium, behaves atypically, with a greater covalent character in its metal-carbon bonds.

Beryllium organometallic compounds should be accorded the respect due all beryllium compounds because of the extreme toxicity of beryllium (see Section 5.4). Dimethylberyllium, $Be(CH_3)_2$, is a white solid having needle-like crystals. When heated to decomposition, it gives off highly toxic beryllium oxide fumes. Diethylberyllium, $Be(C_2H_5)_2$, with a melting point of 12°C and a boiling point of 110°C, is a colorless liquid at room temperature and is especially dangerous because of its volatility.

Magnesium

The organometallic chemistry of magnesium has been of the utmost importance for many decades because of **Grignard reagents**, the first of which was made by Victor Grignard around 1900 by the reaction:



(11.6.1)

Iodomethane Methylmagnesium iodide

Grignard reagents are particularly useful in organic chemical synthesis for the attachment of their organic component ($-CH_3$ in the preceding example) to another organic molecule. The development of Grignard reagents was such an advance in organic chemical synthesis that in 1912 Victor Grignard received the Nobel Prize for his work.

Grignard reagents can cause damage to skin or pulmonary tissue in the unlikely event that they are inhaled. These reagents react rapidly with both water and oxygen, releasing a great deal of heat in the process. Ethyl ether solutions of methylmagnesium bromide (CH_3MgBr) are particularly hazardous because of the spontaneous ignition of the reagent and the solvent ether in which it is contained when the mixture contacts water, such as water on a moist laboratory bench top.

The simplest dialkyl magnesium compounds are dimethylmagnesium, chemical formula $Mg(CH_3)_2$, and diethylmagnesium, $Mg(C_2H_5)_2$. Both are pyrophoric compounds that are violently reactive to water and steam and that self-ignite in air, the latter even in carbon dioxide (like the elemental form, magnesium in an organometallic compound removes O from CO_2 to form MgO and release elemental carbon). Diethylmagnesium has a melting point of 0°C and is a liquid at room temperature. Diphenylmagnesium, $Mg(C_6H_5)_2$, is a feathery solid, somewhat less hazardous than the dimethyl and diethyl compounds. It is violently reactive with water and is spontaneously flammable in humid air, but not dry air.

Unlike the caustic oxides and hydroxides of group IA metals, magnesium hydroxide ($Mg(OH)_2$), formed by the reaction of air and water with magnesium organometallic compounds, is a relatively benign substance that is used as a food additive and ingredient of milk of magnesia.

Calcium, Strontium, and Barium

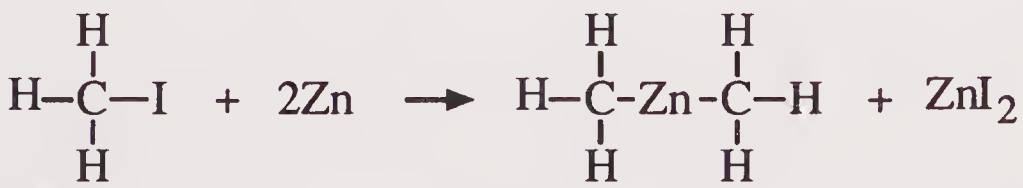
It is much more difficult to make organometallic compounds of Ca, Sr, and Ba than it is to make those of the first two group 2A metals. Whereas organometallic compounds of beryllium and magnesium have metal-carbon bonds with a significant degree of covalent character, the Ca/Sr/Ba organometallic compounds are much more ionic. These compounds are extremely reactive to water, water vapor, and atmospheric oxygen. There are relatively few organometallic compounds of calcium, strontium, and barium; their industrial uses are few, so their toxicology is of limited concern. Grignard reagents in which the metal is calcium rather than magnesium (general formula RCa^+X^-) have been prepared, but are not as useful for synthesis as the corresponding magnesium compounds.

11.7. COMPOUNDS OF GROUP 2B METALS

It is convenient to consider the organometallic compound chemistry of the Group 2B metals immediately following that of the 2A metals because both have two 2*s* electrons and no partially filled *d* orbitals. The group 2B metals — zinc, cadmium, and mercury — form an abundance of organometallic compounds, many of which have significant uses. Furthermore, cadmium and mercury (both discussed in Chapter 10) are notably toxic elements, so the toxicological aspects of their organometallic compounds is of particular concern. Therefore, the organometallic compound chemistry of each of the 2B metals will be discussed separately.

Zinc

A typical synthesis of a zinc organometallic compound is given by the reaction below in which the Grignard-type compound CH_3ZnI is an intermediate:



$$\begin{array}{l} H \\ | \\ H-C-I \\ | \\ H \end{array} + 2Zn \longrightarrow \begin{array}{l} H \quad H \\ | \quad | \\ H-C-Zn-C-H \\ | \quad | \\ H \quad H \end{array} + ZnI_2 \qquad (11.7.1)$$

Dimethylzinc has a rather low melting temperature of -40°C and it boils at 46°C. At room temperature it is a mobile, volatile liquid that undergoes self-ignition in air and

reacts violently with water. The same properties are exhibited by diethylzinc, $(C_2H_5)Zn$, which melts at -28°C and boils at 118°C. Diphenylzinc, $(C_6H_5)Zn$, is considerably less reactive than its methyl and ethyl analogs; it is a white crystalline solid melting at 107°C. Zinc organometallics are similar in many respects to their analogous magnesium compounds (see Section 11.6), but do not react with carbon dioxide, as do some of the more reactive magnesium compounds. An example of an organozinc compound involving a π-bonded group is that of methylcyclopentadienylzinc, shown in Figure 11.4.

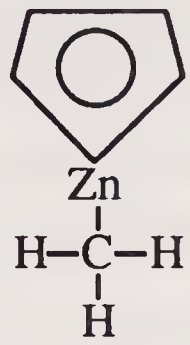


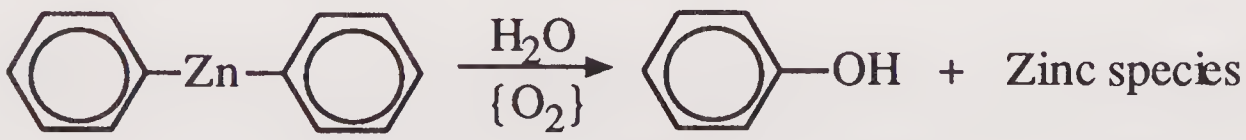
Figure 11.4. Methylcyclopentadienylzinc. The monomer shown exists in the vapor phase. In the solid phase a polymeric form exists.

Zinc organometallic compounds should be accorded the same caution in respect to toxicology as that of organometallic compounds in general. The combustion of highly flammable organozinc compounds such as dimethyl and diethyl compounds produces very finely divided particles of zinc oxide fumes as illustrated by the reaction:

$$2(CH_3)_2Zn + 8O_2 \rightarrow 2ZnO + 4CO_2 + 6H_2O \qquad (11.7.2)$$

Although zinc oxide is used as a healing agent and food additive, inhalation of zinc oxide fume particles causes zinc **metal fume fever** characterized by elevated temperature and "chills." The toxic effect of zinc fume has been attributed to its flocculation in lung airways, which prevents maximum penetration of air to the alveoli and perhaps activates endogenous pyrogen in blood leukocytes.[4] An interesting aspect of this discomfiting but less-than-deadly affliction is the immunity that exposed individuals develop to it, but which is lost after only a day or two of nonexposure. Thus workers exposed to zinc fume usually suffer most from the metal fume fever at the beginning of the work week, and less with consecutive days of exposure as their systems adapt to the metal fume.

Diphenylzinc illustrates the toxicity hazard that may obtain from the organic part of an organometallic compound upon decomposition. Under some conditions this compound can react to release toxic phenol (see Chapter 14):



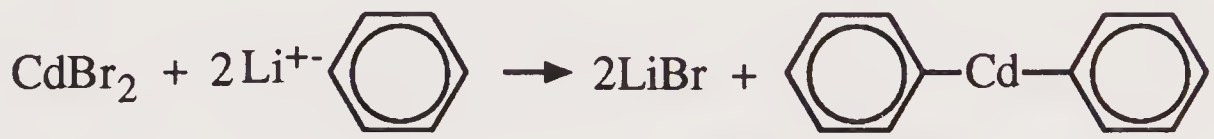
(11.7.3)

A number of zinc compounds with organic constituents (e.g., zinc salts of organic acids) have therapeutic uses. These include antidandruff zinc pyridinethione, antifungal zinc undecylenate used to treat athlete's foot, zinc stearate and palmitate (zinc soap), and antibacterial zinc bacitracin. Zinc naphthenate is used as a low-toxicity wood preservative and zinc phenolsulfonate has insecticidal properties, and was once used as an intestinal antiseptic. The inhalation of zinc soaps by infants has been

known to cause acute fatal pneumonitis[5] characterized by lung lesions similar to, but more serious than, those caused by talc. Zinc pyridine thione (zinc 2-pyridinethiol-1-oxide) has been shown to cause retinal detachment and blindness in dogs;[6] this effect is an apparently species-specific effect because laboratory tests at the same and even much higher dosages in monkeys and rodents do not show the same effect.

Cadmium

In the absence of water, cadmium halides, CdX_2, react with organolithium compounds as shown by the following example:



(11.7.4)

Dimethylcadmium, $(CH_3)_2Cd$, is an oily liquid at room temperature and has a very unpleasant odor. The compound melts at -4.5°C and boils at 106°C. It decomposes in contact with water. Diethylcadmium is likewise an oil; it melts at -21°C and boils at 64°C and reacts explosively with oxygen in air. Dipropylcadmium, $(C_3H_7)_2Cd$, is an oil that melts at -83°C, boils at 84°C, and reacts with water. The dialkyl cadmium compounds are distillable, but decompose above about 150°C, evolving toxic cadmium fume.

The toxicology of cadmium organometallic compounds is of particular concern because of the high toxicity of cadmium. The organometallic compounds of cadmium form vapors that can be inhaled and that can cross membranes because of their lipid solubility. The reaction of cadmium organometallic compounds with water can release highly toxic fumes of cadmium and CdO. Inhalation of these fumes can cause chronic cadmium poisoning and death. The toxicological aspects of cadmium are discussed in Section 10.4.

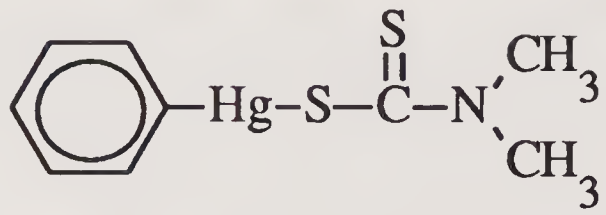
Mercury

In 1853 E. Frankland made the first synthetic organomercury compound by the photochemical reaction below:

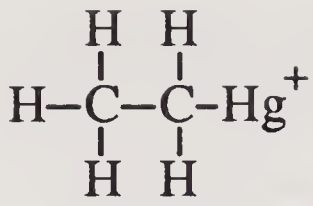
$$2Hg + 2CH_3I + h\nu(\text{sunlight}) \rightarrow (CH_3)_2Hg + HgI_2 \qquad (11.7.5)$$

Numerous synthetic routes are now available for the preparation of a variety of mercury organometallic compounds.

In the late 1800s and early 1900s numerous organomercury pharmaceutical compounds were synthesized and used. These have since been replaced by more effective and safe nonmercury substitutes. Organomercury compounds have been widely used as pesticidal fungicides (see Figure 11.5), but these applications are now declining because of the adverse effects of mercury in the environment.



Phenylmercurydimethyldithiocarbamate (slimicide for wood pulp and mold retardant for paper)



Ethylmercury chloride (seed fungicide)

Figure 11.5. Two organomercury compounds that have been used for fungicidal purposes.

The most notorious mercury compounds in the environment are monomethylmercury (CH_3Hg^+) salts and dimethylmercury ($(CH_3)_2Hg$). The latter compound is both soluble and volatile and the salts of the monomethylmercury cation are soluble. These compounds are produced from inorganic mercury in sediments by anaerobic bacteria through the action of methylcobalamin, a vitamin B12 analog and intermediate in the synthesis of methane:

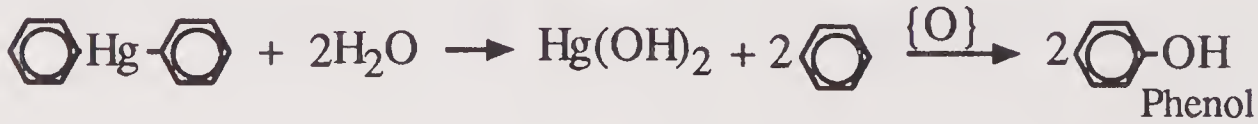
$$HgCl_2(s) \xrightarrow{\text{Methylcobalamin}} CH_3Hg^+(aq) + 2Cl^- \qquad (11.7.6)$$

The preceding reaction is favored in somewhat acidic water in which anaerobic decay, which often produces CH_4, is occurring. If the water is neutral or slightly alkaline, dimethylmercury formation is favored; this volatile compound may escape to the atmosphere. Discovered around 1970, the biosynthesis of the methylmercury species in sediments was an unpleasant surprise, in that it provides a means for otherwise insoluble inorganic mercury compounds to get into natural waters. Furthermore, these species are lipid-soluble, so that they undergo bioaccumulation and biomagnification in aquatic organisms. Fish tissue often contains more than 1000 times the concentration of mercury as does the surrounding water.

The toxicity of mercury is discussed in Section 10.4. Some special considerations apply to organomercury compounds, the foremost of which is their lipid solubility and resulting high degree of absorption and facile distribution through biological systems. The lipid solubilities and high vapor pressures of the methylmercuries favor their absorption by the pulmonary route. These compounds also can be absorbed through the skin, and their uptake approaches 100 % (compared to less than 10% for inorganic mercury compounds) in the gastrointestinal tract.

With respect to distribution in the body, the methylmercury species behave more like mercury metal, Hg(0), than inorganic mercury(II), Hg^{2+}. Like elemental mercury, methylmercury compounds traverse the blood-brain barrier and affect the central nervous system. However, the psychopathological effects of methylmercury compounds (laughing, crying, impaired intellectual abilities) are different from those of elemental mercury (irritability, shyness).

Mono- and diphenylmercury have toxicological effects much like those of inorganic mercury(II) because of their rapid hydrolysis in the body:



(11.7.7)

11.8. ORGANOTIN COMPOUNDS

Global production of organotin compounds is on the order of 40,000 metric tons per year and consumes about 7–8 percent of the tin used each year. Of all the metals, tin has the greatest number of organometallic compounds in commercial use.[6] Major industrial uses include applications of tin compounds in fungicides, acaricides, disinfectants, antifouling paints, stabilizers to lessen the effects of heat and light in PVC plastics, catalysts, and precursors for the formation of films of SnO_2 on glass. Tributyl tin chloride and related tributyl tin (TBT) compounds have bactericidal, fungicidal, and insecticidal properties and are of particular environmental significance because of growing use as industrial biocides. In addition to tributyl tin chloride, other tributyl tin compounds used as biocides include the hydroxide, the naphthenate, bis(tributyltin) oxide, and tris(tributylstannyl) phosphate. A major use of TBT is in boat and ship hull coatings to prevent the growth of fouling organisms.[7] Other applications include preservation of wood, leather, paper, and textiles.[8] Because of their antifungal activity TBT compounds are used as slimicides in cooling tower water.

In addition to synthetic organotin compounds, methylated tin species can be produced biologically in the environment. Figure 11.6 gives some examples of the many known organotin compounds.

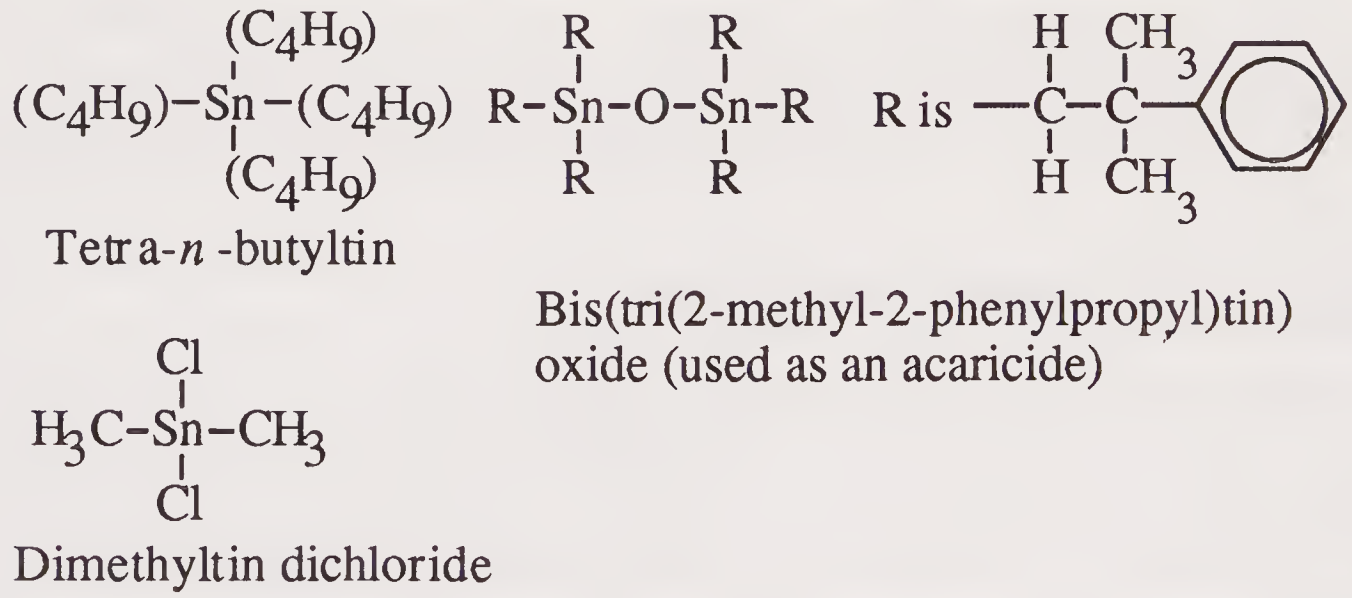


Figure 11.6. Examples of organotin compounds.

Toxicology of Organotin Compounds

Many organotin compounds have the general formula, R_nSnX_{4-n}, where R is a hydrocarbon group and X is an inorganic entity, such as a chlorine atom, or an organic group bonded to tin through a noncarbon atom (for example, acetate bonded to Sn through an O atom).[8] As a general rule, in a series of these compounds, toxicity is at a maximum value for n = 3. Furthermore, the toxicity is generally more dependent upon the nature of the R groups than upon X.

Organotin compounds are readily absorbed through the skin, and skin rashes may result. Organotin compounds, especially those of the R_3SnX type, bind to proteins, probably through the sulfur on cysteine and histidine residues. Interference with mictochondrial function by several mechanisms appears to be the mode of biochemical action leading to toxic responses.

11.9. ORGANOLEAD COMPOUNDS

The toxicities and environmental effects of organolead compounds are particularly noteworthy because of the widespread use and distribution of tetraethyllead as a gasoline additive (see structure in Figure 11.2). Although more than 1000 organolead compounds have been synthesized, those of commercial and toxicological importance are largely limited to the alkyl (methyl and ethyl) compounds and their salts, examples of which are shown in Figure 11.7.

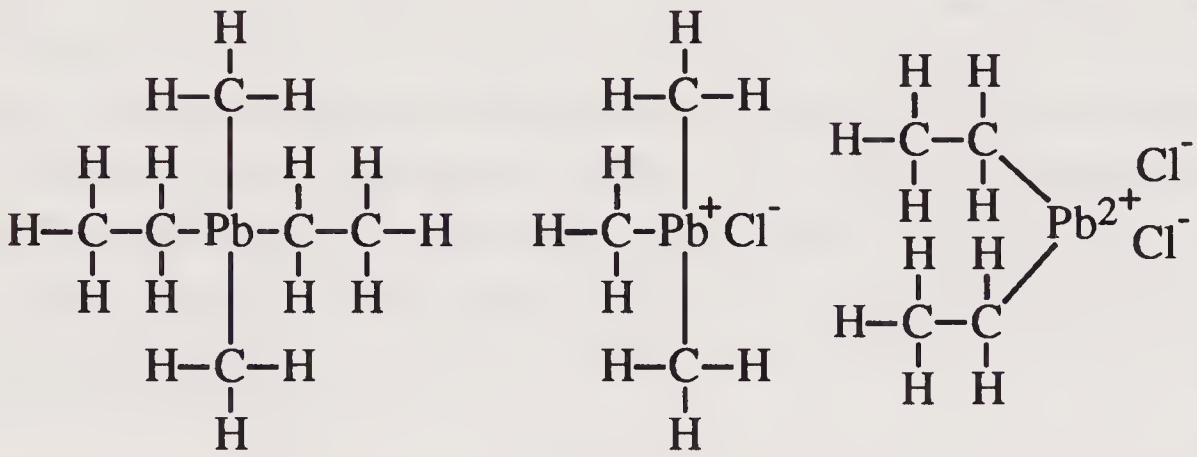


Figure 11.7. Alkyllead compounds and salts.

In addition to manufactured organolead compounds, the possibility exists of biological methylation of lead, such as occurs with mercury (see Section 11.7). However, there is a great deal of uncertainty regarding biological methylation of lead in the environment.

Toxicology of Organolead Compounds

Because of the large amounts of tetraethyllead used as a gasoline additive, the toxicology of this compound has been investigated much more extensively than that of other organolead compounds and is discussed briefly here. Tetraethyllead is a colorless, oily liquid with a strong affinity for lipids and is considered highly toxic by inhalation, ingestion, and absorption through the skin. The toxicological action of tetraethyllead is different from that of inorganic lead. As one manifestation of this difference, chelation therapy is ineffective for the treatment of tetraethyllead poisoning. Symptoms of tetraethyllead poisoning reflect effects upon the central nervous system. Among these symptoms are fatigue, weakness, restlessness, ataxia, psychosis, and convulsions. In cases of fatal tetraethyllead poisoning, death has occurred as soon as one or two days after exposure. Fatalities have been comparatively rare, considering the widespread use of tetraethyllead. Recovery from poisoning by this compound tends to be slow. Its toxic action appears to involve its metabolic conversion to the triethyl form.

11.10. ORGANOARSENIC COMPOUNDS

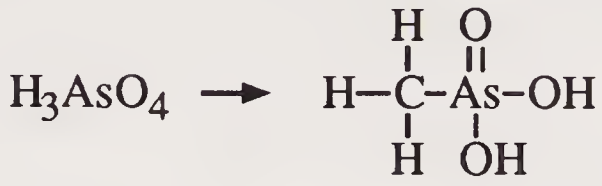
There are two major sources of organoarsenic compounds — those produced for commercial applications and those produced from the biomethylation of inorganic arsenic by microorganisms. Many different organoarsenic compounds have been identified.

Organoarsenic Compounds from Biological Processes

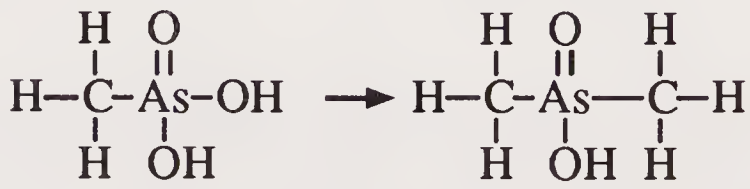
The reactions that follow illustrate the production of organoarsenic compounds by bacteria.[6] In a reducing environment, arsenic(V) is reduced to arsenic(III):

$$H_3AsO_4 + 2H^+ + 2e^- \rightarrow H_3AsO_3 + H_2O \qquad (11.10.1)$$

Through the action of methylcobalamin in bacteria, arsenic(III) is methylated to methyl- then to dimethylarsinic acid:

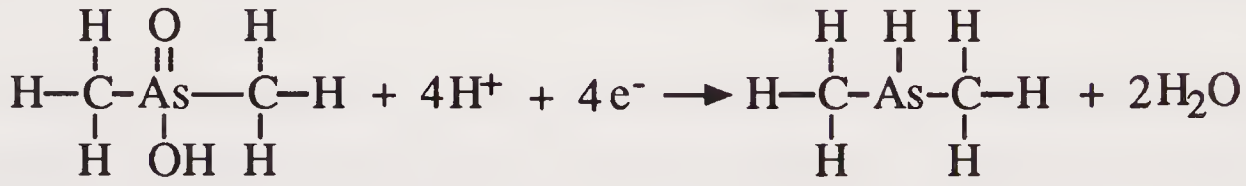


(11.10.2)



(11.10.3)

Dimethylarsinic acid can be reduced to volatile dimethylarsine:



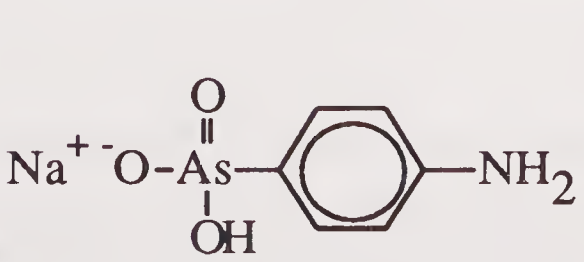
(11.10.4)

Methylarsinic acid and dimethyarsinic acid are the two organoarsenic compounds that are most likely to be encountered in the environment.

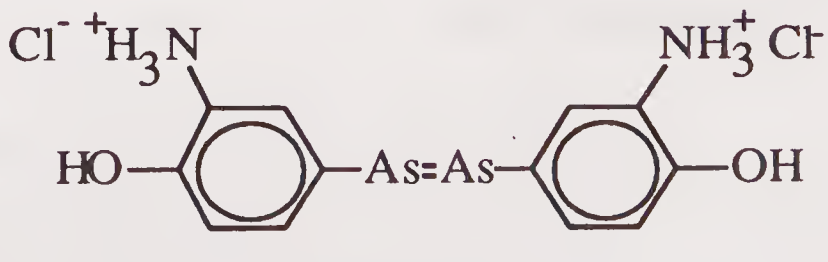
Biomethylated arsenic was responsible for numerous cases of arsenic poisoning in Europe during the 1800s. Under humid conditions, arsenic in plaster and wallpaper pigments was converted to biomethylated forms, as manifested by the strong garlic odor of the products, and people sleeping and working in the rooms became ill from inhaling the volatile organoarsenic compounds.

Synthetic Organoarsenic Compounds

Although now essentially obsolete for the treatment of human diseases because of their toxicities, organoarsenic compounds were the first synthetic organic pharmaceutical agents and were widely used in the early 1900s. The first pharmaceutical application was that of atoxyl (the sodium salt of 4-aminophenylarsinic acid), which was used to treat sleeping sickness. The synthesis of Salvarsan by Dr. Paul Ehrlich in 1907 was a development that may be considered the beginning of modern **chemotherapy** (chemical treatment of disease). Salvarsan was widely used for the treatment of syphilis.

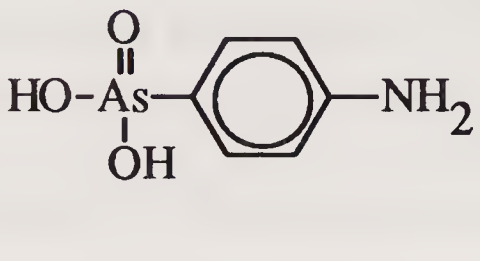


Atoxyl

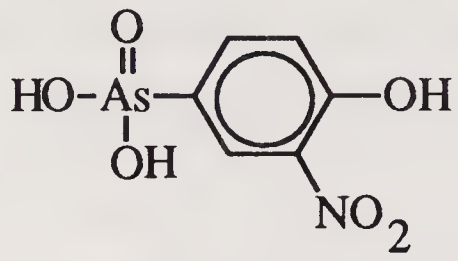


Salvarsan

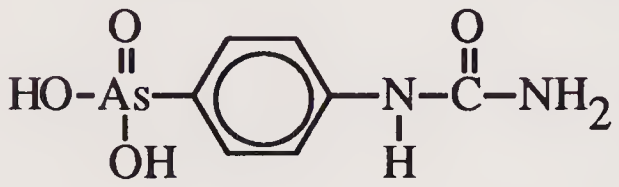
Organoarsenic compounds are used as animal feed additives. The major organoarsenic feed additives and their uses are summarized in Figure 11.8.



Arsanilic acid



3-Nitro-4-hydroxyphenyl-arsinic acid (Roxarsone)



N-carbamoylarsinic acid (Carbarsone)

Figure 11.8. Major organoarsenic animal feed additives. Arsanilic acid and Roxarsone are used to control swine dysentery and increase the rate of gain relative to the amount of feed in swine and chickens. Carbarsone and nitarsone (4-nitrophenylarsanilic acid) act as antihistomonads in chickens.

Toxicities of Organoarsenic Compounds

The toxicities of organoarsenic compounds vary over a wide range. In general, the toxicities are less for those compounds that are not metabolized in the body and that are excreted in an unchanged form. Examples of such compounds are the animal feed additives shown in Figure 11.8. Metabolic breakdown of organoarsenic compounds to inorganic forms is correlated with high toxicity. This is especially true when the product is inorganic arsenic(III), which, for the most part, is more toxic than arsenic(V). The toxicity of arsenic(III) is related to its strong affinity for sulfhydryl (–SH) groups. Detrimental effects are especially likely to occur when sulfhydryl groups are adjacent to each other on the active sites of enzymes, enabling chelation of the arsenic and inhibition of the enzyme.

To a certain extent toxic effects of dimethylarsinic acid (cacodylic acid) have been observed because of its applications as a herbicide and former uses of its sodium salt for the treatment of human skin disease and leukemia. It is most toxic via ingestion because the acidic medium in the stomach converts the compound to inorganic arsenic(III). A portion of inorganic arsenic in the body is converted to dimethylarsinic acid, which is excreted in urine, sweat, and exhaled air, accompanied by a strong garlic odor. Roxarsone has a relatively high acute toxicity to rats and dogs.[9] Among the effects observed in these animals are internal hemorrhage, kidney congestion, and gastroenteritis. Rats fed fatal doses of about 400 ppm in the diet exhibited progressive weakness prior to death.

11.11. ORGANOSELENIUM AND ORGANOTELLURIUM COMPOUNDS

Organo compounds of the two group 6A elements, selenium and tellurium, are of considerable environmental and toxicological importance. Organoselenium and organotellurium compounds are produced both synthetically and by microorganisms. The selenium compounds are the more significant because of the greater abundance of this element.

Organoselenium Compounds

The structures of three common organoselenium compounds produced by organisms are given in Figure 11.9. Some organisms convert inorganic selenium to dimethylselenide. Several genera of fungi are especially adept at this biomethylation process, and their activities are readily detected from the very strong "ultragarlic" odor of the product. The bioconversion of inorganic selenium(II) and selenium(VI) to dimethylselenide and dimethyldiselenide occurs in animals such as rats, and the volatile compounds are evolved with exhaled air. Another organoselenium compound produced by bacteria is dimethylselenone. Some synthetic organoselenium compounds have selenium as part of a ring, such as is the case with the cyclic ether, 1,4-diselenane.

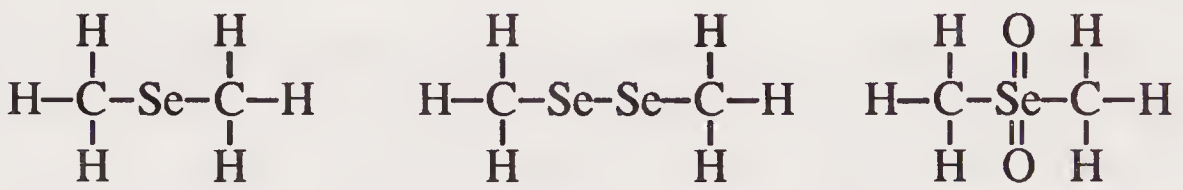


Figure 11.9. Example organoselenium compounds.

Inorganic selenium compounds are rather toxic, and probably attach to protein sulfhydryl groups, much like inorganic arsenic. In general, organoselenium compounds are regarded as being less toxic than inorganic selenium compounds.

Organotellurium Compounds

Inorganic tellurium is used in some specialized alloys, to color glass, and as a pigment in some porcelain products. The breath of workers exposed to inorganic tellurium has a garlic odor, perhaps indicative of bioconversion to organotellurium species. Dimethyltelluride can be produced by fungi from inorganic tellurium compounds. Tellurium is a rather rare element in the geosphere and in water, so that biomethylation of this element is unlikely to be a major environmental problem. In general, the toxicities of tellurium compounds are less than those of their selenium analogs.

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QUESTIONS

1. How is carbon involved in defining what an organometallic compound is? How is electronegativity involved in this definition? How does an organometalloid differ from an organometallic?
2. What are the three major kinds of organic groups, or ligands, bonded to a metal in an organometallic compound? How might the bonding of an alkyl ligand to an element with a very low electronegativity, such as potassium, differ from the bonding to an element with a higher electronegativity, such as arsenic?
3. What is a carbanion? How are carbanions involved in organometallic compounds? How can neutral cyclopentadiene form a carbanion?
4. What would be the expected reactions of $C_2H_5^-Na^+$with water? How might this species react with oxygen in air? What toxic effects might result from these kinds of reactions?
5. Match the following pertaining to bonding in organometallic compounds:

(a) Sigma-covalent	1. Mixed organometallic
(b) Dative covalent	2. Formed by benzene, cyclopentadiene
(c) Bonds with π-electrons	3. Shared electrons all contributed by one atom
(d) CH_3HgCl	4. Electron density is concentrated between the two nuclei

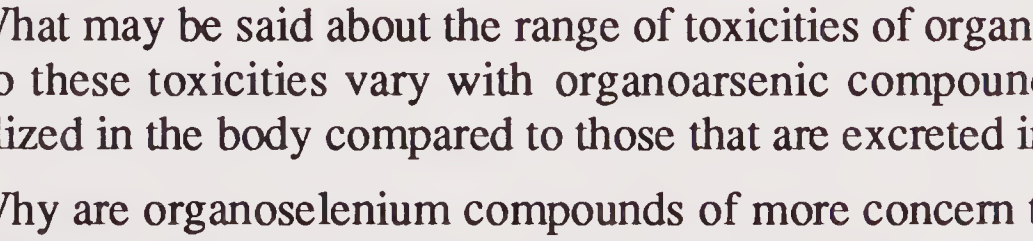
6. Discuss the historical aspects of organometallic compound toxicity, including organoarsenicals used as pharmaceutical agents, gasoline antiknock additives, and compounds used in applications such as catalysis and chemical synthesis.
7. Which group 1A organometallic compounds are more stable compared to other organometallic compounds of group 1A metals, most likely to exist as liquids or low-melting-point solids, and generally more soluble in organic solvents?
8. In general, how should the toxicities of lithium organometallic compounds be regarded? Do they have any unique toxicity characteristics?

9. What are alkoxide compounds? In what sense are they organometallic compounds? In what respects are they not organometallic compounds? What does the reaction,

$$K^{+-}OCH_3 + H_2O \rightarrow KOH + CH_3OH$$

show about alkoxides?

10. What are Grignard reagents? In what sense are they mixed organometals?
11. Diethylmagnesium, $Mg(C_2H_5)_2$, is described as a pyrophoric compound that is violently reactive to water and steam and that self-ignites in air, burning even in a carbon dioxide atmosphere. Describe the siaganificance of this description in terms of such factors as reactivity, susceptibility to hydrolysis or oxidation, and potential toxic effects.
12. Describe what is shown by the following reaction:



13. Describe a specific toxic reaction that may result from the following combustion reaction:

$$2(CH_3)_2Zn + 8O_2 \rightarrow 2ZnO + 4CO_2 + 6H_2O$$

14. Why is the toxicology of cadmium organometallic compounds of particular concern?
15. Describe one chemical and one biochemical means of synthesis of $(CH_3)_2Hg$. In what sense was the discovery of biosynthesis of methylmercury species an "unpleasant surprise" in environmental chemistry?
16. List some special considerations that apply to organomercury compounds. How do their properties and pathways in the body compare to Hg(0) and Hg^{2+}?
17. Describe the biocidal properties and uses of tributyl tin chloride and related tributyl tin compounds.
18. What are some of the biocidal uses of tributyl tin compounds?
19. In what sense are the toxicities and environmental effects of organolead compounds "particularly noteworthy."
20. What is some of the evidence that the toxicological action of tetraethyllead is different from that of inorganic lead? What are some of the symptoms of tetraethyllead poisoning?
21. What are the two major sources of organoarsenic compounds? Give some examples of organoarsenic compounds produced by these two routes.
22. What may be said about the range of toxicities of organoarsenic compounds? How do these toxicities vary with organoarsenic compounds that are readily metabolized in the body compared to those that are excreted in an unchanged form?
23. Why are organoselenium compounds of more concern than organotellurium compounds despite the close chemical similarity of selenium and tellurium?

12

Toxic Inorganic Compounds

12.1. INTRODUCTION

In Chapter 10 elements were discussed that as a rule tend to be toxic in their various forms. Chapter 12 covers toxic inorganic compounds of elements that are not themselves generally regarded as being toxic. These elements include for the most part the lighter nonmetals located in the upper right of the periodic table (Figure 1.1), and exclude the heavy metals. Most of the elements involved in the inorganic compounds discussed in this chapter are those that are essential for life processes. Any division between "toxic" and "nontoxic" elements is by nature artificial in that most of the heavy metals have compounds of relatively low toxicity, and there are deadly compounds that contain elements essential for life. The toxicities of inorganic compounds are covered in detail in a reference work on the subject.[1]

Chapter Organization

In general this chapter is organized in the order of increasing atomic number of the elements that are covered. Inorganic compounds of carbon, atomic number 6, are discussed first, followed by toxic inorganic compounds of nitrogen, atomic number 7. The next element, oxygen, occurs in so many different inorganic compounds that it is not discussed in a separate category except for its toxic elemental form, ozone. The halogens — fluorine, chlorine, bromine, and iodine — are discussed as a group because of their chemical similarities. The other major elements whose toxic inorganic compounds are discussed are silicon, phosphorus, and sulfur.

12.2. TOXIC INORGANIC CARBON COMPOUNDS

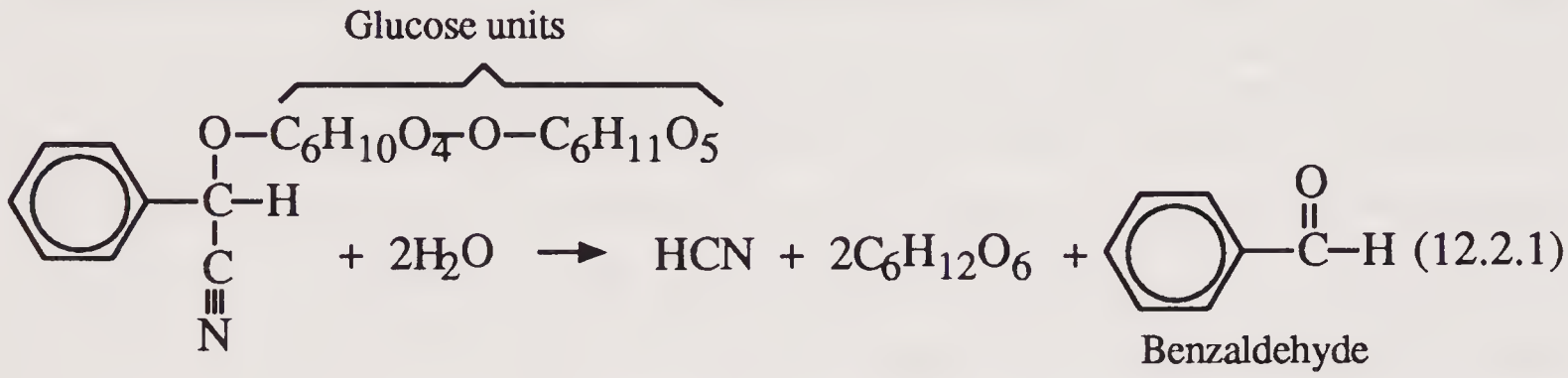
Cyanide

Cyanide, either in the form of gaseous **hydrogen cyanide** (HCN) or **cyanide ion** (CN^- present in cyanide salts such as KCN) is a notably toxic substance. Cyanide is a rapidly acting poison[2] and the fatal oral dose to humans is believed to be only 60–90 mg. Hydrogen cyanide and cyanide salts have numerous uses; examples are as ingredients of pest poisons, fumigants, metal (silver) polishes, and photographic chemical solutions. Therefore, exposure to cyanide is certainly possible. Hydrogen cyanide is used as a fumigant to kill pests such as rodents in warehouses, grain storage bins, greenhouses, and holds of ships, where its high toxicity and ability to penetrate obscure spaces are advantageous. Cyanide salt solutions are used to extract some metals

such as gold from ores, in metal refining, in metal plating, and for salvaging silver from exposed photographic and X-ray film. Cyanide is used in various chemical syntheses. Polyacrylic polymers may evolve HCN during combustion, adding to the toxic gases that are usually responsible for deaths in fires. Sodium nitroprusside, $Na_2Fe(NO)(CN)_5$, used intravenously in humans to control hypertension, can hydrolyze in the body to release cyanide and cause cyanide poisoning.

The Romans used cyanide from natural seed sources, such as apple seeds, for executions and suicides. The seeds of apples, apricots, cherries, peaches, plums, and some other fruits contain sources of cyanide. Other natural sources of cyanide include arrowgrass, sorghum, flax, velvet grass, and white clover.

Cyanide in plants is bonded to a glycoside (sugar-like substance) called amygdalin. The cyanide is released by enzymatic or acidic hydrolysis, such as occurs in the stomach:



(12.2.1)

Biochemical Action of Cyanide

Cyanide deprives the body of oxygen by acting as a **chemical asphyxiant** (in contrast to simple asphyxiants that simply displace oxygen in respired air). In acting as an asphyxiant, cyanide inhibits an enzyme (see enzyme inhibition, Section 9.3) involved in a key step in the oxidative phosphorylation pathway by which the body utilizes oxygen in cell mitochondria. The inhibited enzyme is ferricytochrome oxidase (Fe(III)-oxid), an iron-containing metalloprotein that acts as an acceptor of electrons and is converted to ferrouscytochrome oxidase (Fe(II)-oxid) during the oxidation of glucose. The ferrouscytochrome oxidase that is formed transfers the electrons to molecular oxygen and produces energetic ATP from ADP (see Section 4.3), regenerating Fe(III)-oxid that can repeat the cycle. The overall process is represented as the following:

$$\text{Fe(III)-oxid} + \text{Reducing agent} \rightarrow \text{Fe(II)-oxid} + \text{Oxidized reducing agent} \qquad (12.2.2)$$

$$\text{Fe(II)-oxid} + 2H^+ + {}^1/_2O_2 \xrightarrow[\text{ADP}]{\text{ATP}} \text{Fe(III)-oxid} + H_2O \qquad (12.2.3)$$

Cyanide bonds to the iron(III) of the ferricytochrome enzyme, preventing its reduction to iron(II) in the first of the two reactions above. The result is that ferrouscytochrome oxidase, which is required to react with O_2, is not formed and utilization of oxygen in cells is prevented, leading to rapid cessation of metabolic processes. The decreased utilization of oxygen in tissue results in a buildup of oxyhemoglobin in venous blood, which gives the skin and mucous membranes a characteristic red color (flush).

The metabolic pathway for the detoxification of cyanide involves conversion to the less toxic thiocyanate by a reaction requiring thiosulfate or colloidal sulfur as a substrate:

$$CN^- + S_2O_3^{2-} \xrightarrow[\text{Rhodanase}]{} SCN^- + SO_3^{2-} \qquad (12.2.4)$$

This reaction is catalyzed by *rhodanase* enzyme, also called *mitochondrial sulfur transferase*. Although not found in the blood, this enzyme does occur abundantly in liver and kidney tissue. As a result of this reaction, thiosulfate can be administered as an antidote for cyanide poisoning.

Nitrite, NO_2^-, administered intravenously as sodium nitrite solution or inhaled as amyl nitrite, $C_5H_{11}NO_2$, an ester which hydrolyzes to NO_2^- in the blood, functions as an antidote to cyanide poisoning. This occurs because nitrite oxidizes iron(II) in blood hemoglobin (HbFe(II)) to methemoglobin (HbFe(III)), a brown substance that is ineffective in carrying oxygen to tissues. (This reaction is the mechanism of nitrite toxicity; excessive formation of methemoglobin causes oxygen deprivation that can be fatal.) Methemoblogin in the blood, however, has a high affinity for cyanide and removes it from ferricytochrome oxidase enzyme that has been inhibited by binding of cyanide (Fe(III)-oxid-CN),

$$\text{HbFe(III)} + \text{Fe(III)-oxid-CN} \rightarrow \text{HbFe(III)-CN} + \text{Fe(III)-oxid} \qquad (12.2.5)$$

freeing the ferricytochrome oxidase enzyme so that it can participate in its normal metabolic functions. Additional treatment with thiosulfate results in elimination of the cyanide:

$$\text{HbFe(III)-CN} + S_2O_3^{2-} \rightarrow SCN^- + \text{HbFe(III)} + SO_3^{2-} \qquad (12.2.6)$$

Carbon Monoxide

Carbon monoxide, CO, is a toxic industrial gas produced by the incomplete combustion of carbonaceous fuels. It is used as a reductant for metal ores, for chemical synthesis, and as a fuel. As an environmental toxicant it is responsible for a significant number of accidental poisonings annually. Observable acute effects of carbon monoxide exposure in humans cover a wide range of symptoms and severity. These include impairment of judgement and visual perception at CO levels of 10 parts per million (ppm) in air; dizziness, headache, weariness (100 ppm); loss of consciousness (250 ppm); and rapid death (1,000 ppm). Chronic effects of long-term low-level exposure to carbon monoxide are not well known, but are suspected of including disorders of the respiratory system and the heart.[3]

Biochemical Action of Carbon Monoxide

Carbon monoxide enters the blood stream through the lungs and reacts with hemoglobin (Hb) as follows where O_2Hb is oxyhemoglobin and COHb is carboxyhemoglobin:

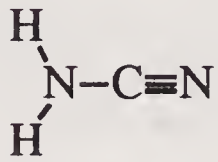
$$O_2 + CO \rightarrow COHb + O_2 \qquad (12.2.7)$$

Carboxyhemoglobin is several times more stable than oxyhemoglobin and ties up the hemoglobin so that it cannot carry oxygen to body tissues.

Cyanogen, Cyanamide, and Cyanates

Cyanogen, NCCN, is a colorless, violently flammable gas with a pungent odor. It may cause permanent injury or even death in exposed individuals. Fumes produced by the reaction of cyanogen with water or acids are highly toxic.

Cyanamide (shown here):



and calcium cyanamide (CaNCN) are used as fertilizers and raw materials. Calcium cyanamide is employed for the desulfurization and nitridation of steel. Inhalation or oral ingestion of cyanamide causes dizziness, lowers blood pressure, and increases rates of pulse and respiration.[4] Calcium cyanamide acts as a primary irritant to the skin and to nose and throat tissues.

Cyanic acid, HOCN, (bp 23.3°C, mp -86°C) is a dangerously explosive liquid with an acrid odor. The acid forms cyanate salts, such as NaOCN and KOCN. During decomposition from heat or contact with strong acid, cyanic acid evolves very toxic fumes.

12.3. NITROGEN OXIDES

The two most common oxides of nitrogen are **nitric oxide** (NO) and **nitrogen dioxide** (NO_2), designated collectively as NO_x. Nitric oxide is produced in combustion processes from organically bound nitrogen endogenous to fossil fuels (particularly coal, heavy fuel oil, and shale oil) and from atmospheric nitrogen under the conditions that exist in an internal combustion engine as shown by the two following reactions:

$$2N(\textit{fossil fuel}) + O_2 \rightarrow 2NO \qquad (12.3.1)$$

$$N_2 + O_2 \xrightarrow[\text{engine}]{\text{Internal combustion}} 2NO \qquad (12.3.2)$$

Under the conditions of photochemical smog formation[5] nitric oxide is converted to nitrogen dioxide by the following overall reaction:

$$2NO + O_2 \xrightarrow[\text{processes}]{\text{Organics, photochemical}} 2NO_2 \qquad (12.3.3)$$

This conversion consists of complex chain reactions involving light energy and unstable reactive intermediate species. The conditions required are stagnant air, low humidity, intense sunlight, and the presence of reactive hydrocarbons, particularly those from automobile exhausts. Of the NO_x constituents, NO_2 is generally regarded as the more toxic, although all nitrogen oxides and potential sources thereof (such as nitric acid in the presence of oxidizable organic matter) should be accorded the same respect as nitrogen dioxide.

Effects of NO_2 Poisoning

Inhalation of NO_2 causes severe irritation of the innermost parts of the lungs resulting in pulmonary edema and fatal bronchiolitis fibrosa obliterans. Inhalation for even very brief periods of time of air containing 200–700 ppm of NO_2 can be fatal. The biochemical action of NO_2 includes disruption of some enzyme systems, such as lactic dehydrogenase. Nitrogen dioxide probably acts as an oxidizing agent similar to, though weaker than, ozone, which is discussed in Section 12.4. Included is the formation of free radicals, particularly the hydroxyl radical HO·. Like ozone, it is likely that NO_2 causes **lipid peroxidation**. This is a process in which the C=C double bonds in unsaturated lipids are attacked by free radicals and undergo chain reactions in the presence of O_2, resulting in their oxidative destruction.[6]

Nitrous Oxide

Nitrous oxide, once commonly known as "laughing gas," is used as an oxidant gas and in dental surgery as a general anesthetic. It is a central nervous system depressant and can act as an asphyxiant.

12.4. OZONE

Ozone (O_3) is a reactive and toxic form of elemental oxygen. It is used as an oxidant for chemical synthesis and for the disinfection of water. In the latter application it avoids the production of potentially toxic organochlorine byproducts. Ozone is also used for the destruction of organic compounds responsible for odors from municipal wastewater treatment plants and industrial operations. For these purposes it is produced by an electrical discharge through dry air. The production of pollutant atmospheric ozone occurs under conditions of photochemical smog formation as discussed in the preceding section. Ozone is also produced from ultraviolet light passing through air by the reactions:

$$O_2 + h\nu \rightarrow O + O \tag{12.4.1}$$

$$O + O_2 + M \leftarrow\rightarrow O_3 + M \tag{12.4.2}$$

where $h\nu$ represents the energy of a photon of ultraviolet radiation and M is an energy-absorbing third body, usually a molecule of O_2 or N_2. The odor of ozone produced by any of these reactions can be detected around inadequately vented instruments, such as spectrofluorometers, that have intense ultraviolet sources.

Toxicity of Ozone

The toxicity of ozone has been summarized in a comprehensive work on the subject.[7] A deep lung irritant, ozone causes pulmonary edema, which can be fatal. It is also strongly irritating to the upper respiratory system and eyes and is largely responsible for the unpleasantness of photochemical smog. A level of 1 ppm of ozone in air has a distinct odor and inhalation of such air causes severe irritation and headache.

Like nitrogen dioxide (see Section 21.5) and ionizing radiation, ozone in the body produces free radicals that can be involved in destructive oxidation processes, such as lipid peroxidation or reaction with sulfhydryl (–SH) groups. Exposure to ozone can cause chromosomal damage. Radical-scavenging compounds, antioxidants, and compounds containing sulfhydryl groups can protect organisms from the effects of ozone.

12.5. THE HALOGENS

This section discusses the toxicities of the elemental **halogens** — fluorine, chlorine, bromine and iodine. As noted in Chapter 10, the elemental forms of the halogens are discussed here because they are chemically and toxicologically similar to many of their compounds, such as the interhalogen compounds. The toxicities of halogen compounds are discussed in the next two sections.

Chlorine

Elemental chlorine (Cl_2, mp -101°C, bp -34.5°C) is a greenish-yellow gas that is produced industrially in large quantities for numerous uses, such as the production of organochlorine solvents (see Chapter 11) and water disinfection. Liquified Cl_2 is shipped in large quantities in railway tank cars and human exposure to chlorine from transportation accidents is not uncommon.

Chlorine was the original poison gas used in World War I. It is a strong oxidant and reacts with water to produce an acidic oxidizing solution by the following reactions:

$$Cl_2 + H_2O \leftarrow\rightarrow HCl + HOCl \qquad (12.5.1)$$

$$Cl_2 + H_2O \leftarrow\rightarrow 2HCl + \{O\} \qquad (12.5.2)$$

where HOCl is oxidant hypochlorous acid and {O} stands for nascent oxygen (in a chemical sense regarded as freshly generated, highly reactive oxygen atoms). When chlorine reacts in the moist tissue lining the respiratory tract, the effect is quite damaging to the tissue. Levels of 10-20 ppm of chlorine gas in air cause immediate irritation to the respiratory tract and brief exposure to 1,000 ppm of Cl_2 can be fatal. Because of its intensely irritating properties, chlorine is not an insidious poison, and exposed individuals will rapidly seek to get away from the source if they are not immediately overcome by the gas.

Fluorine

Fluorine (F_2, mp -218°C, bp -187°C) is a pale yellow gas produced from calcium fluoride ore by first liberating hydrogen fluoride with sulfuric acid, then electrolyzing the HF in a 4:1 mixture with potassium fluoride, KF, as shown in the reaction:

$$2HF(\textit{molten KF}) \xrightarrow[\text{current}]{\text{Direct}} H_2(\textit{cathode}) + F_2(\textit{anode}) \qquad (12.5.3)$$

Of all the elements, fluorine is the most reactive and the most electronegative (a measure of tendency to acquire electrons).[8] In its chemically combined form it always has an oxidation number of -1. Fluorine has numerous industrial uses, such as the manufacture of UF_6, a gas used to enrich uranium in its fissionable isotope, uranium-235. Fluorine is used to manufacture uranium hexafluoride, SF_6, a dielectric material contained in some electrical and electronic apparatus. A number of organic compounds contain fluorine, particularly the chlorofluorocarbons used as refrigerants, and organofluorine polymers, such as DuPont's Teflon.

Given elemental fluorine's extreme chemical reactivity, it is not surprising that F_2 is quite toxic. It is classified as "a most toxic irritant." It strongly attacks skin and the mucous membranes of the nose and eyes.

Bromine

Bromine (Br_2, mp -7.3°C, bp 58.7°C) is a dark red liquid prepared commercially from elemental chlorine and bromide ion in bromide brines by the reaction:

$$Cl_2 + 2Br^- \rightarrow 2Cl^- + Br_2 \qquad (12.5.4)$$

and the elemental bromine product is swept from the reaction mixture with steam. The major use of elemental bromine is for the production of organobromine compounds such as 1,1-dibromoethane, formerly widely used as a grain and soil fumigant for insect control and as a component of leaded gasoline for scavenging lead from engine cylinders.

Bromine is toxic when inhaled or ingested. Like chlorine and fluorine, it is an irritant to the respiratory tract and eyes because it attacks their mucous membranes. Pulmonary edema may result from severe bromine poisoning. The severely irritating nature of bromine causes a withdrawal response in its presence, thereby limiting exposure.

Iodine

Elemental iodine (I_2, solid, sublimes at 184°C) consists of violet-black rhombic crystals with a lustrous metallic appearance. More irritating to the lungs than bromine or chlorine, its general effects are similar to these elements. Exposure to iodine is limited by its low vapor pressure compared to liquid bromine or gaseous chlorine or fluorine.

12.6. HYDROGEN HALIDES

The hydrogen halides are compounds with the general formula HX,where X is F, Cl, Br, or I. They are all gases and all are relatively toxic. Because of their abundance and industrial uses, HF and HCl have the greatest toxicological significance of these gases.

Hydrogen Fluoride

Hydrogen fluoride, (HF, mp -83.1°C, bp 19.5°C) may be in the form of either a clear, colorless liquid or gas. It forms corrosive fumes when exposed to the atmosphere. The major commercial application of hydrogen fluoride is as an alkyl-

ating catalyst in petroleum refining. Hydrogen fluoride in aqueous solution is called **hydrofluoric acid**, which contains 30–60% HF by mass. Hydrofluoric acid must be kept in plastic containers because it vigorously attacks glass and other materials containing silica (SiO_2), producing gaseous silicon tetrafluoride, SiF_4. Hydrofluoric acid is used to etch glass and clean stone

Both hydrogen fluoride and hydrofluoric acid, referred to collectively as HF, are extreme irritants to any tissue they contact. Exposed areas heal poorly, gangrene may develop, and ulcers can occur in affected areas of the upper respiratory tract.

The toxic nature of fluoride ion, F^-, is not confined to its presence in HF. It is toxic in soluble fluoride salts, such as NaF. At relatively low levels, such as about 1 ppm used in some drinking water supplies, fluoride prevents tooth decay. At excessive levels fluoride causes **fluorosis**, a condition characterized by bone abnormalities and mottled, soft teeth. Livestock is especially susceptible to poisoning from fluoride fallout on grazing land as a result of industrial pollution. In severe cases the animals become lame and even die.

Hydrogen Chloride

Hydrogen chloride (HCl, mp -114°C, bp -84.8°C) may be encountered as a gas, pressurized liquid, or aqueous solution called **hydrochloric acid**, commonly denoted simply as HCl. This compound is colorless in the pure state and in aqueous solution. Hydrochloric acid as a saturated solution containing 36% HCl is a major industrial chemical with U.S. production of about 2.3 million tons per year. This chemical is used for chemical and food manufacture, acid treatment of oil wells to increase crude oil flow, and metal processing.

Hydrogen chloride is not nearly as toxic as HF, although inhalation can cause spasms of the larynx as well as pulmonary edema and even death at high levels. Because of its high affinity for water, HCl vapor tends to dehydrate tissue of the eyes and respiratory tract. Hydrochloric acid is a natural physiological fluid found as a dilute solution in the stomachs of humans and other animals.

Hydrogen Bromide and Hydrogen Iodide

Hydrogen bromide (HBr, mp -87°C, bp -66.5°C) and **hydrogen iodide** (HI, mp -50.8°C, bp -35.4°C) are both pale yellow or colorless gases, although contamination by their respective elements tends to impart some color to these compounds. Both are very dense gases, 3.5 g/L for HBr and 5.7 g/L for HI at 0°C and atmospheric pressure. These compounds are used much less than HCl. Both are irritants to the skin and eyes and to the oral and respiratory mucous membranes.

12.7. INTERHALOGEN COMPOUNDS AND HALOGEN OXIDES

Halogens form compounds among themselves and with oxygen. Some of these compounds are important in industry and toxicologically. Some of the more important such compounds are discussed here.

Interhalogen Compounds

Fluorine is a sufficiently strong oxidant to oxidize chlorine, bromine, and iodine, whereas chlorine can oxidize bromine and iodine. The compounds thus formed are called **interhalogen compounds**. The major interhalogen compounds are listed in Table 12.1.

Table 12.1. The Major Interhalogen Compounds

Compound name and formula	Physical properties
Chlorine monofluoride, ClF	Colorless gas, mp -154°C, bp 101°C
Chlorine trifluoride, ClF_3	Colorless gas, mp -83°C, bp 12°C
Bromine monofluoride, BrF	Pale brown gas, bp 20°C
Bromine trifluoride, BrF_3	Colorless liquid, mp 8.8°C, bp 127°C
Bromine pentafluoride, BrF_3	Colorless liquid, mp -61.3°C, bp 40°C
Bromine monochloride, BrCl	Red/yellow highly unstable liquid and gas
Iodine trifluoride, IF_3	Yellow solid decomposing at 28°C
Iodine pentafluoride, IF_5	Colorless liquid, mp 9.4°C, bp 100 °C
Iodine heptafluoride, IF_7	Colorless sublimable solid, mp 5.5°C
Iodine monobromide, IBr	Gray sublimable solid, mp 42°C
Iodine monochloride, ICl	Red–brown solid alpha form, mp 27°C, bp 9°C
Iodine pentabromide, IBr_5	Crystalline solid
Iodine tribromide, IBr_3	Dark brown liquid
Iodine trichloride, ICl_3	Orange–yellow solid subliming at 64°C
Iodine pentachloride, ICl_5	- - -

The liquid interhalogen compounds are usually described as "fuming liquids." For the most part interhalogen compounds exhibit extreme reactivity. They react with water or steam to produce hydrohalic acid solutions (HF, HCl) and nascent oxygen {O}. They tend to be potent oxidizing agents for organic matter and oxidizable inorganic compounds. These chemical properties are reflected in the toxicities of the interhalogen compounds. Too reactive to enter biological systems in their original chemical state, they tend to be powerful corrosive irritants that acidify, oxidize, and dehydrate tissue. The skin, eyes and mucous membranes of the mouth, throat, and pulmonary systems are susceptible to attack by interhalogen compounds. In some respects the toxicities of the interhalogen compounds resemble the toxic properties of the elemental forms of the elements from which they are composed. The byproducts of chemical reactions of the interhalogen compounds — such as HF from fluorine compounds — pose additional toxicological hazards.

Halogen Oxides

The oxides of the halogens tend to be unstable and reactive. Although these compounds are called oxides, it is permissible to call the ones containing fluorine "fluorides" because fluorine is more electronegative than oxygen. The major halogen oxides are listed in Table 12.2. Commercially, the most important of the halogen oxides is chlorine dioxide, which offers some advantages over chlorine as a water disinfectant. It is also employed for odor control and bleaching wood pulp.[9] Because of its extreme instability, chlorine dioxide is manufactured on the site where it is used.

Table 12.2. Major Oxides of the Halogens

Compound name and formula	Physical properties
Fluorine monoxide, OF_2	Colorless gas, mp -224°C, bp -145°C
Chlorine monoxide, Cl_2O	Orange gas, mp -20°C, bp 2.2°C
Chlorine dioxide, ClO_2	Orange gas, mp -59°C, bp 9.9°C
Chlorine heptaoxide, Cl_2O_7	Colorless oil, mp -91.5°C, bp 82°C
Bromine monoxide, Br_2O	Brown solid, Decomp. -18°C
Bromine dioxide, BrO_2	Yellow solid, Decomp. 0°C
Iodine dioxide, IO_2	Yellow solid
Iodine pentoxide, I_2O_5	Colorless oil, Decomp. 325°C

For the most part, the halogen oxides are highly reactive toxic substances. Their toxicity and hazard characteristics are similar to those of the interhalogen compounds, which were described previously in this section.

Hypochlorous Acid and Hypochlorites

The halogens form several oxyacids and their corresponding salts. Of these, the most important is hypochlorous acid (HOCl) formed by the following reaction:

$$Cl_2 + H_2O \leftarrow\rightarrow HCl + HOCl \qquad (12.7.1)$$

Hypochlorous acid and hypochlorites are used for bleaching and disinfection. They produce active (nascent) oxygen ({O}) as shown by the reaction below, and the resulting oxidizing action is largely responsible for the toxicity of hypochloric acid and hypochlorites as irritants to eye, skin, and mucous membrane tissue.

$$HClO \rightarrow H^+ + Cl^- + \{O\} \qquad (12.7.2)$$

Perchlorates

Perchlorates are the most oxidized of the salts of the chloro oxyacids. Although perchlorates are not particularly toxic, ammonium perchlorate (NH_4ClO_4) should be mentioned because it is a powerful oxidizer and reactive chemical produced in large quantities as a fuel oxidizer in solid rocket fuels. Each of the U.S. space shuttle booster rockets contains about 350,000 kg of ammonium perchlorate in its propellant mixture. As of 1988, U.S. consumption of ammonium perchlorate for rocket fuel uses was of the order of 24 million kg/year. In May, 1988, a series of massive explosions in Henderson, Nevada, demolished one of only two plants producing ammonium perchlorate for the U.S. space shuttle, MX missile, and other applications, so that supplies were severely curtailed.[10]

The toxicological hazard of perchlorate salts depends upon the cation in the compound. In general, the salts should be considered as skin irritants and treated as such.

12.8. NITROGEN COMPOUNDS OF THE HALOGENS

Azides

The halogen azides are compounds with the general formula XN_3, where X is one of the halogens. These compounds are extremely reactive and can be spontaneously explosive. Their reactions with water can produce toxic fumes of the elemental halogen, acid (e.g., HCl), and NO_x. The compound vapors are irritants.

Nitrogen Halides

The general formula of the nitrogen halides is N_nX_x, where X is F, Cl, Br or I. A list of nitrogen halides is presented in Table 12.3.

Table 12.3. Nitrogen Halides

Compound name and formula	Physical properties
Nitrogen trifluoride, NF_3	Colorless gas, mp -209°C, bp -129°C
Nitrogen trichloride, NCl_3	Volatile yellow oil, melting below -40°C, boiling below 71°C, exploding around 90°C
Nitrogen tribromide, NBr_3	Solid crystals
Nitrogen triiodide, NI_3	Black crystalline explosive substance
Tetrafluorohydrazine, N_2F_4	—

The nitrogen halides are considered to be very toxic, largely as irritants to eyes, skin, and mucous membranes. Direct exposure to nitrogen halide compounds tends to be limited because of their reactivity, which may destroy the compound before exposure. Nitrogen triiodide is so reactive that even a "puff of air" can detonate it.[4]

Monochloramine and Dichloramine

The substitution of Cl for H on ammonia can be viewed as a means of forming nitrogen trichloride (Table 12.3), monochloramine, and dichloramine. The formation of the last two compounds from ammonium ion in water is shown by the following reactions:

$$NH_4^+ + HOCl \rightarrow H^+ + H_2O + NH_2Cl \qquad (12.8.1)$$

Monochloramine

$$NH_2Cl + HOCl \rightarrow H_2O + NHCl_2 \qquad (12.8.2)$$

The chloramines are disinfectants in water and are formed deliberately in the purification of drinking water to provide **combined available chlorine**. Although combined available chlorine is a weaker disinfectant than water containing Cl_2, HOCl, and OCl^-, it is retained longer in the water system for longer-lasting disinfection.

12.9. INORGANIC COMPOUNDS OF SILICON

Because of its use in semiconductors, silicon has emerged as a key element in modern technology. Concurrent with this phenomenon has been an awareness of the toxicity of silicon compounds, many of which, fortunately, have relatively low toxicities. This section covers the toxicological aspects of inorganic silicon compounds.

Silica

The silicon compound that has probably caused the most illness in humans is **silica**, SiO_2. Silica is a hard mineral substance known as quartz in the pure form and occurring in a variety of minerals such as sand, sandstone, and diatomaceous earth. Because of silica's occurrence in a large number of common materials that are widely used in construction, sand blasting, refractories manufacture, and many other industrial applications, human exposure to silica dust is widespread. Such exposure causes a condition called **silicosis**, a type of pulmonary fibrosis, one of the most common disabling conditions that result from industrial exposure to hazardous substances. Silicosis causes fibrosis and nodules in the lung, lowering lung capacity and making the subject more liable to pulmonary diseases, such as pneumonia. Severe cases of silicosis can cause death from insufficient oxygen or from heart failure.

