Volume 203

Reviews of Environmental Contamination and Toxicology

David M. Whitacre
Editor

Springer

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Coordinating Board of Editors

ISBN 978-1-4419-1351-7 e-ISBN 978-1-4419-1352-4
DOI 10.1007/978-1-4419-1352-4
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2009939269

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife, and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the Earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for Reviews and the Archives are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the *Bulletin* are also reviewed, but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated toxicological consequences. Such articles facilitate the often complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the nearly 50 years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews*) was first published, the number, scope, and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing people worldwide. This fact, and the routine discovery and reporting of new environmental contamination cases, creates an increasingly important function for *Reviews*.

The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. *Reviews* addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities, or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans, and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever-increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific research is to enhance understanding of the environment in ways that allow the public to be better informed. The term “informed public” as used by Thomas Jefferson in the age of enlightenment

conveyed the thought of soundness and good judgment. In the modern sense, being "well informed" has the narrower meaning of having access to sufficient information. Because the public still gets most of its information on science and technology from TV news and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is the newest global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the 21st century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can be either general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, toxicology, and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the editor and the editorial board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of foreign chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Food additives, including pesticides, or their metabolites that may persist into human food and animal feeds are within this scope. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the editor is recommended before volunteered review manuscripts are submitted.

Summerfield, NC, USA David M. Whitacre

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Bioavailability of Xenobiotics in the Soil Environment

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology*,
Reviews of Environmental Contamination and Toxicology 203,
DOI 10.1007/978-1-4419-1352-4_1, © Springer Science+Business Media, LLC 2010

1 Introduction

1.1 What is "Bioavailability"?

When synthetic, xenobiotic compounds such as agrochemicals and industrial chemicals are utilized, they eventually reach the soil environment where they are subject to degradation, leaching, volatilization, sorption, and uptake by organisms. The simplest assumption is that such chemicals in soil are totally available to microorganisms, plant roots, and soil fauna via direct, contact exposure; subsequently these organisms are consumed as part of food web processes and bioaccumulation may occur, increasing exposures to higher organisms up the food chain. However, studies in the last two decades have revealed that chemical residues in the environment are not completely bioavailable, so that their uptake by biota is less than the total amount present in soil (Alexander 1995; Gevao et al. 2003; Paine et al. 1996). Therefore, the toxicity, biodegradability, and efficacy of xenobiotics are dependent on their soil bioavailability, rendering this concept profoundly important to chemical risk assessment and pesticide registration.

Bioavailability is the amount of a chemical in soil able to interact with organisms inhabiting the soil environment. Bioavailability is greatly affected by many factors, including properties of chemicals and soils, aging time in the soil, climate, and the organisms of concern. Bioavailability changes over time, and displays differences when considered in different contexts: degradation vs. efficacy/toxicity, and when indirect exposure occurs through the food web. Thus, "bioavailability" is dependant on context. The following examples illustrate this point:

> [Bioavailability may be described as] "a measure of the potential of a chemical for entry into biological receptors, and it is specific to receptor (e.g., invertebrate and microbe), the route of entry, time of exposure, and the matrix containing the contaminant." (Anderson et al. 1999);

> [Bioavailability is] "the rate at which or extent to which a chemical compound can be transported to the specified biological population. The mechanisms which control contaminant transfer and the indication that the transfer has occurred are specific to the chemical, source media, and specified biological population." (Shor and Kosson 2000); and "Bioavailability refers to the extent to which humans and ecological receptors are exposed to contaminants in soil or sediment." (Ehlers and Luthy 2003).

The differences in these explanations attest to the fact that bioavailability of a chemical is determined as the integration of several dynamic processes, such as advection, sorption/desorption, degradation, volatilization, food web uptake, etc. The best definition may be written as, "Bioavailability of a chemical is defined as the amount of chemical available to be taken up or utilized by an organism/organisms in a defined time and environment." Dissolved and vaporized chemicals are usually completely bioavailable. If chemicals are in contact with soil or sediments, however, sorption reduces bioavailability. The extent of sorption and sequestration varies both with type of chemical and soil. The bioavailability concept can be applied to any environmental compartment: atmosphere, water, soil, and sediments. However, the concept is most important when applied to the contaminants in soils and sediments. These solid environmental matrices greatly affect the bioavailability of chemicals as a result of sorption, sequestration, etc. Even in aqueous environments, sediment has been shown to have a significant affect on chemical degradation rates (Rice et al. 2004). Therefore, soils and sediments will be reviewed and discussed in this chapter in the context of bioavailability.

1.2 Relation to Biodegradation, Bioaccumulation, Ecotoxicity, and Risk Assessment

The interaction of organisms with chemicals in soil consists of two processes: contact with and uptake (absorption) of the chemical, followed by its transport within organisms to site(s) of action. The former process (contact and uptake) relates to bioavailability, whereas the latter process (transport within organisms) delves into the realm of toxicokinetics (Fig. 1). Bioavailability describes the quantitative

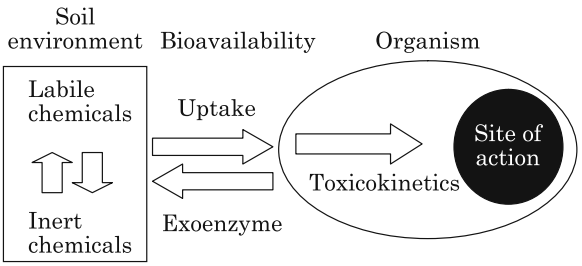

Fig. 1 Exposure of chemicals to organisms consists of two steps: uptake from the soil environment and toxicokinetics in organisms to reach the site of action in organs. For biodegradation, exoenzymes may affect chemicals and, thereby, bioavailability to a greater extent than would be expected from only their soil/water equilibrium.

transport of chemicals from soil/sediment microsites into organisms within a defined period. Toxicokinetics describes the quantitative transport of chemicals to receptors (enzymes, organs, etc.) within organisms. Therefore, bioavailability is explained as the product of interactions among soil (or sediment), chemical, and organism. On the other hand, toxicokinetics is explained as the interaction between chemical and organism. Only bioavailability is affected by the soil (or sediment) environment. Bioavailability and toxicokinetics do, indeed, differ. Therefore, bioavailability is a key determinant of *effective* exposure, and is directly related to the toxicity and degradability of chemicals in soil-inhabiting organisms. Bioavailability is also associated with the potential for bioaccumulation of chemicals in soil-inhabiting organisms. It should be noted that the toxicokinetic behavior of xenobiotics affect both toxicity and degradability. Xenobiotics that are bioavailable to organisms may not be toxic because they are not transported to target organs, or may not be biodegradable because of a lack of degrading enzymes. The ecotoxicity of a chemical in soil/sediment is determined by the integrated processes of bioavailability and toxicokinetics. It is therefore clear that when risks of xenobiotics in soil/sediment are assessed, both bioavailability and toxicokinetics should be taken into account.

In the context of biodegradation, bioavailability has a slightly different meaning, because degradation may occur without uptake of chemicals into organisms, although chemicals are typically degraded after uptake by organisms; exoenzymes excreted from organisms may transform chemicals in the soil environment. Fungi excrete exoenzymes such as cellulase and ligninase. These exoenzymes digest the two macromolecules, cellulose and lignin, respectively. Oligomers produced by the degradation of such macromolecules are absorbed into organisms (Brock et al. 1994). Exoenzymes, especially hydrolytic enzymes from fungi, are often abundant in soils amended with organic material and layers of forest litter (Bumpus et al. 1985; Tien and Kirk 1983, 1984). Phosphatase is also known to be present as cell-free enzyme in soil after lysis of soil microorganisms, and organophosphates are degraded faster in soils with such higher phosphatase activity (Sikora et al. 1990).

1.3 Purpose and Scope

As mentioned, the bioavailability of soil chemicals results from a series of dynamic processes that include desorption, dissolution, diffusion, dispersion, convection, and uptake (Fig. 1). Chemical and soil properties affect all of these processes. Consequently, the experimental determination of bioavailability is not simple. Because of differences in chemical properties, xenobiotics, in the context of bioavailability, can be classified as either metals or organic chemicals.

Many studies have been conducted to characterize the bioavailability of metals in soil. The interaction of soil organisms with metals occurs mainly in soil water. The fraction of metals that is bioavailable can be determined by chemical analysis with differential extraction from soil. Standard estimation methods have been developed for metals (Hund-Rinke and Koerdel 2003), and many reviews on the topic have been published (Amir et al. 2005; Audry et al. 2006; D'Amore et al. 2005; Dercova

et al. 2005; Hooda 2007; Intawongse and Dean 2006; Makovnikova et al. 2006; Michel and Ludwig 2005; Paton et al. 2005; Peijnenburg et al. 2007; Rieuwerts 2007; Sheppard 2005; Young et al. 2005).

In contrast to metals, and despite attempts, no similar adequate methods yet exist to accurately estimate bioavailability of organic chemicals in soil and sediments (Hund-Rinke and Simon 2005; Pollumaa et al. 2004; Semple et al. 2003; Stokes et al. 2005). For this reason, we focus attention, in this review, on organic chemicals rather than metals, and summarize the current status and scientific understanding of bioavailability of organic chemicals in soil and sediments. We address the major factors and processes important to this topic and also address methods for estimating bioavailability. Finally, we make recommendations for probable future research needs in this important area.

To enhance the understanding of what is meant by "bioavailability" of organic chemicals in soil, we will address the following three questions:

(1) How are organisms exposed to chemicals in soil and how are such substances taken up by organisms?
(2) How do critical variables such as organism, chemical, and soil properties affect bioavailability?
(3) How is bioavailability measured and/or modeled?

2 Transport Mechanisms of Molecules Through Membranes

Multiple factors, and their complex interactions, affect soil bioavailability of xenobiotics (Table 1). In this section, we address the properties of organisms important to their uptake of chemicals, a process that profoundly affects bioavailability.

Cellular cytoplasm is separated from the exterior environment by the cytoplasmic membrane. To enter cells, molecules are transported through the cytoplasmic membrane. The cytoplasmic membrane consists of a lipid (mainly phospholipid) bilayer and membrane proteins. This barrier protects the living cytoplasm, but also is selectively permeable to molecules, nutrients, ions, and also xenobiotics, in both directions (Amdur et al. 1993; Brock et al. 1994; Connell and Miller 1984; Tinsley 1979). There are four major mechanisms by which chemicals are transported through membranes: passive diffusion, facilitated transport, active transport, and phagocytosis (endocytosis). The differential functioning of these transport mechanisms has clear implications for the study of bioavailability.

2.1 Passive Diffusion

Most cellular membranes have pores with diameters much smaller than 4 nm. Water and dissolved molecules with molecular weights <100 (e.g., nitrogen gas) can pass through these pores. Normally, cells have higher internal than external

Table 1 Factors influencing bioavailability of chemicals in the soil environment (Alexander 1994; Hund-Rinke and Simon 2005; Pollumaa et al. 2004; Semple et al. 2003; Stokes et al. 2005)

Group	Factors
Chemical properties	Water solubility, vapor pressure, *n*-octanol–water partition coefficient (hydrophobicity), molecular size, molecular shape, single vs. multiple components
Soil environment	Solid phase (sand, clay, humus), liquid phase (water content), gaseous phase (water vapor, O_2, N_2, CO_2), dissolved components (inorganic and organic nutrients, surfactants), physical parameters (humidity, pH, temperature)
Properties of organisms	Uptake mechanisms, motility (active vs. passive), growth pattern (surface growth of bacteria, penetration by mycelia of fungi, actinomycetes, roots, and animals), morphology (cell, mycelium, roots, amoeba, worms), size (bacterial cell<mycelia<protozoa<plant roots = worms), biosurfactant, chemotaxis, predation, simultaneous and successive coordination (aerobic/anaerobic, metabolic consortia, cometabolism), acclimation (enrichment, mutation and selection, induction), growth kinetics (maintenance energy, maximum growth rate, substrate affinity)
Climatic or agricultural activities	Temperature, precipitation, drying/wetting cycle, tillage, fertilization of inorganic chemicals, soil amendment with farmyard manure
Interaction and processes	Sorption/desorption, dissolution, dispersion, sequestration/aging, bound residue, convection, leaching

solute concentrations so that water molecules are transported into cells by osmosis. The hydrophobicity of the central layer of cytoplasmic membranes constitutes a strong barrier to ionic, but not non-ionic compounds. Small compounds that are hydrophobic and weakly polar such as alcohols, fatty acids, benzene, and non-ionic pesticides can penetrate and dissolve in the membrane's lipid phase. Such transport is controlled by diffusion through the lipid bilayer, and its rate is proportional to the concentration gradient between the cytoplasm and outside environment. The majority of pesticides and xenobiotics are uncharged and are lipophilic organic chemicals that are transported by passive diffusion; passive diffusion is the most important mechanism by which chemicals enter cells (Nikaido 1993).

2.2 Facilitated Transport

Strongly polar compounds (e.g., ions and amino acids) cannot diffuse freely through membranes. For example, glycerol permeates a phospholipid bilayer artificial membrane at rates 10^5 times higher than does potassium ion (Brock et al. 1994). However, some highly polar compounds can be bound to membrane transport proteins and, thereby, be transported into the cell. Transport results from a conformational change in a transporter protein. Each discrete compound type binds to a

specific corresponding transporter. The process is not energy consuming; the driving force of transport is diffusion along concentration gradients, though the diffusion rate is faster when a transporter protein is involved (facilitated diffusion). Heavy metals are transported into cells by facilitated diffusion or active transport, described below. However, cationic organic chemicals are poorly transported through membranes (Nikaido and Vaara 1985). There are no specific transporter proteins for ionic organic xenobiotics.

2.3 Energy-Dependent (Active) Transport

Certain polar compounds (e.g., phosphate and potassium ions) are transported into cells against a concentration gradient at the expense of energy (Brock et al. 1994). These compounds are also bound to specific transporter proteins. Adenosine triphosphate (ATP) or proton motive force is the energy source for such transport.

2.4 Phagocytosis (Endocytosis)

Some cells surround solid particles with a cell membrane and then absorb the substrate. This phenomenon is called phagocytosis in *amoeba* and endocytosis in plant roots. Endocytosis has been observed to absorb organic compounds such as peptides.

3 Uptake Mechanisms of Chemicals by Organisms and Their Kinetics

3.1 Kinetics of Chemical Transport Through Membranes by Passive Diffusion

Mass transfer through membranes is a diffusion process. Using a double-layer model, based on Fick's first law, and assuming an equilibrium lipid–water partition coefficient $K_{\mathrm{lip}} = C_{\mathrm{lip}}/C_{\mathrm{w}}$ at the lipid membrane interface, the rate of transport (J) of chemical compounds through the lipid membrane is written as follows (Parsons et al. 1987):

$$J = \frac{D_{\mathrm{d}} D_{\mathrm{lip}} K_{\mathrm{lip}}}{\delta_{\mathrm{d}} \cdot D_{\mathrm{lip}} \cdot K_{\mathrm{lip}} + \delta_{\mathrm{lip}} D_{\mathrm{d}}} \cdot A_{\mathrm{lip}} \cdot \Delta C \tag{1}$$

where

J = rate of transport of the chemical through the lipid membrane
D_{d} = diffusion coefficient of the chemical through aqueous diffusion layer at vicinity of membrane
D_{lip} = diffusion coefficient of the chemical through the lipid membrane
K_{lip} = partition coefficient of the chemical between the lipid membrane and the aqueous diffusion layer

δ_d = thickness of the aqueous diffusion layer
δ_{lip} = thickness of the lipid membrane
A_{lip} = area of the lipid membrane
ΔC = the difference in concentration of the chemical between the outside and inside of the cell. Assuming the concentration of the chemical compound inside the cell is zero, $\Delta C = C_w$
C_w = chemical concentration in the aqueous environment.

For convenience, a list and description of the equation symbols used in this chapter, with corresponding units, has been added as an Appendix. The effect of interface area was also reported by Geller (1979), who stated that accumulation ratios of atrazine in bacterial cells of *Acinetobacter*, *Cytophaga*, and *Pseudomonas* species were proportional to their surface areas. Of note is that the passive diffusion rate is inversely related to molecular size, although Eq. (1) does not account for it. In fact, large molecular size of a chemical decreases uptake by biota (Xiang and Anderson 1994).

For hydrophilic compounds, K_{lip} is low. That is, $\delta_d \cdot D_{lip} \cdot K_{lip} \ll \delta_d \cdot D_d$. Therefore

$$J = \frac{D_{lip} \cdot K_{lip}}{\delta_{lip}} \cdot A_{lip} \cdot C_w \tag{2}$$

Because δ_{lip} and A_{lip} of the lipid membrane are constants, transport rates of hydrophilic compounds are proportional to the partition coefficient (K_{lip}), the diffusion coefficient through the lipid membrane (D_{lip}), and the exterior concentration (C_w). K_{lip} can often be estimated from other measures of hydrophobicity, for example the *n*-octanol–water partition coefficient. In general, the diffusion coefficient through the lipid membrane decreases with increasing molecular weight or size of a compound (Mitragotri 2002; Xiang and Anderson 1994).

For hydrophobic compounds, K_{lip} is high. That is, $\delta_d \cdot D_{lip} \cdot K_{lip} \gg \delta_d \cdot D_d$. Therefore

$$J = \frac{D_d}{\delta_d} \cdot A_{lip} \cdot C_w \tag{3}$$

The transport rates of hydrophobic compounds are proportional to the diffusion coefficient of a chemical through the aqueous layer at the exterior adjacent vicinity of the membrane, and are independent of the lipid–water partition coefficient. However, as the hydrophobicity of compounds increases, C_w may become limited by low water solubility.

3.2 Uptake by Microorganisms

Bacterial and fungal cells have no special structures with which to acquire nutrients from their exterior environment. The cytoplasmic membranes of bacterial and fungal cells are covered with cell walls. Nutrients and other chemicals must pass

through the cell walls and then through the cell membranes that exist within the cell walls. The cell wall is composed mainly of the peptide glycan in bacteria and chitin in fungi. The structure of these cell walls resembles a net that maintains cell shape, although the walls are punctuated with many large holes that are permeable to nutrients and other chemicals. Gram-positive bacteria have a thick cell wall. Gram-negative bacteria (*Proteobacteria*) have a thin cell wall covered with an outer membrane made up of a lipid bilayer and lipopolysaccharides. In this outer membrane, porins, barrel proteins that cross outer membrane, are present and act as large pores through which nutrients and chemicals may rather freely pass (Nikaido 1993). Inside the cell wall, the cytoplasmic membrane (or the inner membrane in *Proteobacteria*) protects the cell contents and allows permeation of chemicals and nutrients by diffusion. A concentration gradient between the outside and inside of the cell is the driving force for uptake. Smaller cells have a higher specific surface area and respond more rapidly to changes than do larger cells (Koch 1990).

The lowest concentration of a substrate on which microorganisms can survive has been theoretically determined (Schmidt et al. 1986). Bacteria cannot survive when the diffusion rate of a needed substrate decreases to less than that amount required to maintain energy for life. The bacterial doubling time τ is expressed as follows:

$$\tau = \left[1/Y_{\max}(Rd^2 - Rb^2)/2\right] / \{D \cdot C_{\mathrm{w}}\} / \left[\rho - (m_{\mathrm{c}}/\ln 2) \cdot (Rd^2 - Rb^2)/2\right] \quad (4)$$

where

τ = bacterial doubling time
$Y_{\max}$ = maximum growth yield
Rd = radius of cell at cell division
Rb = radius of cell at initial stage
D = diffusion coefficient of chemicals in solution
C_{w} = concentration of chemicals in solution
ρ = density of dried cell
m_{c} = cell maintenance coefficient.

In aqueous solutions of organic molecules, where D is about 1×10^{-5} cm^2/sec, C_{w} is calculated to be 0.2 μg/L. In soil, D in the range from 10^{-2} to 10^{-3} cm^2/sec results in a chemical concentration in soil solution from 20 to 200 μg/L. Multiple substrates may contribute to successful microbial survival even if each is present below the critical concentration limit.

3.3 Uptake by Plant Roots

Chemicals enter the food chain by first being absorbed into plants. Non-ionic xenobiotic compounds (with log K_{ow} values of ~1 to 3 and molecular weights <300) can enter plant roots by passive diffusion or cotransport, and can then

move upward in the transpiration stream (McFarlane et al. 1987; Riviere 2000; Trapp et al. 1994). Where K_{ow} = *n*-octanol-water partitioning coefficient. Usually, only natural compounds utilize chemical-specific carriers and active transport to enter plants. Compounds, once absorbed, move in plants via the apoplastic system. Diffusion of chemicals into roots may be described theoretically as follows:

$$N_{dr} = (K_{aw} \cdot D_{a,eff} + D_{w,eff}) \cdot (C_w - C_r/K_{rw}) \cdot 2L\pi/[\ln(R_2/R_1)] \quad (5)$$

where (see also Eq. 29)

N_{dr} = the sum of the diffusive flux of chemical to the roots in air- and water-filled pores
K_{aw} = partition coefficient of air to water (Henry's law constant)
K_{rw} = partitioning coefficient between roots and water
$D_{a,eff}$ = effective diffusion coefficient in air-filled soil pores
C_w = concentration in the external (soil) solution
C_r = concentration in the root
L = total length of the roots
R_1 = radius of the roots
R_2–R_1 = diffusion length
R_2 = radius of a deficiency zone surrounding the roots.

Because R_2 is difficult to estimate, default values of R_2 and R_1 are used.

If transpiration stream flow Q_w is measured, uptake and transport may be expressed as follows:

$$N_{tr} = Q_w(1 - \text{TSCF})C_w \quad (6)$$

where

N_{tr} = uptake and transport
Q_w = flow of transpiration water
TSCF= transpiration stream concentration factor.

TSCF is the ratio of the chemical concentration in the transpiration stream to the concentration of chemical in the external solution (Trapp et al. 1994). N_{tr} is usually assessed indirectly from the mass of chemical accumulated in shoots for a known volume of water transpired. TSCF is independent of time if the chemical involved is stable in the plant. TSCF values are also known to be independent of chemical concentration in the external solution and to have a maximum value of 1.0 for passive uptake (Shone and Wood 1974). The relationship of TSCF to log K_{ow} values has been reported as follows (Trapp et al. 1994):

$$\text{TSCF} = 0.784 \bullet \exp[-(\log K_{ow} - 1.78)^2/2.44] \quad (7)$$

As observed by others, this function reaches a maximum near 0.8 at K_{ow}=100 (Briggs et al. 1982). For example, cyclodiene insecticide residues are found in the peel or at the surface of plant roots with little or none stored within the plant. Organophosphate and carbamate pesticides are more labile and readily sorbed by plants growing in soil containing organophosphates and carbamates.

The root concentration factor (RCF*)* is also defined as the ratio of concentration in roots to concentration in external solution (Shone and Wood 1974). RCF values are generally independent of concentration when solutions are in dilute forms (Leroux and Gredt 1977).

Chemical properties that most influence chemical uptake by plant roots are lipophilicity/water solubility (e.g., *n*-octanol–water partitioning coefficient; Briggs 1981; Briggs et al. 1982), acidity/basicity (pK_a; Bromilow and Chamberlain 1995), and vapor pressure (Bromilow and Chamberlain 1995). Root uptake increases with lipophilicity of the chemical. It is often observed that RCF values are static at approximately 1 for a log K_{ow} less than 1, and increases to 100 as log K_{ow} increases to 4 (Briggs et al. 1982). Although chemicals enter plant roots largely by passive diffusion with undissociated acids, entry through an ion-trap mechanism is also possible by acid exudation from roots (Briggs et al. 1987).

Some compounds diffuse through soil primarily via the vapor phase, and plant roots absorb them after their solubilization in soil solution; these are governed by Henry's law, rather than by systemic transport (Bromilow and Chamberlain 1995; Riviere 2000). Henry's law constant (H') is calculated by

$$H' = \frac{P \cdot M}{S \cdot RT_K} \tag{8}$$

where

P = vapor pressure at absolute temperature T
S = water solubility at absolute temperature T
M = molecular weight
R = gas constant
T_K = absolute temperature.

When H' is larger than 10^{-4}, movement occurs by diffusion in air. Examples are ethylene dibromide, carbon tetrachloride, trichloroethylene, naphthalene, DDT, DDE, trifluralin, nitrobenzene, and dioctyl phthalate (Bromilow and Chamberlain 1995; Riviere 2000). When H' is less than 10^{-6}, diffusional movement occurs only in water; examples are carbofuran, aldicarb, simazine, metalaxyl, prochloraz, and hexazinone. For H' values between 10^{-4} and 10^{-6}, diffusion in both air and water occurs; examples are dibutyl phthalate, parathion, dieldrin, deltamethrin, and diuron (Bromilow and Chamberlain 1995; Riviere 2000). It has not been demonstrated, however, that vapor-phase diffusion within soil leads to absorption by plant roots exposed to the vapor.

In addition to physical and chemical properties, uptake of xenobiotics is influenced by other factors, including:

- Anatomical features and physiological processes of plants, such as differences among root systems (Eijsackers 1994; Trapp et al. 1994).
- Excretion of solubilizing agents (e.g., monocots (barley) excreting mugineic acid as a chelating agent of iron, and then incorporating the Fe–mugineic acid complex) (Alam et al. 2001; Bernards et al. 2002; Chaignon et al. 2002; Figueira et al. 2001; Negishi et al. 2002; Singh et al. 2000; Walker 2002).
- Environmental parameters such as temperature, humidity, and soil water content.
- Changes in bioavailability of xenobiotics caused by enzymes excreted from plant roots and actions of root-associated microbes (El-Shatnawi and Makhadmeh 2001; Lodewyckx et al. 2002; Lovell et al. 2001; Robinson et al. 2001).
- Effects on bioavailability from mycorrhizae, the symbiotic structures of fungal mycelia and plant roots, associated with nearly all plants except for the Brassicaceae. Arbuscular mycorrhizal fungi are known to improve the uptake of phosphorus, nitrogen, zinc, copper, and sulfur (Paul and Clark 1988; Weissenhorn et al. 1995a, b). Ectomycorrhizae, formed by white-rot fungi, cover host plant roots, and the exoenzymes excreted from them degrade a wide variety of organic chemicals in soils, which cannot be degraded by the host plant. The degradation by ectomycorrhizae reduces the availability of organic chemicals to the host plant (often a tree), which has been observed in 2,4,6-trinitrotoluene (Koehler et al. 2002).

3.4 Uptake by Soil Fauna

Because invertebrates are important in the soil environment, many chemical uptake studies with such organisms have been reported. Soil invertebrates feed on OM, fungi, bacteria, and protozoa and thus contribute to nutrient cycling and energy flow within the soil ecosystem. Earthworms are among the most important of soil fauna because of their abundance. For example, *Lumbricus* constitutes 80% of invertebrate biomass in floodplain soils, and comprises a major part of the diet of moles (*Talpa europaea*), badgers (*Meles meles*), shrews (*Soricidae*), and other predators (Hendriks et al. 1995). Thus, soil fauna may affect how chemicals are distributed in the environment.

Soil invertebrates, especially earthworms, are classified by feeding behavior and habitat as being either: *anecic*, *epigeic*, or *endogeic*. *Anecic* fauna (e.g., *Lumbricus terrestris*) burrow deeply into soil, but forage at night on the surface for decaying grass or litter or animal residues to bring into their burrows; *Epigeic* fauna (e.g., *Lumbricus rubellus*) are surface-dwelling, and move through the upper litter layer consuming freshly decayed litter and animal residues. *Endogeic* fauna (e.g., *Nicordrilus caliginosa*) are soil dwelling, and move through the upper organic-rich mineral layers, ingesting soil and extracting nutrients from degraded OM. They

leave finely dispersed organic particles. These examples of feeding behavior and differential habitat are important variables for oral uptake of xenobiotics.

Hard- and soft-bodied fauna may absorb organic chemicals by different routes (Eijsackers 1994). Soft-bodied organisms such as Protozoa, Nematoda, and Lumbricidae rely on contact with soil pore water to remain hydrated; therefore, chemical uptake occurs through the skin from pore water (dissolved organic chemicals) as well as by feeding. Hard-bodied organisms with tracheal systems obtain organic chemicals by feeding, but also absorb vaporized contaminants that exist in the soil atmosphere, or those that are dissolved in pore water. Intake of xenobiotics by feeding is a major route for Carabidae and Aranea. Antennae and legs may also be in contact with chemicals. Soil vertebrates absorb chemicals through the skin, lungs, and via food; the latter is typically most important.

Equilibrium partitioning theory (EqP theory) has been applied to chemical uptake by invertebrates in sediments (Di Toro et al. 1991; Felsot and Lew 1989). Biological effects on organisms can be predicted from the chemical concentration in pore water of sediments for relatively hydrophilic compounds; for hydrophobic chemicals, effects of a chemical can be predicted from the chemical's concentration in sediment organic matter (OM). The ability to make such predictions is based on the assumption that an equilibrium of the chemical(s) is established by diffusion among organisms, pore water, and the OC of sediment (Felsot and Lew 1989). EqP theory accurately describes the uptake of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) by *L. terrestris* L. (Krauss et al. 2000) and phthalate congeners by *Eisenia foetida* (Hu et al. 2005b). However, EqP theory failed to accurately portray acute and chronic toxicity for lindane, in soils, when studied using the soil invertebrates *E. foetida*, *Enchytraeus albidus*, and *Folsomia candida* (Lock et al. 2002). The relative difference of lindane toxicity between the soils differed among the invertebrates, and did not correlate with organic carbon (OC) in the sediment. The results indicated that the pore-water contaminant fraction was not always the toxicological by bioavailable fraction. Apparently EqP theory is considered to be unreliable under certain conditions.

Because soil fauna feed bacteria, fungi, and soil OM, as well as protozoa, bioaccumulation of xenobiotics may occur through the food web. Based on the EqP theory concept, a biota–soil accumulation factor (BSAF) has been described by Ma et al. (1998) to characterize bioaccumulation of a chemical in biota. Assuming steady-state conditions of ingestion and excretion and ignoring possible metabolism of the chemicals, BSAF can be calculated by

$$\mathrm{BSAF} = \frac{C_{\mathrm{org}} \cdot F_{\mathrm{om}}}{C_{\mathrm{s}} \cdot F_{\mathrm{lip}}} \tag{9}$$

where

BSAF= biota–soil accumulation factor
C_{org} = concentration in the worm
C_{s} = concentration in soil solid phase
F_{lip} = weight fraction of lipid in the organism
F_{om} = weight fraction of OM.

By substituting for F_{om} with the equilibrium soil sorption Coefficient of Chemical (K_p), the equation for bioaccumulation factor (BAF) results:

$$\mathrm{BAF} = \frac{C_w \cdot K_p}{C_s \cdot F_{lip}} \tag{10}$$

where

BAF= bioaccumulation factor

K_p = equilibrium soil sorption Coefficient of Chemical.

A state of equilibrium in the equation is assumed. Soil OM and soil OC (typically what is measured in soil tests) are related by

$$K_p = 0.58 F_{om} K_{oc} \tag{11}$$

where

K_{oc} = equilibrium sorption coefficient of chemical on a basis of soil OC.

For hydrophobic organic chemicals with log K_{ow} between 1.5 and 7.5, the following equation (Sabljic et al. 1995) is proposed for estimating log K_{oc}:

$$\log K_{oc} = 0.81 \bullet \log K_{ow} + 0.10 \tag{12}$$

The coefficient values varied (among compounds tested and researchers) from 0.52 to 1 of the pre-logarithmic coefficient, and from –0.779 to +1.377 for the coefficient of the second term (Fetter 1999).

BSAF provides a starting point for characterizing the bioaccumulation of persistent organic compounds such as organochlorines, PAHs, and organotin compounds (Van Brummelen et al. 1996). BSAFs may also help characterize the effects of phthalate congeners on *E. foetida* in soil (Hu et al. 2005b). Concentrations of xenobiotics observed in the body of soil fauna are often a linear function of exposure time (Custer et al. 1996). In the cases of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, BSAF values do not increase with increasing chlorine atom substitution, although *n*-octanol–water partition coefficients increased with degree of chlorine substitution (Van Der Oost et al. 1996). These suggest that bioavailability balances affinities among chemicals, sediments, and organisms, or that molecules with large numbers of chlorines are simply too large to pass through the skin or integument of soil fauna.

3.5 Passive Diffusion: A Common but Variable Mechanism Among Organisms

The major mechanism by which organic compounds, transport through membranes in all species, from microbes to soil fauna, is by passive diffusion. The driving force for such transport is the concentration of xenobiotics in soil solution. Exceptions are

exoenzymes excreted from certain organisms, and metal cations transported through membranes from soil mainly by facilitated diffusion and active transport.

Bioavailability of chemicals also differs among organisms as a result of the mobility and habitat of soil organisms. Microorganisms need water, therefore, in non-saturated soil microorganisms remain near hydrated microsites. On the other hand, fungi and plant roots can penetrate dry microsites. Soil fauna such as earthworms are mobile in soil aggregates. For fungi, plant roots, and soil fauna, chemicals sequestrated in the soil aggregate can be bioavailable.

4 Properties of Xenobiotics and Soils, and Their Interactions

Xenobiotics may exist as free constituents (i.e., dissolved form) in soil solution, may be degraded by chemical and biological processes, or may be sorbed onto soil particles. These three processes determine the availability of xenobiotics to biota. The degradation of xenobiotics is proof that chemicals were actually bioavailable to organisms; moreover, degradation reduces xenobiotic bioavailability to other organisms.

The most significant interaction between soils and xenobiotics that affects bioavailability is sorption, followed by aging and bound residue formation. Sorption can be greatly affected by xenobiotic properties including water solubility, vapor pressure, molecular size, and *n*-octanol–water partition coefficient. Soil properties also affect adsorption rates. In this regard, key soil properties include OM, pH, and cation exchange capacity (Felsot and Lew 1989). Climatic and tillage practices also affect bioavailability and will be addressed in this section.

