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David M. Whitacre *Editor*

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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 everincreasing 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 twenty-first 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 David M. Whitacre

Contents

The Fate of Arsenic in Soil-Plant Systems

Eduardo Moreno-Jiménez, Elvira Esteban, and Jesús M. Peñalosa

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1 Introduction

1.1 The Properties of Arsenic

Arsenic (As) is an element belonging to the group V-A, and it demonstrates characteristics of a metalloid. Because arsenic more easily forms anions, its non-metal properties dominate. When arsenic is in an oxidation state of +5, it acts similar to phosphorus, a fact that has many implications for the way in which it reacts in

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soil, as well as its potential toxicity in plants. The most common oxidation states of As are −3, 0, +3, and +5. Arsines and metal arsines are those in which As has an oxidation state of −3, and these forms are very unstable under oxidizing conditions. Under aerobic conditions, the oxidation state of As tends to be +5, and when this occurs at a pH between 2 and 3, arsenic acid (H_3AsO_4) is formed. When the pH rises to values between 3 and 11, this compound disassociates to $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Smedley and Kinninburgh 2002). Under anaerobic conditions, the predominant As species is H_3AsO_3. Arsenic also biomethlyates easily (Barán 1995).

Arsenic is widely distributed throughout the environment and can be detected in the lithosphere in concentrations between 1.5 and 2 mg kg^{-1}, making it the 52nd most abundant element (Adriano 2001). Arsenic forms a part of more than 245 minerals that include arsenates (60%), sulfides, and sulfosals (20%), as well as other compounds such as arseniurates, arsenites, oxides, and silicates (20%) (Mandal and Suzuki 2002). The majority of arsenic deposits in the earth's surface are found as sulfurous minerals (arsenopyrite).

1.2 The Presence of and Exposure to Arsenic in the Environment

Inorganic arsenic is present in soil, water, air, and food such that humans are constantly exposed to this contaminant (Mandal and Suzuki 2002). The range at which arsenic is present in soils varies normally between 0.2 and 40 mg kg^{-1}, while in urban areas the concentration in atmospheric air is approximately 0.02 μg m^{-3}. On a global level, drinking contaminated water is the major source of exposure to this contaminant (Smedley and Kinninburgh 2002). It is estimated that some 30 million people are exposed to waters contaminated with arsenic, a quarter of them showing symptoms associated with long-term exposure in at least five South Asian countries: Bangladesh, India, Nepal, Tailandia, and Myanmar (Caussy 2003). The World Health Organization (WHO) recommends a maximum level of arsenic in waters of 10 μg L^{-1} (Bissen and Frimmel 2003); however, the concentration of arsenic in surface waters is greater than 2,000 μg L^{-1} in certain regions of Bangladesh and India (Tripathi et al. 2007). Soil and water are the main sources of human exposure to arsenic at any given location, either by consumption (greatest in children; Rodríguez et al. 2003), inhalation, or direct skin contact (DEFRA 2002). Because arsenic accumulates in vegetables, fruits, and other plants that grow in contaminated soils, another important pathway of exposure is the transfer of arsenic within the food chain (Meharg and Hartley-Whitaker 2002).

In terms of anthropogenic sources, annual global production of arsenic is estimated to be between 75 and 100×10^3 t (Adriano 2001). Natural sources (those in which human intervention does not play an important role) will depend, in many cases, on the geochemistry of each site, principally of the site's lithology and dispersion pathways. The major human activities that produce As are mining, metallurgy, agriculture, forestry, fossil fuel treatment plants, urban waste, and cattle farming

(Adriano 2001; Fitz and Wenzel 2002). In both mining and metallurgy, arsenic is produced as a by-product of little value, making it an unwanted waste. There are important arsenic deposits in some components of the earth's surface, which give way to an elevated concentration of arsenic in the adjacent environment (up to 3% As in the soil) that rapidly decreases with distance from the contaminated sites (Zhang and Selim 2008). Also, some industrial activities may be a source of As; for example, when old glass manufacturing industries disposed of rich As wastes in the early twentieth century (Madejón and Lepp 2007). Many biocides, used to control diseases in agriculture and forestry, are composed of As (Lepp 1981). Similarly, the use of fertilizers is also a source of arsenic in soils (Matschullat 2000). Urban wastes derived from treatment plants and compost, often used as organic amendments, may contain arsenic in varying quantities (Beesley and Dickinson 2010). Lastly, fossil fuel combustion also produces quantities of arsenic that may lead to long-term accumulation from the gases emitted to the surrounding areas (Matschullat 2000). All of these factors release arsenic into the environment and can result in its accumulation in soils. When present in soils, As is generally observed to be more abundant in its inorganic form, with As(V) as the predominant species found under aerobic conditions (Atker and Naidu 2006). In soils, organic As species are usually found as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Takamatsu et al. 1982; Mestrot et al. 2011).

2 The Dynamics of Arsenic in Soil

Some authors believe that base concentrations of 10–40 mg kg^{-1} of arsenic exist in areas where the lithology has no known unnatural sources of contamination (Fitz and Wenzel 2002; Mandal and Suzuki 2002); however, estimates of the average concentration that exists in the pedosphere are only 5–8 mg kg^{-1} (Matschullat 2000). In the Andalusia region of Spain, values of 33 mg kg^{-1} of As have been documented to occur in soil, although amounts are highly dependent on the soil horizon, the type of soil and the lithology (Martín et al. 2007). In rare instances, either because of natural or anthropogenic sources, some soils are known to contain extraordinarily high levels of arsenic, i.e., values of 0.1 and 2% arsenic (Chang et al. 2005; Ongley et al. 2007; King et al. 2008). Such places pose a risk to human health as well as to ecosystem health, and if deemed necessary, these areas must be managed to reduce probable exposure risks.

An important variable in the study of As in soils is the heterogeneity in which it can appear, such that regions displaying high concentrations may be adjacent to regions that have much lower levels. In such cases, contaminant hot spots must be identified, because they will pose the hardest-to-manage threats (Dickinson et al. 2009). Several authors have described events in which As levels are greater in surface soil horizons (Adriano 2001; Clemente et al. 2008). When this occurs, it would indicate that contamination took place after the genesis of the soil in which it appears. However, this phenomenon depends on the source and method by which arsenic

made its way into the soil. In one review (Fitz and Wenzel 2002), the authors described how, in the European Union, there may be up to 1.4 million soil sites that are contaminated with metals, metalloids, and/or organic compounds. These authors also estimated that, in the USA, approximately 41% of the soil sites cataloged as being contaminated were cataloged as such because of arsenic. Similarly, in Australia, there are more than 10,000 soil sites contaminated by As. Some of these Australian sites are extensive in area and constitute large-scale events of As contamination.

When setting safe levels, environmental legislation or regulation tends to rely on values that reflect total arsenic levels, but the total content of a trace element, as determined by acid digestion, is rarely a good indicator of the element's mobility or potential risk (Allen 2001).

2.1 The Mobility and Solubility of Arsenic in Soils

The solubility of soil contaminants is a key parameter to understanding their probable mobility. The soluble fraction in which contaminants exist is in equilibrium with others that exist in the soil environment. Once dissolved in soil water, elements are often present as different species that have different ionic activities (Sauvé 2001). Typical concentrations of arsenic in the soil solution, under aerobic conditions are <50 nM in non-contaminated soils, while they can reach values of up to 2 μM in contaminated soils (Wenzel et al. 2002; Moreno-Jiménez et al. 2011a); however, an exception was one sample from a semi-flooded mine soil that had up to 40 μM As. In flooded soils (where the predominate species is arsenite), typical concentrations of As in solution vary between 0.01 and 3 μM (Zhao et al. 2009). In one As study, using lupine plants as indicators of As availability, the behavior of the in-soil crop was compared with that same crop grown hydroponically. In this study, an exposure of less than 10 μM of As in soil solution was established, wherein the total concentration of As was more than 2,000 mg kg^{-1} (Moreno-Jiménez et al. 2010b). In comparison to other trace elements, arsenic shows a low solubility in well-aerated soils (Beesley et al. 2010a; Moreno-Jiménez et al. 2010a).

Traditionally, sequential extraction has been used to fractionate trace elements that appear in soil. For arsenic, which is usually present in its anionic form in soils, specific protocols for arsenic extraction have been developed from these methods (Onken and Adriano 1997; Shiowatana et al. 2001; Wenzel et al. 2001). These extraction protocols have enabled researchers to determine that arsenic is often associated with oxides and hydroxides in soil (McLaren et al. 2006). The exchange surfaces of silicates and organic matter tend to be negatively charged, and therefore have a greater tendency to retain cations. In soils that have a low pH (where positive charges predominate), conditions may favor the retention of As in exchange positions. In soils affected by pyritic materials or one that are associated with pyritic mining, arsenic may be found in large proportions within the residual fraction, indicating that it is bound in its mineral form (i.e., associated with sulfides; Conesa et al. 2008).

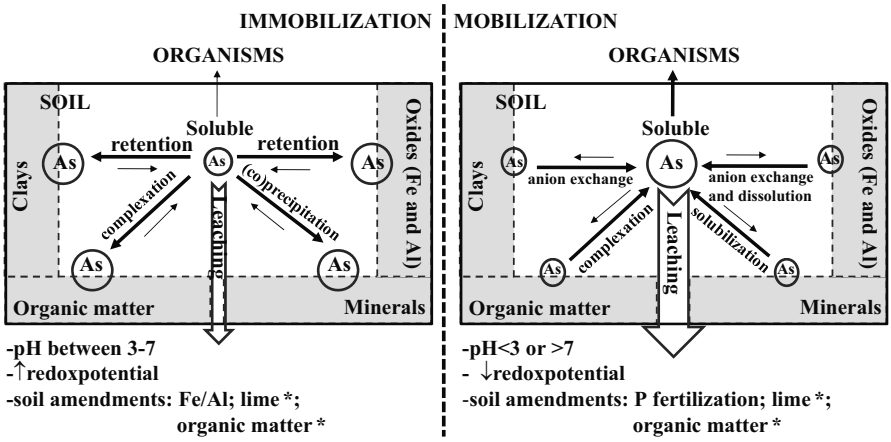

Fig. 1 Soil-As interactions and strategies to manage As availability and mobility in soils. *Asterisk* denotes liming and organic matter application, which may cause either As fixation or release depending on the case

The study of how arsenic is fractioned within soils can provide useful information about its mobility, migration, and potential toxicity. The As fraction retained in a labile form, within the soil matrix (soil and water) will be the most biologically active (bioavailable fraction) and the most mobile (soluble fraction) one. Figure 1 is an illustration that depicts the equilibrium achieved by As among several soil phases. From an ecological and toxicological point of view, the fraction that contains the contaminants in the soil matrix is the most important one and should be used as an indicator when analyzing soil contaminant risks (Mench et al. 2009). Therefore, the soil's solid phase is less important than the liquid phase or the equilibrium established between the solid and liquid phases (Sauvé 2001). To date, there is neither a clear consensus surrounding the concept of bioavailability, nor is there an exact way of defining it, in the context of As. In plants, the bioavailable As fraction would be *the amount of As a plant takes up from the soil*, although this concept has yet to be measured and cannot be predicted (Fitz and Wenzel 2006). The available and unavailable fractions of contaminants tend to be in equilibrium within the soil, but any change in environmental factors (pH, Eh, climate, biology, hydrology, organic matter, etc.), or alterations in mineral content (e.g., from dissolution–precipitation; oxidation–reduction; formation of complexes–disassociation; adsorption–desorption) can alter the availability of an element (Mench et al. 2009). This dynamic behavior notwithstanding, the analysis of soils by many methods have produced interesting results when estimating a contaminant's potential plant bioavailability. The available fraction has generally been measured by correlating amounts of the element found in the soil vs. amounts found within the plants grown in the soil (Feng et al. 2005; Vázquez et al. 2008a). When this approach is used, some neutral salt extraction methods (Vázquez et al. 2008a), or those utilizing organic acid mixtures (Feng et al. 2005) have produced satisfactory results. Moreover, monitoring

programs that rely on rhizosphere suction cups have been employed, and these are designed to measure the available fraction of interstitial water that occurs within samples (Clemente et al. 2010). One factor that is insufficiently understood is the rate of exchange that takes place between unavailable and available fractions, although that rate appears to be rather slow (Cattani et al. 2009).

2.2 Factors Determining Arsenic Availability in Soils

2.2.1 The Effect of pH and Eh

In contrast to what happens with other trace elements, a rise in pH often results in mobilization of arsenic in the soil. In general, a rise in soil pH causes a release of anions from within their exchange positions, such that arsenate and arsenite are released (Smith et al. 1999; Fitz and Wenzel 2002; Beesley et al. 2010b; Moreno-Jiménez et al. 2010a). However, several experiments (mainly with mine soils) have shown that high pH values, in the presence of sulfates and carbonates, can produce either a co-precipitation of arsenic in the subsequently formed oxyhydroxides and sulfates (García et al. 2009), or a precipitate such as calcium arsenate (which is slightly less insoluble than calcium phosphate) (Burriel et al. 1999). For this reason, some soils probably demonstrate their maximum As(V) retention at a pH near 10.5 (Goldberg and Glaubig 1988). In well-aerated alkaline soils, the solubility of As is limited by its precipitation as Ca or Fe arsenates (Xie and Naidu 2006). In soils with a high pH, carbonates can play an important role in the retention of arsenate (Zhang and Selim 2008). When the pH drops below 2.5, As(V) becomes completely protonated (Zhang and Selim 2008), rendering it less likely to be retained by soil particles.

As(V) is the predominant form that exists in soils, in which the pH + pe > 10; in contrast, As(III) is the dominant form found in soils, in which the pH + pe is less than 6 (Sadiq 1997). Under aerobic conditions, sulfides are easily oxidized, and as a consequence arsenic is released into the environment (Adriano 2001); when soil pH is between 3 and 13, the major species found are $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Smedley and Kinninburgh 2002). In reducing environments, arsenic is found as arsenite, the predominant species of which is H_3AsO_3. Poor adsorption of As(III) results from its neutral character in soils (Lakshmipathiraj et al. 2006). Arsenite is more mobile and more toxic than is arsenate. Poor adsorption occurs when the redox potential of the soil is negative (Fitz and Wenzel 2002), and changes in the handling or conditions of soils results in speciation changes of As (Carbonell-Barrachina et al. 2004). Highly reducing conditions can cause As co-precipitation with iron-sulfurs, such as arsenopyrite, or the formation of arsenic sulfides (AsS, As_2S_3). During the oxidation of pyrite, Fe is oxidized from valence II to III, and arsenic is oxidized to arsenate. In contrast, under reducing conditions, Fe and Mn oxides are dissolved, releasing arsenate that is rapidly reduced to arsenite (Gräfe and Sparks 2006).

2.2.2 The Role of Fe, Al and Mn Oxides, and Oxyhydroxides

Soils frequently retain important quantities of Fe, Al and Mn oxides, and oxyhydroxides. The distribution of these solids in the soil depends on both the pH and Eh of the soils involved. Under reducing conditions, the structure of Fe and Mn hydroxides is broken, and arsenic that was fixed to the interior or to the surface of these compounds is released. The activity of arsenic in the soil solution is controlled by reactions of retention and release along the surfaces of Fe, Mn, and Al oxides and hydroxides (Livesey and Huang 1981; Fitz and Wenzel 2002; de Brouwere et al. 2004), and soils with a large quantity of iron had a greater retention capacity of both arsenate and arsenite (Manning and Goldberg 1997), arsenite being retained in lower quantities than arsenate (Fitz and Wenzel 2002). As (V) has a high affinity for the surfaces of iron oxides, where it forms inner-sphere complexes; however, As (V) can also be retained in external-sphere complexes (Waychunas et al. 1993; Cheng et al. 2008). The results of several studies have shown that As(III) can be adsorbed and oxidized along the surfaces of some Fe-oxyhydroxides, such as goethite and ferrihydrite, or those of Mn (such as birmesite; Lin and Puls 2000). In other studies, it was demonstrated that the adsorption of As(V) on goethite, magnetite, and hematite is reduced when the pH is raised (Manning and Goldberg 1997; Giménez et al. 2007). Giménez et al. (2007) found that hematite had the largest sorption capacity, followed by goethite and then magnetite. Arsenate has a high affinity for the surfaces of iron oxides, as phosphate; however, arsenate has a lower affinity for aluminum oxides than phosphate (Adriano 2001). Under reducing conditions, when a large portion of the Fe and Mn oxides have been reduced, gibbsite (which is more thermodynamically stable in anaerobic conditions) is able to absorb some of the As released by other oxides (Mello et al. 2006). The adsorption of arsenic onto oxides depends on the duration of the interaction between As and the oxide, the release of arsenic being more difficult as the interaction time increases (Gräfe and Sparks 2006).

The addition of Fe to the soil in several forms immobilizes As. For example, additions of Fe oxides, iron-rich soils (those reddish in color), inorganic Fe salts or industrial byproducts, rich in Fe, together with $CaCO_3$, have all been used to raise the quantity of soil oxides, which, in turn, immobilizes As (Hartley et al. 2004; Hartley and Lepp 2008; Vithanage et al. 2007).

2.2.3 Concentration of P and Other Elements in the Soil

The phosphate anion, the major species of P present in soils (Marschner 1995), is an analog to arsenate. The application of P to soil results in a release of retained As (Fitz and Wenzel 2002; Cao et al. 2003). This release results from competition between the retention of both anions. Such ions not only compete non-specifically for anionic exchange positions but they also compete in complexation reactions or in the retention by oxides. When exchange positions are involved, there are competitor ions that are less efficient than phosphorus in displacing arsenic. Phosphate and

arsenate are more effectively retained than are other anions, such as Cl^- and NO_3^-, which are rapidly mobilized. This could result from the fact that chlorides and nitrates are adsorbed with little specificity along the colloidal surface, whereas phosphates and arsenates are specifically adsorbed in soil components. Roy et al. (1986) were able to show that the retention capacity of As(V) was lowered in the presence of phosphate ions. In a similar study, the presence of anions other than phosphate had no effect on As(V) retention (Livesey and Huang 1981). Woolson (1973) demonstrated how the application of phosphorous fertilizers in soils, contaminated by arsenic, mobilized up to 77% of the total arsenic found in the soil, and increased the availability of arsenic. It has been shown in numerous other studies that the application of P in soils causes an increase in the extractable fraction of As. This increase, however, is not necessarily reflected by a greater absorption of As by plants, because arsenate and phosphate are competitors also for absorption by plants (Esteban et al. 2003).

In addition to phosphate, As interacts with other anions. Increasing ionic strength of a soil solution is therefore one method used to reduce the quantity of As retained, and provoke competition between anions for exchange positions (Gräfe and Sparks 2006). The action of anions, other than phosphate, appears to be significant only in the absence of phosphate. Therefore, Stachowicz et al. (2008) observed that, in the absence of phosphate, carbonates moderately compete with arsenate for exchange positions, but when phosphorus is present, the effects of carbonates were not significant. Alternatively, cations can alter the retention/mobilization of As in soils. Smith et al. (2002) determined that the presence of Ca^{2+} and Na^+ causes an increase in the retention of As. Similarly, Stachowicz et al. (2008) described how Ca^{2+} and Mg^{2+} can induce the adsorption of phosphate and arsenate in soils.

2.2.4 The Effect of Clay Minerals

In general, the availability of arsenic is greater in sandy than in clay soils (Adriano 2001), although the retention of As in clays is less efficient than with oxides (Gräfe and Sparks 2006). Again, As(III) adsorbs to clay minerals less intensely than does As(V). There are many factors involved in the soil adsorption and desorption processes that affect As mobility. Among these factors is the structure of primary and secondary minerals to which As comes into contact. Another factor is the duration of interaction that exists between the clays of a soil and arsenic; the adsorption of As(V) and As(III) by clay minerals increases with time (Lin and Puls 2000). Zhang and Selim (2008) suggest that isomorphic substitutions of Fe by Al in clays contribute to the adsorption of As. The anionic character of arsenic suggests that the mechanism of clay adsorption of this metalloid may be similar to that of P, through calcium-bridging mechanisms (Fixen and Grove 1990). Frost and Griffin (1977) reported that montmorillonite can adsorb more As(V) and As(III) than does kaolonite, and the difference is derived from the increased surface charge of montmorillonite. Lin and Puls (2000) found that halosite and chlorite clays had a greater capacity to adsorb As(V) than did other clay minerals, and that kaolonite and ilite/montmorillonite, adsorb As (V) to a moderate degree. Arsenic is initially adsorbed on the clay surface,

but with time, it is incorporated into the structure of the mineral. It was demonstrated that Californian soils having a greater percent of clay and appreciable quantities of Fe oxides had a greater As retention capacity (Manning and Goldberg 1997).