Asbestos

Asbestos describes a group of silicate minerals, such as those of the serpentine group, approximate formula $Mg_3P(Si_2O_5)(OH)_4$, which occur as mineral fibers. Asbestos has many properties, such as insulating abilities and heat resistance, that have given it numerous uses. It has been used in structural materials, brake linings, insulation, and pipe manufacture. Unfortunately, inhalation of asbestos damages the lungs and results in a characteristic type of lung cancer in some exposed subjects. The three major pathological conditions caused by the inhalation of asbestos are asbestosis (a pneumonia condition), mesothelioma (tumor of the mesothelial tissue lining the chest cavity adjacent to the lungs), and bronchogenic carcinoma (cancer originating with the air passages in the lungs). Because of these health effects, uses of asbestos have been severely curtailed and widespread programs have been undertaken to remove asbestos from buildings.

Lung cancer from asbestos exposure has a strong synergistic relationship with exposure to cigarette smoke.[11] Long-term exposure to asbestos, alone, increases the incidence of lung cancer about 5-fold, cigarette smoking roughly 10-fold, but the two together more than 50-fold.

Silanes

Compounds of silicon with hydrogen are called **silanes**. The simplest of these is silane, SiH_4. Disilane is H_3SiSiH_3. Numerous organic silanes exist in which alkyl moieties are substituted for H.

In addition to SiH_4, the inorganic silanes produced for commercial use are dichloro- and trichlorosilane, SiH_2Cl_2 and $SiHCl_3$, respectively. These compounds are used as intermediates in the synthesis of organosilicon compounds and in the

production of high-purity silicon for semiconductors. Several kinds of inorganic compounds derived from silanes have potential uses in the manufacture of photovoltaic devices for the direct conversion of solar energy to electricity. In general, not much is known about the toxicities of silanes. Silane itself burns readily in air. Chlorosilanes are irritants to eye, nasal, and lung tissue.

Silicon Halides and Halohydrides

All four **silicon tetrahalides** with the general formula SiX_4 are known to exist. Of these, only silicon tetrachloride, $SiCl_4$, is produced in significant quantities. It is used to manufacture fumed silica (finely divided SiO_2). In addition, numerous **silicon halohydrides** with the general formula $H_{4-x}SiX_x$ have been synthesized. The commercially important compound of this type is trichlorosilane, $HSiCl_3$, which is used to manufacture organotrichlorosilanes and elemental silicon for semiconductors.

Both silicon tetrachloride and trichlorosilane are fuming liquids with suffocating odors. They both react with water to give off HCl vapor.

12.10. INORGANIC PHOSPHORUS COMPOUNDS

Phosphine

Phosphine (PH_3, mp -132°C, bp -88°C) is a colorless gas that undergoes autoignition at 100°C. It is used for the synthesis of organophosphorus compounds. Its inadvertent production in chemical syntheses involving other phosphorus compounds is a potential hazard in industrial processes and in the laboratory. Phosphine gas is very toxic and can be fatal. Symptoms of exposure include fatigue, vomiting, and difficult, painful breathing. Phosphine is a pulmonary tract irritant and central nervous system depressant.

Phosphorus Pentoxide

The oxide most commonly formed by the combustion of elemental white phosphorus and many phosphorus compounds is P_4O_{10}. As an item of commerce this compound is usually misnamed **phosphorus pentoxide**. When produced from the combustion of elemental phosphorus (see Reaction 10.6.1), it is a fluffy white powder that removes water from air to form syrupy orthophosphoric acid:

$$P_4O_{10} + 6H_2O \rightarrow 4H_3PO_4 \qquad (12.10.1)$$

Because of its dehydrating action and formation of acid, phosphorus pentoxide is a corrosive irritant to skin, eyes and mucous membranes.

Phosphorus Halides

Phosphorus forms halides with the general formulas PX_3 and PX_5. Typical of such compounds are phosphorus trifluoride (PF_3), a colorless gas (mp -152°C, bp -102°C), and phosphorus pentabromide (PBr_5) a yellow solid that decomposes at approximately 100°C. Of these compounds the most important commercially is phosphorus pentachloride used as a catalyst in organic synthesis, as a chlorinating

agent and as a raw material to make phosphorus oxychloride ($POCl_3$). Phosphorus halides react violently with water to produce the corresponding hydrogen halides and oxo phosphorus acids as shown by the following reaction of phosphorus pentachloride:

$$PCl_5 + 4H_2O \rightarrow H_3PO_4 + 5HCl \qquad (12.10.2)$$

Largely because of their acid-forming tendencies, the phosphorus halides are strong irritants to eyes, skin, and mucous membranes, and should be regarded as very toxic.

Phosphorus Oxyhalides

Phosphorus oxyhalides with the general formula POX_3 are known for fluoride, chloride, and bromide. Of these, the one with commercial uses is phosphorus oxychloride ($POCl_3$). Its uses are similar to those of phosphorus trichloride, acting in chemical synthesis as a chlorinating agent and for the production of organic chemical intermediates. It is a faintly yellow fuming liquid, mp 1°C, bp 105°C. It reacts with water to form hydrochloric acid and phosphonic acid (H_3PO_3). The liquid evolves toxic vapors and it is a strong irritant to the eyes, skin, and mucous membranes.

12.11. INORGANIC COMPOUNDS OF SULFUR

One of the elements essential for life, sulfur is a constituent of several of the more important toxic inorganic compounds. The common elemental form of yellow crystalline or powdered sulfur, S_8, has a low toxicity, although chronic inhalation of it can irritate mucous membranes.

Hydrogen Sulfide

Hydrogen sulfide (H_2S) is a colorless gas (mp -86°C, bp -61°C) with a foul rotten-egg odor. It is produced in large quantities as a byproduct of coal coking and petroleum refining and massive quantities are removed in the cleansing of sour natural gas. It is a major source of elemental sulfur by a process that involves oxidation of part of the H_2S to SO_2 followed by the Claus reaction:

$$2H_2S(g) + SO_2(g) \rightarrow 2H_2O(l) + 3S(s) \qquad (12.11.1)$$

Hydrogen sulfide is a very toxic substance, which in some cases can cause a fatal response more rapidly even than hydrogen cyanide. It affects the central nervous system, causing symptoms that include headache, dizziness, and excitement. Rapid death occurs at exposures to air containing more than about 1000 ppm H_2S, and somewhat lower exposures for about 30 minutes can be lethal. Death results from asphyxiation as a consequence of respiratory system paralysis. Accidental poisonings by hydrogen sulfide are not uncommon. In the most notorious such case, 22 people were killed in 1950 in Poza Rica, Mexico, when a flare used to "dispose" of hydrogen sulfide from natural gas by burning it to sulfur dioxide became extinguished, releasing large quantities of H_2S and asphyxiating victims as they slept. In 1975 at Denver City, Texas, nine people were killed from hydrogen sulfide blown out of a secondary petroleum recovery well. There are numerous effects of chronic H_2S poisoning, including general debility.

Sulfur Dioxide and Sulfites

Sulfur dioxide (SO_2) is an intermediate in the production of sulfuric acid. It is a common air pollutant produced by the combustion of pyrite (FeS_2) in coal and organically bound sulfur in coal and fuel oil as shown by the two following reactions:

$$4FeS_2 + 11O_2 \rightarrow 2Fe_2O_3 + 8SO_2 \qquad (12.11.2)$$

$$S(organic, in\ fuel) + O_2 \rightarrow SO_2 \qquad (12.11.3)$$

These sources add millions of tons of sulfur dioxide to the global atmosphere annually and are largely responsible for acid rain. Sulfur dioxide is an irritant to the eyes, skin, mucous membranes, and respiratory system. As a water-soluble gas, it is largely removed in the upper respiratory tract (see Section 8.2).

Dissolved in water, sulfur dioxide produces **sulfurous acid**, H_2SO_3; **hydrogen sulfite ion**, HSO_3^-; and **sulfite ion**, SO_3^{2-}. Sodium sulfite (Na_2SO_3) has been used as a chemical food preservative, although some individuals are hypersensitive to it.

Sulfuric Acid

Sulfuric acid is number one in synthetic chemical production. It is used to produce phosphate fertilizer, high octane gasoline, and a wide variety of inorganic and organic chemicals. Large quantities are consumed to pickle steel (cleaning and removal of surface oxides); disposal of spent pickling liquor can be a problem.

Sulfuric acid is of particular concern as an atmospheric pollutant. In times past, air polluted with unquestionably toxic levels of sulfuric acid aerosols (see Section 2.13), such as in severe air pollution that occurred in London and around various smelters in the 1950s and early 1960s, produced toxic effects and even fatalities. At present, sulfuric acid is a major contributor to acid precipitation (see Section 2.11), and it has been described at the most intense common irritant occurring in air polluted with acid substances.[12] Most of the sulfur that becomes atmospheric H_2SO_4 is emitted to the atmosphere as SO_2 from the burning of sulfur-containing fuels (particularly coal). Sulfur dioxide emissions are almost always accompanied by emissions of particulate matter, which often contains metals, such as vanadium, iron, and manganese. These metals can catalyze the oxidation of SO_2 to H_2SO_4,[12] either on particle surfaces or leached into aqueous solution in aerosol droplets:

$$SO_2 + {}^1/_2O_2 + H_2O \rightarrow H_2SO_4(aq) \qquad (12.11.4)$$

The result can be formation of an aerosol mist of droplets containing intensely irritating sulfuric acid.

Sulfuric acid is a severely corrosive poison and dehydrating agent in the concentrated liquid form. It readily penetrates skin to reach subcutaneous tissue and causes tissue necrosis with effects resembling those of severe thermal burns. Sulfuric acid fumes and mists can act as irritants to eye and respiratory tract tissue. Industrial exposure has caused tooth erosion in workers.

At lower levels, inhalation of sulfuric acid from sources such as atmospheric precipitation is damaging to the pulmonary tract. Compared to sulfur dioxide, sulfuric acid is the much more potent lung tissue irritant. Animal studies and limited data from exposed humans indicate that inhalation of H_2SO_4 aerosol increases airway resistance,

and inhibits bronchial clearance of inhaled particles. Asthmatic subjects are sensitive to sulfuric acid inhalation, and the effect may be synergistic with sulfur dioxide. Therefore, particularly for sensitive individuals, exposure to air containing sulfuric acid, sulfur dioxide, and particles — all of which tend to occur together when one is present in a polluted atmosphere — may be particularly damaging to the lungs.

Miscellaneous Inorganic Sulfur Compounds

A large number of inorganic sulfur compounds including halides and salts are widely used in industry. The more important of these are listed in Table 12.4.

Table 12.4. Inorganic Sulfur Compounds

Compound name and formula	Properties
Sulfur	
Monofluoride, S_2F_2	Colorless gas, mp -104°C, bp -99°C, toxicity similar to HF
Tetrafluoride, SF_4	Gas, bp -40°C, mp -124°C, powerful irritant
Hexafluoride, SF_6	Colorless gas, mp -51°C, surprisingly nontoxic when pure, but often contaminated with toxic lower fluorides
Monochloride, S_2Cl_2	Oily, fuming orange liquid, mp -80°C, bp 138°C, strong irritant to eyes, skin, and lungs
Tetrachloride, SCl_4	Brownish/yellow liquid/gas, mp -30°C, Decom. below 0°C, irritant
Trioxide, SO_3	Solid anhydride of sulfuric acid (see toxic effects above), reacts with moisture or steam to produce sulfuric acid
Sulfuryl chloride, SO_2Cl_2	Colorless liquid, mp -54°C, bp 69°C, used for organic synthesis, corrosive toxic irritant
Thionyl chloride, $SOCl_2$	Colorless-to-orange fuming liquid, mp -105°C, bp 79°C, toxic corrosive irritant
Carbon oxysulfide, COS[1]	Volatile liquid byproduct of natural gas or petroleum refining, toxic narcotic
Carbon disulfide, CS_2[1]	Colorless liquid, industrial chemical, narcotic and CNS anesthetic

[1] Carbon oxysulfide and carbon disulfide may also be classed as organic compounds, and their toxicological properties are discussed with organosulfur compounds in Chapter 17.

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QUESTIONS AND PROBLEMS

1. What are the two main toxic forms of cyanide? Which of these is most dangerous by inhalation? Which by ingestion?

2. What is a common natural source of cyanide? How is this form converted to toxic cyanide ion in the body? How did the Romans use this substance?

3. In what sense does cyanide deprive the body of oxygen? How does this differ from the way that methane gas or nitrogen gas deprive the body of oxygen, or the way in which carbon monoxide does so?

4. What is the biochemical action of carbon monoxide? What is the receptor with which carbon monoxide reacts? In what sense is this reaction reversible?

5. In general, how does NO_x enter the atmosphere? How does the more toxic form of NO_x form in the atmosphere?

6. Which organ is most affected by exposure to NO_2? What are the toxic effects, including "bronchiolitis fibrosa obliterans?" In general, what is the biochemical action of NO_2 and how does it involve free radical and lipid peroxidation?

7. What is the major toxic effect of nitrous oxide, N_2O? What might lead you to believe that it is much less toxic than NO_2?

8. What are the major toxicological effects of ozone? What kinds of groups does it attack in the body?

9. What is the main kind of reaction of chlorine in water? In what sense is this reaction tied to chlorine toxicity?
10. What may be said about the toxicity of fluorine compared to that of chlorine? Why is toxic exposure to bromine and iodine usually less of a problem than that to fluorine or chlorine?
11. Of the hydrogen halides, which is the most dangerous? How does this substance occur? What does it do to the body?
12. What are the nature and symptoms of fluorosis? How can this condition result from air pollution?
13. What kind of compound is ClF_3? What kind of compound is ClO_2? What are their chemical and toxicological similarities?
14. What kinds of compounds are NF_3 and NCl_3? What may be said about their chemical properties? What is their major toxicological effect?
15. What would lead you to believe that monochloramine, and dichloramine are not regarded as very toxic, at least in an unconcentrated form? How are these compounds used? How are they related to "combined available chlorine?"
16. What is the chemical nature of silica? What is its major toxicological effect? What are the symptoms of this toxic effect?
17. In addition to silica, there is another silicon-containing mineral that is toxic. What is this mineral? What are its toxic effects? How are its toxic effects synergistic with cigarette smoke?
18. What are silanes? How are they used? Is much known about their toxicities? What are the toxic effects of chlorosilanes?
19. What is the most commonly produced silicon tetrahalide? How is it used? Why might its toxicological properties be similar to those of HCl?
20. Why is PH_3 a particular hazard in the laboratory and in industrial chemical synthesis? Is it very toxic? What are its major toxic effects?
21. What role may be played by particulate matter and by metals, such as vanadium, iron, and manganese, in the production of toxic sulfuric acid?
22. What is the chemical nature of P_4O_{10}? What does it form when exposed to atmospheric moisture? What are its major toxicological effects? Suggest a sequence of reactions by which H_3PO_4 might be formed from PH_3.
23. What is PCl_5? How is it used? How does it react with water, and how is this reaction related to its toxic properties? What are some compounds that are related to PCl_5?
24. What is the most commonly used phosphorus oxyhalide, general formula POX_3? What are its industrial uses? How does it react with water, and how is this reaction related to the fact that it is a strong irritant to the eyes, skin, and mucous membranes?
25. In addition to the "miscellaneous" inorganic sulfur compounds listed in Table 12.3, three sulfur compounds were discussed separately for their toxicities. Of these, which is the most toxic? What is its mode of toxicity?

26. Why does exposure to fatal doses of H_2S still occur? What are some specific incidents in which such exposure has occurred?

27. What is SO_2 like chemically? What does it form in water? Why does it contribute to acid precipitation? In what sense is it less effective as a constituent of acid precipitation than is H_2SO_4?

28. Explain how the following reactions may lead to the occurrence of a major toxic air pollutant:

$$4FeS_2 + 11O_2 \rightarrow 2Fe_2O_3 + 8SO_2$$

$$S(\textit{organic, in fuel}) + O_2 \rightarrow SO_2$$

$$SO_2 + {}^1/_2O_2 + H_2O \rightarrow H_2SO_4(aq)$$

29. What are the major toxic effects of sulfuric acid? How is exposure to sulfuric likely to occur?

13

Toxic Organic Compounds and Hydrocarbons

13.1. INTRODUCTION

The fundamentals of organic chemistry are reviewed in Chapter 1. The present chapter is the first of seven that discuss the toxicological chemistry of organic compounds that are largely of synthetic origin. Since the vast majority of the several million known chemical compounds are organic — most of them toxic to a greater or lesser degree — the toxicological chemistry of organic compounds covers an enormous area. Specifically, this chapter discusses hydrocarbons, which are organic compounds composed only of carbon and hydrogen and are in a sense the simplest of the organic compounds. Hydrocarbons occur naturally in petroleum, natural gas, and tar sands and they can be produced by pyrolysis of coal and oil shale or by chemical synthesis from H_2 and CO.

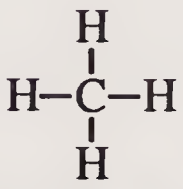
13.2. CLASSIFICATION OF HYDROCARBONS

For purposes of discussion of hydrocarbon toxicities in this chapter, hydrocarbons will be grouped into the five categories of (1) **alkanes**, (2) **unsaturated non-aromatic** hydrocarbons, (3) **aromatic** hydrocarbons (understood to have only one or two linked aromatic rings in their structures), (4) **polycyclic** aromatic hydrocarbons with multiple rings, and (5) **mixed** hydrocarbons containing combinations of two or more of the preceding types. These classifications are summarized in Figure 13.1.

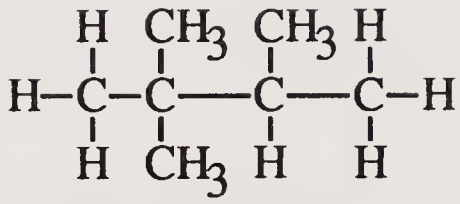
Alkanes

Alkanes, also called **paraffins** or **aliphatic hydrocarbons**, are hydrocarbons in which the C atoms are joined by single covalent bonds (sigma bonds) consisting of two shared electrons (see Section 2.4). As shown by the examples in Figure 13.1 and Chapter 2, Figure 2.5, alkanes may exist as straight chains or branched chains. They may also exist as cyclic structures, for example, as in cyclohexane (C_6H_{12}). Each cyclohexane molecule consists of 6 carbon atoms (each with 2 H atoms attached) in a ring. The general molecular formula for straight- and branched-chain alkanes is C_nH_{2n+2}, and that of a cyclic alkane is C_nH_{2n}. The names of alkanes having from 1 to 10 carbon atoms per molecule are (1) methane, (2) ethane, (3) propane, (4) butane, (5) pentane, (6) hexane, (7) heptane, (8) octane, (9) nonane, and (10) decane.[1] These names may be prefixed by "*n*–" to denote a straight-chain alkane. The same base names are used to designate substituent groups on molecules; for example, a straight-chain 4-carbon alkane group (derived from butane) attached by an end carbon to a molecule is designated as an *n*-butyl group.

Alkanes

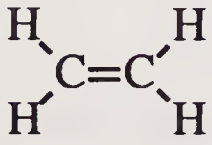


Methane

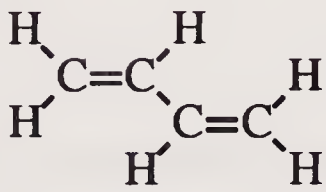


2,2,3,-Trimethylbutane

Unsaturated nonaromatic



Ethylene

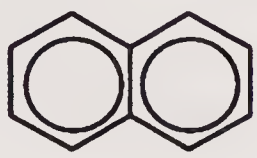


1,3-Butadiene

Acetylene

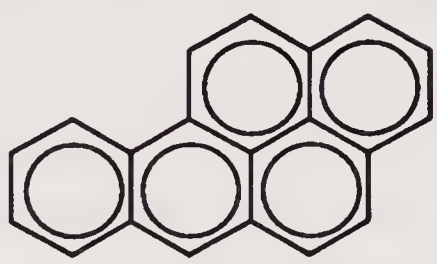
One/two-ring aromatic

Benzene



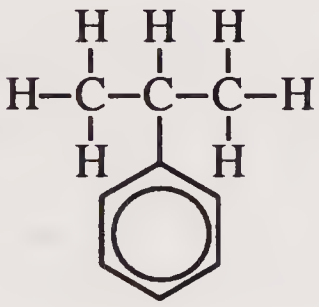
Naphthalene

Polycyclic aromatic

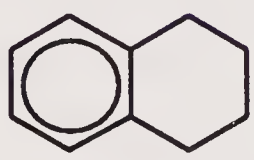


Benzo(a)pyrene

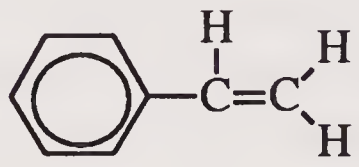
Mixed hydrocarbons



Cumene
(1-methylethylbenzene)



Tetralin
(1,2,3,4-tetrahydronaphthalene)



Styrene

Figure 13.1. Hydrocarbons classified for discussion of their toxicological chemistry.

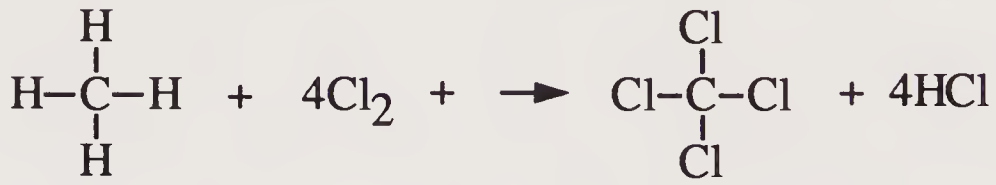
Alkanes undergo a number of chemical reactions, two classes of which should be mentioned here. The first of these is **oxidation** with molecular oxygen in air as shown for the following combustion reaction of propane:

$$C_3H_8 + 5O_2 \rightarrow 3CO_2 + 4H_2O + \text{heat} \qquad (13.2.1)$$

Such reactions can pose flammability and explosion hazards. Another hazard occurs during combustion in an oxygen-deficient atmosphere or in an automobile engine, in which significant quantities of toxic carbon monoxide (CO) are produced.

The second major type of alkane reaction that should be considered here consists of **substitution reactions** in which one or more H atoms on an alkane are replaced by atoms of another element. Most commonly the H is replaced by a halogen, usually chlorine, to yield **organohalide** compounds; when chlorine is the substituent the

product is called an **organochlorine** compound. An example of this kind of reaction is that of methane with chlorine to give carbon tetrachloride, Reaction 13.2.2. Organohalide compounds are of great toxicological significance and are discussed in Chapter 16.

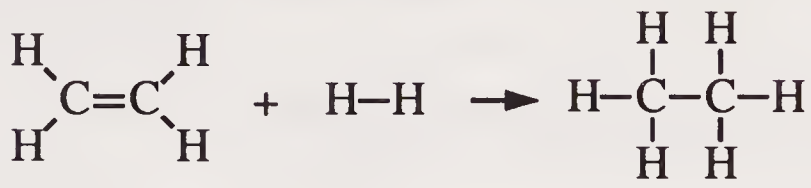


(13.2.2)

Unsaturated Nonaromatic Hydrocarbons

Unsaturated hydrocarbons are those that have multiple bonds, each involving more than 2 shared electrons, between carbon atoms. Such compounds are usually **alkenes** or **olefins** that have double bonds consisting of 4 shared electrons as shown for ethylene and 1,3-butadiene in Figure 13.1. Triple bonds consisting of 6 shared electrons are also possible, as illustrated by acetylene in the same figure.

Alkenes may undergo **addition reactions** in which pairs of atoms are added across unsaturated bonds as shown in the following reaction of ethylene with hydrogen to give ethane:



(13.2.3)

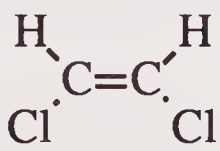
This kind of reaction, which is not possible with alkanes, adds to the chemical and metabolic, as well as toxicological, versatility of compounds containing unsaturated bonds.

Another example of an addition reaction is that of a molecule of HCl gas to one of acetylene to yield vinyl chloride:

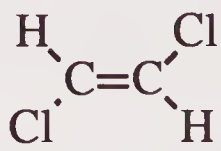
(13.2.4)

The vinyl chloride product is the monomer used to manufacture polyvinylchloride plastic and is a carcinogen known to cause a rare form of liver cancer among exposed workers.

As discussed in Chapter 2, Section 2.6, compounds with double bonds can exist as geometrical isomers exemplified by the two isomers of 1,2-dichloroethylene in Figure 13.2. Although both of these compounds have the molecular formula $C_2H_2Cl_2$, the orientations of their H and Cl atoms relative to each other are different and their properties, such as melting and boiling points, are not the same. Their toxicities are both relatively low, but significantly different. The *cis-* isomer is an irritant and narcotic known to damage the liver and kidneys of experimental animals. The *trans-* isomer causes weakness, tremor and cramps due to its effects on the central nervous system, as well as nausea and vomiting resulting from adverse effects on the gastrointestinal tract.



Cis –1,2–dichloroethylene,
mp -80.5°C, bp 59°C



Trans –1,2–dichloroethylene,
mp -50°C, bp 48°C

Figure 13.2. The two geometrical isomers of 1,2–dichloroethane.

Aromatic Hydrocarbons

Aromatic compounds were discussed briefly in Chapter 1, Section 1.7. The characteristics of **aromaticity** of organic compounds are numerous and are discussed at length in works on organic chemistry. These include a low hydrogen:carbon atomic ratio; C–C bonds that are quite strong and of intermediate length between such bonds in alkanes and those in alkenes; tendency to undergo substitution reactions (see Reaction 13.2.2) rather than the addition reactions characteristic of alkenes; and delocalization of π electrons over several carbon atoms resulting in resonance stabilization of the molecule. For more detailed explanations of these concepts the reader is referred to standard textbooks on organic chemistry. For purposes of discussion here, most of the aromatic compounds discussed are those that contain single benzene rings or fused benzene rings, such as those in naphthalene or benzo(a)pyrene, shown in Figure 13.1.

An example reaction of aromatic compounds with considerable environmental and toxicological significance is the chlorination of biphenyl. Biphenyl gets its name from the fact that it consists of two **phenyl** groups (where a phenyl group is a benzene molecule less a hydrogen atom) joined by a single covalent bond. In the presence of an iron(II) chloride catalyst this compound reacts with chlorine to form a number of different molecules of polychlorinated biphenyls (PCBs), as shown in Figure 13.3. These environmentally persistent compounds are discussed in Chapter 11.

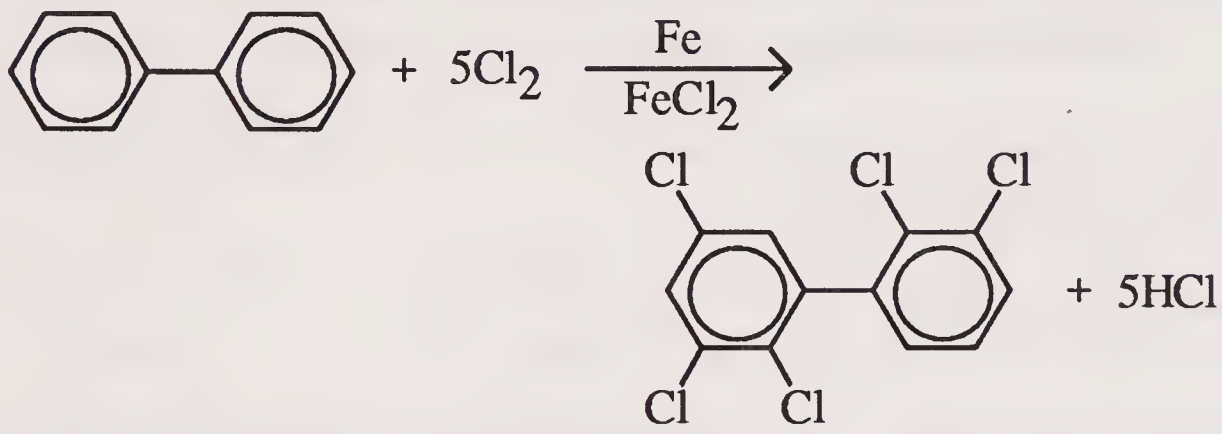


Figure 13.3. An example of a substitution reaction of an aromatic hydrocarbon compound (biphenyl) to produce an organochlorine product (2,3,5,2',3',- pentachlorobiphenyl, a PCB compound). The product is one of 210 possible congeners of PCBs, widespread and persistent pollutants found in the fat tissue of most humans and of considerable environmental and toxicological concern.

13.3. TOXICOLOGY OF ALKANES

Worker exposure to alkanes, especially the lower-molecular-mass compounds, is most likely to come from inhalation. In an effort to set reasonable values for the exposure by inhalation of vapors of solvents, hydrocarbons, and other volatile organic liquids, the American Conference of Governmental Industrial Hygienists sets **threshold limit values** (TLVs) for airborne toxicants.[2] The **time-weighted average exposure** (E) is calculated by the formula:

$$E = \frac{C_a T_a + C_b T_b + \cdots + C_n T_n}{8} \qquad (13.3.1)$$

where C is the concentration of the substance in the air for a particular time T (hours), such as a level of 3.1 parts per million by volume for 1.25 hours. The 8 in the denom-

inator is for an 8-hour day. In addition to exposures calculated by this equation, there are maximum exposures that should not be exceeded at any time, and there may be a relatively high exposure level that may be approached, but not exceeded, for brief periods of time, such as 10 minutes once each day.

"Safe" levels of air contaminants are difficult to set based upon systemic toxicologic effects. Therefore, TLV values often reflect nonsystemic effects of odor, narcosis, eye irritation, and skin irritation. Because of this, comparison of TLV values is often not useful in comparing systemic toxicological effects of chemicals in the workplace.

Methane and Ethane

Methane and ethane are **simple asphyxiants**, which means that air containing high levels of these gases does not contain sufficient oxygen to support respiration.[3] Table 13.1 shows the levels of asphyxiants in air at which various effects are observed in humans. Simple asphyxiant gases are not known to have major systemic toxicological effects, although subtle effects that are hard to detect should be considered as possibilities.

Table 13.1. Effects of Simple Asphyxiants in Air

Percent asphyxiant*	Percent oxygen, O_2*	Effect on humans
0 – 33	21 – 14	No major adverse symptoms
33 – 50	14 – 10.5	Discernible effects beginning with air hunger and progressing to impaired mental alertness and muscular coordination
50 – 75	10.5 – 5.3	Fatigue, depression of all sensations, faulty judgment, emotional instability; in later phases nausea, vomiting, prostration, unconsciousness, convulsions, coma, death
75 – 100	5.3 – 0	Death within a few minutes

* Percent by volume on a "dry" (water vapor-free) basis

Propane and Butane

Propane has the formula C_3H_8 and butane C_4H_8. There are two isomers of butane — *n*-butane and isobutane (2-methylpropane). Propane and the butane isomers are gases at room temperature and atmospheric pressure; like methane and ethane, all three are asphyxiants. A high concentration of propane affects the central nervous system. There are essentially no known systemic toxicological effects of the two butane isomers; behavior similar to that of propane might be expected.

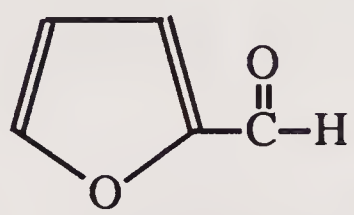
Pentane through Octane

The alkanes with 5 through 8 carbon atoms consist of *n*-alkanes, and there is an increasing number of branched-chain isomers with higher numbers of C atoms per molecule. For example, there are 9 isomers of heptane C_7H_{16}. These compounds are

all volatile liquids under ambient conditions; the boiling points for the straight-chain isomers range from 36.1°C for *n*-pentane to 125.8°C for *n*-octane. In addition to their uses in fuels, such as in gasoline, these compounds are employed as solvents in formulations for a number of commercial products, including varnishes, glues, and inks. They are also used for the extraction of fats.

Once regarded as toxicologically almost harmless, the C_5–C_8 aliphatic hydrocarbons are now recognized as having some significant toxic effects. Exposure to the C_5–C_8 hydrocarbons is primarily via the pulmonary route, and high levels in air have killed experimental animals. Humans inhaling high levels of these hydrocarbons have become dizzy and have lost coordination as a result of central nervous system depression.

Of the C_5–C_8 alkanes, the one most commonly used for nonfuel purposes is *n*-hexane. It acts as a solvent for the extraction of oils from seeds, such as cotton seed and sunflower seed. This alkane serves as a solvent medium for several important polymerization processes and in mixtures with more polar solvents, such as furfural:



Furfural

for the separation of fatty acids.[4] **Polyneuropathy** (multiple disorders of the nervous system) has been reported in several cases of human exposure to *n*-hexane,[5,6] such as Japanese workers involved in the home production of sandals using a glue with *n*-hexane solvent. The workers suffered from muscle weakness and impaired sensory function of the hands and feet. Biopsy examination of nerves in leg muscles of the exposed workers showed loss of myelin (a fatty substance constituting a sheath around certain nerve fibers) and degeneration of axons (part of a nerve cell through which nerve impulses are transferred out of the cell). The symptoms of polyneuropathy were reversible, with recovery taking several years after exposure was ended.

Exposure of the skin to C_5–C_8 liquids causes dermatitis. This is the most common toxicological occupational problem associated with the use of hydrocarbon liquids in the workplace and is a consequence of the dissolution of the fat portions of the skin. In addition to becoming inflamed, the skin becomes dry and scaly.

Alkanes above Octane

Alkanes higher than C_8 are contained in kerosene, jet fuel, diesel fuel, mineral oil, and fuel oil. Kerosene, also called fuel oil No. 1, is a mixture of primarily C_8–C_{16} hydrocarbons, predominantly alkanes with a boiling point range of approximately 175–325°C. Diesel fuel is called fuel oil No. 2. The heavier fuel oils No. 3–6 are characterized by increasing viscosity, darker color and higher boiling temperatures with increasing fuel oil number. Mineral oil is a carefully selected fraction of petroleum hydrocarbons with density ranges of 0.83–0.86 g/mL for light mineral oil and 0.875–0.905 g/mL for heavy mineral oil.

The higher alkanes are not regarded as very toxic, although there are some reservations about their toxicities. Inhalation is the most common route of occupational exposure and can result in dizziness, headache, and stupor. In cases of extreme exposure, coma and death have occurred. Inhalation of mists or aspiration of vomitus containing higher alkane liquids has caused a condition known as aspiration pneumonia. Mineral oils have been reported to be carcinogenic to the skin and scrotum.[7]

Solid and Semisolid Alkanes

Semisolid petroleum jelly is a highly refined product commonly known as "vaseline," a mixture of predominantly C_{16}–C_{19} alkanes. Carefully controlled refining processes are used to remove nitrogen and sulfur compounds, resins, and unsaturated hydrocarbons. Paraffin wax is a similar product behaving as a solid. Neither petroleum jelly nor paraffin is digested or absorbed by the body.

Cyclohexane

Cyclohexane, the 6-carbon ring hydrocarbon with the molecular formula C_6H_{12}, is the most significant of the cyclic alkanes. Under ambient conditions it is a clear, volatile, highly flammable liquid. It is manufactured by the hydrogenation of benzene and is used primarily as a raw material for the synthesis of cyclohexanol and cyclohexanone through a liquid-phase oxidation with air in the presence of a dissolved cobalt catalyst.

Like *n*-hexane, cyclohexane has a toxicity rating of 3, moderately toxic (see Table 1.1 for toxicity ratings). Cyclohexane acts as a weak anesthetic similar to, but more potent than, *n*-hexane. Systemic effects have not been shown in humans.

13.4. TOXICOLOGY OF UNSATURATED NONAROMATIC HYDROCARBONS

Ethylene (structure in Figure 13.1) is the most widely used organic chemical. Almost all of it is consumed as a chemical feedstock for the manufacture of other organic chemicals. Polymerization of ethylene to produce polyethylene is illustrated in Figure 13.4. In addition to polyethylene, other polymeric plastics, elastomers, fibers, and resins are manufactured with ethylene as one of the ingredients. Ethylene is also the raw material for the manufacture of ethylene glycol antifreeze, solvents, plasticizers, surfactants, and coatings.

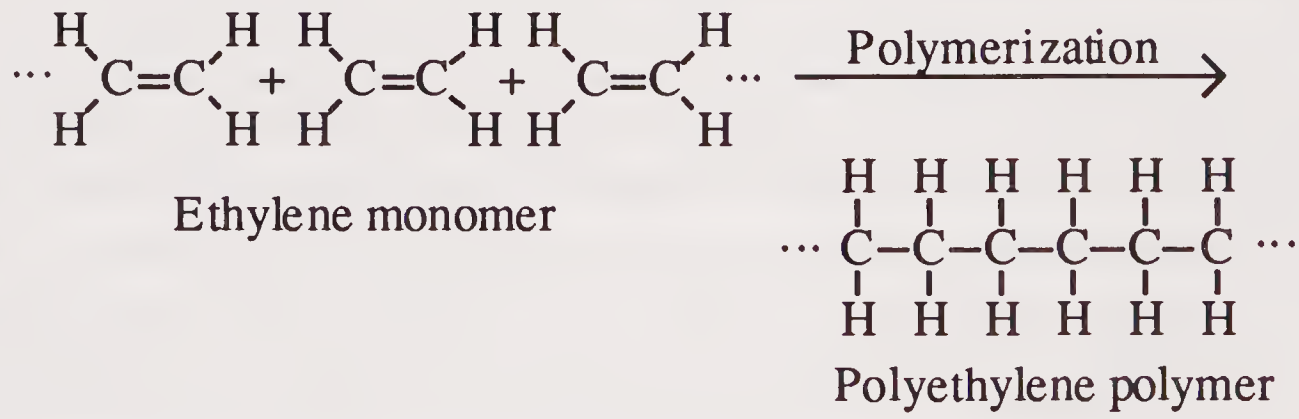
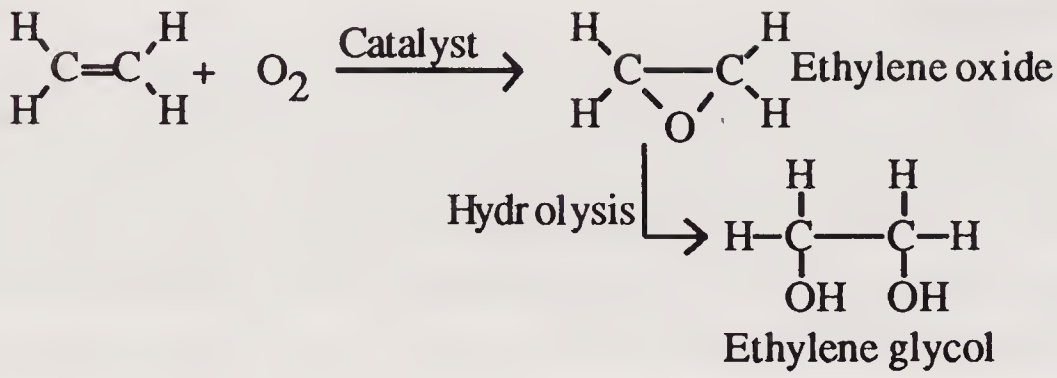
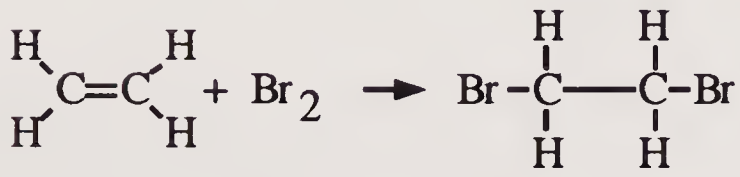


Figure 13.4. Polymerization of ethylene to produce polyethylene.

The boiling point of ethylene is -105°C and under ambient conditions it is a colorless gas. It has a somewhat sweet odor, is highly flammable and forms explosive mixtures with air. Because of its double bond (unsaturation), ethylene is much more active than the alkanes. It undergoes addition reactions as shown in the following examples to form a number of important products:

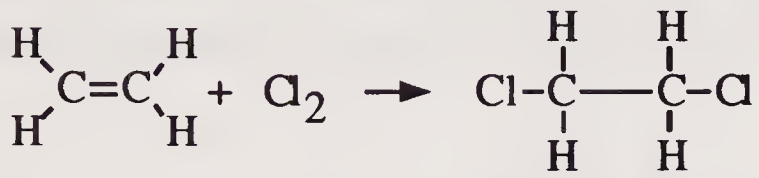


(13.4.1)



(13.4.2)

1,2-Dibromoethane
(ethylene dibromide)



(13.4.3)

1,2–Dichloroethane
(ethylene dichloride)

(13.4.4)

Chloroethane
(ethyl chloride)

The products of the addition reactions shown above are all commercially, toxicologically, and environmentally important. Ethylene oxide is a highly reactive colorless gas used as a sterilizing agent, fumigant, and intermediate in the manufacture of ethylene glycol and surfactants. It is an irritant to eyes and pulmonary tract mucous membrane tissue; inhalation of it can cause pulmonary edema. Ethylene glycol is a colorless, somewhat viscous liquid used in mixtures with water as a high-boiling, low-freezing-temperature liquid (antifreeze and antiboil) in cooling systems. Ingestion of this compound causes central nervous system effects characterized by initial stimulation followed by depression. Higher doses can cause fatal kidney failure due to metabolic oxidation of ethylene glycol to oxalic acid followed by the formation of insoluble calcium oxalate which clogs the kidneys, as discussed in Section 14.2.

Ethylene dibromide has been used as an insecticidal fumigant and additive to scavenge lead from leaded gasoline combustion. During the early 1980s there was considerable concern about residues of this compound in food products and it was suspected of being a carcinogen, mutagen, and teratogen. Ethylene dichloride (bp 83.5°C) is a colorless, volatile liquid with a pleasant odor used as a soil and foodstuff fumigant. It has a number of toxicological effects, including adverse effects on the eye, liver, and kidneys and a narcotic effect on the central nervous system. Ethyl chloride seems to have similar, but much less severe, toxic effects.

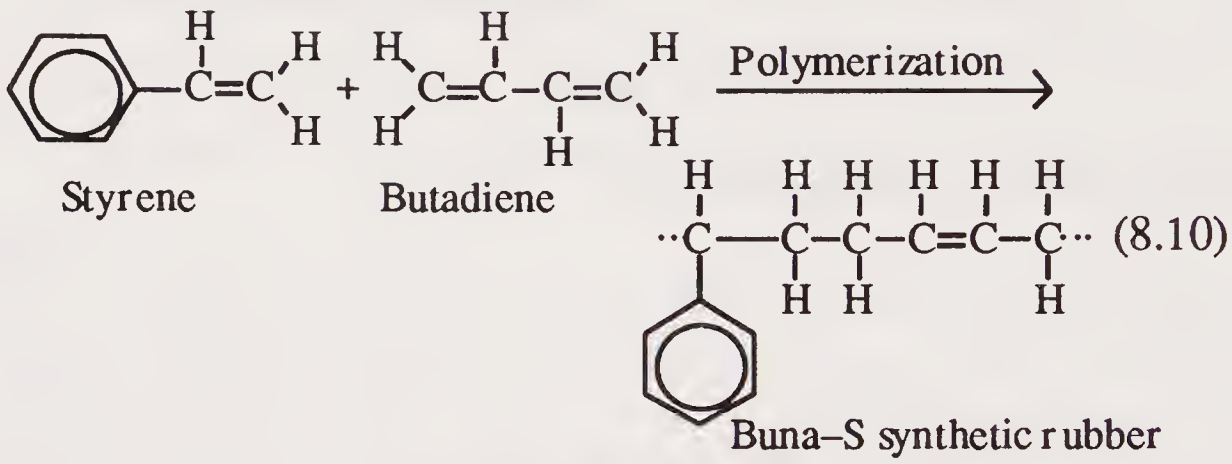
Ethylene, itself, is not very toxic to animals but it is a simple asphyxiant (see Section 13.3 and Table 13.1). At high concentrations it acts as an anesthetic to induce unconsciousness. A highly flammable compound, ethylene forms dangerously explosive mixtures with air. It is phytotoxic (toxic to plants).

Propylene

Propylene (C_3H_6) is a gas with chemical, physical and toxicological properties very similar to those of ethylene. It, too, is a simple asphyxiant. Its major use is in the manufacture of polypropylene polymer, a hard, strong plastic from which are made injection-molded bottles as well as pipes, valves, battery cases, automobile body parts, and rot-resistant indoor-outdoor carpet.

1,3-Butadiene

The dialkene 1,3-butadiene is widely used in the manufacture of polymers, particularly synthetic rubber. The first synthetic rubber to be manufactured on a large scale and used as a substitute for unavailable natural rubber during World War II was a styrene-butadiene polymer.



(8.10)

Butadiene is a colorless gas under ambient conditions with a mild, somewhat aromatic odor. At lower levels the vapor is an irritant to eyes and respiratory system mucous membranes and at higher levels it can cause unconsciousness and even death. The compound boils at –4.5°C, and is readily stored and handled as a liquid. Release of the liquid can cause frostbite-like burns on exposed flesh.

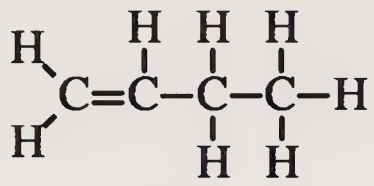
Butylenes

There are four monoalkenes with the formula C_4H_8 (butylenes) as shown in Figure 13.5. All gases under ambient conditions, these compounds have boiling points ranging from -6.9°C for isobutylene to +3.8°C for *cis*-2-butene. The butylenes readily undergo isomerization (change to other isomers). They participate in addition reactions and form polymers. Their major hazard is extreme flammability. Though not regarded as particularly toxic, they are asphyxiants and have a narcotic effect when inhaled.

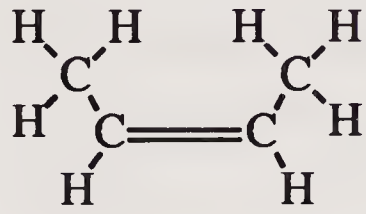
Alpha-Olefins

Alpha-olefins are linear alkenes with double bonds between carbons 1 and 2 in the general range of carbon chain length C_6 through about C_{18}. They are used for numerous purposes. The C_6–C_8 compounds are used as comonomers to manufacture modified polyethylene polymer and the C_{12}–C_{18} alpha-olefins are used as raw materials in the manufacture of detergents. The compounds are also used to manufacture lubricants and plasticizers. In 1986 worldwide consumption of the alpha-olefins was estimated at 800,000 metric tons estimated to rise to 1.7 million metric tons by the

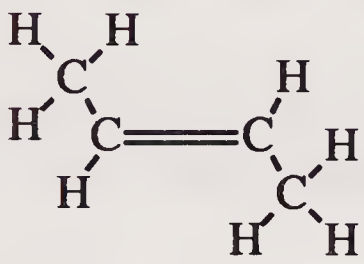
year 2000.[8] With such large quantities involved, due consideration needs to be given to the toxicological and occupational health aspects of these compounds.



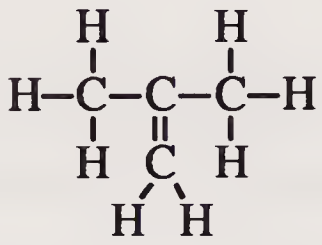
1–Butene



Cis –2–butene



Trans –2–butene

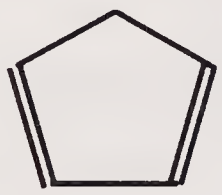


Isobutylene (methylpropene)

Figure 13.5. The four butylene compounds, formula C_4H_8.

Cyclopentadiene and Dicyclopentadiene

The cyclic dialkene cyclopentadiene has the structural formula shown below:



Cyclopentadiene

Two molecules of cyclopentadiene readily and spontaneously join together to produce dicyclopentadiene, widely used to produce polymeric elastomers, polyhalogenated flame retardants and polychlorinated pesticides. Dicyclopentadiene (mp 32.9°C, bp 166.6°C) exists as colorless crystals. It is an irritant and has narcotic effects. It is considered to have a high oral toxicity and to be moderately toxic through dermal absorption.

Acetylene

Acetylene (Figure 13.1) is widely used as a chemical raw material and fuel for oxyacetylene torches. It was once the principal raw material for the manufacture of vinyl chloride (see Reaction 13.2.4), but other synthetic routes are now used. Acetylene is a colorless gas with an odor resembling garlic. Though not notably toxic, it acts as an asphyxiant and narcotic and has been used for anesthesia. Exposure can cause headache, dizziness, and gastric disturbances. Some adverse effects from exposure to acetylene may be due to the presence of impurities in the commercial product.

13.5. BENZENE AND ITS DERIVATIVES

Figure 13.6 shows the structural formulas of benzene and its major hydrocarbon derivatives. These compounds are very significant in chemical synthesis, as solvents, and in unleaded gasoline formulations.

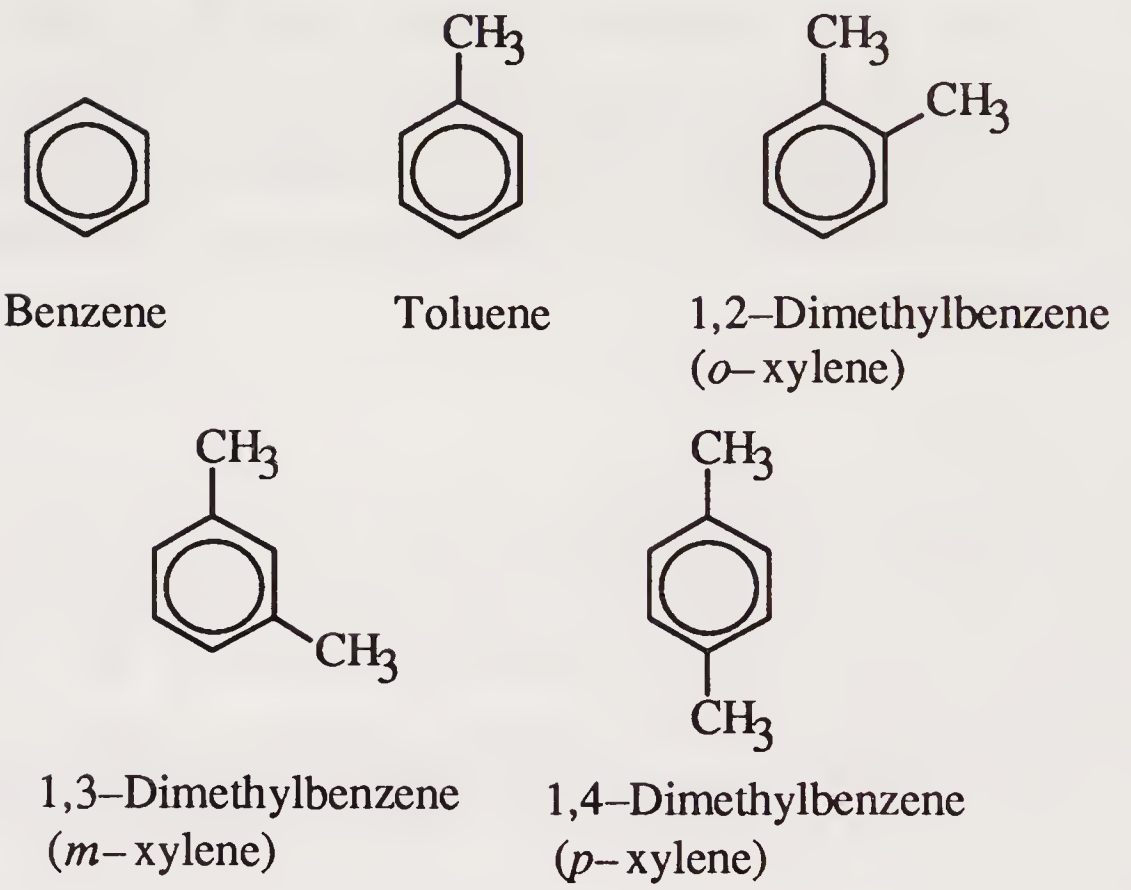


Figure 13.6. Benzene and its most common methyl-substituted hydrocarbon derivatives.

Benzene

Benzene (C_6H_6) is chemically the single most significant hydrocarbon. It is used as a starting material for the manufacture of numerous products including phenolic and polyester resins, polystyrene plastics and elastomers (through intermediate styrene, Figure 13.1), alkylbenzene surfactants, chlorobenzene compounds, insecticides, and dyes. Benzene (bp 80.1°C) is a volatile, colorless, highly flammable liquid with a characteristic odor.

Acute Toxic Effects of Benzene

Benzene has been in commercial use for over a century, and toxic effects of it have been suspected since about 1900.[9] Benzene has both acute and chronic toxicological effects.[10] It is usually absorbed as a vapor through the respiratory tract, although absorption of liquid through the skin and intake through the gastrointestinal tract are also possible. Benzene is a skin irritant, and progressively higher local exposures can cause skin redness (erythema), burning sensations, fluid accumulation (edema) and blistering. Inhalation of air containing about 64 g/m^3 of benzene can be fatal within a few minutes; about 1/10 that level of benzene causes acute poisoning within an hour, including a narcotic effect upon the central nervous system manifested progressively by excitation, depression, respiratory system failure and death.

Chronic Toxic Effects of Benzene

Of greater overall concern than the acute effects of benzene exposure are chronic effects, which are still subject to intense study. As with many other toxicants, subjects suffering from chronic benzene exposure suffer nonspecific symptoms, including fatigue, headache, and appetite loss. More specifically, blood abnormalities appear in people suffering chronic benzene poisoning. The most common of these is a lowered white cell count. More detailed examination may show an abnormal increase in blood

lymphocytes (colorless corpuscles introduced to the blood from the lymph glands), anemia, and decrease in the number of blood platelets required for clotting (thrombocytopenia). Some of the observed blood abnormalities may result from damage by benzene to bone marrow. Because of concerns that long-term exposure to benzene may cause preleukemia, leukemia, or cancer, the allowable levels of benzene in the workplace have been greatly reduced and substitutes such as toluene and xylene are used wherever possible.

Metabolism of Benzene

For a hydrocarbon, the water solubility of benzene is a moderately high 1.80 g/L at 25°C. The vapor is readily absorbed by blood, from which it is strongly taken up by fatty tissues. For nonmetabolized benzene, the process is reversible and benzene is excreted through the lungs. Benzene metabolism occurs in the liver where it undergoes a phase I oxidation reaction to phenol[11] as shown in Figure 13.7.

All monocyclic aromatic hydrocarbons with 6-membered rings other than benzene have substituent groups (such as the methyl group in toluene) upon which phase I reactions may be initiated. However, the oxidative metabolism of benzene requires attachment of oxygen to the aromatic ring as shown in Figure 13.7. This is probably responsible for the unique toxicity of benzene, especially in respect to bone marrow damage. The epoxide likely reacts with cell nucleophiles, damaging or destroying the cells.

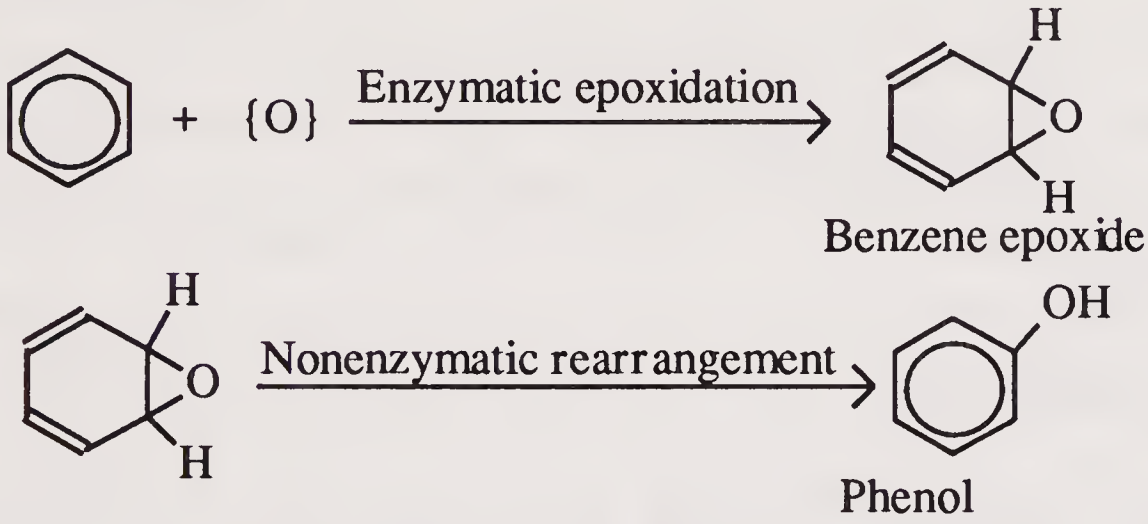


Figure 13.7. Conversion of benzene to phenol in the body.

A phase II conjugation reaction then occurs that converts phenol to water-soluble glucuronide or sulfate, either of which is readily eliminated through the kidneys[12] as illustrated in Figures 13.8 and 13.9 (next page).

Toluene

Toluene is a colorless liquid boiling at 101.4°C. It is classified as moderately toxic through inhalation or ingestion and has a low toxicity by dermal exposure. Concentrations in ambient air up to 200 ppm usually do not result in significant symptoms, but exposure to 500 ppm may cause headache, nausea, lassitude, and impaired coordination without detectable physiological effects. At massive exposure levels, toluene acts as a narcotic, which can lead to coma.

Unlike benzene, toluene possesses an aliphatic sidechain that can be oxidized enzymatically, leading to products that are readily excreted from the body. The metabolism of toluene is thought to proceed via oxidation of the methyl group and formation of the conjugate compound hippuric acid, as shown in Figure 13.10.

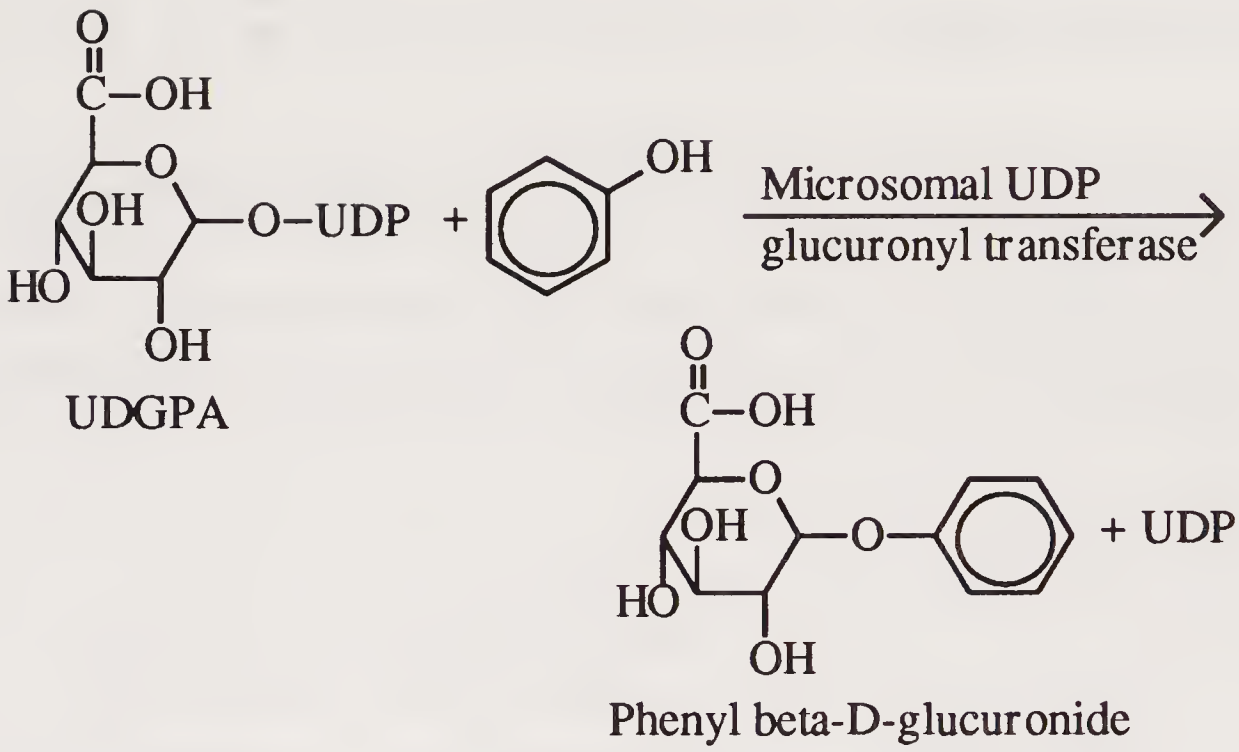


Figure 13.8. Formation of a glucuronide of phenol. The abbreviation UDPGA stands for uridine diphosphate glucuronic acid and UDP for uridine diphosphate.

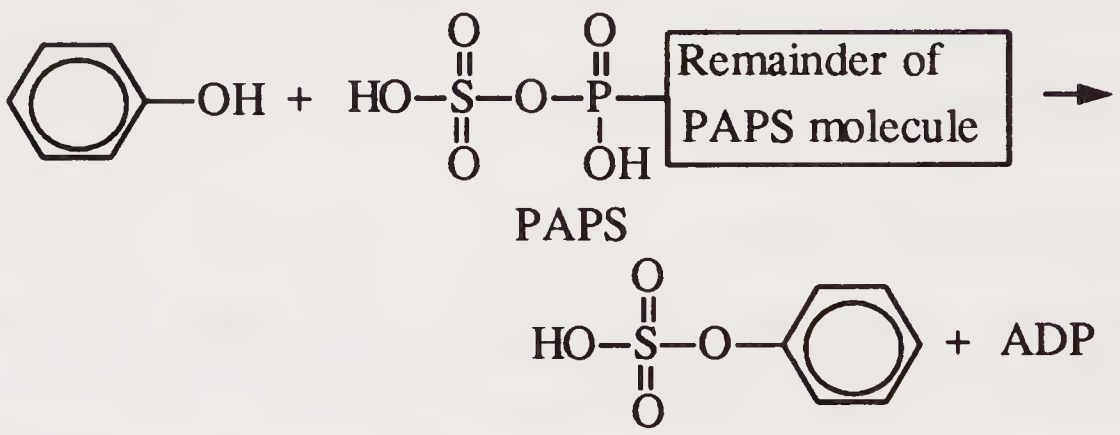


Figure 13.9. Addition reaction for the formation of excretable phenyl sulfate. The abbreviation PAPS is for adenosine 3'-phosphate-5'-phosphosulfate, a somewhat complex structure. ADP is adenosine diphosphate.

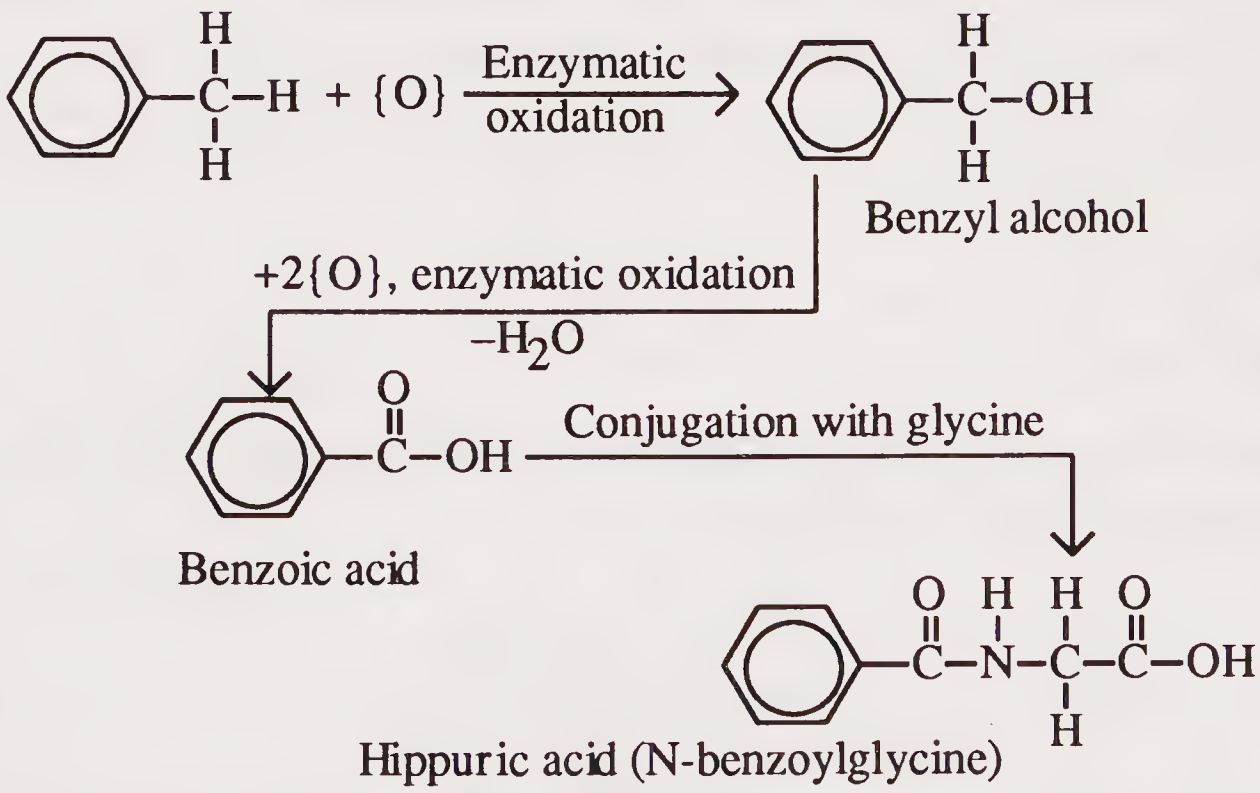


Figure 13.10. Metabolic oxidation of toluene with conjugation to hippuric acid, which is excreted with urine.

13.6. NAPHTHALENE

Naphthalene, also known as tar camphor, and its alkyl derivatives, such as 1-(2-propyl)naphthalene (Figure 13.11), are important industrial chemicals. Used to make mothballs, naphthalene is a volatile white crystalline solid with a characteristic odor. Coal tar and petroleum are the major sources of naphthalene. Numerous industrial chemical derivatives are manufactured from it. The most important of these is phthalic anhydride (Figure 13.11), used to make phthalic acid plasticizers, which are discussed in Chapter 14.