4.1 Sorption Isotherms

The rate of uptake and subsequent degradation of chemicals by organisms is generally determined by the concentration of the chemicals in soil solution. Sorption of xenobiotic compounds to soil constituents reduces the concentration of the xenobiotics in soil solution. Many researchers have observed reductions of chemical uptake and affects on degradation rates as a result of sorption. Some recent examples include alpha-HCH (Rijnaarts et al. 1990), 2,4-D (Ogram et al. 1985), 1,2-dibromoethane (Steinberg et al. 1987), naphthalene (Zhao and Voice 2000), carbon tetrachloride to *Pseudomonas* sp. strain KC (Zhao et al. 1999), PAHs (Breedveld and Sparrevik 2000), and atrazine (Beigel et al. 1999).

Sorption of chemicals to soil has recently been reviewed in detail (Wauchope et al. 2002). Therefore, in this chapter only important equations and parameters concerning sorption are presented in relation to bioavailability. Sorption is often expressed by the Freundlich isotherm equation:

$$\frac{x}{m} = K_{\mathrm{f}} \cdot C^{1/n} \tag{13}$$

where

x = amount of sorbed chemical
m = soil weight
C = concentration of chemical in soil solution
K_f = Freundlich sorption coefficient
n = linearity factor.

Hydrophobic, non-polar chemicals often exhibit a linear sorption ($n = 1$):

$$\frac{x}{m} = K_p \cdot C \tag{14}$$

For non-polar solutes, many studies have shown that most sorption occurs by a simple hydrophobic partition between soil OM and soil water (Chiou 1990). In such cases

$$\frac{x}{m_{oc}} = K_{oc} \cdot C \tag{15}$$

where

m_{oc} = the OC content

This relationship is very widely used to predict the concentration of xenobiotic compounds in soil solution. However, sorption often deviates from this linear equation (Wauchope et al. 2002), because soil OM is not always homogeneous or the dominant sorbent for some soils and chemicals. Clay minerals and metal hydrous oxides can be important sorbents depending on the properties of the solute. When hydrophobic interaction is the major mechanism of sorption, it emulates a partition phenomenon and is reversible in a short time. However, irreversible sorption of pesticides to soil may occur and can be experimentally demonstrated by using ^{14}C-isotopic exchange techniques (Celis and Koskinen 1999a). This phenomenon can be described by a two-compartment model that portends aging or bound residue formation.

Sorption does not always reduce the bioavailability of chemicals or inhibit their degradation. Degradation rates increased during experiments with 2,4-D, 2,4-dichlorophenol, and 4-chlorophenol adsorbed on humified and nonhumified soil OM (Benoit et al. 1999). The concentrations of these chemicals in the aqueous phase were low and concentrations at the surface of suspended particles were high. Thus, bioavailability was higher on the surface than in soil solution (Subba-Rao and Alexander 1982). The direct uptake of sorbed chemicals by microorganisms also occurs (Shor and Kosson 2000). Enhancement is also observed when metal hydrous oxides act as biological catalysts; in such cases degradation rates are higher for chemicals in the sorbed state.

4.2 Properties of Xenobiotics That Affect Their Sorption to Soil

Uneven distribution of electron density in molecules results in molecular polarity or the presence of local charges. Under such conditions, acidic molecules dissociate to form anionic species. Tetra-ammonium salt structures result in a cationic molecular state. Alcohols and phenols are acidic polar structures and nitrogen-containing structures generally have basic polar characteristics. Cationic and basic molecules are strongly sorbed to soil particles because their surface has a net negative charge. Conversely, anionic molecules are repulsed by the soil surface. Therefore, the availability of anionic or acidic molecules to biota is much higher than that of cationic or basic molecules in the soil environment.

The *n*-octanol–water partition coefficient (K_{ow}), soil sorption, bioavailability, and solubility of chemicals may all be correlated. In general, as K_{ow} increases, sorption increases and solubility and bioavailability decrease. Thus, an increase in methanol concentration in soil water, added as co-solvent, increased solubility and decreased sorption of linuron and simazine on a clay soil (Kookana et al. 1990). Similarly, formulation of propoxur as an emulsifiable concentrate resulted in an emulsion which was stable in the soil, thereby greatly increasing the concentration of propoxur in the solution phase (Wybieralski 1992). The presence of several compounds in soil may sometimes increase the concentration of one of them in soil solution. In such cases, multiple compounds compete for sorption to microsites, even in with non-ionic organic chemicals. Competitive sorption was reported between the fungicides carbendazim and iprodion (Leistra and Matser 2004).

4.3 Soil Properties That Affect Sorption of Xenobiotics to Soil

Sorption levels may vary widely among soils, even for the same chemical. Sorption of a chemical to soil is affected most by OM, metal hydrous oxides, and clay minerals; therefore, the overall sorption capacity of soil is dependent on the characteristics of soil OM, soil texture, soil acidity, Fe- and Al-oxide content and clay mineralogy (Johnson and Sims 1993). There is a tendency for organic soils to sorb higher amounts of organic chemicals. The sorption rate is generally fastest for humic acids, followed by amorphous iron and aluminum hydrous oxides and, finally, the clay minerals kaolinite and montmorillonite (Sha'ato et al. 2000).

There is extensive evidence that sorption to soil of a wide range of organic chemicals increases with content of OM (Green 1974; Weed and Weber 1974); therefore, as soil OM increases, bioavailability decreases. For example, the K_p value of imidacloprid was correlated with soil OC content and cation exchange capacity (CEC; Oliveira et al. 2000). K_p values of the herbicides alachlor, atrazine, dicamba, hexazinone, metsulfuron-methyl, simazine, and sulfometuron-methyl had a significant correlation with the OC content of Brazilian soils, although this was not the case for imazethapyr and nicosulfuron (Oliveira et al. 2001). The bioavailability of phenanthrene to mineralization by *Pseudomonas* spp. declined in soil humin (White et al. 1999a). PAHs and PCBs were also mainly bound to the lipid fraction of

humin (Kohl and Rice 1998). The bioavailability (measured by bacterial genotoxicity assay) of benzo(*a*)pyrene, 7,12-dimethylbenz(*a*)anthracene, 9-phenylanthracene, and aldicarb after short-time sorption was correlated with the OM content of soil (Alexander and Alexander 2000). Application of organic material, waste-activated carbon, digested municipal sewage sludge, and animal manure to a sandy soil (OC content 0.8%) increased sorption of alachlor and atrazine and reduced the bioactivity of these herbicides to oat and Japanese millet (Guo et al. 1991).

In addition to OM content, specific surface area of soil is also an important factor in sorption. The type of clay and proportion of small clay particles influence the specific surface area of soil minerals, and therefore, the sorption capacity. The sorption of captan (Alexander and Alexander 2000) and imidacloprid (Ramakrishnan et al. 2000) has been observed to correlate with soil particle size.

As soil moisture decreases, sorption capacity greatly decreases, transferring primary sorption capacity from soil OM to metal hydrous oxides and clay minerals, even for apolar chemicals (Chiou 1990). Hydrophobic chemicals partition into the soil water phase at very low concentrations, which renders sorption equilibrium moisture-dependent. Decreasing soil moisture content also induces the chemical sequestration in soil, and reduces bioavailability compared with BSAF model predictions (Oen et al. 2006).

The CEC of soil is often correlated with the sorption capacity of xenobiotics. Soil OM content and mineral particle size distribution not only determine CEC but also the degree of xenobiotic sorption. Therefore, CEC may correlate to soil sorption capacity even though CEC values are not directly related to the actual sorption capacity, except for cationic chemicals such as heavy metals and the paraquat cation. Cation exchange processes in soil increase the lability of sorbed heavy metals. Heavy metals are displaced with other cations and dissolved into soil pore water. It is well known that heavy metals, extracted with dilute salt solutions (0.1 M $CaCl_2$), are easily removed from soil and made available for uptake into higher plants.

Soil pH affects sorption of chemicals that are dissociated variably at the normal range of soil pH. For example, sorption of pentachlorophenol is much less in alkaline than in acid soils, because it is dissociated (anionic) at high pH, and is nonionic under acidic conditions. Soil pH also influences desorption; for example, more imazethapyr was sorbed at low than high pH, but was readily desorbed (Bresnahan et al. 2000). At higher pH, the situation was reversed, with less imazethapyr sorbed, but no ready tendency to desorb the sorbed entities (Bresnahan et al. 2000). The solubility of heavy metals is greatly influenced by soil pH; the ease with which such metals can be extracted from soil by dilute acid (pH 1–2) is directly proportional to their availability for uptake by soil-dwelling meso/macro fauna (Harmsen et al. 2007). For anionic and neutral compounds, sorption constants for 21 soils were adequately explained to vary as a function of pH (Fontaine et al. 1991).

Soil sorption is a complex process. In addition to interactions with soil OM, clay minerals, and ionic/pH conditions other factors must be considered: soil to water ratio, ionic strength of soil solution, persistence of the xenobiotics, hydrogen-bond complex formation, etc. Soil to water ratio is always changing under actual field conditions, and the change influences the sorption of xenobiotics. For example, the

sorption constant of fluridone at a 1:1 soil/water ratio was higher than when the ratio was 1:5 (Malik and Drennan 1989). Changes in soil to water ratio also produced changes in the ionic strength of soil solution. Increase ionic strength, in soil solution, increased the sorption of four herbicides (Alva and Singh 1991). Calcium saturation changed the Freundlich constant for acifluorfen, probably because a complex with acifluorfen resulted in its precipitation (Pusino et al. 1993). The formation of a hydrogen-bond complex between atrazine and subconstituents of soil OM is important in determining the sorption constant for atrazine (Welhouse and Bleam 1993). The sorption of chemicals was higher in earthworm burrow linings compared to bulk soil (Stehouwer et al. 1993).

4.4 Desorption and Dissolution

The concentration of chemicals in soil water depends on the properties of the sorbate and sorbent, the mechanisms of sorption, the time allowed for establishing an equilibrium, and the degradation rate of the chemical (Guerin and Boyd 1992). These factors also affect the kinetics of desorption, which is a determinant of total bioavailability over time. It has been observed that the rate of desorption is proportional to the rate of uptake of highly hydrophobic chemicals (Jacobsen et al. 2001; Stucki and Alexander 1987; Thomas et al. 1986; Zhao et al. 1999; Zhao and Voice 2000). The process of dissolution of chemicals in non-aqueous soil solvent phases is similar to the process of desorption. Desorption or dissolution flux is generally proportional to the concentration difference of the chemical in the vicinity of particle and soil solution:

$$\text{Flux} = k_{\text{la}} [C_{\text{vic}} - C] \tag{16}$$

where

Flux = desorption/dissolution flux
k_{la} = mass transfer rate coefficient
C_{vic} = concentration of the chemical in the vicinity of soil particles (or chemical crystals/solvents)
C = concentration in soil solution.

When C is high, microorganisms may accelerate desorption by reducing concentrations in soil solution through biodegradation. Incubation of soil-bound residues of atrazine with an atrazine-degrading *Pseudomonas* sp. for 83 d resulted in 30–35% of soil-bound atrazine being converted to extractable residue, compared to only 3% in a sterile control. Under these conditions, the bound atrazine was released from humic material (Khan and Behki 1990). Desorption has also been facilitated with *p*-alkyl amines (Wszolek and Alexander 1979) and parathion (Racke and Lichtenstein 1985). After desorption (or dissolution) of the chemical, its concentration in soil solution can become too low for utilization by microbes, essentially ending desorption or reducing it to a very low rate.

Only the principle of desorption is expressed by the above equation. The kinetic process in soil is complex and poorly understood. A single rate constant often

does not apply over the entire kinetic process (Pignatello and Xing 1996). For example, with longer contact time in soil, more chemical can diffuse into soil aggregates (forming bound residues). This reduces the desorption rate over time (Connaughton et al. 1993). Reduction in bioavailability over time is directly correlated with increasing residue binding and resistance to desorption (White et al. 1999b). Desorption of bound residues is greatly affected by the tortuosity or steric restriction of soil aggregates (Steinberg et al. 1987). Sorption/desorption processes display hysteretic characteristics, i.e., the sorption and desorption rates of xenobiotics in soil, or the equilibria, are not only affected by the present concentration of xenobiotics in soil solution but also by the previous or original concentration. The sorption rate under the increasing xenobiotic concentration in soil solution is usually faster than the desorption rate under the decreasing xenobiotic concentration in soil solution. The hysteretic characteristic is considered to be caused by the strong interaction between the sorbed chemical and the soil or by irreversible sorption. This phenomenon has been demonstrated with ^{13}C-naphthalene using an isotope exchange technique (Sander and Pignatello 2005). The hysteresis has been observed in naphthalene, phenanthrene, *p*-dichlorobenzene (Kan et al. 1994), ethylene dibromide (Steinberg et al. 1987), and trichloroethylene (Pavlostathis and Jaglal 1991). Hysteretic behavior is affected by the combination of sorbate–solvent, concentration, and time (Sander et al. 2005).

The bioavailability of a chemical may be enhanced if it is tested in the presence of chemicals with substantially similar chemical structures, probably as a result of competitive sorption. The rate and extent of degradation of aged phenanthrene in soil by *Pseudomonas* sp. were enhanced by addition of anthracene or pyrene to the soil at the same time as the bacterium was introduced, despite the fact that *Pseudomonas* sp. could not degrade either anthracene or pyrene (White et al. 1999c). In sterile soil, pyrene reduced the K_p value of phenanthrene aged for 123 d in soil, which suggests the competitive displacement of aged chemicals with freshly added chemicals (White et al. 1999c). However, the replacement is often imperfect because a portion of chemical is sorbed to soil irreversibly (Celis et al. 1997; Celis and Koskinen 1999a; 1999b; Cox et al. 1997).

4.5 Effects of Dissolved Organic Matter and Surfactants

Soil water contains a wide range of chemical species in solution. For example, a portion of OM is dissolved in soil solution as dissolved OC (DOC), which is carbon that passes through a 0.45-μm pore size, and is composed of a diversity of components, depending on source and soil conditions. DOC decreases water surface tension and is capable of forming micelles with a hydrophobic interior similar to that of a surfactant (Guetzloff and Rice 1994). When a chemical is bound into the hydrophobic domain of the DOC, dramatic solubility increases occur at DOC concentrations above the critical micelle concentration (CMC). This binding may be reversible (like partitioning) or irreversible (Harms and Bosma 1997). Solubility

increases may also occur below the CMC. Thus, hydrophobic xenobiotics, although mainly sorbed to soil particles (or aggregates), may, in part, sorb to dissolved OC and remain in soil solution.

The amount of a hydrophobic compound available to bacteria may decrease when DOC is present, as a result of competitive interactions between DOC and the bacteria (Robinson and Novak 1994). For example, the degradation rate of PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) was decreased by dissolved or particulate OM (Ressler et al. 1999). Degradation of 2,4,6-trichlorophenol by *Pseudomonas aeruginosa* was reduced in the presence of dissolved humic acid (Robinson and Novak 1994). However, the extent of the decrease is dependent on bacteria present and DOC interaction. Dissolved humic acid decreased the bioavailability of *p,p*'-DDT for bacterial degradation, but fulvic acid did not, although both were extracted from the same soil and both increased apparent solubility (Fujimura et al. 1995). This bioavailability-dampening effect by DOC has also been observed in organisms other than microorganisms; an example is chlorpyrifos bioavailability to the estuarine bivalve *Mercenaria mercenaria* (Bejarano et al. 2005).

When DOC increases the apparent solubility of a chemical, and the interaction between the chemical and DOC is smaller than that which exists between the chemical and the cells, the bioavailability of xenobiotics is expected to increase. Enhanced degradation of PCBs was achieved when solubilization into the hydrophobic domains of soil humic substances was increased (Fava and Piccolo 2002). Soya lecithin, a natural surfactant, also increased the bioavailability of PCBs in soil, when present (Fava and Di 2001). Meredith and Radosevich (1998) demonstrated an enhanced degradation of atrazine by *Agrobacterium radiobacter* ATCC 55551 in the presence of dialyzed Aldrich humic acid. This enhancement was attributed to improved cellar uptake, although similar effects of dialyzed Aldrich humic acid on degradation of quinoline by *Rhodococcus* sp. or naphthalene by *Pseudomonas putida* ATCC 17484 did not occur. Enhanced aerobic degradation of PAHs in contaminated soil was observed in the presence of humic substances and soya lecithin (Fava et al. 2004). Guerrero et al. (2003) demonstrated that suspended solid particles with moderate hydrophobicity such as TOYOPEARL SP^{TM} increased bioavailability of pyrene to a freshwater fingernail clam *Sphaerium corneum*. Cyclodextrin also enhanced degradation of transformer oil in soil (Molnar et al. 2005).

Similar to the situation with DOC, surfactants increase the concentration of hydrophobic xenobiotics in soil solution and increase the desorption/dissolution rate of sorbed chemicals. Soil biodegradation of highly hydrophobic compounds is limited by low water solubility and desorption/dissolution rate. Surfactants have been used to enhance biodegradation, although such enhancement does not always occur (Table 2), probably because chemicals bound to surfactants are less available, and the surfactants may be toxic to microorganisms. The solubility of hydrophobic chemicals is dramatically enhanced above the CMC, and in such cases increased uptake and degradation may occur. However, surfactants may also inhibit the uptake and/or degradation of chemicals (Aronstein and Alexander 1992; Aronstein et al. 1991; Macur and Inskeep 1999). This inconsistency results from the inhibitory

Table 2 Effect of surfactants on bioavailability of organic chemicals in soil

Name	Concentration	Chemical	Organisms or soil	Effect on uptake or degradation	References
LXS-814 Alkylxylylsulfonates, alkyl chain n=8–14,	Below CMC[1]	Naphthalene biphenyl	Bacterium	Decrease	Roch and Alexander (1995)
Neodol 25-3S alkylethoxysulfonate, alkyl chain: n=12–15, ethoxy chain: average × =3	Below CMC	Naphthalene biphenyl	Bacterium	Decrease	Roch and Alexander (1995)
Alfonic 1412-40 alkylethoxylate, alkyl chain: n= 12 and 14, ethoxy chain: average × =2.9	Below CMC	Naphthalene biphenyl	Bacterium	Decrease	Roch and Alexander (1995)
Alfonic 810-60 alkylethoxylate, alkyl chain: n = 8, 10, 12, and 14, ethoxy chain: average × = 4.5	Below CMC	Naphthalene biphenyl	Bacterium	Decrease	Roch and Alexander (1995)
Novel II 1012-62 alkylethoxylate, alkyl chain: n=10, 12, and 14, ethoxy chain: average × =6.7	Below CMC	Naphthalene biphenyl	Bacterium	Decrease	Roch and Alexander (1995)
Triton X-100 (C-8PE-9.5) alkylphenol ethoxylates, alkyl chain: n = 8, ethoxy chain: average × =10	From below to above CMC	Naphthalene, phenanthrene biphenyl	Bacterium, naphthalene-degrading mixed culture, slurry microcosm	No inhibition	Liu et al. (1995), Roch and Alexander (1995), and Tsomides et al. (1995)
Triton X-100	Half of CMC	NAPLs[2]	Bacteria	Inhibition	Stelmack et al. (1999)
Dowfax 8390	Half of CMC	NAPLs	Bacteria	Inhibition	Stelmack et al. (1999)
SDS	Entire range	PAHs,[3] PCBs[4]	Degrading mixed culture	Decrease as alternative nutrient	Tiehm (1994)
SDS	Above CMC	PCBs		Increase	Billingsley et al. (1999)
Alfonic 810-60	10–100μg/g below CMC	Phenanthrene, biphenyl	Phenanthrene- or biphenyl-utilizing microbes	Increase	Aronstein and Alexander (1992), Aronstein et al. (1991)

Table 2 (continued)

Name	Concentration	Chemical	Organisms or soil	Effect on uptake or degradation	References
Novel II 1412-56	10–100μg/g below CMC	Phenanthrene, biphenyl	Phenanthrene- or biphenyl-utilizing microbes	Increase	Aronstein and Alexander (1991, 1992)
Brij 30 (C-12E-4) alkylethoxylate	Above CMC	Naphthalene		No inhibition	Liu et al. (1995)
C11-2E-4 alkyl ethoxylates	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
C-8PE-9.5 alkylphenol ethoxylates	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
C-9PE-10.5 alkylphenol ethoxylates	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
Tween 20 sorbitan monolaurate	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
Tween 80 sorbitan monooleate	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
C-12E-4/C-12E-23(1:1) non-ionic ethoxylates	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
C-12-15 E-3/C-12-15E-9(1:3)	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
CHAPS	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
Octylglucoside	Above CMC	Phenanthrene	Soil	Complete inhibition	Laha and Luthy (1992)
Arkopal N-300, alkylphenolethoxylate		PAH		Increase by surfactant degradation	Tiehm et al. (1997)
Sapogenat T-300, alkylphenolethoxylate		PAH		Increase by surfactant degradation	Tiehm et al. (1997)
Alcohol		PAHs with five rings	Soil slurry reactor	Increase	Lee et al. (2001)
Acetone		PAHs with five rings	Soil slurry reactor	Increase	Lee et al. (2001)
Soya lecithin	15–30 g/kg	PCBs	Soil slurry reactor	Increase	Fava and Di (2001)
Rhamnolipid		PAHs	Bacteria, yeast, and fungi	Increase	Gu and Chang (2001) and Pritchard et al. (1999)
Alasan		PAHs	*Acinetobacter* sp.	Increase	Rosenberg et al. (1999)

[1]CMC: critical micelle concentration; [2]NAPLs: non-aqueous phase liquids; [3]PAHs: polynuclear aromatic hydrocarbons; [4]PCBs: Polychlorinated biphenyls

physiological and physicochemical effects of surfactants on microbial cells. The inhibitory effect is a reversible physiological surfactant–micelle/bacteria interaction, and may result from partial complexing or release of membrane material (Guerin and Jones 1988; Laha and Luthy 1992). Inhibition by non-ionic surfactants decreased with increasing hydrophilicity, and in proportion to increasing ethoxylate chain length of the alkylethoxylate and the alkylphenolethoxylate moieties (Dorn et al. 1993; Wong et al. 1997). Inhibition of a PAH-degrading *Mycobacterium* sp. was observed when surfactants had an average ethoxylate chain length of 9–12 monomers (Tiehm 1994). The observed effect was reversible, because the bacterial ability to degrade PAHs was recovered by dilution of the medium that contained the non-ionic surfactant (Laha and Luthy 1992). It is believed that surfactants do not disrupt the membrane lamellar structure. It has been generally noted that fungi have higher tolerance to surfactants than do bacteria (Pinto and Moore 2000; Zheng and Obbard 2000, 2001).

Both direct and indirect microbial uptake mechanisms have been reported for hydrophobic chemicals bound to surfactants in aqueous solution, and differences between the two may explain some inconsistencies of surfactant effects. In the direct mechanism, hydrophobic chemicals are transferred into microbial cells without first passing through the water phase. In one study, PAHs were transferred directly from inside the surfactant micelles to microbial cells (Guha et al. 1998). Such transfer may derive from fast exit rates of hydrophobic compounds from micelles as attractions are continuously formed and broken (Laha and Luthy 1992; Tiehm 1994). In indirect uptake mechanisms, hydrophobic chemicals are first transferred to the water phase, and subsequent chemical uptake by cells is facilitated by the chemical's relative affinity for cells and surfactant micelles (Fujimura et al. 1995). Therefore, microorganisms with highly hydrophobic cell surface have the advantage in taking up hydrophobic chemicals in soil. Gram-positive bacteria have more hydrophobic cells than do Gram-negative bacteria (Rosenberg et al. 1979).

There are various surfactant types and sizes of hydrophobic domains in micelles; these produce different effects on chemical availability to microbial cells. Triton X-100, a commercially available surfactant, has been observed to only minimally inhibit degradation processes because of its relatively low hydrophobicity (Roch and Alexander 1995; Tsomides et al. 1995). In sand with low OM content, Triton X-100 either adsorbs or solubilizes phenanthrene. However, in topsoil that contains higher OM, Triton X-100 did not act as an adsorbent because of the lower hydrophobicity of the surfactant (Edwards et al. 1994). In a soil solution containing one-half of the critical micelle concentration of Triton X-100, the adhesion of bacteria to the surface of non-aqueous liquid droplets was inhibited. This decreased the uptake efficiency of non-aqueous liquids by bacteria (Stelmack et al. 1999).

Cosurfactants such as alcohols and acetone also increase bioavailability of xenobiotics. Addition of <1% of cosurfactants did not inhibit soil respiration (Kuwatsuka personal communication). The use of cosurfactants may be a better way to increase bioavailability than simply increasing the concentration of surfactants, because surfactants also affect human health.

Some microorganisms, when exposed to PAHs, exude biosurfactants that increase desorption and/or dissolution rates (Banat 1995). Biosurfactants exhibit low interfacial tension and low critical micelle concentration values and are produced by many bacteria, yeast, and fungi (Pritchard et al. 1999). Rhamnolipids are the best-known biosurfactants. The Concentration needed to solubilize phenanthrene was similar between a rhamnolipid and Triton X-100 (Pritchard et al. 1999); both enhanced the mass transfer of hydrophobic compounds (Gu and Chang 2001). *Acinetobacter radioresistens* KA53 excretes alasan, another biosurfactant, and increased the apparent solubility of PAHs by 5–26 times, and more than twofold the biodegradation rate of fluoranthene (Rosenberg et al. 1979, 1999).

There are few reports on the effect of surfactants on more polar compounds. In one case, no enhancement of soil desorption by formulation adjuvant (surfactant) was observed for triticonazole (Beigel et al. 1999).

4.6 Effects of Aging and "Bound Residue"

"Aging" refers to decreases in bioavailability that occur from increased contact time between chemical and soil. Even if no chemical degradation occurs in soil, aging renders chemicals less available for uptake by organisms, less likely to exert toxic effects, and less susceptible to microbial biodegradation (Alexander 2000). The effects of aging has been observed in a wide spectrum of xenobiotics, e.g., PAHs (Beckles et al. 2007; Chung and Alexander 1998; Nam and Alexander 1998), isoproturon (Walker et al. 1999), atrazine (Chung and Alexander 1998; Radosevich et al. 1997), simazine (Scribner et al. 1992), triticonazole (Beigel et al. 1999), and imidacloprid (Koskinen et al. 2001). Carbaryl, although relatively water-soluble and weakly sorbed to soil, also showed reduced bioavailability and microbial biodegradation after aging (Ahmad et al. 2004). The effects of aging have been observed not only in microbial degradation, but also after oral intake of chemicals by mammals, e.g., after oral intake of PAH-contaminated soil by male Fischer 344 rats (Reeves et al. 2001).

It is believed that aging occurs by diffusion of sorbed chemicals into micropores of soil aggregates or particle interstices, a process that segregates the chemical from access by organisms (Gevao et al. 2000; Hatzinger and Alexander 1997). Once entrapped in micropores, diffusion of the chemical back to macropores and bulk soil water is a very slow process. In addition, soil OM may cover openings and block access to micropores. Aging may result from covalent bonding with soil humus to create bound residues after sorption inside micropores. Thus, aging is largely a sequestration phenomenon. Crystallization of hydrophobic chemicals in the soil matrix may also intensify effects of soil aging; this phenomenon occured with anthracene (Willumsen et al. 1997).

The size distribution of micropores is a factor in aging: the smaller the micropore the slower aging proceeds, but the larger the effect. Soils treated with OM have a higher proportion of micropores, which increases chemical aging rates (Nam et al. 1998). When micropore surfaces lack hydrophobic characteristics, aging effects

were small. The role of soil particle hydrophobicity was demonstrated by using beads with different surface characteristics as model soils (Nam and Alexander 1998); results demonstrated that phenanthrene was degraded by the bacterium in the presence of: glass or polystyrene beads with no pores, silica beads with 2.5–15 nm pores, 3-aminopropyl-bonded silica beads with 6-nm pores, diatomite beads with 5.4-nm pores and octadecyl-bonded silica beads with 6-nm pores, but not in the presence of polystyrene beads with 5- or 300–400-nm pores. The concentration of the chemical in soil does not affect extent of aging (Chung and Alexander 1999), though chemical structure does. Such influences were demonstrated with differences in sequestration and microbial-induced biodegradation effects over time with naphthalene and atrazine (Chung and Alexander 1998).

The term "bound residue" is defined as "the portion of chemicals unextractable by methods that do not significantly change the chemical nature of the compounds (Roberts 1984)." The distinction between extractable and non-extractable residues depends on extraction methods and conditions employed (Khan 1982, 1991). Soxhlet extraction, an exhaustive procedure, has often been used to determine bound residues in soil after spiking such soil with isotopically labeled compounds (Northcott and Jones 2000). Covalent bonding is believed to account for the strong attachment between unextractable xenobiotic residues and soil (Dec and Bollag 1997). Soil constituents producing the strong attachment are mostly humic substances, mainly humin. Carbonyl, quinone, and carboxyl groups of humic substances are known to bind xenobiotics with hydrolyzable and non-hydrolyzable bonds. Biotic and abiotic oxidative coupling between xenobiotics and soil OM results in formation of bound residues over even short periods (Bollag 1992; Bollag et al. 1983; Shindo and Huang 1982, 1984, 1985).

Xenobiotics that are unextractable because of occlusion in stable soil nanopores are also regarded to be bound residues (Barriuso and Koskinen 1996). Such unextractability results from entrapment of xenobiotics in small diameter micropores. Compared with Soxhlet extraction, using other extraction methods such as supercritical fluid extraction, high temperature distillation, microwave extraction, and silyation prior to extraction will yield different extractable amounts of xenobiotics in soils and, therefore, different levels of xenobiotic residues in soil (Gevao et al. 2000). This differential extractability is thought to be produced from different penetration rates of extractants into soil micropores. Regardless as to cause of unextractability, or degree of covalent-bond formation or entrapment in small soil pores, unextractable xenobiotic soil residues are considered to be "bound residues"; therefore, the meaning of "aged" and "bound" residues overlaps each other.

A major concern is whether soil-bound xenobiotic residues can be mobilized, become bioavailable and have toxicological and ecological significance. Bound residues can be released by changes in the physico-chemical environment and by activities of soil organisms. However, there is a wide range of opinions concerning whether "bound residues" may eventually become bioavailable or not. The variability in types of soil-bound residues has probably led to this wide range of conclusions. Although bound residues, formed by covalent bonding with soil organic matter, are not considered bioavailable, the exact chemical nature and structure

of this type of bound residue have not been elucidated for all xenobiotics; however, progress in this direction has resulted from use of ^{13}C-labeled xenobiotics (Dec and Bollag 1997; Park et al. 2000). There are also reports that microbial degradation and plant uptake of bound residues from soil can occur. Bound soil residues of anilazine were mobilized as degradation of humic substances, especially fulvic acids, proceeded (Liebich et al. 1999). Cypermethrin residues, bound in soil, were mineralized (25–40%) during 26 weeks of incubation (Roberts and Standen 1981). Mineralization of soil-bound parathion was also observed (Racke and Lichtenstein 1985). After 28 d of incubation 3, 23, and 24% of bound residues of ^{14}C-labeled isoproturon, dicamba, and atrazine, respectively, were extracted by solvents or mineralized to $^{14}CO_2$ (Gevao et al. 2001). These results demonstrate that bound xenobiotics may be mobilized from soil, although only very slowly.

4.7 Environmental and Management Factors

Bioavailability of xenobiotics is also influenced by climate and agricultural management factors (Table 1). Temperature affects the rate of every process involved in bioavailability. This is particularly true of xenobiotics with higher vapor pressures, wherein transport in soil is dramatically increased with increased temperature (Trapp et al. 1994). Conversely, microbial degradation increases with increased temperature, and this decreases availability of xenobiotics to higher plants and soil fauna (Gyldenkaerne and Joergensen 2000; Ouyang 2002). Temperature also affects the biochemical activity, population response, and dwelling area (habitat) of soil fauna, and therefore alters soil bioavailability of xenobiotics (Eijsackers 1994).

Moisture, in the form of precipitation, changes the soil solid to water ratio. Thus, as moisture changes, the distribution of xenobiotics between soil solids and water will vary. Precipitation results in leaching of xenobiotics deeper to subsoils. In deep subsoils, bioavailability decreases because plant roots and soil fauna become sparse (Bromilow and Chamberlain 1995; Gyldenkaerne and Joergensen 2000). Earthworm burrows enhance leaching of xenobiotics. The presence of the earthworm, *L. terrestris* L., doubled the leaching rate of herbicides in soil, clearly demonstrating enhanced preferential flow resulting from presence of earthworm burrows (Farenhorst et al. 2000). Application season is also an important factor that affects the fate of pesticides (leaching or degradation), because of differences in precipitation strength and temperature, especially in the case of weakly sorbed non-persistent pesticides (Boesten and van der Linden 1991).

Soil moisture content affects the bioavailability of the sterol synthesis inhibiting fungicides, flusilazole, propiconazole, epoxiconazole, fenpropimorph, and prochloraz (Roy et al. 2000). Low soil moisture renders surfaces of humic substances more hydrophobic, which favors sorption of hydrophobic fungicides (flusilazole, propiconazole, and epoxiconazole). The herbicide diuron, a rather hydrophilic herbicide, was more highly sorbed in high moisture soil, because its moiety had higher diffusion rates and affinity for hydrophilic regions of humus. Effects of moisture

content in soil are more complex for easily protonated compounds; weakly basic compounds (prochloraz) partition rapidly into the liquid-like humus interior at low soil moisture content; however, increased diffusion at high soil moisture content may cause additional sorption by ion exchange at colloid surfaces. Strongly basic compounds (fenpropimorph) may be adsorbed due to ionic interactions with colloids, and their sorption may be enhanced at high soil moisture content as a result of diffusion (Roy et al. 2000). Soil microorganisms are normally most active where optimum moisture exists (Brock et al. 1994), such as in the vicinity of growing plant roots (Bromilow and Chamberlain 1995) and the habitat of soil fauna (Eijsackers 1994).