2.2.5 Interactions with Organic Matter

Organic matter is of a heterogeneous chemical nature and constitutes a series of organic compounds of variable molecular weights that are differentially polymerized. This soil fraction is dominated elementally by carbon, oxygen, hydrogen, nitrogen, and phosphorus (in this order) and commonly has the following functional groups: carboxyls, carbonyls, alcohols, and amines (Stevenson 1982). The level of polymerization of humic compounds and their molecular weights influence their solubility: as these compounds diminish in size, they have a greater proportion of functional groups (organic, fulvic, and humic acids) and display higher solubility. If more highly polymerized, humic compounds have fewer functional groups, e.g., humins, and will display lower water solubility. The effect organic matter has on trace elements depends on the qualitative composition of the organic matter. An organic fraction that has a large molecular weight will more effectively retain trace elements, whereas a more soluble and lighter fraction tends to dissolve elements, either by chelating (metals) or by displacing (anions) them. Depending on what the predominant compound in the soil is, either of these effects will be observed in the soil.

How soil organic matter affects arsenic is inconsistent: in some studies, the application of organic matter reduced the mobility of arsenic (Gadepalle et al. 2007), whereas, in others As is released after the application of compost (or there is a higher correlation between soluble carbon and soluble arsenic in soils; Mench et al. 2003; Clemente et al. 2008). Weng et al. (2009) have recently studied how fulvic and humic acids in solution are able to reduce the capacity for arsenate retention in goethite through electrostatic competition. Therefore, dissolved organic matter can compete with arsenate and arsenite for soil retention positions (Bernal et al. 2009). Alternatively, some humic acids may form humic-clay complexes that have the capacity to retain As (Saada et al. 2003). Therefore, the relationship between soil arsenic and organic matter is complex and depends on multiple factors that include: the ratio of soluble organic carbon present, and the fractions of insoluble and stable humus, and the concentrations of Fe, Al, and Mn present in the organic matter (Gräfe and Sparks 2006). One important consideration, when adding organic amendments to a soil, is that the bioavailable fraction of As may be high (Beesley and Dickinson 2010), despite the fact that the total concentration of As is usually <30 mg kg^{-1} (Adriano 2001).

2.2.6 Other Factors

Large differences in various soil parameters may exist during the year, and hence, the availability of trace elements in soil may also be variable (Vanderlinden et al. 2006). These variations result from changes in soil physical properties (humidity, aeration,

porosity, temperature, etc.), chemical changes (pH, appearance of precipitates, Eh, etc.), and biological changes (microbial activity, vegetation cover, etc.). Depending on the concentration of As present and the humidity regime in the soil, precipitates of insoluble salts (e.g., Fe and Ca arsenates, or co-precipitates with jarosite, gypsum or calcite) can form (de Brouwere et al. 2004; Zhang and Selim 2008; Cheng et al. 2008; Kreidie et al. 2011). Such precipitates are frequent in mining sites, where high concentrations of As exist in the soil; if As concentrations are high and rainfall takes place the risk that As will leach from soil will increase. The duration of the interaction between arsenic contamination and the soil is another factor that must be considered, since the bioavailability of As decreases as it ages in soil (favoring its retention in less available fractions; Lombi et al. 1999).

3 Arsenic in Plants

3.1 Absorption and Transport

Different arsenic species simultaneously exist in soils (e.g., As(III), As(V), MMA, DMA; Takamatsu et al. 1982). Where the inorganic form of As predominate, the arsenic is primarily taken up into plants via root absorption, in a process analogous to how nutrients and other trace elements are absorbed.

3.1.1 Changes to As Mobility

Plant roots use fairly efficient mechanisms to modify the solubility and availability of mineral elements in the soil (Marschner 1995). Hence, a plant has a direct influence over the biogeochemical conditions in the area of the root or rhizosphere (Mengel and Kirkby 2001). For example, organic exudates, organic molecules of low molecular weight, are able to mobilize nutrients that are available only in low quantities in the soil, thus rendering anions (phosphates) and cations (Fe, Cu) more available. Alternatively, mechanisms by which plants immobilize toxic soil elements, as is the case of aluminum, have also been described (Mariano and Keljten 2003). It is also known that plants are capable of altering the pH of the rhizosphere (Marschner 1995), thanks to the release of organic acids that serve as soil solution buffers. Moreover, most plants establish relationships with microorganisms (fungus and bacteria) at the root level, which also influence the biogeochemical cycle in the rhizosphere. If bacterial activity in the rhizosphere is particularly high, methylation, reduction, or other forms of bacteria-based biochemical activity is favored (Renella et al. 2007). Little is known about the mechanisms by which the availability of toxic soil elements are altered (Kidd et al. 2009), but it is known that the characteristics of the rhizosphere intrinsically depend on the plant species involved.

Although little information exists on the mechanisms by which plants mobilize or immobilize As (Fitz and Wenzel 2002), the analogy drawn between phosphate and arsenate allows us to establish certain parallels between the rhizospheric dynamics of P and As. Many plant species have an active mechanism for pumping organic acids from roots into the rhizosphere, rendering P more mobile (Smith and Read 1997). The mobilization of P induced by organic acids has been attributed to root-influenced pH changes (Raghothama 1999). These organic acids have a low molecular weight (carboxylic acids such as citric and malic acids) and are able to displace phosphate from positions of retention within the soil; they then act to chelate metals that immobilize P, and form metal–chelate complexes with P (Fitz and Wenzel 2002). The soil solubilization of P and its absorption by plants has additionally been attributed to the secretion of flavonoids from roots (Tomasi et al. 2008). Because arsenate and phosphate are chemically analogous, all of these processes are likely to mobilize As; for example, organic acids are capable of displacing arsenate from exchange positions in soils (Redman et al. 2002; Wenzel 2009). Additionally, plant strategies to attack oxides-hydroxides of Fe will also alter the surfaces on which As is retained, and this can potentially solubilize As (Fitz and Wenzel 2002).

Mycorrhizae are associations between plant roots and fungi, wherein the fungi are able to colonize roots, either intra- or extra-cellularly (depending on the type of mycorrhiza formed). Hence, mycorrhizae play an important role in the mineral nutrition and absorption of elements by plants (Marschner 1995; Azcón-Aguilar and Barea 1997; Fitz and Wenzel 2002). Mycorrhizal interactions are produced in approximately 80% of angiosperms and in all gymnosperms (Fitz and Wenzel 2006), and in many cases provide plants with increased resistance to biotic and abiotic stresses (Latch 1993; Schutzendubel and Polle 2002). Phosphorus is particularly important: many mycorrhizae play a fundamental role in P nutrition in the plant (Mengel and Kirkby 2001), and act to increase, by two to three times, the amount of P accumulated in plants (Fitz and Wenzel 2006). Therefore, the study of mycorrhizae is relevant to an understanding of how As/P interact in plants. As absorption is reduced in the presence of mycorrhizae in several plant species, including *Pteris vittata*, lentils, alfalfa, tomatoes, sunflowers, and corn (Trotta et al. 2006; Ahmed et al. 2006; Chen et al. 2007; Liu et al. 2005; Ultra et al. 2007a; Yu et al. 2009). Corn mycorrhizae were able to significantly reduce the amount of arsenate, but not arsenite, absorbed by roots (Yu et al. 2009). In this same experiment, a reduction in arsenate reductase activity also occurred in the root from a fungal infection. Some authors attributed the lower concentrations of As in the plants infected with mycorrhizae to a blockage of absorption (Yu et al. 2009), whereas others attributed it to a dilution effect observed from the increase in overall plant mass (Chen et al. 2007).

3.1.2 Absorption of Arsenic

Root absorption of elements first occurs by diffusion from the soil solution within the root apoplast, followed by the symplast, effecting penetration to the interior of

plant cells (Mengel and Kirkby 2001). At the cellular membrane, ions enter via transport proteins that are often specific for one or several elements of similar chemical characteristics. Aquaporins and phosphate transporters both are involved in the transmembrane transport and absorption of arsenic.

Aquaporins are water channels; however, other non-charged molecules, such as arsenious acid, also enter through them. Various authors have addressed aquaporins in relation to the absorption of As(III) (Isayenkov and Maathuis 2008; Ma et al. 2008). Interestingly, it has been experimentally determined that As(III) and Si both share the same methods of entrance into and transport in rice (Ma et al. 2008). Hence, there is a group of aquaporins (NIPs) that play a fundamental role in the absorption of non-charged molecules, such as glycerol, ammonia, and the boric, arsenious, and silic acids (Zhao et al. 2009).

Physiological studies in plants have illustrated the important role that phosphate carriers play in the absorption of arsenate, and the interaction that occurs between both P and As(V) at the root uptake level (Meharg and Macnair 1992; Esteban et al. 2003). The phosphate/arsenate mechanism of absorption involves the co-transport of the anion with protons, in a stoichiometry of $2H^+$ for each anion (Zhao et al. 2009).

Organic forms of As are absorbed less effectively than are inorganic As forms (Marin et al. 1992; Raab et al. 2007; Zhao et al. 2009), and Si transporters may also be involved (Li et al. 2009a).

3.1.3 Accumulation and Transport

Once inside the cell, arsenate is reduced to arsenite, which consumes reduced glutathione: $AsO_4^{-3} + 2\ GSH \rightarrow AsO_3^{-3} + GSSG$, a reaction that is catalyzed by arsenate reductase (Verbruggen et al. 2009). It has therefore been shown that the majority of arsenic that exists in plant tissue is present as arsenite (Tripathi et al. 2007), regardless of what was in the solution at the time of plant growth. Arsenite has a high affinity for SH groups and tends to be complexed and stored in vacuoles, although it can also be transported via the xylem to other plant tissues. Movement through the xylem is controlled by the flow of the transpiration stream, but is also influenced by membrane transport proteins. Recently, two transporters (Lsi1 and Lsi2) were described between plant endodermis and exodermis, and these mediate the entrance of arsenite into the xylem or its efflux to the external medium (Zhao et al. 2009). These Lsi transporters are principally involved in Si nutrition (Ma et al. 2008).

The transport of As, in most plant species, is generally not very effective, and hence As tends to remain in roots. An exception exists for those plants that are unusually effective at accumulating As in aerial plant parts. Accumulation of arsenite in the vacuole may be one reason why As transport into the xylem is reduced (Zhao et al. 2009). Xylem transport of As has been intensely studied over the past years giving interesting, although somewhat contradictory, results. In many plant species, the reduction of As(V) in roots appears to be a key factor that results in blocking the xylem transport of As. Since phosphate is an anion that is completely

mobile within plants, one would expect As(V) to act similarly (given the analogy between both anions), but this is not the case in any plant species. The influence of reduced As to block its transport was tested in plants (*Arabidopsis thaliana*) mutated to silence arsenate reductase. These plants demonstrated a ratio of $[As]_{Aerial\ tissue}$:$[As]_{Root}$ that was 25 times greater than the wild type (Dhankher et al. 2006). These authors suggest that this change results from a greater proportion of As(V) being available for transport through the xylem in roots, presumably through the same pathways that handle phosphate.

The majority of As that does not accumulate in aerial tissue exists as As(III) (Pickering et al. 2000; Dhankher et al. 2002; Castillo-Michel et al. 2007; Xu et al. 2007). The reducing process in roots may constitute a physiological mechanism by which plants limit the flow of As into aerial tissues, thus protecting them from the effects of this metalloid. Additionally, the majority of As in the root is found as complexes (Vázquez et al. 2005), and there is a negative correlation between the percent of As complexed by –SH groups in roots vs. the amounts translocated to aerial tissue (Huang et al. 2008). In this respect, Raab et al. (2005) proposed that complexed As is not transported through the xylem. In hyper-accumulator plants, some authors have utilized synchrotron and liquid chromatography, coupled to ICP-MS techniques, to determine how As is transported from roots to aerial tissues as As(V), and how As is later stored in leaves as As(III) (Zhao et al. 2003; Pickering et al. 2006; Hokura et al. 2006; Tripathi et al. 2007). Duan et al. (2005), however, reported that arsenate reductase has greater activity in the roots of *P. vittata*, postulating that As transported through the xylem in this fern is primarily in the form of arsenite. Notwithstanding, in both plants that accumulate arsenic and in those that do not, it appears that once inside the cytoplasm, As is stored in vacuoles, thus avoiding interference with normal cellular function (Verbruggen et al. 2009). Other trace elements are generally stored in tissues that are less metabolically active, such as epidermic (Vazquez et al. 1992) or senescing tissues (Ernst et al. 2000).

Baker (1981) established a classification that grouped plants into three categories, based on how much element they accumulated in shoots. The three classes are called excluders, indicators, and accumulators, and these terms will be used henceforth below. Some species are able to hyper-accumulate As: *P. vittata* (up to 22,000 mg As per kg), *Jasione montana* (6,640), *Calluna vulgaris* (4,130), *Agrostis tenuis* and *stolonifera* (10,000), *Pityrogramma calomelanos*, *Mimosa púdica*, *Melastoma malabratrhicum* (8,350) (Wang and Mulligan 2006). Except for these particular plants, most plants accumulate arsenic in their roots. This, however, does not exclude the fact that many plants are adapted to grow in soils that have high As concentrations, without accumulating it. Among such plants are the tolerant excluder ecotypes. The concentration of arsenic in non-accumulator plants rarely exceeds 2 mg As per kg in aerial parts (Horswell and Speir 2006). From an agricultural perspective, areas flooded with waters rich in As may pose a problem, because these crops may contain sufficient As levels to render them dangerous for human consumption (Bhattacharya et al. 2007). In Fig. 2, links between physiological traits in plants and As-phytoaccumulation strategies are presented.

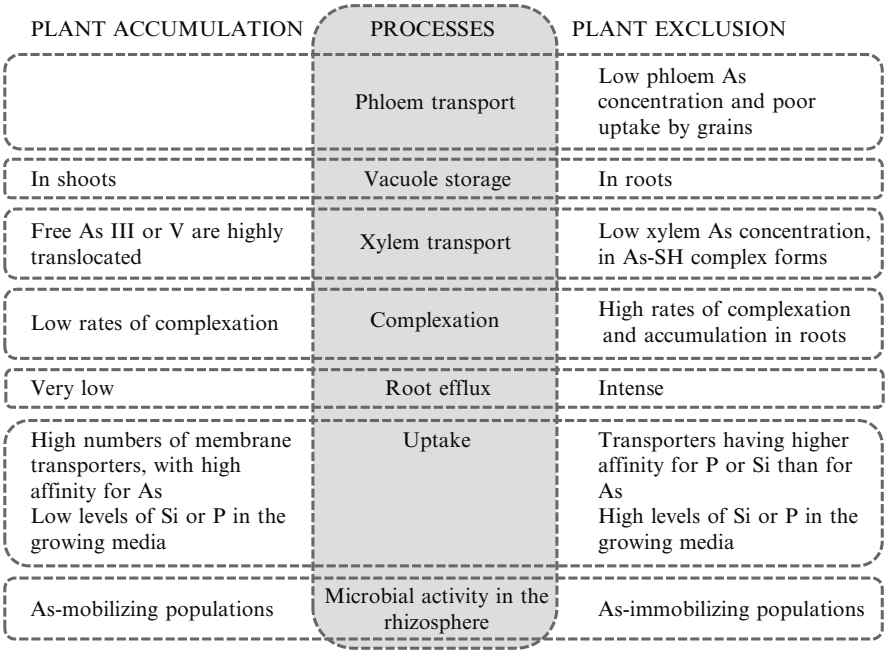

Fig. 2 Plant's traits and physiological mechanisms recognized to be associated with contrasting patterns of As phytoaccumulation: accumulator plants (*left*) vs. exclusory plants (*right*). (*Blank boxes* denote lack of information)

3.2 Arsenic Toxicity in Plants

When present within plant cells, As has various negative physiological effects, many of which have recently been the subject of intense study. Arsenic has no known biological function, although positive effects have been described at low concentrations of arsenate. The reason behind these positive effects has yet to be determined. Although these effects may be attributed to As itself, they may also result from an increase in the absorption of P when As concentrations remain below toxic thresholds (Carbonell-Barrachina et al. 1998). Arsenite is generally more toxic than arsenate, partially because of its greater solubility and mobility. The sensing and signaling of an excess of As in plants and the complex biochemical changes it induces are still unknown, although they have been extensively studied in the recent years (Verbruggen et al. 2009). When the toxicity of trace elements is studied in plants, herbaceous plants and/or crops are generally the target species used. Woody species are uncommonly used in As plant toxicity testing, despite the fact that they are favored when attempts are made to revegetate degraded soils.

The toxicity of organoarsenics has been scarcely studied in plants; however, they appear to block mersistematic tissues and also affect protein synthesis (Horswell and Speir 2006).

3.2.1 Visual Effects

Impairment of plant development is one of the observed symptoms from arsenic toxicity. Arsenate and arsenite both reduce the growth of plant species, such as *Holcus lanatus*, *Lupinus albus,* and *Triticum aestivum,* when grown under hydroponic conditions (Hartley-Whitaker et al. 2001; Vázquez et al. 2005; Geng et al. 2006). Among the symptoms induced by As exposure in plants are reduced root elongation, loss of root branching, chlorosis in leaves, and shrinking or necrosis in aerial plant parts (Carbonell-Barrachina et al. 1998).

3.2.2 Oxidative Stress

Elements that have several oxidation states often serve as good reaction catalysts in which oxidation-reduction is involved (As, Cu, Hg, etc.). In aqueous solutions of near neutral pH, such as in the cytoplasm, radicals can produce H_2O_2, which later produce hydroxyl radical. All reactive oxygen species (ROS) can directly damage biomolecules and cause peroxidation of membrane lipids. Arsenic can also induce oxidative stress in plant cells, a fact reflected as an increase in the concentration of malondialdehyde (MDA) in vegetable tissues; MDA is a metabolic product of the peroxidation of lipids in biological membranes. The stimulation of oxidative stress, associated with the accumulation of arsenic in plants, has been shown to occur in both cultivated plants, and in wild bushes (Hartley-Whitaker et al. 2001; Mascher et al. 2002; Moreno-Jiménez et al. 2008, 2009a). Arsenic also modifies the gene expression involved in cellular homeostasis for redox perturbation (Requejo and Tena 2005) and activates some antioxidant enzymes (Srivastava et al. 2005).

3.2.3 Nutritional Disorders

Plant mineral nutrition is affected in a non-specific manner by many toxic elements. Effects result either from an alteration in transport processes of the cellular membrane, or effects on transpiration. The most notable effect produced by As results from the similarities that exist between arsenate and phosphate. Addition of arsenate causes a decrease in the levels of P in plants, because the entry of both ions is mediated through the same membrane transporter (Meharg and Macnair 1992; Vázquez et al. 2005). Among other changes to nutritional patterns caused by the presence of As in the environment in which plants are grown, is a decrease in the concentrations of Mn, Fe, Cu, N, Zn, and Mg (Mascher et al. 2002; Vázquez et al. 2008b).

3.2.4 Photosynthetic Inhibition

Arsenic causes chlorosis in plant leaves because of the induced decrease in chlorophyll levels (Mascher et al. 2002). There may be other reasons for As-induced chlorosis

(such as iron deficiency), but the primary cause is directly attributed to the inhibition of pigment synthesis (Jain and Gadre 1997). The effects produced result from limited availability of δ-aminolevulinic acid (a precursor to chlorophyll) and from alterations in proteins rich in thiol groups.