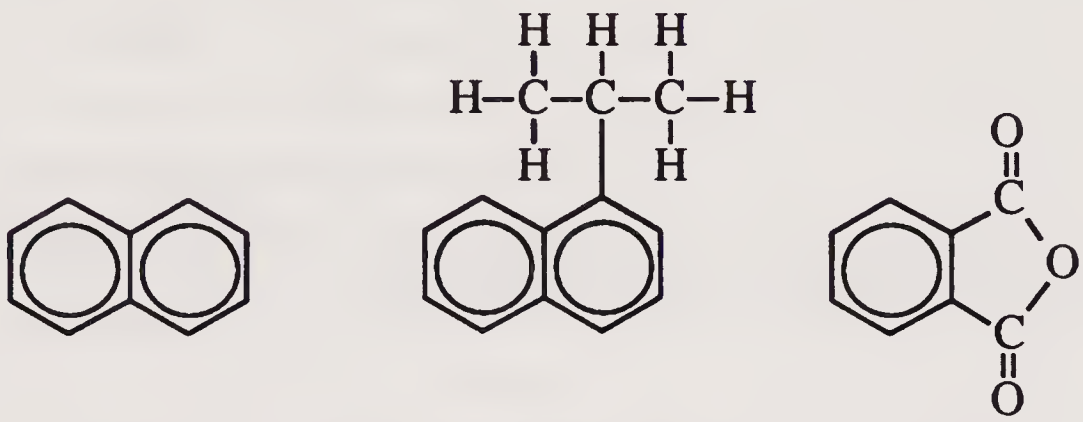
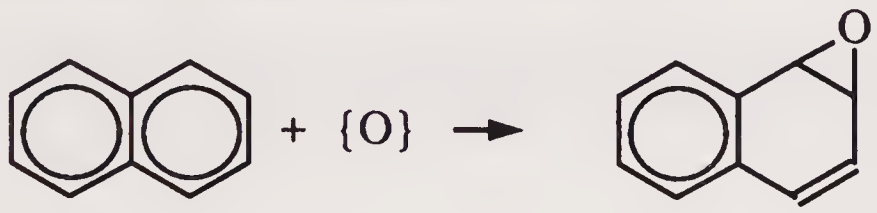


Figure 13.11. Naphthalene and two of its derivatives.

Metabolism of Naphthalene

The metabolism of naphthalene is similar to that of benzene, starting with an enzymatic epoxidation of the aromatic ring:

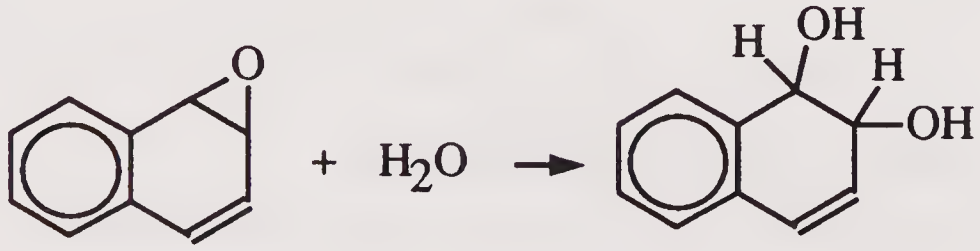


(13.6.1)

followed by a nonenzymatic rearrangement to 1-naphthol:

(13.6.2)

or addition of water to produce naphthalene-1,2-dihydrodiol through the action of epoxide hydrase enzyme:



(13.6.3)

Elimination of the metabolized naphthalene from the body may occur as a mercapturic acid, preceded by the glutathione S-transferase-catalyzed formation of a glutathione conjugate.

Toxic Effects of Naphthalene

Exposure to naphthalene can cause a severe hemolytic crisis in some individuals with a genetically linked metabolic defect associated with insufficient activity of the glucose-6-phosphate dehydrogenase enzyme in red blood cells.[13] Effects include anemia and marked reductions in red cell count, hemoglobin, and hematocrit. Contact of naphthalene with skin can result in skin irritation or severe dermatitis in sensitized individuals. In addition to the hemolytic effects just noted, both inhalation and ingestion of naphthalene can cause headaches, confusion, and vomiting. Kidney failure is usually the ultimate cause of death in cases of fatal poisonings.

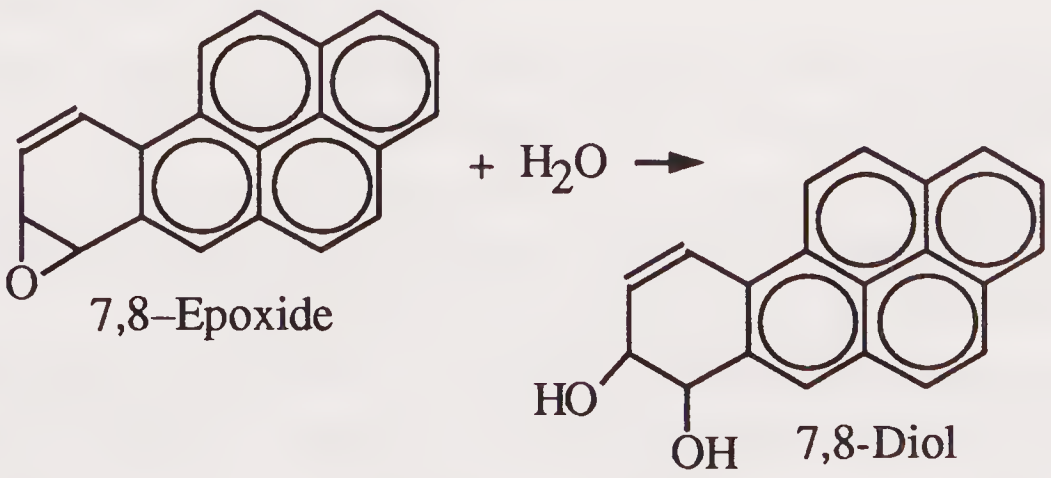
13.7. POLYCYCLIC AROMATIC HYDROCARBONS

Benzo(a)pyrene (Figure 13.1) is the most studied of the polycyclic aromatic hydrocarbons (PAHs). These compounds are formed by the incomplete combustion of other hydrocarbons so that hydrogen is consumed in the preferential formation of H_2O. The condensed aromatic ring system of the PAH compounds produced is the thermodynamically favored form of the hydrogen-deficient, carbon-rich residue. To cite an extreme example, the H:C ratio in methane (CH_4) is 4:1, whereas in benzo(a)pyrene ($C_{20}H_{12}$) it is only 3:5.

There are many conditions of partial combustion and pyrolysis that favor production of PAH compounds, and they are encountered abundantly in the atmosphere, soil, and elsewhere in the environment. Sources of PAH compounds include engine exhausts, wood stove smoke, cigarette smoke and charbroiled food. Coal tars and petroleum residues have high levels of PAHs.

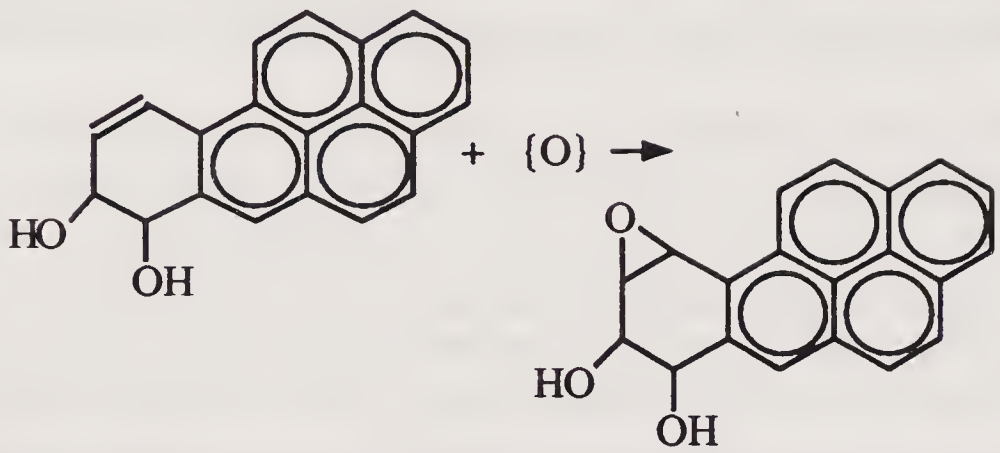
PAH Metabolism

The metabolism of PAH compounds is mentioned here with benzo(a)pyrene as an example. Several steps lead to the formation of the carcinogenic metabolite product of benzo(a)pyrene.[11] After an initial oxidation to form the 7,8-epoxide, the 7,8-diol is produced through the action of epoxide hydrase enzyme as shown by the following reaction:



7,8–Epoxide 7,8-Diol (13.7.1)

The microsomal mixed-function oxidase enzyme system further oxidizes the diol to the carcinogenic 7,8-diol-9,10-epoxide:



(13.7.2)

7,8–Diol–9,10–epoxide of benzo(a)pyrene

Toxicology of PAH Compounds

The most notable toxicologic characteristic of PAH compounds is that some of their metabolites are known to cause cancer. The most studied of these is the 7,8-diol-9,10 epoxide of benzo(a)pyrene shown above. There are two stereoisomers of this metabolite, both of which are known to be potent mutagens.

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QUESTIONS AND PROBLEMS

1. Using compounds other than those shown in Figure 13.1, give examples of each of the following kinds of hydrocarbons: (1) alkanes, (2) unsaturated nonaromatic hydrocarbons, (3) aromatic hydrocarbons, (4) polycyclic aromatic hydrocarbons with multiple rings, and (5) mixed hydrocarbons.

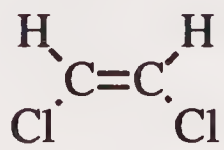
2. What kind of carbon-carbon bond characterizes alkanes? What kind of carbon-carbon bond characterizes other types of hydrocarbons?

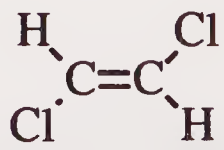
3. Give examples of hydrocarbons having the following general formulas: C_nH_{2n+2}, C_nH_{2n}, C_nH_n, C_nH_x, where x is a number less than n.

4. What are the two most important reactions of alkanes? What kind of additional reaction is possible with alkenes? What may the latter have to do with the toxicological chemistry of alkenes?

5. What kind of reaction is shown by the reaction below. What is the organic reactant? What is the product? What is the special toxicological significance of the product?

6. What structural phenomenon may be shown by the following formulas? What is its toxicological significance?





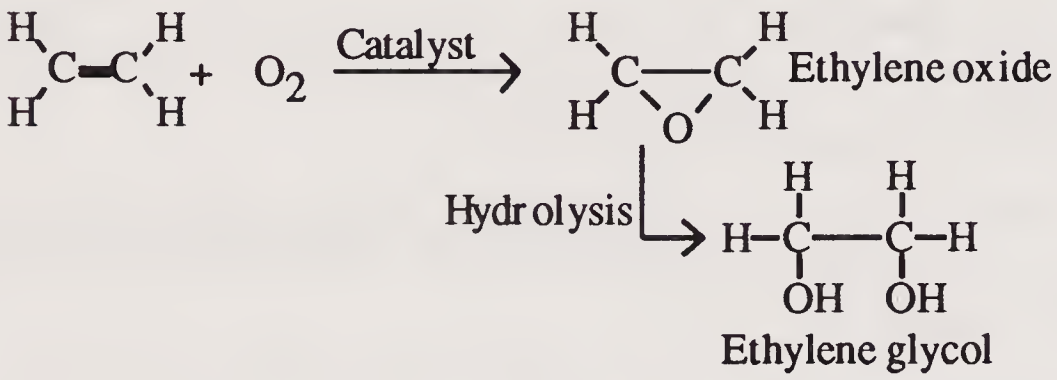
7. Describe the special characteristics of aromaticity.

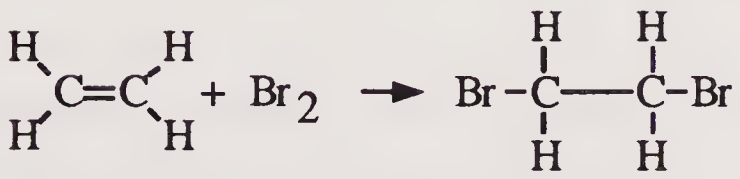
8. Explain the significance of the following formula:

$$E = \frac{C_aT_a + C_bT_b + \cdots + C_nT_n}{8}$$

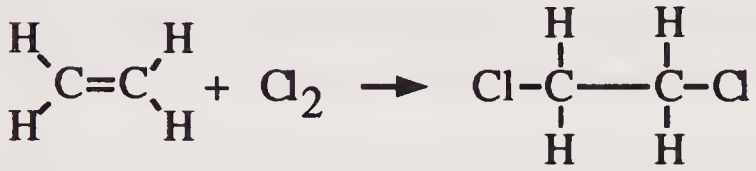
9. What is the main toxicological characteristic of low-molecular-mass alkanes? What condition may be caused by exposure to somewhat higher-molecular-mass alkanes, such as *n*-hexane? How is this condition caused?

10. What is the toxicological significance of the products of each of the following reactions?





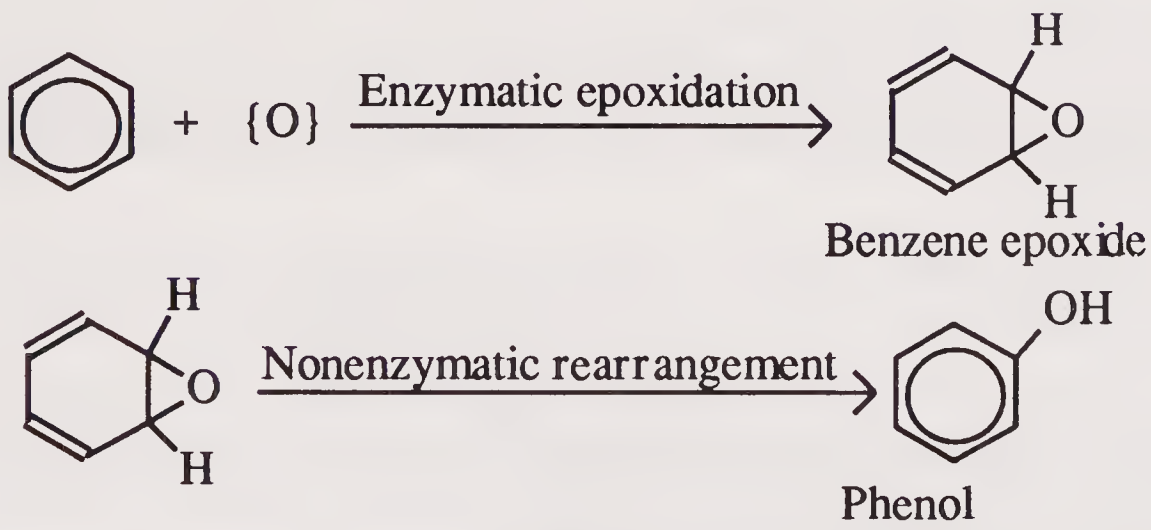
1,2-Dibromoethane
(ethylene dibromide)



1,2–Dichloroethane
(ethylene dichloride)

Chloroethane
(ethyl chloride)

11. Consider the following reactions:



Discuss these reactions in terms of their significance for benzene toxicity and toxicological chemistry, phase I reactions, phase II reactions, and other aspects pertinent to benzene's effects on the body.

12. What is the formula of acetylene? What are its main toxicological effects?

13. What are the major acute toxicological effects of benzene? How does benzene exposure usually occur? How does benzene affect the central nervous system? At what levels of exposure are the acute toxicological effects manifested?

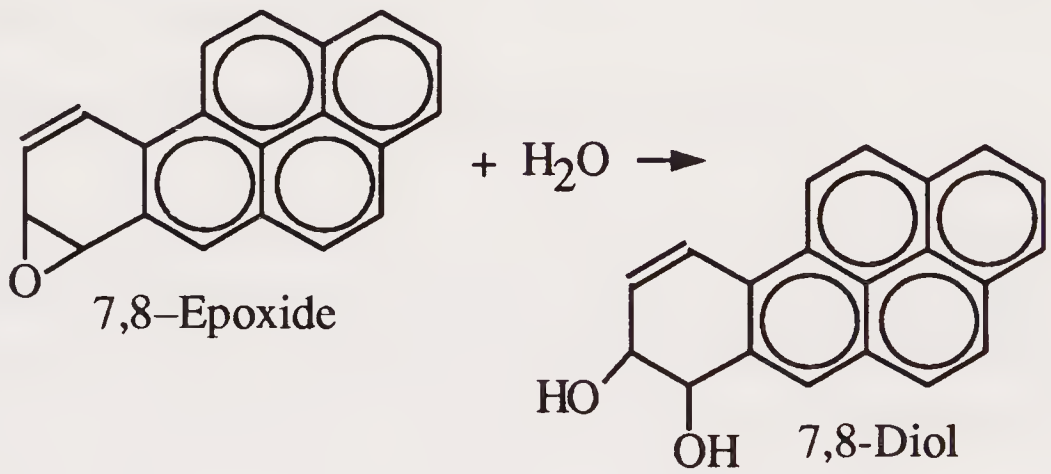
14. What are the chronic toxicological effects of benzene? What kinds of blood abnormalities are caused by benzene exposure? How does benzene toxicity affect white cell count? How does it affect bone marrow?

15. What may be said about the vapor pressure and water solubilities of benzene as they influence its toxicity?

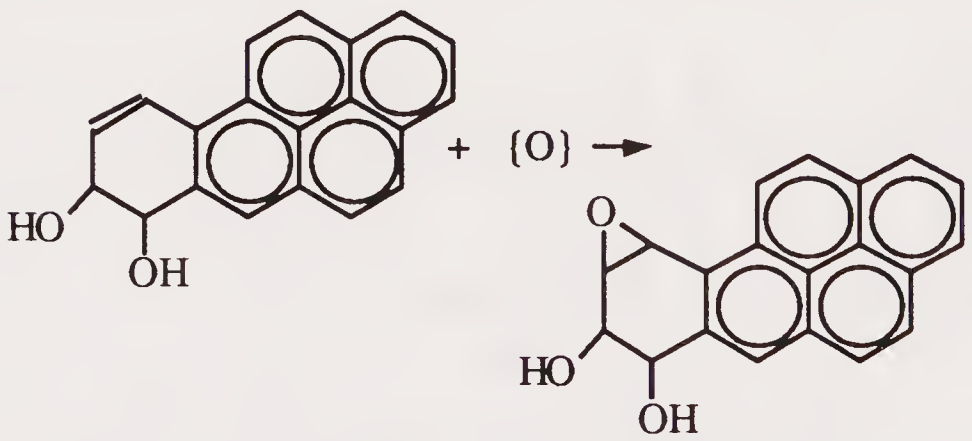
16. In what important respects are the toxicological chemistry and toxicity of toluene quite different from those of benzene? How is hippuric acid formed from toluene?

17. What are the major toxicological chemical and toxicological aspects of naphthalene?

18. Discuss what the following shows about the toxicological chemistry and toxicity of some important polycyclic aromatic hydrocarbons:



7,8–Epoxide 7,8-Diol



7,8–Diol–9,10–epoxide of benzo(a)pyrene

14

Oxygen-Containing Organic Compounds

14.1. INTRODUCTION

A very large number of organic compounds and natural products, many of which are toxic, contain oxygen in their structures. This chapter concentrates on organic compounds that have oxygen covalently bonded to carbon. Organic compounds in which oxygen is bonded to nitrogen, sulfur, phosphorus, and the halogens are discussed in Chapters 15-18.

Oxygen-Containing Functional Groups

As shown in Table 2.1 and Figure 14.1, there are several kinds of oxygen-containing functional groups in organic compounds. In general, the organo-oxygen compounds can be classified according to the degree of oxygenation, location of oxygen on the hydrocarbon moiety, presence of unsaturated bonds in the hydrocarbon structure, and presence or absence of aromatic rings.

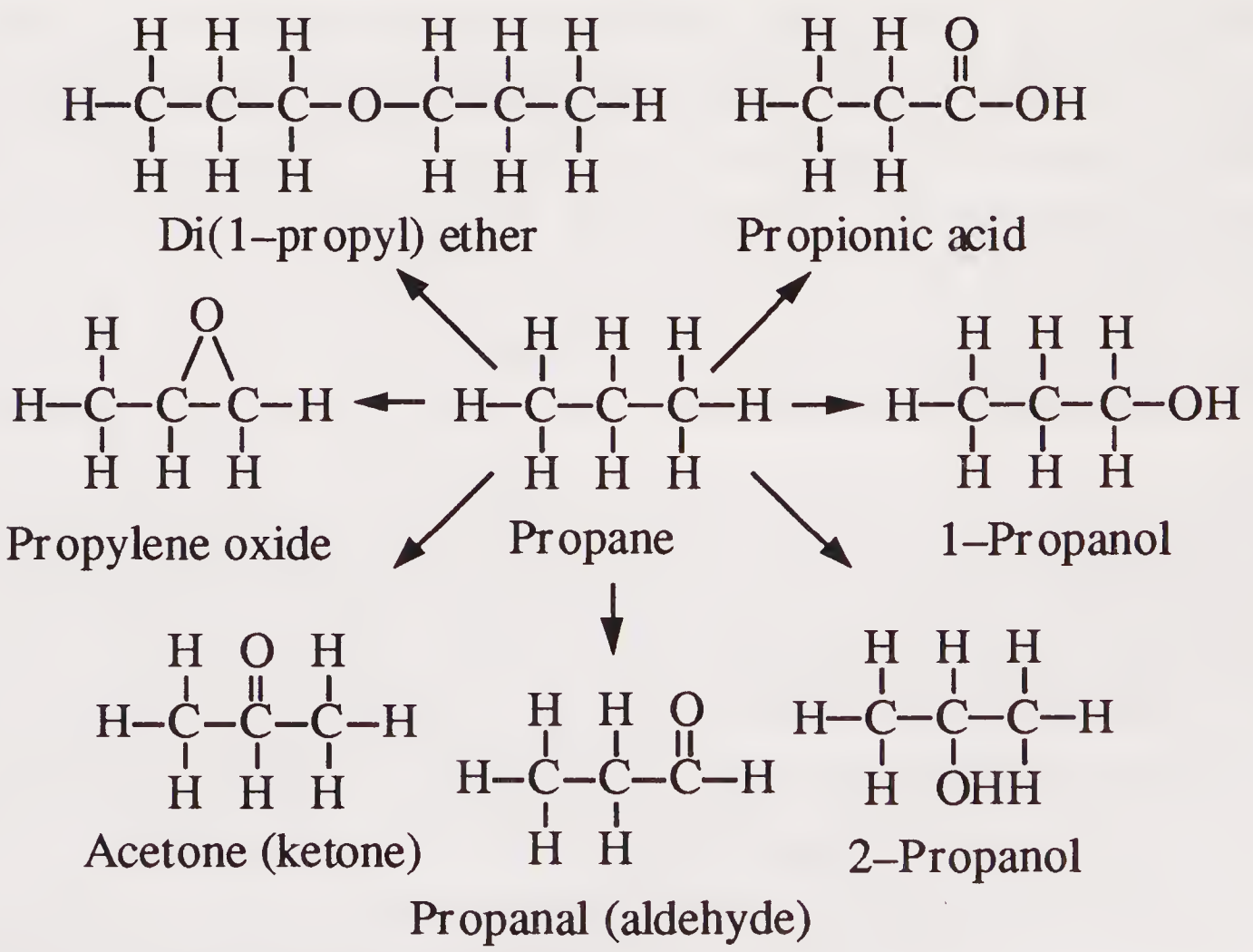


Figure 14.1. Oxygenated derivatives of propane.

Some of the features of organo-oxygen compounds listed above can be seen from an examination of some of the oxidation products of propane in Figure 14.1. The degree of oxygenation increases in the order alcohols-ethers-epoxides<aldehydes-ketones<carboxylic acids.

14.2 ALCOHOLS

This section discusses the toxicological chemistry of the **alcohols**, oxygenated compounds in which the hydroxyl functional group is attached to an aliphatic or olefinic hydrocarbon skeleton. The phenols, which have –OH bonded to an aromatic ring, are covered in Section 14.3. The three lightest alcohols — methanol, ethanol, and ethylene glycol (shown in Figure 14.2) — are discussed individually in some detail because of their widespread use and human exposure to them. The higher alcohols, defined broadly as those containing 3 or more carbon atoms per molecule, are discussed as a group.

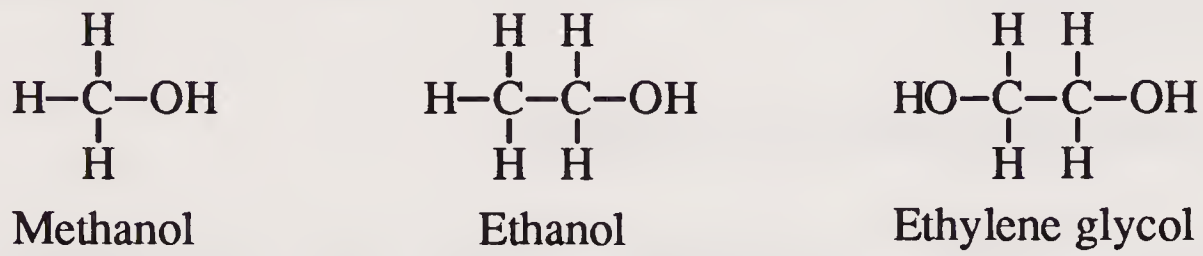


Figure 14.2. Three lighter alcohols with particular toxicological significance.

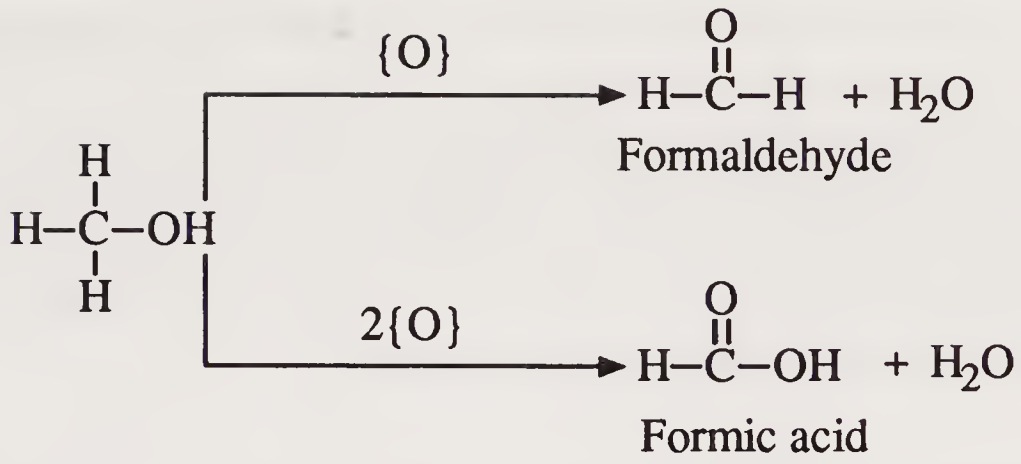
Methanol

Methanol, also called methyl alcohol or wood alcohol, is a clear liquid (mp -98°C, bp 65°C). Until the early 1900s, the major commercial source of methanol was the destructive distillation of wood, a process that yields a product contaminated with allyl alcohol, acetone, and acetic acid. Now methanol is synthesized by the following reaction of hydrogen gas and carbon monoxide, both readily obtained from natural gas or from coal gasification:

$$CO + 2H_2 \xrightarrow[\text{catalyst}]{\text{Metal}} CH_3OH \qquad (14.2.1)$$

The greatest use for methanol is in the manufacture of formaldehyde (see Section 14.5). Additional uses include the synthesis of other chemicals, including acetic acid, applications as an organic solvent, and addition to unleaded gasoline for fuel, antifreeze, and antiknock properties. (Methanol can be used as an "oxygenated additive" to gasoline to raise octane rating and reduce carbon monoxide emissions. For the first time in a major area-wide effort to curtail CO emissions by altering motor fuel composition, oxygenated additives for gasoline were required in Denver, Colorado, during the winter of 1987–1988.)

Methanol has been responsible for the deaths of many humans who ingested it accidentally or as a substitute for beverage ethanol. The fatal human dose is believed to lie between 50 and 250 g. In the body, methanol undergoes metabolic oxidation to formaldehyde and formic acid:



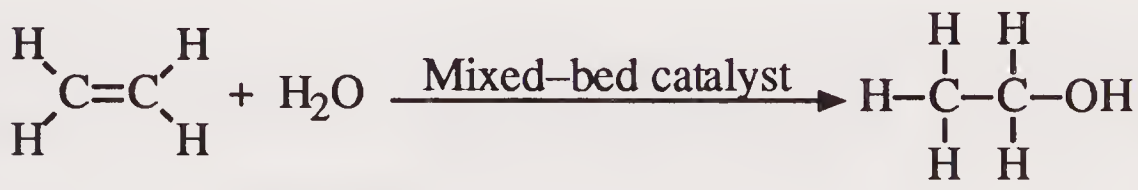
(14.2.2)

The products of this reaction cause acidosis, but the main effects are on the central nervous system and the optic nerve. In cases of acute exposure, an initially mild inebriation is followed in about 10–20 hours by unconsciousness and cardiac depression, and death may occur. For sublethal doses, initial symptoms of visual dysfunction may clear temporarily followed by blindness from deterioration of the optic nerve and retinal ganglion cells.[1] Chronic exposures to lower levels of methanol may result from fume inhalation.

Ethanol

Ethanol (ethyl alcohol, mp –114°C, bp 78°C) is a clear, colorless liquid widely used as a beverage ingredient, synthetic chemical, solvent, germicide, antifreeze, and gasoline additive. It is produced by the fermentation of carbohydrates or by the hydration of ethylene as shown by the two following reactions:

(14.2.3)



(14.2.4)

Ethanol misused in beverages is responsible for more deaths than any other chemical when account is taken of chronic alcoholism, vehicle fatalities caused by intoxicated drivers, and alcohol-related homicides. Chronic alcoholism is a distinct disease arising from generally long-term systemic effects of the ingestion of alcohol and will not be discussed further here.

Ethanol has a range of acute effects, normally expressed as a function of percent blood ethanol.[2] In general these effects are related to central nervous system depression. Mild effects such as decreased inhibitions and slowed reaction times begin to appear at about 0.05% blood ethanol. Most individuals are clinically intoxicated at a level of 0.15–0.3% blood ethanol, in the 0.3–0.5% range stupor may be produced, and at 0.5% and above coma and often death occur.

Metabolically, ethanol is oxidized first to acetaldehyde (Section 14.6), then to CO_2. The overall oxidation rate is faster than for methanol.

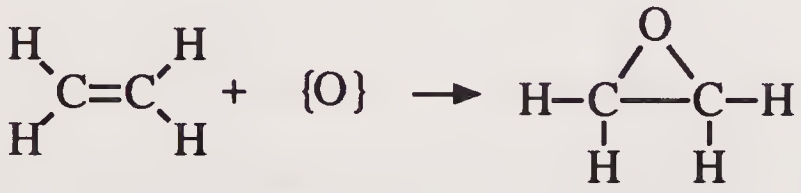
In addition to absorption through the gastrointestinal tract, ethanol can be absorbed by the alveoli of the lungs. Symptoms of intoxication can be observed from inhalation of air containing more than 1000 ppm ethanol.

One of the more serious toxic effects of ethanol is its role as a teratogen when ingested during pregnancy causing **fetal alcohol syndrome.**[3] Fetal alcohol syndrome

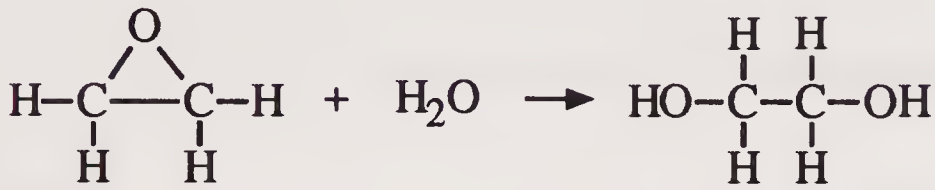
is manifested by a number of effects, some of which are only now being discovered. One of the more obvious of these is the occurrence of defects in head and face structure. Fetal alcohol syndrome is also manifested by central nervous system abnormalities, and it is one of the leading causes of non-genetic mental retardation. It also retards growth, both prenatally and postnatally. Ethanol and its first metabolite, acetaldehyde, rapidly cross the placenta and have adverse effects on its function. Both of these compounds are teratogens, and both are toxic to embryos.

Ethylene Glycol

Although used in cosmetics, chemical synthesis, and other applications, most ethylene glycol is consumed as the major ingredient of antifreeze/antiboil formulations for automobile radiators. Ethylene glycol (mp -13°C, bp 198°C) is synthesized by the oxidation of ethylene to ethylene oxide, followed by hydrolysis of the latter compound:

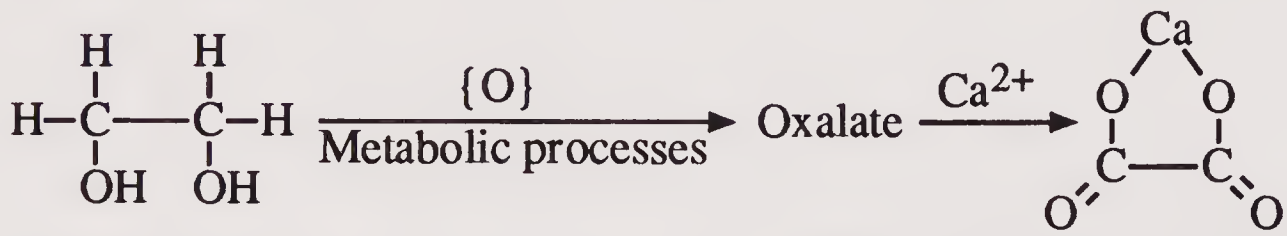


(14.2.5)



(14.2.6)

Toxic exposures to ethylene glycol are rare because of its low vapor pressure, but inhalation of droplets can be very dangerous. About 50 human fatalities attributable to ethylene glycol poisoning have been documented.[4] From the limited amount of information available, the LD_{50} for humans has been estimated at about 110 g. Ingested ethylene glycol initially stimulates the central nervous system, then depresses it. Victims may suffer acedemia from the presence of the intermediate metabolite, glycolic acid. Kidney damage occurs later, and it can be fatal. The kidneys are harmed because of the deposition of solid calcium oxalate resulting from the following overall process:



Ethylene glycol — Calcium oxalate (solid)

(14.2.7)

Important intermediates in this process are glycoaldehyde, glycolate, and glyoxalate.[5] Kidney failure from the metabolic formation of calcium oxalate has been especially common in cat species, which have voracious appetites for ethylene glycol in antifreeze. Deposits of solid calcium oxalate have been observed as well in the liver and brain tissue of victims of ethylene glycol poisoning.

The Higher Alcohols

Numerous alcohols containing three or more carbon atoms are used as solvents and chemical intermediates and for other purposes. Exposure to these compounds can occur and their toxicities are important. Some of the more significant of these alcohols are listed in Table 14.1.

An important alcohol in toxicology studies is ***n*-octanol**, $CH_3(CH_2)_6CH_2OH$. This compound is applied to the measurement of the **octanol-water partition coefficient**, which is used to estimate how readily organic toxicants are transferred from water to lipids, a tendency usually associated with ability to cross cell membranes and cause toxic effects. As just one example, the octanol-water partition coefficient can be used to estimate the tendency of organic compounds to be taken up from water to the lipid gill tissue of fish.[6]

Table 14.1. Some Alcohols with Three or More Carbons

Alcohol name and formula	Properties
2-Propanol, $CH_3CHOHCH_3$	Isopropyl alcohol, used as "rubbing alcohol" and food additive, irritant, narcotic, relatively low toxicity
Allyl alcohol, $CH_2{=}CHCH_2OH$	Olefinic alcohol, pungent odor, strongly irritating to eyes, mouth, lungs
1-Butanol, $CH_3(CH_2)_2CH_2OH$	Butyl alcohol or *n*–butanol, irritant, limited toxicity because of low vapor pressure
1–Pentanol, $CH_3(CH_2)_3CH_2OH$	Amyl alcohol, liquid, bp 138°C, irritant, causes headache and nausea, low vapor pressure and low water solubility reduce toxicity hazard
1–Decanol, $CH_3(CH_2)_8CH_2OH$	Viscous liquid, bp 233°C, low acute toxicity
2-Ethylhexanol, $CH_3(CH_2)_3CH$-$(C_2H_5)CH_2OH$	2–Ethylhexyl alcohol, important industrial solvent, toxicity similar to those of butyl alcohols

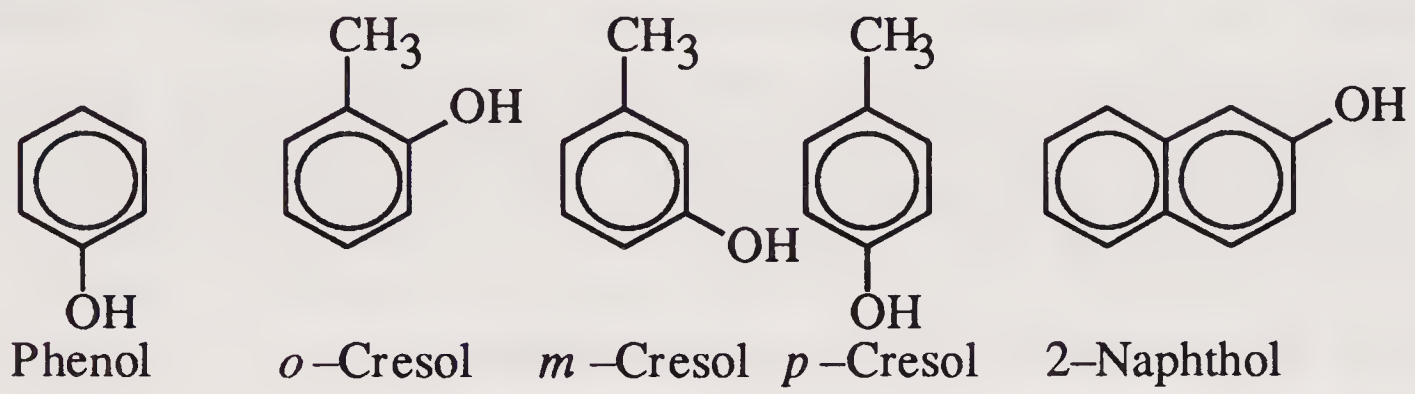
14.3. PHENOLS

Phenols are aryl alcohols in which the –OH group is bonded to an aromatic hydrocarbon moiety. The simplest of these compounds is phenol, in which the hydrocarbon portion is the phenyl group. Figure 14.3 shows some of the more important phenolic compounds. Phenols have properties that are quite different from those of the aliphatic and olefinic alcohols. Many important phenolic compounds have nitro groups ($-NO_2$) and halogen atoms (particularly Cl) bonded to the aromatic rings. These substituents may strongly affect chemical and toxicological behavior; such compounds are discussed in Chapters 15 and 16.

Properties and Uses of Phenols

The physical properties of the phenols listed in Figure 14.3 are summarized briefly in Table 14.2. These phenolic compounds are weak acids that ionize to phenolate ions in the presence of base:

(14.3.1)



Phenol *o*–Cresol *m*–Cresol *p*–Cresol 2–Naphthol

Figure 14.3. Major phenolic compounds.

Phenols are extracted commercially from coal tar into aqueous base as the phenolate ions. The major commercial use of phenol is in the manufacture of phenolic resin polymers, usually with formaldehyde. Phenols and cresols are used as antiseptics and disinfectants in areas such as barns where the phenol odor can be tolerated. Phenol was the original antiseptic used on wounds and in surgery, starting with the work of Lord Lister in 1885.

Toxicology of Phenols

Generally, the phenols have similar toxicological effects. Phenol is a protoplasmic poison, so it damages all kinds of cells. Early medical studies that demonstrated aesepsis with phenol revealed its toxicity as well. Phenol is alleged to have caused "an astonishing number of poisonings"[7] since it came into general use.

Table 14.2. Properties of Major Phenolic Compounds

Compound	Properties
Phenol	Carbolic acid, white solid, characteristic odor, mp 41°C, bp 182°C
m–Cresol	Often occurs mixed with *ortho*- and *para*- analogs as cresol or cresylic acid, light yellow liquid, mp 11°C, bp 203°C
o–Cresol	Solid, mp 31°C, bp 191°C
p–Cresol	Crystalline solid with phenolic odor, mp 36°C, bp 202°C
1–Naphthol	Alpha-naphthol, colorless solid, mp 96°C, bp 282°C
2–Naphthol	Beta-naphthol, mp 122°C, bp 288°C

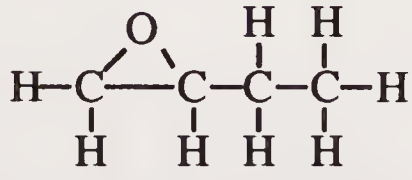
Fatal doses of phenol may be absorbed through the skin. Its acute toxicological effects are predominantly upon the central nervous system. Death can occur as early as one-half hour after exposure. Key organs damaged by chronic exposure to phenol include the spleen, pancreas, and kidneys. Lung edema can also occur.

Some phenol is eliminated from the body as the unchanged molecular compound, although most is metabolized prior to excretion. As noted in Chapter 4, phase II reactions in the body result in the conjugation of phenol to produce sulfates and glucuronides. These water-soluble metabolic products are eliminated via the kidneys.

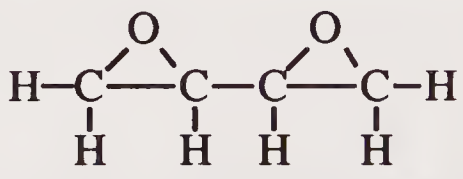
Oral doses of naphthols can be fatal. Acute poisoning by these compounds can cause severe gastrointestinal disturbances, kidney malfunction, circulatory system failure, and convulsions. Naphthols can be absorbed through the skin, one effect of which can be eye damage involving the cornea and lens.

14.4. OXIDES

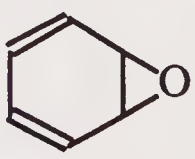
Hydrocarbon **oxides** are significant for both their uses and their toxic effects.[8] As shown for ethylene oxide (1,2-epoxyethane) in Reactions 14.2.5 and 14.2.6 and propylene oxide (1,2-epoxypropane) in Figure 14.1, these compounds are characterized by an **epoxide** functional group consisting of an oxygen atom bridging between two adjacent C atoms. As discussed in Section 4.2, the metabolic formation of such a group is called epoxidation and is a major type of the phase I reactions of xenobiotic compounds. In addition to ethylene and propylene oxides, four other common hydrocarbon oxides are shown in Figure 14.4.



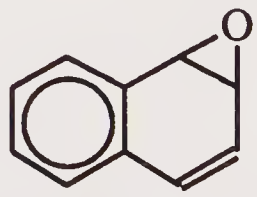
1,2–Butylene oxide
(1,2–epoxybutane)



1,2,3,4–Butadiene epoxide



Benzene–1,2–oxide



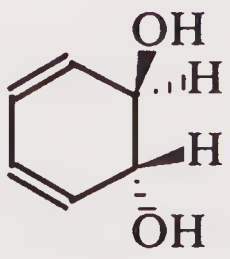
Naphthalene–1,2–oxide

Figure 14.4. Some common epoxide compounds.

Ethylene oxide (mp -111°C, bp 11°C) is a colorless, sweet-smelling, flammable, explosive gas. It is used as a chemical intermediate, sterilant, and fumigant. It has a moderate to high toxicity, is a mutagen, and is carcinogenic to experimental animals. When inhaled, ethylene oxide causes respiratory tract irritation, headache, drowsiness, and dyspnea. At higher levels, cyanosis, pulmonary edema, kidney damage, peripheral nerve damage, and death can result from inhalation of this compound.

Propylene oxide (mp -104°C, bp 34°C) is a colorless, reactive, volatile liquid with uses similar to those of ethylene oxide. Its toxic effects are like those of ethylene oxide, though less severe. The properties of butylene oxide (liquid, bp 63°C) are also similar to those of ethylene oxide. The oxidation product of 1,3-butadiene, 1,2,3,4-butadiene epoxide, is a direct–acting (primary) carcinogen.

As discussed in Section 13.5, benzene-1,2-oxide is an intermediate in the biochemical oxidation of benzene. It is probably responsible for the toxicity of benzene. It is hydrolyzed by the action of epoxide hydratase to the dihydrodiol shown below:

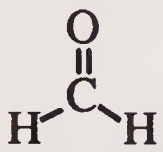


Benzene *Trans* –1,2–dihydrodiol

Naphthalene-1,2-oxide is a metabolic intermediate in the oxidation of naphthalene mediated by cytochrome P-450.

14.5. FORMALDEHYDE

Aldehydes and ketones are compounds that contain the carbonyl (C=O) group. Of these compounds, **formaldehyde**,



Formaldehyde

is uniquely important for several reasons. Among these are that its physical and chemical properties are atypical of aldehydes in some important respects. Furthermore, it is widely used in a number of applications and exhibits toxicological chemical behavior that may differ substantially from that of other common aldehydes. Therefore, formaldehyde is discussed separately in this section. Other aldehydes and ketones are covered in the following section.

Properties and Uses of Formaldehyde

Formaldehyde (mp -118°C, bp -19°C) is a colorless gas with a pungent, suffocating odor. It is manufactured by the oxidation of methanol over a silver catalyst. Because it undergoes a number of important reactions in chemical synthesis and can be made at relatively low cost, formaldehyde is one of the most widely used industrial chemicals. In the pure form it polymerizes with itself to give hydroxyl compounds, ketones, and other aldehydes. Because of this tendency, commercial formaldehyde is marketed as a 37–50% aqueous solution containing some methanol called **formalin**. Formaldehyde is a synthesis intermediate in the production of resins (particularly phenolic resins), as well as a large number of synthetic organic compounds, such as chelating agents. Formalin is employed in antiseptics, fumigants, tissue and biological specimen preservatives, and embalming fluid.

Toxicity of Formaldehyde and Formalin

The fact that formaldehyde is produced by natural processes in the environment and in the body would suggest that it might not be very toxic. However, such is not the case in that formaldehyde exhibits a number of toxic effects.[9]

Exposure to inhaled formaldehyde via the respiratory tract is usually to molecular formaldehyde vapor, whereas exposure by other routes is usually to formalin. Exposure to formaldehyde vapor can occur in industrial settings. In recent years a great deal of concern has arisen over the potential for exposure in buildings from formaldehyde vapor evolved from insulating foams that were not properly formulated and cured or when these foams burn. Hypersensitivity can result from prolonged, continuous exposure to formaldehyde. Furthermore, animal experiments have shown formaldehyde to be a lung carcinogen.[10]

The human LD_{50} for the ingestion of formalin has been estimated at around 45 g. Deaths have been caused by as little as about 30 g and individuals have survived ingestion of about 120 g, although in at least one such case removal of the stomach was required. Ingestion results in violent gastrointestinal disturbances, including vomiting and diarrhea. Formaldehyde attacks the mucous membrane linings of both

the respiratory and alimentary tracts and reacts strongly with functional groups in molecules. Metabolically, formaldehyde is rapidly oxidized to formic acid (see Section 14.7), which is responsible in large part for its toxicity.[2]

14.6. ALDEHYDES AND KETONES

In **aldehydes** the carbonyl group is attached to a C and H atom at the end of a hydrocarbon chain, and in a **ketone** it is bonded to two C atoms in the middle of a hydrocarbon chain or ring. The hydrocarbon portion of aldehydes and ketones may consist of saturated or unsaturated straight chains, branched chains, or rings. The structures of some important aldehydes and ketones are shown in Figure 14.5.

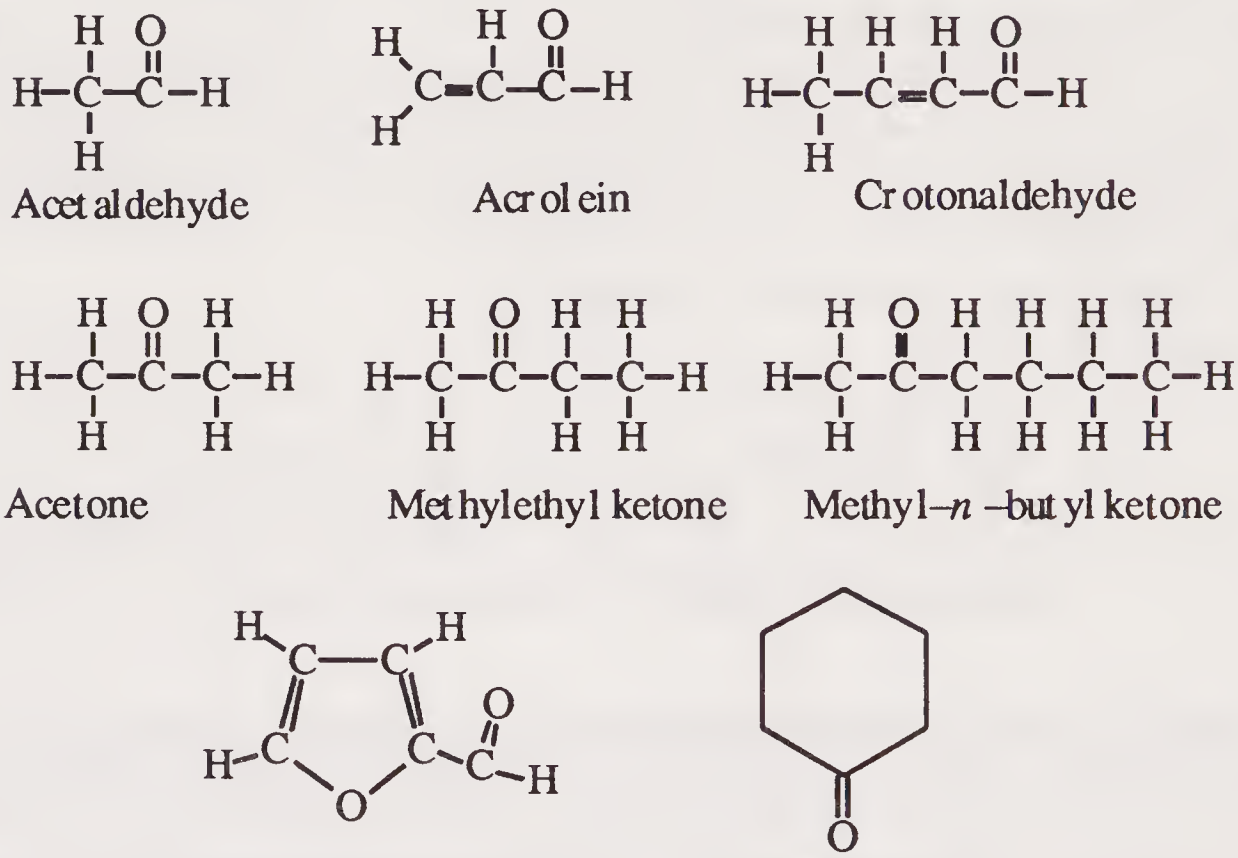


Figure 14.5. Aldehydes and ketones that are significant for their commercial uses and toxicological importance.

Both aldehydes and ketones are industrially important classes of chemicals. Aldehydes are reduced to make the corresponding alcohols and are used in the manufacture of resins, dyes, plasticizers, and alcohols. Some aldehydes are ingredients in perfumes and flavors. Several ketones are excellent solvents and are widely used for that purpose to dissolve gums, resins, laquers, nitrocellulose, and other substances.

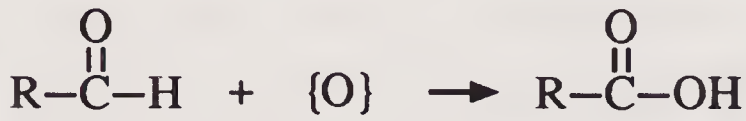
Toxicities of Aldehydes and Ketones

In general, because of their water solubility and intensely irritating qualities, the lower aldehydes attack exposed moist tissue, particularly the eyes and mucous membranes of the upper respiratory tract. Because of their lower water solubility, the lower aldehydes can penetrate further into the respiratory tract and affect the lungs.[11]

The toxicity of formaldehyde was discussed in the preceding section. Acetaldehyde is a colorless liquid (bp 21°C) and acts as an irritant and systemically as a narcotic to the central nervous system. Acrolein, an olefinic aldehyde, is a colorless to light yellow liquid (bp 52°C). It is a very reactive chemical that polymerizes readily. It is quite toxic by all routes of contact and ingestion. It has a choking odor

and is extremely irritating to respiratory tract membranes. It is classified as an extreme lachrymator (substance that causes eyes to water). Because of this property, acrolein serves to warn of its own exposure. It can produce tissue necrosis, and direct contact with the eye can be especially hazardous.[12] Crotonaldehyde is similarly dangerous and can cause burns to the eye cornea.

Metabolically, aldehydes are converted to the corresponding organic acids as shown by the following general reaction:

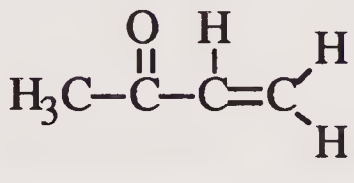


(14.6.1)

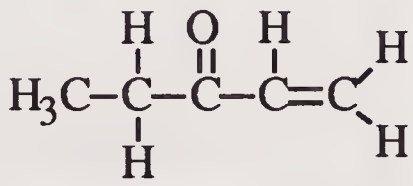
In mammals, the liver enzymes aldehyde dehydrogenase and aldehyde oxidase appear to be mainly responsible for this reaction.[13]

Acetone is a liquid with a pleasant odor. It can act as a narcotic and dissolves fats from skin, causing dermatitis. Methyl-*n*-butyl ketone, a widely used solvent, is a mild neurotoxin. Methylethyl ketone is suspected of having caused neuropathic disorders in shoe factory workers.

Methylvinyl ketone and ethylvinyl ketone,



Methylvinyl ketone



Ethylvinyl ketone

are both classified as α,β-unsaturated ketones. These compounds and α,β-unsaturated aldehydes, of which acrolein discussed above is an example, are mutagenic and, therefore, potentially carcinogenic. Human exposure to these compounds can result from a number of sources, including industrial chemicals (a purpose for which methyvinyl ketone is widely used), metabolites of industrial chemicals, pesticide metabolites, natural products, and pollutants. Ethylvinyl ketone is an especially common contaminant of foods, having been detected in meat, dairy products, fruit juices, kiwi fruit, and other foods. Both of these ketones have been found to form adducts with the guanine moiety in deoxyguanosine nucleoside and in 2'-deoxyguanosine 5'-monophosphate nucleotide (see Figure 3.10 and Section 3.7).[14]

14.7. CARBOXYLIC ACIDS

Carboxylic acids contain the –C(O)OH functional group bound to an aliphatic, olefinic, or aromatic hydrocarbon moiety. This section deals with those carboxylic acids that contain only C, H, and O. Carboxylic acids that contain other elements, such as trichloroacetic acid (a strong acid) or deadly poisonous monofluoroacetic acid, are discussed in later chapters. Some of the more significant carboxylic acids are shown in Figure 14.6.

Carboxylic acids (Figure 14.6) are the oxidation products of aldehydes and are often synthesized by that route. Some of the higher carboxylic acids are constituents of oil, fat, and wax esters from which they are prepared by hydrolysis. Carboxylic acids have many applications. Formic acid is used as a relatively inexpensive acid to neutralize base, in the treatment of textiles, and as a reducing agent. Acetic and propi-

onic acids are added to foods for flavor and as preservatives. Among numerous other applications, these acids are also used to make cellulose plastics. Stearic acid acts as a dispersive agent and accelerator activator in rubber manufacture. Sodium stearate is a major ingredient of most soaps. Many preservative and antiseptic formulations contain benzoic acid. Large quantities of phthalic acid are used to make phthalate ester plasticizers (see Section 14.13). Acrylic acid and methacrylic acid (acrylic acid in which the alpha-hydrogen has been replaced with a $-CH_3$ group; see Figure 14.6) are used in large quantities to make acrylic polymers.

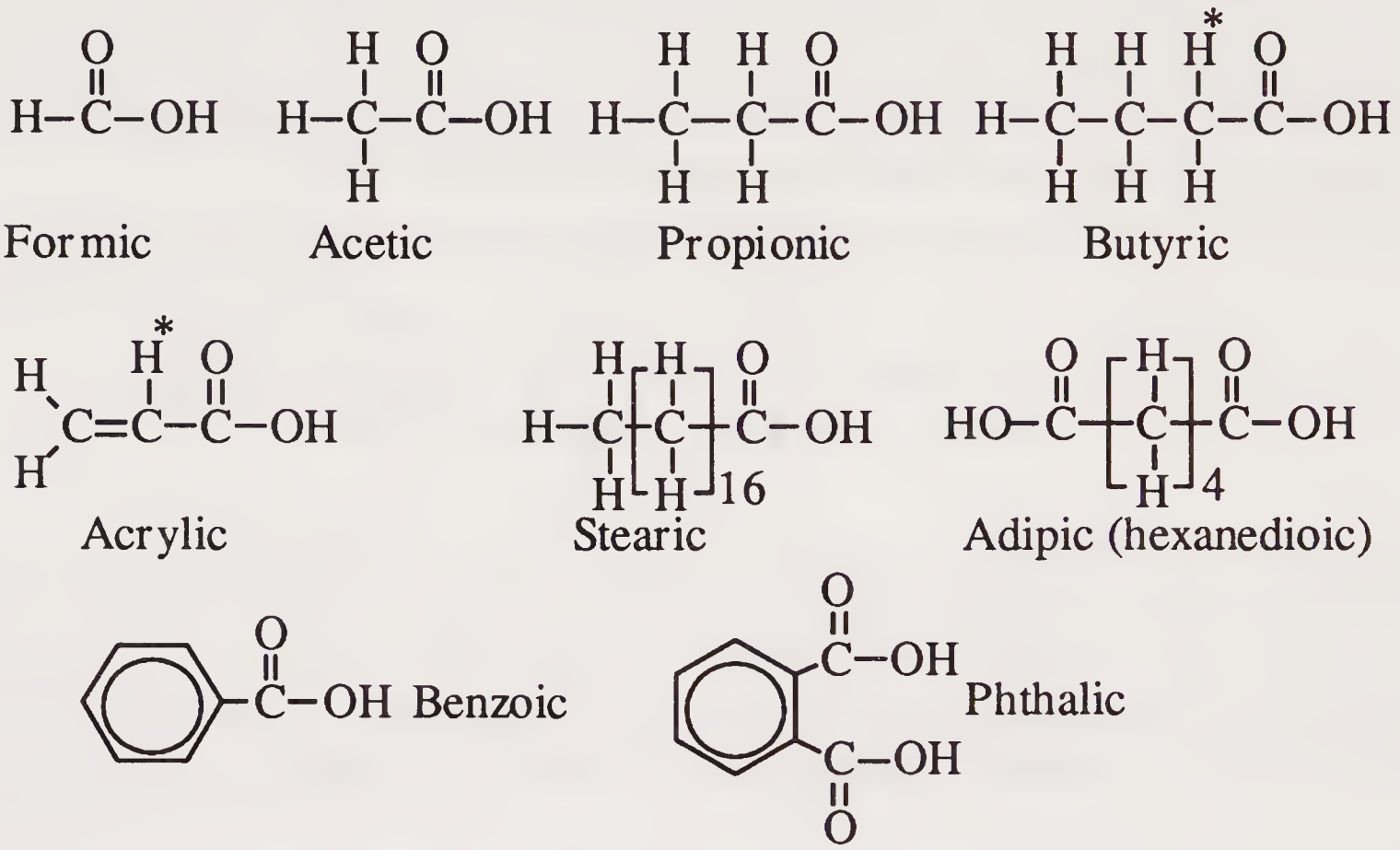


Figure 14.6. Some common carboxylic acids. The positions of the alpha- hydrogens have been marked with an asterisk for butyric and acrylic acids.

Toxicology of Carboxylic Acids

Concentrated solutions of formic acid are corrosive to tissue, much like strong mineral acids. In Europe decalcifier formulations containing about 75% formic acid have been marketed for removing mineral scale. Children ingesting this material have suffered corrosive lesions to mouth and esophageal tissue. Although acetic acid is widely used in food preparation as a 4–6% solution in vinegar, pure acetic acid (glacial acetic acid) is extremely corrosive to tissue that it contacts. Acrylic and methacrylic acids are considered to be relatively toxic, both orally and by skin contact. In general, the presence of more than one carboxylic acid group per molecule, unsaturated bonds in the carbon skeleton, or the presence of a hydroxide group on the alpha-carbon position (see Figure 14.6) increases corrosivity and toxicity of carboxylic acids.

14.8. ETHERS

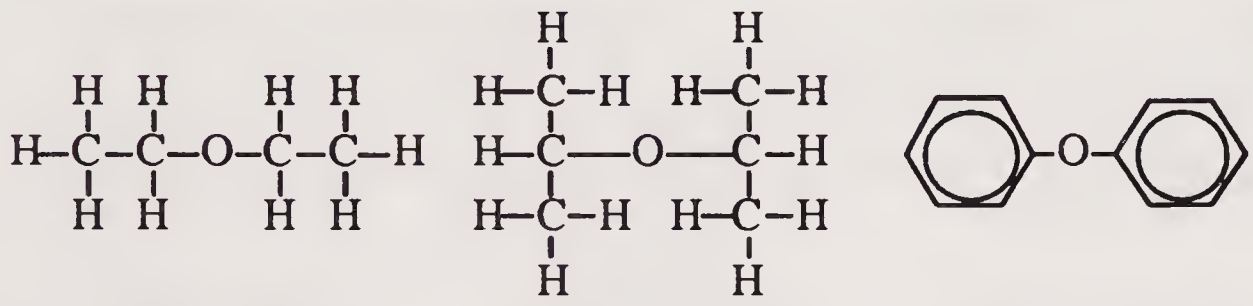
Three important classes of oxygenated organic compounds can be regarded as products of condensation of compounds containing the –OH group accompanied by a loss of H_2O, as shown by the following reaction:

$$R\text{-}OH + HO\text{–}R' \rightarrow R\text{–}O\text{–}R' + H_2O \qquad (14.8.1)$$

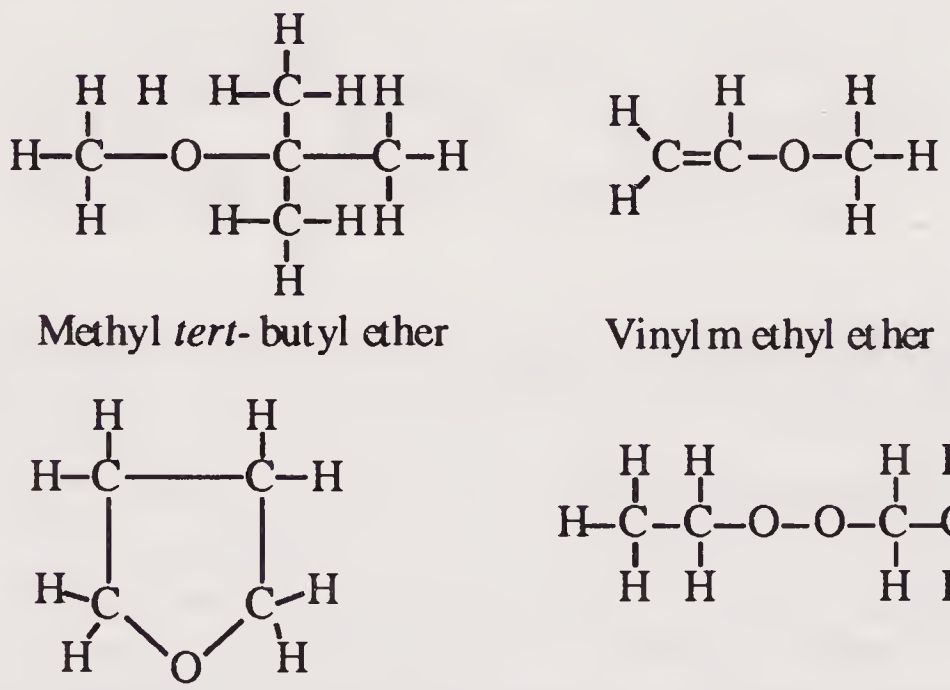
In this reaction, R–OH and HO–R' are either alcohols or carboxylic acids. When both are alcohols, R–O–R' is an ether; when one is an acid and the other an alcohol, the product is an ester; and when both are acids, an acid anhydride is produced. Ethers are discussed in this section, and the other two classes of products are discussed in the two sections that follow.

Examples and Uses of Ethers

An ether consists of two hydrocarbon moieties linked by an oxygen atom as shown in Figure 14.7. Although diethyl ether is highly flammable, ethers are generally not very reactive. This property enables their uses in applications where an unreactive organic solvent is required. Some ethers form explosive peroxides when exposed to air as shown by the example of diethyl ether peroxide in Figure 14.7.



Diethyl ether | Di-isopropyl ether | Diphenyl ether



Methyl *tert*-butyl ether | Vinyl methyl ether

Tetrahydrofuran (cyclic ether) | Peroxide from diethyl ether

Figure 14.7. Structures of some common ethers.

Ethers are prominent members of a class of organic substances widely used as solvents, and including hydrocarbons, chlorinated hydrocarbons, and alcohols as well as ethers.[15] Because of the widespread use of such solvents, human exposure is particularly likely.

Diethyl ether (mp -116°C, bp 34.6°C) is the most commercially important ether. It is used as a reaction medium, solvent, and extractant. The production of methyl *tert*-butyl ether has increased markedly in recent years because of its application as an antiknock ingredient of unleaded gasoline.

Toxicities of Ethers

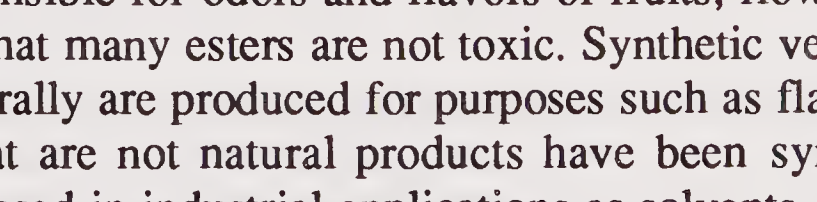
Because of its volatility, the most likely route of exposure to diethyl ether is by inhalation. About 80% of this compound that gets into the body is eliminated unmetabolized as the vapor through the lungs. Diethyl ether is a central nervous

system depressant, and for many years was the anesthetic of choice for surgery. At low doses it causes drowsiness, intoxication, and stupor. Higher exposures result in unconsciousness and even death.