Wetting and drying induce cycles of reduction and oxidation of soil constituents, including ferric hydrous oxides/ferrous irons and OM. At anaerobic spots in soils ferric hydrous oxides are reduced. Portions of soil OM are released by reduction of ferric hydrous oxides to soluble ferrous ion. This also releases into the soil solution chemicals sorbed to/in ferric hydrous oxides or OM. Biodegradability or assimilation by *E. foetida* of di(2-ethylhexyl) phthalate and phenanthrene was increased as a result of wetting and drying cycles (White et al. 1998). Alternatively, wetting and drying during soil aging may reduce bioavailability; an example is reduced mineralization by *Pseudomonas* sp. of naphthalene (White et al. 1997). Karimi-Lotfabad et al. (1996) reported that anthracene was oligomerized by ion exchange to higher molecular weight aromatic compounds on clays that contained surface transition metals. This reaction was inhibited by addition of water, and air-drying of the soil appeared to cause polymerization of anthracene on the soil surface and decrease bioavailability.

Tillage and slurrying (slurrying is used in Japanese paddy fields to pulverize soil crusts under flooded conditions before rice is transplanted) increase the bioavailability of chemicals that have been sequestrated in soil aggregates. Slurrying rendered aged phenanthrene in soil available to a phenanthrene-degrading *Pseudomonas* sp. (White et al. 1999a). Without tillage, atrazine degradation declined and atrazine became unavailable because of soil sequestration (Radosevich et al. 1997). In a soil slurry, PAHs transferred to silicon oil (a water-immiscible, non-biodegradable, and biocompatible liquid) were efficiently degraded by PAH-degrading microbes compared to the slurry system without silicon oil (Villemur et al. 2000). Tillage also eases penetration of plant roots into soil, increasing root density and rendering chemicals more bioavailable.

Soil amendments also affect soil bioavailability of chemicals. Chemical fertilization of soil may induce an exchange of cations with heavy metals and make them bioavailable. Conversely, an increase in the ionic strength in soil solution would increase sorbed amounts of organic chemicals to soil, thereby reducing bioavailablity. Amending soil with manure increases microbial density and soil fauna, thereby increasing soil sorption (Weber and Weed 1974; Weed and Weber 1974); microbial degradation rates of xenobiotics also increase despite increased soil sorption (Alexander 1994). Addition of polyacrylamide gel to soil for prevention of soil erosion also reduced the formation of bound residues from 2,4-D and atrazine (Watwood and Kay-Shoemake 2000).

5 Bioassays to Measure Bioavailability

Bioavailability may be measured directly (chemical analysis) or estimated using bioassays or simulation models. Bioassays can provide insights into bioavailability, distribution and environmental behavior of contaminants, and their interrelation with organisms and soils. Although often laborious and time-consuming, bioassays must be used to verify results produced from chemical analyses, use of mathematic models, and similar methods.

Microorganisms, plants, and invertebrates are used as test organisms in bioassays. Most methods that utilize microorganisms to study bioavailability are performed at the community (consortia) level, and focus on the biodegradation of chemicals or emphasize functional effects on C, N, P, or S cycles. In plant bioassays, survival, growth rate, and seed formation of individual species have been examined. Achieving consistent results from plant bioassays is difficult because of the variability that exists in root uptake systems and physiognomic responses among species. Invertebrate bioassays focus on lethality, reproduction, and more recently, uptake, accumulation, and excretion mechanisms. Bioassays may address issues at the biochemical/biophysical, organismal, population, or community level. At the population and community levels, species composition and population dynamics have been investigated for dominant groups in Lumbricidae, Collembola, Acari, and Carabidae. Few studies of plant–microbe interactions, known to be important in ecosystems, have been performed (Eijsackers 1994).

5.1 Microorganisms

The extent to which degradation occurs is often used as an indicator of chemical bioavailability in soil. Extent and rates of microbial degradation are determined by incubating a chemical in soil samples with or without inoculation of microbes capable of degrading the studied chemical in intact soils, compared with corresponding sterile soils. The resulting biodegradation rate in soil can be compared with the biodegradation rate of the chemical under control conditions to elucidate a probable full bioavailability value for the chemical. Examples are phenanthrene mineralization in soil (Schwartz and Scow 1999), 2,4-D degradation by a *Pseudomonas* sp. in chlorite-2,4-D complexes (McGhee et al. 1999), the degradation of polyaromatic hydrocarbons by *Mycobacterium* sp. (Boldrin et al. 1993; Tiehm and Fritzsche 1995), and atrazine mineralization by bacteria (Radosevich et al. 1997). Chemical bioavailability in aged and field soils may be estimated using a chemical-mineralizing bacterium (Godskesen et al. 2005; Knightes and Peters 2003; Radosevich et al. 1997; Schwartz and Scow 1999). Mineralization has been measured using a ^{14}C respirometer capable of detecting off-gassing from ^{14}C-labeled chemicals (Reid et al. 2001) as was performed using phenanthrene.

Microorganisms sometimes degrade chemicals more extensively than amounts in the aqueous phase would suggest, although results of most mineralization studies

demonstrate that only a portion of chemicals dissolved in soil solution are subject to microbial degradation, e.g., 2,4-D (Burns 2001). There are several possible explanations for this:

(1) The equilibrium is constantly reestablished as the soluble substrate is metabolized.
(2) Microbial exudates alter pH at microsites.
(3) Microbes produce surfactants that render hydrophobic xenobiotics more water-miscible.
(4) Microbes produce extracellular enzymes that are capable of transforming chemicals sorbed to soil particles.
(5) Degradation of sorbed chemicals occurs on solid particle surfaces.

Reasons for degradation would differ depending on the chemical, soil, and how they interact.

Guerin and Boyd (1992) found that microorganism-dependent bioavailability of naphthalene sorbed to soil greatly differed between two bacterial species (*P. putida* ATCC 17484 and a gram-negative soil isolate, NP-Alk). One reason for this may be the difference in hydrophobicity of microbial cell surfaces. Chemical partitioning between humic substances and bacterial cells has been documented (Fujimura et al. 1995; Katayama et al. 1993). The cell surfaces of Gram-positive bacteria are generally more hydrophobic than those of Gram-negative bacteria (Rosenberg and Doyle 1990). In Gram-negative bacteria, cell surface hydrophobicity varies with the proportion of lipopolysaccharides that cover the outer membrane (Nikaido and Vaara 1985).

The bioavailability of a chemical can be measured directly by extracting bacterial cells from soil to determine degree of sorption. Direct extraction of bacteria from soil by dispersion and centrifugation suggested that about 30% of DDT, freshly added to soil, was available (Fujimura and Katayama 1997). However, microbial cells cannot always be separated from soil, particularly clayish soils.

Toxicity assays for soil microbial activities such as mineralization, nitrification, respiration, and substrate-induced respiration have been standardized in OECD and ISO guidelines (Table 3). Toxicity to microbial biomass is also available as an ISO guideline. Positive test results in these assays (e.g., Djomo et al. 2004; Juvonen et al. 2000) show that chemicals are bioavailable. Growth tests of various soil microorganisms to characterize bioavailability are also proposed (Hund 1997; Iannacone and Gutierrez 1999; Krogh et al. 2003; Lang et al. 1992; Martensson 1992; Megharaj et al. 1992; Yarden et al. 1993). The degree of bioavailability can be estimated only when the dose–response curve is provided for each compound. However, dose–response curves of soil activity differ among soils because of differences in the soil microbial communities they harbor. The dose–response curve obtained in a natural soil produces an integrated result of the responses of specific indigenous microorganisms and the bioavailability of the chemical in that soil. Thus, toxicity assays that rely on soil microbial activities are considered to be qualitative rather than quantitative when used to estimate bioavailability. Using artificial soil made up

Table 3 Bioassays with soil microorganisms for estimating bioavailability of chemicals

Organisms	Chemicals	Tests	Endpoints	References
Soil microorganisms	Contaminated soils	Litter bag test (incubation: 28 d)	Nitrification inhibition, N-mineralization inhibition	OECD216 (2000) and ISO14238 (1997)
Soil microorganisms	Contaminated soils	Nitrification test	Nitrification inhibition	ISO/DIS15685 (2001)
Soil microorganisms	Contaminated soils	Respiration test	Respiration inhibition	ISO17155 (1997)
Soil microorganisms	Contaminated soils	Substrate-induced respiration test	Respiration inhibition	ISO14240-1 (1997) and OECD217 (2000)
Soil microorganisms	Contaminated soils	Microbial biomass test (fumigation-extraction)	Biomass decrease	ISO14240-2 (1997)
Phenanthrene-degrading bacterium	Phenanthrene	Degradation test in aged soil	Mineralization rate	Schwartz and Scow (1999)
Pseudomonas sp.	2,4-D	Degradation in chlorite-2,4-D complexes	Mineralization rate	McGhee et al. (1999)
Mycobacterium sp.	Polyaromatic hydrocarbons	Degradation test	Mineralization rate	Tiehm and Fritzsche (1995) and Boldrin et al. (1993)
Atrazine-mineralizing isolate	Atrazine	Degradation test in aged soil	Mineralization rate	Radosevich et al. (1997)
Pseudomonas putida P106 *Rhodococcus erythropolis* NY05	Biphenils	Mineralization test	Initial mineralization rates	Feng et al. (2000)
Chlorella vulgaris (microalgae)	Chlorpyrifos, lindane	Growth test Metabolism test	Growth, photosynthesis, respiration	Iannacone and Gutierrez (1999)
Nitrosomonas, *Nitrosospira* (autotrophic nitrifier)	Organic contaminants in general	Growth test	Growth rate, microcolony formation combined with fluorescent in situ hybridization	Brandt et al. (2001) and Hesselsoe et al. (2001)
Dunaliella salina (halotolerant unicellular algae)	Herbicides	Plant indicator test	Changes in the chlorophyll content Photosynthesis inhibition	Yarden et al. (1993)

Table 3 (continued)

Organisms	Chemicals	Tests	Endpoints	References
Pseudomonas putida, *Bacillus cereus*	2-Naphthol, *p*-nitrophenol	Enzyme activity test Growth test	Dehydrogenase activity and growth (microcolony formation)	Gunkel et al. (1993)
Pseudomonas putida	Polyaromatic hydrocarbons	Solid-phase genotoxicity assay (rifampicin resistance) of chemicals sorbed to soil	Rate of spontaneous mutation	Alexander and Alexander (1999, 2000) and Alexander et al. (1999)
Vibrio fischeri	PAH	Microtox^{TM} test (bioluminescence test) Microbics, Carlsbad, CA.	Mortality	Haeseler et al. (1999)
Escherichia coli PQ37 (*sulA::lacZ*)	PAH	SOS Chromotest (genotoxicity test)	Beta-galactosidase activity, growth measured by the activity of constitutive alkaline phosphatase	Haeseler et al. (1999)
Genetically modified *Pseudomonas fluorescens* strain HK44	Naphthalene	Bioluminescence (*lux*) test	Bioluminescence intensity	King et al. (1990) and Sayler et al. (1999)
A immobilized recombinant bioluminescent bacterium	PAHs-contaminated soil	Biocensors (reporter gene system, *lac::luxCDABE*)	Biosensors	Gu and Chang (2001)
DDT-contaminated soil	DDT	Bacterial cells extraction test using dispersion and centrifugation method	Amount of DDT in cells	Fujimura and Katayama (1997)
Pseudomonas putida P. 106. *Rhodococcus erythropolis* NY05	Biphenyl	Biphenyl mineralization test	Initial mineralization ratio	Yucheng et al. (2000)

of a prescribed mixture of quartz sand, kaoline, and peat moss may give a standard dose–response curve; however, the microbial community in the artificial soil would be different from those of natural soils. More research into this area and these relationships is needed. Measures of bioavailability also differ between bioassays. For example, nitrification inhibition occurs at lower concentrations of chemical than N-mineralization inhibition (Somerville and Greaves 1987). The difference represents different susceptibility among microbial species. A model population of microorganisms is needed as is a model soil; these would be useful as standards when comparing different chemicals in different studies.

A solid-phase genotoxicity assay for chemicals sorbed to soil has been proposed as another way to measure bioavailability (Alexander and Alexander 1999, 2000; Alexander et al. 1999). In this assay, the mutation rate of a *P. putida* strain to rifampicin resistance is measured. In actual testing, the ratio of induced to spontaneous mutants correlated with the concentration of PAHs in soil. Similarly, the Microtox^{TM} test and SOS chromotest using *Vibrio fischeri* and *Escherichia coli* PQ37, respectively, have also been proposed (Haeseler et al. 1999); herein, aqueous solutions extracted from soil are used to conduct a dose–response study. The range of chemicals to which such testing may apply is narrow, although these tests are highly useful for PCBs and other hydrophobic pollutants.

The bioluminescence of genetically modified *Pseudomonas fluorescens* strain HK44 was used for detecting available naphthalene in soil (Sayler et al. 1999). The *P. fluorescens* parent strain was isolated from a site contaminated with PAHs. A plasmid that contains a salicylate inducible operon and the gene cassette for bacterial bioluminescence (*lux*) from *V. fischeri* was incorporated into the parent strain (King et al. 1990). When naphthalene is metabolized to salicylate, the *lux* gene is transcribed and expresses bioluminescence, which signals the presence of bioavailable naphthalene in soil. The bacterial luminescence test is also used in ecotoxicology, wherein toxicity is measured as inhibition of luminescence by *V. fischeri*. This test using *V. fischeri* strain NRRL B-11177 has been standardized, and is commercially available to measure water quality (ISO11348-1 1998). Soils and sediments require higher sensitivity than water for detection of similar endpoints. Studies using reporter gene technique have generally been applied to slurries or soil extracts, although Toba and Hay (2005) were able to detect 2,4-dichlorophenoxyacetate (2,4-D) in soil (as solid phase), using *Ralstonia eutropha* JMP 134-32, a luxCDABE-based 2,4-D whole cell bioreporter. Polaroid film is now used for detection of bioluminescence and provides improved accuracy by employing computer-assisted quantification (Tamminen and Virta 2007). This method allows in situ measurement of bioavailability of chemicals in soil. However, the methods have limitations for quantitative estimation of bioavailability. Bioluminescence strength is affected by oxygen concentration, temperature, and relative humidity (Sayler et al. 1999). Biosensors have also been constructed for detecting the toxicity of PAH-contaminated soil by using an immobilized recombinant bioluminescent bacterium GC2 that harbors the reporter gene system *lac::luxCDABE* (Gu and Chang 2001). Soil bioavailability of PAH (phenanthrene in this experiment) was shown by the decrease in bioluminescence, which was also

well correlated with the concentration of PAH in the soil aqueous phase. Surfactants increase the mass transfer rate of PAH from sorbed soil to aqueous phase, and therefore increase the bioavailability of PAH in soil; this was clearly observed with the immobilized recombinant bacterium GC2 system. The bioluminescence test also revealed that the surfactants Arkopal N-300 and Sapogenat T-300 decreased the toxicity of PAH in soil by increasing availability and enhancing the degradation rate (Tiehm et al. 1997). Two other tests are available: Lumistox™, a commercial toxicity test using luminescence or color (growth inhibition of *V. fischeri* luminescent bacteria), and MetPlate™ that employs enzyme inhibition in a bacterial strain by heavy metals in aqueous samples. Soil microbial activity influences the toxicity of contaminated soil in the Lumistox™ and MetPlate™ bioassays (Brohon and Gourdon 2000). Brouwer et al. (1990) made it possible to measure the toxicity of hydrophobic chemicals by placing a luminescent bacterium in direct contact with sediment. This luminescent bacterium was utilized to measure bioavailability of propiconazole (Tilt 250 EC), dimethoate (Roxion), and chlorsulfuron (Glean 20 DF) (Ahtiainen et al. 2003). Although not yet widely applicable, these methods require less effort and time to produce needed toxicity and degradation assay results.

5.2 Higher Plants

Uptake of chemicals by whole plant roots is relatively easy to study, and many such studies have been performed (Nash 1974). Usually, a radiolabeled xenobiotic is applied to soils and amounts absorbed into plants, and accumulated in plant parts are measured after a given time (Bromilow and Chamberlain 1995). Cultivation of plants on contaminated soil is one of the best ways to estimate bioavailability of chemicals in soil (Riviere 2000), but is laborious and time consuming. *Brassica napus* (Cruciferae), *Glycine max* (Leguminosae), *Kochia scoparia* (Chenopodiaceae), *Lotus corniculatus* (Leguminosae), and *Setaria faberi* (Gramineae) have been used to study bioavailability of atrazine, metolachlor, and pendimethalin (Anhalt et al. 2000).

Uptake of xenobiotics by plants growing in contaminated soil varies with species of plant and growth stage. For an example, cucurbits (Cucurbitaceae) took up more dieldrin and endrin than did 16 other families of arable crops; the highest uptake occurred in zucchini (Otani et al. 2007). Residues of aldrin and heptachlor in peanut, soybean, oat, barley, and corn seeds were directly related to the oil content of seeds (Nash 1974). The plant growth stage has been shown to influence plant residue concentration. In soybeans, residues increased during the active growth stage and then decreased after maturation of seeds. In cotton, however, the opposite trend occurs; the xenobiotic concentration increased during the seed maturation stage. During active plant growth the concentration of pesticides does not usually increase because growth rates outstrip pesticide sorption rates. In addition, metabolism in plants often exceeds plant sorption. The total amount of chemical absorbed by a plant, over its

growing season, may increase, particularly for persistent xenobiotics such as the chlorinated hydrocarbon insecticides. These complexities can be modeled to estimate amounts of chemicals in soil solution and plant growth rates. Weeks or months are required when field measurements of crop or plant bioavailability are undertaken. Such studies could be waived more often if adequate models were available to extrapolate results from laboratory to field.

Toxicity tests with endpoints focusing on germination and growth, in various monocotyledon and dicotyledon plants, have been proposed in OECD guidelines (OECD208 1984) and ISO guidelines (ISO11269-1 1993; ISO11269-2 1995) (Table 4). In such studies, calibration curves are built by incorporation of standard toxic compound dilutions in soil, and assays are performed to glean estimates for testing chemical concentrations in the same soil. If such data were combined with aquatic culture toxicity data (where bioavailability is 100%) one could estimate and relate soil solution concentrations to the corresponding toxic effects and also estimate the degree of sorption of the chemical.

Indirect biological measurements have been used to assess bioavailability. A bioassay using corn seedlings susceptible to a nematode was used to evaluate the effect of a nematicide (Pattison et al. 2000). The challenge in this system is to obtain a reliable dose–response curve.

5.3 Earthworms and Other Soil Fauna

Uptake and/or accumulation of various hydrophobic chemicals by earthworms (mostly *Eisenia* sp.) from soil has been successfully examined by many researchers (Table 5) using various methods (Lanno et al. 2004). Chemicals tested include organochlorine insecticides, other pesticides, PAHs, and chloroaromatic compounds (Table 5). Residue uptake was observed, even from long-term aged soils (Verma and Pillai 1991). For example, uptake by *E. foetida* was 30, 12, 34, and 20% for DDT, DDE, DDD, and total DDT residues, respectively, after aging for 49 yr. The availability of dieldrin, aged 49 yr in soil, was 28% (concentration in *E. foetida)* or 43% (assimilated amount) (Morrison et al. 2000).

Various other soil fauna can be used for toxicity testing. Toxicity tests, using the earthworm (*E. foetida*) and Collembola (*F. candida*), have been standardized by OECD guidelines (OECD207 1984) and ISO guidelines (ISO11267 1999; ISO11268-1 1993; ISO11268-2 1998; ISO11268-3 1999). For marine sediments, benthic organisms such as the Polychaete, *Nereis diversicolor*, and a netted dog whelk, *Hinia reticulata*, have been used as test organisms (Ruus et al. 2005). In OECD guideline tests, survival of earthworms is observed for 14 d. Other endpoints, in addition to mortality, have been used. These include weight loss, reduced burying ability, and curling. These endpoints were recorded as symptomatic effects characteristic of acetylcholinesterase inhibition in a bioavailability assay of diazinon in composts (Leland et al. 2001). Acetylcholinesterase and lactate dehydrogenase activities have been used as endpoints for soil isopods (Ribeiro et al. 1999). Schaefer (2003) also reported using avoidance as a test for the earthworm *E. foetida.*

Table 4 Bioassays that use higher plants for estimating bioavailability of chemicals

Organisms	Chemicals	Test	Endpoints	References
Various higher plants	Pesticides registered	Uptake test using ^{14}C-labeled compound	^{14}C incorporation into edible parts of plants	Many
Hordeum vulgare L. (Barley)	General contaminated soils	Inhibition of root growth	Root growth	ISO11269-1 (1993)
Various higher plants including monocotyledons[1] and dicotyledons[2]	General contaminated soils	Emergence and growth test	Emergence and growth	ISO11269-2 (1995)
Allium cepa (onions roots)	Metolachlor, atrazine, cyanazine and 2,4-D	Genotoxicity test using *Allium*-AA	Anaphase aberration frequencies	Kong and Ma (1999)
Tradescantia	Metolachlor, atrazine, cyanazine and 2,4-D	Genotoxicity test using *Tradescantia*-MCN *Tradescantia*-SHM	Micronucleus frequencies Pink mutation events	Kong and Ma (1999)
Allium cepa (onions)	Chlorsulfuron, metsulfuron (sulfonylurea herbicides)	Growth test	Growth rate	Junnila et al. (1994)
Wheat cv. Yecora	Imazapyr (herbicide)	Growth test	Growth rate	Vizantinopoulos and Lolos (1994)
Allium cepa (onion roots)	Chlorpyrifos, lindane	Growth test	Root growth inhibition	Iannacone and Gutierrez (1999)
Avena sativa (oats)	Cyanazine	Growth test	Growth rate	Blanco et al. (1994)
Cassia obtusifolia (sickle pod)	Chlorimuron (herbicide)	Growth test	Root length	Vencill and Banks (1994)
Lemna minor (duckweed)	Folpet (fungicide)	Growth inhibition test	Growth inhibition and chlorophyll content	Teisseire et al. (1997)
Oat bioassay (*Avena sativa* L.)	Cyanazine (herbicide)	Growth test	Phytotoxicity	Garcia Blanco et al. (1994)
Sicklepod	Chlorimuron	Root elongation test	Sicklepod root length	Vencill and Banks (1994)
Allium, *Tradescantia*	Metalochlor, atrazine, extrazine and 2,4-D	Allium root anaphase aberration (Allium-AA), *Tradescantia* micronucleus (Trad-MCN), *Tradescantia* stamen hair mutation (Trad-SHM)	Genotoxicity Micronuclei frequency in the Trad-MCN test, pink mutation events in the Trad-SHM test anaphase aberrations (AA), in the Allium-AA test	Kong and Ma (1999)

Table 4 (continued)

Organisms	Chemicals	Test	Endpoints	References
Onion	Chlorsulfuron metsulfuron	Onion growth test	Number and height of viable plants per pot, the number of leaves, and the fresh and dry weight of aerial shoots and roots per pot	Junnila et al. (1994)
Corn	Imazaquin	Corn "HyPerformer HS 9773" 7-d shoot height	Corn shoots heights measured from the soil line to the tip of the longest leaf 7 d after planting	Baughman and Shaw (1996)
Rice	Flumetsulam	Rice root length bioassay	Rice root length	Shaw and Murphy (1997)
Lentil	Metsulfuron	Lentil bioassay	Lengths of roots and shoots	Szmigielska et al. (1998)

[1] Monocotyledons: *Secale cereale* L. (Rye), *Lolium perenne* L. (Ryegrass, perennial), *Oryza sativa* L. (Rice), *Avena sativa* L. (Oat, common or winter), *Triticum aestivum* L. (Wheat, soft), *Hordeum vulgare*, L. (Barley, spring or winter), *Sorghum bicolor* (L.) Moench (Sorghum, common), *Zea mays* L. (Sweetcorn).
[2] Dicotyledons: *Sinapis alba* (Mustard, white), *Brassica napus* (L.) ssp. Napus (Rape), *Raphanus sativus* L. (Radish, wild), *Brassica rapa* spp. (DC.) Metzg (Turnip, wild), *Brassica campestris* L. var chinensis (Chinese cabbage), *Trifolium ornithopodioides* (L.) (Birdsfoot fenugreek), *Lactuca sativa* L. (Lettuce), *Lepidium sativum* L. (Cress, garden), *Lycopersicon esculentum* Miller (Tomato), *Phaseolus aureus* Roxb. (Bean).

Table 5 Bioassays that use soil fauna for estimating bioavailability of chemicals

Organisms	Chemicals or Substances	Tests	Endpoints	References
Earthworms (mostly *Eisenia* spp. or *Lumbricus* spp.)	Chlorobenzenes, chlorophenols, DDT, dieldrin, heptachlor, HCH, carbofuran, aldicarb, polychlorodibenzo-*p*-dioxins, phenanthrene, anthracene, fluoranthene, pyrene, atrazine, phenanthrene, naphthalene, telodrin, polychlorinated biphenyls, hexachlorobenzene, nonylphenol, 4-(2-dodecyl)-benzene sulfonate	Uptake test	Concentration in the tissues	Belfroid et al. (1994), Beyer and Gish (1980), Briggs and Lord (1983), Davis (1971), Edwards and Jeff (1974), Heida et al. (1986), Jager et al. (2005), Kelsey and Alexander (1997), Knuutinen et al. (1990), Stenersen and Oeien (1980), Tang et al. (1998), van Gestel and Ma (1988), Verma and Pillai (1991) and Maenpaa and Kukkonen (2006)
Eisenia fetida (earthworm)	DDT, DDE, DDD, dieldrin	Uptake test	Concentration in the tissues	Morrison et al. (2000)
Eisenia fetida (earthworm)	Various contaminated soils	Toxicity test (Acute)	Mortality	ISO11268-1 (1993) and OECD207 (1984)
Earthworms	Polycyclic aromatic hydrocarbons	Toxicity test	Mortality	Loehr (1996)
Eisenia fetida (earthworm)	Malathion	Toxicity test	Mortality	Kuperman et al. (1999)
Eisenia fetida (earthworm)	Imidaclorprid, RH-5849	Genotoxicity	Genotoxicity (sperm deformity), mortality, Earthworm comet assay	Zang et al. (2000)
Eisenia fetida (earthworm)	Various contaminated soils	Reproduction test	Number of earthworms and cocoons	ISO11268-2 (1998)
Eisenia fetida *Eisenia andrei* (earthworm)	Crude oil, 2,4,6-trinitrotoluene	Behavior test	Avoidance, mortality, reproduction	Loureiro et al. (2005) and Schaefer (2003)

Table 5 (continued)

Organisms	Chemicals or Substances	Tests	Endpoints	References
Eisenia foetida Savigny (earthworm)	Soil amended with Diazinon-spiked peat compost	Toxicity study	Mortality and symptomatic effects characteristic of acetylcholinesterase inhibition (wt loss, reduced burying ability, and curling)	Leland et al. (2001)
Diabrotica virgifera (Mexican corn rootworm)	Carbofuran	Toxicity test	Mortality	Sutter and Steward (1995)
Enchytraeus albidus (Oligochaeta)	Malathion	Reproduction test	Reproduction effects (no. adults, no. cocoons)	Kuperman et al. (1999)
Folsomia candida (Collembola)	Contaminated soils	Reproduction test	Number of adults	ISO11267 (1999)
Folsomia candida (Collembola)	Dinoseb	Toxicity test	Reproduction, mortality, induction of heat shock protein (hsp70), response of metabolism biomarkers (glycogen, total protein content)	Staempfli et al. (2002, 2007)
Panagrellus redivivus (nematode)	Chlorpyrifos, lindane	Toxicity test Growth test Enzyme test	Mortality, Growth and maturation, Inhibition of alkaline phosphatase activity	Iannacone and Gutierrez (1999)
Meloidogine javanica (nematode)	Ethoprofos	Toxicity test	Mortality	Karpouzas et al. (1999)

Table 5 (continued)

Organisms	Chemicals or Substances	Tests	Endpoints	References
Larvae of *Chironomus riparius* (Insecta)	Nonylphenol, 4-(2-dodecyl)-benzene sulfonate	Toxicity test	Lethal water concentration, Lethal body residue	Maenpaa and Kukkonen (2006)
Helix aspersa aspersa, Helix aspersa maxima (snails)	Phenanthrene, trichlorophenol, pentachlorophenol	Growth test	Growth inhibition	de Vaufleury and Bispo (2000)
Xenopus laevis (anuran)	Oil	Teratogenesis assay-Xenopus (FETAX)-frog embryo	Mortality, Teratogenicity and growth inhibition.	Prati et al. (2000)
Ceridaphnia dubia (water flea)	Hydrocarbons	Toxicity test for water extracts from soils	Mortality	Loehr (1996)
Termites (*Reticulitermes flavipes* Kollar)	Isofenphos, bifenthrin, chlorpyrifos, cypermethrin, fenvalerate, permethrin	Behavior test	Behavior: Termite tunneling	Gold et al. (1996)
Rats (species not mentioned)	PAHs	Genotoxicity test, induction of 7-ethoxyresorufin *O*-deethylase (EROD) activity	EROD activity measurement, DNA adducts	Fouchecourt et al. (1999)

Although toxicity tests using insect–soil systems have traditionally been performed as summarized by ISO and OECD guidelines, the toxicity tests should be considered as qualitative rather than quantitative, because chemical and soil interactions are not constant over the entire experimental period. Recently, changes in bioavailability of xenobiotics during the discrete test period have been documented; examples are aging (decrease in bioavailability) of creosote constituents (Charrois et al. 2001) and PAHs (Chung and Alexander 1999) in soils. Charrois et al. (2001) assessed the influence of dichloromethane-extractable organic compounds on bioavailability in soil by observing the duration of earthworm survival. A dose–response curve can be obtained for quantitative evaluation by using an artificial soil and a single representative soil fauna (ISO11268-1 1993; ISO11268-2 1998; ISO11268-3 1999).

The toxicity to mammals of xenobiotics in soil has been examined using rats. Exposure to PAH and PCB residues in a polluted soil induced mono-oxygenase enzymes in liver and lungs of rats. Soil properties, especially the OM content and particle size distribution, influenced bioavailability (Billeret et al. 2000). In another report, Fouchecourt et al. (1999) observed three endpoints in tested rats: (1) presence of PAHs in liver and lung; (2) induction of cytochrome P450-dependent monooxygenases and 7-ethoxyresorufin-*O*-deethylase (EROD) activity in liver and lung; and (3) an increase in DNA adducts. Bordelon et al. (2000), in a genotoxicity study, dosed rats with coal tar-contaminated soils and showed that toxicity was reduced by sorption.

There are many bioassays for estimating bioavailability of chemicals in soil. However, very few of these have been standardized. One exception is the confined rotational crop study that utilizes radiolabeled pesticide, a study required for pesticide registration.

6 Chemical Methods to Measure Bioavailability

6.1 Chemical Extraction Methods

Many experiments have been conducted to compare measures of chemical bioavailability resulting from bioassays vs. chemical extraction methods. Exhaustive solvent extraction is used to measure the total amount of extractable xenobiotics. Such rigorous extraction methods, when compared to mechanisms of biological uptake, tend to overestimate bioavailability. For example, Reid et al. (2000) reported that a dichloromethane-Soxhlet extraction and a butanol-shaking extraction both overestimated phenanthrene bioavailability in soils by an average of more than 60%. Thus, less exhaustive techniques have been sought to better emulate the bioavailable pool in soil.

Sequential and/or selective extraction procedures from matrices have been developed for estimating bioavailability of metals. The Standards, Measurements and Testing Programme (formerly BCR) of the European Commission proposed a three-step sequential extraction procedure for such sediment analysis: 0.11 M CH_3COOH

solution (exchangeable fraction), 0.5 M $NH_2OH.HCl$ solution (pH = 1.5) (fraction bound to hydroxides of Fe and Mn) and then 25.0 ml of 1.0 M $CH_3COO\ NH_4$ solution (pH = 2.0), after digestion with 30% H_2O_2 solution (fraction bound to OM and sulphide) (Rauret et al. 1999). This method has been used successfully for measurement of metal availability (Almeida et al. 2005).

Such sequential and/or selective methods are under development (Dean and Scott 2004) for organic compounds, including Priority Organic Pollutants (POPs). The following extractants have been used successively for organic pesticides: 0.01 M $CaCl_2$ for aqueous phase concentrations, followed by acetonitrile and 1 M HCl for sorbed phase concentrations. Carrizosa et al. (2000) estimated bentazone availability in a bentazone-clay system by extraction with a $CaCl_2$/methanol solution. Using this same extractant, Koskinen and his colleague successfully estimated bioavailability of atrazine (Barriuso et al. 2004) and simazine (Regitano et al. 2006) in aged soil (corresponding to bacterial mineralization). Cox et al. (1998) reported that, in aged soils, the fraction of imidacloprid in the initial extract with 0.01 M $CaCl_2$ decreased, whereas the fraction increased in subsequent extracts with acetonitrile and HCl. However, this sequential extraction has not been compared with any bioassay results.

Mild extraction methods that mimic chemical uptake by soil organisms are more suitable for estimating bioavailability. For example, a mixture of water and hexane, which is water immiscible, may better emulate natural bioavailability because the mixture may not penetrate pores of amorphous OM (Schwartz and Scow 1999). Extraction with hydroxypropyl-beta-cyclodextrin, a macromolecule with a hydrophilic exterior and hydrophobic cavity, may offer an improvement because its hydrophobic cavity may trap organic compounds in soil solution (Dean and Scott 2004; Wang et al. 1998). To determine suitability, results of mild extractions must be compared with bioassay results. Using aqueous hydroxypropyl-*p*-cyclodextrin extraction, availability of PAHs to earthworms (*L. rubellus*) was overestimated by twofold, but use of this solvent successfully predicted availability to the mineralizing microbe (*Pseudomonas* sp.) (Hickman and Reid 2005).