3.2.5 Metabolic and Genetic Alterations

Theoretically, arsenate can substitute for phosphate in some metabolic processes. Within the cytoplasm, arsenate competes with phosphate in phosphorylation reactions, such as the synthesis of ATP. Herein, ADP-As is formed, altering the energetics of the cell cycle because of its instability (Verbruggen et al. 2009). Arsenite demonstrates a high affinity for –SH groups of biomolecules (enzymes and proteins), inhibiting their function (Meharg and Hartley-Whitaker 2002). As(III) and (AsV) are mutagenic compounds that can alter the genome (Lin et al. 2008). For this reason, the presence of repair biomolecules in the chromatin may be able to increase a plant's tolerance to arsenic (Verbruggen et al. 2009).

3.3 Mechanisms of Arsenic Resistance in Plants

There is a difference between plant resistance and plant tolerance to toxic elements. Resistance to toxic elements is generally defined by a plant's capacity to support an excess of a toxic element present in the environment. By comparison, tolerance is the exceptional capacity of a plant to survive in a soil that is toxic to other plants, demonstrated by the interaction between genotype and environment (Hall 2002). Ernst et al. (2008) have established another classification, based on the tolerance/sensitivity a plant has to trace elements: (1) hypotolerant, sensitive, or hypersensitive plants are those with genetically modified phenotypes that are extremely vulnerable to one or many metals or metalloids; (2) basal tolerant – would be equivalent to resistance, meaning genetic resistance of one species (also called non-metallic populations or populations with constitutive tolerance); (3) hypertolerants are populations that show a low sensitivity to one or more elements due to adaptive mechanisms (also called metalophytes). Plants resist the effects of toxicity by using several biological mechanisms, all of which are known to be detoxification mechanisms. There is still insufficient research information available to explain what cellular mechanisms plants use to detect the presence of As. Moreover, what signals are triggered after As is detected and detoxification mechanisms initiated is also not known, other than those cellular signals derived from the tissue damage produced (Verbruggen et al. 2009).

3.3.1 Mycorrhization

Mycorrhizae are able to modify tolerance patterns in host plants (Sharples et al. 2000; González-Chávez et al. 2002; Leung et al. 2006). In regard to the effects that

mycorrhizae cause in their hosts, plants can be categorized as follows: (1) plants that respond positively to mycorrhization, with increases in biomass and improvements in mineral nutrition (AM-r plants), and (2) plants in which mycorrhization has no effect (n-AM-r plants). Smith et al. (2010) discussed how mycorrhization effectively alleviated the toxic effects of As on AM-r plants, whereas literature references on those same effects on n-AM-r plants are variable. In wild rye, for example, some plants infected with mycorrhizae (n-AM-r) showed no improved tolerance to As (Knudson et al. 2003), while in other cases, many plants infected with mycorrhizae did show tolerance improvement. When tolerance was improved in plants infected with mycorrhizae, there were several different reasons for the improvement. Xu et al. (2009), for example, attributed improvement to greater absorption of P and a reduced concentration of As in *Medicago truncatula* plants, while Yu et al. (2009) explained this same effect as an inhibition in the absorption and speciation of As in plants infected with mycorrhizae.

3.3.2 Immobilization Within the Rhizosphere

It has been shown that, in flooded soils, iron plaques are formed in areas surrounding the roots. These plaques are able to retain high concentrations of As, and act as the first filter at the root level. This mechanism is especially efficient in rice paddies or where plants grown in liquid media (Hansel et al. 2002; Liu et al. 2004, 2006). In the rhizosphere of aerated soils, redox reactions favor the formation of iron plaques that retain As in the areas directly surrounding roots. Fe, retained in the apoplastic area surrounding roots of plants grown in well-aerated soils, could act in a similar manner as that of flooded soils (Doucleff and Terry 2002).

3.3.3 Exclusion

Exclusion reduces the entrance of As via changes in the mechanisms of root absorption. Tolerant populations of the plant *H. lanatus* lack the high affinity uptake system for phosphate absorption (Meharg and Macnair 1992). For this reason, these plants had reduced absorption of both phosphate and arsenate, and were better adapted to environments having high concentrations of As (Meharg and Hartley-Whitaker 2002). *Arabidopsis thaliana* plants, in the presence of As(V), slowed gene expression associated with phosphate absorption; simultaneously, the plant induced transcriptional gene expression that was stimulated by As, suggesting that there are distinct transcriptional pathways that regulate the deficiency of P and that the responses to As are interconnected (Catarecha et al. 2007).

3.3.4 Active Efflux

Arsenic can be pumped out of plant cells (Xu et al. 2007), mainly in the form of arsenite. Although this mechanism has yet to be clearly established, the status of the

information that exists on the subject was recently summarized by Zhao et al. (2009). Active efflux of arsenite has been observed to occur in various plant species grown under hydroponic conditions: wheat, barley, corn, tomato, *H. lanatus,* and *A. thaliana*. Physiological evidence indicates that the expulsion process is active and depends on a proton gradient created by cellular metabolism. It has yet to be demonstrated that increased expulsion correlates directly with improved plant tolerance within a given plant population; however, this relationship has been demonstrated in microorganisms (Bhattacharjee and Rosen 2007). Studies performed in soil have provided evidence that arsenite accumulates in areas directly surrounding the roots of sunflower and corn (Ultra et al. 2007a, b; Vetterlein et al. 2007). It is estimated that up to 50–80% of the As absorbed by roots may be secreted via active efflux in non-accumulating plants (Verbruggen et al. 2009). In contrast, it has been demonstrated that hyper-accumulating plants do not show As efflux to the environment via roots (Zhao et al. 2009).

3.3.5 Complexation

Plants take advantage of the high affinity arsenite has for –SH groups to deactivate the toxic effects of As. Therefore, there are many ligands in the cytosol for which trace elements have a high affinity. In certain plant species, As(III) complexes exist that have different biomolecules rich in thiol groups such as glutathione and phytochelatines (Meharg and Hartley-Whitaker 2002). For example, it has been shown that the presence of some trace elements, such as Ag, As, Cd, Cu, Hg, Se, or Pb in plants, provoke the synthesis of phytochelatines. Phytochelatines (PCs) are small peptides, rich in cysteine, that have the general structure (y-Glu-Cys)*n*-Gly, where $n = 2–11$. PCs are bound to trace elements via thiolate bonds that are synthesized from glutathione (GSH) and catalyzed by the enzyme PCsynthase. PCs are able to effectively complex inorganic arsenic that accumulates in plants, and GS_3-As(III) complexes have been identified to exist in certain plants (Pickering et al. 2000). Many plant species have responded to the presence of As in the environment, such as lupin, *H. lanatus*, sunflower or *Silene vulgaris* (Sneller et al. 2000; Hartley-Whitaker et al. 2001; Vázquez et al. 2005; Raab et al. 2005; Aldrich et al. 2007; Moreno-Jiménez et al. 2008, 2009a). *Arabidopsis* plants that have a greater capacity to synthesize PCs were more tolerant than were the wild types (Dhankher et al. 2002); moreover, resistant clones of *H. lanatus* showed a greater production of PCs in response to As than sensitive plants (Hartley-Whitaker et al. 2001). The foregoing points indicate that PCs, and probably GSH, play a role in the detoxification of As. However, this mechanism requires great metabolic effort and, in some instances, greater tolerance has not been correlated to greater levels of phytochelatines or thiols. Therefore, in hyper-accumulating plants, this detoxification method appears to be limited in comparison to the high levels of As that accumulate (Zhao et al. 2003). And, there is some evidence that the proportion of As complexed by SH in the root is negatively correlated with the translocation of As to the aerial portion of the plant (Huang et al. 2008).

3.3.6 Compartmentalization

Once arsenite is complexed, it is most probably stored in the vacuoles of root cells, which reduce its mobility in the cytoplasm and its translocation through the xylem. It is believed, however, that vacuolar compartmentalization, and thereby detoxification of As, is important in all plant organs (Pickering et al. 2006). Once inside the vacuole, where the pH is approximately 8, the rupture of the complex could take place and the ligand could then be used to complex more arsenite. Although there is no experimental data to show that the entrance of PC-As or GSH-As through the tonoplast takes place under in vivo conditions (Tripathi et al. 2007), it is known to occur under in vitro conditions (Dhankher et al. 2006). The entrance of such arsenite forms that are complexed to thiol groups is most likely facilitated via ABC- (ATP-binding cassette superfamily) type transporters (Verbruggen et al. 2009).

Some authors have suggested that As is retained in cell walls (Doucleff and Terry 2002; Vázquez et al. 2007), although this has not clearly been established. Cell wall retention has been demonstrated to be an effective detoxification mechanism for other trace elements, such as Cd and Hg (Zornoza et al. 2002; Moreno-Jiménez et al. 2007).

4 Practical Applications for Mitigating Arsenic's Effects

4.1 Phytoremediation

Phytoremediation is the use of plants to remediate organic or inorganic contaminates of soils and water (Salt et al. 1995). Chaney et al. (1997) defined soil phytoremediation as the use of plants, amendments, or agricultural techniques to eliminate, retain, or reduce the toxicity of soil contaminants. From the onset, phytoremediation was seen as a competitive technology for recuperating soils. It has promised and has produced positive results, which makes it attractive to both the commercial and scientific communities (Peuke and Rennenberg 2005). The benefits that phytoremediation has shown over competitive techniques (physico-chemical site-cleaning methods) are as follows: (1) it is inexpensive, (2) it is less invasive, and (3) it is well received both environmentally and socially. Phytoremediation has been successfully used to clean arsenic-contaminated soils and water.

4.1.1 Phytoextraction

Phytoextraction is the technique plants use to accumulate significant quantities of a contaminant in their tissues, and these plants can later be harvested or collected for appropriate disposal or management (McGrath and Zhao 2003). This technology has been studied and applied to events connected with Cd, Zn, Pb, Ni, and As

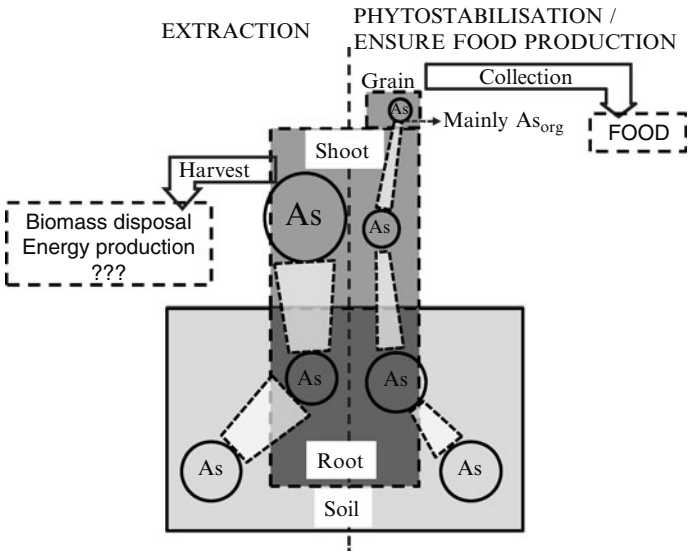

Fig. 3 Optimizing arsenic management in soil-plant systems. (The size of *circles* and of "As" symbols represent the concentration magnitude of the metalloid. The *discontinuous polygons* represent the flux of As, with the broadest, being the most intense flux. The *white discontinuous squares* represent the endpoint of the biomass. In grain, As in organic form ensures the lower ecotoxicologial risk)

contamination. Except for Ni, which has economic value (the technique with Ni clean-up is called phyto-mining; Chaney et al. 2007), the other forms of contaminated plant biomass pose a disposal problem (Robinson et al. 2006). In some cases, phytoextraction has been assisted by mobilizing agents, although the experiments have shown that one must be careful to control leaching, because there is high risk that the contaminant could disperse into water sources (Nowack et al. 2006). McGrath and Zhao (2003) estimated that, if one plant produced 10 t of biomass per hectare, it would need to accumulate a contaminant (soil/plant concentration ratio) by 20-fold to reduce it to 50% of the original soil level, after ten plant cycles. Phytoextraction of As (Fig. 3) has been tested using in-container studies that utilize the hyper-accumulating plant species *P. vittata*, although other hyper-accumulating plants are available (Meharg 2005). A recent and cogent review was published that was specific to As accumulation in *P. vittata* (Xie et al. 2009).

Pteris vittata has root-level transporters that have high affinity and capacity for As absorption (Poynton et al. 2004). In addition, this species shows a limited root complexing of As (Zhao et al. 2003) and elevated concentrations of As in sap (Su et al. 2008). Together, these characteristics trigger an extraordinary level of translocation of As towards the aerial portion of the plant (Tu and Ma 2002). Although As is usually not that bioavailable from soils, the rhizosphere of *P. vitatta* appears to be uniquely capable of mobilizing As, even from soil fractions that are barely available (Fitz et al. 2003). A reduction in redox potential, and an increase in the amount of

organic carbon released in the rhizosphere of ferns may be what enhances As uptake by the plant. Moreover, following phytoextraction with this plant, the available As fraction was reduced. Unfortunately, the distribution and ecological niche of *P. vittata* is restricted, which confines its application to specific zones. In addition, there is the problem of having to dispose of the harvested (contaminated) plant tissue. Recently, two reviews have been authored, in which the necessity of improving the genetics of phytoextracting plants through molecular plant biology was described (Tripathi et al. 2007; Zhu and Rosen 2009).

It has recently been determined that the As fraction retained in labile form is recharged at a slow rate, which impedes phytoextraction (Cattani et al. 2009). With respect to assisted phytoextraction of As, results have revealed that As absorption by plants increased after application of phosphate (Tassi et al. 2004) or biodegradable chelating agents such as HIDS (Hydroxyiminodisuccinic Acid) and EDDS (Ethylenediamine-*N*,*N*′-disuccinic Acid) (Azizur-Rahman et al. 2009). In this case, it is necessary to carefully evaluate the consequence of diffuse contamination, when As becomes more soil mobile (Peñalosa et al. 2007).

4.1.2 Phytostabilization

Phytostabilization is the mixed use of plants and agricultural practices to reduce mobilization and transfer of contaminants (Chaney et al. 1997). When plants are present in the soil, contaminants are stabilized, making them less susceptible to erosion and wind dispersion. Plants accumulate contaminants in the root, which further impedes their transfer and mobility. When this occurs, auto-sustainable plant species that have a prolonged life cycle and are adapted to such environmental conditions are especially interesting. The concept of natural attenuation consists of an ecosystem's and soil's capacity to auto-regulate and react slowly to chemical attacks, which can potentially reduce the risks associated with the presence of the contaminating element (Adriano et al. 2004). Assisted natural attenuation refers to the use of techniques such as replanting or the application of amendments to accelerate the process of natural attenuation (Madejón et al. 2006). For As, it has been demonstrated that both natural attenuation as well as phytostabilization could be useful field techniques (Vázquez et al. 2006; Madejón and Lepp 2007; Domínguez et al. 2008; Moreno-Jiménez et al. 2010a, 2011b).

The authors of some studies, however, have described plants that have little influence on the available As fraction, particularly when As exists at high soil concentrations (King et al. 2008). Phytostabilization is aided when organic or inorganic amendments are used; these materials improve soil properties by retaining metalloids or preventing their solubilization (de la Fuente et al. 2009). Kumpiene et al. (2008) reviewed different amendments that could immobilize As in soils. They concluded that the amendments could be both useful and effective in managing arsenic contamination. Among useful inorganic amendments are those rich in iron (red sludge, rolling mill scale, etc.), fly ash, clays or liming materials (Kumpiene et al. 2008; de la Fuente et al. 2010), whereas organic amendments capable of immobilizing

As are those that have high stability of the humus (de la Fuente et al. 2009). In either case, it is necessary to monitor contaminants over time to assure that the phytostabilization process has been a success (Vangronsveld et al. 2009).

Recently, phyto-attenuation has been described as the process by which a plant extracts the most available fraction of an element from the soil, which can later be harvested in such a way that it reduces the risks associated with inorganic soil contamination (Meers et al. 2010). Phytostabilization of arsenic in roots has been confirmed as a useful remediation technique, because there is low risk of As being remobilized, even when the roots themselves are mineralized (Vázquez et al. 2008c; Moreno-Jiménez et al. 2009b).

4.1.3 Phytofiltration

The presence of As in water presents one of the greatest of environmental risks to human health; As presents both a direct risk through consumption, as well as through indirect risk from contaminated irrigation waters. Phytofiltration is a technique that uses plants/roots to decontaminate water (Raskin et al. 1997). Aquatic, semi-aquatic, and terrestrial plants, and plant biomass (Haque et al. 2007) have all been used to eliminate or reduce arsenic contaminated water. Ideally, plants used in phytofiltration must be effective in capturing the contaminant from water, and must also grow rapidly. Plants that are not adapted to grow in anoxic conditions or those that are unable to grow rapidly in such conditions, may require the water in which they grow to be artificially aerated. Blastofiltration is the use of plant seedlings instead of plants. Seedling use increases the surface-area ratio of contact/volume of water, allowing better contaminant absorption in plant material (Raskin et al. 1997). Anawar et al. (2008) recently reviewed the use of phytofiltration in water, concluding that this method could be an effective alternative. Two strategies to deal with As contamination by this method have been studied: (1) employing plants that need a support structure and (2) using species of plants that float on water. The first strategy relies on *P. vittata* to eliminate As from solutions by growing the plants under hydroponic conditions in contaminated water (Malik et al. 2009). Plants can purify solutions that retain up to 200 $\mu g\ L^{-1}$ of As, and can lower the concentration present to less than 10 $\mu g\ L^{-1}$, in only 24 h (Huang et al. 2004). In other experiments, design parameters were optimized to achieve maximal As uptake by stabilizing the pH below 5.2 (Tu and Ma 2003). In the second strategy, plants of the genus *Lemna* and the macrophyte *Spirodela polyrhiza* were able to reduce the concentration of As in contaminated waters (Azizur Rahman et al. 2007; Sasmaz and Obek 2009).

4.2 Reducing Arsenic Transfer to Edible Plant Tissues

The main sources of arsenic exposure to humans are through consuming contaminated water (Hurtado-Jiménez and Gardea-Torredey 2006) or food (Meharg et al. 2008). In some countries, there is a high risk of exposure to arsenic from eating contaminated foods (Meharg et al. 2009).

4.2.1 Presence of Arsenic in Rice

Rice is the principal source of human nutrition in much of the world. It is commonly grown in naturally or artificially flooded soils. When rice is grown under conditions of poor aeration, arsenic is primarily found as As(III), which is highly mobile. In this form, As is easily absorbed by rice plants, whose capacity to accumulate As is greater than most other crops, such as wheat or barley (Williams et al. 2007). As is stored in rice tissues at increasing concentrations in the following parts: external iron plaque > root > straw > husk > grain (Liu et al. 2006). Therefore, formation of an external iron plaque inhibits, in part, the accumulation of As in the rice plant (Liu et al. 2006).

Regardless of order, the concentrations of As that appear in edible portions of rice are very high, especially considering the quantities of rice eaten by humans. Meharg et al. (2009) have shown that eating rice is the main source of As exposure in many countries, and increases risks associated with chronic arsenic exposure. Liao et al. (2010) described how consuming contaminated rice is associated with skin disease in children. The average concentrations of As in rice are between 0.05 and 0.95 mg kg^{-1}, although some samples exceed 1 mg As per kg (Williams et al. 2005). It has been estimated that the quantity of arsenic consumed per person, per day, in some countries, may be up to 0.9 mg (Butcher 2009). According to the WHO, this intake amount approaches the maximum tolerable limit for daily ingestion of As (Williams et al. 2005).

Experiments in recent years have shown that the development of agricultural and genetic techniques may help control or reduce such risks (Tripathi et al. 2007; Zhao et al. 2009). One approach to risk reduction involves selecting varieties of rice that accumulate less As in their edible parts (Williams et al. 2005). Alternatively, the same end may be achieved by using genetic techniques (Tripathi et al. 2007). Lemont, Azucena, and Te-qing are rice cultivars that apparently accumulate less As in their seeds (Norton et al. 2009). Genetic modification studies are also being conducted to evaluate methods by which As accumulation in edible tissues can be reduced, or As volatilization increased from edible tissues (Tripathi et al. 2007).