Compared to other classes of organic compounds, the ethers have relatively low toxicities. This characteristic can be attributed to the low reactivity of the C–O–C functional group arising from the high strength of the carbon-oxygen bond. Like diethyl ether, several of the more volatile ethers affect the central nervous system. Hazards other than their toxicities tend to be relatively more important for the ethers. These hazards are flammability and formation of explosive peroxides (especially with di-isopropyl ether).

14.9. ACID ANHYDRIDES

The most important carboxylic **acid anhydride** is acetic anhydride, the structure of which is shown below:



Acetic anhydride

Annual world production of this chemical compound is on the order of a million metric tons. In chemical synthesis it functions as an acetylating agent (addition of $CH_3C(O)$ moiety). Its greatest single use is to make cellulose acetate and it has additional applications in manufacturing textile sizing agents, the synthesis of salicylic acid (for aspirin manufacture), electrolytic polishing of aluminum, and the processing of semiconductor components.

Toxicological Considerations

In contrast to the relative safety of many ethers and esters, acetic anhydride is a systemic poison and especially corrosive to the skin, eyes, and upper respiratory tract. Levels in the air of as low as 0.4 mg/m^3 adversely affect eyes, and contamination should be kept to less than 1/10 that level in the workplace atmosphere. Blisters and burns that heal only slowly result from skin exposure. Acetic anhydride has a very strong acetic acid odor that causes an intense burning sensation in the nose and throat that is accompanied by coughing. It is a powerful lachrymator. Fortunately, these unpleasant symptoms elicit a withdrawal response in exposed individuals.

14.10. ESTERS

Esters, such as those shown in Figure 14.8, are formed from an alcohol and acid as shown in Reaction 14.10. Esters exhibit a wide range of biochemical diversity and large numbers of them occur naturally. Fats, oils, and waxes are esters, as are many of the compounds responsible for odors and flavors of fruits, flowers, and other natural products. It follows that many esters are not toxic. Synthetic versions of many of the esters that occur naturally are produced for purposes such as flavoring ingredients. A number of esters that are not natural products have been synthesized for various purposes. Esters are used in industrial applications as solvents, plasticizers, lacquers, soaps, and surfactants. Figure 14.8 shows some representative esters.

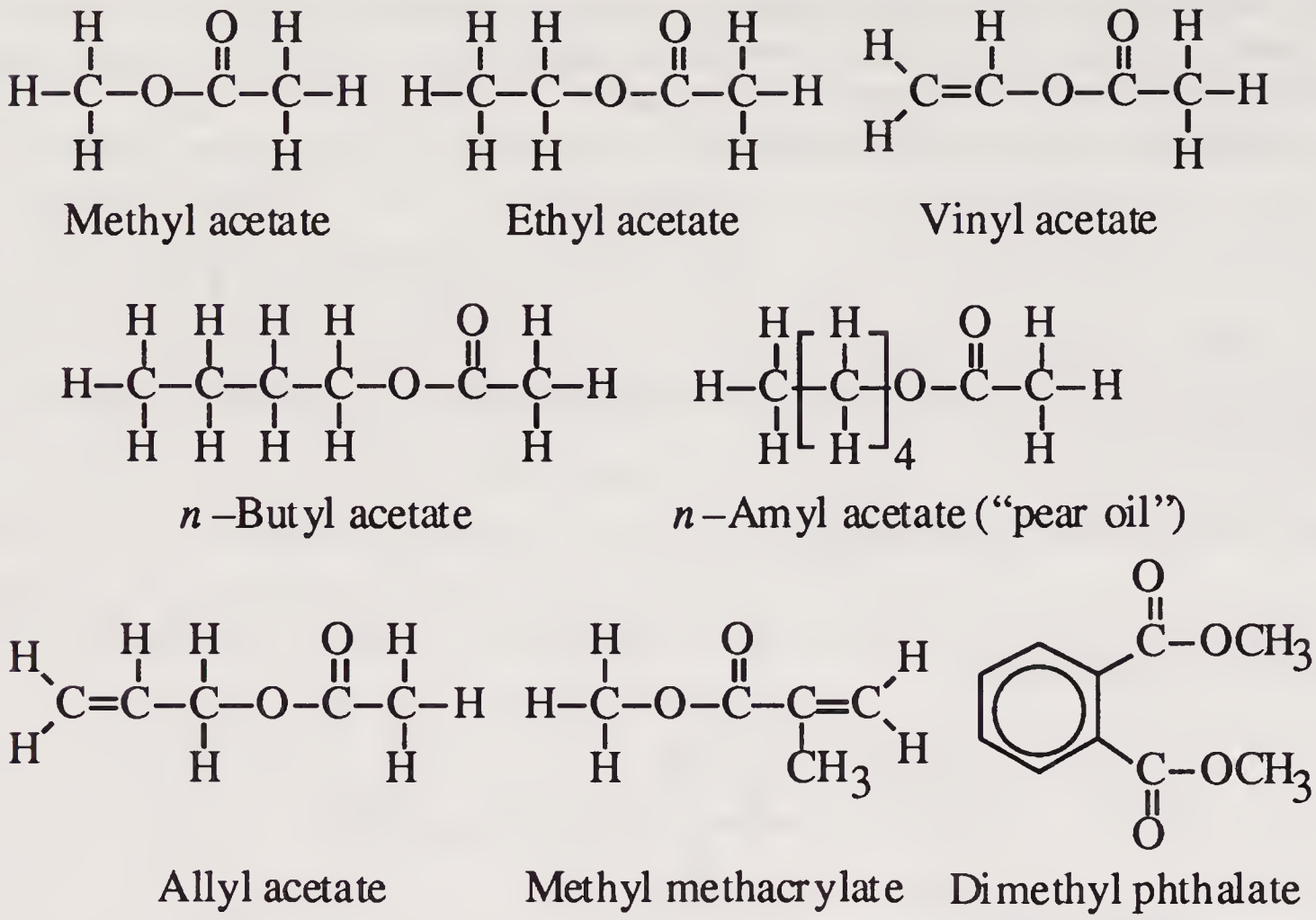
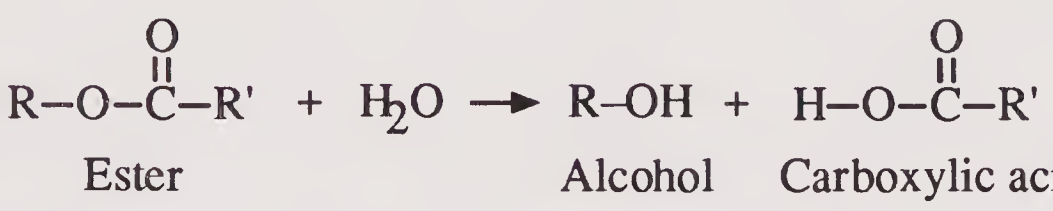


Figure 14.8. Some typical esters.

Methyl acetate is a colorless liquid with a pleasant odor. It is used as a solvent and as an additive to give foods a fruit-like taste. Ethyl acetate is a liquid with a pleasant odor. Liquid vinyl acetate polymerizes when exposed to light to yield a solid polymer. Both *n*-butyl acetate and *n*-amyl acetate are relatively higher-boiling liquids compared to the esters mentioned above. Amyl acetate has a characteristic odor of bananas and pears. Methyl methacrylate is the monomer used to make some kinds of polymers noted for their transparency and resistance to weathering. Among their other applications, these polymers are used as substitutes for glass, particularly in automobile lights. Dimethyl phthalate is the simplest example of the environmentally important phthalate esters. Other significant members of this class of compounds are diethyl, di-*n*-butyl, di-*n*-octyl, bis(2-ethylhexyl), and butyl benzyl phthalates.[16] Used in large quantities as plasticizers to improve the qualities of plastics, these compounds have become widespread environmental pollutants. The higher-molecular-mass phthalate compounds, especially, tend to be environmentally persistent.

Toxicities of Esters

The most common reaction of esters in exposed tissues is hydrolysis:



(14.10.1)

To a large extent, therefore, the toxicities of esters tend to be those of their hydrolysis products. Two physical characteristics of many esters that affect their toxicities are relatively high volatility, which promotes exposure by the pulmonary route, and good solvent action, which affects penetration and tends to dissolve body lipids. Many volatile esters exhibit asphyxiant and narcotic action. As expected for compounds that

occur naturally in foods, some esters are non-toxic (in reasonable doses). However, some of the synthetic esters, such as allyl acetate, have relatively high toxicities. As an example of a specific toxic effect, vinyl acetate acts as a skin defatting agent.

Although environmentally persistent, most of the common phthalates have low toxicity ratings of 2 or 3. Phthalate ester toxicities and metabolism have been summarized in a book on the subject.[17]

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QUESTIONS AND PROBLEMS

1. Explain the basis of the statement that "the degree of oxygenation increases in the order alcohols-ethers-epoxides<aldehydes-ketones<carboxylic acids."

2. In what respects are the chemical and toxicological chemical characteristics of methanol unique? What are some of the particular toxicological hazards of methanol?

3. What is the metabolic pathway of methanol degradation? How does this result in acidosis?

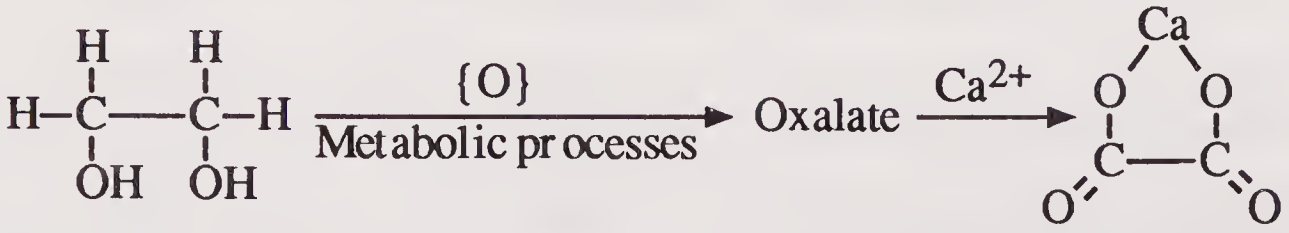
4. What are the major acute toxicological effects of ethanol? How is ethanol exposure usually measured or expressed? What is a particular chronic toxicological effect of long-term ethanol ingestion?

5. What are the metabolic products of ethanol oxidation in the body? How does the rate of ethanol metabolism compare to that of methanol metabolism?

6. What is the name of the long-chain alcohol $CH_3(CH_2)_6CH_2OH$? What is its water solubility? Referring back to Chapter 6, how is this alcohol used to describe bioaccumulation effects? What is the name of the parameter obtained using this alcohol to describe such effects?

7. Recall that in general, the organo-oxygen compounds can be classified according to the degree of oxygenation, location of oxygen on the hydrocarbon moiety, presence of unsaturated bonds in the hydrocarbon structure, and presence or absence of aromatic rings. Using these criteria, discuss how each of the compounds shown in the structure below are classified.

8. What toxicological effect may result from the reaction below? Which organ is most susceptible to damage as a result?

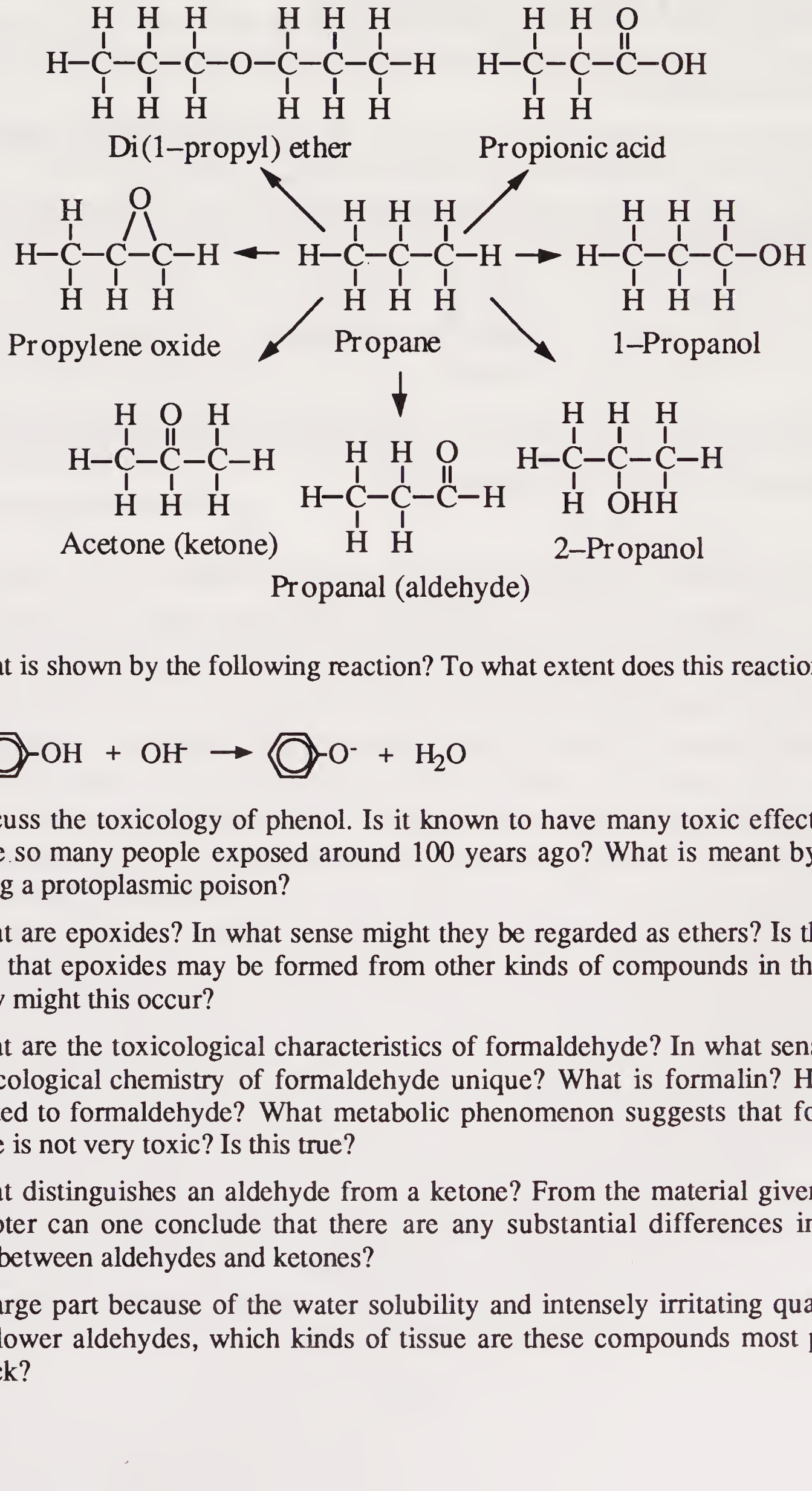


Ethylene glycol

Calcium oxalate (solid)

9. Match the following pertaining to bonding in organometallic compounds:

(a) $CH_3CHOHCH_3$	1. Olefinic alcohol
(b) $CH_2{=}CHCH_2OH$	2. *n*–Butanol
(c) $CH_3(CH_2)_2CH_2OH$	3. Used in bioaccumulation studies
(d) $CH_3(CH_2)_6CH_2OH$	4. "Rubbing alcohol," food additive



10. What is shown by the following reaction? To what extent does this reaction occur?

(14.3.1)

11. Discuss the toxicology of phenol. Is it known to have many toxic effects? Why were so many people exposed around 100 years ago? What is meant by phenol being a protoplasmic poison?

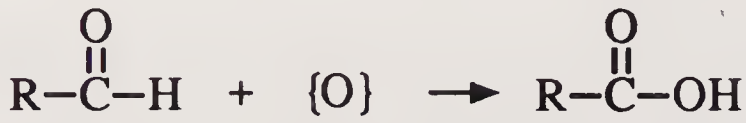
12. What are epoxides? In what sense might they be regarded as ethers? Is there any way that epoxides may be formed from other kinds of compounds in the body? How might this occur?

13. What are the toxicological characteristics of formaldehyde? In what sense is the toxicological chemistry of formaldehyde unique? What is formalin? How is it related to formaldehyde? What metabolic phenomenon suggests that formaldehyde is not very toxic? Is this true?

14. What distinguishes an aldehyde from a ketone? From the material given in this chapter can one conclude that there are any substantial differences in toxicities between aldehydes and ketones?

15. In large part because of the water solubility and intensely irritating qualities of the lower aldehydes, which kinds of tissue are these compounds most prone to attack?

16. Explain what is shown by the following general reaction in terms of the metabolism of an important class of toxic compounds:

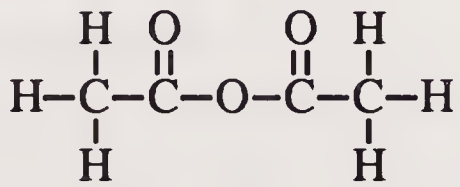


17. Why is it reasonable to believe that many carboxylic acids have only limited toxicities? Give some examples of carboxylic acids that are quite toxic, however.

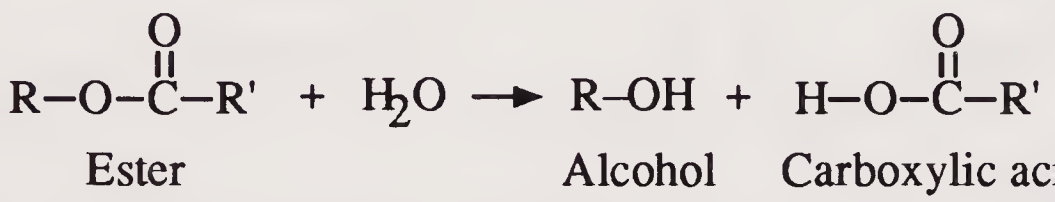
18. Ethers are often "used in applications where an unreactive organic solvent is required." In what sense are ethers unreactive? How is this reflected in their toxicological chemistry?

19. What is the most likely route of exposure to diethyl ether? How is much of the diethyl ether that enters the body by this route subsequently eliminated?

20. What are some of the important chemical and toxicological characteristics of the compound shown below:



21. In general, what are the toxicological characteristics of esters. Why is it reasonable to believe that many esters are not particularly toxic? What does the reaction below imply about the toxicities of esters?



15

Organonitrogen Compounds

15.1. INTRODUCTION

Nitrogen occurs in a wide variety of organic compounds of both synthetic and natural origin. This chapter discusses organic compounds that contain carbon, hydrogen, and nitrogen. Many significant organic nitrogen compounds contain oxygen as well, and these are covered in later parts of the chapter. Not the least of the concerns regarding organonitrogen compounds is that a significant number of these compounds (including some aromatic amines and nitrosamines) are carcinogenic.[1]

15.2. NON-AROMATIC AMINES

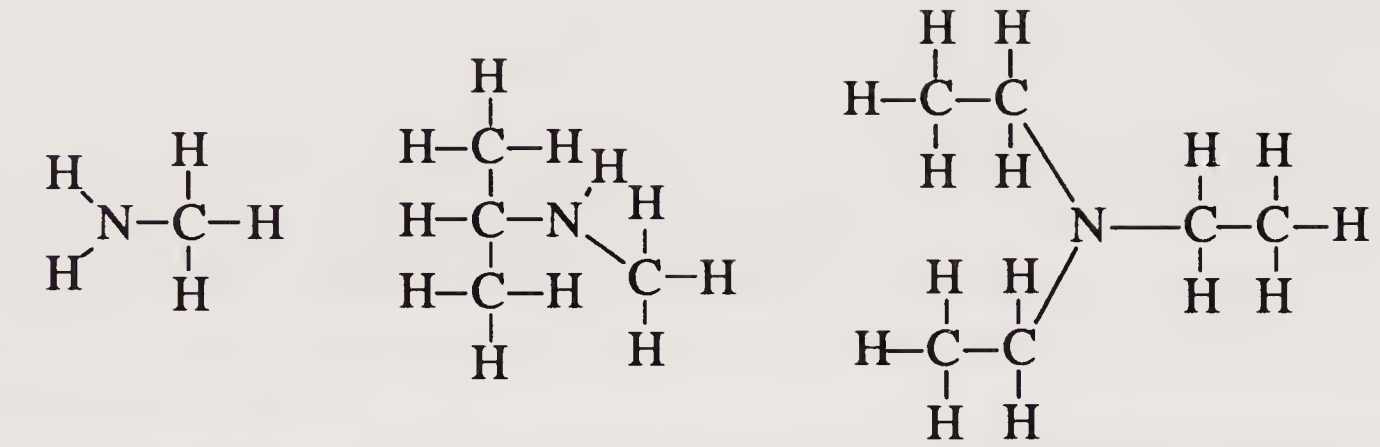
Lower Aliphatic Amines

Amines may be regarded as derivatives of ammonia, NH_3, in which 1 to 3 of the H atoms have been replaced by hydrocarbon groups. When these groups are aliphatic groups of which none contains more than 6 C atoms, the compound may be classified as a **lower aliphatic amine**. Among the more commercially important of these amines are mono-, di-, and trimethylamine; mono-, di-, and triethylamine; dipropylamine, isopropylamine, butylamine, dibutylamine, diisobutylamine, cyclohexylamine, and dicyclohexylamine. Example structures are given in Figure 15.1 (next page).

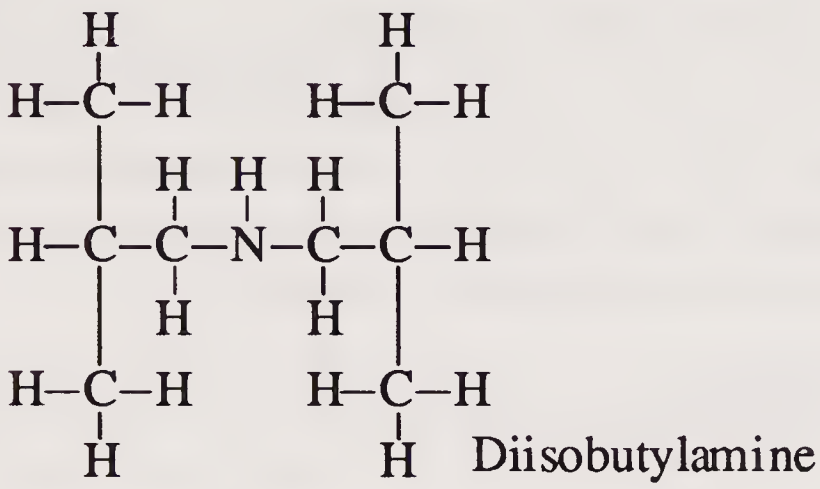
The structures in Figure 15.1 indicate some important aspects of amines. Methylamine, methyl-2-propylamine, and triethylamine are primary, secondary, and tertiary amines, respectively. A primary amine has 1 hydrocarbon group substituted for H on NH_3, a secondary amine has 2, and a tertiary amine has 3. Dicyclohexylamine has two cycloalkane substituent groups attached and is a secondary amine. All of the aliphatic amines have strong odors. Of the compounds listed above as commercially important aliphatic amines, the methylamines and monoethylamine are gases under ambient conditions, whereas the others are colorless volatile liquids. The lower aliphatic amines are highly flammable. They are used primarily as intermediates in the manufacture of other chemicals, including polymers (rubber, plastics, textiles), agricultural chemicals, and medicinal chemicals.

The lower aliphatic amines are generally among the more toxic substances in routine, large-scale use. One of the reasons for their toxicity is that they are basic compounds and raise the pH of exposed tissue by hydrolysis with water in tissue as shown by the following reaction:

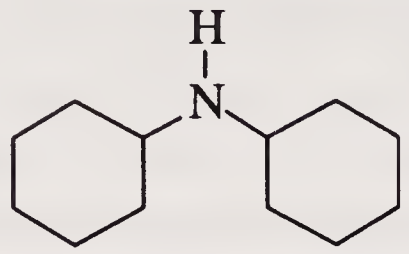
$$R_3N + H_2O \rightarrow R_3NH^+ + OH^- \qquad (15.2.1)$$



Methylamine Methyl–2–propylamine Triethylamine



Diisobutylamine



Dicyclohexylamine

Figure 15.1. Examples of lower aliphatic amines.

Furthermore, these compounds are rapidly and easily taken into the body by all common exposure routes.[2] The lower amines are corrosive to tissue and can cause tissue necrosis at the point of contact. Sensitive eye tissue is vulnerable to amines. These compounds can have systemic effects upon many organs in the body. Necrosis of the liver and kidneys can occur and exposed lungs can exhibit hemorrhage and edema. The immune system may become sensitized to amines.

Of the lower aliphatic amines, cyclohexylamine and dicyclohexylamine appear to have received the most attention for their toxicities. In addition to its caustic effects on eyes, mucous membranes, and skin, cyclohexylamine acts as a systemic poison. In humans the symptoms of systemic poisoning by this compound include nausea to the point of vomiting, anxiety, restlessness, and drowsiness. It adversely affects the female reproductive system. Dicyclohexylamine produces similar symptoms, but is considered to be more toxic. It is appreciably more likely to be absorbed in toxic levels through the skin, probably because of its less polar, more lipid-soluble nature.

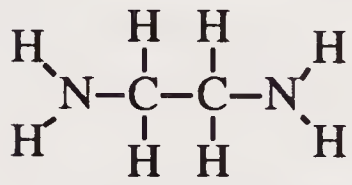
Fatty Amines

Fatty amines are those containing alkyl groups having more than 6 carbon atoms. The commercial fatty amines are synthesized from fatty acids that occur in nature and are used as chemical intermediates. Other major uses of fatty amines and their derivatives include textile chemicals (particularly fabric softeners), emulsifiers for petroleum and asphalt, and flotation agents for ores.

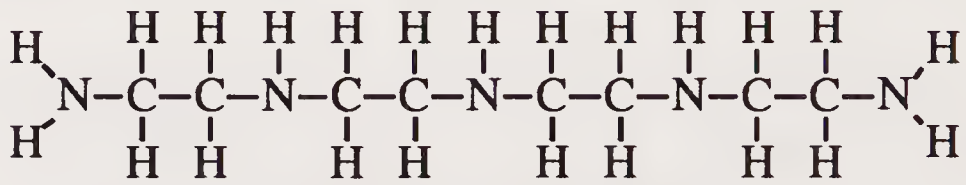
Some attention has been given to the toxicity of octadecylamine, which contains a straight-chain, 18-carbon alkane group, because of its use as an anticorrosive agent in steam lines. There is some evidence to suggest that the compound is a primary skin sensitizer.

Alkyl Polyamines

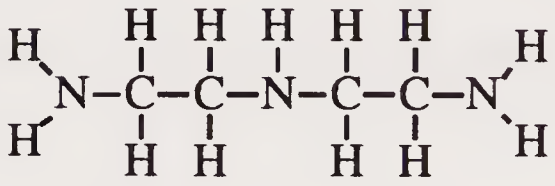
Alkyl polyamines are those in which two or more amino groups are bonded to alkane moieties. The structures of the four most significant of these are shown in Figure 15.2. These compounds have a number of commercial uses, such as for solvents, emulsifiers, epoxy resin hardeners, stabilizers, and starting materials for dye synthesis. They also act as chelating agents; triethylenetetramine is especially effective for that purpose. Largely as a result of their strong alkalinity, the alkyl polyamines tend to be skin, eye, and respiratory tract irritants. The lower homologues are relatively stronger irritants.



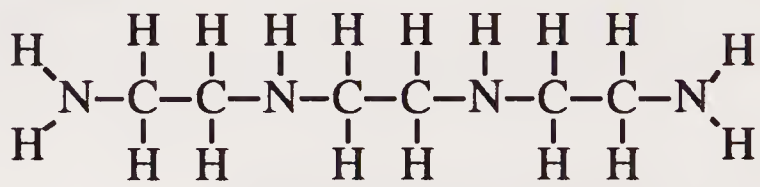
Ethylenediamine



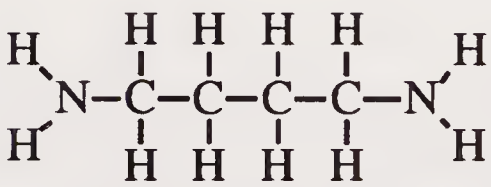
Tetraethylenepentamine



Diethylenetriamine



Triethylenetetramine



Putrescine (odorous product of decayed flesh)

Figure 15.2. Alkyl polyamines in which two or more amino groups are bonded to an alkane group.

Of the common alkyl polyamines, ethylenediamine is the most notable because of its widespread use and toxicity. Although it has a toxicity rating of only 3, it can be very damaging to the eyes and is a strong skin sensitizer. The dihydrochloride and dihydroiodide salts have some uses as human and veterinary pharmaceuticals. The former is administered to acidify urine and the latter as an iodine source. Putrescine is a notoriously odorous naturally occurring substance produced by bacteria in decaying flesh.

Cyclic Amines

Four simple amines in which N atoms are contained in a ring structure are shown in Figure 15.3. Of the compounds shown in Figure 15.3, the first three are liquids under ambient conditions and have the higher toxicity hazards expected of liquid toxicants. All four compounds are colorless in the pure form, but pyrrole darkens upon standing. All are considered to be toxic via the oral, dermal, and inhalation routes. Because of its low volatility, there is little likelihood of inhaling piperazine, except as a dust.

15.3. CARBOCYCLIC AROMATIC AMINES

Carbocyclic aromatic amines are those in which at least one substituent group is an aromatic ring containing only C atoms as part of the ring structure, and with one of the C atoms in the ring bonded directly to the amino group. There are numerous

compounds with many industrial uses in this class of amines. They are of particular toxicological concern because several have been shown to cause cancer in the human bladder, ureter, and pelvis, and are suspected of being lung, liver, and prostate carcinogens.

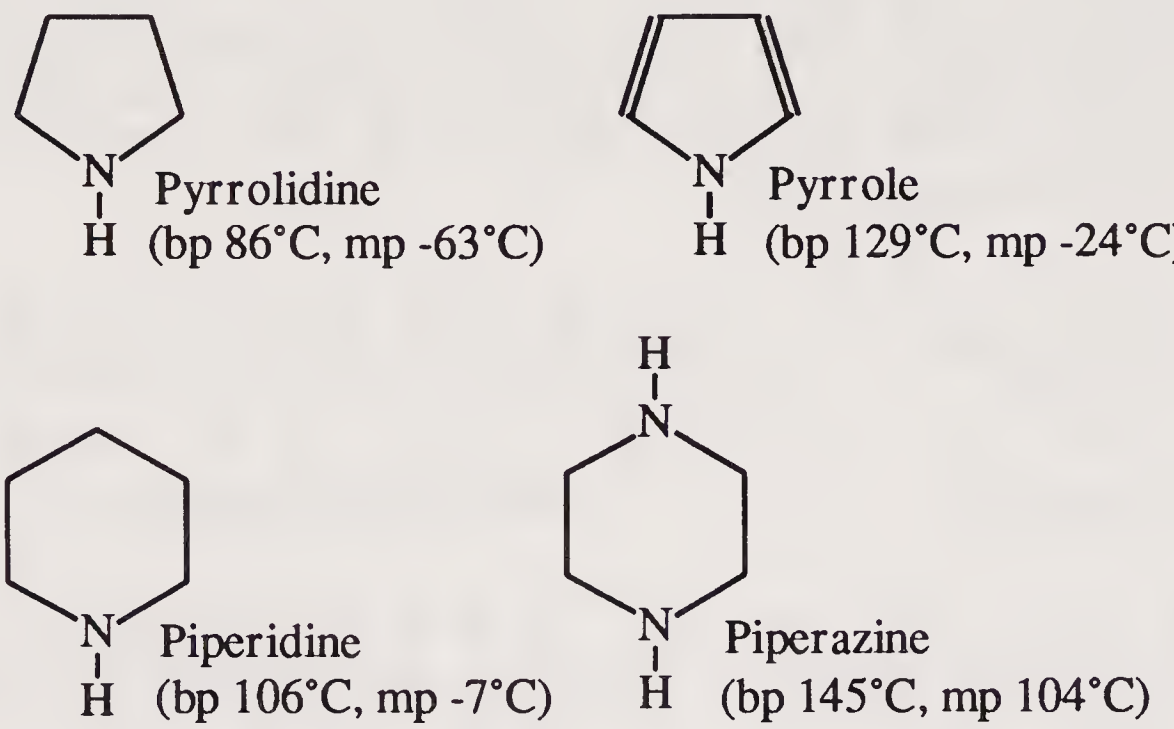
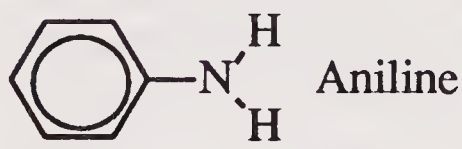


Figure 15.3. Some common cyclic amines.

Aniline

Aniline (structure below) has been an important industrial chemical for many decades. Currently it is most widely used for the manufacture of polyurethanes and rubber, with lesser amounts consumed in the production of pesticides (herbicides, fungicides, insecticides, animal repellants), defoliants, dyes, antioxidants, antidegradants, and vulcanization accelerators.[3] It is also an ingredient of some household products such as polishes (stove and shoe), paints, varnishes, and marking inks. Aniline is a colorless liquid with an oily consistency and distinct odor; aniline freezes at -6.2°C and boils at 184.4°C.



Aniline is considered to be very toxic, with a toxicity rating of 4. It readily enters the body by inhalation, ingestion, and through the skin.[3] In its absorption and toxicological characteristics, aniline resembles nitrobenzene, which is discussed in Section 15.5.

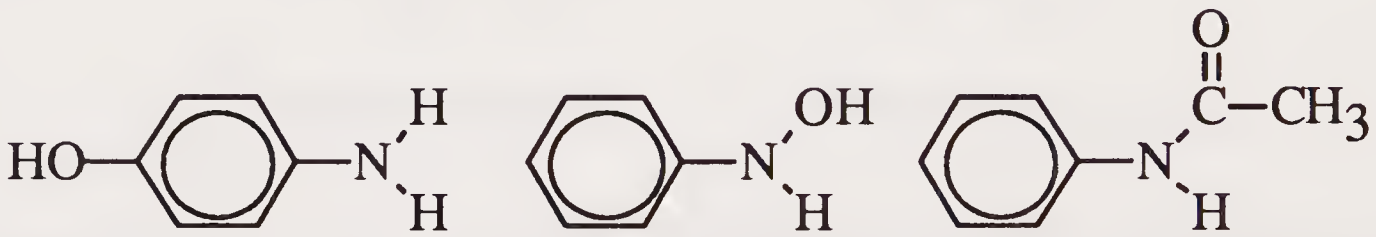
The most common effect of aniline in humans is methemoglobinemia caused by the oxidation of iron(II) in hemoglobin to iron(III) with the result that the hemoglobin can no longer transport oxygen in the body. This condition is characterized by cyanosis and a brown-black color of the blood. Unlike the condition caused by reversible binding of carbon monoxide to hemoglobin, oxygen therapy does not reverse the effects of methemoglobinemia. The effects can be reversed by the action of methemoglobin reductase enzyme as shown by the following reaction:

$$\text{HbFe(III)} \xrightarrow{\text{Methemoglobin reductase}} \text{(HbFe(II)} \qquad (15.3.1)$$

Rodents (mice, rats, rabbits) have a higher activity of this enzyme than do humans, so that extrapolation of rodent experiments with methemoglobinemia to humans is usually inappropriate. Methylene blue can also bring about the reduction of HbFe(III) to HbFe(II) and is used as an antidote for aniline poisoning.

Methemoglobinemia has resulted from exposure to aniline used as a vehicle in indelible laundry-marking inks, particularly those used to mark diapers. This condition was first recognized in 1886, and cases were reported for many decades thereafter. Infants who develop methemoglobinemia from this source suffer a 5–10% mortality rate. The skin of infants (particularly in the genital area, see Section 8.2) is more permeable to aniline than that of adults and infant blood is more susceptible to methemoglobinemia.

Aniline must undergo biotransformation to cause methemoglobinemia because pure aniline does not oxidize iron(II) in hemoglobin to iron(III) in vitro. It is believed that the actual toxic agents formed from aniline are aminophenol and phenyl N-hydroxylamine shown in Figure 15.4. The hepatic detoxification mechanisms for aniline are not very effective. The metabolites of aniline excreted from the body are N-acetyl, N-acetyl-*p*-glucuronide, and N-acetyl-*p*-sulfate products, likewise shown in Figure 15.4.



p –Aminophenol Phenyl N–hydroxylamine N–acetyl metabolite

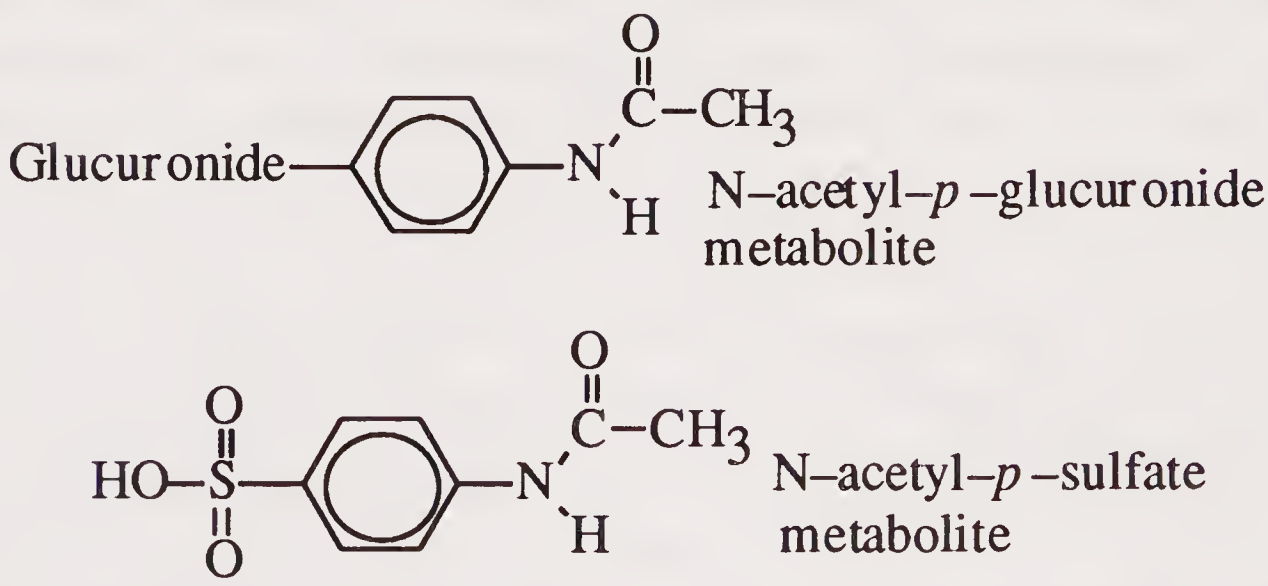
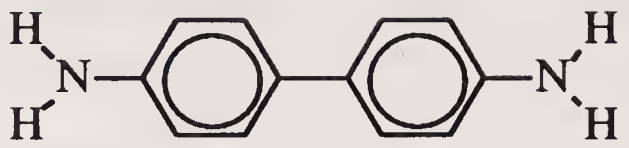


Figure 15.4. Metabolites of aniline that are toxic or excreted.

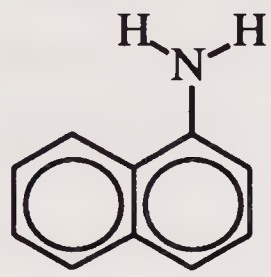
Benzidine

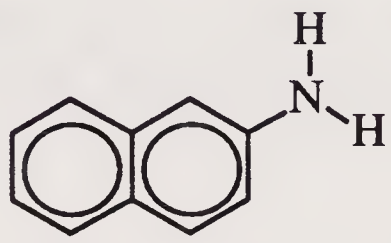
Benzidine, *p*-aminodiphenyl, is a solid compound that can be extracted from coal tar. It is highly toxic by oral ingestion, inhalation, and skin sorption and is one of the few proven human carcinogens. Its systemic effects include blood hemolysis, bone marrow depression, and kidney and liver damage.


Benzidine

Naphthylamines

The two derivatives of naphthalene having single amino substituent groups are **1-naphthylamine** (alpha-naphthylamine) and **2-naphthylamine** (beta-naphthylamine). Both of these compounds are solids (lump, flake, dust) under normal conditions, although they may be encountered as liquids and vapors. Exposure can occur through inhalation, the gastrointestinal tract, or skin. Both compounds are highly toxic and are proven human bladder carcinogens.

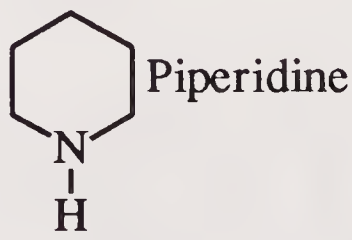

1–Naphthylamine


2–Naphthylamine

15.4. PYRIDINE AND ITS DERIVATIVES

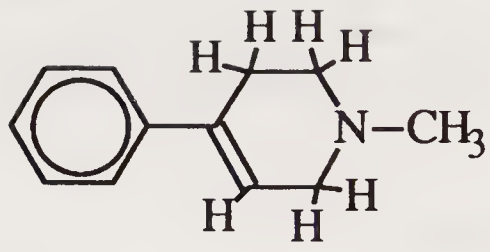
Pyridine is a colorless liquid (mp -42°C, bp 115°C) with a sharp, penetrating odor that can perhaps best be described as "terrible." It is an aromatic compound in which an N atom is part of a 6-membered ring. The most important derivatives of pyridine are the mono-, di-, and trimethyl derivatives; the 2-vinyl and 4-vinyl derivatives; 5-ethyl-2-methylpyridine (MEP); and piperidine (hexahydropyridine, below):


Pyridine


Piperidine

Pyridine and its substituted derivatives are recovered from coal tar. They tend to react like benzene and its analogous derivatives because of the aromatic ring. The major use of pyridine is as an initiator in the process by which rubber is vulcanized. Although considered moderately toxic with a toxicity rating of 3, pyridine has caused fatalities. Symptoms of pyridine poisoning include anorexia, nausea, and fatigue. Its major psychopathological effect is mental depression.

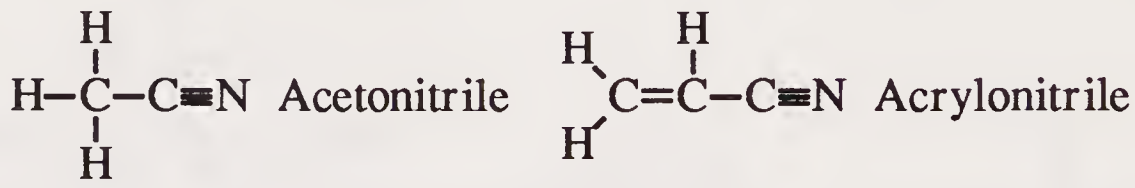
A notably toxic pyridine derivative is 1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP), which has the structural formula shown below:



This compound is a protoxicant that readily crosses the blood-brain barrier, where it is acted upon by the monoamine oxidase enzyme system to produce a positively charged neurotoxic species that cannot readily cross the blood-brain barrier to leave the brain. The result has been described as "selective neuronal death of the dopaminergic neurons in the zona compacta of the substantia nigra."[4] The symptoms of this disorder are very similar to Parkinson's disease, one of several common and devastating neurodegenerative diseases.

15.5. NITRILES

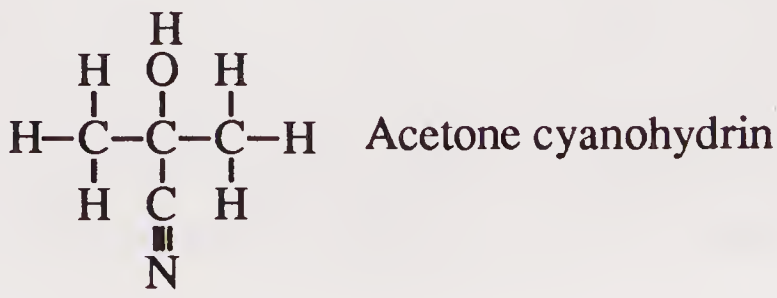
Nitriles are organic analogs of highly toxic hydrogen cyanide, HCN (see Section 12.2), where the H is replaced by a hydrocarbon moiety. The two most common nitriles are acetonitrile and acrylonitrile, shown below:



Acetonitrile (mp -45C, bp 81°C) is a colorless liquid with a mild odor. Because of its good solvent properties for many organic and inorganic compounds and its relatively low boiling point, it has numerous industrial uses, particularly as a reaction medium that can be recovered.[5] Acetonitrile has a toxicity rating of 3–4; exposure can occur via the oral, pulmonary, and dermal routes. Although it is considered relatively safe, it is capable of causing human deaths, perhaps by metabolic release of cyanide.

Acrylonitrile is a colorless liquid with a peach-seed odor used in large quantities in the manufacture of acrylic fibers, dyes, and pharmaceutical chemicals. Containing both nitrile and C=C groups, acrylonitrile is a highly reactive compound with a strong tendency to polymerize. It has a toxicity rating of 5 with a mode of toxic action resembling that of HCN. In addition to ingestion, it can be absorbed through the skin or by inhalation of the vapor. It causes blisters and arythema on exposed skin. During metabolic processes acrylonitrile releases cyanide, and its major acute toxic effect is to inhibit enzymes responsible for respiration in tissue, thereby preventing tissue cells from utilizing oxygen. It is a suspect carcinogen.

Acetone cyanohydrin (structure below) is an oxygen-containing nitrile that should be mentioned because of its extreme toxicity and widespread industrial applications. It is used to initiate polymerization reactions and in the synthesis of foaming



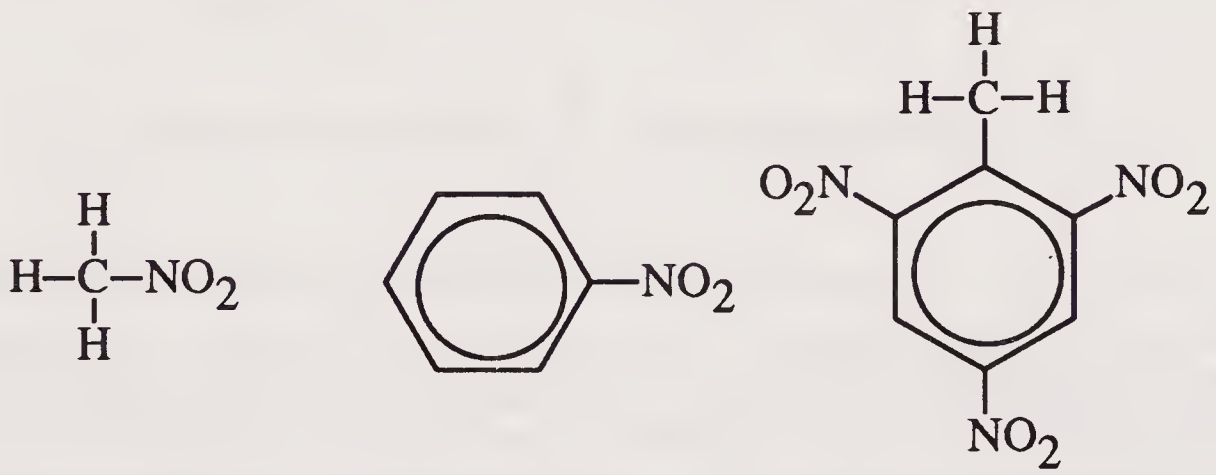
agents, insecticides, and pharmaceutical compounds. A colorless liquid readily absorbed through the skin, in the body it decomposes to hydrogen cyanide, to which it should be considered toxicologically equivalent (toxicity rating 6) on a molecule-per-molecule basis.

15.6. NITRO COMPOUNDS

The structures of three significant **nitro compounds**, which contain the $-NO_2$ functional group, are given in Figure 15.5.

The lightest of the nitro compounds is **nitromethane**, an oily liquid (mp -29°C, bp 101°C). It has a toxicity rating of 3. Symptoms of poisoning include anorexia, diarrhea, nausea, and vomiting. The organs that are most susceptible to damage from it are the kidneys and liver.

Nitrobenzene is a pale yellow oily liquid (mp 5.7°C, bp 211°C) with an odor of bitter almonds or shoe polish (mentioned in Section 3.9 as a symptom of nitrobenzene poisoning). It is produced mainly for the manufacture of aniline. It can enter the body through all routes and has a toxicity rating of 5. Its toxic action is much like that of aniline, including the conversion of hemoglobin to methemoglobin, which deprives tissue of oxygen. Cyanosis is a major symptom of nitrobenzene poisoning.



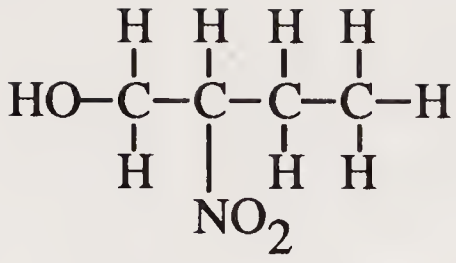
Nitromethane Nitrobenzene Trinitrotoluene (TNT)

Figure 15.5. Some of the more important nitro compounds.

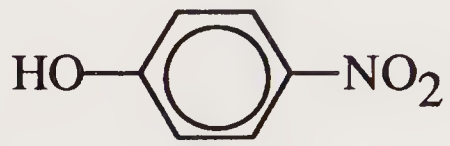
Trinitrotoluene (TNT) is a solid material widely used as a military explosive. It has a toxicity rating of 3–4. It can damage the cells of many kinds of tissue, including those of bone marrow, kidney, and liver. Extensive knowledge of the toxicity of TNT was obtained during the crash program to manufacture huge quantities of it during World War II. Toxic hepatitis developed in some workers under age 30 exposed to TNT systemically, whereas aplastic anemia was observed in some older victims of exposure. In the U.S. during World War II, 22 cases of fatal TNT poisoning were documented[6] (many more people were blown up during manufacture and handling).

Nitro Alcohols and Nitro Phenols

Nitro alcohols are nonaromatic compounds containing both –OH and $-NO_2$ groups. A typical example of such a compound is **2-nitro-1-butanol**, shown below. These compounds are used in chemical synthesis to introduce nitro functional groups or (after reduction) amino groups onto molecules. They tend to have low volatilities and moderate toxicities. The aromatic nitrophenol, ***p*-nitrophenol**, is an industrially important compound with toxicological properties resembling those of phenol and nitrobenzene.



2–Nitro–1–butanol

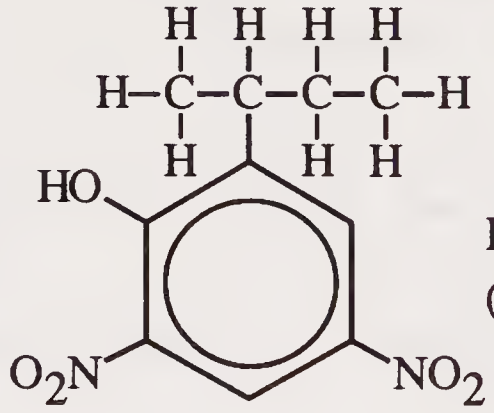


p– Nitrophenol

Dinoseb

Dinoseb is a nitrophenolic compound once widely used as a herbicide and plant desiccant that is noted for its toxic effects. The chemical name of this compound is 4,6-dinitro-2-*sec*-butylphenol, and its structure is shown on the next page. Several derivatives of it have been marketed as herbicides.

Dinoseb has a toxicity rating of 5 and is strongly suspected of causing birth defects in the children of women exposed to it early in pregnancy, as well as sterility in exposed men. In October 1986 the Environmental Protection Agency imposed an emergency ban on the use of the chemical, which was partially rescinded for the northwestern U. S. by court order early in 1987. In June, 1988, the E.P.A. allowed limited use of dinoseb through 1989, primarily in the northwestern U. S. for use on peas, chickpeas, lentils, and raspberry crops.[7]



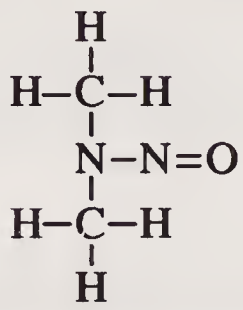
Dinoseb
(4,6–dinitro–2–*sec*–butylphenol)

15.7. NITROSAMINES

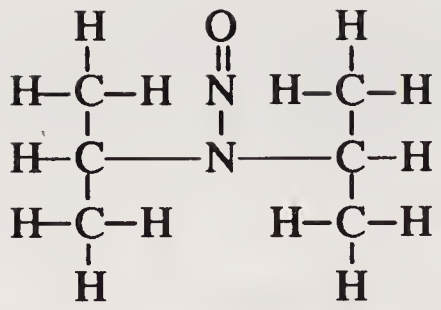
N-nitroso compounds, commonly called **nitrosamines**, are a class of compounds containing the N–N=O functional group. They are of particular toxicological significance because most that have been tested have been shown to be carcinogenic. The structures of some nitrosamines are shown in Figure 15.6.

Some nitrosamines have been used as solvents and as intermediates in chemical synthesis. They have been found in a variety of materials to which humans may be exposed, including beer, whiskey, and cutting oils used in machining.

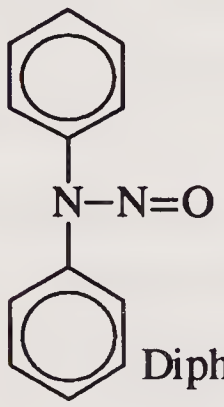
By far the most significant toxicological effect of nitrosamines is their carcinogenicity, which may result from exposure to a single large dose or from chronic exposure to relatively small doses. Different nitrosamines cause cancer in different organs. The first nitrosamine extensively investigated for carcinogenicity was dimethylnitrosamine, once widely used as an industrial solvent. It was known to cause liver damage and jaundice in exposed workers[8] and studies starting in the 1950s subsequently revealed its carcinogenic nature. Dimethylnitrosamine was found to alkylate DNA, which is the mechanism of its carcinogenicity (the alkylation of DNA as a cause of cancer is noted in the discussion of biochemistry of carcinogesis in Section 9.3).



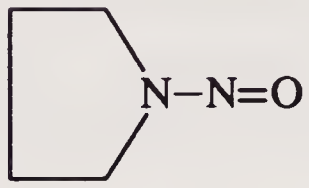
Dimethylnitrosamine
(N–nitrosodimethylamine)



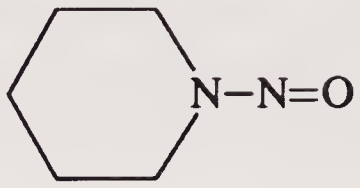
Diisopropylnitrosamine



Diphenylnitrosamine



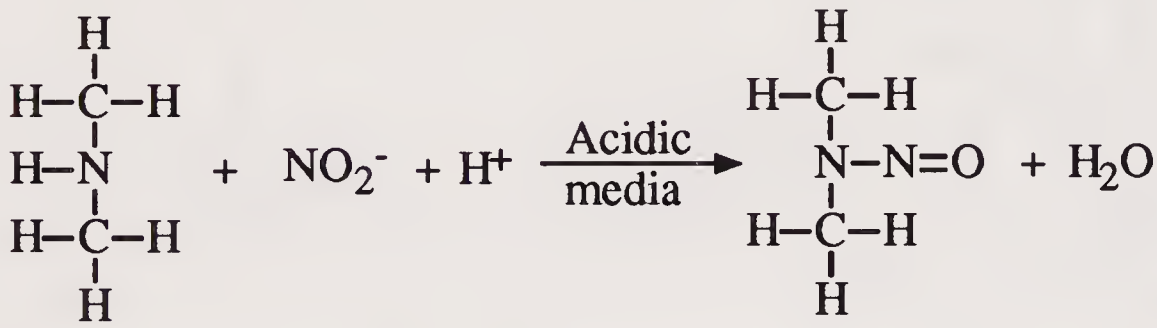
N–nitrosopyrrole



N–nitrosopiperidine

Figure 15.6. Examples of some important nitrosamines.

The common means of synthesizing nitrosamines is the low-pH reaction of a secondary amine and nitrite as shown by the following example:

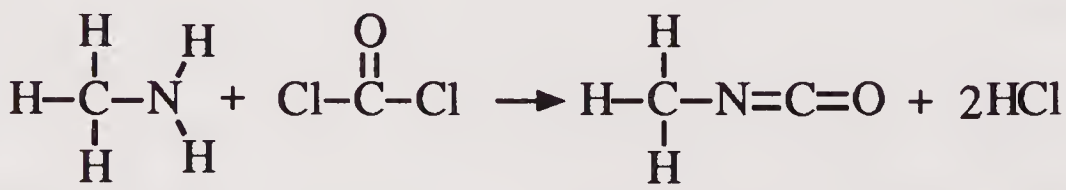


(15.7.1)

The possibility of this kind of reaction occurring in vivo and producing nitrosamines in the acidic medium of the stomach is some cause for concern over nitrites in the diet. Because of this possibility nitrite levels have been reduced substantially in foods such as cured meats that formerly contained relatively high nitrite levels.

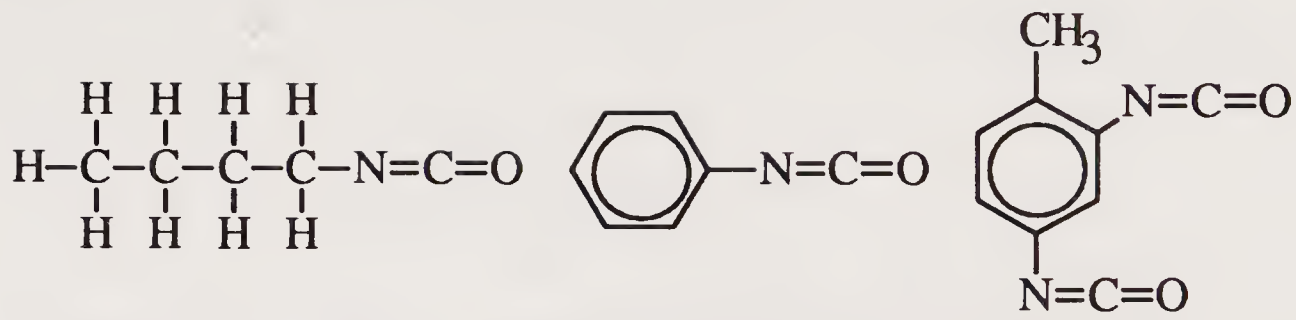
15.8. ISOCYANATES AND METHYL ISOCYANATE

Isocyanates are compounds with the general formula R–N=C=O. They have numerous uses in chemical synthesis, particularly in the manufacture of polymers with carefully tuned specialty properties. Methyl isocyanate is a raw material in the manufacture of carbaryl insecticide. Methyl isocyanate (like other isocyanates) can be synthesized by the reaction of a primary amine with phosgene in a moderately complex process represented by Reaction 15.8.1 and structures of three significant isocyanates are given in Figure 15.7.



Methylamine Phosgene Methyl isocyanate

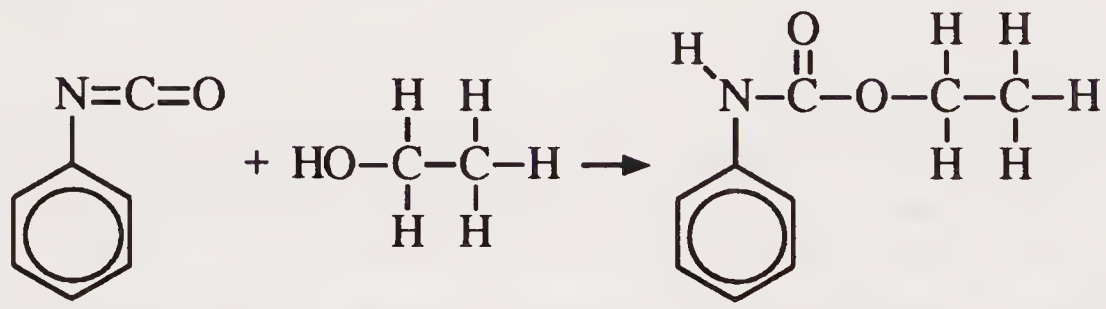
(15.8.1)



n –Butyl isocyanate Phenyl isocyanate 2,4–Toluene diisocyanate

Figure 15.7. Examples of isocyanate compounds.

Both chemically and toxicologically, the most significant property of isocyanates is the high chemical reactivity of the isocyanate functional group. Industrially, the most significant such reaction is with alcohols to yield urethane (carbamate) compounds, as shown by Reaction 15.5. Multiple isocyanate and –OH groups in the reactant molecules enable formation of polymers. The chemical versatility of isocyanates and the usefulness of the products — such as polymers and pesticides — from which they are made have resulted in their widespread industrial production and consumption.



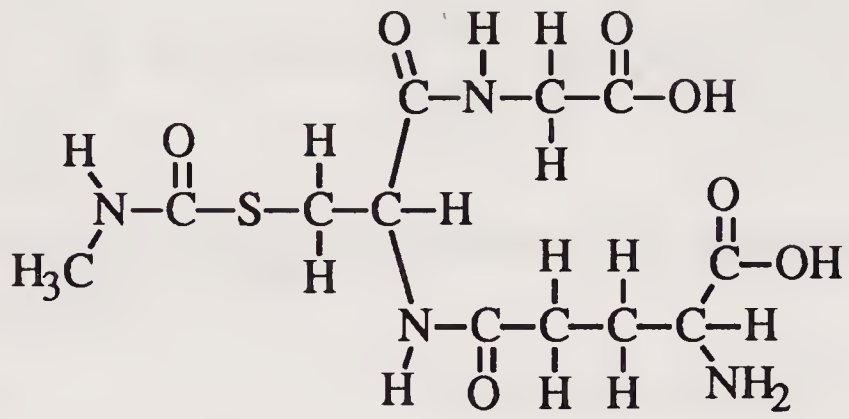
(15.8.2)

Phenyl isocyanate A carbamate or urethane compound

Methyl isocyanate was the toxic agent involved in the most catastrophic industrial accident of all time, which took place in Bhopal, India, on December 2, 1984. This accident occurred when water got into a tank of methyl isocyanate causing an exothermic reaction that built up pressure and ruptured a safety valve. This resulted in the release to the atmosphere of 30-40 tons of the compound over an approximately 3 hour period. Subsequent exposure of people resulted in approximately 3,500 deaths and almost 100,000 injuries.

Most of the deaths at Bhopal resulted from devastating pulmonary edema which caused respiratory failure leading to cardiac arrest. The major debilitating effects of methyl isocyanate on the Bhopal victims were on the lungs, with survivors suffering long-term shortness of breath and weakness from lung damage. However, victims also suffered symptoms of nausea and bodily pain and numerous toxic effects have been observed in the victims. Changes in the immune systems (effects on numbers of T cells, T-helper cells, and lympophyte mitogenesis responses) of victims exposed to methyl isocyanate were also observed.[9] The tendency of the compound to function as a systemic poison was somewhat surprising in view of its chemical reactivity with water — its half-life is only about 2 minutes in aqueous solution — and appears to be the result of its ability to bind with small-molecule proteins and peptides. The most prominent among these is glutathione, a tripeptide described as a conjugating agent in Section 4.6;[10] binding to hemoglobin may also be possible. Isocyanate reacts

reversibly with –SH groups on glutathione, probably to form S-(N-methylcarbamoyl)-glutathione (structure below) :



This complex can be transported to various organs in the body where it releases isocyanate.

15.9. PESTICIDAL COMPOUNDS

A large number of organic compounds used as pesticides contain nitrogen. Space does not permit a detailed discussion of such compounds, but two general classes of them are cited here.

Carbamates

Pesticidal organic derivatives of carbamic acid, for which the formula is shown in Figure 15.8, are known collectively as **carbamates**. Carbamate pesticides have been widely used because some are more biodegradable than the formerly popular organochlorine insecticides and have lower dermal toxicities than most common organophosphate pesticides.

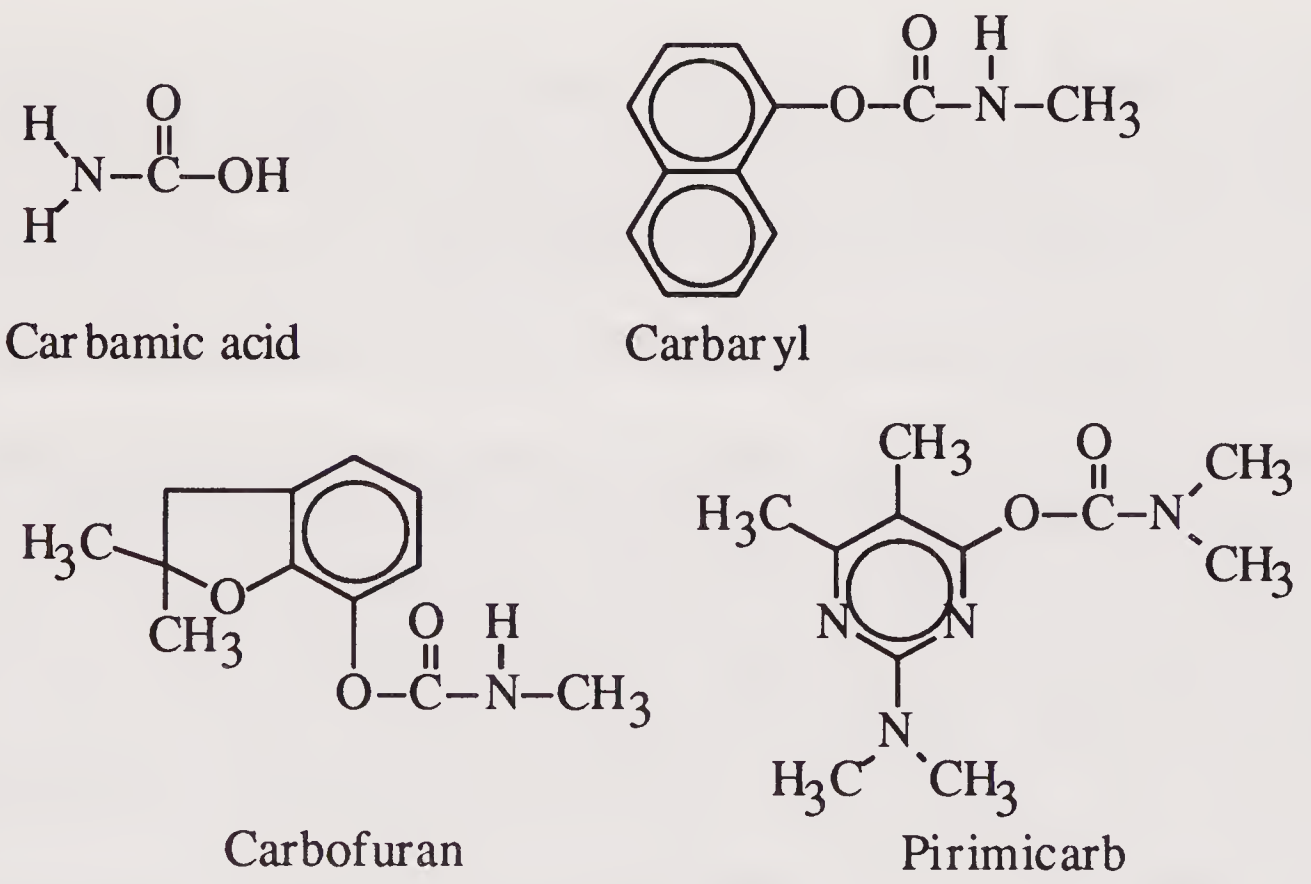


Figure 15.8. Carbamic acid and three insecticidal carbamates.