Extraction with supercritical carbon dioxide is also proposed for use in estimating bioavailability (Kreitinger et al. 2007a, b; Nilsson et al. 2002, 2006). Extraction using supercritical carbon dioxide under mild conditions (60 min, 40°C and 120 bar) resulted in the removal of 54% of PCB from naturally contaminated limnic sediment. These results agreed well with the 60% PCB-bioavailability value obtained in a bioassay with chironomid larvae (Nilsson and Bjorklund 2005; Nilsson et al. 2002, 2006). The mild supercritical fluid extraction of PCB from sediment also gave a good estimation and was compatible with amounts of PCB bioavailable to eels (*Anguilla anguilla*) in sediment (Nilsson and Bjorklund 2005; Nilsson et al. 2002, 2006). The mild supercritical fluid extraction under a different condition (60 min, 50°C and 350 bar) also gave a good estimate of PCB bioavailability to earthworms (*E. foetida*) in soil (Hallgren et al. 2006; Nilsson et al. 2002).

Several reports with promising results have been published on this relatively new line of research, mild extraction (Table 6). Methods regarded to be mild included sequential solvent extraction, solid phase extraction, and supercritical fluid CO_2

Table 6 Selective extraction methods for estimating the bioavailablity of xenobiotics, and bioassays used for verification of bioavailability

Extractants or extraction specifications	Xenobiotic(s)	Bioassay for verification purposes	References
Sequential extraction with *n*-butanol, ethyl acetate, and propanol	Fluoranthene, pyrene	Uptake amount by the earthworm *Eisenia foetida*	Tang and Alexander (1999)
Sequential extraction with *n*-butanol, ethyl acetate, and propanol	Anthracene	Amount retained by wheat and barley	Tang and Alexander (1999)
Sorption to C_{18} membrane disks placed in suspensions of soils and extraction with aqueous tetrahydrofuran or the modified methods	DDT, DDE, DDD, explosives (TNX, MNX)	Uptake amount by the earthworm *Eisenia foetida*	Tang et al. (1999) and Zhang et al. (2006)
Hexane/water	Phenanthrene	Bacterial degradation kinetics	Schwartz and Scow (1999)
Solid-phase extraction for 168 h, followed by 3 h of persulfate oxidation	Total petroleum hydrocarbon	Biodegradation in slurry reactor	Cuypers et al. (2001)
Persulfate oxidation for 3 h	PAHs	Biodegradation in soil	Cuypers et al. (2000)
Acetonitrile	Trinitrotoluene	Activities of nitrification, nitrogen-fixation, and dehydrogenase	Gong et al. (1999)
Supercritical fluid CO_2 (120 bar, 50°C)	PAHs	One year bioremediation, acute toxicity to *Eisenia foetida* and *Enchytraeus albidus*	Hawthorne and Grabanski (2000) and Hawthorne et al. (2005)
Supercritical fluid CO_2	PAHs	Accumulation in earthworm *Aporrectodea caliginosa*	Kreitinger et al. (2007b)
Supercritical fluid CO_2 (400 bar, 40°C)	PCBs	Chironomid larvae in a limnic sediment	Nilsson and Bjorklund (2005) and Nilsson et al. (2006)
Hydroxypropyl-beta-cyclodextrin (HPCD)	PAHs (phenanthrene, pyrene, and benzo(*a*)pyrene) PCB phenols	Biodegradation	Allan et al. (2006), Cuypers et al. (2002), Doick et al. (2005), (2006), Fava et al. (2003), Papadopoulos et al. (2007) and Reid et al. (2000)

Table 6 (continued)

Extractants or extraction specifications	Xenobiotic(s)	Bioassay for verification purposes	References
Polymeric TENAX^{TM} beads	Phenanthlene PCBs	Biodegradation in soil Accumulation in benthivorous carp (*Cyprinus carpio*)	Braida et al. (2004) and Moermond et al. (2004)
Polymeric TENAX^{TM} beads	PCBs, DDE, permethrin, chlorpyrifos, and phenanthrene	Accumulation in freshwater oligochete (*Lumbriculus variegatus*)	You et al. (2006, 2007a. b)
30-μm poly(dimethylsiloxane) -coated solid-phase microextraction fibers	Hexachlorobenzene, Telodrin, PCBs	Accumulation in earthworms (*Eisenia andrei* or *Aporrectodea caliginosa*)	Van der Wal et al. (2004)
30-μm poly(dimethylsiloxane) -coated solid-phase microextraction fibers	PCBs, DDE, permethrin, chlorpyrifos, phenanthrene	Accumulation in freshwater oligochete (*Lumbriculus variegatus*)	You et al. (2006, 2007a, b)
Supelpak-2, an Amberlite XAD-2 macroreticular styrene-divinylbenzene copolymerresin	PAHs	Accumulation in tall fescue (*Festuca arundinacea*), annual ryegrass (*Lolium multiflorum*), yellow sweet clover (*Melilotus officinalis*)	Parrish et al. (2005)
Water	Propyzamide, simazine, linuron, atrazine, diuron, terbutryn	Plant toxicity bioasay	Stalder and Pestemer (1980)
Methanol or methanol:water (1:1)	Pentachlorophenol, Butachlor, myclobutanil	Uptake by *Eisenia foetida* or *Eisenia andrei*	Hu et al. (2005a) and Yu et al. (2005)
0.01 M $CaCl_2$ and aqueous methanol	Atrazine	Bacterial mineralization	Barriuso et al. (2004)

extraction. Estimation of bioavailability for organic chemicals can be achieved by careful selection of solvent, although, in the future, the range of compounds studied should be expanded beyond those in the non-polar category, which constitute the great majority investigated thus far. Further research to compare an array of mild extraction techniques is needed, because differences in estimated bioavailability have been reported among techniques including: solid-phase microextraction, semipermeable membrane devices, leaching with various solvent mixtures, testing effects of additives, and sequential leaching of PAHs in soil (Bergknut et al. 2007).

Results of mild extraction may be confounded by apparent xenobiotic aging (Kelsey and Alexander 1997; Kelsey et al. 1997). As xenobiotic–soil contact time increased, sequential extraction with methanol:water (1:1), 1-butanol and finally dichloromethane-Soxhlet extraction showed changes in extracted amounts of [^{14}C]pyrene. Fractions extracted in methanol:water and 1-butanol decreased during the 24-week incubation. A comparison between microbial mineralization and the amount of ^{14}C activity extracted by the sequential extractants, methanol:water significantly underestimated the mineralized fraction, whereas, 1-butanol overestimated the mineralization (Macleod and Semple 2000). These findings suggest that frequent mild extractions may be required to accurately track changes in bioavailability with time.

Humans are primarily exposed to chemicals in soil through soil ingestion. Such soil ingestion rates vary widely. Values used in exposure estimates by the USEPA are 200-mg soil/d for children (age 1–6) and 100-mg soil/d for others (US Environmental Protection Agency 1991, 1997). The human gastrointestinal tract has a range of pH values: 1.2–1.4 in the stomach and 6.9–8.6 in the small intestine. When extracting soil to simulate such conditions, both acidic (0.1 M NaCl, 0.1 M HCl, 0.01 M NH_4Ac, pH = 1.0) and neutral extractants (0.2 M NaCl, pH = 6.7) are used. Less naphthalene was desorbed by the acidic (<3% of 20 μg/g) than neutral extractant (<30% of 20 μg/g) (Jin et al. 1999). A more elaborate extraction method has been employed with pH-adjusted saline utilizing pepsin (1% wt/v; gastric fluid), pH-adjusted saline (1% wt/v) with pancreatin (3% wt/v), amylase (1% wt/v), and bile salts (0.075% wt/v) (intestinal fluid) to aged or spiked soils contaminated with the following pesticides and other substances in soil: POPs (lindane, endosulfan I, endrin, DDE, DDD, and endosulfan II), phenols (cresol, trichlorophenol, and pentachlorophenol), and base neutral compounds (hexachloroethane, acenaphthene, dibenzofuran, fluorene, and hexachlorobenzene) (Scott and Dean 2005). The extracted amounts of the xenobiotics were always less when using gastric fluid (less than several %) vs. intestinal fluid (less than 25%). This indicated that over 75% of the contaminants in soil would not be available for absorption in the human gastrointestinal tract.

6.2 Estimations Based on K_p or K_{oc}

Some success has been reported for estimating bioavailability using sorption coefficients. Zhao and Voice (2000) measured the true rate of biodegradation in the liquid-phase with both liquid- and sorbed-phase naphthalene. The phytotoxicity of

atrazine and simazine correlated with K_p values in nine Danish soils (Streibig 1979). However, as previously discussed, K_p values vary widely under real soil conditions.

Efforts are underway to more accurately determine K_p values. K_p values have been measured by a batch soil-slurry shaking method as described in OECD guideline 106 (OECD106 2000), wherein a water phase (often 0.01 M $CaCl_2$) is added to obtain lower dry soil to water wt ratios (usually 1:5). However, K_p values are overestimated by the batch soil-slurry shaking method when soil to water ratios are low, probably because soil sorption sites are not all exposed to the chemicals under test conditions; this does not occur in the actual soils except in rice paddy fields, wherein such slurry conditions may exist. It is desirable to measure sorption using soil with actual water content and without shaking to properly determine K_p or K_{oc} values. Use of low-density (i.e., 0.25 g mL^{-1}) supercritical carbon dioxide allowed extraction of atrazine, linuron, and triadimefon from the water phase in field-moist or unsaturated soils; this allowed estimation of sorption coefficients (K_p) under realistic soil moisture conditions without shaking (Berglof et al. 2000a, b; Rochette and Koskinen 1996,1998). Centrifugation is another method for measuring the sorption of chemicals in soils with high soil to water ratios (Walker and Jurado-Exposito 1998). This method has been applied to sorption measurements with isoproturon, diuron (Waker and Jurado-Exposito 1998), and metsulfuron-methyl (Waker and Jurado-Exposito 1998; Kah and Brown 2007), imidacloprid, carbofuran (Yazgan et al. 2005), 2,4-D, dicamba, fluroxypyr, fluazifop-P, and flupyrsulfuron-methyl (Kah and Brown 2007) in soils.

Radosevich et al. (1997) measured atrazine concentration in soil solutions by high performance liquid chromatography, after the solutions were retrieved by centrifugation. Observed microbial degradation rates in the soils were 25–227 times slower than expected from atrazine degradation rates in solution. This result suggests that a significant fraction of the solution-phase atrazine was sequestered from microbial attack, and that the unavailable fraction increased with soil residence time.

Hysteretic desorption may skew estimation of bioavailability when based on K_p or K_{oc} values. Leaching for imidacloprid was greatly overestimated, as compared to K_{oc}, when determined at field application rates (Cox et al. 1997).

7 Simulation Modeling to Estimate Bioavailability

Factors such as BSAF (Eq. 9) and TSCF (Eq. 7) have been proposed to predict the bioavailability of chemicals in soil. Most concentration coefficients assume an implicit equilibration among chemical, organisms, and the soil. Whereas equilibrium factors may explain chemical bioavailability in some cases, such as desorption-resistant phenanthrene to oligochaete *Ilyodrilus templetoni* (Lu et al. 2003), biotic uptake is actually a time-dependent non-equilibrium process. Therefore, time-course modeling is essential to accurately describe the dynamics of bioavailability. Such modeling must account for differences among organisms, i.e., microorganisms vs. plants and animals. In microorganisms, modeling of degradation kinetics has been used to estimate bioavailability. Only chemical uptake has been modeled in plants and animals.

Bioassays, of course, give direct measurements of bioavailability, although not every variable can be tested. Models are needed that are capable of estimating chemical bioavailability by extrapolating results of bioassays.

7.1 Degradation Models: Bioavailability to Microorganisms

The extent and rate of microbial degradation of a chemical in soil depends on the degree to which the chemical is bioavailable to microorganisms. The best description of such degradation is usually expressed by first-order kinetics, and sometimes by zero-order kinetics. Both are derived from the Monod equation:

$$-\frac{dC_w}{dt} = \mu_{max}\frac{C_w}{Y(K_s + C_w)}X \tag{17}$$

where

$-\frac{dC_w}{dt}$ = substrate decomposition rate
X = microbial density
C_w = substrate concentration in soil solution
t = time
K_s = half-saturation constant
Y = growth yield
μ_{max} = maximum specific growth rate.

Generally, nutrient deficiency limits soil microbial density, unless the chemical serves as a carbon source for microorganisms.

If $C_w \ll K_s$, Eq. (17) can be simplified to reflect first-order kinetics:

$$-\frac{dC_w}{dt} = \mu_{max}\frac{C_w}{YK_s}X \approx kC_w \quad \text{where} \quad k = \frac{\mu_{max}X}{YK_s} \tag{18}$$

In this situation, the microbial biomass is assumed to be nearly constant because of low substrate concentration (low C_w). Because microbial activity is dependent on both soil temperature and humidity (Boesten and van der Linden 1991), k also can be expressed as follows:

$$k = f_T f_0 k_{ref} \tag{19}$$

where

k= first-order degradation rate in soil
f_T = a factor for the influence of soil temperature
f_o = a reduction factor for soil humidity
k_{ref} = k at reference conditions.

Temperature dependence is described by the Arrhenius equation: the value of f_T doubles when a temperature increase of 10°C from the reference conditions occurs.

The reduction factor for soil moisture content is described as follows (Boesten and van der Linden 1991):

$$f_0 = \min\left[1, \left(\frac{\theta}{\theta_{\text{ref}}}\right)^B\right] \tag{20}$$

where

min= "the minimum of"
θ = the volumetric water content
θ_{ref} = the θ at the reference conditions
B = a constant.

Zero-order kinetics has been observed when 4,6-dinitro-2-methylphenol was incubated in soil at a concentration range from 5 to 2500 μg/kg-soil, glyphosate at 90 mg/kg-soil and maleic hydrazide at 120 mg/kg-soil, respectively (Alexander 1994). A chemical concentration much greater than K_s ($K_s \ll C_w$) induces zero-order kinetics when growth of degrading microorganisms is not significant in soil. It has been suggested that such growth is suppressed by oxygen limitation, deficiency of essential nutrients, or a too-large degrading microbial biomass. Hydrophobic chemicals may display zero-order kinetics because solubilization/desorption is rate-limiting for microbial degradation.

The Monod equation does not always provide a good fit when depicting degradation of low concentrations of chemicals (Alexander 1994). Logistic and logarithmic models are better in such cases, particularly when testing is conducted in aqueous environments.

Many soil studies have demonstrated that very tiny amounts of chemicals persist in soil, even after long intervals. In such cases, residual chemicals may be sequestered or sorbed at soil sites inaccessible to microorganisms.

Sorption of chemicals in soil is often incorporated into first-order kinetic equations:

$$-\frac{dC_w}{dt} = \frac{k}{1 + W_c \cdot K_p} C_w \tag{21}$$

where

W_c = soil to water ratio
K_p = equilibrium on a basis of soil

K_p values are affected by various factors. If the amount of chemical bioavailable to cells is known, the first-order kinetic equation may be further developed:

$$-\frac{dC_w}{dt} = \frac{k}{1 + (S_w/S_m)} C_w \tag{22}$$

where

S_w = substrate amount in solution or soil
S_m = chemical amount in cells.

Attempts to estimate such quantities of bioavailable chemicals in cells have utilized centrifugation-extraction of bacterial cells (Fujimura and Katayama 1997) and bioluminescent bacteria (Sayler et al. 1999).

There are examples in which microbial populations have directly used chemicals sorbed to soil particles (Alexander 1994). A microbial consortium mineralized biphenyl sorbed to polyacrylic beads faster than the compound's desorption rate. There was no excretion of biosurfactant. These results suggested the direct utilization of biphenyl by attachment of microorganisms to the beads (Calvillo and Alexander 1996). Some of PAH-degrading bacterial strains, three strains of *Burkholderia* sp., a strain of *Delftia* sp., and a strain of *Sphingomonas* sp., degraded phenanthrene sorbed to humic acid, but other isolated PAH-degrading bacteria did not. The sorbed-PAH-degrading bacteria were able to directly access phenanthrene sorbed by humic acids, and did not rely on desorption for substrate uptake (Vacca et al. 2005). However, direct utilization is rare, and in most cases microorganisms use only a chemical dissolved in the soil solution.

Where chemicals are sorbed or sequestered in soil, first-order kinetics are used to describe simultaneous multiple degradation reactions with different rates. Such kinetics also applies to the presence of multiple degrading microorganisms. Kinetics, using compartment models for sorbed chemicals, may be expressed as follows (Ogram et al. 1985):

$$-\frac{\partial C}{\partial t} = k_{\mathrm{w}} C N_{\mathrm{w}} W + k_{\mathrm{sw}} C N_{\mathrm{s}} m \tag{23}$$

$$N_{\mathrm{s}} = K_{\mathrm{b}} \cdot N_{\mathrm{w}} \tag{24}$$

where

N_{s} = bacterial cell number sorbed to unit weight of soil
N_{w} = bacterial cell number present in unit volume of soil solution
K_{b} = sorption coefficient of bacterial cells
W = volume of soil solution
m = weight of soil
k_{w} = degradation rate constant in soil solution by cells in soil solution
k_{sw} = degradation rate constant in soil solution by cells sorbed to soils.

This kinetic equation can be applied to various cases, e.g., as a kinetic model for a chemical present at both accessible and inaccessible soil sites.

In other cases, two-compartment models provide a better fit for data. For 2,4-D degradation by a *Pseudomonas* sp., where the rates of sorption—desorption of 2,4-D and bacteria were high, only 2,4-D in solution was degraded by bacteria that were both attached to the soil and suspended in soil solution (Ogram et al. 1985). A two-compartment model was also used to determine the availability of atrazine and terbuthylazine to the *Pseudomonas* sp. strain ADP. In this experiment, desorption was the rate-limiting step in mineralization of terbuthylazine (Jacobsen et al. 2001). There was a decrease in the degradation rate over time, either because imidacloprid's rate of desorption from or diffusion out of soil particles was slower than the degradation rate in aqueous phase (Koskinen et al. 2001).

It is also possible to incorporate other mechanisms into a model relating to chemical bioavailability. Changes in the concentration of soil solution chemicals occur by many processes: macropore dispersion, advection, dissolution, biodegradation, intraparticle diffusion, and volatilization (Ghoshal and Luthy 1996; Ghoshal et al. 1996; Ramaswami et al. 1997; Ramaswami and Luthy 1997a, b). The change in concentration of chemicals in soil solution can be expressed as functions of these processes in the following differential form:

$$\frac{\partial C}{\partial t} = D_{\mathrm{x}} \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} + k_{\mathrm{la}}^{\mathrm{Chem}} \left[C_{\mathrm{eq,t}} - C \right] - k_{\mathrm{bio}} C + k_{\mathrm{la}}^{\mathrm{soil}} \left[C_{(r=R,t)} - C \right] - k_{\mathrm{la}}^{\mathrm{wg}} \left(C - \frac{C_{\mathrm{g}}}{H'} \right) \tag{25}$$

where

C = concentration of chemical in macropore with location x and time t
D_{x} = dispersion coefficient
v = pore water velocity
k_{bio} = first-order biodegradation rate constant
$k_{\mathrm{la}}^{\mathrm{chem}}$ = mass transfer coefficient
$k_{\mathrm{la}}^{\mathrm{soil}}$ = mass transfer coefficient
$k_{\mathrm{la}}^{\mathrm{wg}}$ = mass transfer coefficient
H' = equilibrium constant (dimensionless Henry's law coefficient)
$C_{(r=R,t)}$ = the concentration of chemicals at the surface of spherical soil aggregates.

In the right side of the equation, the six terms express macropore dispersion, advection, dissolution, biodegradation, intraparticle diffusion, and volatilization of chemicals, respectively. In these processes, the intraparticle diffusion of chemicals is the major rate-limiting step of chemical transfer. The intraparticle diffusion of chemicals is modeled with an assumption that the soil particles are spherical (Scow 1993):

$$-\frac{\partial Ce}{\partial t} I = D_{\mathrm{eff}} \left(\frac{\partial^2 Ce}{\partial r^2} + \frac{2}{r} \frac{\partial Ce}{\partial r} \right), \; 0 < r < R_{\mathrm{s}} \tag{26}$$

$$I = 1 + \frac{\rho_{\mathrm{s}} \cdot K_{\mathrm{p}}}{\varepsilon} \tag{27}$$

where

I = retardation factor
ρ_{s} = bulk density of soil aggregate dried
ϵ = pore ratio of soil
R_{s} = diameter of soil aggregate

The effective diffusion coefficient D_{eff} is defined as follows:

$$D_{\text{eff}} = D_{\text{m}} n^2 / \left[\varepsilon + K_{\text{p}} \rho_{\text{s}} (1 - \varepsilon)\right] \tag{28}$$

where

D_{m} = molecular diffusion coefficient
ϵ = pore ratio of soil
ρ_{s} = density of dry solid material.

A model linking sorption with desorption, biodegradation, and mineralization, in many instances, accurately predicted atrazine mineralization in soil by three atrazine-degrading bacteria (*Pseudomonas* sp. strain ADP, *A. radiobacter* strain J14a, and *Ralstonia* sp. strain M91-3) that utilized atrazine as a sole N source; there was a presumption that atrazine degradation only occurred in soil solution (Park et al. 2003).

Models to estimate bioavailability can be constructed that follow the above-mentioned principles. Several leaching models that take account of microbial degradation have been introduced for regulatory purposes: CHEMRANK, CMLS, PATRIOT, PRE-AP, PRZM2, GLEAMS, CALF, LEACHM, PELMO, etc. (Cleveland 1996). In these models, microbial degradation is considered to occur only for chemicals dissolved in the soil solution, and the degradation rate is described with first-order kinetics. In reality, many factors affect the kinetics of degradation. Therefore, the parameters used in the models, as determined by laboratory experiments in defined homogenous systems, often do not match field conditions; results predicted by the models may, therefore, deviate substantially from actual leaching experience. The models leave certain factors out: diffusion of chemicals into macropores, multiple sorptions of chemicals to soil constituents, presence of other organic substances, presence of multiple degrading microorganisms, the O_2 supply, the supply of nutrients and growth factors, effects of predatory protozoa that consume degrading microorganisms, characteristics of microcolonies of degrading microorganisms, etc. (Cleveland 1996). Further model enhancement is required to describe the availability of chemicals under field soil conditions. Recently, a numerical soil/water microcosm system model has been reported, which is based on diffusion mass balance equations (Fick's second law), local sorption–desorption (a linear isotherm), irreversible sequestration (pseudo-first-order kinetics), and biodegradation (Monod kinetics) (Liu et al. 2007a, b).

7.2 Uptake Models: Bioavailability to Plants

Conceptual models have been proposed for the uptake of xenobiotic organic chemicals into plants. A plant uptake model must describe the dynamics of uptake of xenobiotics from soil, soil solution, and the soil atmosphere, in addition to metabolism and accumulation in roots, stems, leaves, and fruits. Trapp et al. (1994)

combined such individual processes to create the PLANTX model. PLANTX accounts for: diffusion of chemical in soil water to roots and from air pores to roots, transfer of the chemical into roots with the transpiration stream, translocation of the chemical into stems and leaves via the transpiration stream, partitioning of the chemical into the stem, transport of the chemical into fruit via the assimilation stream, diffusive exchange of the chemical between air and leaves via stomata and cuticle, and the metabolism of the chemical and its dilution by growth. PLANTX can be applied to different plant species and most organic chemicals in soil. Mass balance equations are assigned to the four plant compartments: roots, stems, leaves and fruit, within which reactions are assumed to be homogeneous.

Root mass exchange is expressed as follows:

$$V_{\mathrm{r}}\frac{\partial C_{\mathrm{r}}}{\partial t} = \left(K_{\mathrm{aw}} \cdot D_{\mathrm{a,eff}} + D_{\mathrm{w,eff}}\right) \cdot \left(C_{\mathrm{w}} - \frac{C_{\mathrm{r}}}{K_{\mathrm{rw}}}\right) \cdot \frac{2 \cdot L \cdot \pi}{\ln\left(R_2/R_1\right)} + Q_{\mathrm{w}} \cdot (1 - \mathrm{TSCF}) \cdot C_{\mathrm{w}} - \lambda_{\mathrm{r}} \cdot V_{\mathrm{r}} \cdot C_{\mathrm{r}} \tag{29}$$

where

V_{r} = root volume
C_{r} = concentration of chemical in the root
K_{aw} = partition coefficient between air and water (dimensionless Henry's law constant)
$D_{\mathrm{a,eff}}$ = effective diffusion coefficient in air-filled soil pores
$D_{\mathrm{w,eff}}$ = effective diffusion coefficient in water-filled pores
C_{w} = concentration in the external soil solution
K_{rw} = the partitioning coefficient between roots and water
L = total length of the roots
R_1 = radius of the roots
R_2 = the radius of a deficiency zone surrounding the roots
Q_{w} = the flow of transpiration water
TSCF = transpiration stream concentration factor
λ_{r} = the first-order metabolic rate constant in the root.

Mass exchange for shoots is expressed as follows:

$$V_{\mathrm{st}}\frac{\partial C_{\mathrm{st}}}{\partial t} = Q_{\mathrm{w}} \cdot \left(C_{\mathrm{w}} \cdot \mathrm{TSCF} - \frac{C_{\mathrm{st}}}{K_{\mathrm{stxy}}}\right) + Q_{\mathrm{p}} \cdot \left(\frac{C_{\mathrm{le}}}{K_{\mathrm{lew}}} - \frac{C_{\mathrm{st}}}{K_{\mathrm{stxy}}}\right) - \lambda_{\mathrm{st}} \cdot V_{\mathrm{st}} \cdot C_{\mathrm{st}} \tag{30}$$

where

V_{st} = stem volume
C_{st} = concentration of chemical in the stem
K_{stxy} = the partition coefficient between stem and xylem sap
Q_{p} = the flow of the assimilation stream
C_{le} = concentration in the leaves
K_{lew} = the partition coefficient between leaves and water in the assimilation stream
λ_{st} = the first-order metabolic rate constant in the stem.

Similarly, mass exchange for leaves is expressed as follows:

$$V_{\mathrm{le}}\frac{\partial C_{\mathrm{le}}}{\partial t} = Q_{\mathrm{w}} \cdot \frac{C_{\mathrm{st}}}{K_{\mathrm{stxy}}} + A_{\mathrm{le}} \cdot g_{\mathrm{total}} \cdot \left(C_{\mathrm{a}} - \frac{C_{\mathrm{le}}}{K_{\mathrm{lea}}}\right) - Q_{\mathrm{p}} \cdot \frac{C_{\mathrm{le}}}{K_{\mathrm{lew}}} - \lambda_{\mathrm{le}} \cdot V_{\mathrm{le}} \cdot C_{\mathrm{le}} \quad (31)$$

where

C_{le} = concentration in the leaves
V_{le} = volume of the leaves
A_{le} = leaf area
g_{total} = the total conductance of chemical in the foliage/atmosphere system
C_{a} = concentration of chemical in air
K_{lea} = the partition coefficient between leaves and air ($=K_{\mathrm{lew}}\ K_{\mathrm{aw}}{}^{-1}$)
λ_{le} = the first-order metabolic rate constant in the leaves.

Finally, mass exchange for fruit is

$$V_{\mathrm{f}}\frac{\partial C_{\mathrm{f}}}{\partial t} = Q_{\mathrm{p}} \cdot \frac{C_{\mathrm{st}}}{K_{\mathrm{stxy}}} - \lambda_{\mathrm{f}} \cdot V_{\mathrm{f}} \cdot C_{\mathrm{f}} \quad (32)$$

where

V_{f} = volume of the fruit
C_{f} = concentration of chemical in the fruit
λ_{f} = the first-order metabolic rate constant in the fruit.

Plant growth results in dilution of xenobiotics in the plant body. In the absence of metabolism

$$C_0 \cdot V_0 = C_{\mathrm{t}} \cdot V_{\mathrm{t}} \quad (33)$$

where

C_0 and V_0 = concentration and volume at time zero
C_t and V_t = concentration and volume at time t.

These systems of differential equations are solved numerically. This model has the advantage of requiring only a few common input parameters. Partition coefficients and exchange rates are calculated from these minimal data. For example, the partition coefficient of the chemical between plant tissue and soil solution is calculated from lipid and water fractions of the plant and the lipophilicity of the chemical. The estimation of exchange rates is based on the fugacity concept (Riederer 1990). The required input parameters for this estimation of exchange rates are the *n*-octanol–water and cuticle–water partition coefficients, the aqueous solubility, and the saturated vapor pressure of the chemicals. The uptake of chemicals from soil solution into shoots with the transpiration stream is governed by the TSCF. Metabolism is assumed to follow first-order kinetics. By combining the advection and dispersion model for soil, the PLANTX model can also be applied to field

soils. The model has been expanded to deal with gaseous deposition in addition to soil uptake processes, volatilization from leaves, transformation and degradation, and growth (Trapp and Matthies 1995). The uptake of bromacil and terbuthylazine has been successfully simulated with this model (Gayler et al. 1995; Trapp et al. 1994).

Trapp (2000) also applied the model to ionizable organic compounds. The flux of ionized chemicals through biological membranes is described by the Nernst–Planck equation. When the gradient of electric potential is constant through a membrane, the net current is zero and individual ion fluxes reach steady state. Then, the Flux of the ion can be described as follows:

$$\text{Flux} = P_{\text{M}} \frac{N}{e^{\text{N}} - 1} \left[a_0 - a_{\text{i}} \cdot e^{\text{N}} \right], N = \frac{zEF}{RT_{\text{K}}}, P_{\text{M}} = \frac{DK_{\text{lip}}}{\Delta x} \tag{34}$$

where

a_0 = activity of ion on the outside of the membrane
a_{i} = activity of ion on the inside of the membrane
z = valency (for monovalent acid: –1)
E = membrane potential
F = Faraday constant (96484 cal mol^{-1})
R = universal gas constant (8.314 J mol^{-1} K^{-1})
T_{K} = absolute temperature (K)
D = diffusion coefficient of chemical through membrane
K_{lip} = partition or distribution coefficient between solution and membrane
Δx = diffusion length
P_{M} = permeability of the chemical.

The model adds the flux of ionized chemical to the flux of neutral chemical; the latter is described by Fick's law of diffusion. The expanded model has been applied to simulate the kinetics of cyanide degradation after its uptake into plants (Trapp et al. 2001).

In PLANTX, the plant conception is simplified, in that the mechanisms of translocation of chemicals into plants are not addressed by the model, and the four compartments (roots, stem, leaves, and fruits) are regarded to be homogeneous. Many other phenomena are ignored by the model, such as the mycorrhiza-enhanced availability of chemicals and the accumulation of PAHs into the rhizosphere (Liste and Alexander 2000).

A one-dimensional mathematical model for the coupled transport of water, heat, and solutes in the soil–plant–atmosphere continuum (CTSPAC) has also been proposed (Boersma et al. 1988, 1991; Lindstrom et al. 1991; Ouyang 2002). CTSPAC consists of a Soil submodel and a Plant submodel. The Soil submodel represents solute transport in the vadose zone with continuous space and time, and accounts for: (1) simultaneous transport of water, heat, and solutes in the soil slab, (2) dynamic coupling boundary conditions at both the atmosphere–soil and vadose zone–groundwater interfaces, (3) introduction of chemicals by rain, surface air,

groundwater, and initially distributed sources in the soil layers, and (4) balance rules for mass, momentum, and heat.

The Soil submodel equation for solute transport and fate is expressed as follows:

$$V_{\text{revs}}\left\{\frac{\partial}{\partial t}\left[\left(\theta+\left(\varepsilon-\theta\right)H_{\text{c}}\right)C_{\text{l}}+\left(1-\varepsilon\right)S_{\text{s}}\right]+\left[\theta+\left(\varepsilon-\theta\right)H\prime\right]\Lambda C_{\text{w}}\right.$$
$$\left.+\frac{\partial}{\partial z}\left[\theta q_{\text{cl}}+\left(\varepsilon-\theta\right)q_{\text{cv}}\right]\right\} \tag{35}$$
$$=\begin{cases} A_{\text{pr}}(z)q_{\text{ws}}(z,t)C_{\text{l}}(z,t) & \text{for } q_{\text{w}}\le 0 \\ A_{\text{pr}}(z)q_{\text{ws}}(z,t)C_{\text{pr}}(z,t) & \text{for } q_{\text{w}}>0 \end{cases}+A_{\text{pr}}q_{\text{rt}}+V_{\text{revs}}Q_{\text{so}}(z,t)$$

where

V_{revs} = representative elementary soil volume
θ = volumetric water content
ϵ = soil porosity
H' = Henry's law constant
C_{l} = concentration of solute in the liquid phase
S_{s} = average concentration of solute in the sorbed phase
Λ = cumulative first-order loss coefficient
q_{cl} and q_{cv} = solute fluxes in liquid and vapor phases, respectively
$A_{\text{pr}}(z)$ = effective soil–root contact area (cm^2) at soil depth, z
$q_{\text{ws}}(z,t)$= the water flux due to root extraction at soil depth z and time t
$C_{\text{pr}}(z,t)$ = solute concentration inside the plant root at soil depth z and time t
q_{rt} = solute diffusive flux through the soil near the root–soil interface, then through root membranes and finally through plant cells into the xylem vessels
Q_{so} = the sources of solute.

Solute fluxes in the liquid (q_{cl}) and air (q_{cv}) phases, and at the root–soil interface (q_{rt}) are

$$q_{\text{cl}}=-D_{\text{cl}}\frac{\partial C_{\text{l}}}{\partial z}+v_{\text{l}}C_{\text{l}} \tag{36}$$

$$q_{\text{cv}}=-D_{\text{cv}}\frac{\partial C_{\text{v}}}{\partial z} \tag{37}$$

$$q_{\text{rt}}=-\frac{D_{\text{c}}(z,\theta)}{\Delta x_{\text{mb}}(z)}\left(C_{\text{l}}(z,t)-C_{\text{PR}}(z,t)\right) \tag{38}$$

where

q_{cl}, q_{cv}, and q_{rt} = solute flux in liquid phase, air phase and root–soil phase
D_{cl} and D_{cv} = diffusion coefficients of chemicals in the water and air phases
D_{c} = effective molecular diffusion coefficient across the root cell membrane
v_{l} = velocity of liquid phase water.