Work to mitigate As levels in crops through changes in agricultural practices have also been attempted (Fig. 3). Using As-contaminated water for crop irrigation has increased As uptake from soils into plants for decades (Williams et al. 2006; Kahn et al. 2009). Using clean irrigation water or purifying the water before use in irrigation would obviously reduce food levels of arsenic. Improving aeration of soils would help immobilize any As present and would reduce its plant availability (Xu et al. 2008). As(III) is absorbed through plant roots via water channels that also are involved in the absorption of boron and silicon (Ma et al. 2008). Competition between these elements and arsenite may therefore exist, which would explain why rice fertilized with Si had lower concentrations of As (Guo et al. 2009; Li et al. 2009b). Finally, consumers can reduce their As exposure risk by boiling rice in abundant water (Raab et al. 2009), or by substituting rice with other grains, such as wheat or corn, if possible (Signes-Pastor et al. 2009).

4.2.2 Selecting Crops for Low As Levels

Reducing the oral intake of As in humans by reducing the amounts consumed in crops would greatly improve food safety. Some crops accumulate higher levels of As than others do. Since As is generally retained in plant roots (Moreno-Jiménez et al. 2008; Zhang et al. 2009), tubers and edible roots may accumulate As, and it would be most wise to closely evaluate these where they are heavily consumed. Potato plants (Moyano et al. 2009) and other tubers grown in As-contaminated soils, such as radishes (Warren et al. 2003), carrots, garlic and onions (Huang et al. 2006; Zhao et al. 2009), have shown significant levels of As. In contrast to crops showing high As levels, de la Fuente et al. (2010) reported only low or moderate levels of As in potatoes, carrots, and sugar beets.

Other plant organs are also subjected to As accumulation. Huang et al. (2006) described the following pattern of As concentrations in the edible parts of the following edible crops: celery > mustard > spinach > lettuce > taro > bokchoi > cowpea > cauliflower > eggplant. De la Fuente et al. (2010) reported the following pattern in several crops: red cabbage ~ curly endive > barley ~ wheat ~ sugar beet ~ leek > cabbage ~ green pepper. Zhao et al. (2009) found As concentrations to be high in lettuce, eggplant, and green onions, whereas Gulz et al. (2005) found As concentrations in sunflower and rape to be greater than those of corn. Warren et al. (2003) reported concentrations of As to be greater in lettuce and broccoli than in spinach, but above all, these authors found the greatest concentrations of As to occur in radishes. The percentage of inorganic As in edible crops was reported to be high, although the concentration found posed no serious risk for human consumption (de la Fuente et al. 2010). Gulz et al. (2005) advised against using sunflower and rape seeds to produce cooking oil, because As levels in these crops surpassed the maximum levels permitted in Switzerland of 0.2 mg As per kg.

4.2.3 Using Soil Amendments and Mycorrhizae

Applying inorganic amendments can affect the mode of uptake and level of available As in soils. Particularly effective are amendments rich in Fe, because iron oxides effectively retain As (Mench et al. 2003; Warren et al. 2003; Hartley and Lepp 2008; de la Fuente et al. 2010). Fertilization with P also alters the absorption of As, because phosphate displaces arsenate in the soil and mobilizes it. Gunes et al. (2008) reported an increase in As accumulation when P was applied; however, the application of phosphorus-based fertilizers reduced the As plant levels (Khattak et al. 1991; Pigna et al. 2009).

Raising the soil pH increases As plant bioavailability (Smith et al. 1999; Fitz and Wenzel 2002), although some studies indicate that application of $CaCO_3$ to acidic soils reduced As availability (Simón et al. 2005).

The application of organic amendments increases the soil mobility of, and therefore, As plant uptake (Mench et al. 2003; Hartley and Lepp 2008; Renella et al. 2007; Clemente et al. 2010), but some studies show that available As is stabilized with the

application of organic matter to soil (Gadepalle et al. 2007). The fact that organic matter is both stable and is highly polymerized may help to explain such contradictory results. Combining Fe-enriched amendments with organic materials should be a future focus of research, since these amendments provide a good environment for plant growth and reduce or prevent the transfer of harmful As or other metal concentrations to edible plant tissues.

Ninety percent of higher plants interact with mycorrhizae (González-Chávez et al. 2002; Leung et al. 2006; Chen et al. 2007) and mycorrhizae appear to affect the soil behavior of As. Existing data suggest that plants infected with mycorrhizae have a greater ratio of P/As in their tissues, and this bestows greater As tolerance on them (Smith et al. 2010). In some studies, the reduction of As in plants that were infected by mycorrhizae was attributed to two effects: (1) a slower rate of root absorption of As (Yu et al. 2009) and (2) a dilution effect from accentuated plant growth (Smith et al. 2010). Further research is needed to find improved plant–microorganism combinations that minimize As accumulation in plant tissues destined for human consumption.

4.3 Alternatives: Using Contaminated Crops for Non-food Purposes

An alternative to consuming As-contaminated food is to use As-contaminated biomass for non-food purposes (Vangronsveld et al. 2009). Such biomass could be used in energy production or as primary material inputs for industrial products (Thewys 2008; Dickinson et al. 2009; Padey et al. 2009; Mench et al. 2010). In either case, the use of phytotechnologies is viable for managing contaminated soils and as means to return them to economic profitability (Thewys and Kuppens 2008). The examples in which such alternative uses were actually put into practice are few, but, when used, the results have proven satisfactory; notwithstanding, improvements to these techniques are required (Thewys and Kuppens 2008). Several experiments were conducted in contaminated soils using plant species such as *Salix* spp., corn, sunflower, tobacco, poplar, *Brassica* ssp., *P. vittata*, etc. (Vangronsveld et al. 2009). Energy from such biomass could be in the form of biogas generation, direct incineration, pyrolysis, biomass gasification, fermentation into biofuels, etc. (Mench et al. 2010). Degraded sites can be managed to produce energy and at the same time reduce the environmental risk associated with arsenic. For this purpose, contaminated soils may be used to amend wastes (compost, biochar, or iron oxides), thus recycling the materials. Both renewable energy production and human waste recycling are key factors for the future global environmental agenda (Dickinson et al. 2009). Crops grown in contaminated soils could also be used to produce other non-food goods. Examples are: cosmetics, industrial products, essential oils, paper, cardboard, wood, plant fibers, etc. (Mench et al. 2010). Regardless of the end use to which contaminated plant biomass is put, avoiding unacceptable environmental impact is crucial, particularly when arsenic or other contaminants are present in plant biomass.

5 Summary

Arsenic is a natural trace element found in the environment. In some cases and places, human activities have increased the soil concentration of As to levels that exceed hazard thresholds. Amongst the main contributing sources of As contamination of soil and water are the following: geologic origin, pyritic mining, agriculture, and coal burning. Arsenic speciation in soils occurs and is relatively complex. Soils contain both organic and inorganic arsenic species. Inorganic As species include arsenite and arsenate, which are the most abundant forms found in the environment. The majority of As in aerated soils exists as $H_2AsO_4^-$ (acid soils) or $HAsO_4^{2-}$ (neutral and basic). However, H_3AsO_3 is the predominant species in anaerobic soils, where arsenic availability is higher and As(III) is more weakly retained in the soil matrix than is As(V). The availability of As in soils is usually driven by multiple factors. Among these factors is the presence of Fe-oxides and/or phosphorus, (co)precipitation in salts, pH, organic matter, clay content, rainfall amount, etc. The available and most labile As fraction can potentially be taken up by plant roots, although the concentration of this fraction is usually low.

Arsenic has no known biological function in plants. Once inside root cells, As(V) is quickly reduced to As(III), and, in many plant species, becomes complexed. Phosphorus nutrition influences As(V) uptake and toxicity in plants, whilst silicon has similar influences on As(III). Plants cope with As contamination in their tissues by possessing detoxification mechanisms. Such mechanisms include complexation and compartmentalization. However, once these mechanisms are saturated, symptoms of phytotoxicity appear. Phytotoxic effects commonly observed from As exposure includes growth inhibition, chlorophyll degradation, nutrient depletion and oxidative stress. Plants vary in their ability to accumulate and tolerate As (from tolerant hyperaccumulators to sensitive excluders), and some plants are useful for soil reclamation and in sustainable agriculture.

The status of current scientific knowledge allows us to manage As contamination in the soil-plant system and to mitigate arsenic's effects. Phytoremediation is an emerging technology suitable for reclaiming As-contaminated soils and waters. Phytoextraction has been used to clean As-contaminated soils, although its applicability has not yet reached maturity. Phytostabilization has been employed to reduce environmental risk by confining As as an inert form in soils and has shown success in both laboratory experiments and in field trials. Phytofiltration has been used to treat As-enriched waters. Such treatment removes As when it is accumulated in plants grown in or on water. In agricultural food production, appropriate soil management and plant variety/species selection can minimize As-associated human diseases and the transfer of As within the food chain. Selecting suitable plants for use on As-contaminated soils may also enhance alternative land use, such as for energy or raw material production.

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Evaluating the Toxicity of Selected Types of Nanochemicals

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1 Introduction

Nanotechnology deals with particles/materials having at least one dimension of size in the range of 1–100 nm. Such particles/materials are generally termed as nanoparticles (NPs), nanochemicals or nanomaterials (NMs). Hence, nanotechnology involves the synthesis of NPs and NMs and their manipulation to generate materials or devices that are used for various applications (Kumar and Yadav 2009; Mohanpuria et al. 2008). Nanotechnology is one of the fastest growing fields, and has a wide range of industrial, medical, agricultural, and military applications (Jaiswal and Simon 2004; Nel et al. 2006; Kumar and Yadav 2009). Because of the breadth of such applications, the production of NPs is increasing daily, and investments in nanotechnology are increasing rapidly worldwide. The annual global production of NPs is expected to reach 10^4–10^5 tons (t) per year, after 2010 (Science Policy Section 2004). It has been estimated that nanotechnology-based products will constitute a $1 trillion business by the year 2015. About two million workers would be required to achieve this expected economic goal.

Unfortunately, no proper regulatory framework currently exists to address potential problems associated with the introduction of nanotechnology (Roco 2005; Harrison 2007). Some have suggested that voluntary risk assessment, performed by industry, is a prime need in the interim for premarket safety evaluation of NMs. Although some producers have taken the lead in this direction, others have failed to follow (Helland et al. 2008). Therefore, national efforts are underway to evaluate the need for amending the existing regulatory framework and to develop a new one (Harrison 2007).

Although NPs are currently put to a large diversity of applications as a result of their unique properties, they also have associated limitations. In particular, some of the useful characteristic properties that render NPs utilitarian also may confer higher toxicity on them. Size, surface area, and surface reactivity have been found to affect the toxicity behavior of NPs. NPs have a high surface area-to-volume ratio, in comparison to their bulk counterparts. This leads to high reactivity, ultimately causing enhanced toxicity of NPs. When NPs have similar surface reactivity, the toxicity has been reported to decrease with a particle size increase (Karakoti et al. 2006; Oberdörster et al. 2005a). For example, the well-known small particle size, and large surface area of NPs can produce ROS (see Table 1 for a list of the abbreviations and acronyms used in this review) (Nel et al. 2006; Nowack and Bucheli 2007). Toxicity tends to be increased when NPs are properly dispersed and less aggregated. Solubility, charge, and shape are other factors that influence the degree that NPs will be toxic to animals and the environment (Brunner et al. 2006; Rouse et al. 2008). Any modification in NMs morphology also may affect their toxicity behavior. For example, morphological changes that render NMs unrecognizable to phagocytic cells can lead to toxic responses (Hamilton et al. 2009). As plans for their introduction proceeds, steps are necessary to ensure that NMs and NPs should be environmental friendly (Oberdörster et al. 2005b). Hence, the toxicity that NPs may display will require careful evaluation and testing, if potentially unexpected hazards are to be avoided (Oberdörster et al. 2005a).

Table 1 Abbreviations and acronyms used in this chapter

Abbreviations	Acronyms
AAS	Atomic absorption spectrophotometer
Al	Aluminum
Al_2O_3	Aluminum oxide
AMs	Alveolar macrophages
Alamar Blue	7-Hydroxy-3H-phenoxazin-3-one 10-oxide
AP	Alkaline-phosphatase
B	Boron
BRL	Buffalo rat liver
Bw	Body weight
Beas-2B	Human bronchial epithelial cell
Co	Cobalt
Co_3O_4	Cobalt oxide
Cu	Copper
CuO	Copper oxide
CFDA-AM	5-Carboxyfluorescein diacetate, Acetoxymethyl ester
CLSM	Confocal laser scanning microscopy
CD	Carbon dots
CB	Carbon black
CeO_2	Ceria/Cerium oxide
$Ca_3(PO_4)$	Tricalcium phosphate
CLSM	Confocal laser scanning microscopy
CNS	Central nervous system
CdTe	Cadmium telluride
CNT	Carbon nanotubes
C_{60}	Fullerenes
dia.	Diameter
DFP	Diisopropyl fluorophosphate
DAF-2DA	4,5-Diaminofluorescein diacetate
DCF	2′7′-Dichlorofluorescein
DHR	Dihydrorhodamine-1,2,3
ERK	Extracellular signal-regulated kinase
EXAFS	Extended X-ray absorption fine structure
Fe_2O_3 and Fe_3O_4	Iron oxide
Fe_2O_3	Ferric oxide
GST	Glutathione transferase
GSH	Reduced glutathione
GPx	Glutathione peroxidase
G	Graphene layers
GR	Glutathione reductase
H_2DCFDA	Dichlorofluorescein diacetate
HDCF-DA	2′,7′-Dichlorodihydrofluorescein diacetate
HGPR	Hypoxanthine–guanine phosphoribosyltransferase
Taurine	$H_2NCH_2CH_2SO_3H$
HD	Hydrodynamic diameter
ICP-OES	Inductively coupled plasma-optical emission spectrometry

(continued)

Table 1 (continued)

Abbreviations	Acronyms
IR	Infrared
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-AES	Inductively coupled plasma atomic emission spectrometry
LDH	Lactate dehydrogenase
LPO	Lipid peroxidation
MDCK	Madin Darby canine kidney
MPA	3-Mercaptopropionic acid
MnO_2	Manganese oxide
Mn	Manganese
MgO	Magnesium oxide
MoO_3	Molybdenum
MTT	3-(4,5-Dimethyl-Thiazol-2-Yl) 2,5-181 diphenyl tetrazolium bromide
MWCNTs	Multiwalled carbon nanotubes
NPs	Nanoparticles
ND	Nanodiamond
Ni	Nickel
NO	Nitric oxide
Pd	Palladium
Pt	Platinum
PI	Propidium iodide
PEG	Polyethylene glycol
QDs	Quantum dots
ROS	Reactive oxygen species
RT-PCR	Real time reverse transcriptase-polymerase chain reaction
Sb_2O_3	Antimony oxide
STEM	Scanning–transmission electron microscope
SDS	Sodium dodecyl sulfate
SOD	Superoxide dismutase
Sb_2O_3	Antimony oxide
SiO_2	Silicon oxide
SNOMS	Single nanoparticle optical microscopy and spectroscopy
SEM	Scanning electron microscope
SWCNTs	Single walled carbon nanotubes
SPION	Superparamagnetic iron oxide nanoparticles
TiO_2	Titanium dioxide
THF	Tetrahydrofuran
TEM	Transmission electron microscope
WC	Tungsten carbide
WC-Co	Tungsten carbide–cobalt
XTT	3′-[1-(Phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-Nitro) benzene-sulfonic acid hydrate
ZnO	Zinc oxide
ZrO_2	Zirconia
ZnS	Zinc sulfide

Several NP toxicological studies have already been performed on various cell, and tissue types, and in animals. Understanding the degree of in vivo toxicity of NPs also offers the probability of assisting in the development of efficient NPs-based drug delivery, and imaging systems that have minimal or no side effects. Unfortunately, once NPs are absorbed into the blood stream, they may be recognized as antigenic molecules and may produce immunological responses. The adsorption of complement factors (auxiliary factors in serum, acting upon an antibody-coated cell to cause cell death) onto NPs can render them recognizable by macrophages and can induce toxicity by activation of downstream complement pathways (Moghimi et al. 2001). In addition to NPs, NMs made up of these particles can produce toxic effects. It has been suggested that NMs may release their constituent NPs throughout their life. Doubts have been generated about the potential safety of NPs, largely because of the uncertainty surrounding how they will be fabricated, disposed of and used (Wiesner et al. 2009).

It is certain that some engineered NPs will be able to produce toxic effects; however, currently there is neither conclusive reports, data nor scenarios that portray NPs as actually having produced a major safety problem; moreover, there presently is no rational scientific approach for addressing any future safety problems that may occur (Nel et al. 2006). One recent review represented NPs as being toxic, as well as nontoxic to the central nervous system; therefore, Yang et al. (2010a) suggest that there is a need to initiate nanotoxicity studies to determine not only the toxicity of such forms but also to delineate the reasons behind and solutions for nanotoxicity. Yan et al. (2011) categorized NM types into the following categories: metallic (NMs of metals, transition metals, their compounds or composites, QDs, etc.), carbon (SWCNTs, MWCNTs, fullerenes, grapheme, etc.), silicon, and organic (agglomeration or assembly of organic molecules, biomolecules or biomacromolecules, etc.). Among the defined types (metallic, carbon, and silicon NMs), primary uses are to fulfill industrial applications (Miller et al. 2010; Yan et al. 2011; Li et al. 2007a).

The purpose of this review is to provide an overview of the current status of knowledge and insights on the animal toxicity and behavior of metal oxide NPs, metallic NPs, quantum dots, silica NPs, carbon nanotubes, and related carbon NMs.

2 Toxicity of Metal Oxide NPs

Metal oxide NPs are increasingly being used as industrial catalysts, and the increasing use has resulted in elevated levels of them being found in the environment. Unfortunately, only limited data are available on the environment or organismal effects of metal oxide NPs (Fahmy and Cormier 2009). A survey of the in vitro and in vivo toxicity studies that have been conducted on metal oxide NPs are presented in Table 2.