Carbaryl has been widely used as an insecticide on lawns or gardens. It has a low toxicity to mammals. **Carbofuran** has a high water solubility and acts as a plant systemic insecticide. It is taken up by the roots and leaves of plants so that insects

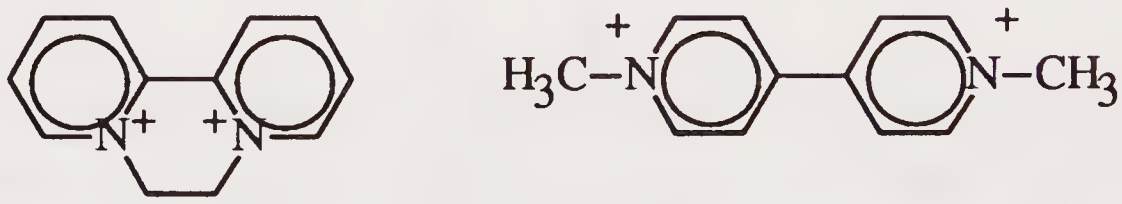
feeding on the plant material are poisoned by the carbamate compound in it.

Pirimicarb has been widely used in agriculture as a systemic aphicide. Unlike many carbamates, it is rather persistent, with a strong tendency to bind to soil.[11]

The toxic effects of carbamates to animals are due to the fact that these compounds inhibit acetylcholinesterase. Unlike some of the organophosphate insecticides (see Chapter 18), they do so without the need for undergoing a prior biotransformation and are therefore classified as direct inhibitors. Their inhibition of acetylcholinesterase is relatively reversible. Loss of acetylcholinesterase inhibition activity may result from hydrolysis of the carbamate ester, which can occur metabolically. In general, carbamates have a wide range between a dose that causes onset of poisoning symptoms and a fatal dose (see discussion of dose-response in Section 8.3). Although pirimicarb has a high systemic mammalian toxicity, its effects are mitigated by its low tendency to be absorbed through the skin.

Bipyridilium Compounds

As shown by the structures in Figure 15.9, a bipyridilium compound contains 2 pyridine rings per molecule. The two important pesticidal compounds of this type are the herbicides **diquat** and **paraquat**; other members of this class of herbicides include chlormequat, morfamquat, and difenzoquat. Applied directly to plant tissue, these compounds rapidly destroy plant cells and give the plant a frost-bitten appearance. However, they bind tenaciously to soil, especially the clay mineral fraction, which results in rapid loss of herbicidal activity so that sprayed fields can be planted within a day or two of herbicide application.



Diquat Paraquat

Figure 15.9. The two major bipyridilium herbicides (cation forms).

Paraquat, which was registered for use in 1965, is the most used of the bipyridilium herbicides. With a toxicity rating of 5, it is reputed to have "been responsible for hundreds of human deaths."[12] Exposure to fatal or dangerous levels of paraquat can occur by all pathways, including inhalation of spray, skin contact, ingestion, and even suicidal hypodermic injections. Despite these possibilities and its widespread application, paraquat is used safely without ill effects when proper procedures are followed.

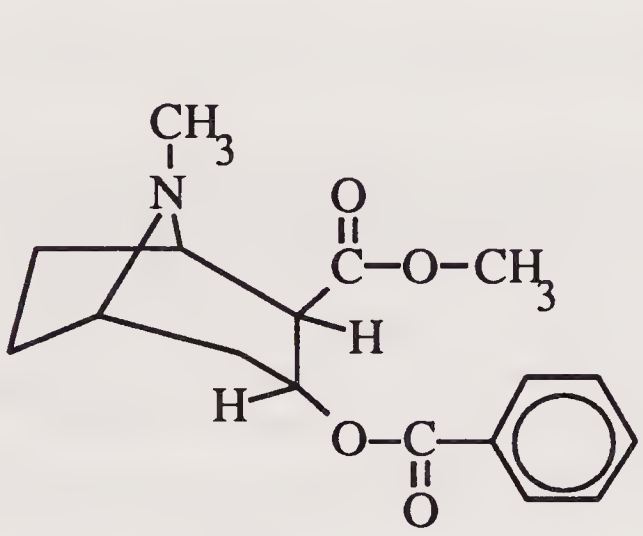
Because of its widespread use as a herbicide, the possibility exists of substantial paraquat contamination of food.[13] Drinking water contamination by paraquat has also been observed. The chronic effects of exposure to low levels of paraquat over extended periods of time are not well known. Acute exposure of animals to paraquat aerosols causes pulmonary fibrosis, and the lungs are affected even when exposure is through nonpulmonary routes. Paraquat affects enzyme activity. Acute exposure may cause variations in the levels of catecholamine, glucose, and insulin.

Although paraquat can be corrosive at the point of contact, it is a systemic poison that is devastating to a number of organs. The most prominent initial symptom of poisoning is vomiting, sometimes followed by diarrhea. Within a few days, dyspnea,

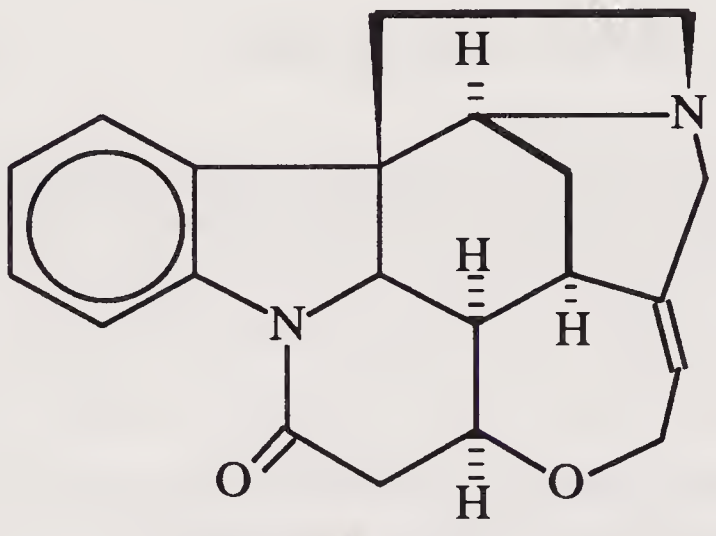
cyanosis, and evidence of impairment of the kidneys, liver, and heart become obvious. In fatal cases, the lungs develop pulmonary fibrosis, often with pulmonary edema and hemorrhaging.

15.10. ALKALOIDS

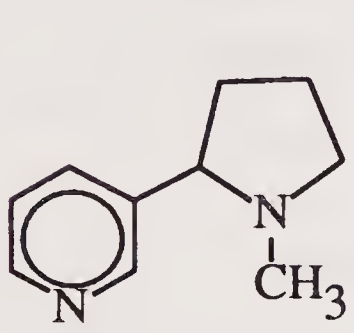
Alkaloids are compounds of biosynthetic origin that contain nitrogen, usually in a heterocyclic ring. These compounds are produced by plants in which they are usually present as salts of organic acids.[14] They tend to be basic and to have a variety of physiological effects. One of the more notorious alkaloids is cocaine and alkaloidal strychnine is a deadly poison. The structural formulas of these compounds and three other alkaloids are given in Figure 15.10.



Cocaine

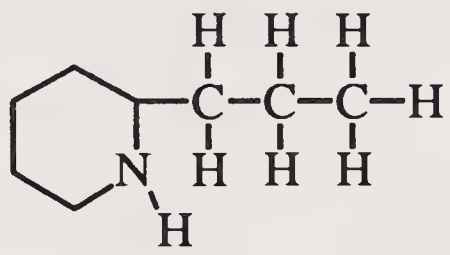


Strychnine



Nicotine

Caffeine



Coniine

Figure 15.10. Structural formulas of typical alkaloids.

Among the alkaloids are some well-known (and dangerous) compounds. Nicotine is an agent in tobacco that has been described as "one of the most toxic of all poisons and (it) acts with great rapidity."[15] In 1988 the U.S. Surgeon General declared nicotine to be an addictive substance. Coniine is the major toxic agent in poison hemlock (see Chapter 19). Alkaloidal strychnine is a powerful, fast-acting convulsant. Cocaine is currently the illicit drug of greatest concern. Quinine and sterioisomeric quinidine are alkaloids that are effective antimalarial agents. Like some other alkaloids, caffeine contains oxygen. It is a stimulant that can be fatal to humans in a dose of about 10 grams.

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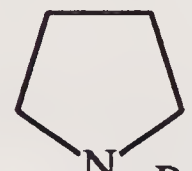
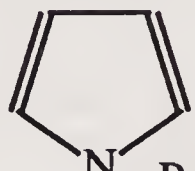
QUESTIONS

1. Describe the sense in which amines may be regarded as derivatives of ammonia, NH_3. Distinguish among primary, secondary, and tertiary amines.

2. How are the compounds shown in the following figure characterized or described? What are their main toxicological characteristics?

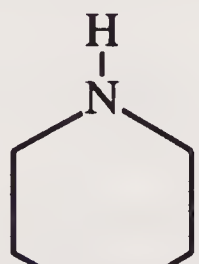


Pyrrolidine
(bp 86°C, mp -63°C)



Pyrrole
(bp 129°C, mp -24°C)

Piperidine
(bp 106°C, mp -7°C)



Piperazine
(bp 145°C, mp 104°C)

3. What is the structural formula of aniline? What are its major uses? Why is human exposure to aniline likely to be relatively common? How is aniline taken into the body?

4. Which other nitrogen-containing nonamine organonitrogen compound does aniline most resemble in its toxicological characteristics? What is its most common manifestation of toxicity? How does this affect the subject?

5. What are fatty amines? From which raw materials that occur in nature are they commonly synthesized?

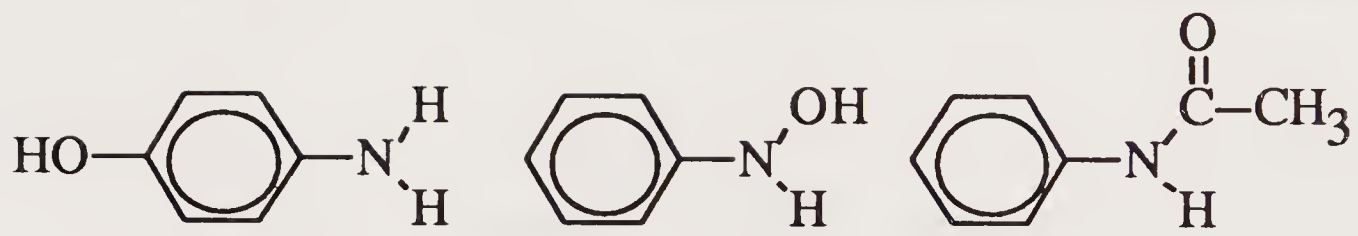
6. What are alkyl polyamines?

7. Of the following, the statement that is **not** true is: (a) The lower amines are corrosive to tissue and can cause tissue necrosis at the point of contact. (b) The most common toxic effect of the lower aliphatic amines is that they cause methemoglobinemia. (c) Sensitive eye tissue is vulnerable to amines. (d) Necrosis of the liver and kidneys can occur from exposure to amines, and exposed lungs can exhibit hemorrhage and edema. (e) The immune system may become sensitized to amines.

8. Explain what the reaction below shows about the toxicity of amines.

$$R_3N + H_2O \rightarrow R_3NH^+ + OH^-$$

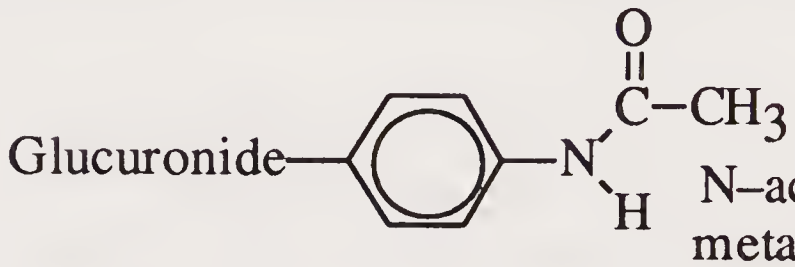
9. Consider the compounds with the structural formulas shown below. Which of these are believed to be the actual toxic agents involved in aniline poisoning? Which are the forms eliminated from the body?



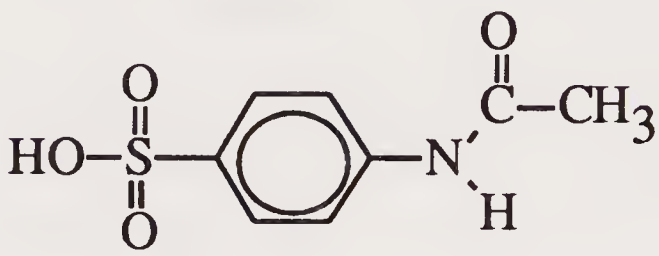
p –Aminophenol

Phenyl N–hydroxylamine

N–acetyl metabolite



N–acetyl–*p* –glucuronide metabolite



N–acetyl–*p* –sulfate metabolite

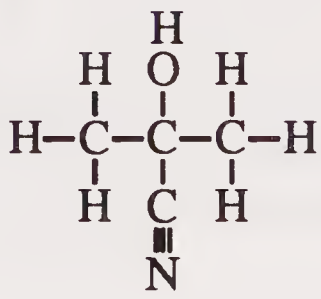
10. What are the two naphthylamines? How does exposure to these compounds occur? What is their toxic effect of most concern?

11. What is the structural formula of pyridine. Is it highly toxic? In what respect is it like benzene?

12. Of which common inorganic compound are nitriles analogs. Which common natural product produces this highly toxic inorganic compound? How does this occur?

13. Acetonitrile is not highly toxic. What does this say about its toxicological chemistry and metabolism in the body?

14. What are two reasons that the compound below is of particular concern?



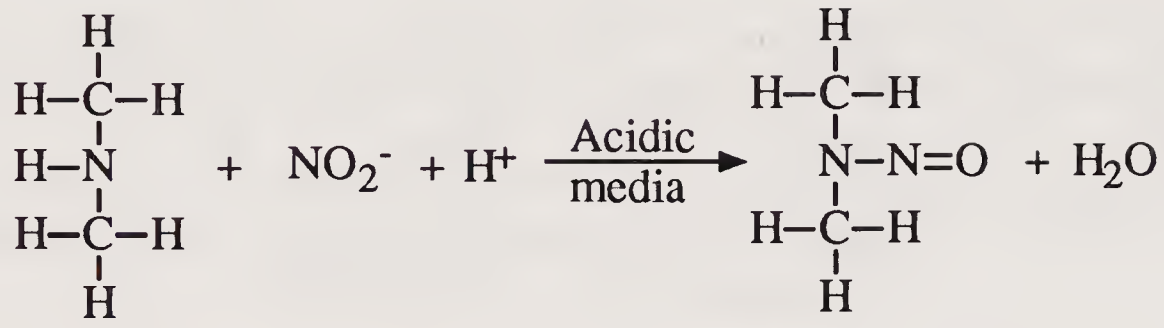
Acetone cyanohydrin

17. Of which class of compounds is the N–N=O functional group characteristic? What is their most importanat toxicological characteristic?

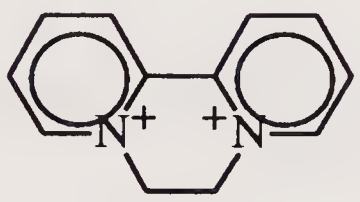
16. Which class of compounds has the general formula R–N=C=O? Which of these is most notorious for an incident of poisoning? What happened?

17. What is the general formula of carbamates? Of which inorganic compound are they derivatives? How are carbamates used?

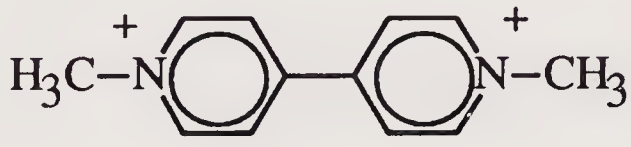
18. What does the reaction below illustrate?



19. For what purposes are the compounds below used? What are their toxicity characteristics?

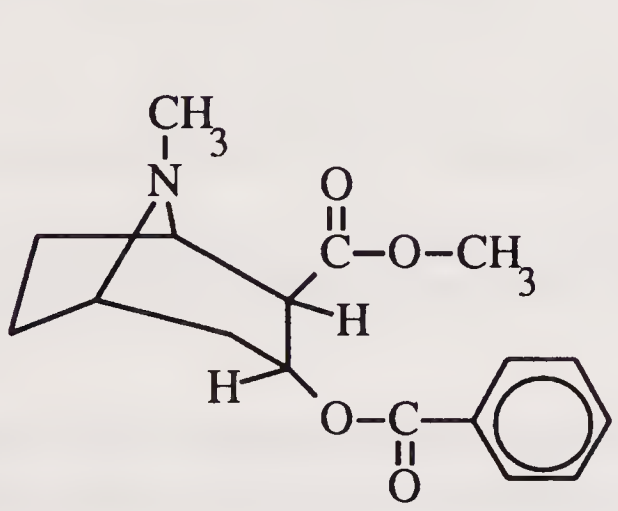


Diquat

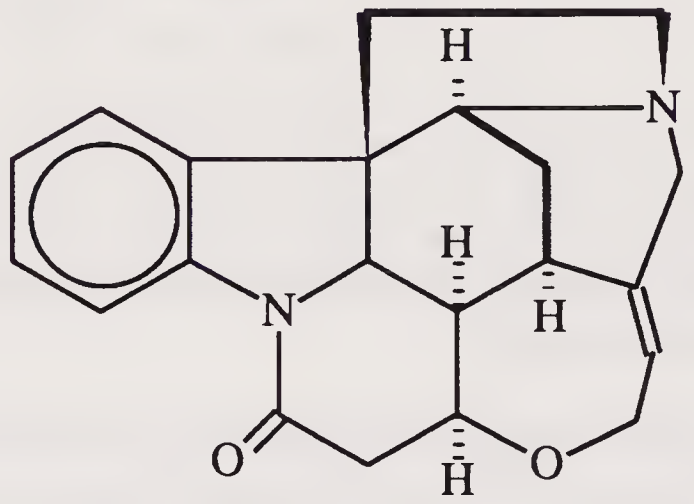


Paraquat

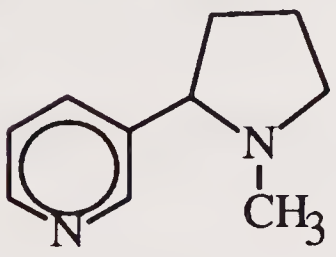
20. What kinds of compounds are the following? What are their sources? What may be said about their toxicities?



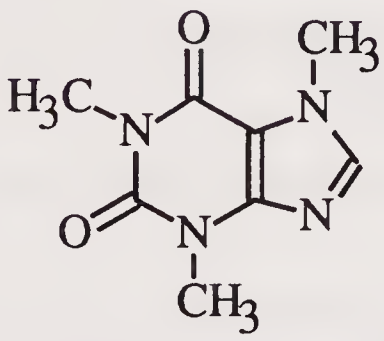
Cocaine



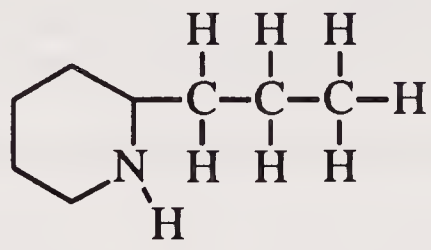
Strychnine



Nicotine



Caffeine



Coniine

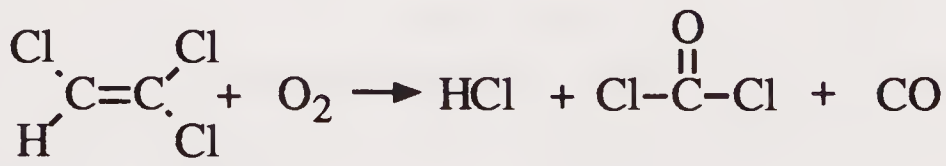
16

Organohalide Compounds

16.1. INTRODUCTION

Organohalide compounds are halogen–substituted hydrocarbons with a wide range of physical and chemical properties produced in large quantities as solvents, heat transfer fluids, chemical intermediates, and for other applications.[1] They may be saturated (alkyl halides) unsaturated (alkenyl halides), or aromatic (aryl halides). The major means of synthesizing organohalide compounds were shown by examples in Chapter 13 and include substitution halogenation, addition halogenation, and hydrohalogenation reactions illustrated in Equations 13.4.2, 13.4.3, and 13.4.4, respectively. Most organohalide compounds are chlorides (chlorocarbons and chlorohydrocarbons), but they also include compounds of fluorine, bromine, and iodine, as well as mixed halides, such as the chlorofluorocarbons.

The chemical reactivities of organohalide compounds vary over a wide range. The alkyl halides are generally low in reactivity, but may undergo pyrolysis in flames to liberate noxious products, such as HCl gas. Alkenyl halides may be oxidized, which in some cases produces highly toxic phosgene, as shown by the following example.



Trichloroethylene Phosgene (16.1.1)

The toxicities of organohalide compounds vary widely. For example, dichlorodifluoromethane ("Freon–12") is generally regarded as having a low toxicity, except for narcotic effects and the possibility of asphyxiation at high concentration. Vinyl chloride (see Section 16.3), however, is a known human carcinogen. The polychlorinated biphenyls are highly resistant to biodegradation and are extremely persistent in the environment.

16.2. ALKYL HALIDES

Alkyl halides are compounds in which halogen atoms are substituted for hydrogen on an alkyl group. The structural formulas of some typical alkyl halides are given in Figure 16.1. Most of the commercially important alkyl halides are derivatives of alkanes of low molecular mass.

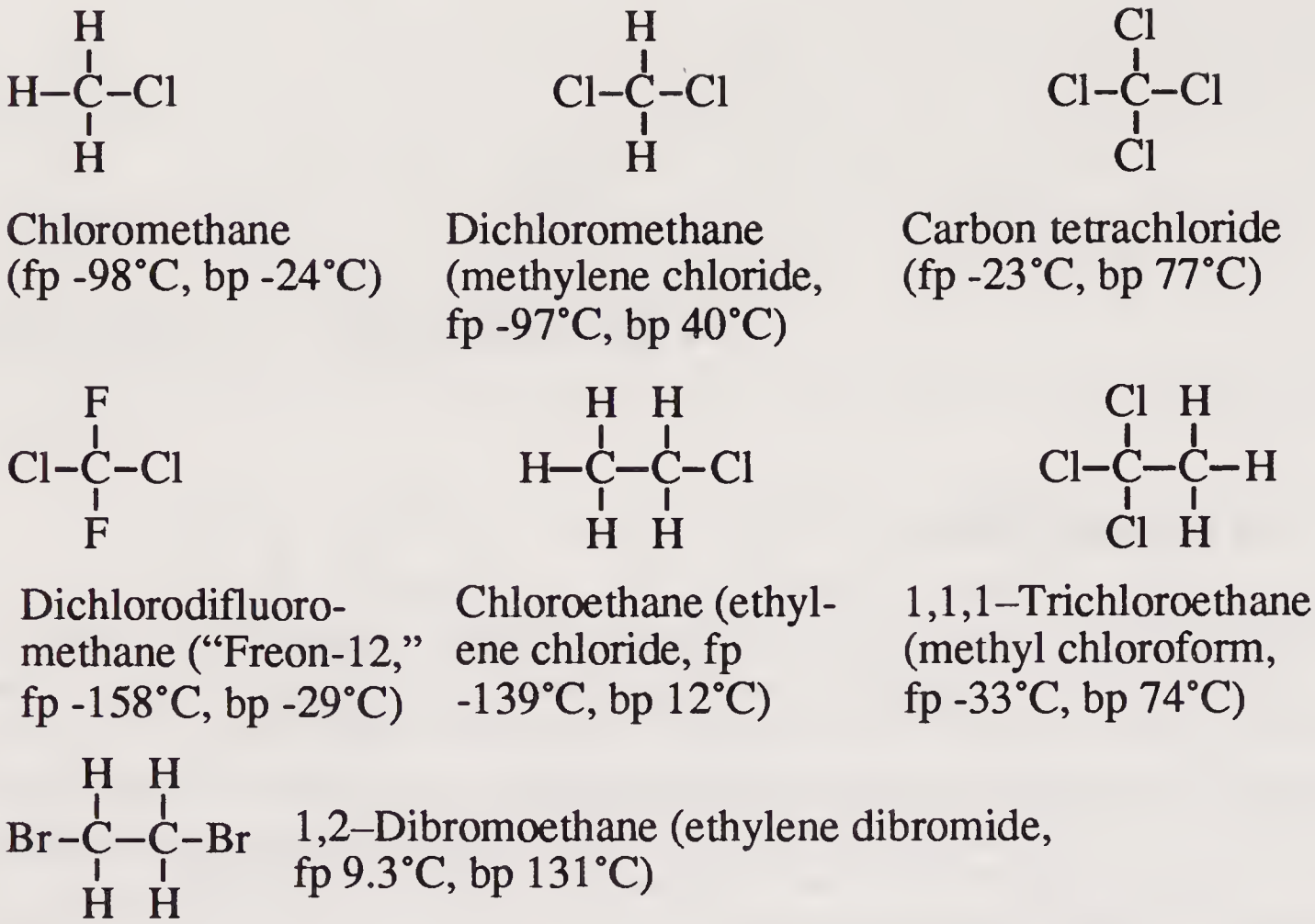


Figure 16.1. Some typical low–molecular–mass alkyl halides.

A brief discussion of the uses of the compounds listed in Figure 16.1 will provide an idea of the versatility of the alkyl halides. Volatile chloromethane (methyl chloride) was once widely used as a refrigerant fluid and aerosol propellant; most of it now is consumed in the manufacture of silicones. Dichloromethane is a volatile liquid with excellent solvent properties for nonpolar organic solutes. It has been applied as a solvent for the decaffeination of coffee and in paint strippers, as a blowing agent in urethane polymer manufacture, and to depress vapor pressure in aerosol formulations. Once commonly sold as a solvent and stain remover, carbon tetrachloride is now largely restricted to uses as a chemical intermediate under controlled conditions, primarily to manufacture chlorofluorocarbon refrigerant fluid compounds, of which dichlorodifluoromethane is an example. Chloroethane is an intermediate in the manufacture of tetraethyllead and is an ethylating agent in chemical synthesis. One of the more common industrial chlorinated solvents is 1,1,1–trichloroethane. Insecticidal 1,2–dibromethane has been used in large quantities to fumigate soil, grain, and fruit and as a lead scavenger in leaded gasoline. It is an effective solvent for resins, gums, and waxes and serves as a chemical intermediate in the syntheses of some pharmaceutical compounds and dyes.

Toxicities of Alkyl Halides

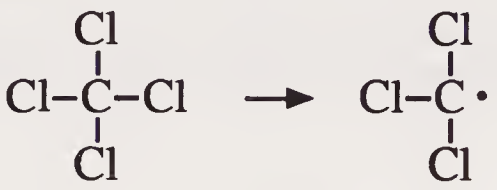
The toxicities of alkyl halides vary a great deal with the compound. Although some of these compounds have been considered to be almost completely safe in the past, there is a marked tendency to regard each with more caution as additional health and animal toxicity study data become available. Perhaps the most universal toxic effect of alkyl halides is depression of the central nervous system. Chloroform, $CHCl_3$, was the first widely used general anesthetic, although many surgical patients were accidentally killed by it.

Carbon Tetrachloride and Lipid Peroxidation

Of all the alkyl halides, carbon tetrachloride has the most notorious record of human toxicity, especially for its toxic effects on the liver.[2] For many years it was widely used in consumer products as a degreasing solvent, in home fire extinguishers, and other applications. However, numerous toxic effects, including some fatalities, were observed and in 1970 the U. S. Food and Drug Administration (FDA) banned the sale of carbon tetrachloride and formulations containing it for home use.

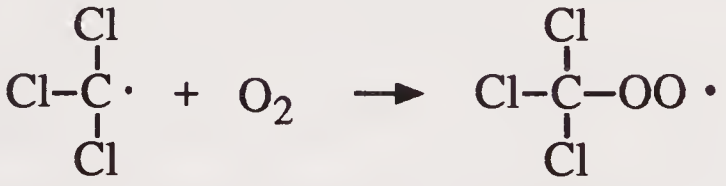
Carbon tetrachloride is toxic through both inhalation and ingestion. Toxic symptoms from inhalation tend to be associated with nervous system, whereas those from ingestion often involve the gastrointestinal tract and liver. Both the liver and kidney may be substantially damaged by carbon tetrachloride.

The biochemical mechanism of carbon tetrachloride toxicity has been investigated in detail.[3] The cytochrome P-450-dependent monooxygenase system acts on CCl_4 in the liver to produce the $Cl_3C\cdot$ free radical.



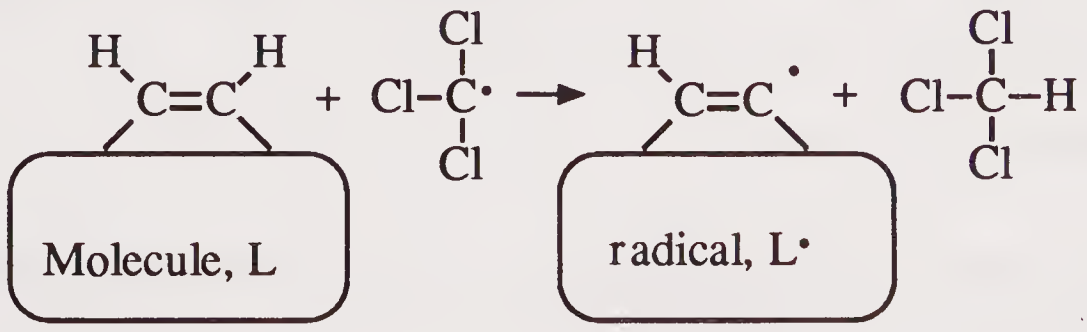
(16.2.1)

This product can combine with molecular oxygen to yield highly reactive $Cl_3COO\cdot$ radical.



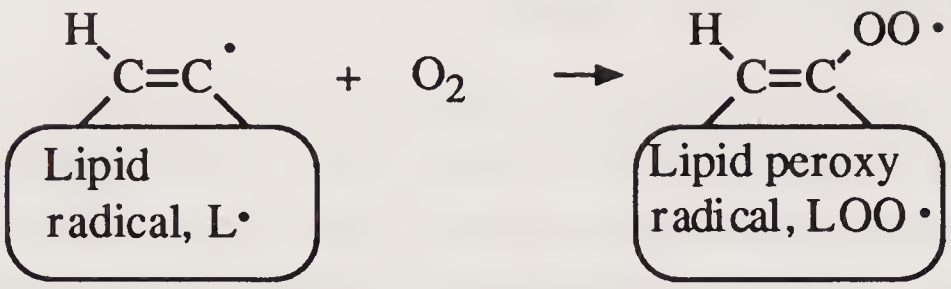
(16.2.2)

These radical species, along with others produced from their subsequent reactions, can react with biomolecules, such as proteins and DNA. The most damaging such reaction is **lipid peroxidation**, a process that involves the attack of chemically active species on unsaturated lipid molecules, followed by oxidation of the lipids through a free radical mechanism. It occurs in the liver and is a major mode of action of some hepatotoxicants, which can result in major cellular damage.[4] The mechanism of lipid peroxidation is not known with certainty. It is known that the methylene hydrogens attached to doubly–bonded carbon atoms in lipid molecules are subject to abstraction by free radicals as shown by the following:



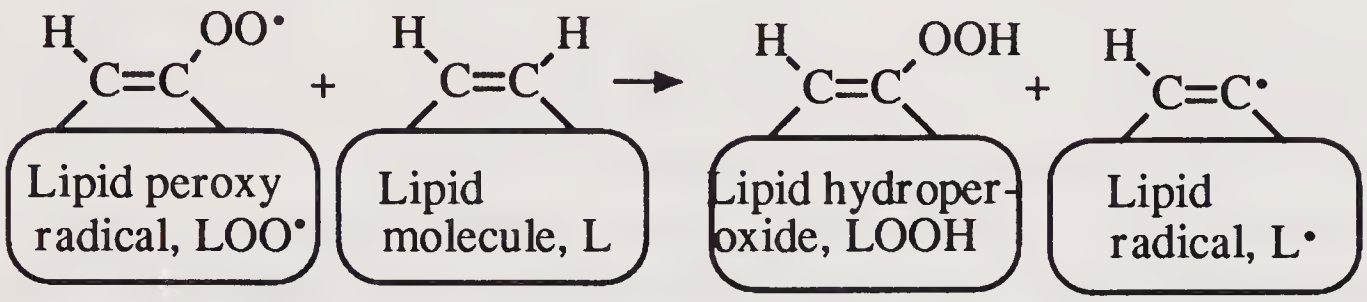
(16.2.3)

Reaction of the lipid radical with molecular oxygen yields peroxy radical species.



(16.2.4)

This species can initiate chain reaction sequences with other molecules as follows:



(16.2.5)

Once inititated, chain reactions such as these continue and cause massive alteration of the lipid molecules. The LOOH molecules are unstable and decompose to yield additional free radicals. The process terminates when free radical species combine with each other to form stable species.

Other Alkyl Halides

Dichloromethane has long been regarded as one of the least acutely toxic alkyl halides. More volatile than most commonly used solvents, this compound has been used in large quantities as a degreasing solvent, paint remover, aerosol propellant additive, and grain fumigant. As a result, human exposure has been relatively high. In 1987, however, the U. S. Occupational Safety and Health Administration (OSHA) considered a move to substantially lower the permissible human exposure limit on evidence that dichloromethane is a probable human carcinogen.[5]

Generally considered to be among the least toxic of the alkyl halides, 1,1,1–trichloroethane is widely used. For that reason, any toxic effects are of concern. A much more toxic alkyl halide is 1,2-dibromoethane. It is a severe irritant, damages the lungs when inhaled in high concentrations, and is a potential human carcinogen. Because of these effects, its use has been severely curtailed.

16.3. ALKENYL HALIDES

The **alkenyl**, or **olefinic organohalides** contain at least one halogen atom and at least one carbon–carbon double bond. The most significant of these are the lighter chlorinated compounds, such as those illustrated in Figure 16.2.

Uses of Alkenyl Halides

The alkenyl halides are used for numerous purposes. Some of the more important applications are discussed here.

Vinyl chloride is consumed in large quantities to manufacture polyvinyl chloride plastic, a major polymer in pipe, hose, wrapping, and other products. Vinyl chloride is a highly flammable volatile gas with a sweet, not unpleasant odor.

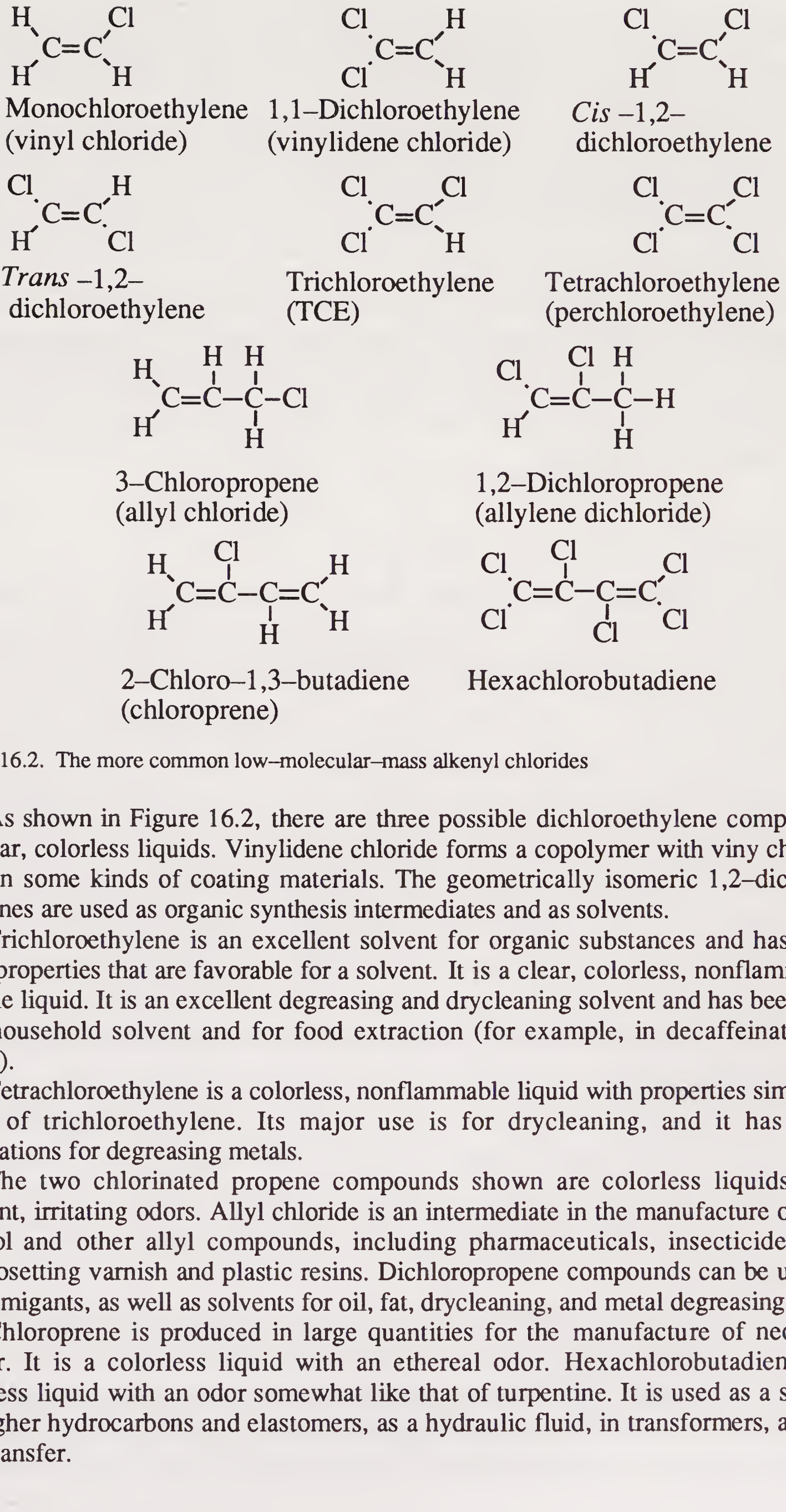


Figure 16.2. The more common low–molecular–mass alkenyl chlorides

As shown in Figure 16.2, there are three possible dichloroethylene compounds, all clear, colorless liquids. Vinylidene chloride forms a copolymer with viny chloride used in some kinds of coating materials. The geometrically isomeric 1,2–dichloroethylenes are used as organic synthesis intermediates and as solvents.

Trichloroethylene is an excellent solvent for organic substances and has some other properties that are favorable for a solvent. It is a clear, colorless, nonflammable, volatile liquid. It is an excellent degreasing and drycleaning solvent and has been used as a household solvent and for food extraction (for example, in decaffeination of coffee).

Tetrachloroethylene is a colorless, nonflammable liquid with properties similar to those of trichloroethylene. Its major use is for drycleaning, and it has some applications for degreasing metals.

The two chlorinated propene compounds shown are colorless liquids with pungent, irritating odors. Allyl chloride is an intermediate in the manufacture of allyl alcohol and other allyl compounds, including pharmaceuticals, insecticides, and thermosetting varnish and plastic resins. Dichloropropene compounds can be used as soil fumigants, as well as solvents for oil, fat, drycleaning, and metal degreasing.

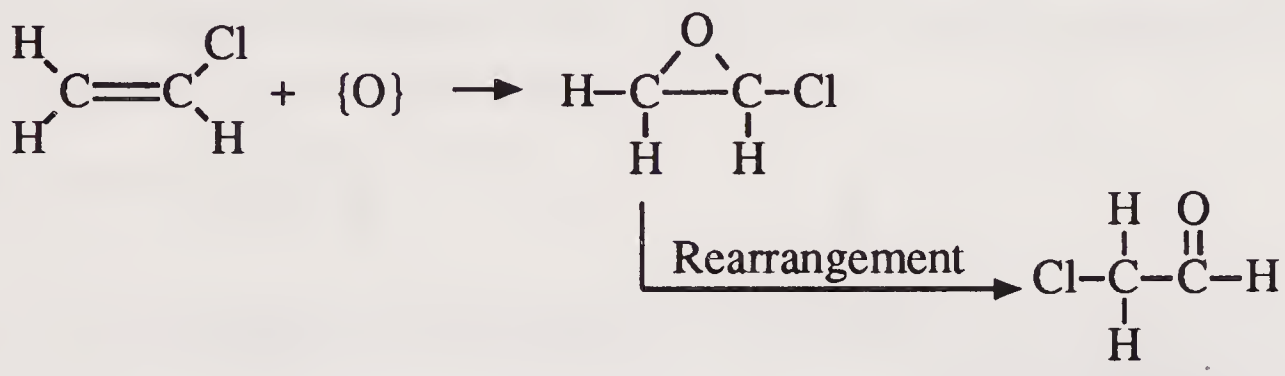
Chloroprene is produced in large quantities for the manufacture of neoprene rubber. It is a colorless liquid with an ethereal odor. Hexachlorobutadiene is a colorless liquid with an odor somewhat like that of turpentine. It is used as a solvent for higher hydrocarbons and elastomers, as a hydraulic fluid, in transformers, and for heat transfer.

Toxic Effects of Alkenyl Halides

Because of their widespread use and disposal in the environment, the toxicities of the alkenyl halides are of considerable concern. They exhibit a wide range of acute and chronic toxic effects.

Many workers have been exposed to vinyl chloride because of its use in polyvinylchloride plastic manufacture. The central nervous system, respiratory system, liver, and blood and lymph systems are all affected by exposure to vinyl chloride. Among the symptoms of poisoning are fatigue, weakness, and abdominal pain. Cyanosis may also occur. Vinyl chloride was abandoned as an anesthetic when it was found to induce cardiac arrhythmias.

The most notable effect of vinyl chloride is its carcinogenicity. It causes a rare angiosarcoma of the liver in chronically exposed individuals, observed particularly in those who cleaned autoclaves in the polyvinyl chloride fabrication industry. The carcinogenicity of vinyl chloride results from its metabolic oxidation to chloroethylene oxide by the action of the cytochrome P–450 monooxygenase enzyme system in the liver[3] as follows:



(16.3.1)

Chloroacetaldehyde

The epoxide has a strong tendency to covalently bond to protein, DNA, and RNA and it rearranges to chloroacetaldehyde, a known mutagen. Therefore, vinyl chloride produces two potentially carcinogenic metabolites. Both of thse products can undergo conjugation with glutathione to yield products that are eliminated from the body.

Based upon animal studies and its structural similarity to vinyl chloride, 1,1–dichloroethylene is a suspect human carcinogen. Although both 1,2–dichloroethylene isomers have relatively low toxicities, their modes of action are different. The *cis* isomer is an irritant and narcotic, whereas the *trans* isomer affects both the central nervous system and the gastrointestinal tract, causing weakness, tremors, cramps, and nausea.

Trichloroethylene has caused liver carcinoma in experimental animals and is a suspect human carcinogen. Numerous body organs are affected by it. As with other organohalide solvents, skin dermatitis can result from dissolution of skin lipids by trichloroethylene. Exposure to it can affect the central nervous and respiratory systems, liver, kidneys, and heart. Symptoms of exposure include disturbed vision, headaches, nausea, cardiac arrhythmias, and burning/tingling sensations in the nerves (paresthesia).

Tetrachloroethylene damages the liver, kidneys, and central nervous system. Because of its hepatotoxicity and experimental evidence of carcinogenicity in mice, it is a suspect human carcinogen.

The chlorinated propenes are obnoxious compounds. Unlike other compounds discussed so far in this section, their pungent odors and irritating effects lead to an avoidance response in exposed subjects. They are irritants to the eyes, skin, and res-

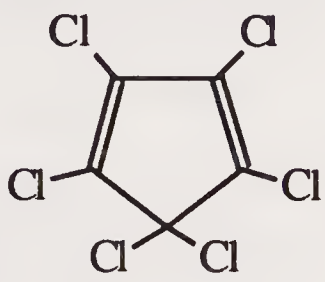
piratory tract. Contact with the skin can result in rashes, blisters, and burns. Chronic exposure to allyl chloride is manifested by aching muscles and bones; it damages the liver, lungs, and kidney and causes pulmonary edema.

Chloroprene is an eye and respiratory system irritant . It causes dermatitis to the skin and alopecia, a condition characterized by hair loss in the affected skin area. Affected individuals are often nervous and irritable.

Ingestion and inhalation of hexachlorobutadiene inhibits cells in the liver and kidney. Animal tests have shown both acute and chronic toxicities. The compound is a suspect human carcinogen.

Hexachlorocyclopentadiene

As shown by the structure below, hexachlorocyclopentadiene is a cyclic alkenyl halide with two double bonds:



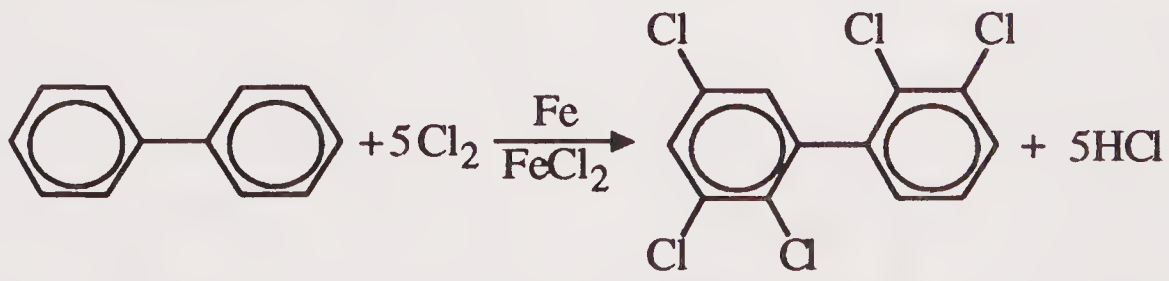
Hexachlorocyclopentadiene

It was once an important industrial chemical used directly as an agricultural fumigant and as an intermediate in the manufacture of insecticides. Hexachlorocyclopentadiene and still bottoms from its manufacture are found in hazardous waste chemical sites, and large quantities were disposed at the Love Canal site. The pure compound is a light yellow liquid, fp 11°C, bp 239°C, with a density of 1.7 g/cm^3 and a pungent, somewhat musty odor. With two double bonds, it is a very reactive compound and readily undergoes substitution and addition reactions. Its photolytic degradation yields water–soluble products.

Hexachlorocyclopentadiene is considered to be very toxic, with a toxicity rating of 4. Its fumes are strongly lacrimating, and it is a skin, eye, and mucuous membrane irritant. In experimental animals it has been found to damage most major organs, including the kidney, heart, brain, adrenal glands, and liver.

16.4. ARYL HALIDES

Figure 16.3 gives the structural formulas of some of the more important aryl halides. These compounds are made by the substitution chlorination of aromatic hydrocarbons as shown, for example, by the reaction below for the synthesis of a polychlorinated biphenyl:



(16.4.1)

This is a substitution reaction of an aromatic hydrocarbon compound (biphenyl) to produce an organochlorine product (2,3,5,2',3',- pentachlorobiphenyl, a PCB compound). The product is one of 210 possible congeners of PCBs, widespread and persistent pollutants found in the fat tissue of most humans and of considerable environmental and toxicological concern.

Properties and Uses of Aryl Halides

Aryl halides have many uses, which have resulted in substantial human exposure and environmental contamination. Some of their major applications are summarized here.

Monochlorobenzene is a flammable clear liquid, fp -45°C, bp 132°C, used as a solvent, solvent carrier for methylene diisocyanate, pesticide, heat transfer fluid, and in the manufacture of aniline, nitrobenzene, and phenol. The 1,2– isomer of dichlorobenzene (*ortho*– dichlorobenzene) has been used as a solvent for degreasing hides and wool and as a raw material for dye manufacture, the 1,4– isomer (*para*–dichlorobenzene) is also used in dye manufacture and as a moth repellant and germicide, and all three isomers have been used as fumigants and insecticides. The 1,2– and 1,3– (*meta*) isomers are liquids under ambient conditions, whereas the 1,4– isomer is a white sublimable solid. Used as a solvent, lubricant, dielectric fluid, chemical intermediate, and formerly as a termiticide, 1,2,4–trichlorobenzene is a liquid, fp 17°C, bp 213°C.

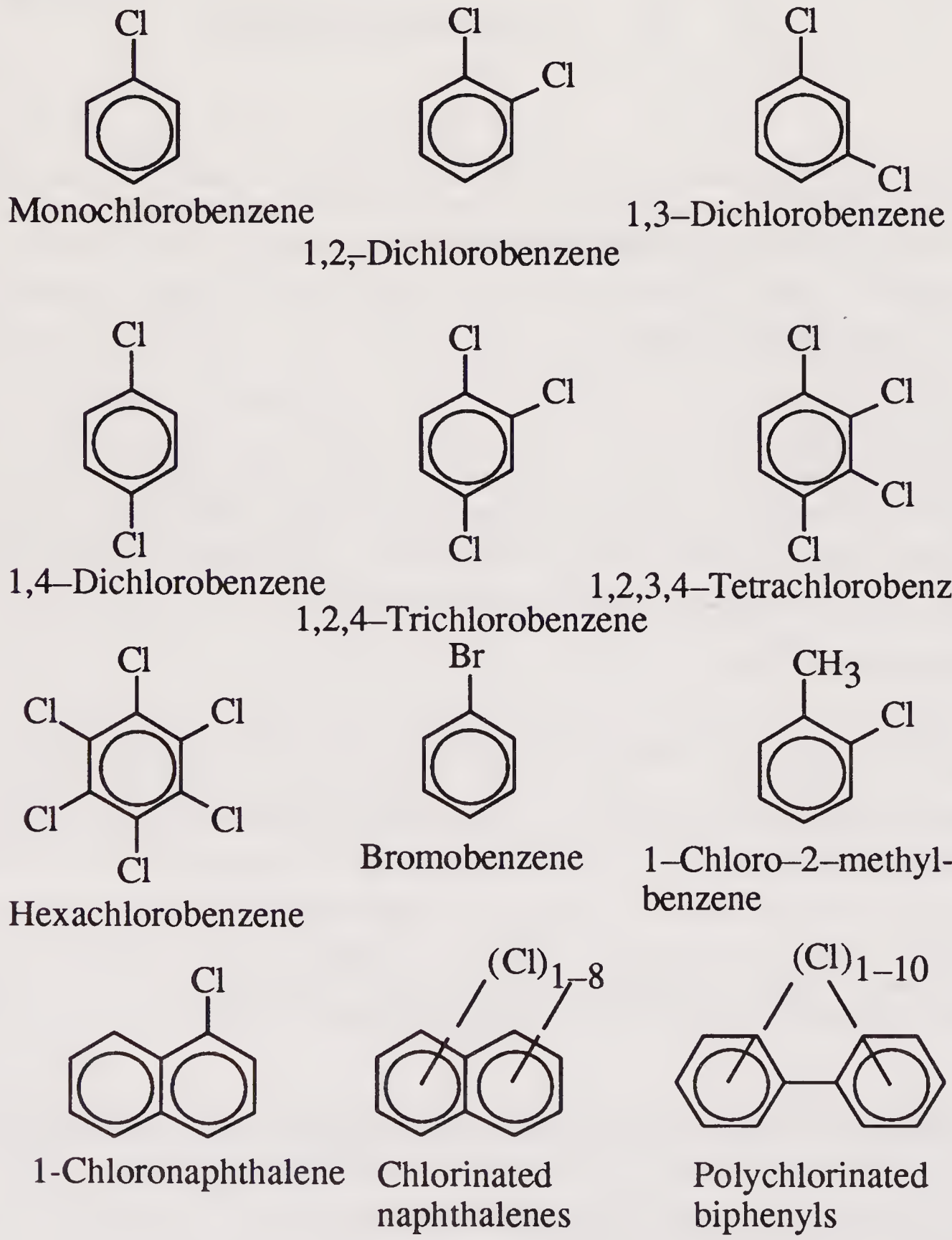


Figure 16.3. Some of the more important aryl halides.

Hexachlorobenzene (perchlorobenzene) is a high–melting solid consisting of white needles and used as a seed fungicide, wood preservative, and intermediate for organic synthesis. Bromobenzene (fp -31°C, bp 156°C) serves as a solvent and motor oil additive, as well as an intermediate for organic synthesis. Most 1-chloro-2-methylbenzene is consumed in the manufacture of of 1-chlorobenzotrifluoride.

There are two major classes of halogenated aryl compounds containing two benzene rings. One class is based upon naphthalene and the other upon biphenyl, as shown by the examples in Figure 16.3. For each class of compounds, the individual members range from liquids to solids, depending upon the degree of chlorination. These compounds are manufactured by chlorination of the parent compounds and have been sold as mixtures with varying degrees of chlorine content. The desirable properties of the chlorinated naphthalenes, polychlorinated biphenyls, and polybrominated biphenyls, including their physical and chemical stabilities, have led to many uses, such as for heat transfer, and hydraulic fluids, dielectrics, and flame retardants. However, for environmental reasons these uses have been severely curtailed.

Toxic Effects of Aryl Halides

Exposure to monochlorobenzene usually occurs by inhalation or skin contact. It is an irritant and affects the respiratory system, liver, skin, and eyes. Ingestion of this compound has caused incoordination, pallor, cyanosis, and eventual collapse, effects similar to those of aniline poisoning (see Section 15.3).

Exposure to the dichlorobenzenes is also most likely to occur through inhalation or contact. These compounds are irritants and tend to damage the same organs as monochlorobenzene. The 1,4- isomer has been known to cause profuse rhinitis (running nose), nausea, weight loss associated with anorexia, jaundice, and liver cirrhosis. The di– and tetrachlorobenzenes are considered to be moderately toxic by inhalation and ingestion.

Hexachlorobenzene is a notorious compound in the annals of toxicology because of a massive poisoning incident involving 3,000 people in Turkey during the period 1955–1959.[6] The victims ate seed wheat that had been treated with 10% hexachlorobenzene to deter fungal growth. As a consequence, they developed **porphyria cutanea tarda**, a condition in which the skin becomes blistered, fragile, photosensitive, and subject to excessive hair growth. In addition to the skin damage, the victims' eyes were damaged in severe cases and many suffered weight loss associated with anorexia. Wasting of skeletal muscles was also observed. The possibility exists that many of these effects were due to the presence of manufacturing byproduct impurity polychlorinated dibenzodioxins (see Section 16.6).

Bromobenzene can enter the body through the respiratory tract, gastrointestinal tract, or skin. Little information is available regarding its human toxicity. It has been shown to damage the livers of rats used in animal tests.

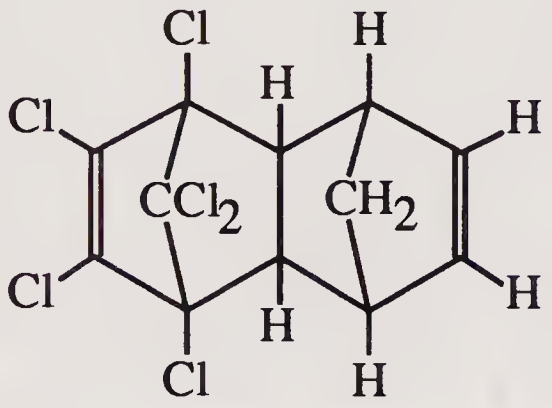
Wide variations have been reported in the toxicities of the chlorinated naphthalenes, raising the possibility that some of the effects observed were due to impurities introduced during manufacture. Humans exposed to the more highly halogenated fractions by inhaling the vapors have developed chloracne rash and have suffered from debilitating liver necrosis. In the 1940s and early 1950s several hundred thousand cattle died from polychlorinated naphthalene-contaminated feed.

Polychlorinated biphenyls (PCBs) are of concern because of their widespread environmental occurrence and extreme persistence.[7] Their polybrominated biphenyl analogs (PBBs) were the cause of massive livestock poisoning in Michigan in 1973 because of the addition of PBB flame retardant to livestock feed during its formulation.

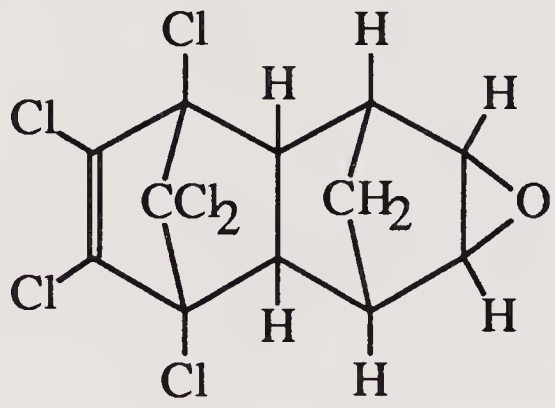
16.5. ORGANOHALIDE INSECTICIDES

Organohalide compounds were the first of the widely used synthetic organic pesticides. In this section organohalide insecticides are discussed and in Section 16.6 other pesticides of the organohalide chemical type are covered.

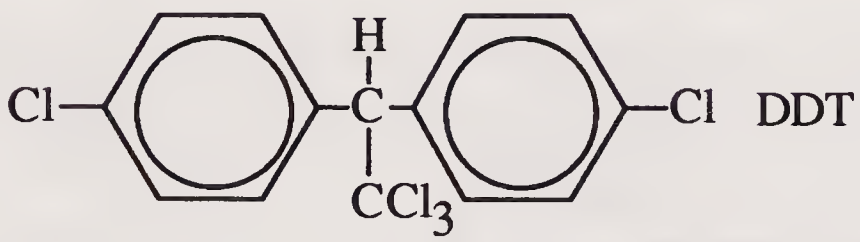
Figure 16.4 shows the structural formulas of some of the more common organohalide insecticides. Most of the insecticidal organohalide compounds contain



Aldrin

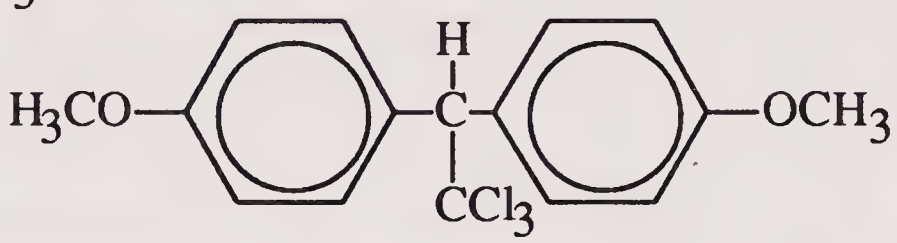


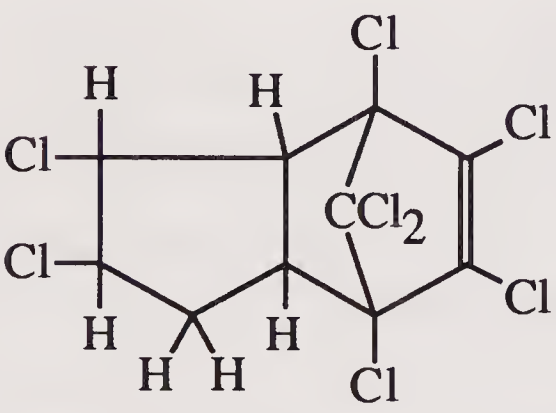
Dieldrin



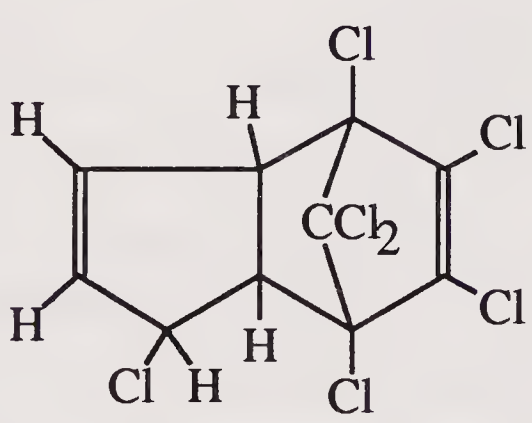
DDT

Methoxychlor

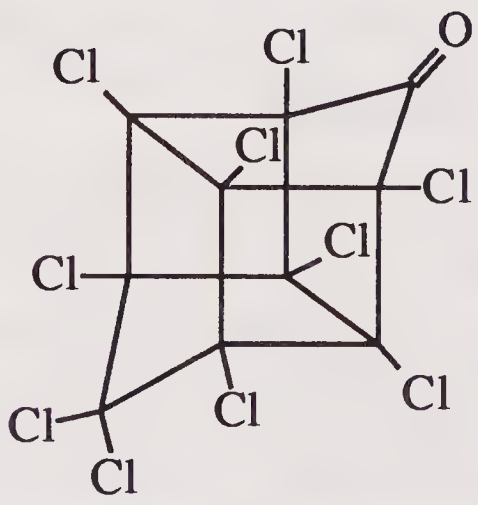




Chlordane



Heptachlor



Chlordecone (Kepone); Mirex is structurally similar with 2 Cl's in place of =O.

Figure 16.4. Some typical organohalide insecticides.

chlorine as the only halogen. Ethylene dibromide and dichlorobromopropane are insecticidal, but are more properly classified as fumigants and nematocides.

As seen from the structural formulas in Figure 16.4, the organochlorine insecticides are of intermediate molecular mass and contain at least one aromatic or nonaromatic ring. They can be placed in four major chemical classes. The first of these consists of the chloroethylene derivatives, of which DDT and methoxychlor are the prime examples. The second major class is composed of chlorinated cyclodiene compounds, including aldrin, dieldrin, and heptachlor. The most highly chlorinated members of this class, such as chloredecone, are manufactured from hexachlorocyclopentadiene (see Section 16.3). The benzene hexachloride stereoisomers make up a third class of organochlorine insecticides and the third group, known collectively as toxaphene, constitutes a fourth.

Toxicities of Organohalide Insecticides

Organohalide insecticides exhibit a wide range of toxic effects and varying degrees of toxicity. Many of these compounds are neuropoisons and their most prominent acute effects are upon the central nervous system, manifested by symptoms of CNS poisoning including tremor, irregular jerking of the eyes, changes in personality, and loss of memory. Some of the toxic effects of specific organohalide insecticides and classes of these compounds are discussed below.

Despite its role in the establishment of the modern environmental movement as the basis of Rachel Carson's classic book *Silent Spring*, the acute toxicity of DDT to humans is very low. It was applied directly to people on a large scale during World War II for the control of typhus and malaria. Symptoms of acute DDT poisoning are much the same as those described previously for organohalide insecticides in general and are for the most part neutrotoxic in nature. In the environment, DDT undergoes bioaccumulation in the food chain, with animals at the top of the chain most affected. The most vulnerable of these are predator birds that produce thin–shelled, readily broken eggs from ingestion of DDT through the food chain. The other major insecticidal chloroethane–based compound, methoxychlor, is a generally more biodegradable, less toxic compound than DDT,[8] and has been used as a substitute for it.

The toxicities of the chlorinated cyclodiene insecticides, including aldrin, dieldrin, endrin, chlordane, heptachlor, endosulfan, and isodrin are relatively high and similar to each other. They appear to act on the brain, releasing betaine esters and causing headaches, dizziness, nausea, vomiting, jerking muscles, and convulsions. Some members of this group are teratogenic or toxic to fetuses. In test animals, dieldrin, chlordane, and heptachlor cause liver cancer. For several years the use of aldrin, dieldrin, and heptachlor has been prohibited in the U. S., and chlordane was restricted to underground applications for termite control. In 1987, even this use of chlordane was discontinued.

A significant number of human exposures to the insecticides derived from hexachlorcyclopentadiene (Mirex and Kepone) have occurred. Use of these environmentally damaging compounds has been restricted in the U. S. to eradication of fire ants in the southeastern states. The manufacture of Kepone in Hopewell, Virginia, during the 1970s resulted in the discharge of about 53,000 kg of this compound to the James River through the city sewage system. Toxic effects of Kepone include central nervous system symptoms (irritability, tremor, hallucinations), adverse effects on sperm, and damage to the nerves and muscles. The compound causes liver cancer in rodents and is teratogenic in test animals. Studies of exposed workers have shown that kepone absorbed by the liver is excreted through the bile, then reabsorbed from the gastrointestinal tract, thereby participating in the enterohepatic circulation system as illustrated in Figure 8.5.

Hexachlorocyclohexane

Hexachlorocyclohexane, often confusingly called benzene hexachloride (BHC), consists of several stereoisomers with different orientations of H and Cl atoms. The gamma isomer is shown in Figure 16.5. It is an effective insecticide, constituting at least 99% of the commercial insecticide **lindane**.

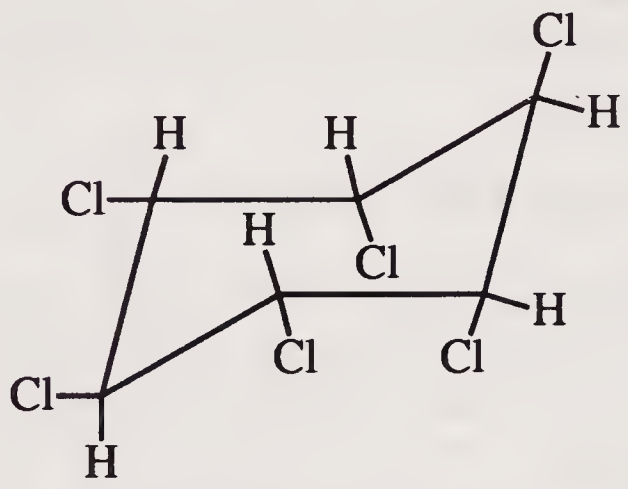
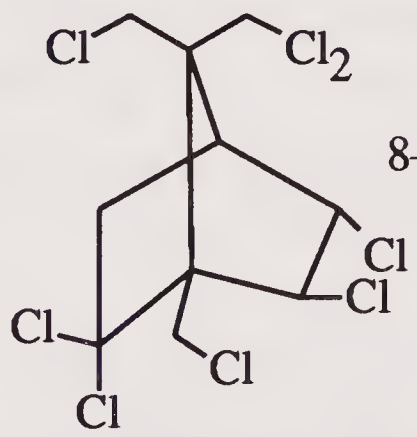


Figure 16.5. The gamma isomer of hexachlorocyclohexane (lindane).