In the Plant submodel, the plant is divided into compartments of similar tissue structure and function. Important compartments and processes are: (1) water uptake by roots, (2) water transport driven by the gradient of total water potential through roots, stem and leaves in both xylem and phloem, (3) chemical transport in phloem driven by a gradient of positive pressure, and (4) water vapor flow from intercellular spaces to the atmosphere for evapotranspiration. The Plant submodel describes the transport and storage of a solute in plants as follows:

$$\frac{d\left[V_{-i}\left(1+B_{-i}\right)C_{-i}\right]}{dt} = \frac{D_i}{\Delta x_i}A_i\left(C_{-i}-C_i\right) - Q_i C_{-i} - \lambda_i M_{-i} \tag{39}$$

where

$-i$ = the compartment just below compartment i
V_i = sugar molar volume in compartment i
B_i = sorption coefficient for compartment i (dimensionless) that expresses immobilization of the solute by reversible sorption to cell walls or large molecules in compartment i
C_i = concentration of sugar in compartment i
D_i = diffusion coefficient across membrane along the flow path i
A_i = contact area between compartment i and the adjacent compartment
Q_i = water and water vapor flow rate
λ_1 = rate constant for all other first-order loss processes in compartment i that expresses immobilization of solute by incorporation into structural materials or loss of solute due to metabolism
M_i = solute mass in compartment i.

The Soil and Plant submodels are coupled with atmospheric conditions, which are non-linear and dynamic top boundary conditions. These include a daily cycle of soil temperature determined by the energy balance at the soil surface, a daily cycle of leaf evapotranspiration, and daily variations in air temperature and relative humidity. The Plant submodel was calibrated under constant soil water content and soil temperature using the soil experimental data in the report by Aitchison et al. (2000), who studied the phytoremediation of 1,4-dioxane by hybrid poplar trees in both hydroponic and soil experiments. A comparison of measured and predicted amounts of 1,4-dioxane in soil, roots, stem, and leaves for the soil experiment showed good agreement.

In a simulation study using the calibrated model, the following scenario was defined: phytoremediation of 1,4-dioxane by a poplar cut from a contaminated sandy soil in response to daily cycles of water flow and heat flux for a simulation period of 7 d. Simulation indicated that the 1,4-dioxane concentration was high in leaves and low in roots, with stem concentration in between. About 30% of soil 1,4-dioxane was removed within 7 d by root uptake. Leaves were an important compartment for 1,4-dioxane accumulation and transpiration. The same model, with modification, was successfully applied to estimate phytoremediation of 2,4,6-trinitrotoluene from a contaminated site by a poplar tree (*Populus fastigiata*) (Ouyang et al. 2005).

Results suggested that this model is useful in estimating bioavailability of chemicals. However, plant growth was not considered in the model. In addition, this is a one-dimensional model, which limits its application to field-scale transport and chemical fate.

The more polar a molecule, the more readily it reaches the root, passes through the epidermis, and is translocated throughout the plant. Plants, specifically plant roots, are not very discriminating toward small (molecular wt <500) organic molecules, except when they are polar. Non-polar molecules tend to adsorb to root surfaces rather than pass through the epidermis.

7.3 Uptake Models: Bioavailability to Soil Fauna

A comprehensive model, based on chemical and physical pesticide properties and biotic and abiotic factors, has been developed that predicts exposure of soil-dwelling organisms to pesticides under various test conditions, e.g., different soil types (Gyldenkaerne and Joergensen 2000). Soil-dwelling fauna may be exposed directly to a chemical during application. Fauna may be exposed by consuming contaminated food, by inhaling (respiring) contaminants, and by direct contact of tarsi and the cuticula with the soil solution. Fauna may eliminate chemicals from their bodies by enzymatic reaction, enterobacterial degradation, or simple excretion. Therefore, the total amount of chemical inside the organism at a given time can be expressed as the summation of these processes as follows:

$$P(t) = P_{\text{topical}} + \int_0^t C_{\text{F}} F_{\text{c}} \partial t + \int_0^t C_{\text{A}} A_{\text{c}} \partial t + \frac{1}{\rho_{\text{s}}} \int_0^t C_{\text{W}} U \partial t - \int_0^t k_{\text{e}}(t) \partial t \qquad (40)$$

where

$P(t)$ = total amount of chemical inside the organism
P_{topical} = the amount of chemical contacting the organisms at the time of chemical application (t=0)
C_{f} = concentration of chemical in the food
F = food consumption
C_{a} = chemical concentration in air
A_{c} = air consumption
C_{w} = chemical concentration in the soil solution
U = uptake rate from the soil
k_{e} = elimination rate constant.

Elimination rates follow first-order kinetics. The concentrations of chemical in the soil, air, and soil solution can be estimated by a soil model that accounts for sorption, degradation, solute transport, volatilization, and uptake by soil fauna. The

soil model for fauna, unlike the one for microbes, also incorporates uptake by fauna as a component of the model.

Little work has been reported on uptake of chemicals by eating contaminated prey. Beetles, for instance, are not very selective in choosing between contaminated and non-contaminated prey, and they may eat contaminated prey species that have been killed and have fallen to the ground. In studies using the beetle *Nebria brevicollis* (F.), and the deltamethrin-treated aphid *Metopolophium dirhodum* (Walk.) (Wiles and Jepson 1993a, b), it appeared that consuming contaminated prey was an important cause of faunal mortality. However, in another study using the spider *Oedothorax apicatus*, Mullie and Everts (1991) observed no significant effect of deltamethrin contamination on prey consumption. Environmental factors such as accessibility of fauna to prey that change the prey consumption rate (Dixon and McKinley 1992) may explain the discrepancy among the studies. Faunal behavior may also account for the discrepancy. For example, carabids only eat fresh or newly killed aphids. The maximum consumption rate of prey (aphids) by carabids that have an unlimited access to prey may be expressed (Winder et al. 1994) as a function of body weight and temperature:

$$\mathrm{Log}_{10}(F_{\mathrm{a}}) = \frac{2.36\log_{10}(T) + 0.495\log_{10}(W_{\mathrm{b}}) - 3.423}{24} \tag{41}$$

where

F_{a} = consumption rate
T = temperature (°C)
W_{b} = weight of carabids.

The model fits data from another study well (Wiles and Jepson 1993a).

A simple model for actual aphid consumption ($A_{\mathrm{ac,\ actual}}$, mg/hr) is given by Gyldenkaerne and Joergensen (2000)

$$A_{\mathrm{ac,actual}} = \begin{cases} t \le t_{\mathrm{fm}} & A_{\mathrm{ac}} k_{\mathrm{fp}} \\ t < t_{\mathrm{fe}} & A_{\mathrm{ac}} \frac{t_{\mathrm{fe}} - t}{t_{\mathrm{fm}} - t_{\mathrm{fe}}} k_{\mathrm{fp}} \\ t > t_{\mathrm{fe}} & A_{\mathrm{ac}} = 0 \end{cases} \tag{42}$$

where

A_{ac} = consumption rate of aphids
t_{fm} = the time when maximum contaminated food uptake occurs
t_{fe} = the time when the uptake of contaminated prey ends
k_{fp} = the fraction of the prey containing pesticides.

The model assumes a maximum uptake until t_{fm}, after which uptake decreases linearly until t_{fe}. After t_{fe}, uptake becomes zero.

The concentration of chemicals in prey should be estimated in the context of the prey's behavior and application and subsequent behavior of the chemicals concerned. The concentration in pesticide-contaminated aphids will depend on rate of

topical application and plant stem sap concentration. Absorption rates of chemicals from the gut are fast: The first-order kinetic rate constants of absorption were 0.72–1.39 (hr^{-1}) for tobacco hornworm larvae (*Manduca sexta* (L.)) and 0.34–0.45 (hr^{-1}) for cockroach (*Blaberus Craniifer* (Burm.)), respectively (Shah et al. 1972). Thus, chemicals in consumed food are quite bioavailable unless food is taken up with soil/sediment solid particles.

Chemicals are absorbed by beetles from air by passive diffusion through the respiratory system and the cuticula. The respiration rate depends on temperature and body size (Gyldenkaerne and Joergensen 2000), and pesticide uptake $P_G(t)$ at time t is defined by

$$P_g(t) = \frac{10^{mc+mp\cdot T}}{O_a} W_b^r \cdot C_a(t) \tag{43}$$

where

$P_g(t)$ = pesticide uptake at time t
mc = a constant
mp = temperature dependence
O_a = relative oxygen content in the air
W_b = weight of carabids
r = constant
C_a = pesticide concentration in the air
W_b^r = respiration rate.

The uptake of chemicals from soil solution is related to the surface contact area:

$$\int_0^t C_w U \partial t = C_w(t) c_b t_b \tag{44}$$

where

C_w = uptake of chemical from soil solution
U = uptake rate from the soil
C_b = contact area with soil
T_b = transfer coefficient.

These differential equations are solved numerically using a Runge–Kutta method. Most of these are widely accepted soil-related parameters. What constitutes appropriate parameters for any test is usually determined from controlled laboratory studies. Validation of the model that expresses the total amount of chemical inside the organisms (Eq. 40) has been tested for soil-dwelling beetles exposed to insecticides (Gyldenkaerne and Joergensen 2000). The model described dose–response curves for four pesticides: lindane, parathion, fenvalerate, and metamidophos, rather well. If the exposure was combined with the toxicity of the pesticides, the model predicted pesticide bioavailability for different soil types, and different times of release

of beetles to the soil environment along with pesticide-contaminated aphids. Uptake from the soil was the most important route among the four considered uptake routes. It was also the most difficult route to estimate because of the large variation in pesticide parameters and soil properties. Uptake from prey (food) can be significant. Uptake through respiration was negligible. In actual field exposure, spatial factors would also be important to chemical bioavailability.

8 Recommendations for Further Research

Chemical bioavailability is determined by a dynamic process in which a chemical is taken up by (an) organism(s) in a specified time and place. Estimation of bioavailable amounts is critical to assessing chemical risk, predicting biodegradation rates, and achieving a better understanding of prospective ecological effects. To date, there are four main approaches to estimating bioavailability of chemicals: bioassays, chemical analyses with mild extraction, estimating relationship from sorption coefficients, and use of simulation models. Each approach has advantages and disadvantages (Table 7). Bioavailability is affected by many factors, and even using the same approach or method may produce different predictions of degree of bioavailability. Therefore, one cannot accurately determine bioavailability by employing a single method for all chemicals, organisms, or soils. Therefore, further research is needed to achieve improvements for estimating bioavailability of chemicals. The following comprises our priority recommendations for further research in this area:

(1) Efforts are needed to standardize bioassays. This action is important in that it would allow better comparison of bioavailability results from one experiment to another. The results of bioassays that measure actual chemical accumulation into organisms and degradation by organisms are usually achieved as an integrated reflection of (a) bioavailability, (b) toxicity of the chemical, and (c) physiology and other characteristics of species of test organism. Standardization will improve comparisons of chemical bioavailability among different organisms in the context of physiology, uptake mode, behavior, and distribution in the soil (or sediment) environment; it would also make bioavailability extrapolations from one organism to another less problematic. In addition, it is important to maintain standardization of experimental conditions (e.g., moisture content) and materials (e.g., nature of soil/sediment), because they may dramatically affect bioavailability.
(2) Rapid bioavailability assay methods are needed. Bioassays provide direct evidence of bioavailability, but are time consuming, laborious, and expensive, especially when studying higher plants and animals. Faster assays are desirable, even for biological assays. Development of alternative methods such as biosensors may permit achieving good estimates of bioavailability while reducing labor and time. In addition, we encourage expanded use of chemical analyses that employ mild extraction methods, and further enhancement of mathematical modeling to achieve estimates of bioavailability.

Table 7 Summary of the laboratory/mathematical methods used to evaluate bioavailability of chemicals in soil

No.	Methods	Advantages	Disadvantages or Improvement Required
1	Bioassays	Real availability measured	Physiology and habitat affect the results. Needed are standardized procedure and interpretation as bioavailability test
a	Microbial degradation	Well established	Multiple substances are not measured correctly
b	Uptake (plant, invertebrates)	Well established for plants, real availability measured, multiple chemicals evaluated	Time consuming and laborious Needs standardization for invertebrates
c	Toxicity (plant invertebrates)	Useful without information on pollutants	Does not directly show bioavailability. Needs dose–response curve and standardization
d	Genotoxicity and enzyme activities	Well established, commercially available, useful without information on pollutants	Does not directly show bioavailability Needs dose–response curve and standardization
e	Behavior (invertebrates)	Useful without information on pollutants	Does not directly show bioavailability Needs dose–response curve
2	Mild extraction methods	Quick in predicting bioavailable amount at the moment measured	Needs to be correlated with the bioassay results Does not show availability changes with time (needs linearity between mild extraction and bioavailability with time)
3	K_p, K_{oc}	Rapid, can utilize accumulated data, good indicator	Deviates from actual bioavailability Need to determine K_p values under realistic soil moisture Needs to be correlated with bioassay results
4	Modeling	Inexpensive once developed, describes non-equilibrium state	Requires huge background data for validation Applicability limited to certain chemicals and situations Deviates from actual bioavailability Needs to be correlated with bioassay results and important parameters for extrapolation must be identified
a	Microbial degradation	Well established (but still not predictable for new soils)	Quantitative measurement of aging and “bound residue”
b	Plant uptake	Successful at one-dimensional level	Needs development for other plants and chemicals in various different soils at three-dimension levels
c	Invertebrate uptake	Promising results with beetles	Needs development for other soil invertebrates and chemicals in various soils

(3) Further efforts are needed to validate and compare results from non-biological and rapid biological estimation methods with conventional (and more laborious) bioassay results. Rapid methods may only represent bioavailability at the moment of measurement, whereas conventional bioassays produce bioavailability results that are dependent on longer exposure times in which equilibria may not have been reached. Validation studies must consider such basic differences between methods, and find ways to better bridge the differences.
(4) Elaborate on attempts to use the soil sorption coefficient K_p, and the soil OC sorption coefficient K_{oc} to estimate bioavailability. These values may compare well with analyses of total amounts of chemical gleaned from exhaustive extraction experiments. Many K_p and K_{oc} values already exist in the literature for a wide range of chemicals and soils. Unfortunately, these coefficients are typically measured in soil slurries and may not represent typical and actual soil moisture levels. To be relevant, realistic soil moisture levels should be used when determining a chemical's sorption coefficients.
(5) Mathematical models that describe bioavailability should be developed, particularly for individual microorganisms, plants, and invertebrates. These individual models are needed, because of the large differences that exist in physiology and behavior among these groups of organisms. Simulation models are useful in describing the behavior of xenobiotics in soil at states of non-equilibrium. The output is enhanced when these models account for all significant processes that may affect an organism's bioavailability. Individual processes, such as fate of xenobiotics, sorption/desorption, volatilization, dissolution, intraparticle-aggregate sorption–diffusion (aging), and biodegradation, should be investigated and incorporated into these models. Physiological properties such as transpiration in plants and habitat characteristics for invertebrates are also important factors that should be considered. Although the key equations should be incorporated into such models, they should be kept as simple as possible to make them utilizable actually. Whenever possible these models should be validated against existing reliable bioavailable data. Because obtaining accurate bioavailability data is difficult, particularly with aged xenobiotic residues, efforts to validate the models are needed.
(6) Experimental work is required to reconcile experiments where scale differences are large. For example, when laboratory experiments are conducted with soil plugs in bottles, the dynamics of chemical movement with infiltrating soil water is not considered. In the field, by contrast, transport of chemicals in the soil matrix with infiltrating water will have a very important impact on bioavailability. Such transport is often described by convection–dispersion models. Notwithstanding, field-scale modeling is complicated by the heterogeneity of field soils. Therefore, ecotoxicological assessment of bioavailability at the field level is not yet practical, although it is desired and needed.
(7) Conduct more studies on the interaction of multiple xenobiotics in soils, and with soil components. Currently, the ability to assess the ecological implications of bioavailability, when more than one xenobiotic is present (e.g., the simultaneous presence of petroleum hydrocarbons and heavy metals in soil) is in its infancy.

Table 8 A possible tier approach to determine the bioavailability of chemicals in soils

	Aim: Bioavailability related to	
Tiers	Degradability[1]	Ecotoxicological effect or efficacy[2]
Primary level	Chemical structure Chemical properties	Exhaustive extraction analysis Estimation from K_p and K_{oc}
Secondary level	Degradation assay using soil plugs Degradation model	Mild extraction analysis Genotoxicity/enzyme assay
Tertiary level	Degradation assay in microcosm – mesocosm with degradation model Mild extraction analysis Soil microbial analysis	Toxicity/behavior assay
Ecological level	Residue analysis in field Ecological models	Uptake assay Ecological models

[1] Triggers to next step for degradability constitute rates that are lower than a threshold-degrading rate.
[2] Triggers to next step for ecotoxicological effect or efficacy are exceedence of threshold concentration as determined by chemical analysis or prediction by models.

(8) The methods used to estimate chemical bioavailability should be selected and organized to ensure that they are suitable to their primary purpose: (a) bioavailability as it affects degradability, and (b) bioavailability as it affects the ecotoxicology of chemicals. A potential tier approach for estimating chemical bioavailability is presented in Table 8, and relies on approaches that range from the simple to sophisticated. Utilizing a compilation of standardized measurement/estimation methods is important to this tier system, especially standardization of the biological endpoints used to evaluate bioavailability. Dealing with the ecological level tier is more challenging, because organisms occupy different ecological niches, and bioavailability of a chemical to each organism has different importance to the ecosystem as a whole.

9 Summary

It is often presumed that all chemicals in soil are available to microorganisms, plant roots, and soil fauna via dermal exposure. Subsequent bioaccumulation through the food chain may then result in exposure to higher organisms. Using the presumption of total availability, national governments reduce environmental threshold levels of regulated chemicals by increasing guideline safety margins. However, evidence shows that chemical residues in the soil environment are not always bioavailable. Hence, actual chemical exposure levels of biota are much less than concentrations present in soil would suggest. Because "bioavailability" conveys meaning that combines implications of chemical soil persistency, efficacy, and toxicity, insights on the magnitude of a chemicals soil bioavailability is valuable. However, soil bioavailability of chemicals is a complex topic, and is affected by chemical properties, soil properties, species exposed, climate, and interaction processes.

In this review, the state-of-art scientific basis for bioavailability is addressed. Key points covered include: definition, factors affecting bioavailability, equations governing key transport and distributive kinetics, and primary methods for estimating bioavailability. Primary transport mechanisms in living organisms, critical to an understanding of bioavailability, also presage the review.

Transport of lipophilic chemicals occurs mainly by passive diffusion for all microorganisms, plants, and soil fauna. Therefore, the distribution of a chemical between organisms and soil (bioavailable proportion) follows partition equilibrium theory. However, a chemical's bioavailability does not always follow partition equilibrium theory because of other interactions with soil, such as soil sorption, hysteretic desorption, effects of surfactants in pore water, formation of "bound residue", etc. Bioassays for estimating chemical bioavailability have been introduced with several targeted endpoints: microbial degradation, uptake by higher plants and soil fauna, and toxicity to organisms. However, these bioassays are often time consuming and laborious. Thus, mild extraction methods have been employed to estimate bioavailability of chemicals. Mild methods include sequential extraction using alcohols, hexane/water, supercritical fluids (carbon dioxide), aqueous hydroxypropyl-beta-cyclodextrin extraction, polymeric TENAX^{TM} beads extraction, and poly(dimethylsiloxane)-coated solid-phase microextraction. It should be noted that mild extraction methods may predict bioavailability at the moment when measurements are carried out, but not the changes in bioavailability that may occur over time.

Simulation models are needed to estimate better bioavailability as a function of exposure time. In the past, models have progressed significantly by addressing each group of organisms separately: microbial degradation, plant uptake via evapotranspiration processes, and uptake of soil fauna in their habitat. This approach has been used primarily because of wide differences in the physiology and behaviors of such disparate organisms. However, improvement of models is badly needed, particularly to describe uptake processes by plant and animals that impinge on bioavailability. Although models are required to describe all important factors that may affect chemical bioavailability to individual organisms over time (e.g., sorption/desorption to soil/sediment, volatilization, dissolution, aging, "bound residue" formation, biodegradation, etc.), these models should be simplified, when possible, to limit the number of parameters to the practical minimum.

Although significant scientific progress has been made in understanding the complexities in specific methodologies dedicated to determining bioavailability, no method has yet emerged to characterize bioavailability across a wide range of chemicals, organisms, and soils/sediments. The primary aim in studying bioavailability is to define options for addressing bioremediation or environmental toxicity (risk assessment), and that is unlikely to change. Because of its importance in estimating xenobiotic degradability and toxicity in contaminated soils and sediments, further research is needed to more comprehensively address the key environmental issue of "bioavailability of chemicals in soil/sediment."

Acknowledgments This study was supported by IUPAC as project No.1999-041-1-600.

Appendix: A Description of Equation Symbols, and the Units They Use

Symbol	Description	Unit
δ_d	Thickness of the aqueous diffusion layer	L
δ_{lip}	Thickness of the lipid membrane	L
ΔC	Difference in concentration of the chemical between the outside and inside of the cell; assuming the concentration of the chemical compound inside the cell is zero, $\Delta C = C_w$	ML^{-3}
$-\frac{dC_w}{dt}$	Substrate decomposition rate	$ML^{-3}T^{-1}$
θ_{ref}	θ at the reference conditions	–
k_{la}^{Chem}	Mass transfer coefficient	T^{-1}
k_{la}^{soil}	Mass transfer coefficient	T^{-1}
k_{la}^{wg}	Mass transfer coefficient	T^{-1}
$C_{(r=R,t)}$	Concentration of chemicals at the surface of spherical soil aggregates	ML^{-3}
ρ	Density of dried cells	ML^{-3}
ρ_s	Density of dry solid material	ML^{-3}
λ_r	First-order metabolic rate constant in the root	T^{-1}
λ_{st}	First-order metabolic rate constant in the stem	T^{-1}
λ_{le}	First-order metabolic rate constant in the leaves	T^{-1}
λ_f	First-order metabolic rate constant in the fruit	T^{-1}
Δx	Diffusion length	L
θ	Volumetric water content	–
ϵ	Soil porosity	–
Λ	Cumulative first-order loss coefficient	T^{-1}
λ_f	Rate constant for all other first-order loss processes in compartment i that express immobilization of solute by incorporation into structural materials or loss of solute from metabolism	T^{-1}
μ_{max}	Maximum specific growth rate	–
τ	Bacterial doubling time	T
A_{ac}	Consumption rate of aphid	MT^{-1}
$A_{ac,\ actual}$	Actual consumption rate of aphid	MT^{-1}
A_l	Area of the lipid membrane	L^2
A_c	Air consumption	L^3T^{-1}
A_0	Activity of ion on the outside of the membrane	ML^{-3}
a_i	Activity of ion on the inside of the membrane	ML^{-3}
A_i	Contact area between compartment i and the adjacent compartment	L^2
A_{le}	Leaf area	L^2
$A_{pr}(z)$	Effective soil–root contact area at the soil depth, z	L^2
B	Constant	–
BAF	Bioaccumulation factor	–
B_i	Sorption coefficient for compartment i (dimensionless) that expresses the immobilization of the solute by reversible sorption to cell walls or large molecules in compartment i	–
BSAF	Biota–soil accumulation factor	–

Symbol	Description	Unit
C	Concentration of chemical in soil solution	ML^{-3}
C_0	Concentration at time zero	ML^{-3}
C_a	Concentration of chemical in air	ML^{-3}
C_b	Contact area with soil	L^2
C_f	Concentration of chemical in the fruits of food	ML^{-3}
C_i	Concentration of sugar in compartment i	ML^{-3}
C_{le}	Concentration in the leaves	ML^{-3}
C_l	Concentration of solute in liquid phase	ML^{-3}
C_{org}	Concentration in the worm	MM^{-1}
$C_{pr}(z,t)$	Solute concentration inside the plant root at the soil depth z and time t	ML^{-3}
C_r	Concentration in the root	ML^{-3}
C_s	Concentration in soil solid phase	–
C_{st}	Concentration of chemical in the stem	ML^{-3}
C_t	Concentration at time t	ML^{-3}
C_{vic}	Concentration of the chemical in the vicinity of soil particles (or chemical crystals/solvents)	ML^{-3}
C_w	Chemical concentration in the aqueous environment or in solution	ML^{-3}
D	Diffusion coefficient of chemical through membrane or in solution	M^2T^{-1}
$D_{a,eff}$	Effective diffusion coefficient in air-filled soil pores	M^2T^{-1}
D_c	Effective molecular diffusion coefficient across the root cell membrane	M^2T^{-1}
D_{cl}	Diffusion coefficient in the water phases	M^2T^{-1}
D_{cv}	Diffusion coefficient in air phases	M^2T^{-1}
D_d	Diffusion coefficient of the chemical through aqueous diffusion layer at vicinity of membrane	M^2T^{-1}
D_{eff}	Effective diffusion coefficient	M^2T^{-1}
D_i	Diffusion coefficient across membrane along the flow path i	M^2T^{-1}
D_l	Diffusion coefficient of the chemical through the lipid membrane	M^2T^{-1}
D_m	Molecular diffusion coefficient	M^2T^{-1}
$D_{w,eff}$	Effective diffusion coefficient in water-filled pores	M^2T^{-1}
D_x	Dispersion coefficient	M^2T^{-1}
E	Membrane potential (V)	C
F	Faraday constant (96,484 cal mol^{-1})	$ML^{-1}N^{-1}$
F_c	Food consumption	MT^{-1}
F_a	Consumption rate	MT^{-1}
F_{lip}	Weight fraction of lipid in the organism	–
Flux	Dissolution flux	$ML^{-3}T^{-1}$
f_o	Reduction factor for soil humidity	–
F_{om}	Weight fraction of OM	–
f_T	Factor for the influence of soil temperature	–
g_{total}	Total conductance of chemical in the foliage/atmosphere system	LT^{-1}
H'	Henry's law constant	–
I	Retardation factor	–
J	Rate of transport of the chemical through the lipid membrane	MT^{-1}

Symbol	Description	Unit
k	First-order degradation constant in soil	T^{-1}
k_{fp}	Fraction of the prey containing pesticides	–
K_{aw}	Partition coefficient of air to water (the dimensionless Henry's law constant)	–
K_b	Sorption coefficient of bacterial cells	L^3M^{-1}
k_{bio}	First-order biodegradation rate constant	T^{-1}
k_e	Elimination rate constant	T^{-1}
K_f	Freundlich sorption coefficient	L^nM^{-n}
K_{lip}	Partition coefficient of the chemical between the lipid membrane and the aqueous diffusion layer	–
k_{la}	Mass transfer rate coefficient	T^{-1}
K_{lea}	Partition coefficient between leaves and air ($=K_{lew}\ K_{aw}{}^{-1}$)	–
K_{lew}	Partition coefficient between leaves and water in the assimilation stream	–
K_{oc}	Equilibrium sorption coefficient on a basis of soil OC	L^3M^{-1}
K_{ow}	*n*-Octanol–water partitioning coefficient	–
K_p	Equilibrium soil sorption coefficient of chemical	L^3M^{-1}
k_{ref}	k at reference conditions	T^{-1}
K_{rw}	Partitioning coefficient between roots and water	–
K_s	Half-saturation constant	–
K_{stxy}	Partition coefficient between stem and xylem sap	–
k_{sw}	Degradation rate constant in soil solution by cells sorbed to soils	T^{-1}
k_w	Degradation rate constant in soil solution by cells in soil solution	T^{-1}
L	Total length of the roots	L
m_c	Cell maintenance coefficient	–
m	Soil weight	M
M	Molecular weight	M
mc	Constant	–
M_i	Solute mass in compartment i	M
min	"The minimum of"	–
m_{oc}	OC content	–
mp	Temperature dependence	–
n	Linearity factor	–
N_{dr}	Sum of the diffusive flux of chemical to the roots in air- and water-filled pores	MT^{-1}
N_s	Bacterial cell number sorbed to unit weight of soil	–
N_{tr}	Uptake and transport	MT^{-1}
N_w	Bacterial cell number present in unit volume of soil solution	–
O_a	Relative oxygen content in the air	ML^{-3}
P	Vapor pressure	$ML^{-1}T^{-2}$
P_M	Indicates the permeability of the chemical	LT^{-1}
$P_g(t)$	Pesticide uptake at time t	MT^{-1}
P_t	Total amount of chemical inside the organism	M
$P_{topical}$	Amount of chemical hitting the organisms at the time of chemical application ($t = 0$)	M
q_{cl} and q_{cv}	Solute fluxes in liquid and vapor phases, respectively	$ML^{-2}T^{-1}$
q_{cl}, q_{cv} and q_{rt}	Solute flux in liquid phase, air phase, and root–soil phase	$ML^{-2}T^{-1}$

Symbol	Description	Unit
Q_i	Water and water vapor flow rate	L^3T^{-1}
Q_p	Flow of assimilation stream	L^3T^{-1}
q_{rt}	Solute diffusive flux through the soil near the root–soil interface, then through root membranes and finally through plant cells into the xylem vessels	$ML^{-2}T^{-1}$
Q_{so}	Sources of solute	$ML^{-3}T^{-1}$
Q_w	Flow of transpiration water	L^3T^{-1}
$q_{ws}(z,t)$	Water flux due to root extraction at the soil depth z and time t	$ML^{-2}T^{-1}$
R_s	Diameter of soil aggregate	L
R	Universal gas constant (8.314 J mol^{-1} K^{-1})	$ML^2T^{-2}N^{-1}\theta^{-1}$
r	Constant	–
R_1	Radius of the roots	L
R_2	Radius of a deficiency zone surrounding the roots	L
R_2–R_1	Diffusion length	L
Rb	Radius of cell at initial stage	L
Rd	Radius of cell at cell division	L
S	Water solubility	ML^{-3}
S_m	Chemical amount in cells	M
S_w	Substrate amount in soil or solution	M
S_s	Average concentration of solute in the sorbed phase	ML^{-3}
t	Time	T
t_{fm}	Time when maximum contaminated food uptake occurs	T
t_{fe}	Time when the uptake of contaminated prey ends	T
T_K	Absolute temperature (K)	θ
T	Temperature (°C)	θ
T_b	Transfer coefficient	MTL^{-2}
TSCF	Transpiration stream concentration factor	–
U	Uptake rate from the soil	MT^{-1}
v	Pore water velocity	LT^{-1}
V_0	Volume at time zero	
V_f	Volume of the fruits	L^3
V_i	Sugar molar volume in compartment i	L^3
V_{le}	Volume of the leaves (m^3)	L^3
v_l	Velocity of liquid phase water	LT^{-1}
V_r	Root volume	L^3
V_{revs}	Representative elementary soil volume	L^3
V_{st}	Stem volume	L^3
V_t	Volume at time t	L^3
W	Volume of soil solution	L^3
W_b	Weight of carabid	M
W_b^r	Respiration rate	MT^{-1}
Wc	Soil to water ratio	–
x	Amount of sorbed chemical	M
X	Microbial density	–
Y	Growth yield	–
Y_{max}	Maximum growth yield	–
z	Valency (for monovalent acid: –1)	–

Symbol	Description	Unit
x, y, z	Coordinates	M
i	Compartment	–
–i	Compartment before compartment *i*	–

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Effects of Pesticides on Freshwater Diatoms

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Contents

1 Introduction

Human activities have resulted in release of a large range of toxic contaminants into aquatic ecosystems. The assessment of environmental pesticide residue concentrations in Europe and North America has shown significant contamination of streams and groundwater in both agricultural and urban settings (Gilliom et al. 1999; IFEN

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology*,
Reviews of Environmental Contamination and Toxicology 203,
DOI 10.1007/978-1-4419-1352-4_2,

2006; Devault et al. 2007; Gilliom 2007; Sprague and Nowell 2008). According to data recently available in France and the United States, water quality benchmarks for aquatic life were exceeded in half of the sites studied (IFEN 2006; Gilliom 2007). Gilliom et al. (2007) reported that the pollution of streams was relatively concentrated in time with frequent short pulses of higher pesticide concentrations. Numerous publications have shown that pesticide concentrations exceeded 10 μg/L, and even reached values approaching 700 μg/L in samples collected after heavy rains or during high river flow (Spalding and Snow 1989; Carder and Hoagland 1998; Schulz 2001; Ferenczi et al. 2002). Such events have elevated concerns about environmental risk.

Enforcement of new legislation in Europe (Water Framework Directive 2000/60) has been undertaken to restore the quality of the most contaminated water resources and to meet "good ecological status" by 2015. The main purpose of these rules was to establish levels of pesticide pollution that may have noxious effects on aquatic ecosystems. The majority of ecotoxicity data are only available for single pesticides, and have not been adapted for use in estimating the collective toxicity of pesticides in stream water. The combined toxicity of such pollution is much more complex, given the large range of toxic molecules (pesticides and their metabolites) that may exist in streams and other water bodies (Gilliom et al. 1999; Gilliom 2007). It has been reported that the toxicity of pesticide mixtures is greater than that of individual pesticides (Lydy et al. 2004; Belden et al. 2007). Therefore, pesticide pollution may constitute a major threat to the health and productivity of aquatic ecosystems. At the base of the trophic food chain, primary producers such as diatoms, which represent a source of food for numerous other organisms, may be seriously affected by pesticide exposure. Moreover, such pollutants may significantly disturb the equilibrium of the trophic food chain (Stevenson and Pan 1999).

When rapid water flow limits the development of other algae, diatoms are often the dominant remaining primary producers (Ghosh and Gaur 1998). Therefore, these microalgae are particularly relevant when the harmful effects of pesticide pollution are investigated in aquatic ecosystems. In addition, herbicides (e.g., isoproturon, diuron, atrazine, and *s*-metolachlor), which are particularly toxic to algae, represent the major pesticide class that contaminate rivers, owing primarily to the large amounts used in agriculture and on urban areas, and sometimes owing to their environmental persistence (Agence de l'eau Adour-Garonne 2006; Gilliom 2007). Numerous studies have been performed to study the effects of pesticides on individual species and on species communities. Indeed, if diatom communities are recognized as useful bioindicators of herbicide-contaminated water, their efficiency to detect such toxic pollutants has yet to be demonstrated (Dorigo et al. 2004).