Table 2 A survey of the in vitro and in vivo toxicity studies that have been performed on metal oxide NPs

NP types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
TiO_2 (<100 nm)	10–100 μg ml^{-1}	In vitro	NIH 3T3 cells and human fibroblast HFW cells	Cell viability tested by MTT and trypan blue exclusion assays; ROS levels measured using H_2DCFDA; TiO_2 measured using AAS; apoptosis assay utilized annexin V-FITC	Long-term exposure leads to chromosomal instability and cell transformation	Huang et al. (2009b)
TiO_2 (10 and 200 nm)	0, 5, and 10 μg ml^{-1}	In vitro	A human bronchial epithelial (BEAS-2B) cell line	Oxidative DNA damage measured by comet assay and cytotoxicity by MTT assay	Size- and shape dependent oxidative stress	Gurr et al. (2005)
TiO_2 (21-nm)	5–40 μg ml^{-1}	In vitro	BEAS-2B cells, A human bronchial epithelial cell line	Cell viability measured by MTT assay; ROS levels measured using DCFH-DA; oxidative stress and inflammation-related gene expression analyzed; apoptosis tested by analysis of caspase-3 activity and chromosome condensation	Oxidative stress and apoptosis induced	Park et al. (2008)
TiO_2 (25-nm)	20–100 μg ml^{-1}	In vitro	Human lymphocytes	Apoptosis tested by Caspase-8 activity assay; expression levels of phosphorylated stress-responsive p38 and JNK/SAPK analyzed by Western blotting	Apoptosis induced by p38/JNK and caspase-8-dependent bid pathways	Kang et al. (2009)
TiO_2 (<100 nm)	5–4,000 μg ml^{-1}	In vitro	U937 human monoblastoid cells	DNA fragmentation tested; forward and side scatter for cellular size and density analyzed, respectively; apoptosis analyzed by PI dye membrane impermeability testing	Toxic; induced both apoptotic and necrotic modifications	Vamanu et al. (2008)

TiO_2 (75 nm)	0.02–2 μg ml^{-1}	In vitro	H596 human lung tumor cells	Cytotoxicity evaluated using MTT assay	Cytotoxic	Magrez et al. (2009)
TiO_2 (20–30 nm)	0–2.5 μg ml^{-1}	In vitro	Neonatal rat ventricular cardiomyocytes	Changes in cellular electrophysiology, intercellular gap junctional coupling and ROS generation measured; myofibrillar structure investigated using LM and TEM	Induced cardiac cell damage	Helfenstein et al. (2008)
TiO_2 (25–70 nm)	0–1,000 μg ml^{-1}	In vitro	Mouse testis Leydig (TM3) cell line	Cell viability assessed by hemocytometer using trypan blue exclusion method; steroidogenic acute regulatory (StAR) and HO-1 gene expression investigated	Cell viability, proliferation, and gene expression affected	Komatsu et al. (2008)
TiO_2 (20 nm)	0–5 μg ml^{-1}	In vitro	Human U937 monocytes	CellTiter 96® Aqueous nonradioactive cell proliferation assay used for cell viability; determined ROS using H2-DCF-DA; RT-PCR analysis of MMP-2 and MMP-9 and their inhibitors (TIMP-1 and TIMP-2)	Dose-dependent toxicity	Wan et al. (2008)
TiO_2	0.05–5 μg ml^{-1}	In vitro and in vivo	Hemocytes, *Mytilus galloprovincialis*	Catalase and GST activity assayed	Induced lysosomal membrane destabilization; change in oxidative stress biomarkers in the digestive gland	Canesi et al. (2010)

(continued)

Table 2 (continued)

NP types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Co_3O_4 (45 nm)	20–120 μg ml^{-1}	In vitro	Human endothelial-like cells (ECV-304) and hepatoma cells (HepG2)	CellTiter-Glo luminescent cell viability assayed; uptake study performed by SEM and TEM; ROS measured using DCFH-D; cytotoxicity determined by cellular ATP	Induced concentration and time dependent impairment of cellular viability	Papis et al. (2009)
CuO (43 nm)	62 μM	In vitro	Immortalized human keratinocytes (SIK)	Measured OH radical formation using benzoate probe; evaluated HO1 induction by RT PCR	Induced oxidant stress	Rice et al. (2009)
CuO	0.01–100 μM	In vitro	Human neuroglioma (H4) cells	Cell viability assay using Hoechst and ethidium homodimer-1 dye	Dose-dependent toxicity induced	Li et al. (2007a)
CuO (20–40 nm), TiO_2 (20–100 nm), ZnO (20–200 nm), $CuZnFe_2O_4$ (10–100 nm), Fe_3O_4 (30–60 nm), Fe_2O_3 (20–40 nm)	0–80 μg ml^{-1}	In vitro	Human lung epithelial cell line (A549)	Cytotoxicity evaluated using trypan blue; measured oxidative DNA lesions by comet assay and ROS by DCFH-DA	CuO, ZnO, TiO_2, and $CuZnFe_2O_4$ NPs induced DNA damage and toxic; Fe_3O_4 and Fe_2O_3 induced no or low toxicity	Karlsson et al. (2008)
Fe_3O_4:Oleic acid (9–16 nm)	14–46 μg ml^{-1}; 2,200–22,000 μg kg^{-1}	In vitro and in vivo	Human fibrosarcoma (HT-1080) cells, Mouse hepatoma (MG-22A) and normal fibroblasts (NIH 3T3); mice	Cytotoxicity assayed with MTT; investigated antitumor properties using MF 3c on mice with implanted tumor S-180	Tumor specific toxicity	Zablotskaya et al. (2009)

Fe_3O_4 (20–30 nm)	0.5–500 μg ml^{-1}		Human cervical adenocarcinoma epithelial cells (HeLa), human lung epithelial normal (L-132) and carcinoma (A549) cells	MTT or WST-1 assayed, and trypan blue exclusion method used to test cell viability; analyzed apoptosis using annexin V-FITC and PI; monitored ROS by H_2DCFDA and LDH leakage using CytoTox 96 nonradioactive cytotoxicity assay	Specifically toxic to cancer cell lines <250 μg ml^{-1}	Choi et al. (2009)
Fe_3O_4 (86 nm)	0.2–20 mM	In vitro	Primary mouse connective tissue cells (L929 fibroblast)	Cell toxicity assayed with MTT; cell morphology tested using SEM and florescence microscopy and TEM	Shape- and size-dependent toxicity induced; toxicity decreases with increase in hydrodynamic size	Mahmoudi et al. (2009)
Mn (40 nm)	1–100 μg ml^{-1}	In vitro	*Rattus norvegicus* PC-12 cell line	Cell toxicity assay with MTT; cell morphology tested by inverted light microscopy, modified with CytoViva150 ultra resolution imaging system	Moderate toxicity induced	Hussain et al. (2006)
ZnO (30 nm)	0.001–5 μg ml^{-1}	In vitro	Human epidermal cell line (A431)	Cell viability assayed by MTT and neutral red uptake method; DNA damage assessed using comet assay; oxidative stress assayed using markers (GSH level, catalase activity, SOD activity)	Genotoxicity and oxidative stress induced	Sharma et al. (2009)

(continued)

Table 2 (continued)

NP types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
ZnO (20–70 nm)	5–10 μg ml^{-1}	In vitro	Immortalized human bronchial epithelial cells (BEAS-2B)	MTS assayed, and LDH assayed using carboxy-H2DCFDA; ROS measured using carboxy-H2DCFDA and intracellular calcium using Fura-2AM; oxidative stress evaluated by gene expression	Cytotoxicity induced; alteration in the expression of genes involved in oxidative stress and apoptosis resulted cytotoxicity	Huang et al. (2010)
ZnO (90–168 nm)	100–100,000 μg ml^{-1}; 1,000 and 5,000 μg kg^{-1} bw	In vitro and In vivo	Rat lung epithelial cells, primary alveolar macrophages, and alveolar macrophages-L2 lung epithelial cell cocultures; Rats	Inhalation exposure tested using twin screw volumetric feeder, via intratracheal instillation; LDH assay and enzyme immunometric assay of tumor necrosis factor-r (TNF-R) made; histopathology analyzed using light microscopy; BALF biochemical assays performed using clinical chemistry analyzer	Minor cytotoxic responses in vitro; in vivo study also reveals cytotoxicity; metal fume fever types of responses in the lungs of rats	Warheit et al. (2009)
ZnO (20–80 nm)	0.1 μg ml^{-1}	In vitro	Rat hippocampal CA3 pyramidal neurons	Enzymes digested and mechanically dispersed to isolate neurons; whole cell currents measured using EPC10 patch-clamp amplifier	Depolarization-induced neuronal injury induced	Zhao et al. (2009)
ZnO (<100 nm), TiO_2 (<25 nm), and MgO (<50 nm)	1–50 μg ml^{-1}	In vitro	Human astrocytoma (U87) cells and human fibroblasts (HFF-I)	MTT assayed for cellular viability; cell morphology tested using light microscopy; necrosis and apoptosis determined by Annexin V-FITC Kit and fluorescent microscopy	All NPs induced apoptosis and necrosis like cell death	Lai et al. (2008)

Al_2O_3 (13 nm) and TiO_2 (12–142 nm)	10–100 μg ml^{-1}	In vitro	Type II lung epithelium cell line (A549)	LDH, WST-1, and XTT assayed-cytotoxicity; intracellular NP accumulation and distribution studied using TEM	Cytotoxic	Simon-Deckers et al. (2008)
Fe_2O_3 (12 nm), ZnO (19 nm), CeO_2 (6 nm), ZrO_2 (11 nm), TiO_2 (8 nm) and $Ca_3(PO_4)_2$ (21 nm)	0–30 ppm	In vitro	Human mesothelioma (MSTO-211H) and rodent fibroblast (3T3) cell lines	MTT assay performed; evaluated cell proliferation using DNA Hoechst assay	Solubility governs toxicity; more soluble NPs more toxic	Brunner et al. (2006)
SiO_2 (80 nm), Fe_2O_3 (20–40 nm) and CuO (30 nm)	4–400 μg cm^{-2}	In vitro	Human laryngeal epithelial cells (hep-2 cells)	Tested cell viability using alamar blue assay; measured cellular ROS by H_2DCFDA; analyzed SOD, catalase, GR and GPx enzymes activity; detected LPO by competitive ELISA	CuO cytotoxic in a dose-dependent manner; SiO_2 and Fe_2O_3 nontoxic, induced reversible ROS generation	Fahmy and Cormier (2009)
Fe_3O_4, (20–30 nm), Fe_2O_3 (55–65 nm), Sb_2O_3 (41–91 nm)	5–100 μg ml^{-1}	In vitro	Primary cultures of human hematopoietic progenitor cells	CFU assays performed; analyzed cell antigenic profile by flow cytometer; quantitative PCR used for β-globin gene expression analysis; analyzed cell viability using trypan blue exclusion dye with hemocytometer and STEM	Sb_2O_3 NPs toxic; Fe_3O_4 and Fe_2O_3 NPs nontoxic; primary cell cultures give better idea of toxicity as compared to immortalized cell lines	Bregoli et al. (2009)

(continued)

Table 2 (continued)

NP types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
MoO_3 (30, 150 nm), Fe_3O_4 (30, 47 nm), TiO_2 (40 nm)	5–250 μg ml^{-1}	In vitro	Rat liver-derived cell line (BRL 3A)	Assays performed: MTT, LDH leakage; GSH level estimation by glutathion estimation kit, and ROS estimated by H_2DCFDA; external morphology evaluated by phase contrast inverted microscopy	All NPs toxic at higher doses; at lower dose MoO_3 moderately toxic, Fe_3O_4 and MnO_2 least or nontoxic	Hussain et al. (2005)
TiO_2 (30 nm)	2.0 ppm	In vivo	*Daphnia magna*	Changes in postabdominal claw curling, change in heart beat rate and feeding and postabdominal curling rates measured	Did not cause any behavioral and physiological change	Lovern et al. (2007)
CuO (30 nm) and ZnO (70 nm)	0.006–20 μg ml^{-1} and ZnO 10 μg ml^{-1}	In vivo	*Daphnia magna* and *Thamnocephalus platyurus*	TEM analysis of NP distribution in vivo performed; acute immobilization assay performed in *D. magna*, acute mortality tested in *T. platyurus*; solubilized Zn^{2+} and Cu^{2+} tested, using recombinant bioluminescent Zn and Cu sensor bacteria	Both CuO and ZnO NPs toxic; natural water mitigate the toxic effects of CuO NPs but not of ZnO NPs	Blinova et al. (2010)
TiO_2 and ZnO (<100 nm)	0.01–100 μg ml^{-1}	In vivo	*Daphnia magna*	Daphnia mobility used to determine death; viability evaluated using U.S. Environmental Protection Agency (EPA) 1994 procedure; chronic toxicity tested (reproductive output) using T-LiteTM SF-S	Tio_2 less toxic as compared to ZnO; reproduction more sensitive and affected even at 10-fold lower concentrations	Wiench et al. (2009)

TiO_2 (21 nm)	10 μg ml^{-1}	In vivo	*Cyprinus carpio*	As and TiO_2 analyzed using atomic fluorescence spectrometer and ICP-OES, respectively. EXAFS analyzed for As binding on TiO_2	TiO_2 presence lead to more As accumulation	Sun et al. (2009)
TiO_2 (5, 23 and 154 nm)	1,000 and 5,000 μg kg^{-1}	In vivo	Rats	Intratracheal instillation used; body and lung weight measured; BALF cells and inflammatory biomarkers assessed; histopathology evaluated in lung and other tissues	Smaller NPs induced greater inflammation in the short-term study; in long-term inflammation recovered regardless of size	Kobayashi et al. (2009)
TiO_2 (100–150 nm)	1,000–5,000 μg kg^{-1}; 57,000 μg per eye; 0–100 μg ml^{-1}	In vivo	Rats; Rabbit; *Oncorhynchus mykiss* (rainbow trout)	Instilled intratracheally in rats; analyzed for inflammatory markers and cell proliferation of BALF; histopathologically analyzed; acute eye irritation tested in rabbits; 96-h static tests in *O. mykiss,* according to OECD 203 testing guideline	Very low toxicity in rats; nontoxic to *O. mykiss*; nonirritating to rabbit skin	Warheit et al. (2007)
TiO_2 (20–30 nm)	100,000 μg m^{-3}	In vivo	Rats	Head–nose exposed to dust aerosols for 6 h per day on 5 consecutive days; total BALF cells counted with hematology analyzer; BALF total protein LDH, alkaline phosphatase activity analyzed with reaction rate analyzer; histological examinations used TEM; analysis of TiO_2 in tissue used ICP-AES	Low toxicity	van Ravenzway et al. (2009)

(continued)

Table 2 (continued)

NP types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
TiO_2 anatase (155 nm) and rutile (80 nm)	~500 μg per mice	In vivo	Mice	Intranasal instillation; TiO_2 contents determined in the sub-brain regions by ICP-MS; pathological changes observed by optical microscopy and TEM	Rutile had lower adverse effects on the CNS than did anatase	Wang et al. (2008a)
TiO_2 (20–200 nm)	5,000–50,000 μg kg^{-1}	In vivo	Mice	Instilled intratracheally; serum biochemistry and cytokine measured with serum chemical analyzer; immunophenotyping done on splenocyte by flow cytometry; IgE determined using ELISA; histopathology and immunohistochemistry studied by avidin–biotin affinity system and light microscopy; microarray analysis of genes expression conducted with antigen presentation	Toxic; chronic inflammatory responses and granuloma formation in lungs	Park et al. (2009)
TiO_2 NPs (25 and 80 nm)	5,000 μg kg^{-1}	In vivo	Mice	Single oral gavage administration; histopathologically examined using optical microscopy; biochemical assays (ALT/AST, BUN, and LDH) utilized biochemical autoanalyzer; TiO_2 measured in tissues using ICP-MS	Gender-specific inflammation in female mice	Wang et al. (2007)

TiO_2 (4–90 nm)	400 μg cm^{-2}	In vivo	Mice	Ti quantification by AAS; NP penetration tested in modified Franz equipment; TEM imaging made of skin biopsies and tape strips	Induced pathological changes in skin and liver; long-term exposure can induce skin aging	Wu et al. (2009)
TiO_2 (<25 and <75 nm)	10–1,000 μg g^{-1} dry food	In vivo	*Porcellio scaber*, (Latreille)	GST and catalase activity, eight change and feeding capacity measured	No adverse effects on mortality	Drobne et al. (2009)
TiO_2 (Anatase, ~8 nm)	4,100 μg	In vivo	Mice	Quantitative determination of air components using IR connected to the chamber	Nontoxic; caused removal of toxic DFP	Besov et al. (2010)
Dextran-coated iron oxide NPs	1,000–4,000 μg kg^{-1} bw	In vivo	Mice	SDS gel electrophoresis analysis of binding of plasma proteins to NPs performed; Western blotting analysis made of protein binding to NPs	Thrombosis induced in tumor blood vessels	Simberg et al. (2009)
Fe_2O_3 NPs (22 and 280 nm)	800–20,000 μg kg^{-1} bw	In vivo	Rats	Instilled Intratracheally; LDH and ACP measured in BALF by colorimetric assay; evaluated blood coagulation using automated analyser; examined histopathology using light microscopy	Toxic; induced oxidative stress in lung	Zhu et al. (2008)
ZnO (50–70 and <1,000 nm)	0.001–30,000 μg ml^{-1}	In vitro and in vivo	Immortalized rat L2 lung epithelial cells, rat lung AMs and cocultures of these two cells; Rats	In vitro LDH release and MTT assay evaluation included the following: intratracheal instillation; BALF total cell number; LDH release using olympus lactate dehydrogenase reagent and inflammatory response analysis that relied on enzyme immunometric assay kit	Cytotoxic in vitro; nontoxic in vivo, reversible inflammatory response induced	Sayes et al. (2007)

(continued)

Table 2 (continued)

NP types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
CeO_2 (20–30 nm), NiO (10–20 nm), TiO_2 (5–40), MgO (20), Al_2O_3 (2–300)	0–500 cm^2 ml^{-1}	In vivo	Rats	Instillation and BAL assessment; biochemistry content (LDH and protein) and analysis of BALF	Low cytotoxicity	Lu et al. (2009)
ZnO (20 nm), Al_2O_3 (60 nm) and TiO_2 (50 nm)	0.4–8.1 μg ml^{-1}	In vivo	*Caenorhabditis elegans*	NPs were exposed to *C. elegans* in an aqueous medium; viability evaluated via dissecting microscopy; metal toxicity evaluated on dissolved metal ion by ICP-MS	Toxic; reproductive capability more affected as compared to mortality	Wang et al. (2009b)
TiO_2 (<25 nm) and ZnO NPs (85 nm)	20 μl of 1,000 μg ml^{-1}	In vivo	*Porcellio scaber*	NPs applied orally; digestive gland tubes analyzed used fluorescent microscopy; acridine orange/ethidium bromide (AO/EB) assay performed to evaluate membrane destabilization potential of the NPs	Cell membrane destabilization potential	Valant et al. (2009)

2.1 The In Vitro Toxicity of Metal Oxide NPs

2.1.1 TiO_2 NPs

Bulk TiO_2 (titanium dioxide) particles are nontoxic and are used in the cosmetic, food, and drug industries, among others. Very little is known of the behavior of TiO_2 NPs, and therefore, they may adversely affect human health (Li et al. 2008). To the present, the biological effects that have been produced by TiO_2 NPs have shown controversial results. A comprehensive study paradigm has been suggested for successful investigation of their molecular mechanisms (Huang et al. 2009a). TiO_2 NPs have the tendency to cause toxicity by different routes, depending on dose, and on the concentration of the dose. TiO_2 NPs have been reported to cause abnormal sedimentation, hemagglutination, and hemolysis of erythrocytes (Li et al. 2008). Independent studies have shown that TiO_2 NPs have the tendency to increase levels of cellular nitric oxide, hydrogen peroxide, and reactive oxygen species (ROS) in a human bronchial epithelial cell line (BEAS-2B), and the effect was size- and shape dependent (Gurr et al. 2005). In a similar study, exposure of these NPs to BEAS-2B revealed increased ROS, and decreased amounts of reduced glutathione (GSH). Moreover, induction of oxidative stress and inflammation-related genes resulted in cell death from an apoptotic process (Park et al. 2008).

TiO_2 NPs also interfered with chromosome segregation, centrosome duplication, cytokinesis, and functional regulation of mitotic checkpoint protein PLK1. Short-term exposure to NPs was reported to enhance cell survival, cell proliferation, ERK signaling activation, and ROS production. Similarly, long-term exposure has produced disturbances in cell cycle progression, duplicated genomes segregation, chromosomal instability, and cell transformation in cultured human fibroblast HFW cells (Huang et al. 2009a). TiO_2 NPs have also been observed to induce apoptosis via a mitochondrial pathway and necrosis in cultured human lymphocytes and in U937 human monoblastoid cells (Kang et al. 2009; Vamanu et al. 2008).

The chemical composition of NPs is also known to be a critical factor that affects their cytotoxicity. TiO_2-based nanofilaments (titanate nanotubes and nanowires) with a $Na_xTiO_{2+\delta}$ composition have shown a strong dose-dependent cytotoxic effect on H596 human lung tumor cells. Structural imperfections from substitution of Na^+ with H^+ in the $Na_xTiO_{2+\delta}$ nanofilaments strongly enhanced their cytotoxic action. Moreover, CNTs that have comparable morphology induced lower toxicity than did nanofilaments (Magrez et al. 2009). Helfenstein et al. (2008) reported dose-dependent toxicity of these NPs on neonatal rat ventricular cardiomyocytes. These NPs have also induced toxicity in a mouse testis Leydig cell line (TM3). Therefore, we can conclude that exposure to these NPs can impair the male mouse reproductive system (Komatsu et al. 2008).