The toxic effects of lindane are very similar to those of DDT. Degeneration of kidney tubules, liver damage associated with fatty tissue, and hystoplastic anemia have been observed in individuals poisoned by lindane.

Toxaphene

Toxaphene is insecticidal chlorinated camphene and consists of a mixture of more than 170 compounds containing 10 C atoms and 6–10 Cl atoms per molecule and often represented by the empirical formula $C_{10}H_{10}Cl_8$. The structural formula of one of the molecules contained in toxaphene, 8–octachlorobornane, is given below. Toxaphene was once the most used insecticide in the U. S. with annual consumption of about 40 million kg.



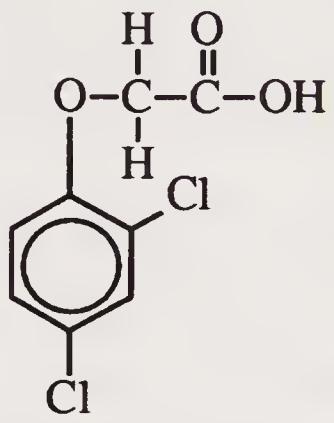
8–Octachlorobornane

The many compounds found in formulations of toxaphene vary widely in their toxicities. One of the most toxic of these compounds is 8–octachlorobornane, shown above. Toxaphene produces convulsions of an epileptic type in exposed mammals.

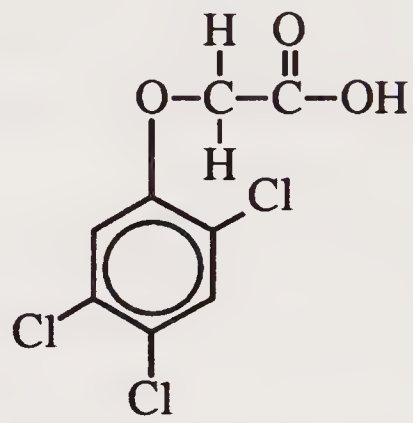
16.6. NONINSECTICIDAL ORGANOHALIDE PESTICIDES

The best known noninsecticidal organohalide pesticides are the **chlorophenoxy** compounds. These consist of 2,4–dichlorophenoxyacetic acid (2,4–D), 2,4,5–trichlorophenoxyacetic acid (2,4,5–T or Agent Orange), and a closely related

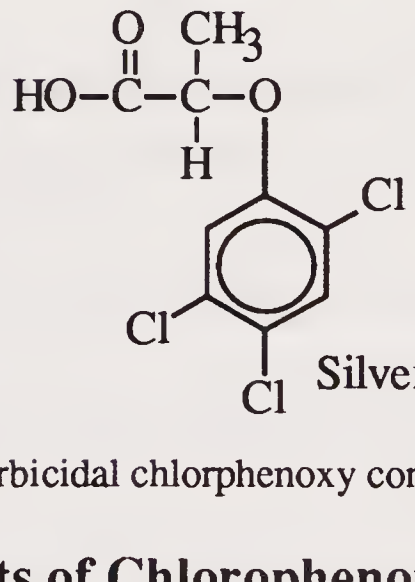
compound Silvex. These compounds, their esters, and their salts have been used as ingredients of a large number of herbicide formulations. Formulations of 2,4,5–T have become notorious largely by a manufacturing byproduct, 2,3,7,8-tetrachloro-*p*-dioxin (TCDD, commonly known as "dioxin."). The structural formulas of these compounds are shown in Figure 16.6.



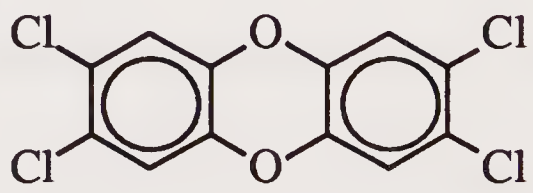
2,4–Dichlorophenoxy-acetic acid (and esters)



2,4,5–Trichlorophenoxy-acetic acid (and esters)



Silvex



2,3,7,8–Tetrachloro–*p*–dioxin

Figure 16.6. Herbicidal chlorphenoxy compounds and TCDD manufacturing byproduct.

Toxic Effects of Chlorophenoxy Herbicides

The oral toxicity rating of 2,4–dichlorophenoxyacetic acid is 4, although the toxicities of its commercially marketed ester and salt forms are thought to be somewhat lower. Large doses have been shown to cause nerve damage, such as peripheral neuropathy, as well as convulsions and even brain damage. A National Cancer Institute study of Kansas farmers who had handled 2,4–D extensively has shown an occurrence of nonHodgkins lymphoma 6 to 8 times that of comparable unexposed populations.[9] The toxicity of Silvex appears to be somewhat less than that of 2,4–D and to a large extent it is excreted unchanged in the urine.

Although the toxic effects of 2,4,5–T may even be somewhat less than those of 2,4–D, observations of 2,4,5–T toxicity have been complicated by the presence of manufacturing byproduct TCDD. Experimental animals dosed with 2,4,5–T have exhibited mild spasticity. Some fatal poisonings of sheep have been caused by 2,4,5–T herbicide. Autopsied carcasses revealed nephritis, hepatitis, and enteritis. Humans absorb 2,4,5–T rapidly and excrete it largely unchanged through the urine.

Toxicity of TCDD

TCDD belongs to the class of compounds called **polychlorinated dibenzo-dioxins**, which have the same basic structure of TCDD, but different numbers and arrangements of chlorine atoms on the ring structure. These compounds exhibit varying degrees of toxicity. Classified as a supertoxic compound, TCDD is unques-

tionably extremely toxic to some animals. Its acute LD_{50} to male guinea pigs is only 0.6 micrograms per kilogram of body mass. Because of its production as a manufacturing byproduct of some commercial products, such as 2,4,5–T, possible emission from municipal incineration, and widespread distribution in the environment from improper waste disposal (for example, as the infamous "dioxin" spread from waste oil at Times Beach, Missouri) or discharge from industrial accidents (Seveso, Italy), TCDD has become a notorious environmental pollutant. However, the degree and nature of its toxicity to humans are both rather uncertain. It is known to cause a human skin condition called chloracne.

A study conducted by the Hamburg Department of Health Center for Chemical Worker's Health of 1583 German pesticide workers exposed to TCDD during the period 1952-1984 has shown a statistically significant elevated cancer mortality.[10] Workers exposed to TCDD wastes containing 60 mg of TCDD per kg of plant waste at a plant run by Hamburg-Moorfleet were shown to have a 39% higher cancer mortality rate compared to other Germans. The key findings of this study were the following:

- Higher levels of TCDD were found in fat tissue of workers with greater exposure to TCDD (296 ng TCDD per kg body mass compared to 83 ng/kg for less exposed workers and 10 ng/kg for the general population).
- An approximately three-fold increased risk of cancer among workers exposed to particularly high TCDD concentrations prior to 1954 when when changes were made in plant operations to decrease byproduct TCDD production.
- Increased cancers among workers with longer exposures in excess of 20 years.
- No evidence of soft tissue sarcoma, which had been associated with TCDD exposure in other cases.

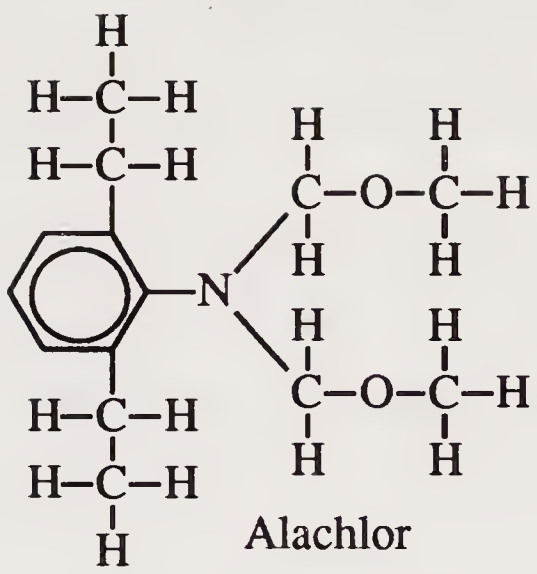
The results of this epidemiological study support the thesis that exposure to high levels of TCDD may cause human cancer.

Alachlor

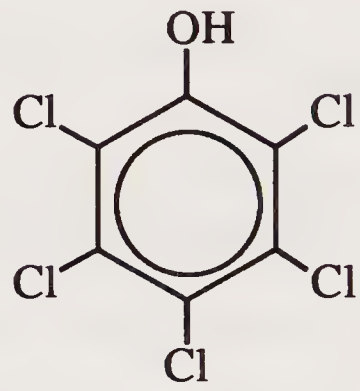
Widely marketed as Monsanto's Lasso® herbicide, Alachlor (Figure 16.7) has become a widespread contaminant of groundwater in some corn and soybean producing areas. It seems to be efficiently absorbed through the skin. Allergic skin reactions and skin and eye irritation have been reported in exposed individuals. The U. S. Environmental Protection Agency has estimated a lifetime cancer risk of 1 in 100,000 from drinking water containing 2 ppb of alachlor,[11] although this risk estimate has been disputed by manufacturers.

Chlorinated Phenols

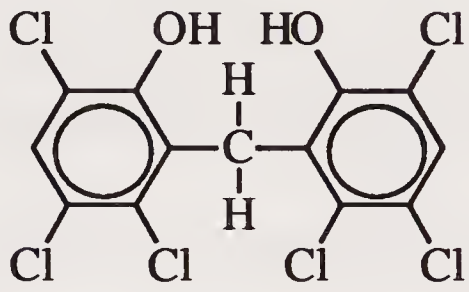
The chlorinated phenols, particularly **pentachlorophenol** (Figure 16.7) and the trichlorophenol isomers, have been widely used as wood preservatives. Applied to wood, these compounds prevent wood rot through their fungicidal action and prevent termite infestation because of their insecticidal properties. Both cause liver malfunction and dermatitis. Contaminant polychlorinated dibenzodioxins may be responsible for some of the observed effects.



Alachlor



Pentachlorophenol



Hexachlorophene

Figure 16.7. Structural formulas of alachlor, pentachlorophenol, and hexachlorophene

Hexachlorophene

Hexachlorophene (Figure 16.7) has been used as an agricultural fungicide and bacteriocide, largely in the production of vegetables and cotton. It is most noted for its use as an antibacterial agent in personal care products, now discontinued because of toxic effects and possible TCDD contamination.

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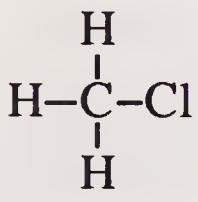
10. Hanson, David, "Study is Second to Link Dioxin, Worker Cancer." *Chemical and Engineering News*, Oct. 28, 1991, p. 6.

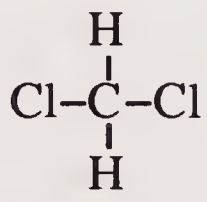
11. "EPA Proposal on Alachlor Nears," *Science*, **233**, 1143–1144 (1986).

QUESTIONS AND PROBLEMS

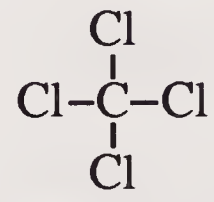
1. Give an example of each of the following: An alkyl halide, an alkenyl halide, and and an aryl halide. Give an example of each of the following kinds of reactions for forming an organohalide compound: Substitution halogenation, addition halogenation, and hydrohalogenation.
2. List some chemical and/or toxicological properties of each of the following compounds.



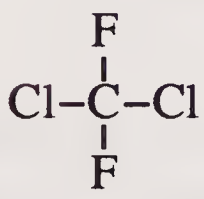
Chloromethane (fp -98°C, bp -24°C)



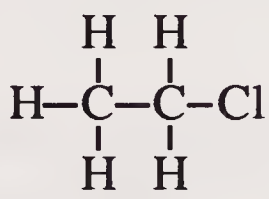
Dichloromethane (methylene chloride, fp -97°C, bp 40°C)



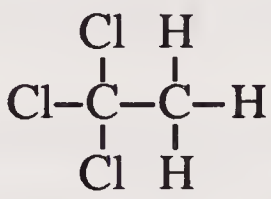
Carbon tetrachloride (fp -23°C, bp 77°C)



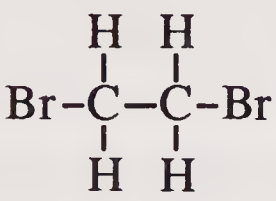
Dichlorodifluoromethane ("Freon-12," fp -158°C, bp -29°C)



Chloroethane (ethylene chloride, fp -139°C, bp 12°C)

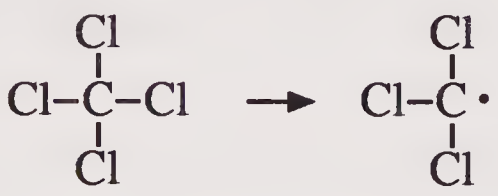


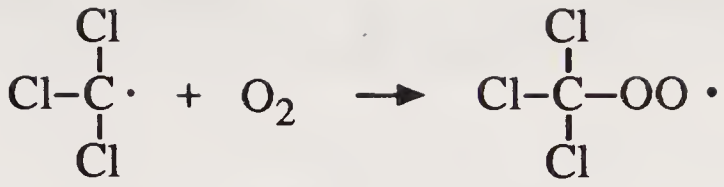
1,1,1–Trichloroethane (methyl chloroform, fp -33°C, bp 74°C)

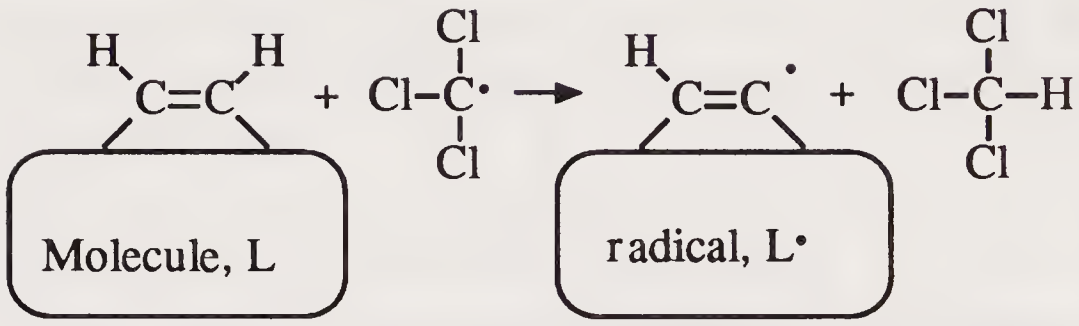


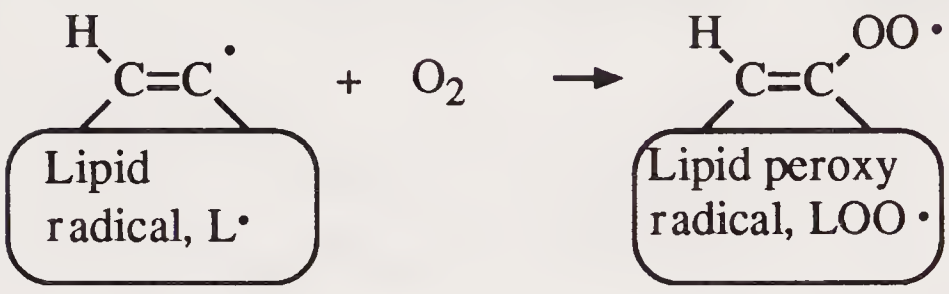
1,2–Dibromoethane (ethylene dibromide, fp 9.3°C, bp 131°C)

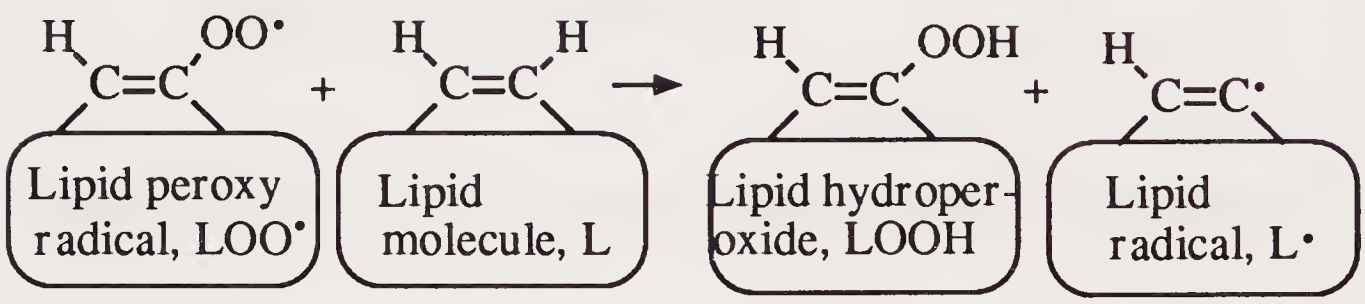
3. Explain the statement that, "carbon tetrachloride has the most notorious record of human toxicity, especially for its toxic effects on the liver."
4. Explain the special toxicological significance of vinyl chloride.
5. Explain what is shown by the following sequence of reactions:



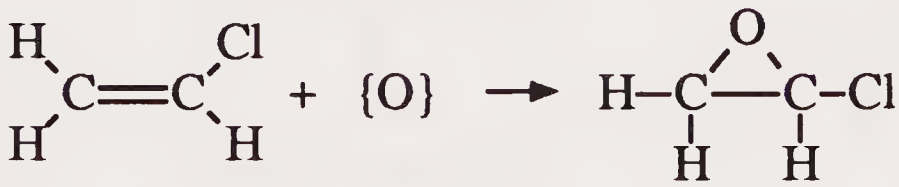


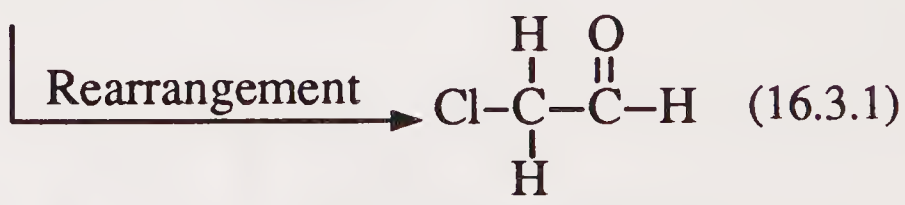






6. What are the possible dichloroethylene compounds?
7. What is shown by the reaction below? What is its toxicological chemical significance?





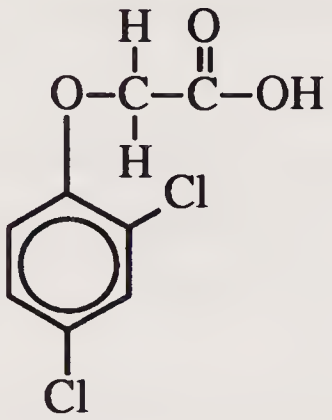
(16.3.1)

Chloroacetaldehyde

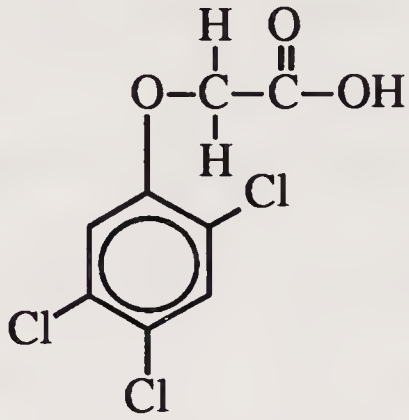
8. What are two former uses of the compound below? Why is it of particular importance in the area of hazardous wastes?

Hexachlorocyclopentadiene

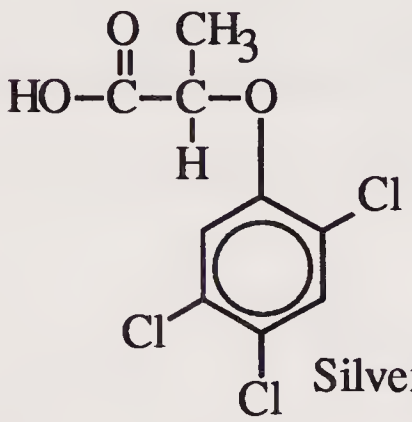
9. For what purposes were three of the compounds below used? Which of the compounds shown is an undesirable byproduct of the manufacture of the other three?



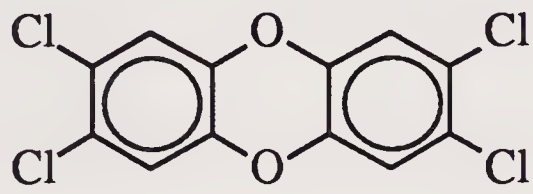
2,4–Dichlorophenoxyacetic acid (and esters)



2,4,5–Trichlorophenoxyacetic acid (and esters)

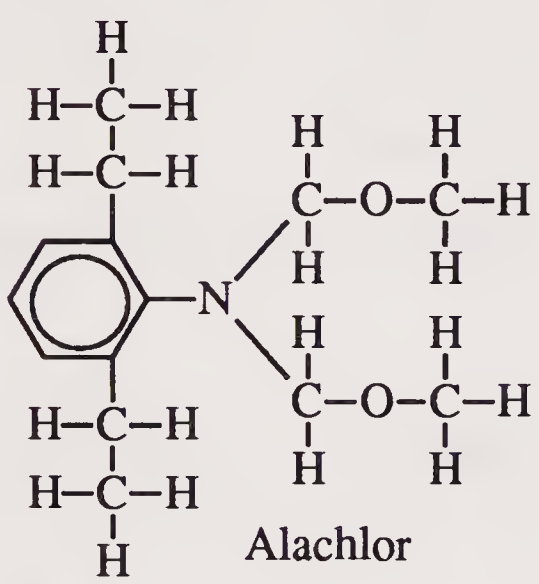


Silvex

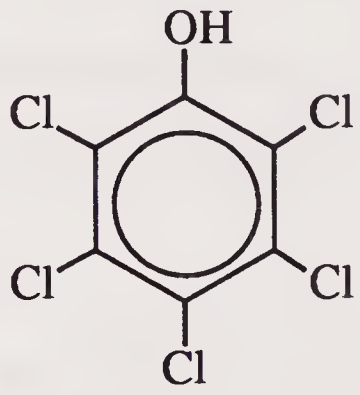


2,3,7,8–Tetrachloro–*p*–dioxin

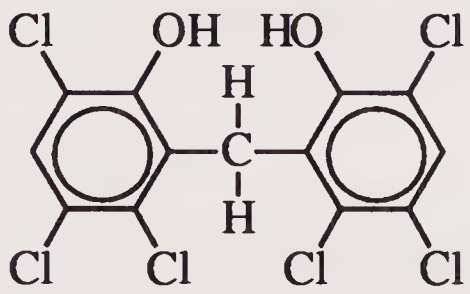
10. What are the compounds shown below? What are their toxicity characteristics?



Alachlor

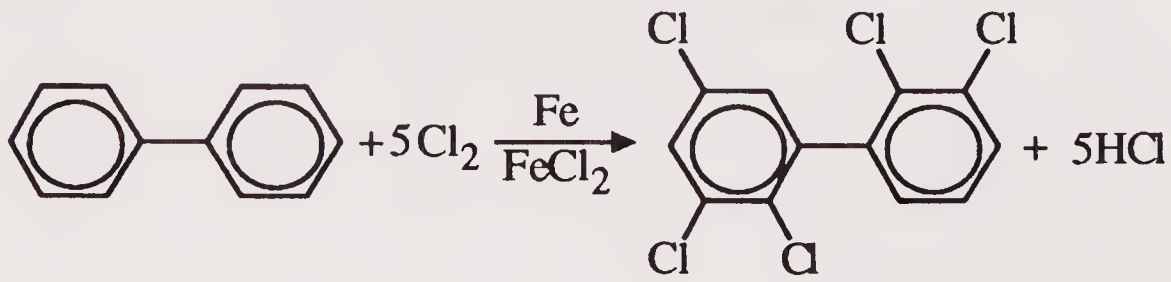


Pentachlorophenol



Hexachlorophene

11. What is shown by the reaction below? How many different kinds of compounds (congeners) may be made with this kind of reaction?



12. What is the nature of the malady called porphyria cutanea tarda? Which chemicals may cause this condition?
13. Is the acute toxicity of DDT very high? Why was there sufficient concern over this insecticide to cause its use to be banned?
14. What are the toxic effects of Kepone? How is it involved in the enterohepatic circulation system? Why is it a notorious substance in the annals of environmental pollution?
15. What is the compound commonly known as "dioxin"? Why is it a particularly notorious environmental pollutant? What is known about its toxicity to humans? What about its toxicity to specific kinds of rodents?

17

Organosulfur Compounds

17.1. INTRODUCTION

Sulfur is directly below oxygen in the periodic table. The sulfur atom has 6 valence electrons as shown in Figure 17.1 and its electron configuration is $\{Ne\}3s^23p^4$. Because of their very similar valence shell electron configurations, oxygen and sulfur behave somewhat alike chemically. However, unlike oxygen, the sulfur atom has three underlying $3d$ orbitals, and its valence shell can be expanded to more than 8 electrons. This makes sulfur's chemical behavior more diverse than that of oxygen. For example, sulfur has several common oxidation states, including -2, +4, and +6, whereas most chemically combined oxygen is in the -2 oxidation state.

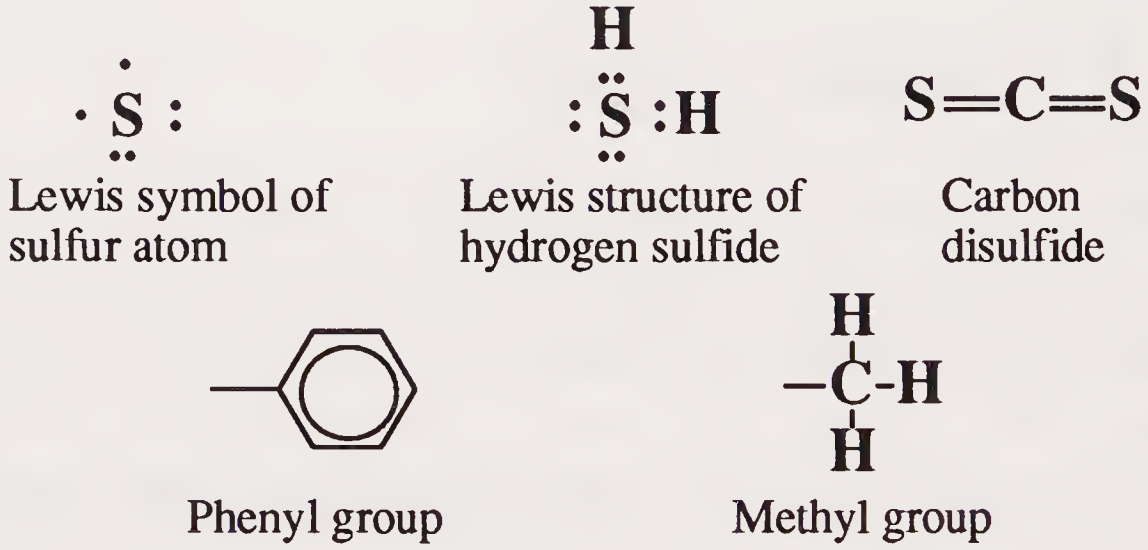


Figure 17.1. Sulfur atom, compounds, and substituent groups.

Classes of Organosulfur Compounds

The hydride of sulfur is H_2S (Figure 17.1), a highly toxic gas discussed in Section 7.11. Substitution of alkyl or aryl hydrocarbon groups such as phenyl and methyl (Figure 17.1) for H on hydrogen sulfide leads to a number of different organosulfur compounds. These include thiols (R–SH) and thioethers (R–S–R). Because of the availability of $3d$ orbitals, sulfur that is bonded to hydrocarbon moieties can also be bonded to oxygen, adding to the variety of organosulfur compounds that can exist.

Despite the high toxicity of H_2S, not all organosulfur compounds are particularly toxic. Many of the compounds have strong, offensive odors that warn of their presence, which reduces their hazard.

Reactions of Organic Sulfur

Organic sulfur undergoes a number of toxicological chemical reactions. These include the following:

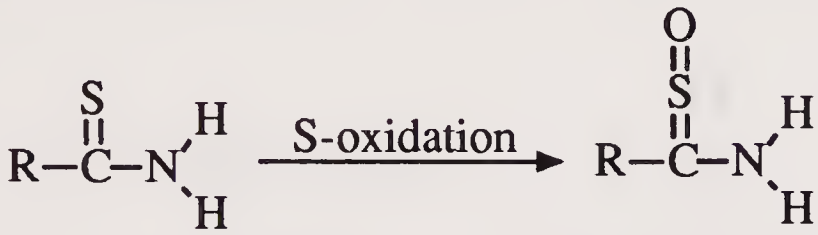
- Oxidation of sulfur
- Reduction of sulfur
- Removal of sulfur from a molecule
- Addition of sulfur-containing groups

Examples of these kinds of reactions, some of which are very important in xenobiotic metabolism, are given below.

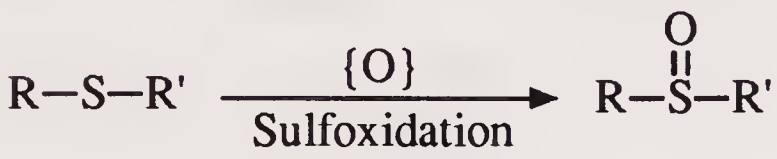
Oxidation of sulfur is called **S-oxidation**. Thiols can be oxidized to form disulfides:

$$2\text{R-SH} + \{\text{O}\} \rightarrow \text{R-S S-R} + \text{H}_2\text{O} \qquad (17.1.1)$$

The same kind of reaction occurs with aminothiols, such as in the oxidation of cysteamine, $\text{H}_2\text{NCH}_2\text{CHSH}$, to cystamine, $\text{H}_2\text{NCH}_2\text{CH}_2\text{S SCH}_2\text{CH}_2\text{NH}_2$. S-oxidation may also involve sulfur on thioamides:

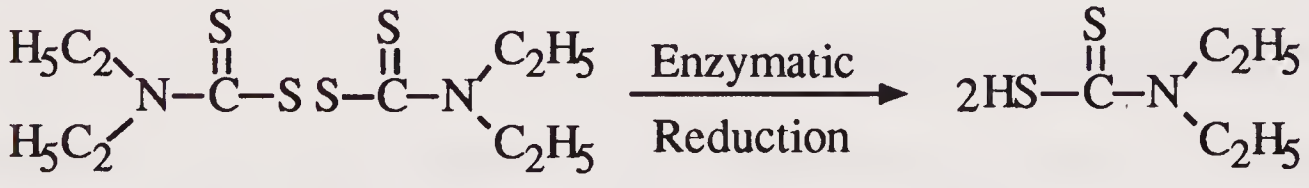


(17.1.2)



(17.1.3)

An example of sulfur reduction is **disulfide reduction** as shown by the following reaction:

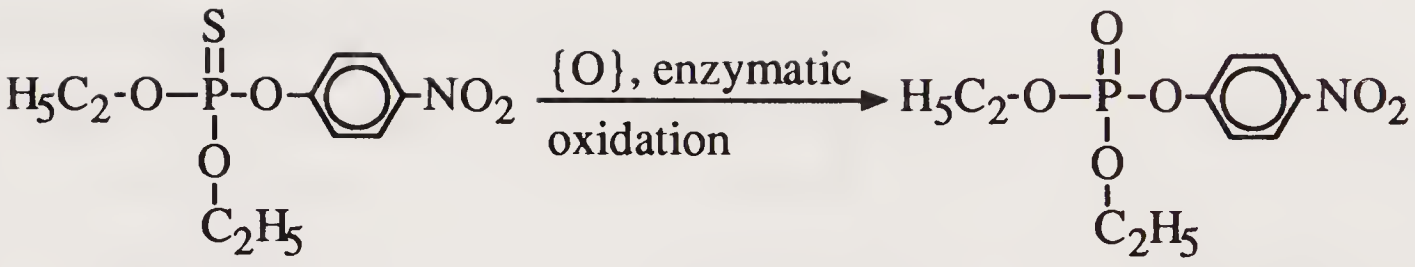


(17.1.4)

Disulfiram Dithiocarb

This reaction converts disulfiram, a therapeutic agent for the treatment of alcohol abuse discussed in Section 17.3, to dithiocarb (diethylthiocarbamate) a substance that strongly binds metals and is used for the treatment of nickel carbonyl poisoning.[1]

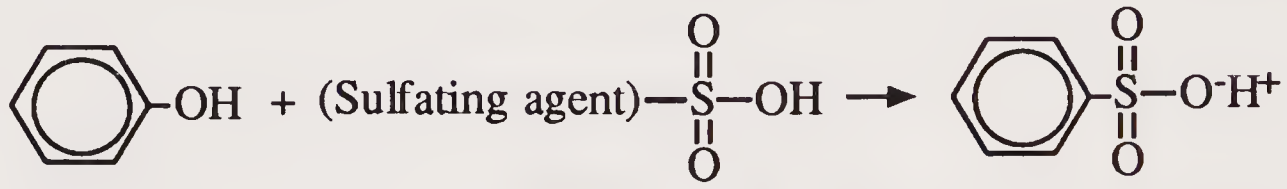
Desulfuration is the term given to removal of sulfur from a molecule. One of the most common desulfuration reactions occurs with sulfur bonded to phosphorus. A common desulfuration reaction is the enzyme-mediated conversion of parathion to paraoxon (see discussion of organophosphate insecticides in Section 18.7):



(17.1.5)

Parathion Paraoxon

The most significant instance of addition of a sulfur-containing group is the phase II conjugation to sulfate of a xenobiotic compound or its phase I metabolite (see Section 4.6) by the action of adenosine 3'-phosphate-5'-phosphosulfate, a sulfotransferase enzyme that acts as a sulfating agent.



(17.1.6)

Phenol Phase II sulfate conjugate

17.2. THIOLS, SULFIDES, AND DISULFIDES

Substitution of alkyl and aryl groups for H on H_2S yields **thiols** and **sulfides** (thioethers). Structural formulas of examples of these compounds are shown in Figure 17.2.

Thiols

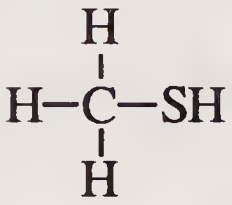
Thiols are also known as mercaptans. The lighter alkyl thiols, such as methanethiol, are fairly common air pollutants with odors that may be described as "ultragarlic." Inhalation of even very low concentrations of the alkyl thiols in air can be very nauseating and result in headaches. Exposure to higher levels can cause increased pulse rate, cold hands and feet, and cyanosis. With extreme cases, unconsciousness, coma, and death may occur. The biochemical action of alkyl thiols likely is similar to that of H_2S and they are precursors to cytochrome oxidase poisons.

Gaseous methanethiol and volatile liquid ethanethiol (bp 35°C) are intermediates in pesticide synthesis and odorants placed in lines and tanks containing natural gas, propane, and butane to warn of leaks. Information about their toxicities to humans is lacking, although these compounds and 1-propanethiol should be considered dangerously toxic, especially by inhalation. Both 1- and 2-butanethiol are associated with skunk odor. Also known as amyl mercaptan, 1-pentanethiol (bp 124°C) is an allergen and weak sensitizer which causes contact dermatitis.

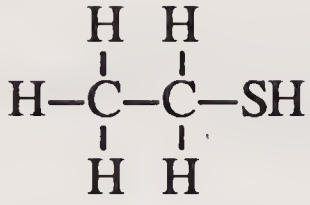
A typical alkenyl mercaptan is 2-propene-1-thiol, also known as allyl mercaptan. It is a volatile liquid (bp 68°C) with a strong garlic odor. It has a high toxicity and is strongly irritating to mucous membranes when inhaled or ingested.

Alpha-toluenethiol, also called benzyl mercaptan (bp 195°C) is very toxic orally. It is an experimental carcinogen.

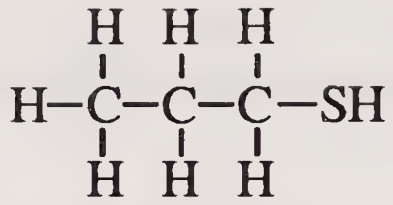
The simplest of the aryl thiols is benzenethiol (phenyl mercaptan), bp 168°C. It has a severely "repulsive" odor. Inhalation causes headache and dizziness and skin exposure results in severe contact dermatitis.



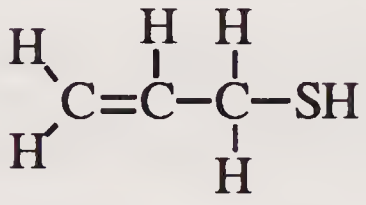
Methanethiol



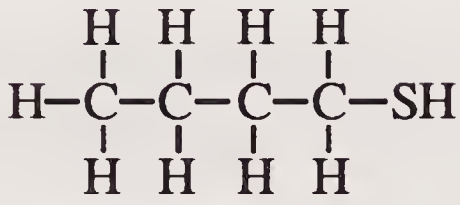
Ethanethiol



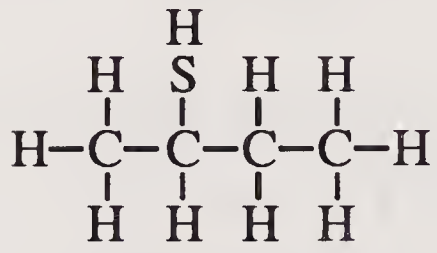
1–Propanethiol



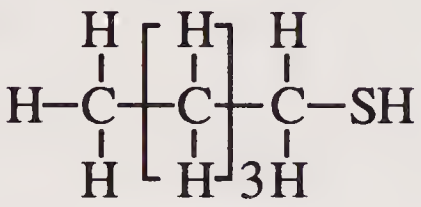
2–Propene–1–thiol



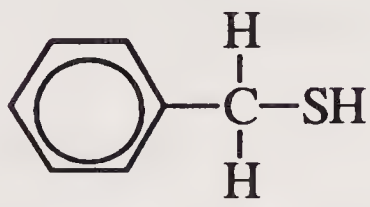
1–Butanethiol



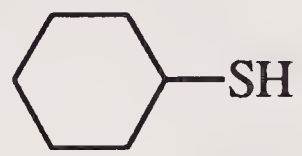
2-Butanethiol



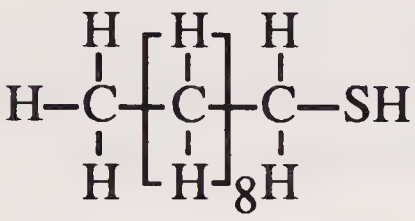
1–Pentanethiol



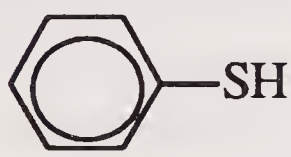
Alpha-toluenethiol



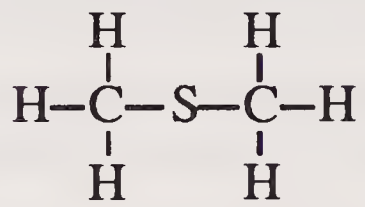
Cyclohexanethiol



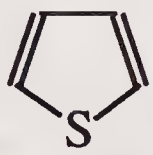
1-Decanethiol



Benzenethiol



Dimethyl sulfide



Thiophene (an unsaturated cyclic sulfide)

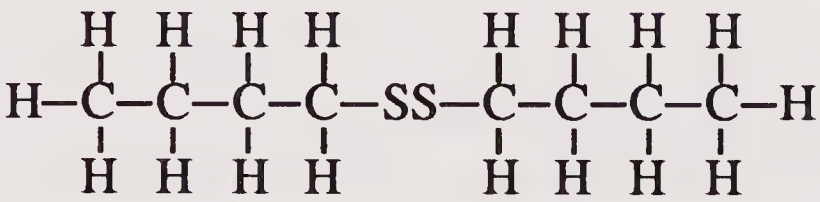
Thiophane

Figure 17.2. Common low-molecular-mass thiols and sulfides. All are liquids at room temperature, except for methanethiol, which boils at 5.9°C.

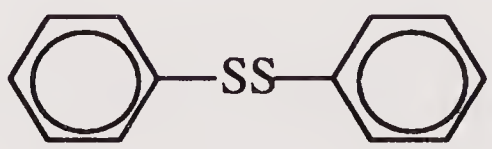
Sulfides and Disulfides

Dimethyl sulfide is an alkyl sulfide or thioether. It is a volatile liquid (bp 38°C) that is moderately toxic by ingestion. Thiophene is the most common cyclic sulfide. It is a heat-stable liquid (bp 84°C) with a solvent action much like that of benzene. It is used in the manufacture of pharmaceuticals and dyes, as well as resins that also contain phenol or formaldehyde. Its saturated analog is tetrahydrothiophene, or thiophane.

The organic disulfides contain the –SS– functional group as shown in the following two examples:



n –Butyldisulfide



Diphenyldisulfide

These compounds may act as allergens that produce dermatitis in contact with skin. Not much information is available regarding their toxicities to humans, although animal studies suggest several toxic effects, including hemolytic anemia.

Carbon Disulfide and Carbon Oxysulfide

Carbon disulfide (CS_2) is one of the most significant sulfur compounds because of its widespread use and toxicity. This compound has 2 sulfur atoms each separately bonded to a carbon atom. This compound is a volatile colorless liquid (mp -111°C, bp 46°C). Unlike most organosulfur compounds, it is virtually free of odor. Although its uses are declining, it has numerous applications in chemical synthesis, as a solvent to break down cellulose in viscose rayon manufacture, and in the manufacture of cellophane. It has also been used as an insecticide and fumigant.

Acute doses of carbon disulfide inhaled at 100–1000 ppm irritate mucous membranes and affect the central nervous system, usually causing excitation as a first noticeable effect, followed by restlessness, depression, and stupor. It is a much stronger anesthetic than chloroform (Section 16.2), causing unconsciousness and even death in cases of high exposure. Symptoms experienced during recovery from severe acute carbon disulfide poisoning resemble those that occur following intoxication from ingestion of ethanol in alcoholic beverages.

Chronic carbon disulfide poisoning by absorption through the skin or respiratory tract involves the central and peripheral nervous systems and may cause anemia. Symptoms include indistinct vision, neuritis, and a bizarre sensation of "crawling" on the skin. Psychopathological symptoms may be varied and severe, including excitation, depression, irritability, and general loss of mental capabilities to the point of insanity. Parkinsonian paralysis may result from chronic carbon disulfide poisoning.

Replacement of one of the S atoms on carbon disulfide with an O atom yields **carbon oxysulfide** (COS), a volatile liquid boiling at 50°C. It can decompose to liberate toxic hydrogen sulfide. Carbon oxysulfide vapor is a toxic irritant. At high concentrations this compound has a strong narcotic effect.

17.3. ORGANOSULFUR COMPOUNDS CONTAINING NITROGEN OR PHOSPHORUS

Several important classes of organosulfur compounds contain nitrogen or phosphorus. These compounds are discussed in this section.

Thiourea Compounds

Thiourea is the sulfur analog of urea. Substitution of hydrocarbon moieties on the N atoms yields various organic derivatives of thiourea, as illustrated in Figure 17.3.

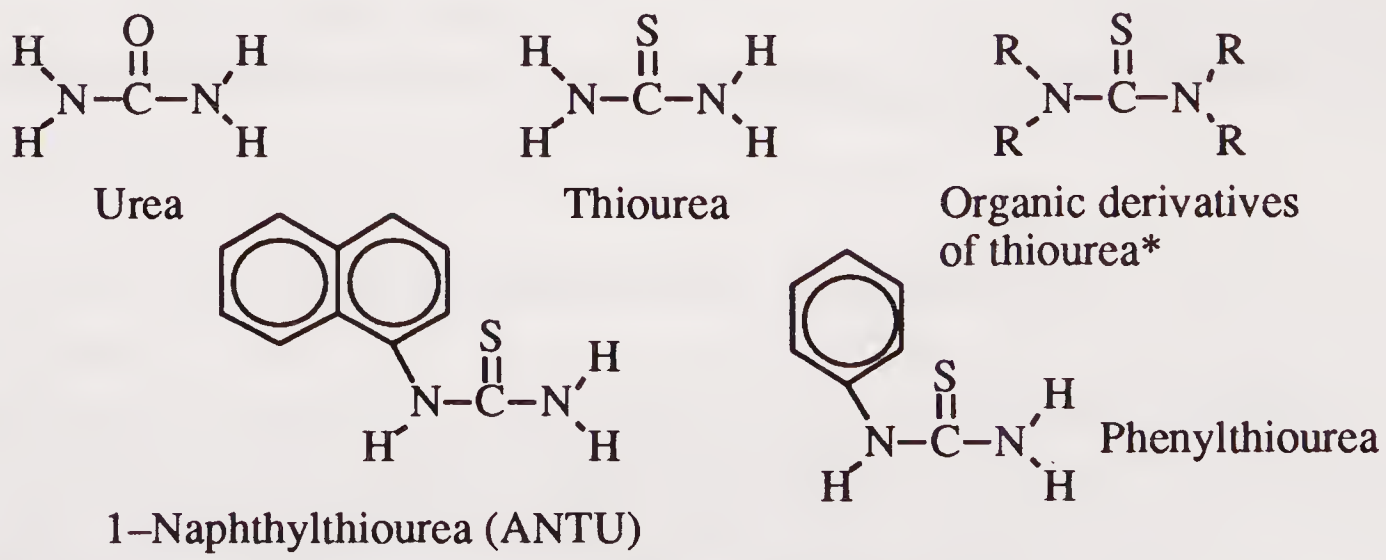


Figure 17.3. Structural formulas of urea, thiourea, and organic derivatives of thiourea.
* At least one R group is an alkyl, alkenyl, or aryl substituent.

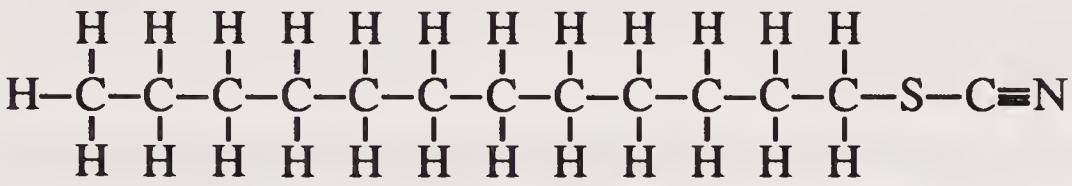
Thiourea has been used as a rodenticide. It has a moderate to high toxicity to humans, affecting bone marrow and causing anemia. It has been shown to cause liver and thyroid cancers in experimental animals.

Phenylthiourea is likewise a rodenticide. Its toxicity is highly selective to rodents relative to humans, although it probably is very toxic to some other animals. The compound is metabolized extensively, and some of the sulfur is excreted as sulfate in urine.

Commonly called ANTU, **1-naphthylthiourea** is a virtually tasteless rodenticide that has a very high rodent:human toxicity ratio. The lethal dose to monkeys is about 4,000 mg/kg. One suicidal adult male human ingested about 80 g of 30% ANTU rat poison along with a considerable amount of alcohol. He vomited soon after ingestion and survived without significant ill effects.[2] Dogs, however, are quite susceptible to ANTU poisoning.

Thiocyanates

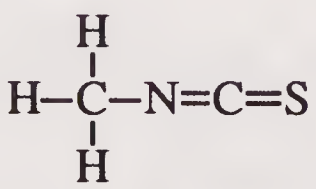
Organic **thiocyanates** are derivatives of thiocyanic acid (HSCN) in which the H is replaced by hydrocarbon moieties, such as the methyl group. Dating from the 1930s and regarded as the first synthetic organic insecticides, these compounds kill insects upon contact. Because of their volatilities, the lower-molecular-mass methyl, ethyl, and isopropyl thiocyanates are effective fumigants for insect control. Insecticidal lauryl thiocyanate (below) is not volatile and is used in sprays in petroleum-based solvents and in dusting powders.



Lauryl thiocyanate

The toxicities of the thiocyanates vary widely by compound and route of administration. Some metabolic processes liberate HCN from thiocyanates. As discussed in Chapter 12, HCN is highly toxic so its generation in the body can result in death. Therefore, methyl, ethyl, and isopropyl thiocyanates should be regarded as rapid-acting, potent poisons.

The **isothiocyanate** group is illustrated in the structure of methylisothiocyanate below:

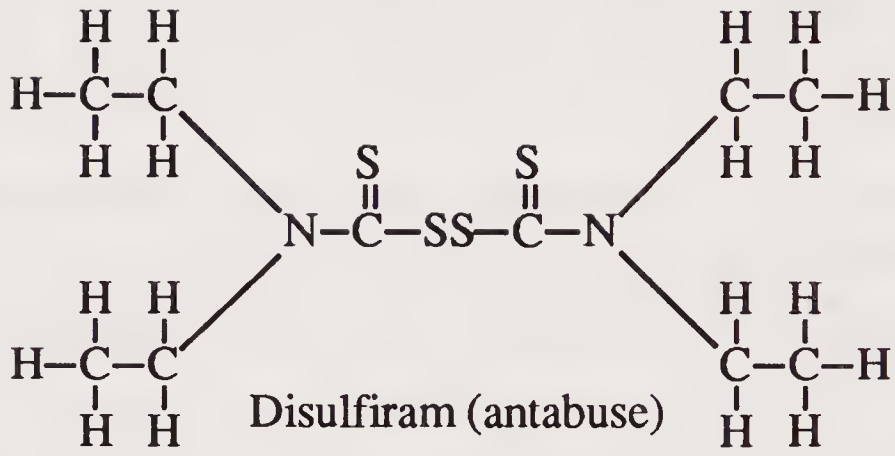


Methylisothiocyanate

Other compounds in this class include ethyl, allyl, and phenyl isothiocyanates. Methylisocyanate, also known as methyl mustard oil, and its ethyl analog have been developed as military poisons. Both are powerful irritants to eyes, skin, and respiratory tract. When decomposed by heat, these compounds emit sulfur oxides and hydrogen cyanide.

Disulfiram

Disulfiram,



Disulfiram (antabuse)

is a sulfur- and nitrogen-containing compound with several industrial uses, including applications as a rubber accelerator and vulcanizer, fungicide, and seed disinfectant. It is most commonly known as antabuse, a therapeutic agent for the treatment of alcohol abuse which causes nausea, vomiting, and other adverse effects when ethanol is ingested.

Cyclic Sulfur/Nitrogen Organic Compounds

The structural formulas of several cyclic compounds containing both nitrogen and sulfur are shown in Figure 17.4. Basic to the structures of these compounds is the simple ring structure of **thiazole**. It is a colorless liquid (bp 117°C). One of its major uses has been for the manufacture of sulfathiazole, one of the oldest of the sulfonamide class of antibacterial drugs. The use of sulfathiazole is now confined to the practice of veterinary medicine because of its serious side effects.[3]

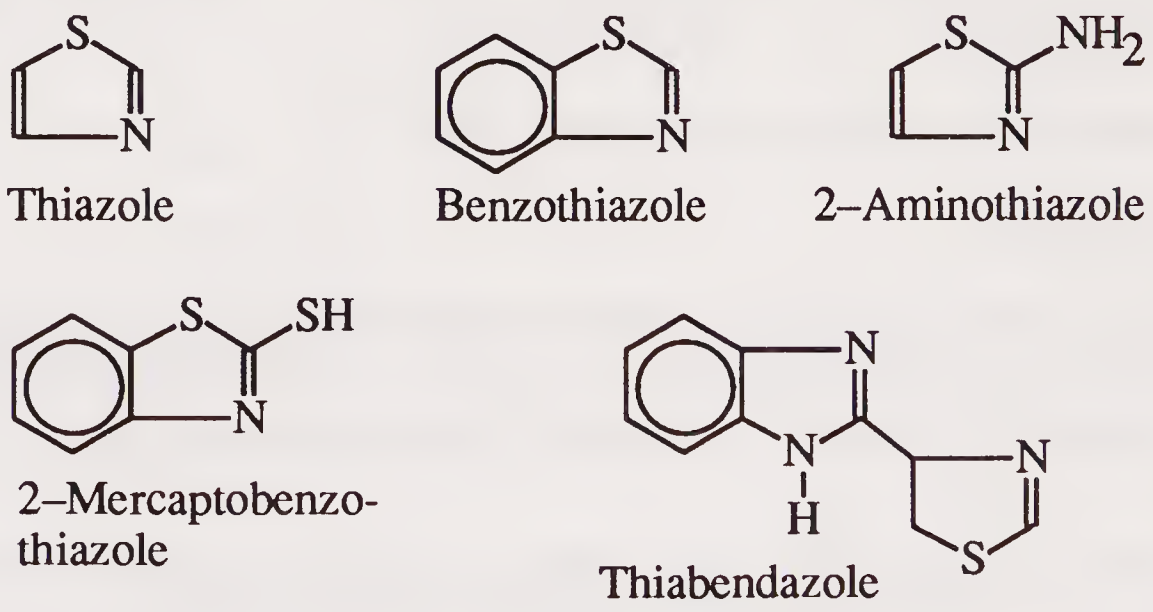


Figure 17.4. Cyclic compounds containing nitrogen and sulfur.

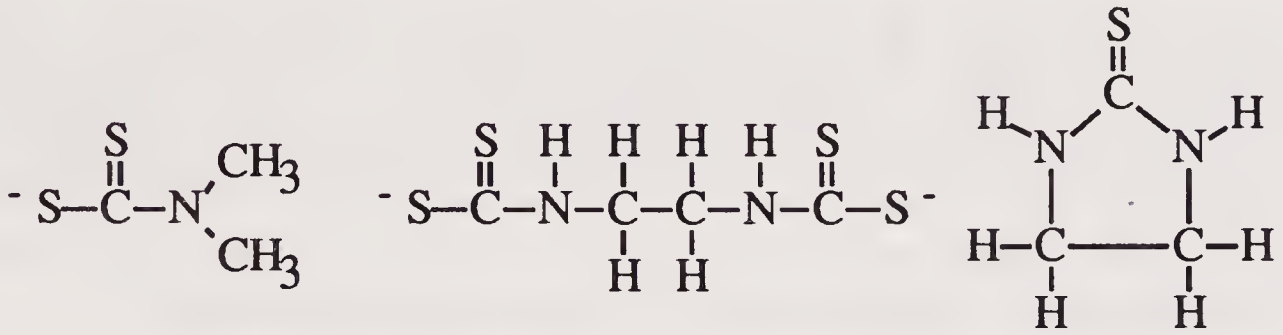
Several derivatives of thiazole have commercial uses. One of these is **2-aminothiazole**, which has shown a high toxicity to experimental animals. **Benzothiazole** is another related compound used in organic synthesis. Its human toxicity is not known, although it has a high toxicity to mice. **Thiabendazole** (2-(4'-thiazoyl)benzimidazole) is a systemic fungicide that can be carried through a plant and onto plant leaves. Rats and dogs tolerate a relatively high dose of this chemical, although it tends to make the latter vomit. Acting as an adjuvant (a substance added to a drug or insecticide to give it a desired form and enhance its action and effective-

ness), 2-mercaptobenzothiazole is mixed with dithiocarbamate fungicides (see below) to increase their potency. It is an allergen that causes type IV (cell-mediated) hypersensitivity.[4] This condition is manifested by contact dermatitis and a delayed hypersensitive reaction that follows a latent period after exposure. This cell-mediated process results from the sensitization of T lymphocytes.

Dithiocarbamates

Dithiocarbamate fungicides consist of metal salts of **dimethylthiocarbamate** and **ethylenebisdithiocarbamate** anions as shown in Figure 17.5. These fungicides are named in accordance with the metal ion present. For example, the manganese salt of dimethyldithiocarbamate is called maneb, and the zinc and sodium salts are zineb and nabam, respectively. The iron salt of ethylenebisdithiocarbamate is called ferbam, and the zinc salt of this ion is called ziram. These salts are chelates (Section 2.4) in which two S atoms from the ethylenebisdithiocarbamate anion are bonded to the same metal ion in a ring structure.

The dithiocarbamate fungicides have been popular for agricultural use because of their effectiveness and relatively low toxicities to animals. However, there is concern over their environmental breakdown products, particularly ethylenethiourea (2-imidazolidinethione, Figure 17.5), which is toxic to the thyroid and has been shown to be mutagenic, carcinogenic, and teratogenic in experimental animals.

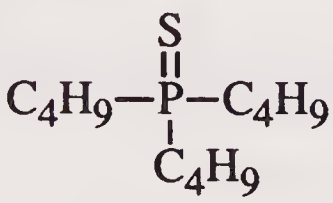


Dimethyldithiocarbamate anion | Ethylenebisdithiocarbamate anion | Ethylenethiourea

Figure 17.5. Dithiocarbamate anions and ethylenethiourea.

Phosphine Sulfides

A number of toxicologically important organic compounds have sulfur bound to phosphorus. The simplest of these are the phosphine sulfides, containing only carbon, hydrogen, phosphorus, and sulfur as illustrated by the example below:



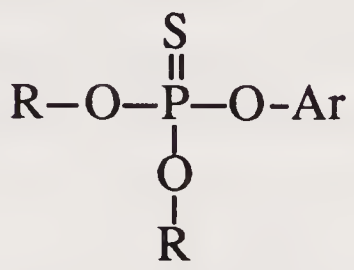
Tributylphosphine sulfide

Phosphine sulfides tend to be toxic. When burned, they give off dangerous phosphorus oxide and sulfur oxide fumes.

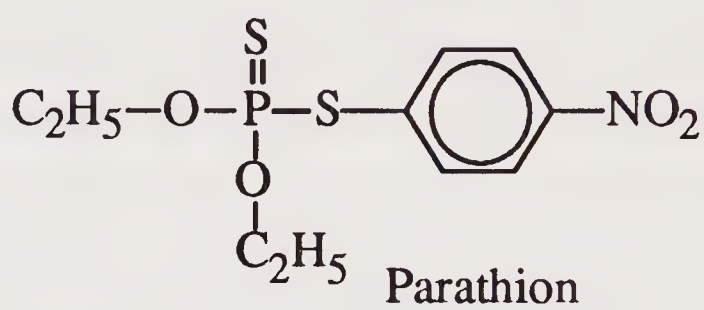
Phosphorothionate and Phosphorodithioate Esters

The most toxicologically significant organic compounds that contain both phosphorus and sulfur are the thiophosphate esters, which are used as insecticidal acetylcholinesterase inhibitors. The general formulas of insecticidal **phosphorothionate** and **phosphorodithioate** esters are shown in Figure 17.6, where R is usually a methyl ($-CH_3$) or ethyl ($-C_2H_5$) group and Ar is a moiety of more complex structure,

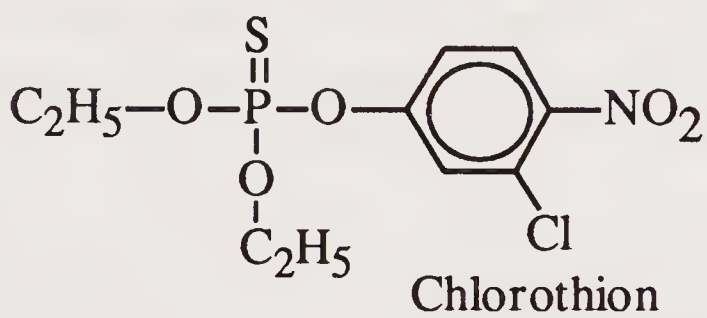
frequently aromatic. Phosphorothionate and phosphorodithioate esters contain the P=S (thiono) group, which increases their insect:mammal toxicity ratios and decreases their tendency to undergo nonenzymatic hydrolysis compared to their analogous compounds that contain the P=O functional group. The metabolic oxidative desulfuration conversion of P=S to P=O in organisms (see Section 17.1) converts the phosphorothionate and phosphorodithioate esters to species that have insecticidal activity.



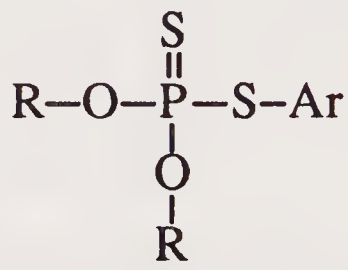
General formula of phosphorothionate insecticides



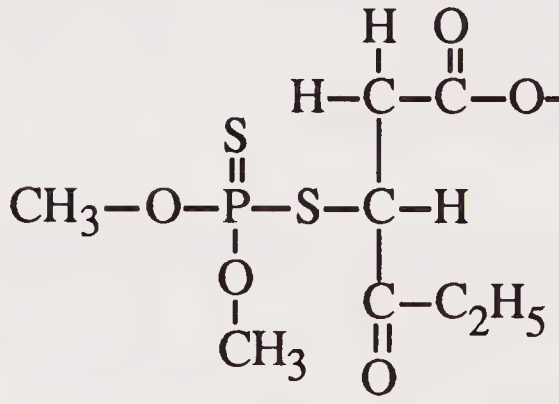
Parathion



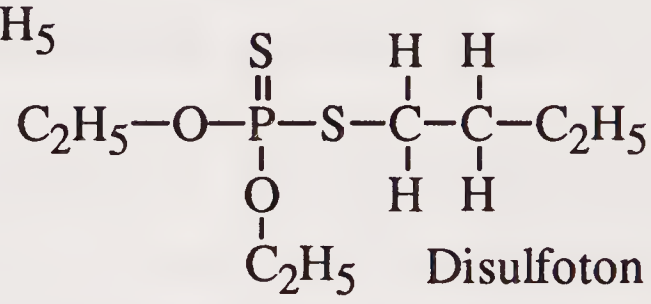
Chlorothion



General formula of phosphorodithioate insecticides



Malathion

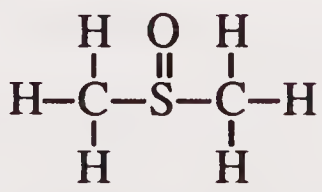


Disulfoton

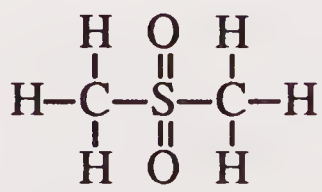
Figure 17.6. General formulas and specific examples of phosphorothionate and phosphorodithioate organophosphate insecticides.

17.4. SULFOXIDES AND SULFONES

Numerous important organic compounds contain oxygen bonded to sulfur. Among these compounds are the **sulfoxides** and **sulfones**, shown by the examples in Figure 17.7.



Dimethylsulfoxide (DMSO)



Dimethylsulfone

Sulfolane

Figure 17.7. Sulfoxides and sulfones.

Dimethylsulfoxide (DMSO) is a liquid with numerous uses and some very interesting properties. Mixed with water, it produces a good antifreeze solution. It is also employed to remove paint and varnish and as a hydraulic fluid. It has some potential pharmaceutical applications, for example, as an anti-inflammatory and bacteriostatic agent. It has the ability to carry solutes into the skin's stratum corneum (see Figure 8.3) from which they are slowly released into the blood and lymph system. This phenomenon has some pharmaceutical potential, as well as some obvious hazards. Dimethylsulfoxide has a remarkably low acute toxicity, with an LD_{50} of 10–20 *grams* per kg in several kinds of experimental animals. DMSO applied to the skin rapidly spreads throughout the body, and the subject experiences a taste in the mouth resembling that of garlic and quickly develops a garlic odor in the breath. Some DMSO is excreted directly in the urine and it also undergoes partial metabolism to dimethylsulfide and dimethylsulfone (Figures 17.2 and 17.7, respectively).

Although dimethylsulfone has some commercial uses, **sulfolane** (Figure 17.7) is the most widely used sulfone.[5] It is a polar aprotic (no ionizable H) solvent with a relatively high dielectric constant, and it dissolves both organic and inorganic solutes. When ionic compounds are dissolved in sulfolane, the cations are solvated (bound by the solvent) rather strongly. However, the anions are left in a relatively unsolvated form, which tends to increase their reactivities substantially.[6] The major commercial use of sulfolane is in an operation called BTX processing in which it selectively extracts benzene, toluene, and xylene from aliphatic hydrocarbons. It is also the solvent in the Sulfinol process by which thiols (Section 17.2) and acidic compounds are removed from natural gas. Sulfolane is used as a solvent for polymerization reactions and as a polymer plasticizer. Exposure to sulfolane can cause eye and skin irritation, although its overall toxicity is relatively low.

17.5. SULFONIC ACIDS, SALTS, AND ESTERS

Sulfonic acids contain the $-SO_3H$ group attached to a hydrocarbon moiety. For many applications these acids are converted to salts, such as sodium 1-(*p*-sulfophenyl)decane, a biodegradable detergent surfactant. Its structural formula and those of two sulfonic acids are shown in Figure 17.8.

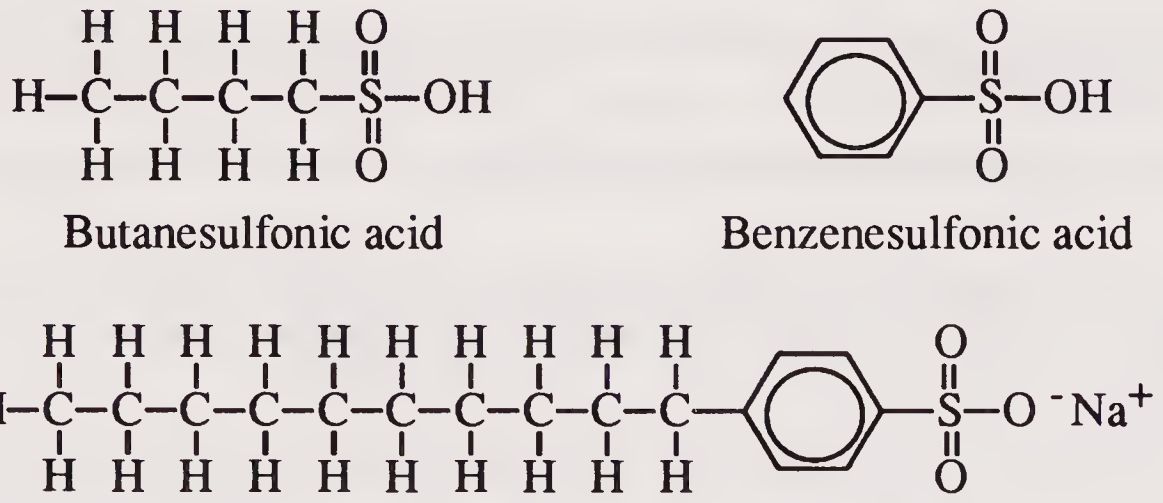


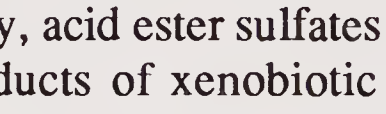
Figure 17.8. Sulfonic acids and a sulfonate salt.