The purpose of this chapter is to provide a broad bibliographical review of articles that address the effects of pesticides and certain other xenobiotics on diatoms. In this review, we emphasize the following targets of pesticide action: (i) cytology and cell ultrastructure, (ii) cell metabolism, and, finally, (iii) effects on community species composition.

2 Effects on Cytology and Cell Ultrastructure

Organelles are strongly interlinked in diatoms. A single alteration can seriously perturb all cellular organelles. The study of different intracellular-component responses to toxic agents may help to understand how diatoms respond when exposed to pesticides. Nevertheless, the literature that deals with intracellular toxic effects in diatoms is quite limited. In this review, we have chosen to focus attention on the effects to major organelles (intracellular cytoskeleton, nucleus, and cell wall) of diatoms that may be linked to pesticide exposure.

2.1 The Cytoskeleton

The cytoskeleton (microtubules, actin filaments, and microfilaments) is involved in cell division and manages the internal layout of the cell. Many herbicides have been developed that can or do affect the cytoskeleton. Disturbance of cytoskeleton organization may induce several types of cell injuries, especially during mitosis. Thus, Coss and Pickett-Heaps (1974) have shown that IPC (isopropyl *N*-phenyl carbamate), a carbamate herbicide, produced mitotic spindle effects in the green alga *Oedogonium cardiacum*. Similarly, diatoms and green algae, exposed to the cytoskeleton inhibitors colchicine and cytochalasin D, induced a disruption of mitotic spindles (Coombs et al. 1968; Pickett-Heaps and Spurck 1982; Edgar and Pickett-Heaps 1984; Pickett-Heaps et al. 1984; Cohn and Pickett-Heaps 1988; Puiseux-Dao 1989; Sampson and Pickett-Heaps 2001). Colchicine is known to block the binding of tubulins α and β, and thus, the synthesis of microtubules (Puiseux-Dao 1989). According to Coss and Pickett-Heaps (1974), colchicine and IPC have similar mechanisms of action. Similarly, the work of Spurck and Pickett-Heaps (1994) demonstrated an abnormal arrangement of chromosomes during metaphase for two diatom species (*Surirella robusta*, *Hantzschia amphioxys*) caused by the drug, diazepam; the primary effect observed in these two species was disturbed organization of the mitotic spindle. However, in the case of a moderate exposure to diazepam, these authors reported a restoration of the cytoskeleton integrity.

2.2 The Nucleus and DNA

A few studies have been conducted to determine the toxic effects of chemicals on the diatom cellular nucleus. Cassoti et al. (2005) observed dispersion of DNA in cells of the marine diatom, *Thalassiosira weissflogii*, when these organisms were exposed to the aldehyde, 2-*trans*,4-*trans*-decadienal. Debenest et al. (2008) also observed DNA dispersion in diatom cells exposed to the plant growth regulator, maleic hydrazide. Multinuclear cells were reported to occur in the diatom *Navicula pelliculosa*, when these cells were exposed to colchicine (Coombs et al. 1968; Duke and Reimann 1977) or when green algae (*O. cardiacum*) were exposed to IPC

(Coss and Pickett-Heaps 1974). The well-known genotoxic plant growth regulator, maleic hydrazide, induced micronucleus and multinuclear cells in a multispecific diatom culture (Debenest et al. 2008). As previously observed by Cassoti et al. (2005), DNA fragmentation was observed for diatoms exposed to certain genotoxic agents (cadmium, 1-methyl-3-nitro-1-nitrosoguanidine and benzo[*a*]pyrene) (Aoyama et al. 2003; Desai et al. 2006). Similar genotoxic effects were also reported for diatoms exposed to UV radiation. Cells exposed to genotoxic agents show increases in thymine dimers; such dimers induce chromosome bridges and produce nuclear alterations (Buma et al. 1995, 1996; Holzinger and Lutz 2006). Two interpretations were proposed to explain the subsequent effects of these alterations on DNA. Rijstenbil (2001) suggested that oxidative stress, induced by genotoxic agents, may alter DNA integrity (clastogenic action). This toxic mechanism has been widely demonstrated to occur in microorganisms and higher plants exposed to genotoxic pesticides. As observed for higher plants, multinuclear cells may also be produced when the diatom cytoskeleton is affected by chemicals or UV light; such effects may result in abnormal migration of the chromosomes during cell division (Grant et al. 1992; El Hajjouji et al. 2007). Moreover, in diatoms exposed to microtubule inhibitors (colchicine, IPC), abnormal mitotic spindles were noted, as were multinuclear cells (Coombs et al. 1968; Coss and Pickett-Heaps 1974).

2.3 The Siliceous Cell Wall

The main character of diatoms is their siliceous cell wall (frustule). Many authors have identified abnormal morphology or disturbed ornamentation for some diatom cells, potentially as a result of exposure to xenobiotics (Feldt et al. 1973; Thomas et al. 1980; Adshead-Simonsen et al. 1981; Fisher et al. 1981; Yang and Duthie 1993; McFarland et al. 1997; Dickman 1998; Gomez and Licursi 2003; Cattaneo et al. 2004; Stoermer 2004; Debenest et al. 2008).

Numerous toxic agents have been reported as potential inducers of diatom cell wall (frustule) abnormalities. In situ studies identified abnormal frustules in samples contaminated by heavy metals (Feldt et al. 1973; McFarland et al. 1997; Dickman 1998). Abnormal forms were also observed within diatom communities exposed to cadmium, copper, mercury, and zinc (Thomas et al. 1980; Adshead-Simonsen et al. 1981; Fisher et al. 1981; Rijstenbil et al. 1994; Ruggiu et al. 1998; Gold et al. 2003; Gomez and Licursi 2003; Cattaneo et al. 2004).

Few publications exist, however, that describe xenobiotic induction of abnormal cell walls. Schmitt-Jansen and Altenburger (2005) reported abnormal forms of diatoms exposed to high concentrations (up to 312 μg/L) of isoproturon, a herbicide widely used in agriculture. The growth regulator, maleic hydrazide, a known genotoxin, has induced abnormal frustules, completely destroying the ornamentation of some (Debenest et al. 2008). Abnormal frustule morphology was also observed in diatoms exposed to microtubule inhibitors such as colchicine (Duke et al. 1977; Edgar and Pickett-Heaps 1984; Van Den Hoek et al. 1995).

Oxidative stress due to radiation or toxic exposure may also be implicated in the induction of frustule abnormalities (Rijstenbil et al. 1994; Rijstenbil 2001).

Environmental factors, such as nutrient deficiencies and pH, may also play a role in the development of abnormal frustules (Dickman 1998). In particular, severe silica deficiencies show evidence of inducing these abnormalities (Thomas et al. 1980; McFarland et al. 1997). Among other mentioned causes for induction of abnormal frustules are the mechanical effects of high cell density in crowded communities (Andresen and Tuchman 1991). According to Stoermer (1998), abnormal frustules may constitute clones of a single cell which suffered a genetic mutation which resulted in cell wall distortion.

The cellular mechanisms involved in the genesis of abnormal forms are still poorly understood. Many authors have suggested that silica deficiency is involved in the synthesis of abnormal frustules (Thomas et al. 1980; McFarland et al. 1997). Such deficiency may also result in different intracellular effects from contact with toxic agents. Many researchers believe that abnormal frustule induction may be linked to a disturbance in silica absorption by cells (Fisher et al. 1981; Rijstenbil et al. 1994; McFarland et al. 1997; Cattaneo et al. 2004). Pollutant-induced membrane alteration may occur and would reduce the absorption of silica. Rijstenbil et al. (1994) offered the hypothesis that copper may potentially produce lipid peroxidation effects on membranes (Rijstenbil et al. 1994).

Another scientific interpretation of silica deficiency would implicate the cytoskeleton (microtubules, actin filaments, and microfilaments), which is known to manage the migration of silica deposition vesicles (SDV) for cell wall synthesis (Pickett-Heaps et al. 1979; Round et al. 1990; Pickett-Heaps 1991; Lee and Li 1992; Van Den Hoek et al. 1995). Operational microtubules are important to the proper synthesis of the frustule (Edgar and Pickett-Heaps 1984). Thus, Debenest et al. (2008) suggested that disturbance of the SDV transporters would lead to poor silica supply and thereby induction of frustule abnormalities. Numerous authors have observed abnormal frustules in diatoms treated with microtubule inhibitors (Coombs et al. 1968; Duke and Reimann 1977; Lee and Li 1992).

3 Effects on Cell Metabolism

The metabolism in diatom cells can be disturbed by toxic chemicals at three different levels: (i) photosynthesis, (ii) fatty and amino acids synthesis, and (iii) nutrients absorption.

3.1 Photosynthesis

Numerous herbicides such as the *s*-triazines and substituted ureas (phenylureas and sulphonylureas) are widely used in agriculture, and exert their action by disrupting photosynthesis. The active constituents of these molecules bind with a protein called the D1 protein, block electron transfer, and thus inhibit a redox reaction: the

Hill reaction (Berard and Pelte 1996; Peres et al. 1996; Dorigo and Leboulanger 2001; Leboulanger et al. 2001; Berard et al. 2003b; Dorigo et al. 2004). A large body of research has been published that concerns the impact of such molecules on algae. It has been observed that atrazine, in a concentration range from 1 to 5 μg/L, affected the photosynthesis of phytoplankton (De Noyelles et al. 1982). At higher concentrations (20, 25, and 500 μg/L), a more pronounced inhibition of photosynthesis was reported (De Noyelles et al. 1982; Weiner et al. 2007). Irgarol, another *s*-triazine that is used as an algaecide in copper-based antifoulant paints for controlling fouling organisms on the hulls of marine vessels, was also confirmed to inhibit algal photosynthetic activity (Dahl and Blanck 1996; Nystrom et al. 2002; Berard et al. 2003b). Subchronic studies with this algacide produced photosynthesis inhibition at low concentrations (0.063–0.25 μg/L) (Dahl and Blanck 1996). Kasai and Hanazato (1995) reported reduced photosynthetic activity in phytoplankton communities exposed to another *s*-triazine chemical, simetryn, but the induced effects occurred at higher concentrations (0.1 and 1 mg/L) than for Irgarol (Kasai and Hanazato 1995). Effects were also produced when diatoms and green algae were exposed to other photosynthesis inhibitors (diquat, hexazinone, and the sulfonylureas such as chlorsulfuron and metsulfuron) (Peterson et al. 1997; Nystrom et al. 1999). A dose-dependent inhibition of photosynthesis was also reported for periphytic algae exposed to high concentrations (8–1800 mg/L) of glyphosate (Goldsborough and Brown 1988).

3.2 Synthesis of Protein, Lipids, and Carbohydrates

Exposure of several species of diatoms to atrazine is known to significantly reduce protein synthesis, including proteins D1 and D2, which play an important role in photosynthesis (Weiner et al. 2007). Nicosulfuron was also reported to inhibit amino acid (valine and isoleucine) synthesis and thereby disrupt protein production in algae (Rimet et al. 1999). Atrazine exposure also induced lipid accumulation in cultured diatoms, this accumulation being also observed in diatoms cultured in nutrient-deficient conditions (Weiner et al. 2007).

Disturbances in carbohydrate synthesis may also occur and may cause loss of mobility in some diatom species (*Hantzschia* sp.) exposed to phenylurea herbicides such as linuron (Pipe and Cullimore 1984). Polysaccharides secreted by these microalgae are known to be involved in their normal mobility (Round et al. 1990; Van Den Hoek et al. 1995; Bertrand 1999). Cohn and McGuire (2000) have thus proposed to use mobility loss as an indicator of toxic exposure.

3.3 Nutrient Absorption

Herbicides also affect the absorption of nutrients (NO_3, NO_2, and Si) by algae. Krieger et al. (1988) have observed that continuous exposure to high concentrations (134 μg/L) of atrazine reduced the absorption of nitrate, nitrite, and

silica by microalgae. It has also been reported that periphytic algae, exposed to atrazine, behaved as do algae cultured under nutrient-deficient conditions (Carder and Hoagland 1998). It appears that atrazine disrupts absorption of nutrients by cells. Nevertheless, temperature played a significant role in this cellular mechanism. Krieger et al. (1988) have noted disturbed absorption of nutrients at 10°C, but not at 25°C, in several periphytons exposed to peak concentrations of four herbicides (alachlor, atrazine, metolachlor, and metribuzin).

An increase of nitrite and nitrate concentrations in the medium was also noted during simetryn exposure of phytoplankton communities. Similar results were reported in other experimental systems when treated with photosynthesis-inhibiting herbicides (e.g., *s*-triazines) (Goldsborough and Robinson 1986; Herman et al. 1986; Gurney and Robinson 1989). Peres et al. (1996) related nitrite and nitrate concentration increases to inhibition of periphytons; such organisms not being able to consume and maintain these nutrients in the upper layers of sediment (Peres et al. 1996). Goldsborough and Robinson (1986) attributed this increase to dead cell degradation, which would release nutrients to the medium. But, these observations are limited to the community level and are thus difficult to extrapolate to the cellular level.

4 Effects on Diatom Growth

4.1 Algal Biomass

Algal biomass is measured by evaluating chlorophyll pigment concentrations, using either classical spectrophotometry or liquid chromatography. The measurement of chlorophyll a is one of the most widely used parameters to assess effects of pesticides on algae growth. Numerous authors have shown that exposing algae, including diatoms, to concentrations of atrazine that ranges from 10 to 1000 μg/L produced a decrease in chlorophyll a concentration (De Noyelles et al. 1982; Kosinski and Merkle 1984; Krieger et al. 1988; Jurgensen and Hoagland 1990; Berard 1996; Guasch et al. 1997, 1998; Tang et al. 1997; Carder and Hoagland 1998). Nevertheless, these results were not consistent with other research studies. Some studies failed to find an impact from exposure to atrazine at a concentration of 25 μg/L on algal biomass. Other studies actually demonstrated an increase in chlorophyll a concentration in algae communities exposed to concentration ranges from 10 to 32 μg/L of either atrazine or to atrazine mixed with nicosulfuron (30 μg/L for each molecule) (Lynch et al. 1985; Tang et al. 1997; Seguin et al. 2001a, b). These results are unexpected; the increase in chlorophyll a content may be related to moderate exposure to herbicides, cells being able to maintain sufficient photosynthetic activity (Seguin et al. 2001a). Other herbicides that target photosynthetic activity, such as simetryn or isoproturon, disrupted the development of algal biomass at high concentrations (100–1000 μg/L for simetryn and 40–312 μg/L for isoproturon) (Kasai and Hanazato 1995; Schmitt-Jansen and Altenburger 2005).

At a lower concentration (20 μg/L) of isoproturon Schmitt-Jansen and Altenburger (2005) did not report algal biomass inhibition, whereas Peres et al. (1996) observed effects on biomass with the same molecule at a lower concentration (5 μg/L). In this last study, results covered only diatoms, without taking into account other algal classes (cyanophytes and chlorophytes). Schmitt-Jansen and Altenburger (2005) observed development of green algae at an intermediate concentration (20 μg/L). These algae could be more tolerant to this herbicide than are diatoms. Therefore, no effects were observed on global biomass, increases in green algal biomass apparently compensating for any inhibition of diatom biomass growth.

4.2 Diatom Cell Density

Diatom cell density response patterns, following pesticide exposure, are generally difficult to discern. Many authors have shown that atrazine exposure may decrease cell density in some centric diatom species, and do just the opposite for some pennate diatom species (Tang et al. 1997; Berard and Benninghoff 2001; Berard et al. 2004). For one diatom species (*Cocconeis placentula*) exposed to another *s*-triazine herbicide (simetryn), Goldsborough et al. (1986) observed an increase in cell density, whereas Kasai et al. (1995) reported noxious effects of this herbicide on phytoplankton. Isoproturon, even at low concentrations, had a strong effect on diatom cell density (Peres et al. 1996).

5 Effects on Species Composition

An exposure to a single pesticide, or a mixture of pesticides, may selectively disturb some species more than others and thereby disturb the balance within the community. Toxic agents such as atrazine may reduce the ability of some species to develop, even if the effects are not readily visible, and thus may favor the more tolerant species in an ecosystem (Berard and Pelte 1996).

5.1 Diatom Species

Generally, herbicide exposure alters the diversity of diatom communities. A decrease in diversity in such communities was observed from exposures to atrazine (10–90 μg/L) (Hamala and Kollig 1985; Berard and Pelte 1996; Berard et al. 2004) and isoproturon (40–160 μg/L) (Peres et al. 1996; Schmitt-Jansen and Altenburger 2005). At a lower concentration (2 μg/L), Schmitt-Jansen and Altenburger (2005) reported an increase in diversity, probably from development of tolerance by certain species, and the presence of sensitive species that had not yet disappeared.

In benthic diatom communities, eutrophic species tend to be more tolerant to herbicides than certain others. Survival of species, e.g., *Achnanthes lanceolata frequentissima*, *Achnanthes minutissima*, *Asterionella formosa*, *C. placentula*, *Fragilaria capucina* var. *vaucheriae*, *Gomphonema parvulum*, *Nitzschia palea*, *Navicula lanceolata* and *Synedra acus*, was favored in communities exposed to *s*-triazine herbicides (atrazine and Irgarol), either under controlled (Hamala and Kollig 1985; Goldsborough and Robinson 1986; Berard 1996; Munoz et al. 2001; Berard et al. 2003a) or natural conditions (Guasch et al. 1998; Berard et al. 2003b). *A. lanceolata frequentissima* and *N. palea* were known for their tolerance to atrazine (Kosinski and Merkle 1984; Kasai 1999; Downing et al. 2004). Similar results were reported in communities exposed to isoproturon; eutrophic species such as *Navicula cryptocephala*, *N. Halophila*, *N minima* and *G. parvulum* being more abundant (Peres et al. 1996; Schmitt-Jansen and Altenburger 2005).

Results from numerous studies performed under controlled conditions disclosed that exposure to a herbicide, such as atrazine or isoproturon, promoted the development of smaller organism size in the following species: *A. lanceolata frequentissima*, *A. minutissima*, *Achnanthidium minutissimum*, *C. placentula*, *Navicula minima*, and *Sellaphora seminulum* (Goldsborough and Robinson 1986; Peres et al. 1996; Munoz et al. 2001; Seguin et al. 2001a; Schmitt-Jansen and Altenburger 2005). Several interpretations have been proposed to explain this response. Numerous authors believe that small species are pioneers in colonizing pebbles and all surfaces which permit the development of benthic algae (Korte and Blinn 1983; Sekar et al. 2004). The species' survival potential is based on high growth rate, which allows them to colonize substrates (pebbles, rocks, etc.) and other natural surfaces earlier than certain other species, even under unfavorable conditions such as the presence of toxic pollution (Goldsborough and Robinson 1986; Peres et al. 1996). The colonization pattern of substrates continues to be under discussion in the scientific community (Acs and Kiss 1993). Another explanation could be related to the availability of cell defenses against oxidative stress among the smaller species. Rijstenbil (2001) reported that concentrations of gamma-glutamylcysteinylglycine (GSH), an antioxidant molecule, are 5–10 times higher in smaller than in larger species.

The trophic mode of diatom species also influences their sensitivity to herbicides. Some authors noticed that N-heterotrophic species such as *N. halophila* or *N. minima* were tolerant to photosynthesis inhibitors (simazine, terbutryn, and isoproturon) (Goldsborough and Robinson 1986; Peres et al. 1996). Similarly, Hamala and Kollig (1985) observed that periphytic algae exposed to another well-known photosynthetic inhibitor (atrazine) demonstrated an increase in heterotrophic activity. Some diatom species are able to switch their principal trophic modes (autotrophy) when the environmental conditions are unfavorable to photosynthesis (Hellebust and Lewin 1977). This character would explain their relative tolerance to photosynthesis inhibitors (Hamilton et al. 1988).

5.2 Algal Community

A large body of research has been performed that emphasizes the effects of herbicides such as atrazine on the species composition of algal communities. Berard and Benninghoff (2001) showed that exposure to 10 μg/L of atrazine modified algae community structure. The different algae genera did not present the same sensitivity to this herbicide. Several studies, carried out on periphytic communities, highlighted a shift in the algae composition of communities in support of diatoms (Goldsborough and Robinson 1986; Guasch et al. 1997, 1998). Numerous publications have reported that these microalgae are more tolerant to photosystem II inhibitors (*s*-triazines and phenylurea) (Kosinski and Merkle 1984; Gurney and Robinson 1989; Molander and Blanck 1992; Hoagland et al. 1993; Kasai 1999; Dorigo et al. 2004). Some authors have reported that green algae (chlorophyceae and chrysophyceae) and cyanobacteria are between 4 and 10 times more sensitive than are diatoms to these herbicides (Hoagland et al. 1993; Tang et al. 1997; Guasch et al. 1998; Guasch and Sabater 1998; Navarro et al. 2002; Lockert et al. 2006). Similar observations were made on phytoplankton communities exposed over the long term to metazachlor (Mohr et al. 2008).

The higher tolerance of diatoms may be linked either with the capacity of some species to change trophic mode or with the nature of pigments in diatoms (Plumley and Davis 1980; Hamilton et al. 1988; Peres et al. 1996). Diatoms contain carotenoids and xanthophylls. Several authors reported that these pigments have antioxidant properties, which would enhance the potential tolerance to oxidative stress caused by pesticides (Rijstenbil et al. 1994; Pinto et al. 2003). In this context, herbicide effects on algae community depend on the species composition at the beginning of the exposure period (Herman et al. 1986).

6 Interferences in the Response of Diatoms to Pesticide Exposure

Many ecological and environmental parameters may interfere when diatoms come into contact with pesticides. Thus, under natural conditions, the potential ecological disturbances observed in a population are often difficult to relate to pesticide exposure, with certainty, because, the complex biological matrix (biofilm), where benthic diatoms evolve, may protect these algae against pesticide effects (Peres et al. 1996). Similarly, the dynamics of colonization and the evolution of biofilms have an impact on the response of benthic diatom communities to toxic agent exposures such as atrazine (Guasch et al. 1997). This protective layer may be disturbed by invertebrate or fish grazers, which also affect cell structure and promote toxic effects (Munoz et al. 2001). Nevertheless, the real impact of these grazers on diatom response to toxic exposure remains quite difficult to evaluate. Without protective layers, planktonic diatoms have also developed mechanisms to escape from a toxic environment by deep-diving (Rijstenbil 2001).

Environmental parameters (light exposure, nutrient concentrations, etc.) also interfere in the responses of algal communities to pesticides (Guasch et al. 1997; Guasch and Sabater 1998; Berard and Benninghoff 2001; Navarro et al. 2002). Thus, several scientific studies showed that diatoms were more sensitive to atrazine during light exposure (Guasch et al. 1997; Guasch and Sabater 1998). In the context of light, the response of algae depends on the season of study and on the site where samples were taken. Navarro et al. (2002) noted lower tolerance to atrazine for periphytic communities during the summer. The opposite results were obtained in phytoplankton communities exposed to the same herbicide (Berard and Pelte 1996). Nutrient concentrations also affect the sensitivity of algae to herbicides. Lin et al. (2005) reported that deficiencies in nitrogen and phosphorus may increase the sensitivity of algae (Lin et al. 2005). Under field conditions, the spatial and temporal variations of nutrient concentrations must interfere in the response of algae to toxic agents, especially in the case of agricultural watersheds, where high water pollution by nitrates occurs (Berard and Benninghoff 2001; Navarro et al. 2002).

7 Summary

The study of pesticide effects on algae, and diatoms in particular, was focused on photosynthesis and biomass growth disturbances. Few studies have been performed to investigate the effects of these toxic agents on intracellular structures of diatom cells. Nuclear alterations and cell wall abnormalities were reported for diatoms exposed to toxic compounds. Nevertheless, the cellular mechanisms implicated in the development of such alterations and abnormalities remain unclear. Sensitivity to pesticides is known to be quite different among different diatom species. Eutrophic and small species are recognized for their tolerance to pesticides exposure. More pronounced cell defenses against oxidative stress may explain this absence of sensitivity in species of smaller physical size. Notwithstanding, on the whole, explaining the rationale behind tolerance variations among species has been quite difficult, thus far. In this context, the understanding of intracellular toxicity in diatoms and the relation between these intracellular effects and the disturbance of species composition in communities represent a key target for further research.

The original community species structure determines the response of a diatom community to toxic agent exposure. Diatom communities that have species capable of switching from autotrophic to heterotrophic modes, when photosynthesis is inhibited (e.g., after pesticide exposure), can continue to grow, even in the presence of high pesticide pollution. How diatoms respond to toxic stress, and the degree to which they respond, also depends on cell and community health, on ecological interactions with other organisms, and on general environmental conditions. The general structural parameters of diatom communities (biomass, global cell density) are less sensitive to pesticide effects than are the specific structural parameters of the unicellular organisms themselves (cell density by species, species composition). For benthic species, biofilm development and grazing on this matrix as a source of food for invertebrates and fishes may also modify the response of diatom communities.

Environmental parameters (light exposure, nutrient concentrations, and hydraulic conditions) affect, and often interfere with, the response of diatoms to pesticides. Therefore, the complexity of aquatic ecosystems and the complexity of pesticide pollution in stream water (many molecules in interaction) do not permit researchers to easily detect the effects of such pollutants on diatoms. Clearly more research will be required to address this problem.

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The Toxic Effects of Formaldehyde on the Nervous System

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Contents

1 Introduction

1.1 Physical and Chemical Features

Formaldehyde (FA) (formula: HCHO; IUPAC name: metanal) is a member of the aldehyde family and is one of the simplest organic molecules. FA is an irritating, colorless gas that has a pungent smell (Franklin et al. 2000; Smith 1992; Songur et al. 2003; Yamato et al. 2005). It is rarely found in its original state because it has a short half-life in air and decomposes in light to form a toxic substance. FA is highly soluble in water, as well as in most organic solvents, and is a highly reactive molecule that can be irritating to tissues through direct contact. Furthermore, FA causes cytotoxicity through the formation of strong DNA–protein cross-links, as well as cross-links with other molecules, e.g., amino acids (Cheng et al. 2003; Gurel et al. 2005; Metz et al. 2004).

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology*,
Reviews of Environmental Contamination and Toxicology 203,
DOI 10.1007/978-1-4419-1352-4_3,

FA is easily absorbed through the respiratory and gastrointestinal tracts, is metabolized to formic acid (formate) in the nasal mucosa, liver, and erythrocytes of living organisms, and is then excreted in the urine and feces, or is converted into carbon dioxide and exhaled. There are at least seven enzymes that catalyze the oxidation of FA in animal tissues, namely aldehyde dehydrogenase, xanthine oxidase, catalase, peroxidase, aldehyde oxidase, glyceraldehyde-3-phosphate dehydrogenase, and a specific NAD-dependent formaldehyde dehydrogenase (FDH) (Cooper and Kini 1962; Gurel et al. 2005; Solomons and Cochrane 1984). During this latter reaction, the FDH enzyme requires glutathione as a cofactor. Therefore, inhaled FA certainly affects liver metabolism. FA is a naturally occurring metabolite that is found in varying degrees within cells; however, FA cannot be stored in cells (Barber and Donohue 1998; Sogut et al. 2004).

The binding of FA to proteins and nucleic acids, subsequent to being metabolized, is known as *metabolic binding*. Inhaled FA rapidly forms covalent bonds, through several metabolic pathways, with intracellular DNA, RNA, and protein pools, and these interactions underlay the toxic effects of FA. The direct binding reaction without metabolic breakdown, generally in nasal mucosa, is called *irreversible binding* and results in necrosis, allergy, and mutagenicity in living organisms (Upreti et al. 1987). The gaseous form of FA, at concentrations greater than 6 ppm (part per million), causes injuries and cellular denaturation in the nasal mucosa. For this reason, FA concentrations of 6 ppm or greater have been accepted as the *cytotoxic concentration* for nasal mucosa in rats (Morgan 1997).

1.2 Sources and Uses

FA is produced and used worldwide on a large scale, predominately in industry, for the production of resins, manufacturing of building materials, and as a component of numerous household products. FA is found in nature, domestic air (e.g., sourced from paint, insulating materials, chipboard and plywood, fabrics, furniture, paper), cosmetics, cigarette smoke, and in the polluted atmosphere of cities from the incomplete combustion of organics, photochemical smog, and off-gassing of products containing FA (Aslan et al. 2006; Franklin et al. 2000; Smith 1992; Songur et al. 2008; Usanmaz et al. 2002).

FA is widely found in workplaces. Occupational exposure to FA mainly results from its presence in amino and phenolic resins used in several products, such as plastics, varnishes, and glues. FA is also used as a component in sanitizing products, histological fixative products, and embalming fluids, and serves as an intermediate in chemical synthesis. Occupational exposure databanks (OEDBs) have been described previously as potential sources of FA data for exposure surveillance or occupational epidemiology (Goldman et al. 1992; LaMontagne et al. 2002). From the foregoing, one can observe that nearly all humans, including susceptible children, may be affected by FA exposure.

FA is also an important public health problem, because cigarette smoke contains FA (Tox Probe 2002). In a study conducted in the US, six different brands

of cigarettes were examined and the presence of FA was found at rates of 45.2–73.1 mg/cigarette and 5.1–8.9 mg/puff (Mansfield et al. 1977). In another study, it was reported that the level of FA in side-stream cigarette smoke is 50 times higher than exists in mainstream smoke (Triebig and Zober 1984). The National Research Council (1986) estimates that there is 5–8 times more formaldehyde in side-stream smoke than in mainstream cigarette smoke (Tox Probe 2002). Smoking is common worldwide, and thus, cigarette smoking can be considered as an important factor for both indoor and outdoor FA release.

In addition, FA is widely used in both industrial and medical settings, and as a sterilizing agent, disinfectant, and preservative. Therefore, employees in these setting may be at risk for high levels of exposure to FA. In particular, anatomists, histologists, pathologists, and medical students are the individuals most frequently exposed to FA gas in dissection lectures and laboratories (Cohen et al. 1998; Sarnak et al. 1999). Epidemiological studies of industrial workers, embalmers and pathology anatomists have associated FA exposure with elevated risks for cancers at various sites, including the brain (Coggon et al. 2003; Hayes et al. 1990), nasal cavities (Blair et al. 1990; Coggon et al. 2003), lung (Coggon et al. 2003; Gardner et al. 1993), pancreas (Stone et al. 2001), and lymphohematopoietic system (Hauptmann et al. 2003; Pinkerton et al. 2004). However, these positive findings may have been confused by concomitant exposures and remain controversial.

1.3 Harmful Effects

It is accepted that FA is toxic and slightly carcinogenic over certain concentrations, and the harmful effects of FA increase under room temperature conditions, because the molecule easily evaporates (Feron et al. 1991; Franklin et al. 2000; Gurel et al. 2005; Ozen et al. 2002; Smith 1992; Songur et al. 2003). FA is thought to have weak carcinogenic effects through the formation of protein cross-links and the promotion of cell proliferation in the human respiratory tract. According to the United States National Toxicology Program (US-NTP), European Union (EU), and International Agency for Research on Cancer (IARC), FA is classified as a weak genotoxic, probable carcinogenic agent for humans (category 3). Exposure to FA is a predisposing factor for the occurrence of cancer in the nasal cavity, paranasal sinuses, and leukemia. According to the World Health Organization (WHO), FA does not have a high carcinogenic potential in humans, but inhalation of FA may be linked to nasal or nasopharyngeal cancers (Binetti et al. 2006).

In our studies, FA inhalation was observed to cause a reversible decrease in food and water consumption, and in body weight (and body weight gain) in rats (Ozen et al. 2002, 2003; Songur et al. 2003; Zararsiz et al. 2006). Furthermore, Martin (1990) and Saillenfait et al. (1989) found that inhalation of FA (10–40 ppm) during the gestation period caused a reduction in maternal food consumption, a decrease in body weight gain, and lower birth weight of pups. These toxic effects may occur by central inhibition or most likely from inhibition of nucleic acid and protein synthesis (Ozen et al. 2003).

After administration, FA rapidly diffuses to many tissues, including the brain. In a postmortem study, FA and the metabolites, methanol and formic acid, were found at similar concentrations in the brain (Nishi et al. 1988). The inhaled FA gas had negative effects on the central nervous system (CNS), which appeared acutely in the form of headache, malaise, insomnia, anorexia, and dizziness (Harris et al. 1981; Solomons and Cochrane 1984). There is a relationship between indoor FA concentrations and the sick building syndrome, which is a form of multiple chemical sensitivities (Kim et al. 2002; Sari et al. 2004, 2005). Long-term exposure (e.g., 14–30 year) to FA may cause irreversible neurotoxicity (Kilburn 1994), and is related to neurodegenerative disorders and brain cancer (astrocytoma) (Stroup et al. 1986). In addition, inhaled FA has been shown to cause behavioral and memory disorders in rats and has been classified as "probably neurotoxic" (Pitten et al. 2000).

FA may be found in the cerebrospinal fluid (CSF), since this compound easily passes through the blood–brain barrier, and would thus affect neuroglial and nerve cells (Malek et al. 2003). A recent study indicated that concentrations of FA exceeding 1 ppm may occur in anatomy dissection laboratories, which is a potential problem for medical and dental students (Kawamata and Kodera 2004; Kunugita et al. 2004). Past regulatory amendments lowered the permissible exposure level of 1 ppm for FA to 0.75 ppm as an 8-hr time-weighted average (U.S. Department of Labor Occupational Safety & Health Administration 1992).

In this review, we compile the literature that concerns the neurotoxic effect of FA on neuronal morphology, behavior, and biochemical parameters.