TiO_2 NPs have been shown to affect aquatic animals in various ways. These NPs have induced oxidative stress in *Mytilus* (the marine mussel) gill hemocytes (Canesi et al. 2010), for example, and at a low concentration other NPs caused behavioral and physiological changes in aquatic animals. In one study, TiO_2 NPs did not induce

any behavioral or physiological changes in *Daphnia magna*. In contrast, the fullerenes (C_{60}) and $C_{60}HxC_{70}Hx$ NPs induced an increase in hopping frequency, and appendage movement. It has been suggested that these NPs can increase risks of predation and reproductive decline. Hence, both of these NPs may produce population and food-web dynamic effects in aquatic systems (Lovern et al. 2007). Such safety studies, such as those described above, are apparently useful in showing that NPs, even at low concentrations, can affect aquatic species, and can exert ill effects on humans.

2.1.2 Co_3O_4 NPs

Co-based NPs have a wide range of applications that include catalysis, uses in energy storage devices, biomedicine, contrast enhancement agents for magnetic resonance imaging, and drug delivery. Cobalt oxide (Co_3O_4) NPs have been reported to induce ROS-dependent cellular toxicity to HepG2 and ECV-304 human cell lines. These NPs have the tendency to induce concentration- and time-dependent cellular toxicity through ROS generation. Easy and rapid entry of the NPs has been suggested as a possible reason for their toxicity (Papis et al. 2009).

2.1.3 CuO NPs

Rice et al. (2009) have used a two-step mechanism to access the oxidative stress-causing potential of copper oxide (CuO) NPs. In the first step, hydroxyl radicals, generated under cell-free conditions, were measured in the presence of ascorbate (a ubiquitous antioxidant in mammalian cells). Those exhibiting activity in the first step were subsequently analyzed for toxicity in epithelial cell culture. These NPs induced oxidative stress and remained active in both assays (Rice et al. 2009). CuO NPs induced dose-dependent toxicity in human H4 neuroglioma cells (Li et al. 2007b). These NPs induced cytotoxicity by causing DNA damage and oxidative stress to the human lung epithelial cell line A549. The reason for the toxicity induction is unclear. However, some mechanism other than the release of Cu ions from the NPs has been suggested to be responsible for the toxicity. The CuO NPs have also caused oxidative lesions by significantly increasing intracellular ROS levels (Karlsson et al. 2008).

2.1.4 Iron Oxide (Fe_2O_3/Fe_3O_4) NPs

Iron oxide (Fe_2O_3/Fe_3O_4) NPs that have SiO_2 and CNTs are widely used for various biomedical purposes**.** The magnetic properties of iron oxide NPs render them suitable for such biomedical applications. The Fe_3O_4 NPs of 20–30 nm in diameter have been reported to induce toxicity in a dose-dependent manner to normal human lung alveolar epithelial cells (L-132), and to tumor cell lines (human cervical adenocarcinoma epithelial cells, HeLa, and human lung alveolar carcinoma epithelial cells;

A549) (Choi et al. 2009). Oleic-acid-coated Fe_3O_4 NPs have specifically been characterized as being toxic against mouse hepatoma and human fibrosarcoma tumor cell lines. These NPs have a considerably lower level of cytotoxicity to the normal 3T3 cell line. The characteristic properties of tumor cells, such as colony formation, and changes in form, and size, have been reported to induce enhanced cytotoxicity (Zablotskaya et al. 2009). Polyvinyl alcohol-coated superparamagnetic iron oxide NPs have caused shape- and size-dependent toxicity to primary mouse connective tissue cells. Moreover, it has been observed that toxicity increases for NP forms in the following order: nanobeads > nanoworms > nanospheres. With an increase in hydrodynamic size, cytotoxicity has decreased. Easy internalization of certain comparatively small particles into cells has been suggested as a possible reason for the size-dependent toxic effects (Mahmoudi et al. 2009).

2.1.5 MnO NPs

Manganese oxide (MnO) NPs have been reported to cause dose-dependent toxicity in the *Rattus norvegicus* PC-12 cell line. These NPs have induced ROS levels, and depleted dopamine levels. Dopamine depletion has been concluded to be a specific after effect of MnO NPs exposure, and hence is not nanosize dependent. Interestingly, 15-nm silver (Ag) NPs were more toxic than the MnO NPs; however, they did not cause dopamine depletion, except at higher cytotoxic doses (Hussain et al. 2006).

2.1.6 ZnO NPs

Zinc oxide (ZnO) NPs have been widely used as ingredients in cosmetics, and in other dermatological preparations. Because of their small size, these NPs always have some probability to interact with DNA, and in fact, have been reported to produce DNA damage on a human epidermal cell line (A431). Such genotoxic effects are thought to be mediated through lipid peroxidation (LPO), and oxidative stress mechanisms. Cytotoxicity increases with an increase in NP concentration, and exposure time (Sharma et al. 2009). Similarly, ZnO NPs have been reported to induce cytotoxicity in L2 cells (Sayes et al. 2007). In another study, a concentration- and time-dependent increase in oxidative stress, intracellular [Ca^{2+}] levels, and cell membrane damage occurred to a cultured BEAS-2B cell line. Alteration in gene expression from oxidative stress and apoptosis was considered to be the main reason behind the induced cytotoxicity (Huang et al. 2010). ZnO NPs (8–10 nm) have induced more toxicity to human colon cancer cells (RKO) than has the micrometer-sized ZnO (<44 μm). Both particle types have been found to agglomerate into micrometer-sized particles in cell culture media, and to induce toxicity through apoptotic pathways (Moos et al. 2010). At higher doses these NPs have caused minor cytotoxic responses in rat lung epithelial cells, primary alveolar macrophages, and epithelial cell/macrophages cocultures (Warheit et al. 2009). Zhao et al. (2009)

reported depolarization-induced neuronal injury in rats by the activation of voltage-gated Na^+ channels, after exposure to ZnO NPs. Therefore, it follows that these NPs can probably cause neuronal apoptosis (Zhao et al. 2009).

2.1.7 Comparative Toxicity Studies

Comparative toxicity studies have also been conducted to evaluate the relative toxicity of various NPs. The ZnO, TiO_2, and MgO (magnesium oxide) NPs have a tendency to induce apoptosis- and necrosis-like cell death in human astrocytoma U87 cells and in human fibroblasts. These NPs are cytotoxic in the following order: ZnO > TiO_2 > MgO (Lai et al. 2008). Aluminum oxide (Al_2O_3) and TiO_2 NPs have shown low cytotoxicity against cell model A549 human pneumocytes, in comparison to multiwalled carbon nanotubes (MWCNTs) (Simon-Deckers et al. 2008).

Solubility has been found to play a key role in the cytotoxicity of metal oxide NPs on human mesothelioma (MSTO-211H) and on rodent 3T3 fibroblast cell lines. The more soluble the NPs the higher the induction of toxicity. The cytotoxic response for 3T3 cell lines of all seven NP types has the following order: Fe_2O_3 > ZnO > cerium oxide (CeO_2) ≈ zirconia (ZrO_2) ≈ TiO_2 ≈ tricalcium phosphate ($Ca_3(PO_4)_2$); and for the MSTO-211H cell line the relative cytotoxicity of these seven is as follows: ZnO > ZrO_2 > $Ca_3(PO_4)_2$ ≈ Fe_2O_3 ≈ CeO_2 ≈ TiO_2 (Brunner et al. 2006). The exposure to SiO_2, Fe_2O_3, and CuO NPs of human laryngeal epithelial cells (HEp-2) revealed that only CuO NPs induce cytotoxicity in a dose-dependent manner. In contrast, CuO acts by generating ROS, and blocking the antioxidant defense of the cells. High doses of SiO_2 and Fe_2O_3 have also generated ROS. In cellular systems, ROS are detoxified by antioxidant defense systems (Fahmy and Cormier 2009). Fe_2O_3, Fe_3O_4, and TiO_2 NPs are reported to be nontoxic to primary cultures of hematopoietic progenitor cells of the human bone marrow. However, antimony oxide (Sb_2O_3) NPs have induced toxicity in erythroids during colony development, although they have no effect during erythroid differentiation. No study results have yet demonstrated that NP accumulation in cells causes cytotoxicity. However, NPs have been reported to induce toxicity in cell membranes (Bregoli et al. 2009).

The chemical properties of NPs also influence their cytotoxicity. Hussain et al. (2005) evaluated the toxicity of certain NPs on rat liver-derived BRL cell lines and found that lower doses of molybdenum (MoO_3), ranging from 5 to 50 μg ml^{-1}, were moderately toxic. The same dose of TiO_2 and Fe_3O_4 (30, 47 nm) were either nontoxic or had the least toxicity of any tested NP. At higher doses (100–250 μg ml^{-1}), all NPs exhibited cytotoxicity that resulted in irregular cell shape, and cell shrinkage (Hussain et al. 2005). Auffan et al. (2009) reported that chemically stable metallic oxide NPs (e.g., Fe_2O_3 NPs) were nontoxic under physiological redox conditions, whereas NPs having strong oxidative (e.g., CeO_2, Mn_3O_4, and Co_3O_4) or reductive (e.g., Fe^0, Fe_3O_4, Ag^0, and Cu^0) potential showed a tendency for inducing in vitro cytotoxicity/genotoxicity. It is concluded that electronic and/or ionic transfers occurring during oxido-reduction, dissolution, and catalytic reactions, either within the NPs lattice or on interaction with culture broth, cause cytotoxicity (Auffan et al. 2009).

Ecotoxicological testing of CuO and ZnO NPs on the crustaceans *D. magna* and *Thamnocephalus platyurus,* using artificial freshwater and natural waters as the test medium, produced toxic responses (Blinova et al. 2010). The toxicity of both CuO and ZnO NPs has mainly been induced by the production of solubilized ions (Blinova et al. 2010). In another comparative study on *D. magna*, TiO_2 NPs had lower toxicity than did ZnO. The higher toxicity of ZnO resulted from Zn^+ itself, rather than from the ZnO NPs (Wiench et al. 2009).

Contaminants already present in the environment can affect the toxic behavior of NPs. The carcinogenic contaminant arsenic (As) is widely distributed in the environment and can be highly toxic to humans, even at low doses. Strong adsorption of As on the surface of NPs has been reported to enhance the accumulation of As in carp (*Cyprinus carpio*) by 44% after 25 days of exposure. The order of As accumulation in carp tissues and organs is as follows: viscera > gills > skin and scales > muscle (Sun et al. 2009). This suggests that NPs should be tested for their release and toxic characteristics before being used or released to the environment.

2.2 The In Vivo Toxicity of Metal Oxide NPs

NPs behave entirely differently inside living systems vs. outside those systems. Hence, defining the acute toxicity of newly synthesized NPs before they are released for use is essential. In performing prerelease evaluations, in vitro studies may be easier to conduct, but in-vivo studies are likely to give a more realistic picture of the actual toxicity of NPs to natural (and complex) living systems.

2.2.1 TiO_2 NPs

Variations in toxic responses after intratracheal instillation have resulted from testing different agglomeration levels, and sizes (small/larger) of TiO_2 NPs on rat lungs. Smaller NPs have induced higher levels of inflammation in short-term (1 week postinstillation) studies, whereas inflammatory responses produced in longer term studies were reversible regardless of the size of the NPs. NPs that have the same primary size, but varying agglomeration levels have not shown differences in toxicity (Kobayashi et al. 2009). In another in vivo pulmonary toxicity study, low levels of inflammation and lung tissue toxicity were revealed in rats (Warheit et al. 2007). A 5-day inhalation study (6 h per day, head/nose exposure) in male Wistar rats revealed that surface reactivity is an important factor that influences toxicity. Ravenzwaay et al. (2009) reported that inhalation of TiO_2 NPs by male Wistar rat led to substantial accumulation in the lungs, and small amounts were translocated to the mediastinal lymph nodes. In addition, mild neutrophilic inflammation and activation of macrophages were induced in the lungs. Despite having more surface area deposition in lungs, TiO_2 NPs induced lower toxicity than did quartz dust. The higher toxicity from exposure to quartz dust was thought to result from its higher surface reactivity.

A crystal structure-dependent damage has been shown to occur with TiO_2 NPs on the central nervous system (CNS) in female mice, after intranasal instillation of the material. Anatase (155 nm) TiO_2 NPs have shown to be comparatively more toxic than anatase or TiO_2 and to induce higher inflammatory responses, and increased levels of tumor necrosis factor alpha (TNF-α) and interleukin (IL-1β). Rutile TiO_2 NPs have produced a slightly lower adverse effect on the CNS than did TiO_2. Differences in toxic responses of NPs have been induced by various crystal structures. After 30 days of exposure, TiO_2 deposition declined in the following order: hippocampus > olfactory bulb > cerebellum > cerebral cortex (Wang et al. 2008a). Single intratracheal instillation of TiO_2 has been reported to cause chronic inflammatory responses through the Th2-mediated pathway in mice. This substance also increased the expression levels of genes related to antigen presentation, and chemotaxis induction of immune cells. Further, intratracheal instillation of TiO_2 induced granuloma formation and expressions of proinflammatory proteins in lung tissues (Park et al. 2009). Wang et al. (2007) administrated TiO_2 NPs by single oral gavage (OECD procedure) to adult mice, and the exposed mice showed gender-specific toxicity. Evidence of toxicity in female mice included liver inflammation, myocardial damage, hepatic injury, and renal lesions. However, there were no abnormal pathological changes in the heart, lungs, ovary, or splenic tissues**.**

Acute dermal irritation and oral toxicity studies have been conducted on rabbits. Results reported were that TiO_2 NPs that had a size of ~100 nm were non-skin irritants and were nontoxic (Warheit et al. 2007). However, according to Wu et al. (2009), these NPs can cause skin aging if the exposure is for a longer time period. Sixty-day dermal exposure to TiO_2 NPs in hairless mice resulted in NP absorption by different tissues/organs. Skin and liver displayed the most severe pathological changes, which is believed to have resulted from oxidative stress (Wu et al. 2009). Moreover, further testing showed that these NPs have a very low degree of aquatic hazard when tested on the rainbow trout *Oncorhynchus mykiss* (Warheit et al. 2007). The results of one recent study revealed toxic effect of these NPs on the gills and digestive glands of *Mytilus galloprovincialis* (Canesi et al. 2010). Amounts equal to 10–1,000 μg TiO_2 NPs g^{-1} in dry food were reported to be safe to the terrestrial isopod *P. scaber*, after 3–14 days of dietary exposure. In this study, no adverse effects, e.g., mortality, weight change or a decrease of feeding activity, were observed (Drobne et al. 2009).

TiO_2 has been reported to possess the potential to detoxify another substance. Application of a high concentration of TiO_2 as a NP aerosol was reported to be a powerful method for detoxifying toxic vapors of diisopropyl fluorophosphate (DFP) (Besov et al. 2010).

2.2.2 Iron Oxide NPs

In vivo mouse studies have shown that tumor-targeted superparamagnetic iron oxide nanoparticles (SPION) may act as antitumor agents. The high concentration of SPION in the lumen induced blood vessel thrombosis. Further, entrapment of these

NPs, in the growing intravascular thrombi, led to cell death. Therefore, tumor-targeted SPION can be used to inhibit tumor growth (Simberg et al. 2009). In another such study, the antitumorigenic action of an iron-oxide-containing magnetic fluid in vivo was retained up to the fifth day after administration. However, after that inhibition efficiency was decreased. It has been suggested that the addition of a surfactant that has antitumorigienic properties may offer an approach in the future for prolonging antitumorigenic action (Zablotskaya et al. 2009).

Intratracheal administration of Fe_2O_3 NPs to male Sprague Dawley rats induced oxidative stress in the lungs (Zhu et al. 2008). Such oxidative stress produced follicular hyperplasia, protein effusion, pulmonary capillary vessel hyperaemia, and alveolar lipoproteinosis.

2.2.3 ZnO NPs

In rats, the intratracheal instillation and inhalation exposure of ZnO NPs for 1–3 h induced metal-fume-fever-like responses in lungs that was characterized by short-term lung inflammatory or cytotoxic responses (Warheit et al. 2009). In vivo pulmonary toxicity studies in rats demonstrated potent, but reversible, inflammation after both nano- and fine-sized ZnO exposures (Sayes et al. 2007). NPs tend to form much larger aggregates in water, although very little information on the toxicity of NPs of this sort is available.

The effect of ZnO NPs has also been tested on aquatic species. In one such study, aggregates of ZnO NPs showed a dose-dependent reduction in hatching rate, and induction of pericardial edema in developing zebrafish embryos, and in larvae. The Zn^{2+}-dependent mechanism was responsible for the embryonic toxicity that resulted from ROS generation (Zhu et al. 2009).

2.2.4 Comparative Toxicity Studies

Metal oxide NPs (aluminas 1, 2, 3, 4, SiO_2, MgO, TiO_2, ZnO, carbon black (CB), CeO_2, nickel oxide (NiO), and Co_3O_4) have been reported to induce low in vivo toxicity. NiO and alumina 2 caused significant lung inflammation when instilled into rat lungs. This toxic response resulted from the extra surface reactivity of alumina 2, compared to other NPs, upon application to equal surface areas of rat lungs. Free radicals, generated by NiO, CeO_2, Co_3O_4, CB NPs. NiO, CeO_2, and alumina 2, produced significant hemolytic activity. NiO_2 and alumina 2 also induced inflammogenic responses (Lu et al. 2009). ZnO, Al_2O_3, and TiO_2 NPs' toxicity to the nematode *Caenorhabditis elegans* severely affected reproductive capability and caused mortality.

The dissolution rate for NPs has been slightly faster than for corresponding non-NP (bulk) forms of the particles. The bulk forms have not shown toxic effects. It appears that the solubility of the oxide does influence the toxicity of NPs. Additionally, some unknown NP-dependent mechanism may be involved in causing the toxicity

(Wang et al. 2009a). Using metal oxide NPs, Valant et al. (2009) tested a terrestrial invertebrate (*Porcellio scaber*) via oral exposure, and analyzed changes in cell membrane permeability in single-layer epithelia of digestive glands. Exposures to the same concentrations of TiO_2 and ZnO NPs decreased cell membrane permeability, and lowered the membrane destabilization potential. The compound known as C_{60} proved to be the most permeable and biologically potent of the tested compounds. Moreover, sonicated NPs were reported to be more biologically aggressive than were nonsonicated NPs (Valant et al. 2009).

3 Toxicity of Metallic Nanoparticles

The large-scale production of NPs will inevitably cause risks to human health, and to the environment. It has been suggested by some authors that the chemical stability of metallic NPs largely determine their cytotoxicity. NPs that have the ability to be oxidized/reduced or dissolved have shown the capacity to be toxic to cellular organisms (Auffan et al. 2009). Therefore, prudence suggests that toxicity testing should be performed before releasing and using such NP forms. A survey of the in vitro and in vivo toxicity studies that have been performed on metallic NPs are tabulated in Table 3.

3.1 The In Vitro Toxicity of Metallic NPs

3.1.1 Ag NPs

Ag NPs have recently received considerable attention for use in possible defense and engineering applications. Ag NPs have previously seen wide use as antimicrobial agents (Arora et al. 2008; Chadeau et al. 2010). In addition, Ag NPs have been used to treat clothing to render them resistant to microbes. When used in such applications, NPs come into direct skin contact. Hence, again, such exposures underline the importance of assessing new uses of NPs for potential cytotoxicity. Such an evaluation was undertaken for Ag NP-coated cotton fabric. The treated fabric was determined to be safe to guinea pigs; this NP form did not cause direct skin irritation after application to the skin (Lee et al. 2007a). Moreover, Ag NPs were reported to be safe to human skin carcinoma and human fibrosarcoma cell lines (Arora et al. 2008).

Kim et al. (2009) reported that Ag NPs induced oxidative stress-mediated cytotoxicity in human hepatoma cells. These NPs tended to agglomerate in the cytoplasm and in nuclei, and cause intracellular oxidative stress. Both Ag NPs, and Ag^+ ions induced cytotoxicity, but by different mechanisms. Interestingly, the mRNA level of oxidative stress-related genes was discovered to be regulated variably (Kim et al. 2009). Because these NPs are smaller in size, they can also interact with cellular genetic material. Unfortunately, little is known about the genotoxicity of Ag NPs.