In general, the sulfonic acids are water-soluble and are strong acids because of virtually complete loss of ionizable H^+ in aqueous solution. They have some important commercial applications, such as in the hydrolysis of fats and oils (see Section 4.2

and Figure 4.2) to fatty acids and glycerol. Benzenesulfonic acid is fused with NaOH in the preparation of phenol. Dyes and some pharmaceutical compounds are manufactured from *p*-toluenesulfonic acid. Methanesulfonic acid has been developed as an esterification catalyst in place of sulfuric acid for the synthesis of resins in paints and coatings. A strong acid, one of its major advantages over sulfuric acid is that it is not an oxidizing species.[7]

Benzenesulfonic acid and *p*-toluenesulfonic acid are strong irritants to skin, eyes, and mucous membranes. Solutions of sulfonic acids are strongly acidic and precautions appropriate to the handling of strong acids should be taken with them.

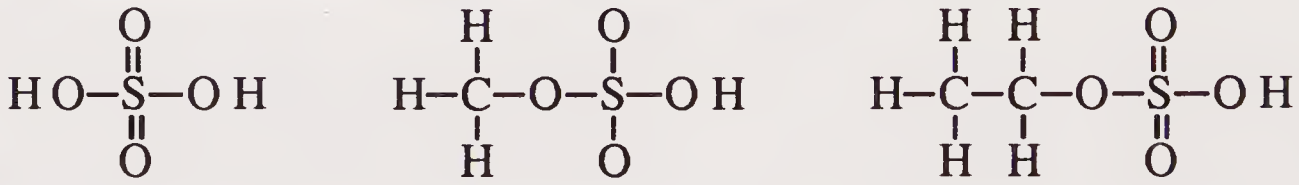
The methyl ester of methylsulfonic acid is methylmethane sulfonate. Its structural formula is the following:


Methylmethane sulfonate

Toxicologically, it is notable for being a primary or direct-acting carcinogen that does not require metabolic conversion to act as a carcinogen.[8]

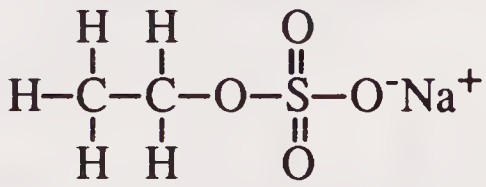
17.6. ORGANIC ESTERS OF SULFURIC ACID

As shown in Figure 17.9, esters of sulfuric acid exist in which either one or both of the ionizable H atoms are replaced by hydrocarbon substituents, such as the methyl group. Replacement of 1 H yields an acid ester and replacement of both yields an ester. Metabolically, acid ester sulfates are synthesized in phase II reactions to produce water-soluble products of xenobiotic compounds (such as phenol) that are readily eliminated from the body (see Sections 4.5 and 9.2).


Sulfuric acid

Methylsulfuric acid

Ethylsulfuric acid


Sodium ethylsulfate

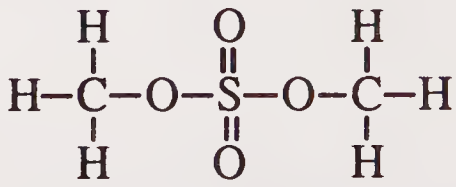

Dimethylsulfate

Figure 17.9. Sulfuric acid and organosulfate esters.

Sulfuric acid esters have several industrial uses, especially as alkylating agents, which act to attach alkyl groups (such as methyl) to organic molecules. Among the products made with sulfuric acid ester reactants are agricultural chemicals, dyes, and drugs.

Methylsulfuric acid is an oily water-soluble liquid. It is a strong irritant to skin, eyes, and mucous tissue. **Ethylsulfuric acid** is likewise an oily liquid and a strong tissue irritant. **Sodium ethylsulfate** is a hygroscopic white crystalline solid.

Dimethylsulfate is a liquid (bp 188°C, fp -32°C). It is colorless, odorless, and highly toxic. It is a primary carcinogen.[8] When skin or mucous membranes are exposed to dimethylsulfate, there is an initial latent period during which few

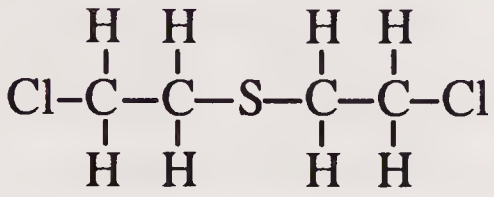
symptoms are observed. After this period, conjunctivitis and inflammation of nasal tissue and respiratory tract mucous membranes develop. Heavier exposures damage the liver and kidney and cause pulmonary edema and cloudiness of the cornea. Death can follow in 3–4 days. The related compound, diethylsulfate, is an oily liquid. It reacts with water to yield sulfuric acid. Like dimethylsulfate, it is a strong irritant to tissue and has proven to be carcinogenic in experimental animals.

17.7. MISCELLANEOUS ORGANOSULFUR COMPOUNDS

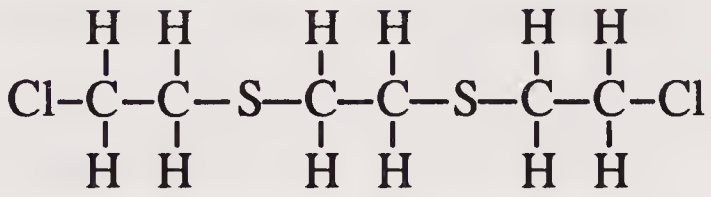
A number of sulfur compounds containing other elements, such as the halides, are used for various purposes. Some examples of such compounds are shown in Figure 17.10 and discussed briefly here.

Sulfur Mustards

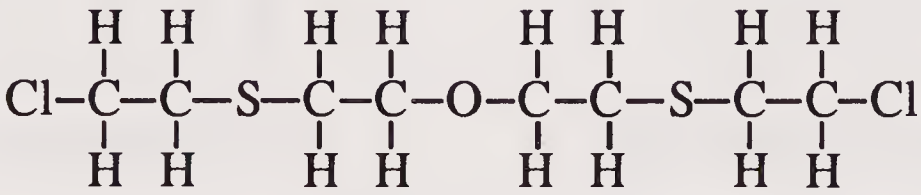
The first three compounds shown in Figure 17.10 are **sulfur mustards**, which are highly toxic military poisons,[9] or "poison gases." These are mustard oil (bis(2-chloroethyl)sulfide), sesquimustard (1,2-bis(2-chloroethylthio)ethane), and O-mustard (bis(2-chloroethylthioethyl)ether). The toxic properties of mustard oil are typical of those of the sulfur mustards. As a military "blistering gas" poison, the vapors of this compound are very penetrating, so that it damages and destroys tissue at some depth from the point of contact. Affected tissue becomes severely inflamed and the resulting lesions often become infected. Death can result from pulmonary lesions. Part of the hazard of mustard oil stems from the speed with which it penetrates tissue, so that efforts to remove it from the exposed area are ineffective after about 30 minutes. The compound is an experimental mutagen[10] and primary carcinogen.[8]



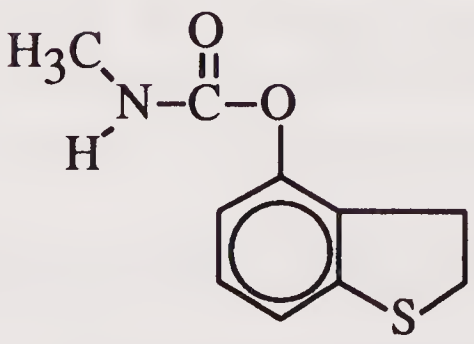
Mustard oil (H)



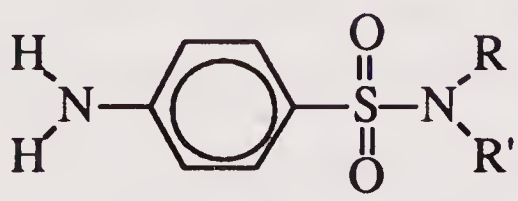
Sesquimustard (Q)



O–Mustard (T)



Mobam insecticide



General formula of sulfa drugs

Figure 17.10. Some miscellaneous organosulfur compounds.

Sulfur in Pesticides

In Section 17.3, rodenticidal thioureas, insecticidal thiocyanates, and fungicidal dithiocarbamates were discussed. Sulfur is a common constituent of other classes of insecticides. These include prominently the organophosphate insecticides discussed in Chapter 18. Mobam (Figure 17.10) is a contact insecticide of the carbamate type, closely related in structure and function to the well-known carbamate insecticide carbaryl (see Figure 15.8). Mobam has been found to have a relatively high toxicity to laboratory mammals and is considerably more toxic than carbaryl.

Sulfa Drugs

The general structure representing sulfa drugs (sulfonamides) is shown in Figure 17.10, where the R groups may be various substituents. In the simplest of these, sulfanilamide, both R groups are H. It was once the most commonly used therapeutic sulfonamide but, because of side effects in humans, is now limited largely to the practice of veterinary medicine. It has a toxicity rating of 3. A large number of therapeutic sulfonamides have been produced. Some of the compounds have a tendency to cause injury to the urinary tract by precipitating in the kidney.

17.8. ORGANICALLY BOUND SELENIUM

Selenium, Se, is directly below sulfur in the periodic table and has a number of chemical similarities to sulfur. There is some evidence to suggest that a significant fraction of environmental selenium, such as selenium in soil, is substituted for sulfur in seleno-amino acids bound in peptides and proteins. This view is supported by a study of selenium in soil humic acid.[11] This study showed that most of the selenium in the soil samples studied could be extracted by base along with the humic and fulvic acid fractions (see Section 2.4). These humic substances are biodegradation residues of plant and animal biomass, and the results suggest that selenium originally added to the soil in the inorganic form was converted to proteinaceous selenium, which was incorporated into humic and fulvic acids. Acidic hydrolysis of these substances appeared to release selenium as seleno-amino acids. Seleno-methionine amino acid has been identified in selenium-rich soil humic matter.

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QUESTIONS

1. What is the inorganic hydride of sulfur? In what sense may organosulfur compounds be viewed as derivatives of this compound? What kind of organosulfur compound is formed by substituting alkyl or aryl hydrocarbon groups such as phenyl and methyl for H on this hydride?

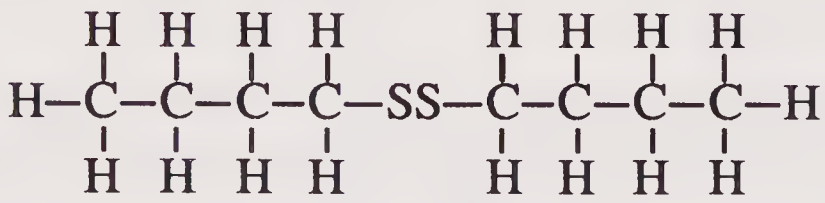
2. What are the most common toxicological chemical reactions involving organosulfur? Give an example of each.

3. What kind of organosulfur reaction is illustrated by the following:

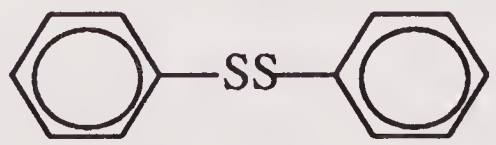
$$2R\text{-}SH + \{O\} \rightarrow R\text{-}S\,S\text{-}R + H_2O \qquad (17.1.1)$$

4. What kinds of compound are known as mercaptans? Give an example formula of a mercaptan. How do these compounds make their presence known? How is this property put to practical use as a safety measure?

5. Despite the similarity of their names, how does carbon disulfide, CS_2, differ from from the compounds shown below:



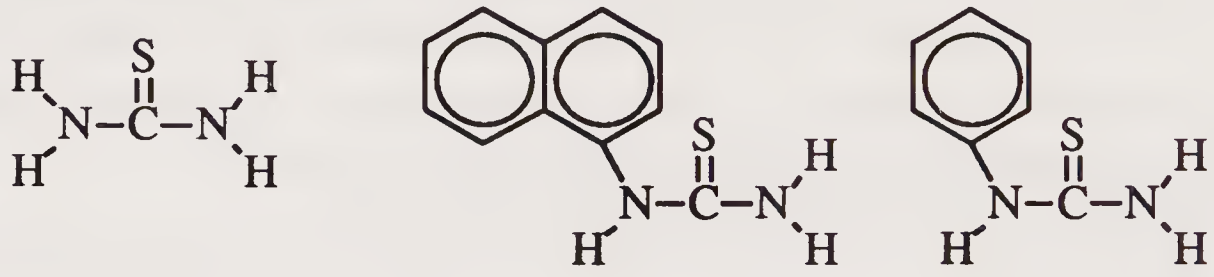
n –Butyldisulfide



Diphenyldisulfide

6. What are the symptoms of chronic carbon disulfide poisoning? How does this compound appear to affect the central nervous system?

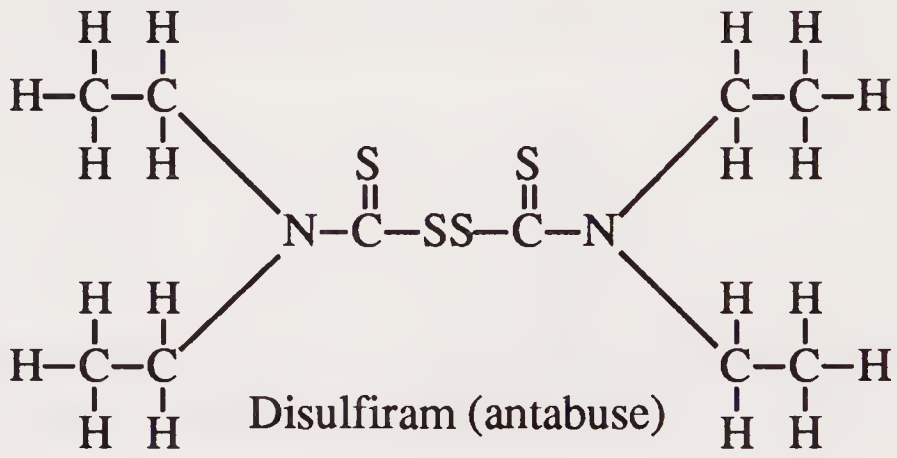
7. To what general class of organosulfur compounds do the following belong? What are some of their uses and toxicological properties?



8. What class of organosulfur compounds was regarded as the "first synthetic organic insecticides?" Give an example of one of these compounds.

9. What is an adjuvant? Explain the application of 2-mercaptobenzothiazole as an adjuvant?

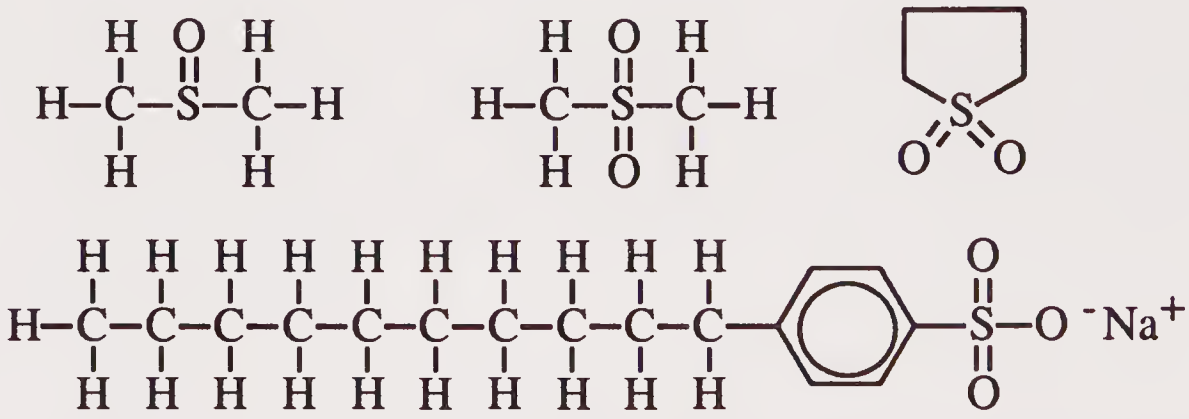
10. What are the therapeutic uses of the compound shown below. What other therapeutically-used compound may be derived from it?



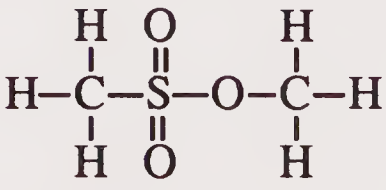
Disulfiram (antabuse)

11. What is done to organophosphate compounds containing the P=O group to increases their insect:mammal toxicity ratios and decrease their tendency to undergo non-enzymatic hydrolysis?

12. To what general classes of compounds do the following belong? What are some of their properties and uses?



13. What is a particularly notorious toxicological property of the following compound?



Methylmethane sulfonate

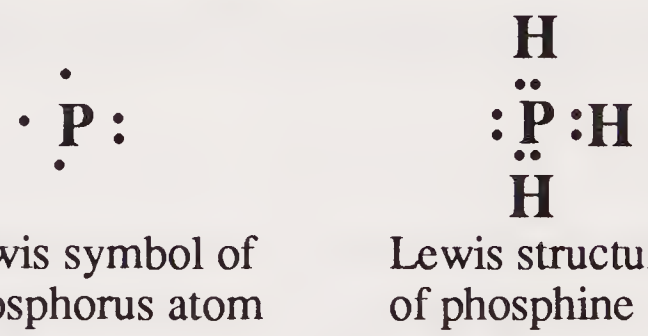
14. Give the properties and uses of sesquimustard, Mobam, and the sulfonamides.
15. What is the basic structure of thiazole? For which major pharaceutical can it serve as a raw material? Why is this pharmaeutical no longer widely used? Give the names, structures and properties or uses of some compounds related to thiazole.
16. Give the names and structures of two dithiocarbamate fungicides. How are they used? How are zinc and iron involved with these fungicides?
17. Which are the simplest of the toxicologically important organic compounds that contain sulfur bound to phosphorus? What would be the formula of the trimethyl member of this group?

18

Organophosphorus Compounds

18.1. INTRODUCTION

Phosphorus is directly below nitrogen in the periodic table. (The relationship of the chemistry of phosphorus to that of nitrogen is somewhat like the sulfur-oxygen relationship discussed in the introduction to Chapter 17.) The phosphorus atom electron configuration is $\{Ne\}3s^23p^3$, and it has five outer-shell electrons as shown by its Lewis symbol in Figure 18.1. Because of the availability of underlying $3d$ orbitals, the valence shell of phosphorus can be expanded to more than 8 electrons.



Lewis symbol of phosphorus atom — Lewis structural formula of phosphine

Figure 18.1. Lewis representations of the phosphorus atom and its hydride, phosphine, showing valence electrons as dots.

There are many kinds of organophosphorus compounds, including those with P-C bonds and those in which hydrocarbon moieties are bonded to P through an atom other than carbon, usually oxygen. These compounds have numerous industrial uses and many of them, especially the organophosphate ester insecticides (see Section 18.7), are economic poisons, that is, they are used to destroy pests that are harmful to crops, fruits and vegetables. Organophosphorus compounds have varying degrees of toxicity. Some of these compounds, such as the "nerve gases" produced as military poisons, are deadly in minute quantities. The organophosphate esters, a class of compounds that contains the organophosphate ester insecticides and the organophosphate military poisons, are of particular toxicological interest because of their ability to inhibit acetylcholinesterase enzyme.

Phosphine

Phosphine (PH_3) is the hydride of phosphorus discussed as a toxic inorganic compound in Section 12.10. The formulas of many organophosphorus compounds can be derived by substituting organic groups for the H atoms in phosphine, and such an approach serves as a good starting point for the discussion of organophosphorus compounds.

18.2. ALKYL AND ARYL PHOSPHINES

Figure 18.2 gives the structural formulas of the more significant alkyl and aryl phosphine compounds. **Methylphosphine** is a colorless reactive gas that is very toxic by inhalation. **Dimethylphosphine** is a colorless, reactive, volatile liquid (bp 25°C). It is toxic by inhalation and ingestion. Both methylphosphine and dimethylphosphine have toxic effects similar to those of phosphine, a pulmonary tract irritant and central nervous system depressant that causes fatigue, vomiting, difficult breathing and even death. **Trimethylphosphine** is a colorless volatile liquid (bp 42°C). It is reactive enough to be spontaneously ignitable and probably has a high toxicity. **Triethylphosphine** probably has a high toxicity and tributylphosphine is a moderately toxic liquid. **Phenylphosphine** (phosphaniline) is a reactive, moderately flammable liquid (bp 16°C) with a high toxicity by inhalation. **Triphenylphosphine** is a crystalline solid (mp 79°C, bp > 360°C) with a low reactivity and moderate toxicity when inhaled or ingested.

The combustion of aryl and alkyl phosphines, such as trimethylphosphine, occurs as shown by the following example:

$$4C_3H_9P + 26O_2 \rightarrow 12CO_2 + 18H_2O + P_4O_{10} \qquad (18.2.1)$$

Such a reaction produces P_4O_{10}, a corrosive irritant toxic substance discussed in Section 12.10, or droplets of corrosive orthophosphoric acid, H_3PO_4.

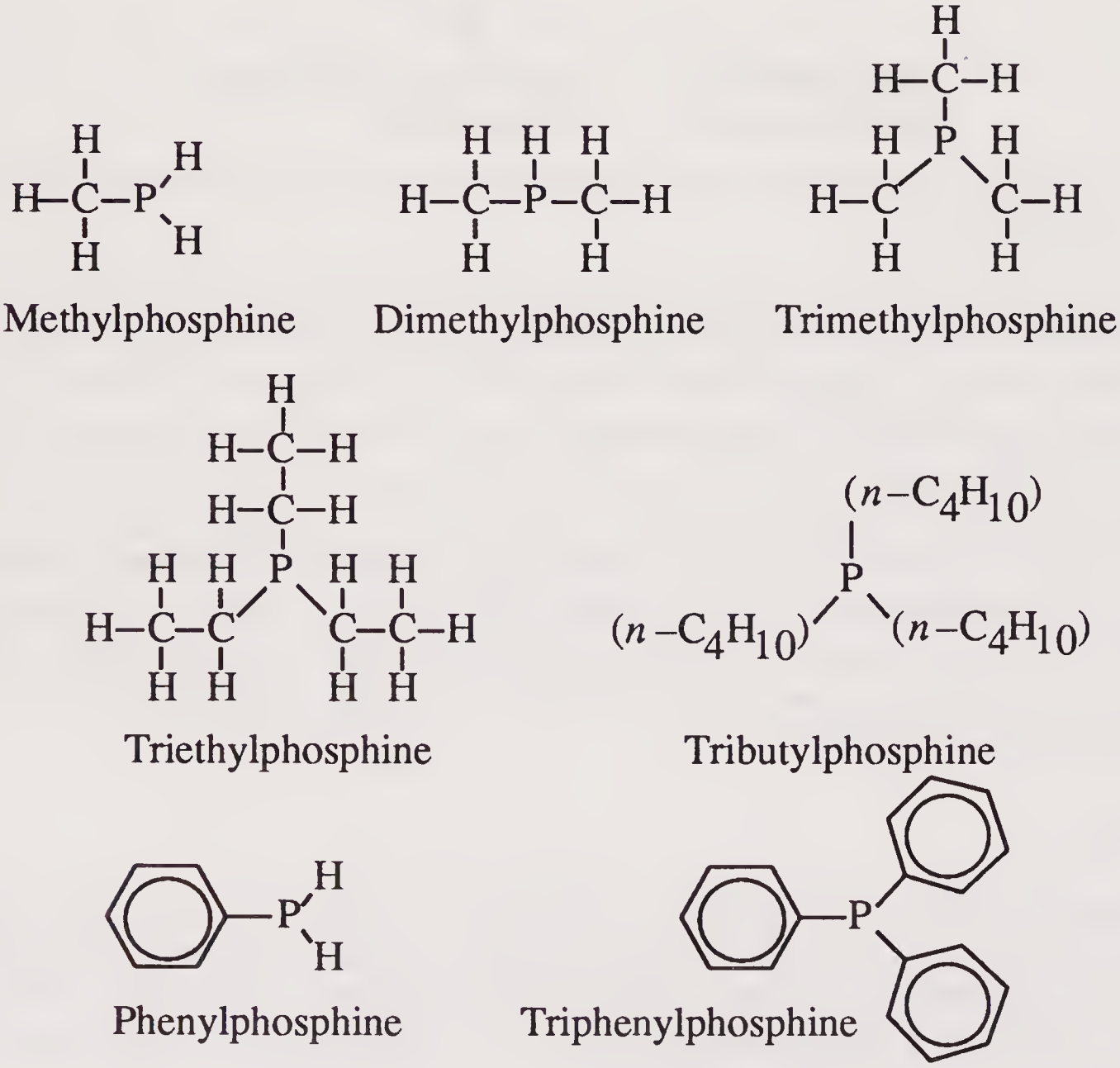
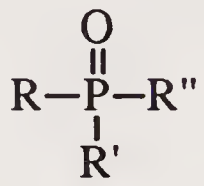


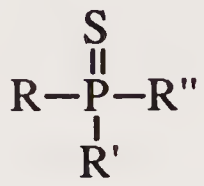
Figure 18.2. Some of the more significant alkyl and aryl phosphines.

18.3. PHOSPHINE OXIDES AND SULFIDES

Phosphine oxides and sulfides have the general formulas illustrated below, where the Rs represent hydrocarbon groups:



Phosphine oxide



Phosphine sulfide

Two common phosphine oxides are **triethylphosphine** oxide (each R is a C_2H_5 group) and **tributylphosphine oxide** (each R is a C_4H_9 group). The former is a colorless, deliquescent, crystalline solid (mp 52.9°C, bp 243°C). The latter compound is a crystalline solid (mp 94°C). Both compounds probably have high toxicities when ingested.

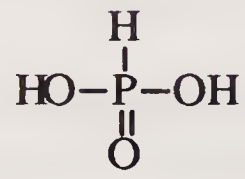
Triethylphosphine sulfide, $(C_2H_5)_3PS$, is a crystalline solid (mp 94°C). Not much is known about its toxicity, which is probably high. **Tributylphosphine sulfide**, $(C_4H_9)_3PS$, is a skin irritant with a moderate toxicity hazard.[1] When burned, both of these compounds give off dangerous fumes of phosphorus and sulfur oxides.

18.4 PHOSPHONIC AND PHOSPHOROUS ACID ESTERS

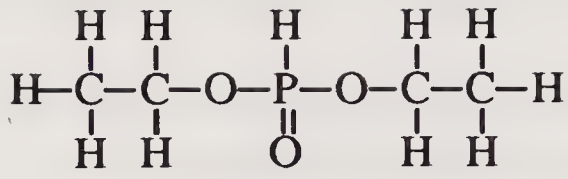
Phosphonic acid esters are derived from phosphonic acid (often erroneously called phosphorous acid), which is shown with some of its esters in Figure 18.3. Only two of the H atoms of phosphonic acid are ionizable, and hydrocarbon groups may be substituted for these atoms to give phosphonic acid esters. It is also possible to have esters in which a hydrocarbon moiety is substituted for the H atom that is bonded directly to the phosphorus atom. An example of such a compound is **benzylphosphonic acid**, diethyl ester, shown in Figure 18.3. This type of compound has the same elemental formula as triesters of the hypothetical acid, $P(OH)_3$, phosphorous acid. Examples of triesters of phosphorous acid such as **trimethylphosphite** are shown in Figure 18.3.

Trimethylphosphite is a colorless liquid (bp 233°C). It is soluble in many organic solvents, but not in water. Little information is available regarding its toxicity or other hazards. **Tributylphosphite** is a liquid, bp 120°C. It decomposes in water, but is probably not very toxic. **Triphenylphosphite** is a white solid or oily liquid (mp 23°C, bp 157°C). It is a skin irritant with a moderate oral toxicity. Although it is not soluble in water, it may hydrolyze somewhat to phenol, which adds to its toxicity. **Tris(2-ethylhexyl)phosphite** (a trialkyl phosphite in which the hydrocarbon moieties are the 2-ethylhexyl group, $-CH_2CH(C_2H_5)C_4H_9$) is a water-insoluble compound (bp 100°C). Its toxicity is largely unknown.

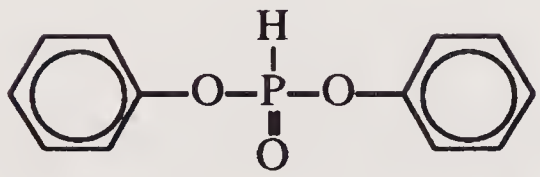
Methylphosphonate $(CH_3O)P(O)H(OH)$ has a moderate oral toxicity and is a skin and eye irritant. **Dibutylphosphonate** (formula $(C_4H_9O)_2P(O)H$) is a liquid boiling at 115°C at 10 mm Hg pressure. Through ingestion and dermally it has a moderately high toxicity. Like other organophosphonates and phosphites, it can decompose to evolve dangerous products when heated, burned, or exposed to reactive chemicals, such as oxidants. Thermal decomposition can result in the evolution of highly toxic phosphine, PH_3. Combustion produces corrosive orthophosphoric acid and oxides of phosphorus.



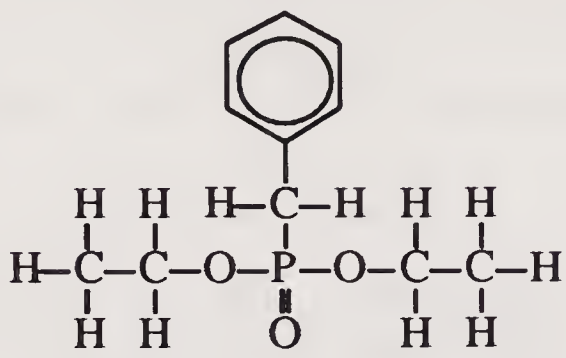
Phosphonic acid



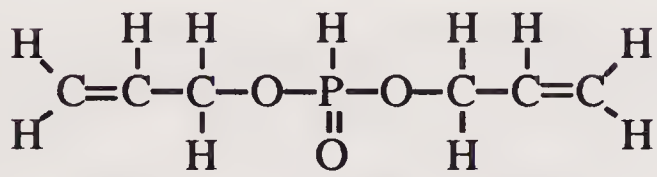
Diethylphosphonate



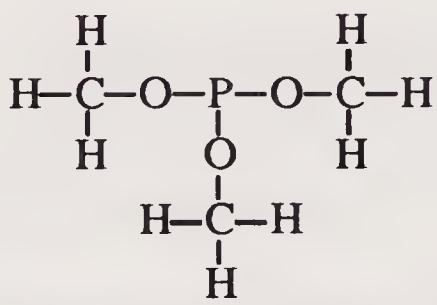
Diphenylphosphonate



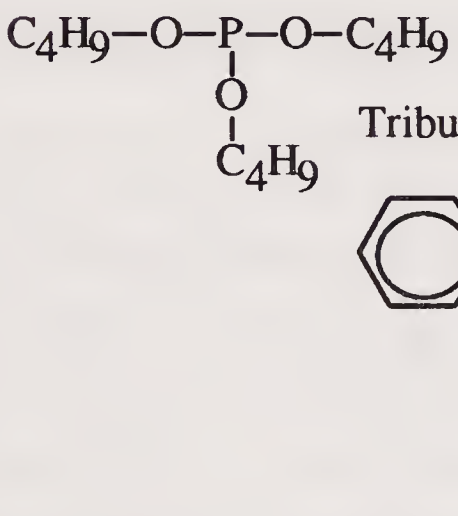
Benzyl phosphonic acid, diethyl ester



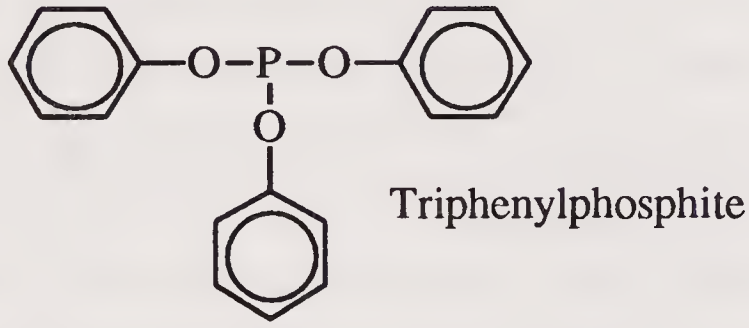
Diallylphosphonate



Trimethylphosphite



Tributylphosphite



Triphenylphosphite

Figure 18.3 Phosphonic acid and esters of phosphonic and phosphorous acids.

Diallylphosphonate, shown in Figure 18.3, has two alkenyl substituent groups. Information is lacking on its toxicity, although compounds with allyl groups tend to be relatively toxic. Incidents have been reported in which this compound has exploded during distillation.[2]

18.5. ORGANOPHOSPHATE ESTERS

Orthophosphates and Polyphosphates

Figure 18.4 shows the structural formula of orthophosphoric acid as well as those of diphosphoric and polyphosphoric acids produced by polymerization of orthophosphoric acid with loss of water. These compounds form esters in which alkyl, alkenyl, and aryl hydrocarbon moieties are substituted for H; most of the more common ones are esters of orthophosphoric acid. In this section only the relatively simple organophosphate esters are discussed. Many economic poisons — particularly insecticides — are organophosphate esters that often contain nitrogen and sulfur. These compounds are discussed in a later section.

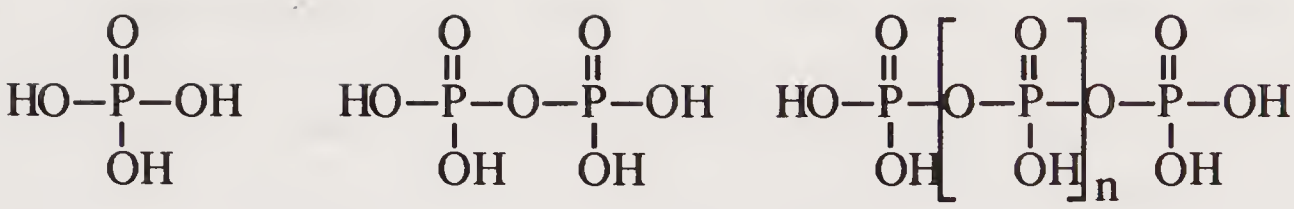


Figure 18.4. Orthophosphoric acid and acids formed by its polymerization.

Orthophosphate Esters

Some of the more significant phosphate esters are shown in Figure 18.5. Trimethylphosphate is the simplest of the organophosphate esters; the structural formulas of the other alkyl esters of orthophosphoric acid are like those of trimethylphosphate, but with alkyl substituent groups other than methyl. Comparatively little information is available about the toxicity of trimethylphosphate, although it is probably moderately toxic orally or through skin absorption.

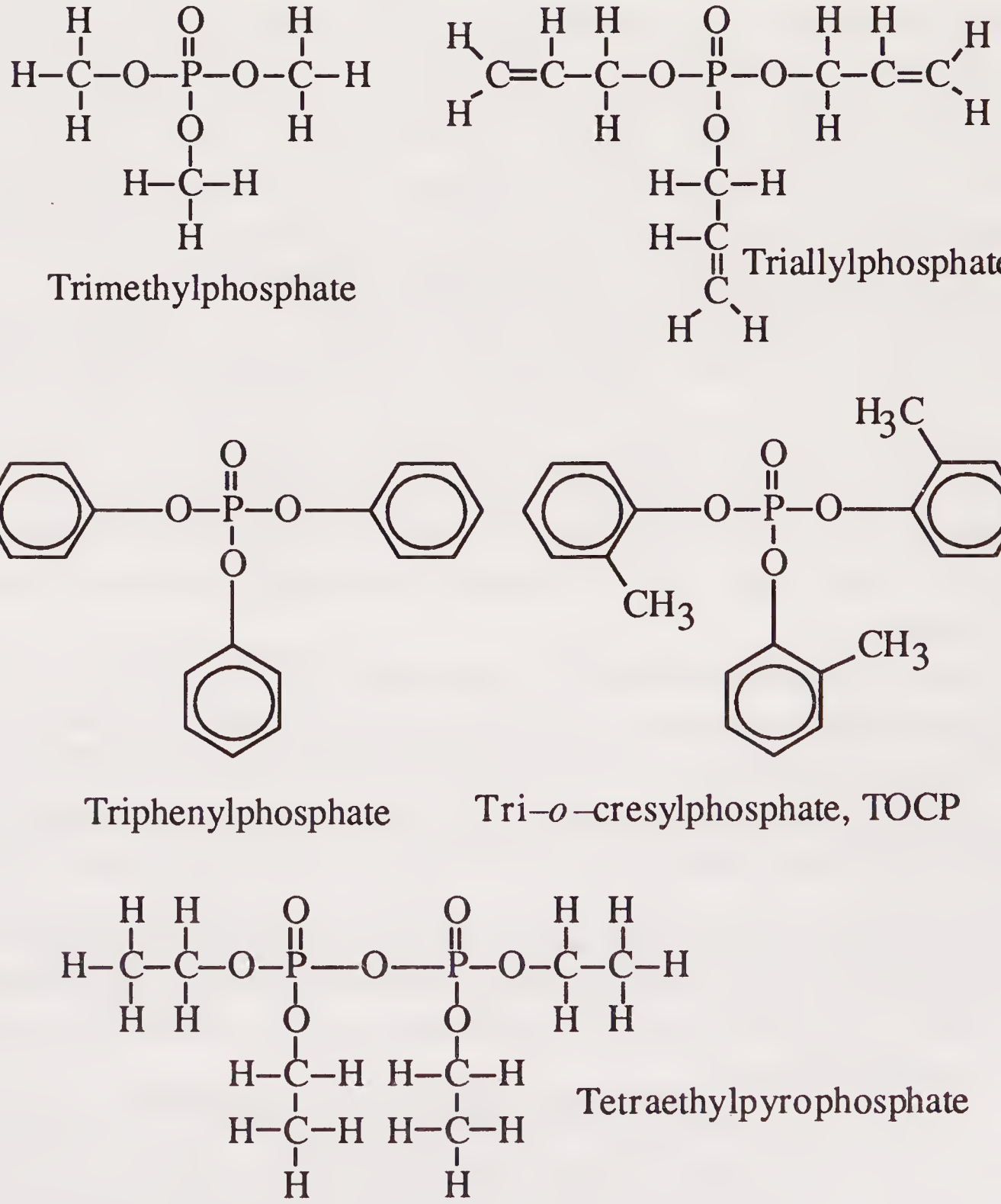


Figure 18.5. Phosphate esters.

Triethylphosphate, $(C_2H_5O)_3PO$, is a liquid (fp -57°C, bp 214°C). It is insoluble in water, but soluble in most organic solvents. Like other phosphate esters, it damages nerves and is a cholinesterase inhibitor. It is regarded as moderately toxic. Two other alkyl phosphates with toxicities probably similar to that of triethyphosphate are **tributylphosphate**, $(n\text{-}C_4H_9O)_3PO$, and **tris(2-ethylhexyl)phosphate**, formula $(C_8H_{17}O)_3PO$.

Triallylphosphate is the phosphate triester of allyl alcohol and contains unsaturated C=C bonds in its structure. This compound is a liquid (fp -50°C). It is regarded as having a high toxicity and produces abnormal tissue growth when administered subcutaneously. It has been known to explode during distillation.

Aromatic Phosphate Esters

Triphenylphosphate is a colorless, odorless, crystalline solid (mp 49°C, bp 245°C). It is moderately toxic. A similar, but much more toxic, compound is **tri-*o*-cresyl-phosphate, TOCP,** an aryl phosphate ester with a notorious record of poisonings.[3] Before its toxicity was fully recognized, TOCP was a common contaminant of commercial **tricresylphosphate**. Tricresylphosphate is an industrial chemical with numerous applications and consists of a mixture of phosphate esters in which the hydrocarbon moieties are *meta* and *para* cresyl substituents. It has been used as a lubricant, gasoline additive, flame retardant, solvent for nitrocellulose, plasticizer, and even a cooling fluid for machine guns. Although modern commercial tricresylphosphate contains less than 1% TOCP, contaminant levels of up to 20% in earlier products have resulted in severe poisoning incidents.

Pure TOCP is a colorless liquid (fp -27°C, bp 410°C). It produces pronounced neurological effects and causes degeneration of the neurons in the body's central and peripheral nervous systems, although fatalities are rare. Early symptoms of TOCP poisoning include nausea, vomiting, and diarrhea accompanied by severe abdominal pain. Normally a 1–3 week latent period occurs after these symptoms have subsided, followed by manifestations of peripheral paralysis as evidenced by "wrist drop" and "foot drop." In some cases, the slow recovery is complete, whereas in others partial paralysis remains.

The most widespread case of TOCP poisoning occurred in the U.S. in 1930 when approximately 20,000 people were affected by the ingestion of alcoholic Jamaican ginger ("Jake") adulterated by 2% TOCP. The peculiar manner in which the victims walked, including "foot drop," slapping the feet on the floor, high stepping, and unsteadiness, gave rise to the name of "jake-leg" to describe the very unfortunate condition.

A major incident of TOCP poisoning affected 10,000 people in Morocco in 1959. The victims had eaten food cooked in olive oil adulterated with TOCP-contaminated lubricating oil. A number of cases of permanent paralysis resulted from ingestion of the contaminated cooking oil.

It is believed that metabolic products of TOCP inhibit acetylcholinesterase. Apparently other factors are involved in its neurotoxicity. Despite the devastating effects of TOCP, the percentage of virtually complete recovery in healthy subjects is relatively high.

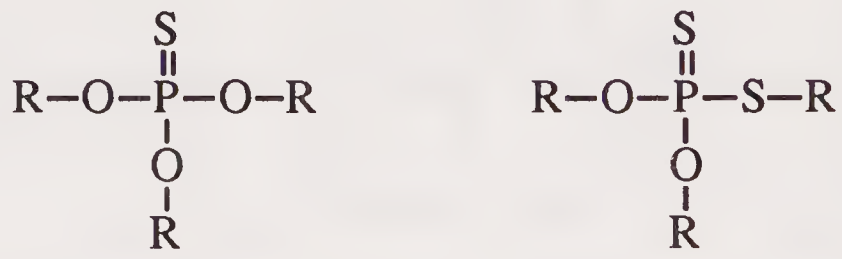
Tetraethylpyrophosphate

Tetraethylpyrophosphate, TEPP, was the first organophosphate compound to be used as an insecticide.[4] This compound was developed in Germany during World War II and was substituted for nicotine as an insecticide. It is a white to amber hygroscopic liquid (bp 155°C) that readily hydrolyzes in contact with water. Because of its tendency to hydrolyze and its extremely high toxicity to mammals, TEPP was used for only a very short time as an insecticide, although it is a very effective one. It was typically applied as an insecticidal dust formulation containing 1% TEPP.

The toxicity of TEPP to humans and other mammals is very high and it has a toxicity rating of 6, supertoxic. TEPP is a very potent acetylcholinesterase inhibitor. (The inhibition of acetylcholinesterase by organophosphate insecticides is discussed in Section 18.7.)

18.6. PHOSPHOROTHIONATE AND PHOSPHORODITHIOATE ESTERS

The general formulas of **phosphorothionate** and **phosphorodithioate** esters are shown in Figure 18.6, where R represents a hydrocarbon or substituted hydrocarbon moiety. Many of the organophosphate insecticides are sulfur-containing esters of these general types, which often exhibit higher insect:mammal toxicity ratios than do their nonsulfur analogs. Esters containing the P=S (thiono) group are not as effective as their analogous compounds that contain the P=O functional group in inhibiting acetylcholinesterase.[5] In addition to their lower toxicities to nontarget organisms, thiono compounds are more stable toward nonenzymatic hydrolysis. The metabolic conversion of P=S to P=O (oxidative desulfuration) in organisms is responsible for the insecticidal activity and mammalian toxicity of phosphorothionate and phosphorodithioate insecticides.



Phosphorothionate Phosphorodithioate

Figure 18.6. General formulas of phosphorothionate and phosphorodithioate esters; each R represents a hydrocarbon or substituted hydrocarbon moiety.

An example of a simple phosphorothionate is tributylphosphorothionate, in which the R groups (above) are *n*-C_4H_9 groups. It is a colorless liquid (bp 143°C). The compound is a cholinesterase inhibitor, as are some of its metabolic products. Examples of phosphorothionate and phosphorodithioate esters with more complex formulas synthesized for their insecticidal properties are discussed in the following section.

18.7. ORGANOPHOSPHATE INSECTICIDES

The organophosphate insecticides were originally developed in Germany during the 1930s and 1940s, primarily through the efforts of Gerhard Schrader and his research group. The first of these was tetraethylpyrophosphate, TEPP, discussed in

Section 18.5. Its disadvantages — including high toxicity to mammals — led to the development of related compounds, starting with **parathion**, *O,O*-diethyl-*O*-*p*-nitrophenylphosphorothionate, which will be discussed in some detail.

Chemical Formulas and Properties

Many insecticidal organophosphate compounds have been synthesized. Unlike the organohalide insecticides that they largely displaced, the organophosphates readily undergo biodegradation and do not bioaccumulate. The most common organophosphate insecticides can be represented by the general formulas for phosphorothionate esters (Figure 18.7), phosphorodithioate esters (Figure 18.8), and their oxygen analogs, phosphate esters (Figure 18.9). In these generalized formulas, R is a methyl ($-CH_3$) or ethyl ($-C_2H_5$) group and Ar is a moiety of more complex structure, frequently aromatic. Example organophosphate insecticides based upon these three types of esters are also shown in these figures.

Phosphorothionate Insecticides

Figure 18.7 gives the structural formulas of three typical phosphorothionate esters and the general formula of this type of organophosphate insecticide.

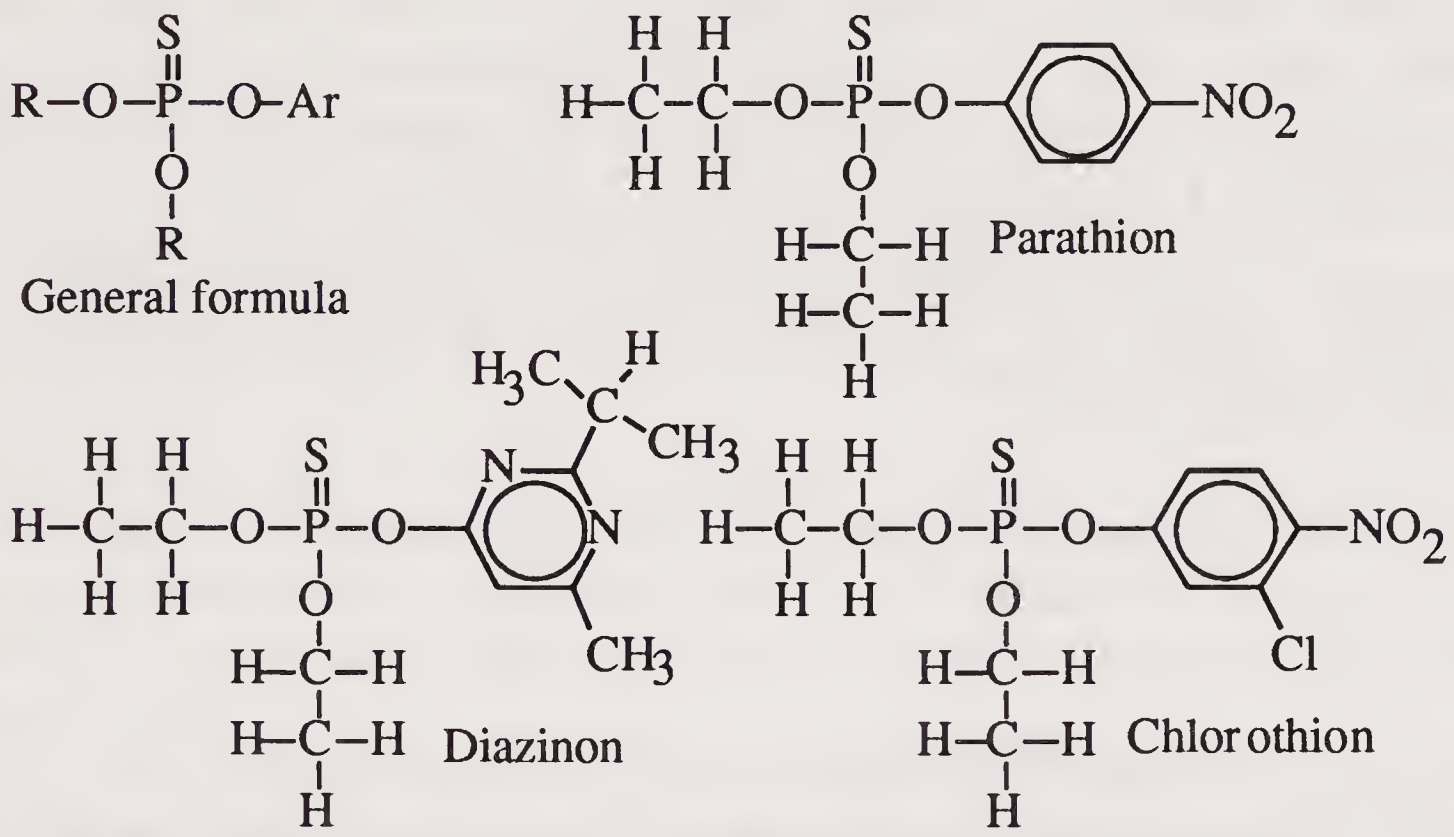


Figure 18.7. Phosphorothionate organophosphate insecticides.

Parathion

Insecticidal parathion is a phosphorothionate ester first licensed for use in 1944. Pure parathion is a yellow liquid that is insoluble in kerosene and water, but stable in contact with water. Among its properties that make parathion convenient to use as an insecticide are stability in contact with neutral and somewhat basic aqueous solutions, low volatility, and toxicity to a wide range of insects. It is applied as an emulsion in water, dust, wettable powder, or aerosol. It is not recommended for applications in homes or animal shelters because of its toxicity to mammals.

Parathion has a toxicity rating of 6 (supertoxic) and methylparathion (which has methyl groups instead of the ethyl groups shown in Figure 18.7) is regarded as

extremely toxic.[6] As little as 120 mg of parathion has been known to kill an adult human and a dose of 2 mg has killed a child. Most accidental poisonings have occurred by absorption through the skin. Since its use began, several hundred people have been killed by parathion. One of the larger poisoning incidents occurred in Jamaica in 1976 from ingestion of parathion-contaminated flour. Of 79 people exposed, 17 died.

In the body, parathion is converted to paraoxon (structure in Figure 18.9), which is a potent inhibitor of acetylcholinesterase. Because this conversion is required for parathion to have a toxic effect, symptoms develop several hours after exposure, whereas the toxic effects of TEPP or paraoxon develop much more rapidly. Symptoms of parathion poisoning in humans include skin twitching, respiratory distress, and, in fatal cases, respiratory failure due to central nervous system paralysis.

Phosphorodithioate Insecticides

Figure 18.8 shows the general formula of phosphorodithioate insecticides and structural formulas of some examples, of which **malathion** is the best known.

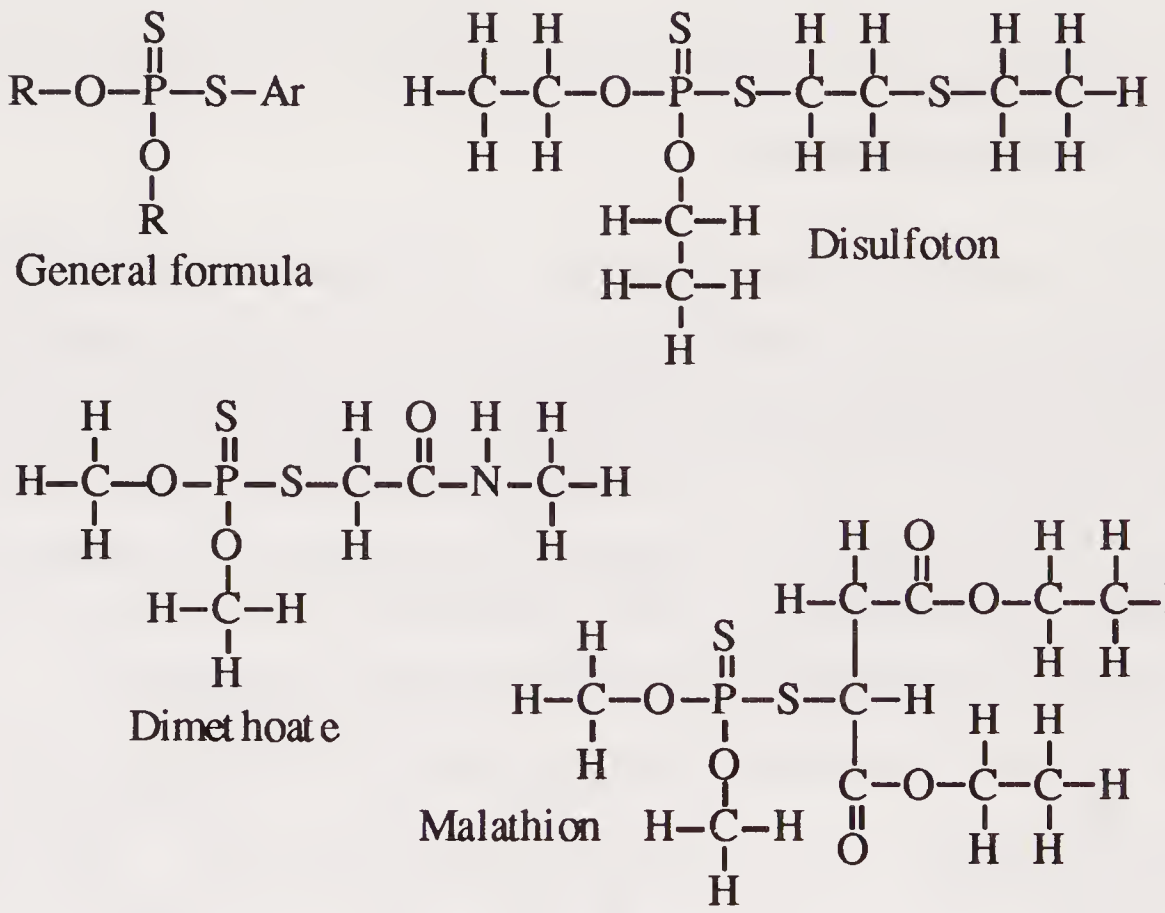
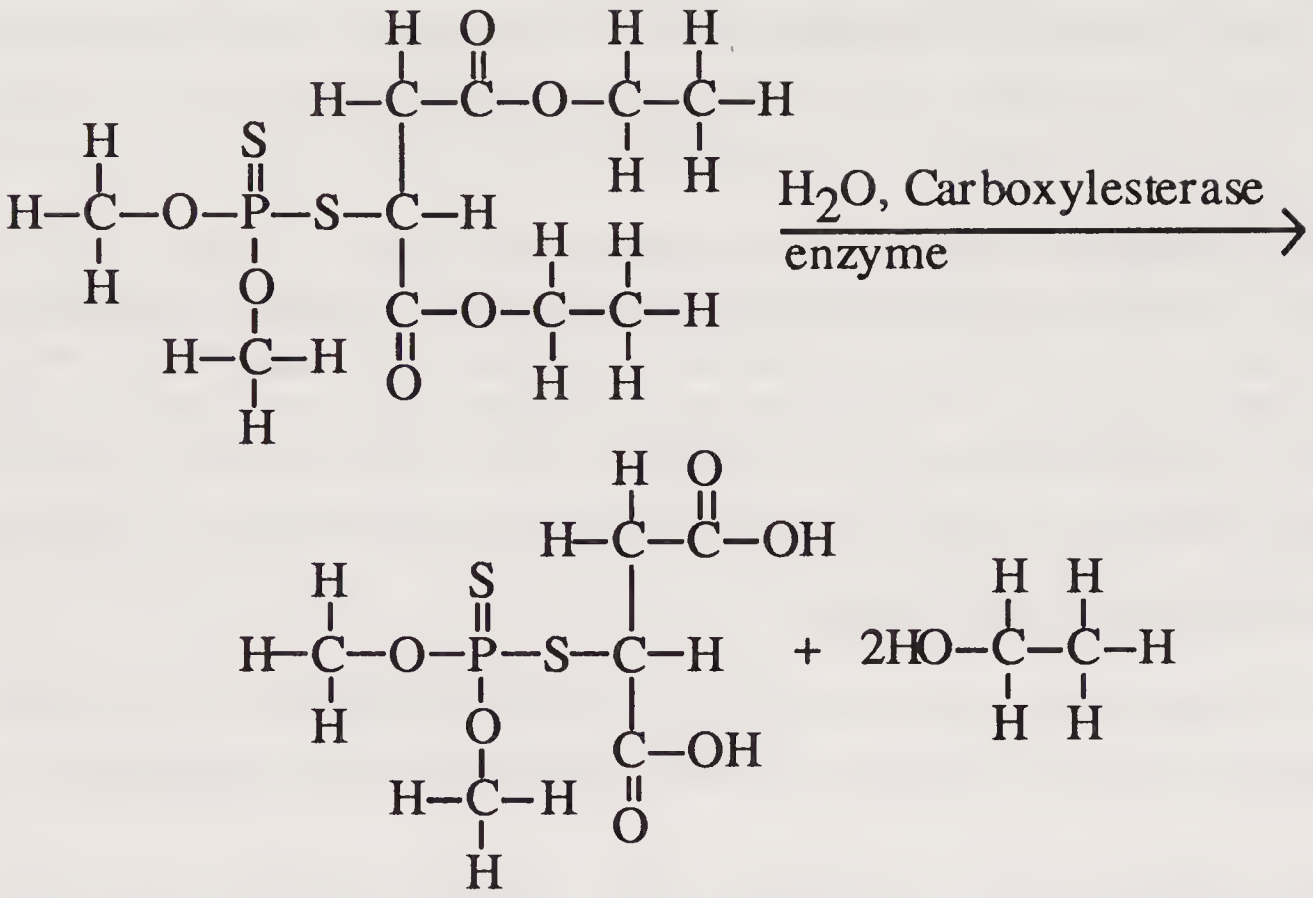


Figure 18.8. Phosphorodithioate organophosphate insecticides.

Malathion shows how differences in structural formula can cause pronounced differences in the properties of organophosphate pesticides. Malathion has two carboxyester linkages which are hydrolyzable by carboxylase enzymes to relatively nontoxic products as shown by the reaction in Equation 18.7.1 (page 410).

The enzymes that accomplish this reaction are possessed by mammals, but not by insects, so that mammals can detoxify malathion, whereas insects cannot. The result is that malathion has selective insecticidal activity. For example, although malathion is a very effective insecticide, its LD50 for adult male rats is about 100 times that of parathion, reflecting the much lower mammalian toxicity of malathion compared to some of the more toxic organophosphate insecticides, such as parathion.

Carboxylase enzymes are inhibited by organophosphates other than malathion. The result of exposure of mammals to malathion plus another organophosphate is potentiation (this term describes enhancement of the action of an active substance by an otherwise inactive substance) of the toxicity of malathion.



(18.7.1)

Phosphate Ester Insecticides

Figure 18.9 shows some organophosphate insecticides based upon the phosphate esters. These compounds do not contain sulfur. One of the more significant of these compounds is paraoxon which, as noted previously, is a metabolic activation product of parathion. It has been synthesized directly and was made by Schrader in 1944 along with parathion. One of the most toxic organophosphate insecticides, paraoxon has a toxicity rating of 6. **Mevinphos** is considered to be an extremely dangerous chemical. **Dichlorvos** has a toxicity rating of 4 and is deactivated by enzymes in the livers of mammals. Its tendency to vaporize has enabled its use in "pest strips."

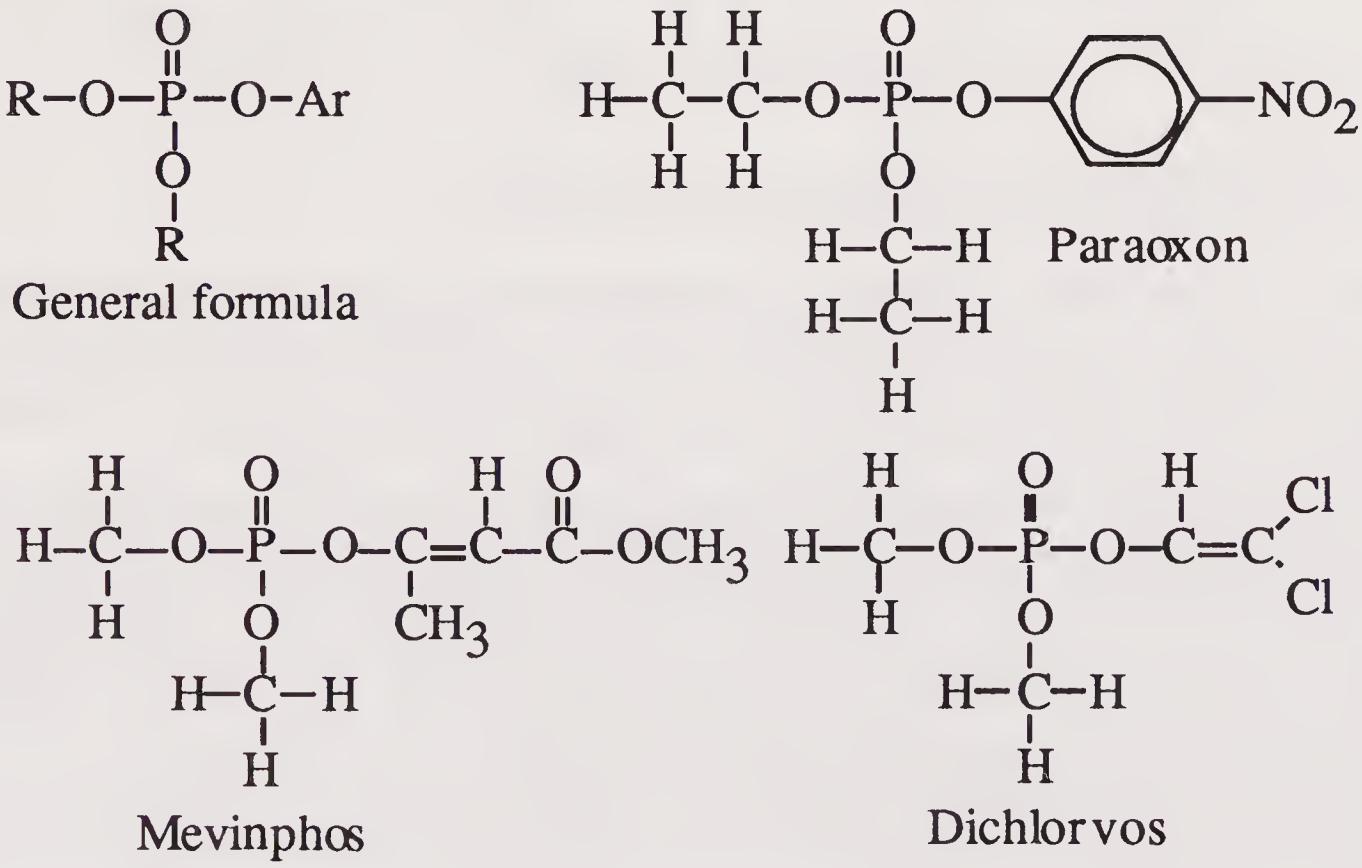


Figure 18.9. Organophosphate insecticides based on phosphate esters.

Toxic Actions of Organophosphate Insecticides

Inhibition of Acetylcholinesterase

The organophosphate insecticides inhibit acetylcholinesterase in mammals and insects.[7] As discussed in Section 9.3 and shown in Section 9.3.1, acetylcholine forms during the transmission of nerve impulses in the body, including the central nervous system, and it must be hydrolyzed by the action of acetylcholinesterase enzyme to prevent excessive stimulation of the nerve receptors. Accumulation of acetylcholine can cause numerous effects related to excessive nerve response. Among these effects in humans are bronchioconstriction resulting in chest tightness and wheezing; stimulation of muscles in the intestinal tract resulting in nausea, vomiting and diarrhea; and muscular twitching and cramps. The central nervous system shows numerous effects from the accumulation of acetylcholine. These include psychological symptoms of restlessness, anxiety, and emotional instability. The subject may suffer from headache and insomnia. In more severe cases, depression of the respiratory and circulatory systems, convulsions, and coma may result. In fatal poisonings, death is due to respiratory system paralysis.

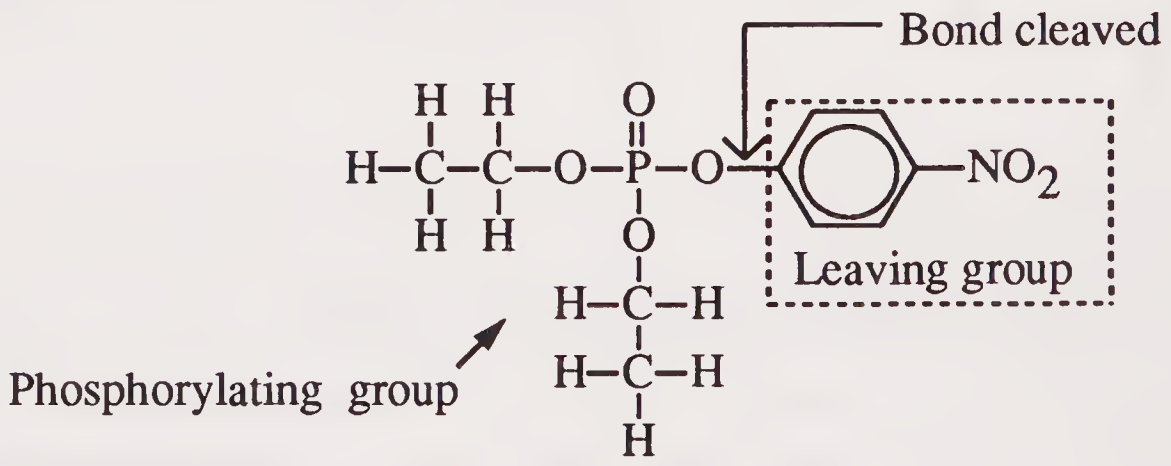
Cholinesterase inhibition occurs when an inhibitor, I, binds to the cholinesterase enzyme, E, to produce an enzyme-inhibitor complex as shown by the following reaction:[7]

$$E + I \leftarrow\!\rightarrow EI \qquad (18.7.2)$$

With some inhibitors the reaction is reversible. With other kinds of compounds, such as the organophosphates, a stable, covalently bound complex, E', is formed from which it is difficult to regenerate the original enzyme, as illustrated by the reaction

$$E + I \leftarrow\!\rightarrow EI \rightarrow E' \xrightarrow{\text{Slowly or not at all}} E + \text{Products} \qquad (18.7.3)$$

An example of irreversible binding is that of paraoxon, which can be viewed as an organophosphate compound containing a phosphorylating group, P, and a leaving group, L, as shown below:



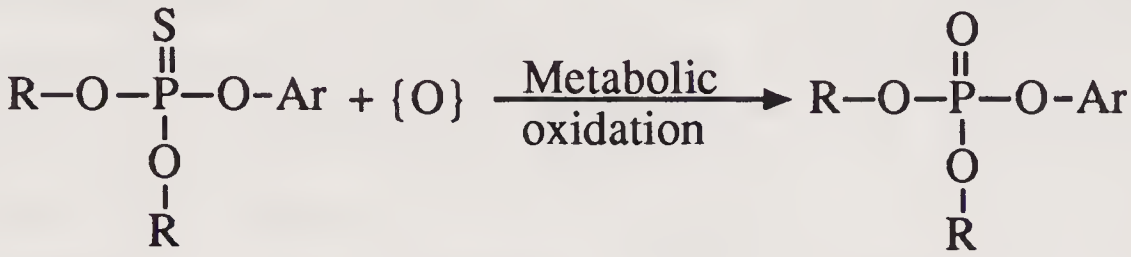
The reaction of this compound with cholinesterase enzyme, E, can be represented by the following reaction:

$$E + PL \rightarrow EP + L \xrightarrow[\text{bound enzyme}]{\text{Slow dissociation of covalently}} E + \text{Products} \qquad (18.7.4)$$

The phosphorylating group bonds to an OH group at the active site of the enzyme.