2 Formaldehyde Neurotoxicity

2.1 The Effect on Various Biochemical Parameters

The first response to a toxic agent is at the chemical level, and morphological changes are observed as damage continues. In our studies on FA neurotoxicity, FA was observed to affect cerebral oxidant/antioxidant systems and cause oxidative damage. Although reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anion, and hydroxyl radical, are essential for many normal biological processes and are produced physiologically, the excessive production and accumulation of ROS can become hazardous to cells and tissues (Bas et al. 2007; Gurel et al. 2005; Sarsilmaz et al. 2003; Tian et al. 2005). ROS are important mediators of cellular injury, play a role in oxidative stress, and can contribute to a variety of diseases, or be present in situations where toxicity is produced. ROS-initiated oxidative stress can be regulated by cellular defense mechanisms, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Halliwell 1997). The brain has a high content of easily peroxidizable unsaturated fatty acids and requires very high amounts of oxygen per unit weight. Additionally, the rates of oxidative metabolic activities in the brain are relatively high and antioxidant enzymes activities are low in the brain. Therefore, the neurons

are more vulnerable to toxic or ischemic occurrences in the CNS (Irmak et al. 2003; Tian et al. 2005).

We performed a study in this topic area and observed that exposure to FA during the adult period (10 mg/kg, 10 days, intra peritoneal (ip)) caused an increase in oxidant substances, such as malondialdehyde(MDA) and protein carbonyl (PC), but resulted in a decrease in the activity of antioxidant enzymes, such as SOD and CAT, in the rat frontal cortex and hippocampus (Gurel et al. 2005). In another study, we found that exposure to FA under similar conditions (10 mg/kg, 14 days, ip*)* led to an increase in the MDA level and a decrease in the activity of SOD and GSH-Px in the rat prefrontal cortex (Zararsiz et al. 2006, 2007). Inhalation of FA during the early postnatal period was also found to cause an increase in the activity of GSH-Px and levels of MDA and NO, and a decrease in t-SOD activity at postnatal day (PND) 30, in the rat cerebellum. In general, the results of FA exposure to rats at PND 90 were similar to those at PND 30. Additionally, we observed that the effect of FA on cerebellar oxidant/antioxidant systems increased in a dose-related manner and continued for a long time (Songur et al. 2008). An increase in MDA levels, which is one of the common findings of our three studies, is an indication of lipid peroxidation and neuronal membrane injury. Thus, fluidity of cell membranes and cell compartmentation is disrupted, and eventually the cell is lysed, if injury is not prevented (Datta and Namasivayam 2003). Exposure to FA has also been demonstrated to lead to an increase in lipid peroxidation products in different tissues (Tang et al. 2003; Teng et al. 2001), and our studies confirm these results. Decreases in GSH-Px and SOD activities provide evidence that these enzymes have acted to protect cells from increased oxidative events. Furthermore, these activities provide evidence for the involvement of glutathione, since FA is metabolized by FDH and this enzyme is dependent on glutathione. Therefore, we postulate that FA causes oxidative damage as a general toxic effect. Also, FA neurotoxicity may be mediated by the activation of free radical producing enzymes and by the inhibition or expenditure of free radical scavenger systems, thereby enhancing the production of ROS.

We have also investigated the effect of FA on certain cerebral trace elements. Subacute (4 week) or subchronic (13 week*)* inhalation of FA (6 and 12 ppm*)* was discovered to lead to a time- and concentration-dependent increase in zinc (Zn) and copper (Cu) levels, and also resulted in a decrease in iron (Fe) levels in the rat cerebral cortex. Zn, Cu, and Fe are involved in important chemical processes in the brain, and levels of these elements in the cerebral tissue reveal the condition of cerebral functions (Ozen et al. 2003). As Zn and Cu are the prosthetic groups of SOD, which is an antioxidant enzyme, elevated levels of these elements in tissue may portend the action of SOD. Therefore, we may regard these elevated levels as an indicator, which confirms the decrease of SOD that was detected in the aforementioned studies (Gurel et al. 2005; Zararsiz et al. 2006, 2007). FDH is utilized in the detoxification of FA in the cerebral cortex. Since FDH requires glutathione and NAD+ as cofactors, the excessive use of FDH results in the utilization of glutathione, which indirectly results in oxidative damage. Oxidative stress coupled to elevated Fe levels may cause negative effects on cerebral cortex (Ozen et al. 2003).

According to the literature, FA is a highly reactive compound and easily reacts with the amino acid residues of proteins. A postulated mechanism for FA neurotoxicity is the production of epoxides, which bind to axonal neuro(micro)filaments, rendering these filaments nonfunctional. Axons then swell, and as axonal neurofilaments are implicated in rapid axonal transport of proteins, axonal transport becomes progressively abnormal (Kilburn 1994). The resulting hydroxymethyl derivatives react with nucleophilic groups and form methylene bridges, resulting in the formation of intramolecular and intermolecular bonds. These bonds not only result in changes in protein function, but also cause changes in polypeptide structure and physiochemical features (Kilburn et al. 1987). The previously mentioned studies by Kilburn are highly significant, since these are among the first studies to explore FA neurotoxicity.

2.2 The Effect on Neuronal Morphology

In a previous study that addressed FA neurotoxicity, inhalation of FA at concentrations of 6 and 12 ppm, during the early postnatal period (PND30), resulted in an increase in pyknotic neuron counts in the rat hippocampus pyramidal cell layer. These increases, in the tested rats, were most significant in the CA3 area and continued throughout the PND60 period. However, there were no significant changes in the PND90 group of rats (Songur et al. 2003). In this same study, FA inhalation also resulted in an increase in immunostaining of heat shock protein 70 kDa (*Hsp70*) in the rat hippocampus, particularly at the exposure concentration of 12 ppm, and for the PND30 group rats. In contrast, Hsp70 immunostaining decreased in the PND60 and PND90 rat groups (Songur et al. 2003). Hsp70 is an intracytoplasmic molecular chaperone that helps repair, connect, and transport proteins. Hsp70 is a component of the cytoprotectant system, which protects the cell in response to cellular damage and stressful perturbations. The increase in Hsp70 production indicates that the cells were exposed to a toxic agent and cellular defense mechanisms were activated (Gilby et al. 1997).

It was observed, in a stereological study performed to confirm the aforementioned investigation, that FA inhalation at 12 ppm concentration during the early postnatal period caused a reversible decrease in the volume of cerebral hemispheres and in the hippocampal pyramidal cell layer. Additionally, FA inhalation caused a decrease in the total pyramidal neuron counts in hippocampal CA regions. The decrease was evident in both PND30 and PND90 group rats, and thus, the damage appeared to be permanent (Sarsilmaz et al. 2007). In comparison to these results, the volumes of the dentate gyrus (DG) were observed to significantly increase in the rat brain after inhalation exposure to both 6 and 12 ppm FA for the PND30 group. This increase in DG volume was also observed at the 6 ppm FA inhalation level for the PND90 rat group. Furthermore, exposure to 12 ppm FA inhalation for the PND90 group caused a decrease in the total number of granular cells of the DG, in comparison to the control group and the 6 ppm FA inhaled rat groups (Aslan et al. 2006).

Drawing on the results of these three studies, FA inhalation, during the early postnatal period at cytotoxic concentrations, appears to result in an increase in apoptosis,

a decrease in neuronal development, and damage to the hippocampal formation. Generally, this damage is positively correlated with the dose and is morphologically reversible. The observed increase in DG volume could be a result of the high rate of neuronal generation in the DG during the early weeks of postnatal life. It could also constitute the neurotoxic effects of FA, which might trigger inflammation of the DG, resulting in a volume increase. The reduction in granule cell number (12%) at the 12 ppm FA inhalation level, compared to the 10% increase in neuronal number at the 6 ppm level, in the PND90 groups, may represent neurogenesis stimulation at the 6 ppm dose, whereas the 12 ppm dose might impair generation of new neurons. Some neuroprogenitor cells are found in the DG, as seen in the subventricular zone and in the olfactory bulb. These cells may also contribute to neuronal formation in response to brain damage (Aslan et al. 2006; Gould et al. 1998; Jin et al. 2004; Lie et al. 2004; Lucassen et al. 2004; Ohnuma and Harris 2003). In particular, granular cells of the DG are more sensitive to FA toxicity and may display a latent neurotoxic effect after FA exposure. If so, this would support the hypothesis that exposure to toxic agents during childhood may lead to diseases later in life.

In another study, it was revealed that exposure to FA (10 mg/kg, 10 days, ip) increased pyknosis and decreased neuronal number in the adult rat frontal cortex and hippocampus (Gurel et al. 2005). Furthermore, FA administration under similar conditions increased apoptosis in the rat prefrontal cortex and caused an increase in the immunoreactivity of Bax, which is a pro-apoptotic protein (Zararsiz et al. 2006, 2007). The Bax protein induces cytochrome C release from the mitochondrial membrane to the cytoplasm, which initiates the apoptotic process through activation of caspases in the cytoplasm (Zararsiz et al. 2006, 2007).

Sorg et al. reported the effect of exposure to repeated low-level formaldehyde on the corticosterone level in rats (Sorg et al. 2001). Sari et al. (2004) found that chronic exposure to low levels of formaldehyde in rats caused an increase in the number of CRH-ir neurons in the hypothalamus (PVN) and ACTH-ir cells in the pituitary gland, with an increase in ACTH-mRNA expression in a dose-dependent manner. In view of these results, FA inhalation was suggested to increase activity of the HPA axis so as to mitigate FA neurotoxicity (Sari et al. 2004).

FA forms strong bonds with proteins and nucleic acids, and the neurotoxic effect of FA is postulated to be a result of the formation of epoxide products, as well as molecular binding that renders axonal neurofilaments nonfunctional (Kilburn 1994). The reason for the noted augmentation of gray matter injury, after FA exposure, may be that there is less FA dehydrogenase in the neural gray than white matter, and almost none in the neural perikarya (Keller et al. 1990).

2.3 The Effect on Behavior

Although FA studies have not focused on the behavioral effects, several symptoms of associated disorders have been observed during studies of FA-exposed rats, such as lethargy, decrease in motor activity, and loss of appetite (Ozen et al. 2003; Songur et al. 2003; Zararsiz et al. 2006, 2007).

There have been reports indicating extensive neurobehavioral impairments, such as malaise, headache, indigestion, balance dysfunctions, sleep disorders, as well as mental and memorial disorders from FA exposure (Kilburn et al. 1987; Kilburn 1994). Moreover, reports of severe fatigue, thirst, convulsion, irritability, lethargy, memory loss, behavioral, and sensory-emotional disorders of people working in industrial areas, who were regularly exposed to FA, are further indicators of FA neurotoxicity (Kilburn 1994; Kilburn et al. 1987). In certain experiments with FA inhalation in rats, FA-exposed animals exhibited a pronounced impairment in open field, maze trail performance, and in CNS function (Kilburn 1994; Malek et al. 2003; Pitten et al. 2000). A labirent test with FA-exposed rats (>2.6 ppm, 10 min/day, 90 days) demonstrated an influence on food-finding abilities, such as a decrease in overall success, increases in food-finding time, and increases in mistakes (Pitten et al. 2000). Inhalation of FA at 11 ppm for 7 days or 1 ppm for 20 days was observed to cause an increase in cocaine-induced locomotor activity and a conditioned fear response to odor (Sorg and Hochstatter 1999), suggesting that FA may cause chemical encephalopathy.

During biochemical and histopathological studies of FA neurotoxicity, disorders linked to FA exposure were related to the duration of exposure and dose, but these disorders were morphologically reversible. However, morphological changes do not correlate with behavioral changes at all times. Therefore, it is not a rule that morphological changes do not necessarily induce behavioral changes, or changes in behavior do not inevitably result in morphological changes. Furthermore, FA exposure during the early postnatal period may lead to disorders and behavioral changes in adults (Ladefoged et al. 1991; Pryor 1991; Slomianka et al. 1992).

3 Conclusion

The reviewed studies have indicated that FA induces several characteristics of neurotoxicity, in addition to systemic toxic effects. The neurotoxic effects produced by FA become more pronounced with increases in concentration and exposure duration, though this is not always the case. Additionally, FA-produced neurotoxicity may vary among different species of organisms and exposure concentrations. The neurotoxic effects in FA studies with animals are extremely pronounced and occurred at concentrations exceeding those that would be acceptable for human studies. Notwithstanding, neurotoxic effects in humans from FA exposure occur at lower concentrations than in rodents, because of different nasal structure and respiration characteristics between the species. It is known that the basis for many psychological diseases in adult humans is dependent on factors that occurred prenatally or during the early postnatal period (Lemaire et al. 2000; Schmitz et al. 2002; Slomianka et al. 1992). Therefore, it is hypothesized that inhalation of FA during the early postnatal period may well predispose to certain neurological diseases in adults.

Complete prevention of FA exposure is impossible for anatomists, histologists, pathologists, medical/dental students, and members of industries utilizing FA.

However, the following suggestions may decrease and/or prevent the systemic and neurotoxic effects from FA exposures that do occur:

1. In anatomy and pathology laboratories, the FA exposure concentration must be maintained so that it is beneath the legal limit. To achieve this, periodic measurement of FA concentrations is required, ambient humidity and temperature must be lowered, air conditioners with special filters must be used, and the ventilation of laboratories must be monitored. Moreover, novel mechanisms must be used to eliminate or reduce FA concentrations, care must be taken during the preparation of FA solutions, and dissections should be performed on downdraft ventilated dissection tables that remove or direct FA vapors down and away from the user.
2. Less toxic novel preservative techniques (e.g., plastination) that meet users' requirements must be developed.
3. Formaldehyde-free household products are preferred and should be offered, when possible. Wood, porcelain, marble, and natural fibers should be used instead of chipboard, melamine, synthetic fibers, and plastics. Products that are released into the atmosphere as FA, such as quaternium 15, dimethyloldimethyl (DMDM), hydantoin, imidiazolidinyl urea, diazolidinyl urea, and bronopol, should not be used. The rate of formaldehyde release depends on the type of resin contained in the product. The use of products made from wood that contains phenol resins, instead of urea resins, will result in a decrease in FA inhalation. Interior paints and materials, which are manufactured using nanotechnology and do not contain FA, are preferred.
4. Temperature and humidity (30–50%) inside homes must be kept at low levels through the use of air conditioners and dehumidifiers. Houses must be ventilated, especially when new items that contain FA are placed inside the house.
5. FA is a component of tobacco smoke; therefore, smoking indoors should not be allowed.
6. Liquid petroleum gas (LPG) catalytic heaters should not be used without venting pipes.
7. Clothes are treated with FA to help make them wrinkle-resistant. Such clothing should be laundered prior to use.
8. The intake of antioxidants and/or neuroprotective agents (e.g., melatonin, fish omega-3 fatty acids, vitamins E and C, erdosteine, or caffeic acid phenethyl ester (CAPE)) is recommended for individuals who are exposed to FA in their work environment.

4 Summary

Formaldehyde (FA) is found in the polluted atmosphere of cities, domestic air (e.g., paint, insulating materials, chipboard and plywood, fabrics, furniture, paper), and cigarette smoke, etc.; therefore, everyone and particularly susceptible children may

be exposed to FA. FA is also widely used in industrial and medical settings and as a sterilizing agent, disinfectant, and preservative. Therefore, employees may be highly exposed to it in these settings. Of particular concern to the authors are anatomists and medical students, who can be highly exposed to formaldehyde vapor during dissection sessions. Formaldehyde is toxic over a range of doses; chances of exposure and subsequent harmful effects are increased as (room) temperature increases, because of FA's volatility.

Many studies have been conducted to evaluate the effects of FA during systemic and respiratory exposures in rats. This review compiles that literature and emphasizes the neurotoxic effects of FA on neuronal morphology, behavior, and biochemical parameters. The review includes the results of some of the authors' work related to FA neurotoxicity, and such neurotoxic effects from FA exposure were experimentally demonstrated. Moreover, the effectiveness of some antioxidants such as melatonin, fish omega-3, and CAPE was observed in the treatment of the harmful effects of FA.

Despite the harmful effects from FA exposure, it is commonly used in Turkey and elsewhere in dissection laboratories. Consequently, all anatomists must know and understand the effects of this toxic agent on organisms and the environment, and take precautions to avoid unnecessary exposure.

The reviewed studies have indicated that FA has neurotoxic characteristics and systemic toxic effects. It is hypothesized that inhalation of FA, during the early postnatal period, is linked to some neurological diseases that occur in adults. Although complete prevention is impossible for laboratory workers and members of industries utilizing FA, certain precautions can be taken to decrease and/or prevent the toxic effects of FA.

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Dermal Toxicity and Environmental Contamination: Electron Transfer, Reactive Oxygen Species, Oxidative Stress, Cell Signaling, and Protection by Antioxidants

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology*,
Reviews of Environmental Contamination and Toxicology 203,
DOI 10.1007/978-1-4419-1352-4_4,

1 Introduction

Many papers have addressed the role of electron transfer (ET) (electron movement from one site to another), reactive oxygen species (ROS), and oxidative stress (OS) in producing cellular insults and, thereby, toxicity in major organs. The present review provides evidence for the same mechanistic theme as it applies to skin toxicants.

Insults to the skin may be mild, serious, or lethal. Various constituents of the skin may be affected by dermal toxicants. Cutaneous damage may also result from inhalation or ingestion of toxins, in addition to direct skin contact. Similarly, substances that induce toxicity through absorption by the skin can also migrate to and adversely affect other organs.

The preponderance of bioactive substances or their metabolites incorporates ET functionalities that, we believe, play an important role in physiological responses. The main groups of such cogent bioactive substances include quinones (or phenolic precursors), metal complexes (or complexors), aromatic nitro compounds (or reduced hydroxylamine and nitroso derivatives), and conjugated imines (or iminium species). In vivo redox cycling with oxygen may occur and give rise to OS through generation of ROS, such as hydrogen peroxide, hydroperoxides, alkyl peroxides, and diverse radicals (hydroxyl, alkoxyl, hydroperoxyl, and superoxide (SO)). In some cases, ET results in interference with normal electrical effects, e.g., in respiration or neurochemistry. Generally, active entities possessing ET groups display reduction potentials (a measure of ease of electron uptake) in the physiologically responsive range, i.e., more positive than 0.5 V. ET, ROS, and OS have been increasingly implicated in the mode of action of drugs and toxins, e.g., anti-infective agents (Kovacic and Becvar 2000), anticancer drugs (Kovacic and Osuna 2000; Kovacic and Somanathan 2007), carcinogens (Kovacic and Jacintho 2001a), reproductive toxins (Kovacic and Jacintho 2001b), nephrotoxins (Kovacic et al. 2002), hepatotoxins (Poli et al. 1989), cardiovascular toxins (Kovacic and Thurn 2005), nerve toxins (Kovacic and Somanathan 2005), mitochondrial toxins (Kovacic et al. 2005), abused drugs (Kovacic and Cooksy 2005a), ototoxins (Kovacic and Somanathan 2008), and various other categories, including human illnesses (Halliwell and Gutteridge 2000).

In this review, we draw lines of evidence to support the concept that the ET–ROS–OS unifying theme, which has been successful in describing the means by which many other classes of toxins induce their effects, can also be applied to dermatotoxins. Such toxin classes include a variety of structurally diverse substances that include medicinals, abused drugs, metals and metal compounds, industrial

chemicals, and natural constituents of plants. There are also numerous literature reports that address the beneficial effects of antioxidants (AOs) on such toxic processes. Therefore, in this review, we address antioxidants and cell signaling as well. Herein, we also reveal that the above-mentioned unifying theme unites a large majority of reported dermal toxins in a common mechanistic framework. This is the first review of which we are aware that comprehensively addresses the role of ET–ROS–OS, in attempts to explain the mechanism of toxic action that occurs with more than 90 cutaneous toxins. We have excluded the dermal effects of heat (burns), cold (frostbite), acids, and caustic substances from this review.

2 Mechanisms of ET and ROS Formation

An understanding of the biochemistry of chemical functionalities involved in ET is important to understanding how ET results in dermatotoxic effects. Hence, we will name certain substances that retain such functionalities and therefore engage in the types of reactions cogent to the toxic mechanisms addressed in this review.

Redox cycling occurs between hydroquinone **1** (Fig. 1) and *p*-benzoquinone **2**, and between catechol and *o*-benzoquinone, with generation of SO via ET to oxygen. In such reactions, semiquinones act as intermediates, and various amino acids can operate as electron donors. SO serves as precursor to a variety of other ROS. Quinones may come from either endogenous (formed in vivo) or exogenous

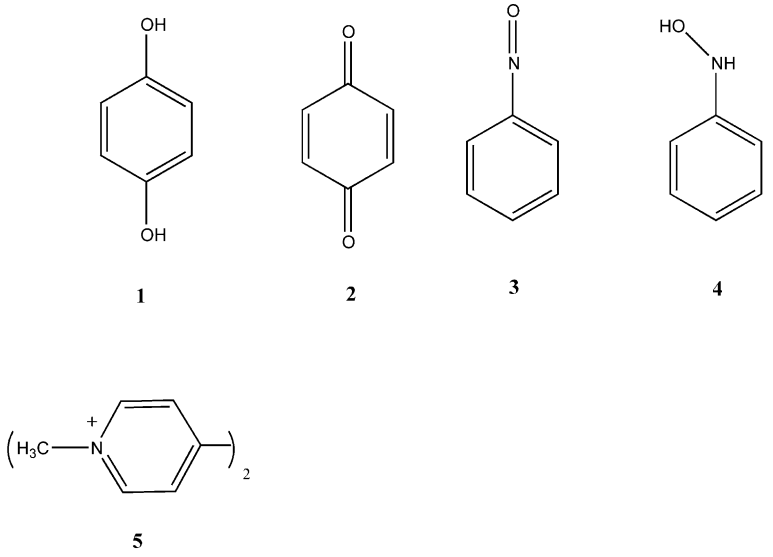

Fig. 1 Structures of ET (electron transfer) functional groups

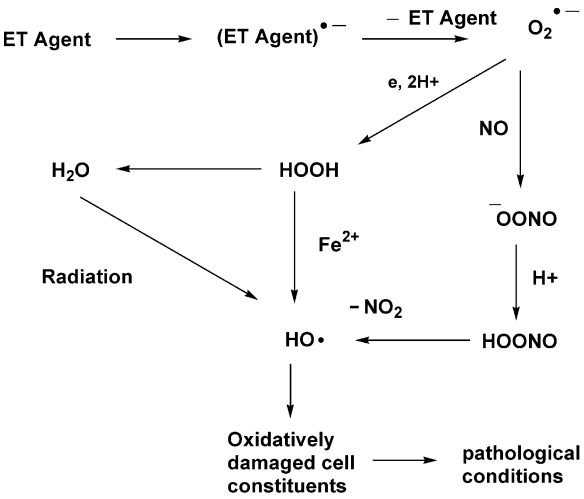

Fig. 2 Schematic of the ET and ROS (reactive oxygen species) forms that may induce dermatotoxicity

(sourced externally) sources. The aromatic nitro compounds are exogenous, and two representatives, the reduced nitroso **3** and hydroxylamine **4** metabolites, are known to enter into redox cycling. Less well known than the foregoing are exogenous conjugated iminium compounds, of which paraquat **5** is a prominent member. Electron uptake yields resonance contributors that serve to stabilize molecules. The process by which ET produces ROS and ultimately possibly induces toxicity is presented as a schematic in Fig. 2.

There are many examples in the literature of the skin-damaging effects of ROS. Ha et al. (2005) reported that OS, arising from contact with hydrogen peroxide, triggered dramatic changes in the expression of proteins in human dermal endothelial cells. Pelle et al. (2005) revealed that keratinocytes may be a source of hydrogen peroxide. Watt et al. (2004) showed that peroxide causes toxicity by three main modes: lipid peroxidation, corrosive damage, and oxygen gas formation. Lipid peroxidation is an important feature in toxicity produced by ROS. Shvedova et al. (2002) demonstrated that selective peroxidation of phosphatidylserine occurred in human epidermal keratinocytes from OS induced by cumene hydroperoxide. Subsequently, Shvedova et al. (2004) showed that COX-2-dependent oxidative metabolism is involved in inflammatory responses and tumor promotion. Bezard et al. (2005) studied carbon radicals from linalyl hydroperoxide and showed their binding to protein as a step in producing skin sensitization. Hanausek et al. (2004) examined various organic peroxides in relation to induction of subchronic effects related to carcinogenesis in mouse skin. In a Japanese study, an increase in the incidence of atopic dermatitis provided evidence for a link between environmental oxidants and protein oxidative damage (Niwa et al. 2003).

In the next sections we will address some specific factors and agents that may act to produce dermatotoxic effects as a result of ET and production of reactive oxygen species.

3 Atmospheric Contaminants and Toxicants

3.1 Radiation

Exposure to solar UV radiation is undoubtedly linked to skin carcinogenesis. It has been well demonstrated that ultraviolet-B (UV-B) radiation, which constitutes about 5% of the solar UV radiation that reaches the surface of the earth, directly activates DNA molecules to generate dipyrimidine photoproducts, such as cyclobutane pyrimidine dimers and pyrimidine–pyrimidone photoadducts; these photoproducts and photoadducts result in mutations and carcinogenesis. Kovacic and Jacintho (2001a) reviewed radiation carcinogenesis, including radiation-induced skin cancer, with emphasis on mechanisms that involve ROS. In a recent summary, Hiraku et al. (2007) treated the topic of mechanisms of UV-A-induced DNA damage, including SO and singlet oxygen formation, in the presence of various photosensitizers. Valencia and Kochevar (2007) showed NOX1-based NADPH oxidase to be the major source of UV-A-induced ROS in human keratinocytes. Kulms et al. (2002) reported that ROS, DNA insult, and death receptor activation contributed to UV-B-induced apoptosis. In addition to the foregoing, several studies have reported effects of UV exposure, photoaging, ROS, and AOs (Scharffetter-Kochanek et al. 2000; Wlaschek et al. 2001; Yasui and Sakurai 2003; Bernstein 2002; Ouédraogo and Redmond 2003; Nishigori et al. 2004).

3.2 Metals and Metal Compounds

Heavy metal compounds usually possess reduction potentials favorable for ET that may lead to OS in the biological domain. Metal toxicities are characterized by generation of ROS, lipid peroxidation, DNA cleavage, and decreases in AO levels. Skin insults commonly result from exposure in metal working industries. The negative effects of metal toxicity may be alleviated in the presence of AOs.

A review by Shi et al. (2004a) provided epidemiological evidence that exposure to certain metals induces cancer. There is support for involvement of OS in production of such cancer. In another review, Harris and Shi (2003) discuss cell signaling by carcinogenic metals, including As, Cr, and Ni. ROS appear to alter signaling pathways and thereby affect growth factor receptors, G-proteins, MAP kinases, and nuclear transcription factors. Some of the following metals either rely on or are linked to ROS: As (Ganyc et al. 2007; Shi et al. 2004b), Fe (Simonart et al. 2002; Leveque et al. 2003; Tyrell et al. 2000), Ni (Kasprzak et al. 2003; Das and Buchner 2007), and Cr (Bagchi et al. 2002). Arsenic exposure results in basal cell and squamous cell carcinomas and produces DNA damage in keratinocytes (Shi et al. 2004b).

Recently, increased work has been undertaken to investigate risks of nanoparticles that contain or are made up of metal and metal compounds. The skin may intentionally or unintentionally be exposed to solid nanoscale particles. Intentional

dermal exposure to nanoscale materials may include the application of lotions or creams containing nanoscale TiO_2 or ZnO as sunscreens or fibrous materials coated with nanoscale substances with water- or stain-repellent properties. Unintentional exposure may result from dermal contact with substances generated during nanomaterial manufacture or combustion. It is still unclear whether nanoparticles penetrate the skin, and to what degree if they do, and induce toxicity. However, serious concerns have been raised regarding dermal penetration, accumulation, and local (to skin) or systemic cytotoxicity, which may result from long-term exposure to metal-containing nanomaterials. Another unanswered question is whether photoactivated nanoparticles may be metabolized to smaller particles that have enhanced toxicity.

3.3 Phenols

Kovacic and Jacintho (2001a) have shown that ET–ROS–OS mechanisms are linked to the toxicity of phenols and are based on metabolic processes that involve phenoxyl radicals, ET quinones, and semiquinones. Zapor (2004) showed that the degree of cytotoxicity of phenol derivatives (catechol, resorcinol, hydroquinone, and phloroglucinol), in mice skin, appears to be related to the number and position of the hydroxyl groups on the corresponding rings.

Studies were performed on members of the Anacardiaceae family that include poison ivy, poison oak, and poison sumac. Poison ivy and associated plants that cause dermatitis produce classic contact-type allergic reactions that are based on cell-mediated hyposensitivity, in which catechols serve as the sensitizing antigen that reaches the Langerhans cells in the epidermis. Among the many plants that produce adverse skin reactions, the biochemical mechanisms of the ones in this category are well delineated at the molecular level. Zug and Marlss (1999) showed that the active toxins are urushiols which are comprised of catechols that contain C_{13}–C_{17} substituents, both saturated and unsaturated. Oxidation converts the catechol moiety to an *o*-quinone (Dupuis 1979; Liberato et al. 1981; Dunn et al. 1986); the quinones are a class that displays reduction potentials favorable for redox cycling with generation of ROS. Nucleophilic attack on the *o*-quinone by protein thiol or amino groups results in protein binding. Xia et al. (2004) showed that the product formed from conjugate addition can also be oxidized to bound *o*-quinone forms.

ROS, such as hydroperoxides, evidently arise from oxidation at side-chain allylic positions (Xia et al. 2004). Hydroperoxide decomposition and concomitant dehydrogenation of phenolic groups may produce radicals, which then couple to form crosslinked polymers. Dupuis (1979) provided further evidence for OS involvement as a result of AO depletion or protection by elevated levels of AOs.

3.4 Quinones

Inbaraj and Chignell (2004) studied the cytotoxicity of juglone (5-hydroxy-1,4-naphthoquinone) and plumbagin (5-hydroxy-3-methyl-1,4-naphthoquinone). Both

of these compounds are natural products and are used in hair dyes and skin coloring. In addition, these two substances are used as a herbal treatment for acne, a treatment for inflammatory diseases, ring worm, and certain fungal and bacterial infections.

Two major mechanisms have been proposed for the cytotoxic action of quinones in a variety of cell systems. First, quinones undergo a one-electron reduction mediated by enzymes such as microsomal NADPH-cytochrome P-450 reductase or mitochondrial NADH-ubiquinone oxidoreductase. This electron reduction yields the corresponding semiquinone radical. Under aerobic conditions, the semiquinone radical then participates in redox cycling to generate the superoxide anion and H_2O_2. Second, quinones are potent electrophiles, capable of reacting with thiol groups in proteins or GSH (glutathione). Depletion of GSH has been associated with menadione-induced cytotoxicity. The anti-infective properties may be the result of redox cycling, which agrees with earlier reports (Kovacic and Becvar 2000).

3.5 Polycyclic Aromatic Hydrocarbons (PAHs)

Occupational and environmental exposures to PAHs may result in inflammatory and/or allergic disorders, asthma, rhinitis, and dermatitis. The molecular mechanisms by which such effects are produced remain to be clarified. Kovacic and Somanathan (2007) and Kovacic and Jacintho (2001a) discussed the long use of PAHs in skin cancer investigations. The primary mechanism of PAH action is through epoxide and quinone metabolites that generate ROS, which then may lead to lipid peroxidation.

Masafumi et al. (2005) provided evidence that hydrocarbon receptor (AhR) target genes may be a central mechanism that explains how PAH-mediated inflammatory diseases occur. Therefore, blocking AhR signals may reduce allergic symptoms.

Nair et al. (2000) provided results showing close correlation between upregulation of lipoxygenase-catalyzed arachidonic acid metabolism and the formation of etheno-cyclic adenosine monophosphate – AMP – (dA) and 3,N^4-ethenodeoxycytidine (dC) adducts in DNA; such upregulation occurred during tumor development by initiation-promotion of mouse skin carcinogenesis using 7,12-dimethylbenz(*a*)anthracene (DMBA). Evidence shows that abundant ROS is generated in this oxidative metabolism process.

Other reports deal with the effects of adducts (Kleiner et al. 2004; Cavalieri et al. 2005). Wang et al. (2003) showed that benzo[*a*]pyrene (BaP) served as a photosensitizer to generate massive amounts of ROS upon irradiation. This irradiation caused oxidative damage, BaP binding to DNA (genetic effect), and activation of signal transduction cascades, thereby leading to carcinogenesis. Gao et al. (2005) demonstrated that the epoxide metabolite of BaP interacts synergistically with UV radiation to generate 8-OH-dG via ROS.

3.6 Mustard Gas

Sulfur mustard, bis-(2-chloroethyl) sulfide (SM), is a bifunctional alkylating agent that has cytotoxic, mutagenic, vesicant, and carcinogenic properties. Sulfur mustard interacts with cellular DNA to form the crosslink, di-(2-guanin-7yl-ethyl)-sulfide, and two monoadducts, 7-(2-hydroxyethylthioethyl) guanine (HETEG) and 3-(2-hydroxyethylthioethyl) adenine (HETEA). DNA modification by SM has been shown to interfere with replication and transcription and is probably responsible for its various toxicities (Matijasevic et al. 2001). Kovacic and Jacintho (2001a) reviewed the toxicity and mode of action of SM, which involves DNA alkylation. Cai et al. (2004) reported the various theories of mustard gas poisoning, including DNA alkylation and involvement of free radicals, NO and calcium. Vijayaraghavan et al. (2005) showed that toxic mustard gas caused serious skin blisters. Such intoxication results in DNA fragmentation and GSH depletion. Simpson and Lindsay (2005) examined various agents that protect against the effects of mustard gas poisoning, including thiols. Buthionine sulfoxime pretreatment increased cell resistance to sulfur mustard, presumably through AO-mediated metabolites. Alkylating agents, including mustard gas, are also used therapeutically (Kovacic and Osuna 2000) in treating a number of cutaneous conditions, including lymphomas.