Table 3 A survey of the in vitro and in vivo toxicity studies that have been performed on metallic NPs

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Ag (7–20 nm)	0.76–50 μg ml^{-1}	In vitro	Human skin carcinoma (A431) and human fibrosarcoma (HT-1080)	Cytotoxicity evaluated by XTT assay; apoptosis tested by DNA fragmentation analysis and caspase-3 colorimetric assays; cell morphology analyzed by inverted phase-contrast microscopy	Nontoxic	Arora et al. (2008)
Ag (<10 nm)	0.7 μg ml^{-1}	In vitro	Human hepatoma (HepG2) cells	Evaluated toxicity by MTT, Alamar blue, and LDH assays; monitored oxidative stress using DCFH-DA; evaluated expression of metal responsive metallothionein by RT-PCR	Oxidative stress-mediated toxicity	Kim et al. (2009)
Ag (25 nm)	50 μg ml^{-1}	In vitro	Mouse embryonic stem (mES) cells and embryonic fibroblasts (MEF)	MTT assay performed; Key double-strand break repair protein Rad51 expression analyzed; apoptosis evaluated by Western blotting of annexin V protein; cell morphology evaluated using laser scanning confocal microscopy	Polysaccharide coated Ag NPs exhibited more severe DNA damage than uncoated Ag NPs; both types induced cell death	Ahamed et al. (2008)
Ag (15 and 100 nm) and Al (30 and 103 nm)	10–250 μg ml^{-1}	In vitro	Rat liver-derived cell line (BRL 3A)	MTT assay, LDH leakage assay performed; estimated GSH levels by glutathion estimation kit and ROS by H_2DCFDA; cell morphology determined by phase contrast inverted microscopy	Ag NPs toxic; Al NPs toxic only at higher doses (50–250 μg ml^{-1})	Hussain et al. (2005)

(continued)

Table 3 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Ag (10–90 nm)	0.1–100 μg ml^{-1}	In vitro	Rat coronary endothelial cells	Cell proliferation tested by MTT and LDH assays	Concentration-dependent toxicity induced	Rosas-Hernandez et al. (2009)
Ag (50–100 nm)	1–10 μg ml^{-1}	In vitro	Rats hippocampal CA1 neurons	Recorded electrophysiology using a modified upright infrared DIC microscopy and conventional patch-clamp techniques; analyzed effects on action potential and action potential firing rate using whole cell current-clamp recordings	Neurotoxic effect	Liu et al. (2009)
Ag (15 nm)	1–100 μg ml^{-1}	In vitro	PC-12 (Neuroendocrine) *Rattus norvegicus* cell line	MTT assay performed to determine cell viability; dichlorofluorescein assay performed for ROS; DA and its metabolites determined using HPLC/electrochemical detection system; cell morphology analyzed using inverted light microscopy modified with the ultra resolution imaging system	Cytotoxic; induced cell shrinkage and irregular membrane borders	Hussain et al. (2006)
Au (18 nm)	0.2–2 nM	In vitro	Human cell line, HeLa	MTT assay performed; TEM analysis performed for internalization study; gene expression in stress-related genes analyzed	Nontoxic	Khan et al. (2007)

Au nanorods (~8-nm)	0.2–0.4 nM	In vitro	Human colon carcinoma cells (HT-29)	MTT assay performed, NP uptake and toxicity tested by ICPMS and TEM	Cytotoxicity caused due to CTAB released by NPs; coating with polymers reduces toxicity	Alkilany et al. (2009)
Au (~5 nm)	1–67 nM ml^{-1}	In vitro	Human cancer cell lines (Panc-1 and Hep3B)	Used MTT assay and PI-fluorescence associated cell sorting to assess cell proliferation and toxicity	Nontoxic	Gannon et al. (2008)
Au (2 nm)	0.38–3 μM	In vitro	Mammalian Cos-1 cells and red blood cells	Determined cell viability by MTT assay; hemolysis assay performed for RBC; vesicle leakage assay performed	NPs coated with cationic and anionic charged material moderately toxic and nontoxic, respectively	Goodman et al. (2004)
Au, spherical and nanorods (5–70 nm)	–	In vitro	Human skin cell line (HaCaT)	Cytotoxicity evaluated using MTT test, absorption spectroscopy and TEM	Nanorods toxic; while spherical NPs and coated nanorods nontoxic	Wang et al. (2008b)
Au (33 nm)	0–120 nM	In vitro	Human carcinoma lung cell line (A549), normal baby hamster kidney (BHK21), and human hepatocellular liver carcinoma cell lines	Analyzed cellular morphology using phase contrast microscopy and fluorescence; used flow-cytometric analysis and MTT assay for cytotoxicity; apoptosis tested by immunoblotting of poly(ADP-ribose) polymerase degradation	Specifically toxic to human carcinoma lung cell line; nontoxic to other two cell lines	Patra et al. (2007)

(continued)

Table 3 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Au NPs (−)	0–100 nM	In vitro	HepG2 human hepatocellular carcinoma cells	Studied toxicity by nuclei count, nuclear area, mitochondrial membrane potential, and intracellular free calcium evaluation with the cell analyzer HCS system	Inhibit cell proliferation	Jan et al. (2008)
Au rods (17 × 39 nm)	$1.38 \times 10\_^{11} 4.6 \times 10^{9}$ p ml^{-1}	In vitro	Epithelial MDCK cells	Evaluated cytotoxicity by electrical cell-substrate impedance analysis and MTS test; NPs localization determined by dark-field microscopy	Nontoxic	Tarantola et al. (2009)
Au (9 nm)	500 µl of 44 ppm	In vitro	Human semen/sperm	Analyzed motility and morphological changes by clinical microscopy	Spermatotoxicity induced; motility of sperms reduced to 25% vs. 95% in control	Wiwanitkit et al. (2009b)
Co (100–500 nm)	0.1–0.2 µM	In vitro	Human peripheral blood leukocytes (PBLs)	Assayed Co uptake; cytokinesis-block micronucleus assay; DNA damage detected by Comet assay	Internalized by human leukocytes; Genotoxic	Colognato et al. (2008)

WC and WC-CO (50 and 300 nm)	100 μg ml^{-1}	In vitro	Human lung, skin, and colon cell lines; rat neuronal and glial cells and oligodendrocyte precursor cell line (OLN-93); Caco-2 human colon adenocarcinoma cells , HaCaT human keratinocyte, A549 human lung carcinoma, and OLN-93 oligodendroglial precursor cells	Analyzed NP uptake by SEM; assessed cell viability using light microscopy, a fluorescent indicator (alamar blue), a cell counting kit and cell membrane integrity was determined using CFDA-AM	WC NPs acutely nontoxic; WC doping with Co markedly increased cytotoxicity	Bastian et al. (2009)
Co (20 nm)	0–5 μg ml^{-1}	In vitro	Human U937 monocytes at	Evaluated cell viability using Celltiter 96® aqueous nonradioactive cell proliferation assay; determined ROS using H_2DCF-DA; analyzed expression and secretion of MMP-2 and MMP-9 and their specific tissue inhibitors by RT-PCR	Toxic; act by increasing the transcription and activities of MMP-2 and MMP-9	Wan et al. (2008)
WC-Co (62 and 145 nm)	8.25, 16.5 and 33 μg ml^{-1}	In vitro	*Oncorhynchus mykiss* gill cell line	Assessed cell viability by the fluorescent dyes alamar blue, CFDAAM and fluorescence reader; analyzed uptake and localization with SEM	Toxic	Kuhnel et al. (2009)

(continued)

Table 3 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Cu (40, 60 and 80 nm)	10–100 μM	In vitro	Neurons of dorsal root ganglion (DRG)	Localization determined by light microscopy, and cell viability by LDH and MTS assay	Induced size- and concentration-dependent toxicity	Prabhu et al. (2010)
Pt (1–5 nm)	1 or 100 μg ml^{-1}	In vitro and In vivo	Human diploid embryonic lung fibroblasts diploid fibroblasts and hepatocarcinoma cell lines and cervical carcinoma cell; Mice	ESR analysis of $O_2^{\bullet -}$ scavenging activity determined using spin trap method; administration was via intraperitoneal route; health was visually monitored	Nontoxic in vitro and in vivo; $O_2^{\bullet -}$ scavenging activity increases with a decrease in particle size	Hamasaki et al. (2008)
Pt (2–150 nm)	0.0001–1,000 ng cm^{-2}	In vitro	Human colon carcinoma cell line	Studied particle uptake by focused ion beam/SEM; determined DNA integrity and cellular ROS generation by comet and DCF assays, respectively; Cell viability evaluated by trypan blue exclusion;	Toxic; Induced decrease in cellular GSH level and DNA damage	Pelka et al. (2009)
Magnetite and Pd/magnetite (20–30 nm)	5–25 μg ml^{-1}	In vitro	Colon adenocarcinoma cells, Caco-2	Cell viability assessed using alamar blue; NP internalization analyzed by SEM; ROS measured using H_2DCFDA	Nontoxic	Hildebrand et al. (2010)
Al (10–20 nm)	1.0–250 μg ml^{-1}	In vitro	Primary porcine arterial endothelial and primary human umbilical vein endothelial cells	Analyzed changes in VCAM-1 and ELAM-1 gene expression using quantitative RT-PCR	Toxic; can induce proinflammatory response and poses risk of cardiovascular disease	Oesterling et al. (2008)

Ag (90–210 nm), Au (50–100 nm) and Co (50–200 nm)	5–100 μg ml^{-1}	In vitro	Primary cultures of human hematopoietic progenitor cells and human hematopoietic origin (K562, HL-60, CEM, CEM-R, Thp-1, Jurkat, and Molt-4) cell lines	CFU assay performed; cell antigenic profile analyzed by flow cytometry; β-globin gene expression tested by quantitative PCR and cell viability by trypan blue exclusion dye using hemocytometer and STEM	Co NPs toxic; Ag and Au NPs nontoxic	Bregoli et al. (2009)
Zn (300 nm), and Fe (100 nm)	0.00024–2.400 μg ml^{-1}; 2.5 g per mice	In vitro and in vivo	Human liver (Huh-7), brain (A-172), stomach (MKN-1), lung (A-549) and kidney (HEK293) cell lines; Mice	MTT assay performed; DNA content assayed; membrane permeability tested using fluorescent phase-contrast microscopy; glutathione assayed; histopathologically observed in heart, liver, spleen, stomach, and intestine	Low cytotoxicity in vitro; in vivo nonspecific hemorrhage, lymphocytic infiltration and medullary congestion induced in heart, spleen and liver	Cha and Heejoon (2007)
Al (100 nm) and B (10–20 nm)	0–250 μg ml^{-1}	In vivo	*Daphnia magna*	Toxicity evaluated by the Microtox test	Toxic	Strigul et al. (2009)
Ag (18–19 nm)	49–515 μg m^{-3}	In vivo	Rat	Exposed whole body in inhalation chamber; observed mortality, body weight, food consumption; tested pulmonary function; organs histopathologically examined	Higher dose induces inflammatory response; lungs and liver major target tissues	Sung et al. (2009)

(continued)

Table 3 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Ag (25 nm)	0–1,000,000 μg kg^{-1}	In vivo	Mice	Dissected regions of the mouse brain and analyzed for oxidative stress and antioxidant defense arrays	Toxic; induced oxidative stress and apoptosis	Rahman et al. (2009)
Ag NPs (5–46 nm)	0–0.71 nM	In vivo	Zebrafish embryos	Determined NP transport mechanism by two-dimensional mean square displacement and diffusion models; characterized biocompatibility and toxicity of NPs embedded in embryos using dark-field SNOMS	Embryo mortality induced at concentration above 0.19 nM	Lee et al. (2007b)
Ag (<100 nm)	0.1 and 0.5 μg ml^{-1}	In vivo	*Caenorhabditis elegans*	Assayed survival and growth using dissecting microscopy; analyzed gene expression changes with DNA microarray; functional analysis of NP responsive genes performed using mutant strains	Toxic	Roh et al. (2009)
PEG-coated Au NPs (4 and 100 nm)	0.170–4,260 μg kg^{-1} bw	In vivo	Mice	Gene expression profiles analyzed with microarray and quantitative RT-PCR; histopathology analyzed	Induced apoptosis, cell cycle and inflammation responsive genes	Cho et al. (2009b)
PEG-coated Au NPs (13 nm)	0–4,260 μg kg^{-1} bw	In vivo	Mice	Detected apoptosis by transferase-mediated dUTP nick end-labeling; used ICP to determine tissue distribution; pharmacokinetics analyzed; analyzed expression of adhesion, chemokines and cytokines with quantitative RT PCR; detected immunohistochemistry using avidin–biotin affinity system	Acute inflammation and apoptosis induced in the liver; accumulation in liver and spleen; long blood circulation time	Cho et al. (2009a)

Au (~13 nm)	0.750 μg ml^{-1}	In vivo	*Mytilus edulis*	Determined toxicity by measuring protein ubiquitination and carbonylation; measured catalase activity, lysosomal membrane stability; gill proteins analyzed by 2D gel electrophoresis	Oxidative stress induced	Tedesco et al. (2008)
Cu (23.5 nm)	108,000–1,080,000 μg kg^{-1} bw	In vivo	Mice	Exposed via oral gavage; morphological and pathological examination performed by optical light microscopy; blood chemistry analyzed by biochemical autoanalyzer	Toxic; heavy injuries on kidney, liver, and spleen	Chen et al. (2006)
Cu (23.5 nm)	108,000–1,080,000 μg kg^{-1}	In vivo	Mice	Evaluated toxicity by ICP-MS and pH detector	High toxicity	Meng et al. (2007a)
Cu (23.5 nm)	70,000 μg kg^{-1} bw	In vivo	Mice	Analyzed blood and plasma electrolytes; examined autopsy and pathology using ICP-MS and optical microscopy; biochemistry assays performed using a ceruloplasmin kit and ICP-MS	Toxic due to surface reactivity of NPs	Meng et al. (2007b)
Cu (25 nm)	50,000–200,000 μg kg^{-1} bw	In vivo	Rats	Analyzed serum with automatic analyzer; used optical light microscopy for evaluating histopathology; urine, serum and organs analyzed by NMR spectroscopy	Hepato- and nephrotoxicity	Lei et al. (2008)

(continued)

Table 3 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Cu (80 nm)	0.25–1.5 μg ml^{-1}	In vivo	Zebrafish (*Danio rerio)*	Expression of stress responsive genes studied by quantitative-PCR; toxicity tested by ICPMS and histopathology study; transcriptome responses measured using microarray	Toxic; gill injury and acute lethality	Griffitt et al. (2007)
Iron oxide (8.4 nm)	0–200 μg ml^{-1} and 10,000 μg kg^{-1} bw	In vitro and in vivo	Rat glioma C6 cells and rats	Intravenous administration; MTT assay for cell viability performed; histopathology analyzed; NP content in tissue analyzed by ICP-OES	Low toxicity in vitro; good bioavailability to brain in vivo	Huang et al. (2009a)
Zn (58 ± 16)	5 g Zn kg^{-1} bw	In vivo	Mice	Gastrointestinal administration; serum assayed by automatic biochemical analyzer; blood element and blood coagulation tested using automatic hematology analyzer and ELISA; histopathology utilized light microscopy	Toxic; severe renal damage occurs in the nanoscale zinc-treated mice	Wang et al. (2006)
Ni (30, 60 and 100 nm)	10 and 1,000 μg ml^{-1}	In vivo	Zebrafish	Tested embryo mortality using immnohistochemistry assay; developmental defects studied by alcan blue staining and evaluating histology; Ni in tissue estimated by ICP-MS	Toxic	Ispas et al. (2009)

Ahamed et al. (2008) reported characteristics of the surface chemistry-based genotoxic effects of these NPs on mouse embryonic fibroblasts, and on stem cells. Better dispersion of polysaccharide functionalized Ag NPs exhibited more apoptosis than did uncoated Ag NPs (Ahamed et al. 2008). The 15- and 100-nm diameter Ag NPs induced toxicity in cells of a rat liver-derived BRL 3A cell line by generating oxidative stress. These same NPs reduced GSH levels, and compromised cellular antioxidant defenses that led to ROS accumulation.

The mechanism by which GSH is depleted through the action of Ag NPs is not yet known (Hussain et al. 2005). Ag NPs of 45 nm in diameter were reported to induce selective, specific, and concentration-dependent cytotoxicity on coronary endothelial cells. Higher concentrations (50–100 μg ml^{-1}) favored cell proliferation, while lower concentrations (1.0–10 μg ml^{-1}) inhibited proliferation via activation, and impaired endothelial nitric oxide (NO) synthase. Furthermore, low and high concentrations of NPs induced vasoconstriction and vasodilatation in isolated rat aortic rings, respectively. This action was mediated by the endothelial cells of the aortic rings (Rosas-Hernandez et al. 2009). Ag NPs, ranging from 32 to 380 nm in diameter, induced neurotoxic effects on rat hippocampal CA1 neurons. The resulting alterations in sodium current that probably led to the observed neuronal dysfunctioning were long lasting (Liu et al. 2009). Ag NPs (15 nm) were reported to be cytotoxic to a PC-12 *Rattus norvegicus* cell line and caused cell shrinkage, but, interestingly, cellular morphology was not affected (Hussain et al. 2006). Ag NPs (6 nm) at concentrations of 1, and 5 μg ml^{-1} inhibited a cancer cell line by 30% and >60%, respectively (Safaepour et al. 2009). The results of the studies conducted on Ag NPs, thus far, have suggested that there is a need to conduct longer-term cytotoxicity evaluations.

3.1.2 Au NPs

Gold (Au) NPs are widely used in applications ranging from chemical sensing to imaging (Kumar and Yadav 2009). Similar to other NPs, the toxicity of nano forms of gold differs from the toxicity of the standard form of gold. When dealing with gold toxicity, it is important to differentiate between cytotoxicity and cellular damage. NMs that are nontoxic can still cause serious cellular damage (Murphy et al. 2008). Au NPs are not considered to be genotoxic to the human cell line, HeLa. The uptake of Au NPs by HeLa cells did not induce any change in gene expression (Khan et al. 2007). The entry of NPs into cells and their cytotoxicity depends both upon the type of material that is absorbed and their relative orientation on nanoscale surfaces.

Chemically synthesized metallic NPs are known to exert cytotoxic effects in the presence of surfactants. The effect appears to be on the surface of the NPs, and surfactants used during NP synthesis are difficult to remove after synthesis. Green synthesis techniques may offer improvements, but may fail to offer an efficient method to manage the shape and size of the synthesized nanoparticles (Alkilany et al. 2009; Kumar and Yadav 2009). Cetyltrimethylammonium bromide (CTAB)-capped nanorods have shown potential cytotoxicity on human colon carcinoma

cells. Free CTAB, released from nanorod surfaces, is a main cause of cytotoxicity. Interestingly, bound CTAB and gold nanorods have been found to be safe. Moreover, polymer coatings over these nanorods have reduced cytotoxicity, regardless of surface charge. This may occur because of reduced desorption of CTAB from the involvement of CTAB-capped gold nanorods (Alkilany et al. 2009).

In an in vitro study, 5-nm diameter Au NPs have produced specific cytotoxicity on two human cancer cell lines, in the presence of noninvasive external radiofrequency (RF) (13.56 MHz). RF fields are used for heating intracellular Au NPs to cause thermal destruction of malignant cells. Au NPs alone are noncytotoxic. However, in presence of RF they become cytotoxic to both cell lines. Both cell lines have shown a concentration-dependent increase in cytotoxicity with NP concentration increases, in presence of external RF (Gannon et al. 2008).

Surface charge also affects the toxicity of Au NPs. Catatonically functionalized mixed-monolayer-protected Au clusters have shown toxicity to mammalian Cos-1 cells, and to red blood cells, whereas Au NPs, coated with cationic and anionic particles, have been moderately toxic or nontoxic to these respective cell lines. This cytotoxic response is concentration dependent. It has been suggested that concentration-dependent electrostatic binding of these NPs on cells have resulted in cell lysis (Goodman et al. 2004). In a similar study, Wang et al. (2008b) reported that Au nanorods were highly toxic to human skin cells, because of the CTAB coating used during their synthesis. Interestingly, spherical Au NPs of different sizes were reported to be nontoxic. CTAB-induced toxicity was further confirmed when gold nanorods were coated with polystyrenesulfonate (PSS) (Wang et al. 2008b). Moreover, Tarantola et al. (2009) reported that polyethyleneglycolated (PEGylated) Au NPs were nontoxic to epithelial MDCK (type II) cells.