Metabolic Activation

Highly purified phosphorothionate and phosphorodithioate insecticides do not inhibit acetylcholinesterase directly. In order for these compounds to inhibit acetylcholinesterase, the following phase I metabolic conversion of P=S to P=O must occur:



(18.7.5)

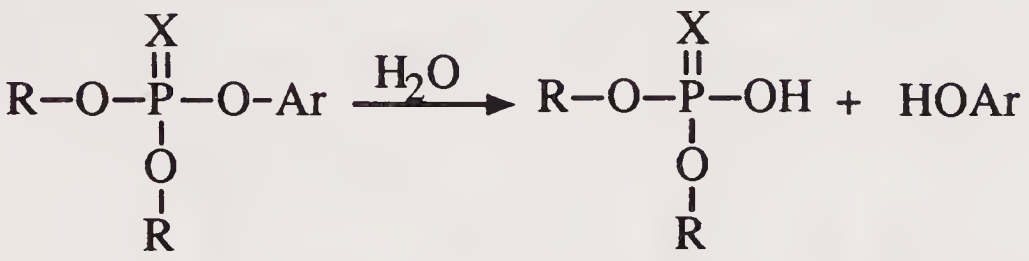
A specific example of this type of reaction is the conversion of parathion to paraoxon mentioned in the preceding section.

Mammalian Toxicities

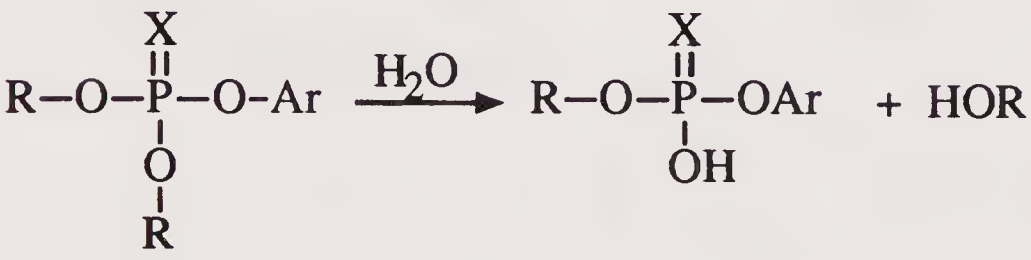
The mammalian toxicities of the organophosphate insecticides vary widely. This may be seen from the LD_{50} values to male rats of organophosphate insecticides, including some of the ones discussed in the preceding section. Where the approximate LD_{50} values (oral, mg/kg) are given in parentheses, a listing of common organophosphate insecticides in descending order of toxicity is TEPP (1) > mevinphos, disulfoton (6-7) > parathion, methylparathion, azinphosmethyl, chlorfenvinphos (13-15) > dichlorvos (80) > diazinon (110) > trichlorfon (215) > chlorothion (880) > ronnel > malathion (1300).

Deactivation of Organophosphates

The deactivation of organophosphates is accomplished by hydrolysis as shown by the following general reactions where R is an alkyl group, Ar is a substituent group that is frequently aromatic, and X is either S or O:



(18.7.6)



(18.7.7)

18.8. ORGANOPHOSPHORUS MILITARY POISONS

Organophosphorus compounds developed for use as military poisons — the "nerve gases" — are among the most toxic synthetic compounds ever made.[8] Three example structures of these compounds are shown in Figure 18.10. Other chemical warfare agents containing organic phosphorus include **Tabun** (O-ethyl N,N-dimethyl-

phosphoramidocyanidate), **Soman** (*o*-pinacolyl methylphosphonofluoridate), and "**DF**" (methylphosphonyldifluoride).

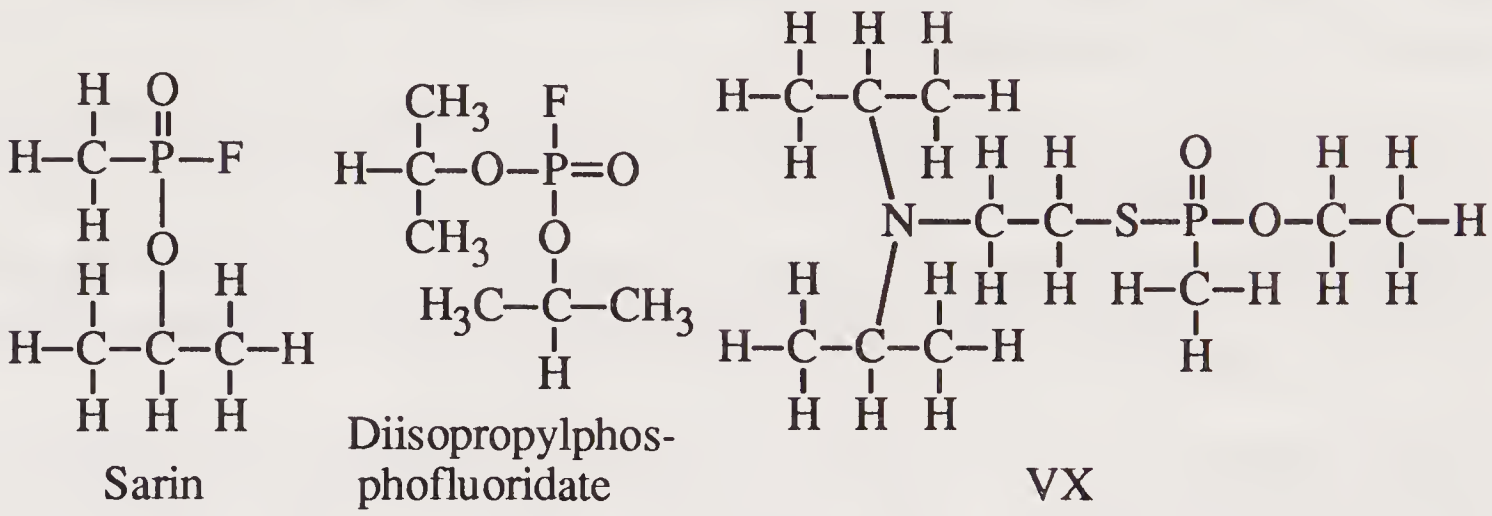
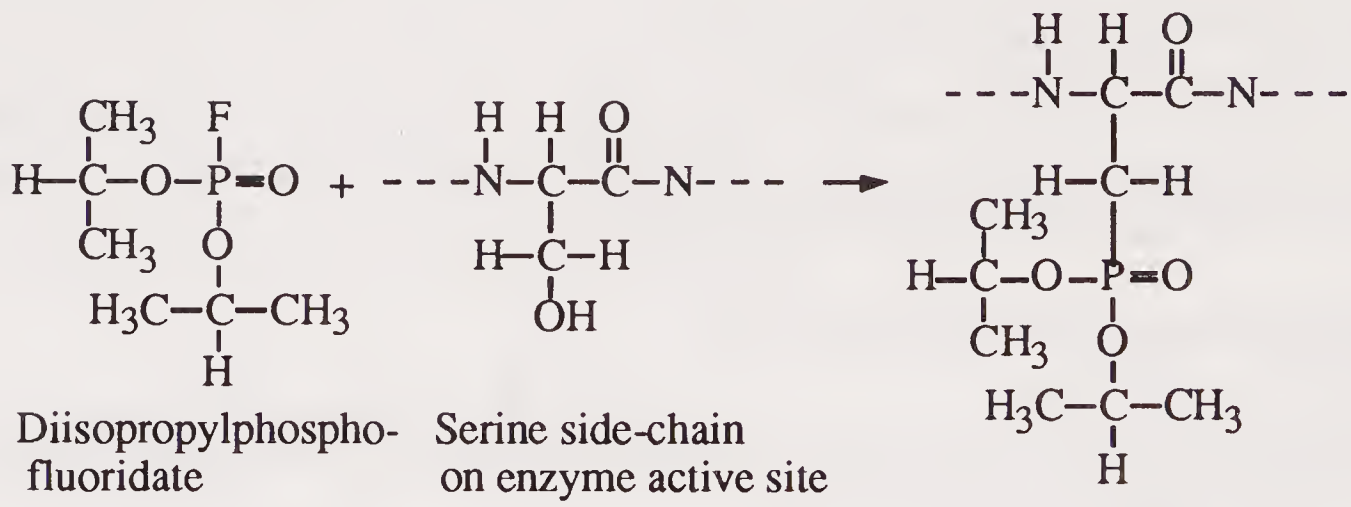


Figure 18.10. Three examples of organophosphate military poisons.

The action of **Sarin** is typical of the organophosphorus military poisons. Its lethal dose to humans may be as low as about 0.01 mg/kg. It is a systemic poison to the central nervous system that is readily absorbed as a liquid through the skin; a single drop so absorbed can kill a human. It is a colorless liquid (fp -58°C, bp 147°C). A compound that is chemically similar to Sarin, diisopropylphosphorfluoridate acts by binding to the active site of acetylcholinesterase enzyme, thereby inhibiting the enzyme. Specifically, the reaction is thought to be with a serine side-chain on the active site as shown by the following reaction:



(18.8.1)

Soman is an irreversible inhibitor of acetylcholinesterase.[9] Unlike the case with some acetylcholinesterase inhibitors, recovery from sublethal poisoning by Soman requires enzyme resynthesis, rather than reversible binding to the enzyme.

Pure Tabun is also a colorless liquid; it has a freezing point of -49°C and decomposes when heated to 238°C. Its toxicity is similar to that of Sarin. Tabun acts primarily on the sympathetic nervous system and it has a paralytic effect on the blood vessels. Its toxic action and symptoms of poisoning are similar to those of parathion, an organophosphate insecticide for which extensive human toxicity data are available. **Diisopropyl fluorophosphate**, $(i\text{-}C_3H_7O)_2P(O)F$, is a highly toxic oily liquid that served as the basis for the development of "nerve gases" in Germany during World War II. The organophosphorus military poisons are powerful inhibitors of acetylcholinesterase enzyme.

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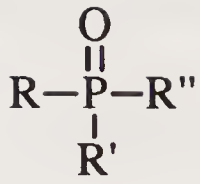
QUESTIONS

1. What may be said about the relationship of phosphorus with nitrogen, oxygen, and sulfur in organophosphorus compounds? Give examples of organophosphorus compounds that contain N, O, or P.
2. To which kinds of nitrogen and sulfur compounds are alkyl and aryl phosphine compounds analogous?
3. What particular hazards are posed by the reaction below?

   $$4C_3H_9P + 26O_2 \rightarrow 12CO_2 + 18H_2O + P_4O_{10}$$

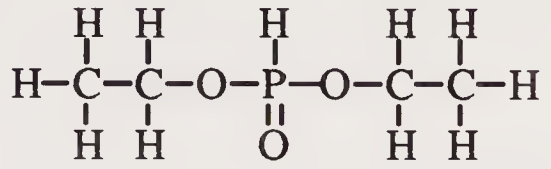
4. What classes of compounds do the general formulas below illustrate? Give examples of each, along with their toxic effects.



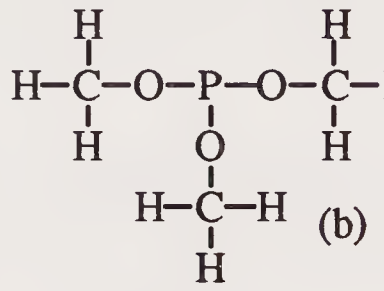
5. Discuss the toxicity characteristics of tri-*o*-cresyl-phosphate, TOCP.

6. Discuss the uses and toxicity characteristics of tricresylphosphate.

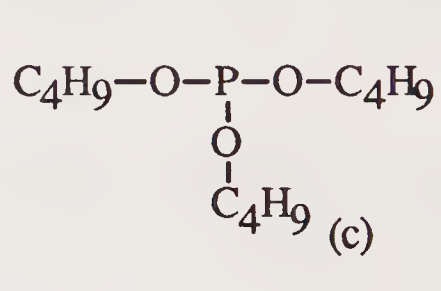
7. What is phosphonic acid? How does it differ from phosphorous acid? From the structures below, pick out the esters of each of these acids.



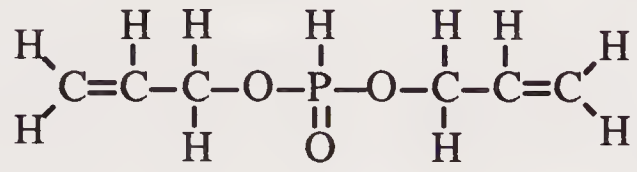
(a)



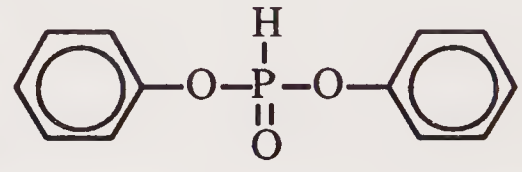
(b)



(c)



(d)

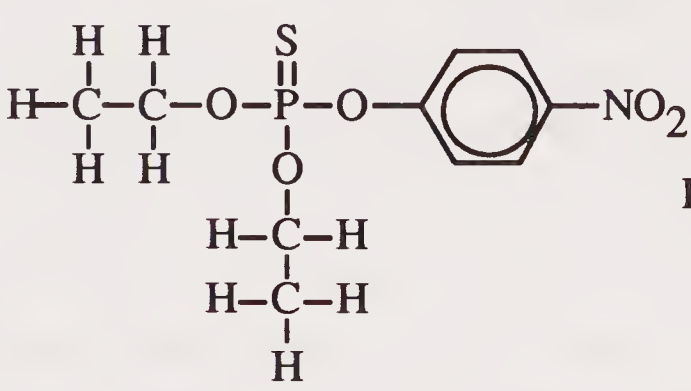


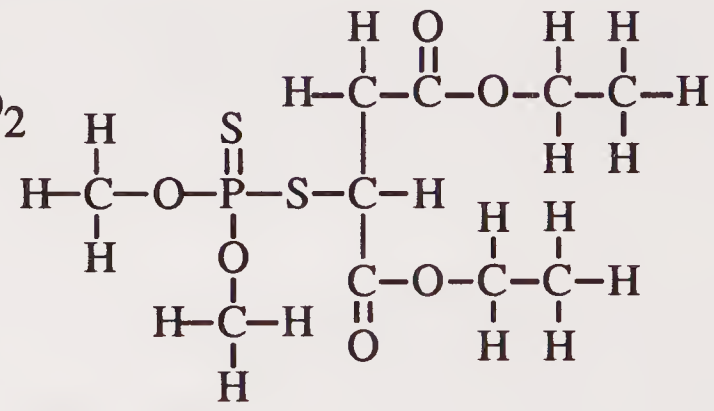
(e)

8. Give an example of a simple ester of each of the compounds below and comment on the toxicities of each of the examples. Which compound was developed in Germany during World War II and was substituted for nicotine as an insecticide?

9. What are the general formulas of phosphorothionate and phosphorodithioate esters? How are the thiono and P=O functional groups involved in these kinds of esters? How are they used? What is oxidative desulfuration and why is it significant with these kinds of compounds?

10. For what purposes have the two compounds below been used? What are their toxicity characteristics and relative toxicities?





11. What is parathion converted to in the body? Of what kind of reaction mentioned in Chapter 17 is this an example? What does it have to do with the uses and toxicity of parathion?

12. Designate which of the following statements is **not** true: ( ) The organophosphate insecticides inhibit acetylcholinesterase in mammals and insects. ( ) These insecticides are toxic because they prevent the formation of acetylcholine produced during the transmission of nerve impulses in the body. ( ) Acetylcholine must be hydrolyzed by the action of acetylcholinesterase enzyme to prevent excessive stimulation of the nerve receptors. ( ) Excessive accumulation of acetylcholine can cause numerous effects related to excessive nerve response.

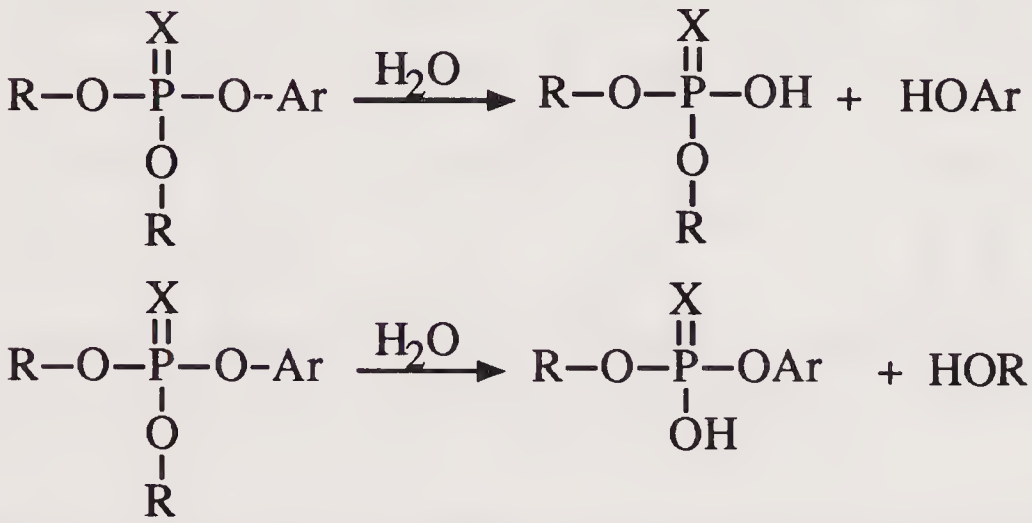
13. Discuss what the following shows regarding the interaction of an organophosphate insecticide with cholinesterase enzyme:

$$E + PL \rightarrow EP + L \xrightarrow[\text{bound enzyme}]{\text{Slow dissociation of covalently}} E + \text{Products}$$

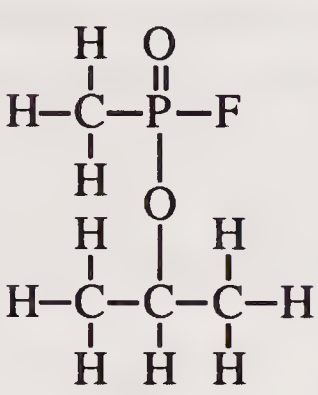
14. Discuss what the following shows regarding cholinesterase inhibition:

$$E + I \leftarrow\!\rightarrow EI \rightarrow E' \xrightarrow{\text{Slowly or not at all}} E + \text{Products}$$

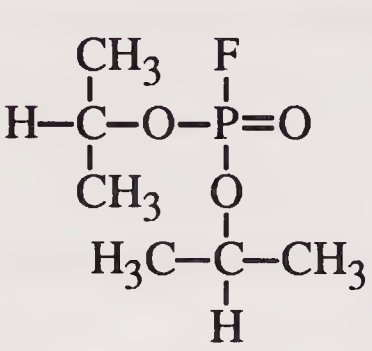
15. Explain what the following shows regarding the deactivation of organophosphates:



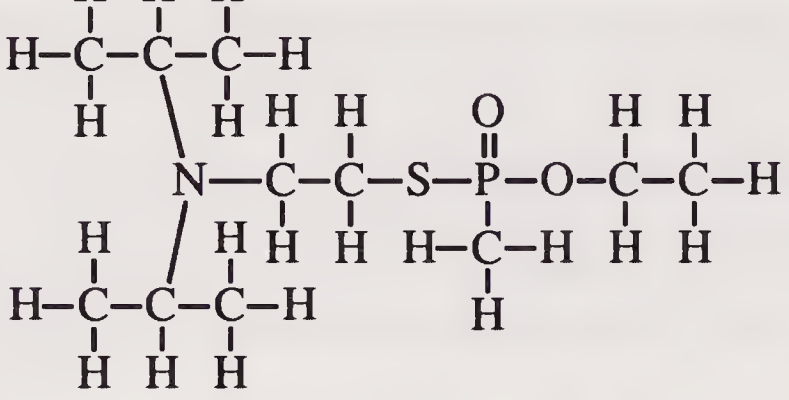
16. What are the uses of the following compounds? What may be said about their toxicities?



Sarin



Diisopropylphosphofluoridate



VX

19

Toxic Natural Products

19.1. INTRODUCTION

Toxic natural products are poisons produced by organisms. They include an enormous variety of materials. Perhaps the most acutely toxic substance known is botulism toxin produced by the anaerobic bacterium *Clostridium botulinum*, and responsible for many food poisoning deaths, especially from improperly canned food. Mycotoxins generated by fungi (molds) can cause a number of human maladies, and some of these materials, such as the aflatoxins, are carcinogenic to some animals. Venoms from wasps, spiders, scorpions, and reptiles — consisting of an exotic variety of biomolecules, including low-molecular-mass polypeptides, proteins, enzymes, steroids, lipids, 5-HT, and glycosides[1] — can be fatal to humans. Each year, in the Orient, tetrodotoxin from improperly prepared puffer fish makes this dish the last delicacy consumed by some unfortunate diners. The stories of Socrates' execution from being forced to drink an extract of the deadly poisonous spotted hemlock plant and Cleopatra's suicide at the fangs of a venomous asp are rooted in antiquity. Many household poisonings result from children ingesting toxic plant leaves or berries. High on this list is philodendron. Other plants that may be involved in poisonings include diefenbachia, jade plant, wandering Jew, Swedish ivy, pokeweed, string-of-pearls, and yew. Pollen from plants causes widespread misery from allergies, and reactions to toxins from plants such as poison ivy can be severe.

Living organisms wage chemical warfare against their potential predators and prey with a fascinating variety of chemical substances. Some organisms produce toxic metabolic by-products that have no obvious use to the organisms that make them. This chapter briefly describes some of the toxic natural products from living organisms with emphasis on those that are toxic to humans.

One distinction should be made at this point that applies particularly to animals. A **poisonous organism** is one that produces toxins. A **poisonous animal** may contain toxins in its tissues that act as poisons to other animals that eat its flesh. **Venoms** are poisons that can be delivered without the need for the organism to be eaten. **Venomous animals** can deliver poisons to another animal by means such as biting (usually striking with fangs) or stinging. The puffer fish — some tissues of which are deadly when ingested — is a poisonous animal, whereas the rattlesnake is a venomous one, although its flesh may be eaten safely. Some organisms — notoriously, the skunk — wage chemical warfare by emitting substances that are not notably toxic, but still effective in keeping predators away. Perhaps these organisms should be classified as noxious species.

19.2. TOXIC SUBSTANCES FROM BACTERIA

The two greatest concerns regarding toxic substances from bacteria are their roles in causing symptoms of bacterial disease and food poisoning. It is useful to consider as one class those bacteria that produce toxins that adversely affect a host in which the bacteria are growing and, as another class, bacteria that produce toxins to which another organism is subsequently exposed, such as by ingestion.

Bacteria are single-celled microorganisms that may grow in colonies and are shaped as spheres, rods, or spirals. They are usefully classified with respect to their need for oxygen, which accepts electrons during the metabolic oxidation of food substances, such as organic matter. **Aerobic bacteria** require molecular oxygen to survive, whereas **anaerobic bacteria** grow in the absence of oxygen, which may be toxic to them. **Facultative bacteria** can grow either aerobically or anaerobically. Anaerobic bacteria and facultative bacteria functioning anaerobically use substances other than molecular O_2 as electron acceptors (oxidants that "take electrons away" from other reactants in a chemical reaction). For example, sulfate takes the place of O_2 in the anaerobic degradation of organic matter (represented as $\{CH_2O\}$) by *Desulfovibrio*, yielding toxic hydrogen sulfide (H_2S) as a product as shown by the following overall reaction.[2]

$$SO_4^{2-} + 2\{CH_2O\} + 2\,H^+ \rightarrow H_2S + 2CO_2 + 2H_2O \qquad (19.2.1)$$

Toxicants such as H_2S produced by microorganisms are usually not called microbial toxins, a term that more properly refers to usually proteinaceous species of high molecular mass synthesized metabolically by microorganisms and capable of inducing a strong response in susceptible organisms at low concentrations. Bacteria and other microorganisms do produce a variety of poisonous substances, such as acetaldehyde, formaldehyde, and putrescine (see Figure 19.2).

In Vivo Bacterial Toxins

Some important bacterial toxins are produced in the host and have a detrimental effect on the host. For example, such toxins are synthesized by *Clostridium tetani*, common soil bacteria that enter the body largely through puncture wounds.

The toxin from this bacterium interferes with neurotransmitters, such as acetylcholine, causing **tetanus**, commonly called lockjaw. Abnormal populations or strains of *Shigella dysenteriae* bacteria in the body can cause a severe form of dysentery because they release a toxin that causes intestinal hemorrhaging and gastrointestinal tract paralysis. Toxin-releasing bacteria responsible for the most common form of food poisoning are those of the genus *Salmonella*. Victims are afflicted with flu-like symptoms and may even die from the effects of the toxin. Diphtheria is caused by a toxin generated by *Cornybacterium diphtheriae*. The toxin interferes with protein synthesis and is generally destructive to tissue.

Bacterial Toxins Produced Outside the Body

The most notorious toxin produced by bacteria outside the body is that of *Clostridium botulinum*. This kind of bacteria grows naturally in soil and on vegetable material. Under anaerobic or slightly aerobic conditions it synthesizes an almost

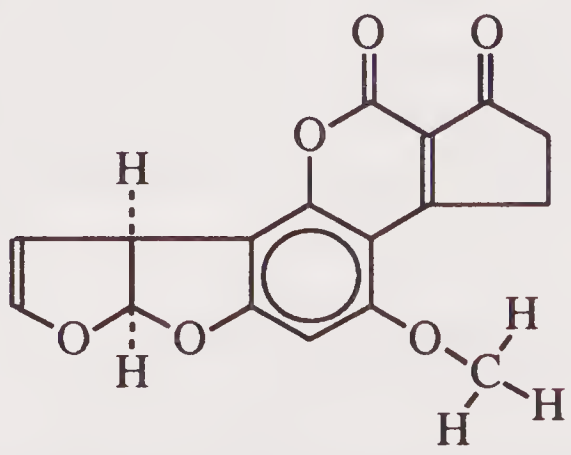
unbelievably toxic product. The conditions for generating this toxin most commonly occur as the result of the improper canning of food, particularly vegetables. Botulinum toxin binds irreversibly to nerve terminals, preventing the release of acetylcholine; the affected muscle acts as though the nerve were disconnected. The toxin actually consists of several polypeptides in the range of 200,000 to 400,000 molecular mass. Fortunately, these proteins are inactivated by heating for a sufficient time at 80–100°C. Botulinum poisoning symptoms appear within 12–36 hours after ingestion, beginning with gastrointestinal tract disorders and progressing through neurologic symptoms, paralysis of the respiratory muscles, and death by respiratory failure.

19.3. MYCOTOXINS

Mycotoxins are toxic metabolites from fungi that have a wide range of structures and a variety of toxic effects.[3] Human and animal exposure to mycotoxins usually results from ingestion of food upon which fungal molds have grown. Among the many kinds of molds that produce mycotoxins are *Aspergillus flavus*, *Fusarium*, *Trichoderma*, *Aspergillus*, and *Penicillium*. Perhaps the most well-known mycotoxins are the **aflatoxins** produced by *Aspergillus*. These molds grow on a variety of food products including corn, cereal grains, rice, apples, peanuts, and milk. Adverse human health effects have occurred during times of short food supply when substandard grain has been consumed for food. One such case occurred in the vicinity of Orenburg in Siberia in 1944 during World War II. Harvest delayed by the war resulted in contamination of barley, millet, and wheat by trichothecenes. Humans that later consumed the grain were afflicted with a number of disorders including gastrointestinal maladies, internal hemorrhaging, and severe skin rash; about 10 percent of those afflicted died.[4] Another major class of mycotoxins consists of the **ergot alkaloids** from *Claviceps*. Several genera of *fungi imperfecti* produce toxic tricothecenes.

Aflatoxins

The most common source of aflatoxins is moldy food, particularly nuts, some cereal grains, and oil seeds. The most notorious of the aflatoxins is aflatoxin B_1, for which the structural formula is shown in Figure 19.1. Produced by *Aspergillus niger*, it is a potent liver toxin and liver carcinogen in some species. It is metabolized in the liver to an epoxide (see Section 4.5). The product is electrophilic with a strong tendency to bond covalently to protein, DNA, and RNA. Other common aflatoxins produced by molds are those designated by the letters B_2, G_1, G_2, and M_1.



Aflatoxin B_1

Figure 19.1. Structural formula of aflatoxin B_1, a mycotoxin.

Other Mycotoxins

The ergot alkaloids have been associated with a number of spectacular outbreaks of central nervous system disorders, sometimes called ergotism. St. Anthony's fire is an example of convulsive ergotism. From examination of historical records, it is now known that outbreaks of this malady resulted for the most part from ingestion of moldy grain products. Although ergotism is now virtually unknown in humans, it still occurs in livestock.

Trichothecenes are composed of 40 or more structurally related compounds produced by a variety of molds, including *Cephalosporium*, *Fusarium*, *Myrothecium*, and *Trichoderma*, which grow predominantly on grains. Much of the available information on human toxicity of trichothecenes was obtained from an outbreak of poisoning in Siberia in 1944, mentioned above.

19.4. TOXINS FROM PROTOZOA

Protozoa are microscopic animals consisting of single eukaryotic cells, and classified on the bases of morphology , means of locomotion, presence of absence of chloroplasts, presence or absence of shells, ability to form cysts. Several devastating human diseases, including malaria, sleeping sickness, and some kinds of dysentery are caused by parasitic protozoa. Parasitic protozoa can cause debilitating, even fatal, diseases in livestock and wildlife.

Toxic substances from two of the major types of unicellular protista — bacteria and fungi — were discussed in the preceding sections. Protozoans are also notable for the production of toxic substances. Most of the protozoans that produce toxins belong to the order **dinoflagellata**, which are predominantly marine species. The cells of these organisms are enclosed in cellulose envelopes, which often have beautiful patterns on them. Among the effects caused by toxins from these organisms are gastrointestinal, respiratory, and skin disorders in humans; mass kills of various marine animals; and paralytic conditions caused by eating infested shellfish.

The marine growth of dinoflagellates is characterized by occasional incidents in which they multiply at such an explosive rate that they color the water yellow, olive-green, or red by their vast numbers. In 1946, some sections of the Florida coast became so afflicted by "red tide" that the water became viscous and for many miles the beaches were littered with the remains of dead fish, shellfish, turtles, and other marine organisms. The sea spray in these areas became so irritating that coastal schools and resorts were closed.

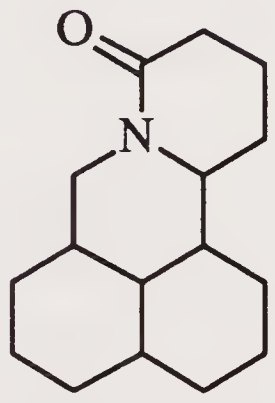
The greatest danger to humans from dinoflagellata toxins comes from the ingestion of shellfish, such as mussels and clams, that have accumulated the protozoa from sea water. In this form the toxic material is called paralytic shellfish poison. As little as 4 mg of this toxin, the amount found in several severely infested mussels or clams, can be fatal to a human. The toxin depresses respiration and affects the heart, resulting in complete cardiac arrest in extreme cases.

19.5. TOXIC SUBSTANCES FROM PLANTS

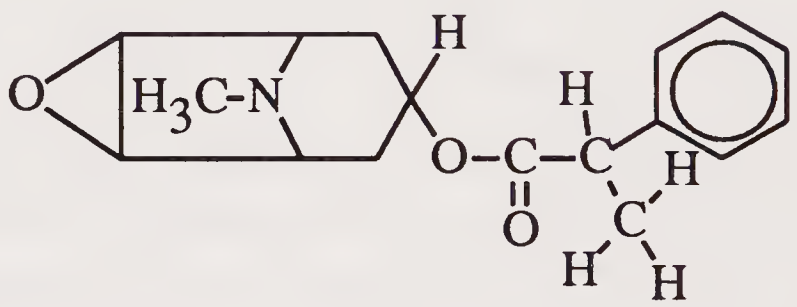
Various plants produce a wide range of toxic substances as reflected by plant names such as "deadly nightshade" and "poison hemlock." Although the use of "poison arrows" having tips covered with plant-derived curare has declined as the

tribes that employed them have acquired the sometimes dubious traits of modern civilization, poisoning by plants is still of concern in the grazing of ruminant animals, and houseplants such as philodendron and yew are responsible for poisoning some children. Taxol from the western yew tree is a neurotoxin, but is proving to be a useful chemotherapeutic agent for the treatment of breast cancer. Plant-derived cocaine causes many deaths among those who use it or get into fatal disputes marketing it.

Toxic substances from plants are discussed here in the five categories of nerve poisons, internal organ poisons, skin and eye irritants, allergens, and metal (mineral) accumulators. As can be seen in Figure 19.2, plant toxins have a variety of chemical structures. Prominent among the chemical classes of toxicants synthesized by plants are nitrogen-containing alkaloids (see Section 15.9) that usually occur in plants as salts. Some harmful plant compounds undergo metabolic reactions to form toxic substances. For example, amygdalin, present in the meats of fruit seeds such as those of apples and peaches, undergoes acid hydrolysis in the stomach or enzymatic hydrolysis elsewhere in the body to yield toxic HCN.



Matrine, a quinolizidine from mescal bean



Scopolamine

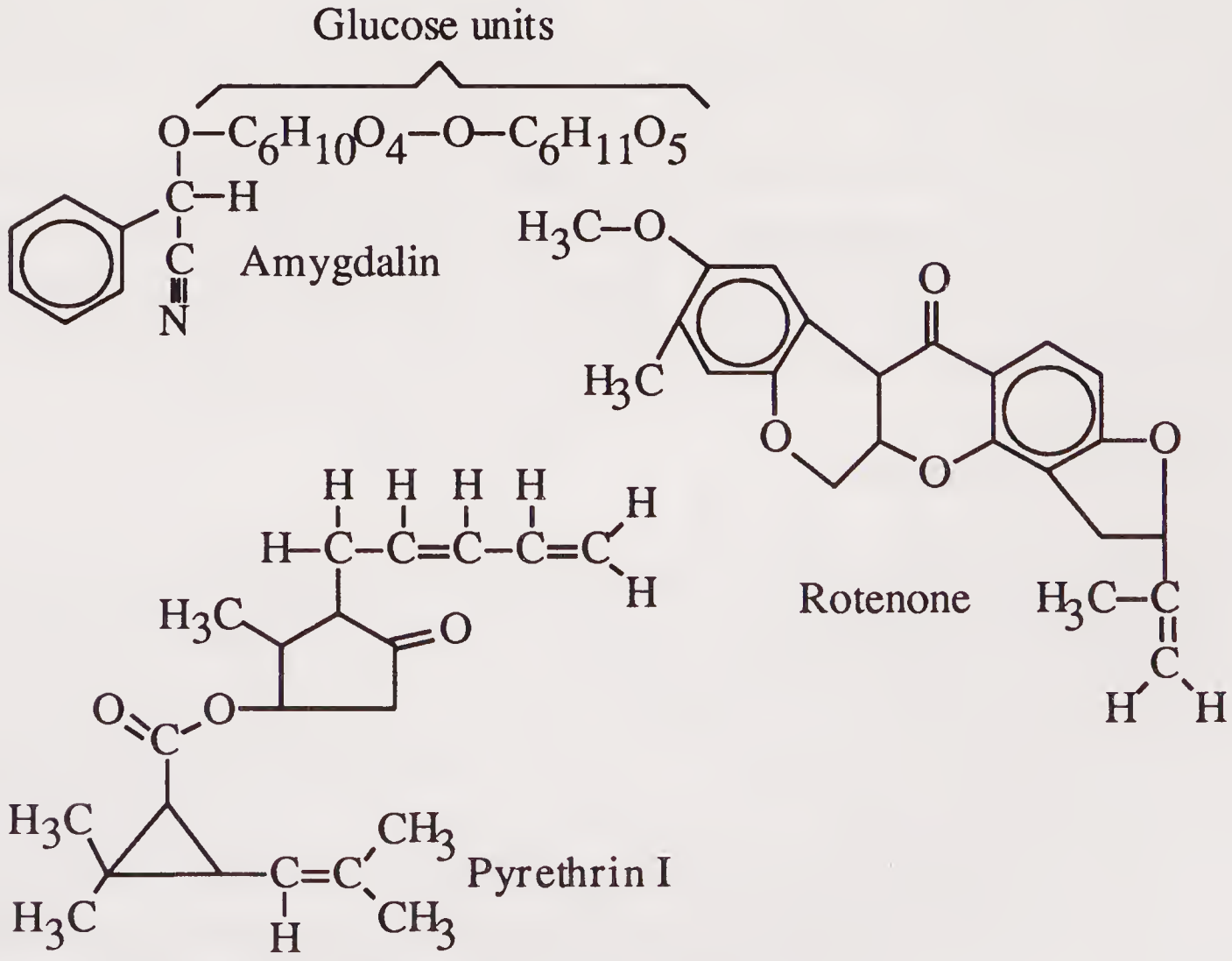


Figure 19.2. Some representative toxic substances from plants.

Nerve Toxins from Plants

Nerve toxins from plants cause a variety of central nervous and peripheral nervous system effects. Several examples are cited here.

Plant-derived **neurotoxic psychodysleptics** affect peripheral neural functions and motor coordination, sometimes accompanied by delirium, stupor, trance states, and vomiting. Prominent among these toxins are the pyrollizidines from peyote. Also included are erythrionones from the coral tree and quinolizidines from the mescal bean (see Figure 19.2).

A plant neurotoxin that is receiving much current publicity because of its effectiveness in the chemotherapeutic treatment of at least one form of cancer is **taxol**, a complex molecule that belongs to the class of taxine alkaloids. Taxol occurs in most tissues of *Taxus breviofolia*, the western yew tree, and is isolated from the bark of that tree (once considered a nuisance tree in forestry, but now, for obvious reasons, in short supply). Ingestion of taxol causes a number of neurotoxic effects, including sensory neuropathy, nausea and gastrointestinal disturbances, impaired respiration and cardiac function. It also causes blood disorders (leukopenia and thrombocytopenia). The mechanism of taxol neurotoxicity involves binding to tubulin, a protein involved in the assembly of microtubules, which assemble and dissociate as part of cell function. This binding of tubulin in nerve cell microtubules stabilizes the microtubules and prevents their dissociation, which can be detrimental to normal nerve cell function.[5]

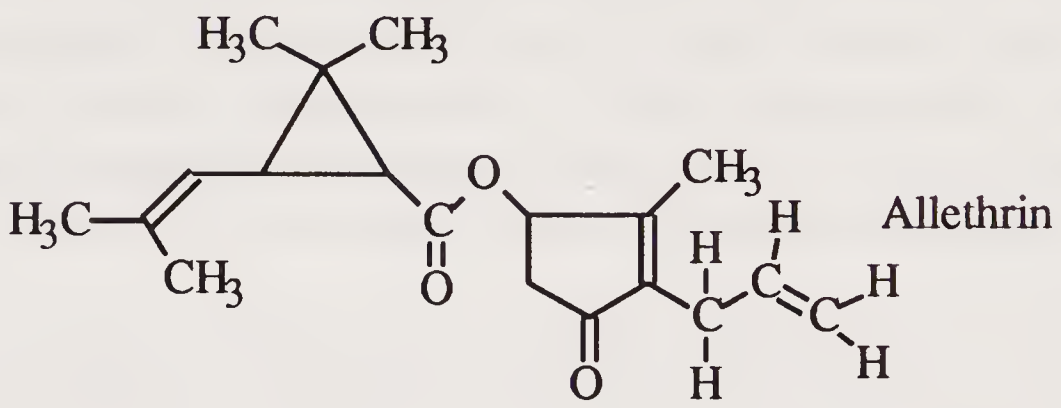
Spotted hemlock contains the alkaloid nerve toxin coniine (see Figure 15.10). Ingestion of this poison is followed within about 15 minutes by symptoms of nervousness, trembling, arrythmia, and bradycardia. Body temperature may decrease and fatal paralysis can occur. The Nightshade family of plants contains edible potato, tomato, and eggplant. However, it also contains the "deadly nightshade," or *Atropa Belladonna* (beautiful woman). This toxic plant contains scopolamine (Figure 19.2) and atropine. Ingestion causes dizziness, mydriasis, speech loss, and delirium. Paralysis can occur. Fatally poisoned victims may expire within half an hour of ingesting the poison.

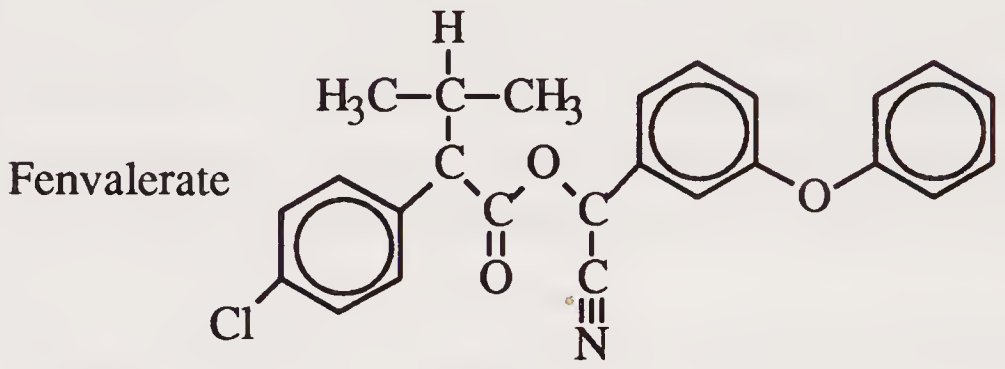
Several nerve toxins produced by plants are interesting because of their insecticidal properties. Insecticidal nicotine is extracted from tobacco. Rotenone (Figure 19.2) is synthesized by almost 70 legumes. This insecticidal compound is safe for most mammals, with the notable exception of swine. The most significant insecticidal plant derivatives, however, are the pyrethrins, discussed below.

Pyrethrins and Pyrethroids

Pyrethrins, and their synthetic analogs, represent both the oldest and newest of insecticides. Extracts of dried of dried chrysanthemum or pyrethrum flowers, which contain pyrethrin I (Figure 19.2), pyrethrin II, jasmolin I and II, and cinerin I and II, have been known for their insecticidal properties for a long time, and may have even been used as botanical insecticides in China almost 2000 years ago. The most important commerical sources of insecticidal pyrethrins are chrysanthemum varieties grown in Kenya. Pyrethrins have several advantages as insecticides, including facile enzymatic degradation, which makes them relatively safe for mammals, ability to rapidly paralyze ("knock down") flying insects, and good biodegradability characteristics.[6]

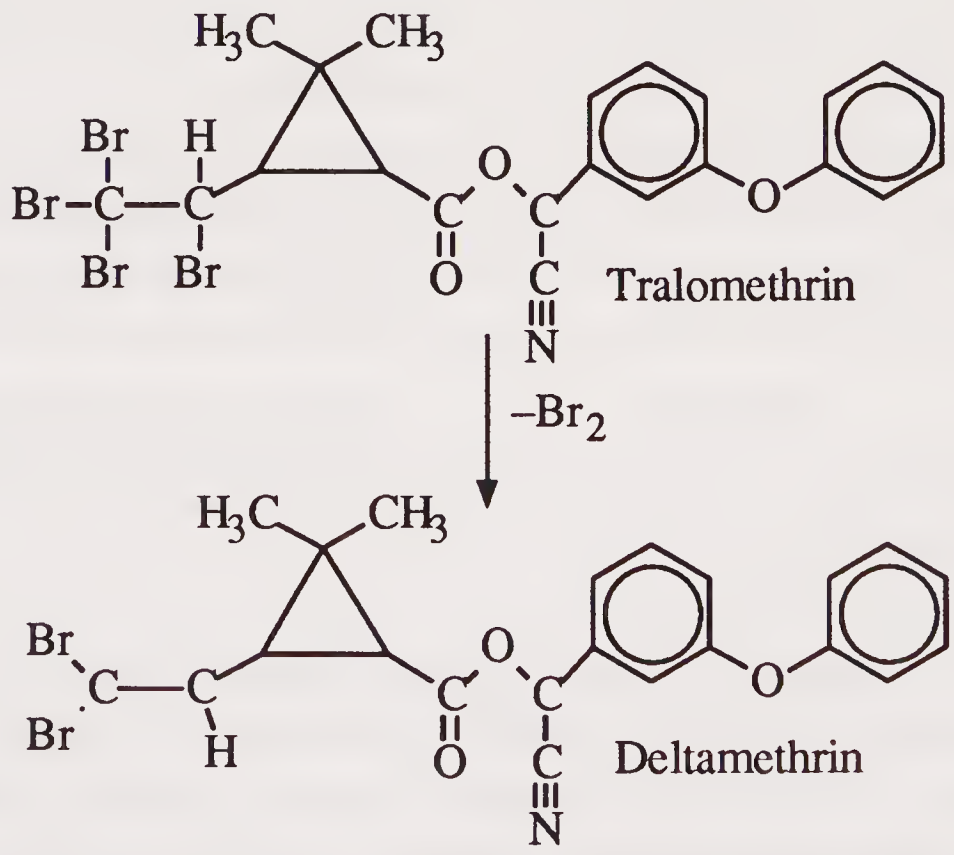
Synthetic analogs of the pyrethrins, **pyrethroids**, have been widely produced as insecticides during recent years. The first of these was allethrin;[7] another common example is fenvalerate (see structures below):





Pyrethroids act as neurotoxins that cause excitation of the nervous system. Organisms may become hypersensitive to stimuli and experience paresthesia (abnormal sensations of burning or prickling of the skin). Tremors and salivation are common symptoms of mammals exposed to toxic levels of pyrethroids.

An interesting microbial reaction of a pyrethroid is the partial dehalogenation of insecticidal tralomethrin to deltamethrin.[8] The product is more potently insecticidal than the original compound.



Internal Organ Plant Toxins

Toxins from plants may affect internal organs, such as the heart, kidney, liver, and stomach. Because of their much different digestive systems involving multiple stomachs, ruminant animals may react differently to these toxins than do monogastric animals. Some major plant toxins that affect internal organs are summarized in Table 19.1.

Eye and Skin Irritants

Anyone who has been afflicted by poison ivy, poison oak, or poison sumac appreciates the high potential that some plant toxins have to irritate skin and eyes. The toxic agents in the plants just mentioned are catechol compounds, such as urishikiol in poison ivy. Contact with the poison causes a characteristic skin rash that may be disabling and very persistent in heavily exposed, sensitive individuals. Lungs may be affected — often by inhalation of smoke from the burning plants — to the extent that hospitalization is required.

Photosensitizers constitute a class of systemic plant poisons capable of affecting areas other than those exposed. These pigmented substances may pass through the liver without being conjugated and collect in skin capillaries. When these areas of the skin are subsequently exposed to light, the capillaries leak. (Since light is required, the phenomenon is called a photosensitized condition.) In severe cases, tissue and hair are sloughed off. St. John's wort or horsebrush causes this kind of condition in farm animals.

Table 19.1. Plant Toxins that Affect Internal Organs of Animals

Toxin	Source	Target organ
Saponins	Alfalfa, cockles, English ivy	Noncardioactive steroid glycosides that cause gastric upset
Pyrrolizidine alkaloids such as festucine	Fescue hay	Liver: obstructs veins
Hypercin	St. John's wort, horsebrush	Liver: releases pigmented molecules to the bloodstream causing photosensitization
Digitoxin	Foxglove	Heart: overdose causes heart to stop, strengthen heartbeat and eliminate fluids present in congestive heart failure
Oxalates	Oak tannin	Kidney: precipitation of CaC_2O_4 obstructs kidney tubules

Allergens

Many plants are notorious for producing allergens that cause allergic reactions in sensitized individuals. The most common plant allergens consist of pollen. The process that leads to an allergic reaction starts when the allergen, acting as a hapten, combines with an endogenous protein in the body to form an antigen. Antibodies are generated that react with the antigen and produce histamine, resulting in an allergic reaction. (See Section 8.12, Immune System Response.) The severity of the symptoms varies with the amount of histamine produced. These symptoms can include skin rash, watery eyes, and running nose. In severe cases, victims suffer fatal anaphylactic shock. An example of an allergic reaction to a plant product — all too familiar to many of its victims — is hay fever induced by the pollen of ragweed or goldenrod.

Mineral Accumulators

Some plants classified as mineral accumulators become toxic because of the inorganic materials that they absorb from soil and water and retain in the plant biomass. An important example of such a plant is *Astragalus*, sometimes called "locoweed." This plant causes serious problems in some western U.S. grazing areas because it accumulates selenium. Animals that eat too much of it get selenium poisoning, characterized by anemia and a condition known descriptively as "blind staggers."

Nitrate accumulation may occur in plants growing on soil fertilized with nitrate under moisture-deficient conditions, such as those that occurred in the the central U.S. during the record-setting spring/summer drought of 1988. In the stomachs of ruminant animals, nitrate (NO_3^-) ingested with plant material is reduced to nitrite (NO_2^-). The nitrite product enters the bloodstream and oxidizes the iron(II) in hemoglobin to iron(III). The condition that results is methemoglobinemia, which was discussed in Section 15.3 in connection with aniline poisoning.

Another toxicological problem that can result from excessive nitrate in plant material is the generation of toxic nitrogen dioxide gas (see Section 12.3) during the fermentation of ensilage composed of chopped plant matter contaminated with nitrate. The toxic effect of NO_2 from this source has been called "silo-filler's disease."

Mushroom Toxins

Although mushrooms are fungal bodies, their toxic effects are often discussed along with those of toxic plants. Some mushrooms, such as *Amanita phalloides*, *Amanita virosa*, and *Gyromita esculenta*, are very toxic, with reported worldwide deaths of the order of 100 per year.[9] In extreme cases, one bite of one poisonous mushroom can be fatal. Accidental mushroom poisonings are often caused by the Death's Head mushroom, because it is easily mistaken for edible varieties.

Some toxins in mushrooms are alkaloids that cause central nervous system effects of narcosis and convulsions. Hallucinations occur in subjects who have eaten mushrooms that contain **psilocybin**. The toxic alkaloid **muscarine** is present in some mushrooms.

Another class of toxins produced by some mushrooms consists of polypeptides, particularly amanitin and phalloidin. These substances are stable to heating (cooking). They are systemic poisons that attack cells of various organs, including the heart and liver. In early 1988, an organ transplant was performed on a woman in the U.S. to replace her liver, which was badly damaged from the ingestion of wild mushrooms that she and a companion had mistakenly collected and consumed as edible varieties.

The symptoms of mushroom poisoning vary. Typical early symptoms involve the gastrointestinal tract and include stomach pains and cramps, nausea, vomiting, and diarrhea. Victims in the second phase of severe poisoning may suffer paralysis, delirium, and coma along with often severe liver damage.

Edible *Coprinus atramentarius* mushrooms produce an interesting ethanol-sensitizing effect similar to that of disulfiram (antabuse, see Section 17.3).[10] Ingestion of alcohol can cause severe reactions in individuals up to several days after having eaten this kind of mushroom.

19.6. INSECT TOXINS

Although relatively few insect species produce enough toxin to endanger humans, insects cause more fatal poisonings in the U.S. each year than do all other venomous animals combined. Most venomous insects are from the order *Hymenoptera*, which includes ants, bees, hornets, wasps, and yellowjackets. These insects deliver their toxins by a stinging mechanism.

Chemically, the toxic substances produced by insects are variable and have been incompletely characterized. In general, *Hymenopteran* venoms are composed of water-soluble, nitrogen-containing chemical species in concentrated mixtures. Although they contain chemical compounds in common, the compositions of insect venoms from different species are variable. The three major types of chemical species are biologically synthesized (biogenic) amines, peptides and small proteins, and enzymes. Of the biogenic amines, the most common is histamine, which is found in the venoms of bees, wasps, and hornets. Wasp and hornet venoms contain serotonin, and hornet venom contains the biogenic amine acetylcholine. Among the peptides and low-molecular-mass proteins in insect venoms are apamin, mellitin, and mast cell degranulating peptide in bee venom; wasp kinin, and hornet kinin. Enzymes contained in bee, wasp, and hornet venom are phospholipase A and hyaluronidase. Phospholipase B occurs in wasp and hornet venom.

Bee Venom

Bee venom contains a greater variety of proteinaceous materials than do wasp and hornet venoms. Apamin in bee venom is a polypeptide containing 18 amino acids and having three disulfide (–SS–) bridges in its structure. Because of these bridges and its small size, the apamine molecule is able to traverse the blood-brain barrier and function as a central nervous system poison. Mellitin in bee venom consists of a chain of 27 amino acids. It can be a direct cause of erythrocyte hemolysis. Symptoms of bradycardia and arrythmia can be caused by mellitin. Mast cell degranulating peptide in bee venom acts on mast cells. These are a type of "white blood cell" believed to be involved in the production of heparin, a key participant in the blood-clotting process. The degranulating peptide causes mast cells to disperse, with an accompanying release of histamine into the system.

Wasp and Hornet Venoms

Wasp and hornet venoms are distinguished from bee venoms by their lower content of peptides. They do contain kinin peptide, which may cause smooth muscle contraction and lowered blood pressure. Two biogenic amines in wasp or hornet venoms (serotonin and acetylcholine) lower blood pressure and cause pain. Acetylcholine may cause malfunction of heart and skeletal muscles.

Toxicities of Insect Venoms

The toxicities of insect venoms are low to most people. Despite this, relatively large numbers of fatalities occur each year from insect stings because of allergic reactions in sensitized individuals. These reactions can lead to potentially fatal anaphylactic shock, which affects the nervous system, cardiovascular function, and respiratory function. The agents in bee venom that are responsible for severe allergic reactions are mellitin and two enzymes of high molecular mass — hyaluronidase and phospholipase A-2.

19.7. SPIDER TOXINS

There are about 30,000 species of spiders, virtually all of which produce venom![11] Fortunately, most lack dangerous quantities of venom, or the means to deliver it. Nevertheless, about 200 species of spiders are significantly poisonous to humans. Many of these have colorful common names, such as tarantula, trap-door spider, black widow, giant crab spider, poison lady, and deadly spider. Space permits only a brief discussion of spider venoms here.

Brown Recluse Spiders

Brown recluse spiders (*Loxosceles*) are of concern because of their common occurrence in households in temperate regions. Many people are bitten by this spider despite its nonaggressive nature. A brown spider bite can cause severe damage at the site of the injury. When this occurs, the tissue and underlying muscle around the bite undergo severe necrosis, leaving a gaping wound up to 10 cm across. Plastic surgery is often required in an attempt to repair the damage. In addition, *Loxosceles* venom may cause systemic effects, such as fever, vomiting, and nausea. In rare cases death results. The venom of *Loxosceles* contains protein and includes enzymes. The mechanisms by which the venom produces lesions are not completely understood.

Widow Spiders

The widow spiders are *Latrodectus* species. Unlike the *Loxosceles* species described above, the bite sites from widow spiders show virtually no damage. The symptoms of widow spider poisoning are many and varied. They include pain, cramps, sweating, headache, dizziness, tremor, nausea, vomiting, and elevated blood pressure. The venom contains several proteins, including a proteinaceous neurotoxin with a molecular mass of about 130,000.[12]

Other Spiders

Several other types of venomous spiders should be mentioned here. Running spiders (*Chiranthium* species) are noted for the tenacity with which they cling to the bite area, causing a sharply painful wound. The bites of cobweb spiders (*Steatoda* species) cause localized pain and tissue damage. Venomous jumping spiders (*Phidippus* species) produce a wheal (raised area) up to 5 cm across in the bite area.

19.8. REPTILE TOXINS

Snakes are the most notorious of the venomous animals. The names of venomous snakes suggest danger — Eastern diamondback rattlesnake, king cobra, black mamba, fer-de-lance, horned puff adder, *Crotalus horridus horridus*. About 10 percent of the approximately 3500 snake species are sufficiently venomous to be hazardous to humans.[13] These may be divided among *Crotilidae* (including rattlesnakes, bushmaster, and fer-de-lance), *Elipidae* (including cobras, mambas, and coral snakes), *Hydrophidae* (true sea snakes), *Laticaudae* (sea kraits), and *Colubridae* (including the boomslang and Australian death adder).

Chemical Composition of Snake Venoms

Snake venoms are complex mixtures that may contain biogenic amines, carbohydrates, glycoproteins, lipids, and metal ions. The most important snake venom constituents, however, are proteins, including numerous enzymes. Approximately 25 different enzymes have been identified in various snake venoms.[11] The most prominent of the enzymes in snake venom are the proteolytic enzymes, which bring about the breakdown of proteins, thereby causing tissue to deteriorate. Some proteolytic enzymes are associated with hemorrhaging. Collagen (connective tissue in tendons, skin, and bones) is broken down by collagenase enzyme contained in some snake venoms. Among the other kinds of enzymes that occur in snake venoms are hyaluronidase, arginine ester hydrolase, lactate dehydrogenase, DNase, L-amino acid oxidase, nucleotidase enzymes, RNase, phospholipase enzymes, phosphoesterase enzymes, and acetylcholinesterase.

Numerous nonenzyme polypeptides occur in snake venom. Some of these polypeptides, though by no means all, are neurotoxins.

Toxic Effects of Snake Venom

The effects of snake bite can range from relatively minor discomfort to almost instant death. The latter is often associated with drastically lowered blood pressure and shock. The predominant effects of snake venoms can be divided into the two major categories of cardiotoxic and neurotoxic effects. Blood clotting mechanisms may be affected by enzymes in snake venom, and blood vessels may be damaged as well.[2] Almost all organs in the victims of poisoning by *Crotilidae* have exhibited adverse effects, many of which appear to be associated with changes in the blood and with alterations in the lung. Clumped blood cells and clots in blood vessels have been observed in the lungs of victims. These effects are caused in part by the action of thrombin-like enzymes, which are constituents of *Crotilidae* (as examples, copperheads, rattlesnakes, Chinese habu) and *Viperidae* (examples are puff adders, European viper, Sahara sand viper) venoms. Thrombinlike enzymes cause the release of fibrinopeptides that result in fibrinogen clot formation. Agents in cobra toxin break down the blood-brain barrier by disrupting capillaries and cell membranes. So altered, the barrier loses its effectiveness in preventing the entry of other brain-damaging toxic agents.

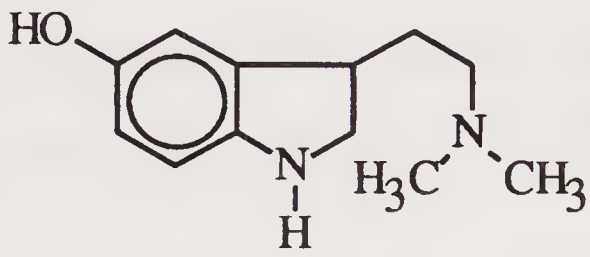
19.9. NONREPTILE ANIMAL TOXINS

Several major types of animals that produce poisonous substances have been considered so far in this chapter. With the exception of birds, all classes of the animal kingdom contain members that produce toxic substances.[11] Those not covered so far in this chapter are summarized here.

Numerous kinds of fishes contain poisons in their organs and flesh. The most notorious of the poisonous fish are "puffers" or "puffer-like" fish that produce tetrodotoxin. This supertoxic substance is present in the liver and ovary of the fish. It acts on nerve cell membranes by affecting the passage of sodium ions, a process involved in generating nerve impulses. The fatality rate for persons developing clinical symptoms of tetrodotoxin poisoning is about 40 percent. Usually associated with Japan, puffer fish poisoning kills about 100 people per year globally. Some of these poisonings are self-inflicted.

Some fishes are venomous and have means of delivering venom to other animals. This is accomplished, for example, by spines on weever fish. The infamous stingray has a serrated spine on its tail that can be used to inflict severe wounds, while depositing venom from specialized cells along the spine. The venom increases the pain from the wound and has systemic effects, especially on the cardiovascular system.

Numerous species of amphibia (frogs, toads, newts, salamanders) produce poisons, such as bufotenin, in specialized skin secretory glands. Most of these animals pose no hazard to humans. However, some of the toxins are extremely poisonous. For example, Central American Indian hunters have used hunting arrows tipped with poison from the golden arrow frog.



Bufotenin, a compound isolated from some amphibian toxins

In addition to the poisonous fishes and sea snakes mentioned previously in this chapter, several other forms of marine life produce toxins. Among these are *Porifera*, or sponges, consisting of colonies of unicellular animals. The sponges release poisons to keep predators away. They may have sharp spicules that can injure human skin, while simultaneously exposing it to poison. Various species of the *Coelenterates*, including corals, jellyfish, and sea anenomes, are capable of delivering venom by stinging. Some of these venoms have highly neurotoxic effects. *Echinoderms*, exemplified by starfish, sea cucumbers, and sea urchins, may possess spines capable of delivering toxins. People injured by these spines often experience severe pain and other symptoms of poisoning. Various molluscs produce poisons, such as the poison contained in the liver of the abalone, *Haliotis*. Some mollusc poisons, such as those of the genus *Conus*, are delivered as venoms by a stinging mechanism.

Arthropods, which consist of a vast variety of invertebrate animals that have jointed legs and a segmented body, are notable for their production of toxins. Of the arthropods, insects and spiders were discussed earlier in this chapter. Some **scorpions**, arachnidal arthropods with nipper-equipped front claws and stinger-equipped, long, curved segmented tails, are notably venomous animals. The stings of scorpions, some of which reach a length of 8 inches, are a very serious hazard, especially to children; most fatalities occur in children under age 3. In Mexico, the particularly dangerous scorpion *Centruroides suffusus* attains a length up to 9 cm. Mexico has had a particularly serious problem with fatal scorpion bites. During the two decades following 1940, it is estimated that over 20,000 deaths occurred from venomous scorpion stings! Some **centipedes** are capable of delivering venom by biting. The site becomes swollen, inflamed, and painful. Some millipedes secrete a toxic skin irritant when touched.

Although the greater hazard from ticks is their ability to carry human diseases, such as Rocky Mountain spotted fever or Lyme disease (a debilitating condition that had become of great concern in some areas during the summer of 1988), some species discharge a venom that causes a condition called tick paralysis, characterized by weakness and lack of coordination. An infamous mite larva, the chigger, causes inflamed spots on the skin that itch badly. The chigger is so small that most people require a magnifying glass to see it, but a large number of chigger bites can cause intense misery in a victim.

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QUESTIONS

1. Distinguish betweeen poisonous organisms and venomous organisms. Give an example of each.

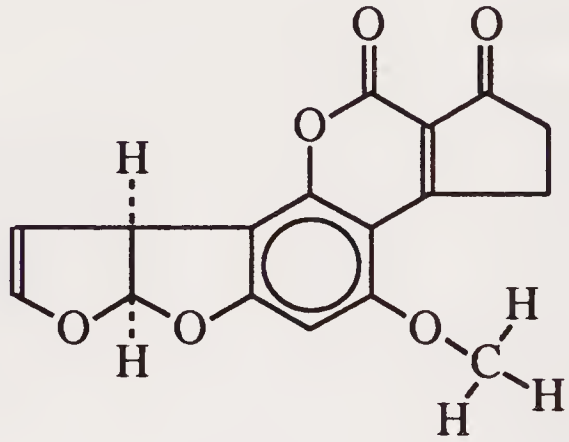
2. What does the following reaction show about "toxic natural products"?

$$SO_4^{2-} + 2\{CH_2O\} + 2\,H^+ \rightarrow H_2S + 2CO_2 + 2H_2O$$

3. What two kinds of bacterial toxins are illustrated by *Shigella dysenteriae* as compared to *Clostridium botulinum*?

4. What kinds of toxins are produced by *Aspergillus flavus*, *Fusarium*, *Trichoderma*, *Aspergillus*, and *Penicillium*? Give some examples of these toxins.

5. What kind of toxic substance is illustrated by the following? What produces it? What are some of its effects?



Aflatoxin B_1

6. What kind of organism causes "red tide"? What are some of the symptoms of poisoning by "red tide toxins"?

7. What is the greatest danger to humans from dinoflagellata toxins? What are the toxic effects of these poisons?

8. A very large number of plant toxins are classified in a diverse group of natural products. What is this group? What are some of the toxic effects of substances belonging to it?

9. Taxol has both harmful and potentially beneficial effects. List and discuss both of these. What is the source of taxol?

10. List and discuss several of the prominent nerve toxins from plants. From which plants do they come? What are some of their important effects?

11. What are the most common plant allergens consisting of pollen? What are some of the major allergic conditions caused by these substances?

12. What is the toxicological significance of *Amanita phalloides*, *Amanita virosa*, and *Gyromita esculenta*? What are psilocybin and muscarine?

13. To which order do most venomous insects belong? What is the greatest danger from insect stings?

14. What is contained in the venom of *Loxosceles*? What are some of the major toxic effects of this venom?

15. What are the major constituents of snake venoms? What are the toxicological effects of snake venoms?

16. What is bufotenin? What kind of organism produces it?

17. What venomous animal is an "arachnidal arthropods" with nipper-equipped front claws and stinger-equipped, long, curved segmented tails"? What are some significant aspects of its hazards?

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