3.7 Acrylamide

Acrylamide is an industrial chemical used to synthesize polyacrylamides for wastewater treatment, paper making, ore processing, and in the manufacture of fabrics and dyes. Recently, it has been detected in fried foods, whose appearance is probably a byproduct of the Maillard reaction. Acrylamide causes cancer, tumors in the central nervous system, oral cavity, thyroid gland, mammary gland, and uterus. Acrylamide may pose a risk to the genetic material after absorption through the skin, through a genetic mechanism that involves heritable translocation (Adler et al. 2004). The mode of toxicity for acrylic monomers entails ROS and is addressed elsewhere (Kovacic and Somanathan 2005).

3.8 Dioxin

Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (TCDD) exposure in experimental animals results in an array of tissue- and species-specific responses, including the following: dermal toxicity, immunotoxicity, hepatotoxicity, carcinogenicity, teratogenicity, neurotoxicity, and endocrine and metabolic alterations. Slezak et al. (2000) have shown that OS occurs in various tissues of TCDD-treated animals and is one of the important mechanisms responsible for toxicity. Shertzer et al. (1998) demonstrated that TCDD exposure in laboratory animals leads to increases in the

production of ROS, lipid peroxidation, DNA and membrane damage, and possible enzyme inhibition.

TCDD is also a multi-site rodent carcinogen and tumor promoter that induces papilloma formation when applied dermally (Wyde et al. 2004). Metabolism of TCDD yields hydroxylated derivatives that may serve as quinone precursors in redox cycling (Kovacic and Somanathan 2005).

3.9 Naphthalenes

Singh and Singh (2004) reported that the dermal toxicity of naphthalene and alkyl derivatives thereof caused severe erythrema and edema. Oxidative metabolism of the naphthalenes is similar to that of benzene, in which hydroxylation and quinone formation takes place, and is followed by redox cycling that results in ROS (Kovacic and Somanathan 2005).

3.10 2,4,6-Trinitro-1-Chlorobenzene (TNCB)

Harada et al. (2005) reported that repeated application of TNCB caused an increase in skin thickness characterized by inflammatory cell infiltration into dermis and epidermis. Metabolism of such aromatic nitro compounds consists of reduction to nitroso and hydroxylamine derivatives, which subsequently can generate ROS through redox cycling (Kovacic et al. 2005).

3.11 Nitric Oxide (NO)

Weller (2003) demonstrated that increased NO is released in the skin following exposure to UV irradiation. Experiments show that melanogenesis is dependent on keratinocyte-generated NO. Wound healing is delayed in NO-synthase null mice. Extensive studies demonstrated participation of NO, in both beneficial and toxic effects, in which ROS and RNS play a role (Jacintho and Kovacic 2003).

3.12 Carvoxime

Bergström et al. (2007) showed that carvoxime, an α,β-unsaturated oxime, elicits a contact allergenic response. Metabolites of this moiety were sensitizers of extreme potency and were highly reactive toward peptides and nucleophilic amino acids. Metabolism of carvoxime yielded epoxides that are capable of alkylation reactions (Kovacic and Jacintho 2001a). The toxicity of carvoxime may also derive from formation of a very reactive nitroso intermediate.

3.13 Trichloroethylene (TCE) and Perchloroethylene (PERC)

Zhu et al. (2005) reported that TCE and PERC, both industrial chemicals, cause skin damage that is characterized by a decrease in cell viability. Exposure to these substances resulted in lipid peroxidation and a decline in AO enzyme activity. Pretreatment with vitamin E attenuated the cytotoxic effects. Kovacic et al. (2002) have discussed the mechanisms of TCE and PERC toxicity that derive from OS.

3.14 Methyl Bromide

Before its use was curtailed by the U.S. EPA and other regulatory entities, methyl bromide was widely used as a soil sterilant and as a general-purpose fumigant to kill a variety of pests including rats and insects. Lifshitz and Gavrilov (2000) reported that methyl bromide exposure caused CNS toxicity, peripheral neuropathy, dermal burns, and vesicles. Interaction of methyl bromide with GSH results in AO depletion (Kovacic and Jacintho 2001a). Methyl bromide induces DNA methylation in rats, and such alkylation is known to generate ROS.

3.15 Reactive Carbonyl Species (RCS)

Roberts et al. (2003) showed RCS, an example of which is the α-dicarbonyls, to have implications for skin damage and carcinogenesis. Moreover, RCS are potent mediators of cellular carbonyl stress, arising from lipid peroxidation, glycation, and DNA strand cleavage. After exposure, adverse effects were prevented by the carbonyl scavenger penicillamine and partly suppressed by the hydroxyl radical scavenger mannitol. Kovacic and Cooksy (2005b), in a recent review, addressed the relationship of RCS to ET–ROS–OS.

3.16 Geraniol

Geraniol and linalool are monoterpenoids that contain an alcohol and two alkene groups in the chain. The alcohol group can be converted endogeneously to the reactive carbonyl species by lipid peroxidation. Formed carbonyl can readily react with amines and other alkylating agents to produce toxic compounds. Similarly, alkenes can undergo epoxidation in the presence of ROS and subsequent reactions with nucleophiles (e.g., primary amines) that lead to epoxide ring opening and production of toxic byproducts. Hagvall et al. (2007) reported that fragrances such as geraniol are a common cause of contact allergy. The autoxidation of geraniol follows two paths, both originating from the allylic hydrogen atom abstraction. Hydrogen peroxide is primarily formed, as are aldehydes from a hydroxyhydroperoxide. These

chemical entities, together with small amounts of a hydroperoxide, are believed to be the major contributors to geraniol-induced allergy.

3.17 Linalool

Sköld et al. (2004) reported that the unsaturated hydrocarbon linalool, a fragrance chemical used in perfumes, is a contact allergen. Autoxidation of the substance yields two hydroperoxides that are believed to act as sensitizers. Other oxidation products include an alcohol and α,β-unsaturated aldehyde.

3.18 Sunscreens

Konaka et al. (1999) reported that titanium dioxide (TiO_2) is a semiconductor that absorbs light at wavelengths below approximately 385 nm (i.e., UV-A or UV-B); such absorption of light results in the creation of an electron–hole pair. The electron–hole pair will interact with water or oxygen at the crystal surface, resulting in the generation of ROS, including hydroxyl radicals, singlet oxygen species, or SO. TiO_2 is an active ingredient in sunscreen lotions. Photoexcitation of sunscreens that contain TiO_2 resulted in generation of ROS, including SO, hydroxyl radical, hydroperoxide radical, and carbon radicals (Brezová et al. 2005). These substances are well-known initiators of several forms of toxic response.

3.19 Fullerenes

Fullerenes are a family of carbon allotropes, molecules composed entirely of carbon C_{60}, in the form of a hollow sphere. Spherical fullerenes are also called buckyballs. Buckyballs can inhibit the growth of common soil bacteria. There is a growing body of evidence that indicates potentially harmful effects from exposure to this substance, and these effects include damage to human skin (Halford 2005). There are reports that the C_{60} species are also involved in ET (El-Khouly 2007) and in generation of ROS, which may be therapeutically useful (Burlaka et al. 2004; Kamat et al. 2000).

4 Therapeutic Drugs

4.1 Doxorubicin

Although doxorubicin (Adriamycin) is a widely used anticancer drug that retains a broad spectrum of anticancer activity, it also produces clinical toxicities. Extravasation of doxorubicin into adjacent soft tissues frequently occurs during

intravenous infusion of the drug into cancer patients. The extravasation can induce progressive tissue necrosis, and ultimately produce ulcers. Moreover, Kim et al. (2005) reported that severe skin toxicity has been associated with doxorubicin during treatment of gynecologic cancers. Doxorubicin belongs to the family of anthracycline antibiotics that bear an anthraquinone and 1,4-dihydroxybenzene ring system in their structural skeleton; this ring system predisposes to redox cycling.

The antibiotic can also be anti-tumorigenic. The mechanism by which doxorubicin produces anti-tumorigenic effects is linked to ET–ROS–OS features, which have been reviewed (Kovacic and Becvar 2000). The primary AO mode of action involves reduction in levels of toxic ROS species through a scavenging process. Examples of AOs that have decreased doxorubicin-induced skin toxicity include butylated hydroxytoluene, an antioxidant used in the food industry, α-tocopherol, and other radical scavengers (Daugherty and Khurana 1985; Korać and Buzadić 2001).

4.2 Methyl Salicylate

Bell and Duggin (2002) reported acute skin toxicity of methyl salicylate that resulted from use in a herbal skin cream for the treatment of psoriasis. Salicylic acid, the hydrolysis product of methyl salicylate, can be a toxic precursor to ROS (Kovacic and Jacintho 2001b).

4.3 Methotrexate

Gaigl et al. (2007) reported that methotrexate, an anticancer drug, produced epidermal toxicity associated with necrolysis. This enzyme inhibitor possesses a reduction potential amenable to ET in vivo (Kovacic and Osuna 2000).

5 Occupational Sources and Other Dermal Toxins

There is extensive coverage of skin diseases by Wigger-Alberti et al. (1999) in their book on occupation sources of exposure. As this book chronicles, occupational dermatotoxins are mainly precursors of ET agents that produce ROS.

Numerous articles deal with ROS and OS as factors in skin diseases, with the role of AOs addressed in some cases. In these articles, some key diseases or afflictions caused or influenced by ROS and OS, and the references that treat the link to AOs, are provided. Fuchs et al. (2001) documented the involvement of inflammation. There is little doubt that chronic inflammation is a risk factor for cancer (Halliwell and Gutterridge 2000). Extensive evidence demonstrates the favorable effects of AOs in carcinogenesis (Kovacic and Jacintho 2001a). Allergy resulting from exposure to metals, such as Ni (Hostynek et al. 2002) and Cr (Hansen et al.

2003), has been investigated. There are other reports that deal with the topic of general dermal toxicity (Briganti and Picardo 2003; Maccarrone et al. 1997; Rhodes 2000; Fuchs and Packer 1993) and associated dermatotoxic mechanistic features, and these address some points covered in the present review.

6 Cell Signaling

Signal transduction is known to occur broadly in living organisms and affects various aspects of the biochemistry in such organisms. A recent review addresses the involvement of radicals, electrons, conduits, and electrochemistry in cell signaling processes (Kovacic and Pozos 2007a). There is a hypothesis that has been advanced for participation of electrostatics in a bridging mechanism that involves receptor–ligand action, phosphates, sulfates and metal ions (Kovacic and Pozos 2007b; Kovacic et al. 2007a; b), and energetics (Kovacic 2008). Some examples of cell signaling in dermatotoxicity have surfaced since 2000. An analog of vitamin E was discovered to modulate UV-induced signaling activation and increased cell viability (Peus et al. 2001). Fuchs et al. (2001) described redox events that occur when inflammation is induced by contact dermatitis; some of the inflammatory-inducing agents are kinases, cytokines, transcription factors, and the T-lymphocyte receptor. One study shows that NF-kB (nuclear factor-kappa beta) activation, by the cytokine TNF-α (tumor necrosis factor-alpha) in keratinocytes, is mediated by ROS (Köhler et al. 2001), and adjunctive therapy with AOs may be of therapeutic value. Cell communication appears to be ROS dependent during antigen presentation in dendritic cells (Matsue et al. 2003). The epoxide metabolite of benzo[*a*]pyrene is the active agent in initiating signaling after exposure to the PAH (Li et al. 2004). This effect entails activation of AP-1 (activator protein-1) and NF-kB in epidermal cells.

7 Antioxidants

Abundant evidence exists that AOs protect against dermal damage, and this evidence supports, for a variety of agents, the proposed involvement of ROS in induction of dermal toxicity. Among the many AOs that have been reported to counter, or protect against, dermal toxicity include the following: melatonin and the other AOs that follow protect against UV skin cancer induction (Fischer et al. 2001): *Ginkgo biloba* (Ozkur et al. 2002), nitroxide Tempol (Bernstein et al. 2001), vitamin C (Humbert et al. 2003), phenols (Katiyar et al. 2001; Pillai et al. 2006; Psotova et al. 2006; Inal et al. 2001; Svobodova et al. 2003), alpha-lipoic acid (Podda et al. 2001), vitamin E (Podda and Grundmann-Kollmann 2001), *N*-acetylcysteine (D'Agostini et al. 2005), *n*-3-polyunsaturated fatty acids (Jackson et al. 2002), gingerol (Kim et al. 2007), and virgin olive oil (Ischihashi et al. 2000). Moreover, Dammak et al. (2007) report that the oil from date seeds reduces oxidative injuries caused by hydrogen peroxide (Dammak et al. 2007), and rosemary extracts may protect skin from harmful

cosmetic effects (Calbrese et al. 2000). A review that addresses the role of AOs in protecting against a broader range of toxins has been published (Kovacic and Somanathan 2006).

8 Summary

Large numbers of chemicals are known to produce diverse types of skin injury, and these substances fit into a wide variety of both organic and inorganic chemical classes. Skin contact with toxins is difficult to avoid, because they are widely distributed, e.g., in industrial substances, agricultural chemicals, household products, and plants. Although various hypotheses have been advanced, there is no universal agreement as to how dermal toxins act to produce their effects. In this review, we provide evidence and numerous literature citations to support the view that oxidative stress (OS) and electron transfer (ET) comprise a portion of a key mechanism, and perhaps unifying theme that underlie the action of dermatotoxins.

We apply the concept that ET and OS are key elements in the induction of dermatotoxic effects to all of the main classes of toxins, and to other toxins, as well. We believe it is not coincidental that the vast majority of dermatotoxic substances incorporate recurrent ET chemical functionalities (i.e., quinone, metal complexes, $ArNO_2$, or conjugated iminium), either per se or as metabolites; such entities potentially give rise to reactive oxygen species (ROS) by redox cycling. However, in some categories, wherein agents cause dermal damage, e.g., peroxides and radiation, it appears that ROS are generated by non-ET routes. As expected, if ET and oxidative process do constitute the mechanistic framework by which most dermal toxins act, then antioxidants (AOs), if present, should prevent or mitigate effects. This is exactly what has been discovered to occur. Because ET and OS either cause or contribute to dermal toxicity, and AOs may offer protection therefrom, policy makers and researchers may be better positioned to prevent human dermatotoxicity.

Acknowledgments Assistance by Dr. Terence O'Grady, UCSD, as well as by Thelma Chavez, is gratefully acknowledged.

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Heavy Metals Alter the Potency of Medicinal Plants

Sekh Abdul Nasim and Bhupinder Dhir

Contents

1 Introduction

Heavy metals, accumulated naturally in soil, surface water or through industrial and mining processes, pose a potential threat to various terrestrial and aquatic organisms (Greeger 1999**;** Larison et al. 2000**;** Dwivedi and Dey 2002; Hsu et al. 2006; Dhir et al. 2008). Exposure to high metal concentrations impinges on the growth and development of plants (Rout and Das 2003; Shanker et al. 2005; Dhir et al. 2009). Such growth effects result from alterations in physiological events such as photosynthesis, respiration, changes in lipid composition, enzyme activity, and distribution of macro and micronutrients at the cellular level (Sheoran et al. 1990; Van Assche and Clijsters 1990; Rout and Das 2003; Shanker et al. 2005). Research also suggests that abiotic factors such as heavy metals may alter the production of bioactive compounds by changing aspects of secondary metabolism (Verpoorte et al. 2002). The secondary metabolites are usually bioactive compounds that include such entities as alkaloids and isoprenoids that do not have a specific role in growth, photosynthesis, reproduction, or other "primary" functions in plants, but contribute to the medicinal value of the plant. Such natural chemical entities are synthesized in secondary metabolic processes that are specific to certain cell types. Because secondary metabolic processes are complex and diverse, the mechanism by which abiotic stress factors produce their effects is not yet well understood.

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology*,
Reviews of Environmental Contamination and Toxicology 203,
DOI 10.1007/978-1-4419-1352-4_5,

In recent decades, the low cost and milder side effects of phytopharmaceuticals and herbal medicines, compared to conventional synthetic drugs, have enhanced their worldwide use. As use of phytopharmaceuticals increases, it becomes essential that their quality should be assured prior to use. There is an extensive literature that addresses the effects of heavy metals on plant physiology and biochemistry. However, thus far, very little attention has been paid to the effects of heavy metals on the therapeutically active constituents and ultramorphological variation in different parts of medicinal plants. The goal of this review is to evaluate, in a preliminary way, the impact of heavy metal stress on the growth and development of medicinal plants, with special attention given to how the active medicinal constituents responsible for medicinal properties in plants may be altered.

2 Effects on Growth and Metabolic Status

Heavy metal accumulation in different parts of oil yielding plants and heavy metal contamination of medicinal plants and market samples of plant-based drugs (Table 1) have been reported in the last decade (Jiang et al. 2001, 2006; Chan 2003; Haider et al. 2004; Rai et al. 2004, 2005a, 2007; Kováčik et al. 2006; Srivastava et al. 2006; Khan et al. 2007; Krejpcio et al. 2007; Mishra et al. 2007; Pandey et al. 2007; El-Rjoob et al. 2008; Jonnalagadda et al. 2008). Heavy metal accumulation in plant parts increases in a concentration- and duration-dependent manner (Khan et al. 2007). Research has revealed that heavy metal accumulation in plant leaves may range between 5 and 10% on a dry weight basis.

Exposure to high concentrations of heavy metals may produce toxic effects on the growth and development of medicinal plants (Jiang et al. 2001; Rai et al. 2007; Rai and Mehrotra 2008). The plant growth changes recorded from exposure to heavy metals include alterations in seed germination potential and curtailment in root and shoot length, biomass production, and leaf area (Thanagavel et al. 1999; Rai et al. 2005b, 2007; Pandey et al. 2007; Street et al. 2007). Germination of seeds has been inhibited after exposure to 180 μM of Cd, and 1 mg L^{-1} each of Cu, Zn, and Hg in several plant species, viz., *Catharanthus roseus* L., *Eucomis autumnalis*, and *Bowiea volubilis* (Pandey et al. 2007; Street et al. 2007). Heavy metal-induced changes in plant growth patterns and metabolic activities affect (and reduce) production of proteins, photosynthetic pigments, sugars, and non-protein thiols. Such effects may result from inhibition of various enzymes involved in biosynthesis of these natural products or, more likely, through impaired substrate utilization (Sanita di Toppi and Gabbrielli 1999; Rai et al. 2004; 2005b, Singh et al. 2006; Kováčik et al. 2006; Rai and Mehrotra 2008).

In contrast to the foregoing, positive effects of heavy metal exposure on growth and development of medicinal plants have also been reported. Enhanced plant yield has been measured in the plant species *Matricaria chamomilla*, *Mentha arvensis*, and *Stevia rebaudiana*, all of which were exposed to heavy metals such as Zn, Co, Pb, and Ni (Misra 1992; Kartosentono et al. 2002; Das et al. 2005; Grejtovský et al. 2006). The affects noted included enhancement in plant height, total number of

Table 1 Heavy metal accumulation observed in medicinal plant species

Plant species	Heavy metal	Values	References
Amaranthus dubius	Cd	150 ppm	Chunilall et al. (2005)
Amaranthus hybridus	Hg	336 ppm	Chunilall et al. (2005)
Agave amaniensis	Cd	900 $\mu g\ g^{-1}$ dry wt	Kartosentono et al. (2002)
	Pb	1390 $\mu g\ g^{-1}$ dry wt	
Costus speciosus	Cd	530 $\mu g\ g^{-1}$ dry wt	Kartosentono et al. (2002)
	Pb	1170 $\mu g\ g^{-1}$ dry wt	
Matricaria chamomilla	Zn	271 mg kg^{-1} dry wt	Grejtovský et al. (2006)
Ocimum tenuiflorum	Cr	419 $\mu g\ g^{-1}$ dry wt	Rai et al. (2004)
Matricaria chamomilla	Zn	271 mg kg^{-1} dry wt	Grejtovský et al. (2006)
Phyllanthus amarus	Cd	82 ppm	Rai et al. (2005b)
		63 ppm	
Hypericum sp.	Cd	0.5 mg kg^{-1} dry wt	Chizzola and Lukas (2006)
Cuminum cyminum	Fe	1.4 mg g^{-1} dry wt	Maiga et al. (2005)
Bombax costatum	Fe	1.5 mg g^{-1} dry wt	Maiga et al. (2005)
Hibiscus sabdariffa	Mn	243 $\mu g\ g^{-1}$ dry wt	Maiga et al. (2005)
Spilanthes oleracea	Zn	62.8 $\mu g\ g^{-1}$ dry wt	Maiga et al. (2005)
Bombax costatum	Zn	67.1 $\mu g\ g^{-1}$	Maiga et al. (2005)
Aesculus hippocastanum	Pb	1480 $\mu g\ g^{-1}$	Caldas and Machado (2004)
Tilia sp.	Zn	13.8–32.5 mg kg^{-1}	Celechovská et al. (2004)
Sambucus nigra	Zn	30.8–49.9 mg kg^{-1}	Celechovská et al. (2004)

branches, shoot biomass, and number of leaves per plant. Moreover, low doses of heavy metals such as Co and Ni have enhanced uptake of essential elements, and improved the macro and micronutrient status (N, P, K, Co, Ni, Mn, and Zn) of plants (Eman et al. 2007).

One important additional feature of heavy metal-ion exposure in plants is that reactive oxygen species (ROS), such as $\cdot O_2^-$, H_2O_2, and $\cdot OH$, may be generated (Gratão et al. 2005). ROS represent intermediates that emerge during the successive reduction of O_2 to H_2O. ROS are highly reactive entities and may damage lipids, proteins, and nucleic acids. It is the protonated form of $\cdot O_2^-$ and hydroperoxyl radical ($\cdot O_2H$) that are mainly involved in lipid peroxidation. The oxidative stress induced by heavy metals may be mitigated by antioxidants and antioxidant enzymes that are present, viz., superoxide dismutase, guaiacol peroxidase, catalase, and accumulation of compatible solutes such as proline (Rai et al. 2004; Sinha and Saxena 2006).

3 Effects on Secondary Metabolite Production

Heavy metal contamination may alter the chemical composition of plants and thereby seriously affect the quality and efficacy of the natural plant products produced by medicinal plant species (Zhu and Cullen 1995). Plants exposed to heavy metal stress show differential responses in synthesis and accumulation of pharmacologically active molecules. Such responses range from negative effects on

secondary metabolite production (Thangavel et al. 1999; Murch et al. 2003; Pandey et al. 2007) in a few plant species, viz., *Matricaria recutita*, to stimulatory effects that result in enhanced metabolite production in other species (Kim et al. 1991; Kasparová and Siatka 2004; Zheng and Wu 2004; Rai et al. 2005b; Michalak 2006; Eman et al. 2007).

Heavy metals are mobile within plants, and because of this mobility may reduce biosynthesis of active constituents in different plant components. Such effects may result from loss or inactivation of specific essential enzymes, or damage to non-essential biosynthetic processes, such as those involved in production of secondary metabolites. Ultimately, heavy metals may reduce the synthesis and accumulation of key bioactive plant molecules (Thangavel et al. 1999; Murch et al. 2003; Pandey et al. 2007). For example, *Hypericum perforatum* seedlings grown in a medium supplemented with 25 or 50 mM Ni lost the capacity to produce or accumulate hyperforin, and demonstrated a 15-/20-fold decrease in the concentration of pseudohypericin and hypericin (Murch et al. 2003).

Increases in heavy metal-induced secondary metabolite biosynthesis have been reported to occur in some medicinal plant species (Table 2). Induction of phenolic compound biosynthesis, in response to Ni, Al, and Cu toxicity, has been noted in wheat, maize and *Phyllanthus tenellus* (Winkel-Shirley 2002; Michalak 2006). An increase in phenolic levels correlated with increased enzyme activity associated with phenolic compound metabolism, suggesting de novo synthesis of phenolics under heavy metal stress.

4 Heavy Metals: Actions and Mechanisms

Heavy metal-induced stimulation of secondary metabolites in medicinal and other plants is significantly influenced by several factors, including growth stage of treated cells, concentration and duration of treatment, and composition of growth medium. In cell suspension cultures, maximum yield enhancement is noted in the mid-exponential and early stationary growth phases. Generally, enhanced secondary metabolite production results from increased synthesis of precursors (Zheng and Wu 2004). Alteration in secondary metabolism may be a strategy of the plant to survive and grow in adverse conditions (including growth in the presence of phytotoxic metals; Cobbett and Goldsbrough 2000).

Heavy metals sometimes act as abiotic elicitors, which, when introduced in small concentrations initiate or improve the biosynthesis of specific compounds (Namdeo 2007). One proposal is that genes involved in synthesis of specific secondary metabolites are activated in response to signaling pathways induced by environmental challenges (Pichersky and Gang 2000). Elicitation-induced stress activates the defensive reactions of the plant, which results in a change in transcription of the genes coding for the enzymes that influence biosynthesis of secondary metabolites (Kasparová and Siatka 2004). Treatment of undifferentiated cells with heavy metal elicitors enhances production of secondary metabolites (Namdeo 2007). It has been proposed that ROS, such as $\cdot O_2^-$, H_2O_2, $\cdot OH$, generated during heavy metal

Table 2 List of medicinal plant species showing increased production of secondary metabolite compounds under heavy metal stress

Plant species	Heavy metal	Compound	Medicinal properties	References
Catharanthus roseus	Cd 0.05–0.4 mM	Ajmalicine	Anticancer, antidiabetic	Zheng and Wu (2004)
Phyllanthus amarus	Cd (0.1–1 mM)	Phyllanthin, Hypophyllanthin	Hepatoprotective, diuretic, stomachic	Rai et al. (2005b)
Matricaria chamomilla	$CuCl_2$ 2%, Cd 60–120 μM Zn 50 mg kg^{-1} soil	Herniarin Umbelliferone essential oil, sesquiterpenes	Anti-inflammatory, spasmolytic effects	Eliasova ét al. (2004), Grejtovský et al. (2001), (2006) and Kováčik et al. (2006)
Salvia miltiorrhiza	Ag 15–40 μM Cu, Zn, Fe, Mn	Tanshinone	Broad spectrum bactericide, dilating coronary artery	Zhang et al. (2004) and Guo et al. (2005)
Ononis arvensis	Ni, Co Cr 6.3 μ mol	Flavonoid	Anti-inflammatory, antiproliferative, anticancer, antioxidant, cardioprotective	Tumova et al. (2001) and Tumova and Blazkova (2002)
Rheum palmatum	Cd 10 μM	Anthracene derivatives	Antioxidant	Kasparova and Siatka (2004)
Thalictrum rugosum	Cu 200–500 μM	Berberine	Antimicrobial in the treatment of dysentery and infectious diarrhea	Kim et al. (1991)
Hibiscus sabdariffa	Co 20 mg kg^{-1} soil Ni 25 mg kg^{-1} soil	Anthocyanins Flavons	Diaphoretic, hepatic and gastric disorders, arteriosclerosis, intestinal infections	Eman et al. (2007)
Ocimum tenuiflorum	Cr 20 μM	Eugenol	Antiseptic, antispasmodic, antibacterial	Rai et al. (2004)
Hypericum perforatum	Cr(VI) 0.1 mM	Hypericin Pseudohypericin	Minor burns, wounds, skin inflammation, nerve pain	Tirillini et al. (2006)
Datura stramonium	Cd 1 mM	Hyoscyamine	Peptic ulcers, diarrhea, and bronchial asthma	Furze et al. (1991)
Mentha arvensis	Zn	Essential oil, menthol	Antibacterial, antifibrile, effective in rhinitis, cough sore throat, colic	Misra (1992)
Costus speciosus	Pb 30 mg L^{-1}	Sitosterol	Purgative, febrifuge, expectorant	Kartosentono et al. (2002)
Pluchea lanceolata	200 μM $ZnSO_4$ or 150 μM $CuSO_4$	Quercetin	Inflammations, bronchitis, psoriasis, cough, piles, antipyretic, analgesic, laxative	Kumar et al. (2004)
Atropa belladonna	Cd	Tropane alkaloids	Diuretic, sedative, antispasmodic	Lee et al. (1998)

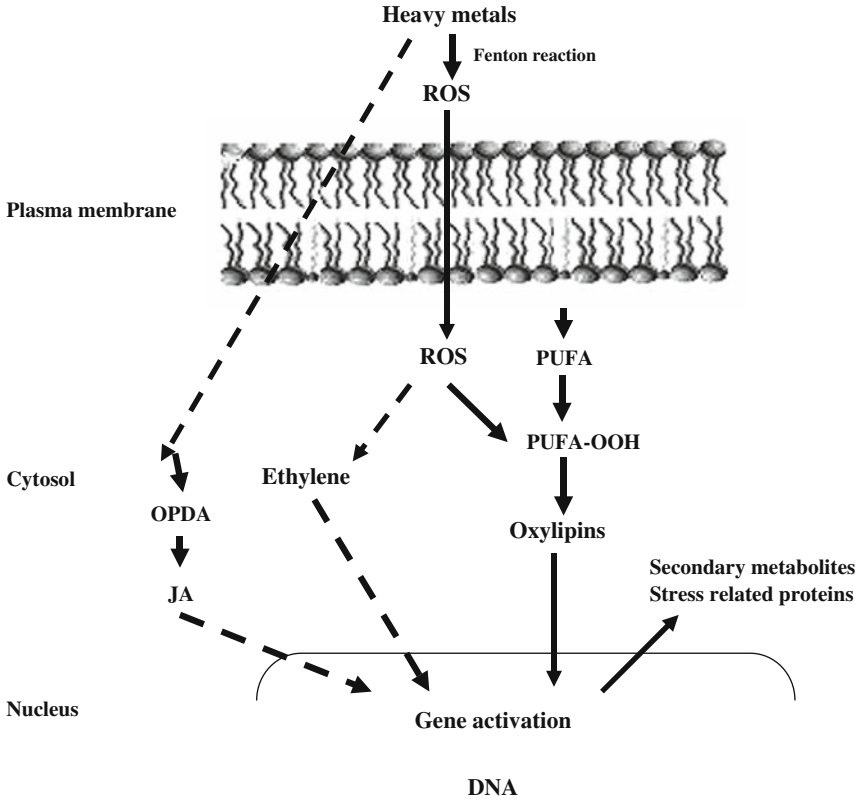

Fig. 1 A model illustrating the role of signaling pathways in heavy metal-induced enhancement of secondary metabolite production. ROS (reactive oxygen species) generated from heavy metal-induced oxidative stress leads to formation of lipid hydroperoxides, which are converted to oxylipins. Oxylipins induce the gene expression involved in the biosynthesis and accumulation of secondary metabolites. Other signaling pathways (*dashed lines*) include ethylene biosynthesis and jasmonic acid (JA) production through the precursor 12-oxo-phytodieonic acid (OPDA), which may also play an indirect role in activation of genes involved in biosynthesis of secondary metabolites. PUFA = polyunsaturated fatty acids. –OOH = hydroperoxide

exposure cause lipid peroxidation and ultimately induce formation of highly active signaling compounds (Fig. 1) (Gratão et al. 2005). Peroxidation of polyunsaturated fatty acids (PUFA) in cell membrane lipids results from non-enzymatic reactions initiated by ROS or enzymatic reactions catalyzed by α-dioxygenase, peroxidases, lipoxygenases, etc. (Mithöfer et al. 2004). The formation of fatty hydroperoxides leads to generation of oxylipins (oxygenated fatty acids), which represent a pool of active signaling molecules that contribute to defense responses in plants and induce expression of the genes involved in the biosynthesis and accumulation of secondary metabolites (Farmer et al. 2003; Mithöfer et al. 2004). Non-regulated

formation of oxylipins, initiated by the presence of heavy metals, may elicit secondary plant metabolism by generation of structurally similar or even identical compounds (Mithöfer et al. 2004).

Heavy metals are also known to induce expression of other signaling molecules, such as jasmonate through a pathway that increases ethylene concentrations, especially by stimulating the activity of ACC (1-aminocyclopropane-1-carboxylic acid) synthase and oxidase (Turner et al. 2002; Maksymiec et al. 2005; Maksymiec 2007). In heavy metal-exposed plants, ethylene has been known to regulate a pathway that accounts for production of the tropane alkaloids, scopolamine, and hyoscyamine (Pitta-Alvarez et al. 2000). Hairy root cultures of *Brugmansia candida* exposed to Ag showed an increase in scopolamine release, in comparison to hyoscyamine production. It is supposed that Ag^{+} may act as an ethylene-blocking agent, causing ethylene to down-regulate hyoscyamine-6-β-hydroxylase (H6H), the enzyme that converts hyoscyamine into scopolamine (Pitta-Alvarez et al. 2000). Osmotic stress induced by Ag^{+} also explains the release of scopolamine as a result of cell lysis (Pitta-Alvarez et al. 2000). Rakwal et al. (1996) indicated that Cu^{2+} induced a fast and strong increase of signal molecules correlated with an increase of secondary metabolites.

5 Summary

There has been increased use of herbal drugs in recent years. Because of increasing demand and wider use, it is essential that the quality of plant-based drugs should be assured prior to use. When heavy metals contaminate the plants from which herbal drugs are derived, they affect both plant growth characteristics and production of secondary plant metabolites.

Plants exposed to heavy metal stress show changes in production of secondary metabolites. High levels of heavy metal contamination in medicinal or other plants may suppress secondary metabolite production. Alternatively, the presence of heavy metals in medicinal plants may stimulate production of bioactive compounds in many plant species. Moreover, some research results suggest that heavy metals may play an important role in triggering plant genes to alter the titers or nature of secondary plant metabolites, although the exact mechanism by which this happens remains unclear. Oxidative stress induced by heavy metals triggers signaling pathways that affect production of specific plant metabolites. In particular, reactive oxygen species (ROS), generated during heavy metal stress, may cause lipid peroxidation that stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhances the medicinal value of the plant. As usual, further research is needed to clarify the mechanism by which heavy metals induce responses that result in enhanced secondary metabolite production.

Acknowledgments The financial assistance from Department of Science and Technology, New Delhi, to Bhupinder Dhir is gratefully acknowledged.

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology,*
Reviews of Environmental Contamination and Toxicology 203,
DOI 10.1007/978-1-4419-1352-4,