Au NPs that lack functionalization (such as being coated with PSS, or PEGylation) have been reported to kill human carcinoma lung cells (of the cell line A549) in a dose-dependent manner; however, these NPs were safe to normal BHK21 (baby hamster kidney) and HepG2 (human hepatocellular liver carcinoma) cell lines (Patra et al. 2007). The exact mechanism by which NPs have cell specificity is not known. Patra et al. (2007) reported that Au NPs inhibit the proliferation and release of intracellular calcium in HepG2 human hepatocellular carcinoma cells. These NPs also induce more toxicity in differentiated NG108-15 murine neuroblastoma cells as compared to undifferentiated cells (Jan et al. 2008). Spermatotoxic evaluation is suggested as one critical toxic endpoint that should be assessed for Au NPs. Spermatoxicity of these NPs on human semen was reported; effects included fragmentation of sperm and loss of motility in 25% of the sperm sample (Wiwanitkit et al. 2009a).

If cytotoxicity is to be better understood, then better insights as to how NPs enter cells is needed. Au NPs (25 nm) penetrate human red blood cells (RBC) by an unknown mechanism, but one that is different from phagocytosis and endocytosis. The size of NPs was a factor critical in regulating their entry into cells, whereas surface charge and material type had no relevant influence (Rothen-Rutishauser et al. 2006). Interestingly, Wiwanitkit et al. (2009b) documented that Au NPs are easily accumulated inside white blood cells (WBCs), either via phagocytosis or direct penetration.

3.1.3 Co NPs

Cobalt (Co) NPs tend to interact with the DNA of leukocytes. These NPs interfered with normal functioning of blood leukocytes and caused genotoxic effects on human peripheral blood leukocytes (Colognato et al. 2008). Wan et al. (2008) evaluated the toxicity of Co NPs on human U937 monocytes, and reported a dose-dependent toxicity of 20 nm-sized Co NPs. Toxicity was induced through oxidative stress that mediated imbalances in the expression, and activity of matrix metalloproteinases (MMPs), and their inhibitors. MMPs are zinc-dependent endopeptidases that are involved in extracellular matrix morphogenesis remodeling, angiogenesis, arthritis, skin ulcers, tumor invasion, metastasis, and wound healing (Wan et al. 2008). Bastian et al. (2009) reported that doping of tungsten carbide (WC) produced toxic WC-Co NPs. WC NPs alone are nontoxic to all the tested cell lines, but the presence of Co enhanced the hazardous effects on those cell lines. Kuhnel et al. (2009) reported similar results, i.e., that WC-Co NPs are more toxic than WC NPs on a rainbow trout (*O. mykiss*) RTgill-W1 cell line. Co NPs are known to kill cancerous cells in presence of external low RF radiation. Xu et al. (2008) found that 7 nm cubic crystalline graphitic carbons coated ferromagnetic Co NPs penetrate through cellular plasma membrane of the cultured HeLa cells, and entered into cytoplasm and the nucleus. Exposure to low RF radiation (350 kHz) caused localized heating of metallic NPs. Such heating led to cell death in a time- and concentration-dependent manner (Xu et al. 2008). Therefore, Co NPs are thus far generally toxic to most tested animal cell lines.

3.1.4 Cu NPs

Cu NPs are used in industrial and healthcare applications. These NPs are quite small and can easily penetrate through the skin and can be inhaled and ingested. They can be transported from nerve endings on the skin and may reach the somatosensory neurons in dorsal root ganglia (DRG). Prabhu et al. (2010) reported neurotoxic effects from Cu NPs on rat DRG neuron cell cultures. Exposure of Cu NPs to DRG neurons resulted in vacuoles formation, detachment from the substratum, and disruption of the neurite network. The toxicity observed with the Cu NPs was size- and concentration-dependent. High concentrations and smaller size NPs produced the maximum toxic effects (Prabhu et al. 2010).

3.1.5 Pt NPs

Platinum (Pt) NPs are reported to be nontoxic to regular as well as cancerous human cell lines. These NPs protect against ROS-induced cell death in HeLa cells. As the size of these NPs increases, the O_2^- scavenging ability was significantly enhanced. This suggests that the Pt NPs act as novel antioxidants (Hamasaki et al. 2008). Pelka et al. (2009) found that exposure to Pt NPs decreased the level of

cellular GSH, and impaired the integrity of DNA in a human colon carcinoma cell line. Interestingly, with the increase in size of the Pt NPs, toxic effects decreased. However, under the same conditions, toxicity increased as incubation time increased (Pelka et al. 2009).

3.1.6 Pd NPs

Palladium (Pd)/magnetite NPs are used as catalysts to remove halogenated organic pollutants from contaminated wastewater. Use of Pd/magnetite NPs at very high concentrations showed only minor toxicity to human skin and colon, and to rainbow trout gills cell lines (Hildebrand et al. 2010).

3.1.7 Al NPs

The exposure of ultrafine particulate air pollutants (diameters <100 nm) increases the chance of cardiovascular diseases through endothelial cell dysfunctioning and inflammation. Oesterling et al. (2008) reported that aluminum (Al) NPs induced inflammatory diseases such as atherosclerosis, which can lead to cardiovascular dysfunction. Exposure to Al NPs increases levels of VCAM-1, ICAM-1, and ELAM-1 inflammatory markers; mRNA levels; and protein expression in porcine pulmonary artery endothelial cells and in human umbilical vein endothelial cells. Moreover, these NPs increased the adhesion of activated monocytes in human endothelial cells (Oesterling et al. 2008). Al NPs also induced dose-dependent toxicity to a rat liver-derived BRL cell lines. Exposure to a lower dose of Al NPs (5–50 μg ml^{-1}) induced little or no toxicity, whereas higher doses (100–250 μg ml^{-1}) produced irregular cell shapes and cell shrinkage (Hussain et al. 2005).

3.1.8 Comparative Toxicity Studies

Bregoli et al. (2009) reported that Au and Ag NPs at 5–100 μg ml^{-1} were nontoxic to primary cultures of human bone marrow hematopoietic progenitor cells. However, Co NPs were toxic at this test concentration (Bregoli et al. 2009). Mild in vitro toxicity was induced by Zn (300 nm), Fe (100 nm), and SiO_2 (10–20, 40–50, 90–110 nm) NPs on several cell lines that included human liver (Huh-7), brain (A-172), stomach (MKN-1), lung (A-549), and kidney (HEK293). The toxicity produced by these NPs was independent of their size and resulted from the presence of inorganic particles (Cha and Heejoon 2007). Al NPs were more toxic than were TiO_2 NPs on *D. magna* (Strigul et al. 2009). Broadly speaking, the toxicity that they produced from exposure to the foregoing NPs generally resulted from their chemical nature or from their dissolution products and/or agglomerates.

3.2 The In Vivo Toxicity of Metallic NPs

3.2.1 Ag NPs

In vivo studies with Ag NPs have shown dose-dependent cytotoxic effects. Sung et al. (2009) revealed that 90-day inhalation exposure to Sprague-Dawley rats with Ag NPs at levels up to 100 mg m^{-3} were safe. However, higher doses induced inflammatory responses, even after 28 days of exposure. Lung and liver were the major affected organs on prolonged exposure of the Ag NPs (Sung et al. 2009). Rahman et al. (2009) showed that intraperitoneal administration of Ag NPs in mice caused free radical-induced oxidative stress, alterations in gene expression, and apoptosis in brain tissue. Conclusions were that these NPs are neurotoxic (Rahman et al. 2009).

Lee et al. (2007b) used zebrafish embryos to screen the biocompatibility and real time transport of Ag NPs (5–46 nm). Ag NPs are transported into and out of embryos through chorion pore canals (CPCs), and these NPs exhibited brownian diffusion. The biocompatibility, toxicity, and type of abnormalities reported from Ag NP exposures in zebrafish were dependant on the exposure dose. Levels that exceeded a concentration of 0.19 nM produced death and deformation of zebrafish (Lee et al. 2007b). The Ag NPs that had diameters smaller than 100 nm exerted toxic effects on *C. elegans* through an oxidative stress-mediated pathway and produced reproductive failure (Roh et al. 2009).

3.2.2 Au NPs

Tissue distribution, acute inflammatory responses, and histopathological changes are some of the cytotoxic responses that were documented to result from Au NP exposures. These nanomaterials have previously been shown to possess limited or no toxicity at the cellular level. Although, generally, Au NPs are considered to be biologically safe and are widely used in various biomedical applications, very little is known about their effects on cells. However, a few reports suggest that the Au NPs may be cytotoxic. Single intravenous administration of 4- and 100-nm size Au NPs influenced apoptosis rate, affected the cell cycle, and induced inflammatory immune- and metabolic-process responses in the liver of mice (Cho et al. 2009a). Cho et al. (2009b) revealed two phases of liver toxicity that resulted from the exposure of Au NPs in mice. The first phase appeared immediately after administration of the NPs and caused increasing liver inflammation as soon as 5 min after initial exposure. The second phase was observed 7 days after administration. After this point, the NPs disappeared from the circulation and became localized in liver and spleen tissues. Seven days posttreatment, apoptosis of hepatocytes significantly increased in a dose-dependent manner. However, accumulation of these NPs in nontarget organs such as kidneys, lung, brain, and testis decreased over time, indicating that the NPs were slowly being cleared (Cho et al. 2009b).

Tedesco et al. (2008) studied the effects of exposure of the Au-citrate NPs to the aquatic filter feeder *Mytilus edulis* over a 24-h period; results indicated that this *NP* induced oxidative stress in digestive gland and in gills.

3.2.3 Cu NPs

In a comparative toxicity study, Chen et al. (2006) reported both nano (23.5 nm) and ionic Cu ($CuCl_2 \cdot 2H_2O$) particles to be moderately toxic. These NPs have caused grave toxicological effects and substantial kidney, liver, and spleen injury in mice, in a sex-dependent manner. In this study, male mice exhibited more severe toxic symptoms than did female mice. In contrast, the micro Cu (17 μm) form was found to be nontoxic (Chen et al. 2006).

It appears that the environment of the exposure to NPs may be critical in defining their toxicity. For example, Meng et al. (2007a) reported that copper becomes extremely reactive at the nanoscale level in the stomach, because it consumes hydrogen ions present in the stomach more quickly than do larger micron-sized ones. This oxidation of Cu NPs led to generation of cupric ions. These ions are highly toxic, causing enhanced mortality of experimental mice (Meng et al. 2007a). The reaction of Cu NPs with acidic substances in the mouse stomach provokes metabolic alkalosis, and thereby poisoning. The cumulative overload of alkalescent substances and heavy metal ions (copper ions) was suggested to be the main reason behind the induction of toxicity (Meng et al. 2007b). In a similar study on rats, oral administration of Cu NPs for 5 days induced toxic effects. Toxicity was increased as the administered dose of the Cu NPs increased. Higher dosages of NPs and bulk Cu, as well, have caused anorexia, diarrhea, lethargy, and significant body-weight loss. Also observed from exposure to both forms was liver damage, scattered hepatocytic necrosis, an increase in kidney weight, and dysfunctioning of renal glomerular filtration (Lei et al. 2008). Cu NPs were also observed to be acutely toxic to the gills of zebra fish (Griffitt et al. 2007).

3.2.4 Iron Oxide NPs

Huang et al. (2009b) reported that carboxymethyl dextran-coated iron oxide NPs are bioavailable in the brain and cause only limited cytotoxic effects. These NPs, when present in the brain, are influenced by applying an external magnetic field. Application of such a field reduces the accumulation of these NPs in the reticuloendothelial system of the liver and in spleen. Interestingly, accumulation was more pronounced in regions that hosted tumors. Use of magnetic fields may therefore be a future technique that can be utilized to mediate drug delivery to the brain for these NPs (Huang et al. 2009b).

3.2.5 Zn NPs

Gastrointestinal exposure of nano- and micro-sized zinc powder produced different responses (Wang et al. 2006). In mice, microscale Zn powder induced more severe

liver damage than did nanoscale Zn. After 2 weeks of administration, the nanoscale Zn-treated mice showed severe symptoms of lethargy, vomiting, and diarrhea, as compared to the microcsale Zn-treated mice. Severe renal damage in the nanoscale Zn-treated mice may have resulted from their higher cytotoxicity (Wang et al. 2006).

3.2.6 Ni NPs

Nickel (Ni) has a wide array of applications in catalysis, sensing, and electronics. Therefore, adequate testing of the toxicity of Ni NPs is needed from both an environmental and organismal standpoint.

Ispas et al. (2009) exposed zebra fish embryos to three different sizes of Ni NPs: 30, 60, and 100 nm in diameter. Larger particle aggregates, comprising 60-nm entities that formed dendritic structures were also tested. Among these, dendritic clusters induced the highest toxicity. Interestingly, all forms of Ni NPs were less toxic than were the soluble Ni forms. Exposure to Ni NPs caused thinning of intestinal epithelium and separation of skeletal muscle fibers. In contrast, exposure of soluble Ni did not cause any intestinal defects, but induced skeletal muscle fiber separation (Ispas et al. 2009).

3.2.7 Comparative Toxicity Studies

Cha and Heejoon (2007) conducted a comparative in vivo acute toxicity of Zn (300 nm), Fe (100 nm), and SiO_2 (10–20, 40–50, 90–110 nm) NPs on mice. Notably, the toxicity that resulted was induced by inorganic particles, and not because of the nanosize of these tested NPs (Cha and Heejoon 2007).

4 Toxicity of QDs

Boasts have been made that bioconjugated QDs are capable of noninvasive, ultrasensitive, and multiplexed imaging of molecular targets in animal models and humans. Gao et al. (2005) has suggested that QDs require thorough testing by methods similar to those used for other NM. Cd NP-containing QDs have shown promise for treatment and diagnosis of cancer and targeted drug delivery (Rzigalinski and Strobl 2009). However, more work is needed to better understand the pharmacology and toxicology of the potentially toxic Cd-based QDs. Without such studies, assessing their risks will be difficult. Cadmium selenide (CdSe) QDs was found to be highly toxic to cultured cells when held under UV illumination for extended time periods (Derfus et al. 2004). However, in vivo studies have shown that stably protected QDs are nontoxic (Ballou et al. 2004). A survey of the in vitro and in vivo toxicity studies that have been conducted on QDs are presented in Table 4.

Table 4 A survey of the in vitro and in vivo toxicity studies that have been performed on the QDs

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
CdSe-core QDs (7 nm)	62.5–1,000 μg ml^{-1}	In vitro	Rat hepatocytes	Evaluated cytotoxicity by MTT assay and mitochondrial activity by colorimetric assay; cell morphology tested by bright field microscopy	Acutely toxic; ZnS and BSA coating reduces toxicity	Derfus et al. (2004)
CdSe/CdS core/shell (39–40 nm)	0.3125–10 nM	In vitro	Pig skin and human epidermal keratinocytes	Imaged QDs in tissue by confocal; quantified QDs using flurometer; analysis Cd by ICP-OES; cell viability by MTT assay; skin sections analyzed by TEM	Toxic	Zhang et al. (2008)
CdSe core and ZnS shell (45 nm)	3 nM	In vitro	Human epidermal keratinocytes (HEK)	Cell viability assayed by alamar blue and Fluorescence microscopy; human cytokines analyzed using the Bio-Plex suspension array system	Toxic	Rouse et al. (2008)
CdSe/CdS/ZnCdS/ZnS multishell QDs (5–6 nm)	6×10^{11}–3×10^{14} p ml^{-1}	In vitro	Epithelial MDCK (type II) cells	Cytotoxicity tested by MTS and NP localization by fluorescence microscopy. Used immunostaining and fluorescence microscopy to monitor alterations in the cell cytoskeleton	Toxic	Tarantola et al. (2009)
Silica-coated CdSe/ZnS QDs (8–10 nm)	8–80 nM	In vitro	Human skin fibroblasts (HSF-42) and lung fibroblast cells	Analyzed cell proliferation, apoptosis, necrosis, and cell cycle distribution using a high content image analyzer; high throughput array (HTA) GeneChip system used to profile HSF-42 gene expression	Nontoxic; minimal impact on cell health and molecular response	Zhang et al. (2006)

CdTe core (3.5–15 nm)	0.1–1 μg ml^{-1}	In vitro	Human intestinal (Caco-2) cell line	Assayed viability/cytotoxicity; evaluated epithelial integrity using transwell membrane inserts and transepithelial electrical resistance (i.e., TEER) measurement	Toxic; caused cell death	Koeneman et al. (2009)
CdSe and CdSe/ZnS (2–24 nm)	2–10 nM	In vitro	NRK fibroblasts, MDA-MB-435S breast cancer cells, CHO cells, RBL cells	Analyzed cells by light microscopy and NP uptake by fluorescence microscopy; ion channel function tested by patch-clamp recordings; cells electrophysiologically characterized; number of adherent cells determined before and after incubation with QDs	Toxic; CdSe coated with ZnS less toxic	Kirchner et al. (2005)
CdSe/ZnS and CdTe	10 μg ml^{-1}	In vitro	Human breast cancer (MCF-7) cell line	MTT assay performed; QD uptake imaged via a fluorescence method	CdSe/ZnS nontoxic; CdTe toxic	Cho et al. (2007)
CdTe (3 nm)	0–100 nM	In vitro	Murine neuroblastoma (NG108-15) and human hepatocellular carcinoma cells	Toxicity evaluated by the Hoechst stain and HCS cell analyzer	Toxic	Jan et al. (2008)
Organic QDs; COOH and NH_2 (PEG) CdTe/CdSe core, ZnS shell QDs (15–21 nm)	40 nM	In vitro	Murine macrophage-like (J774.A1) cells	Cell uptake of QDs studied using confocal microscopy and flow cytometry; cytotoxicity tested using MTT and LDH release assays	COOH QDs, NH_2 (PEG) QDs, nontoxic; organic QDs toxic	Clift et al. (2008)

(continued)

Table 4 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
CdSe/ZnS (<5–15 nm)	10 nM kg^{-1} bw	In vivo	Mice	Intraoperative fluorescence imaging used; QD biodistribution and clearance studied by 99mTc-QDs (~250 μ Ci) using Gamma radioscintigraphy	<5.5 nm HD rapidly eliminated in urine; nontoxic	Choi et al. (2007)
CdSe/ZnS	480 pM	In vivo	Mice	Exposure via intravenous administration; QDs monitored by two-photon spectral microscopy; organs tested for histopathology using two-photon spectral microscopy	Nontoxic	Fitzpatrick et al. (2009)
CdSe/ZnS (10.5–25 nm)	0–100 nM	In vitro and in vivo	Human primary monocytes; mice	Exposure via intravenous administration; cell viability determined using cell count assay and μQuant microplate reader; ROS detected by DHR assay; human and mouse TNF-α and CXCL8 detected by sandwich ELISA; Co-localized lysosome determined by confocal microscopy; cells analyzed using TEM	Induced inflammatory responses in vitro and in vivo	Lee et al. (2009)

4.1 The In Vitro Toxicity of QDs

QDs are extensively used in biomedical research labeling, because they perform better than alternative methods. QDs are often tested using in vitro cell cytotoxicity assays, because such testing is easier and cheaper than conducting in vivo studies.

4.1.1 CdSe QDs

Because QDs are widely used, assessing their cytotoxicity in the context of every material they interact with would be useful and prudent. Little is known about the penetration/permeability of QDs into skin, although it is known that toxicity may be affected by the presence of other interacting materials. Zhang et al. (2008) reported on the exposure of nail-shaped QD 621 [(CdSe core with a cadmium sulfide shell), coated with PEG] to human epidermal keratinocytes. These authors have documented that these QDs can penetrate porcine skin, but only up to the outer stratum corneum layers. However, direct exposure of QDs to skin or keratinocytes has produced inflammatory responses. The cytotoxic and inflammatory effects of QDs on IL-8 and IL-6 were both dose- and time dependent.

4.1.2 CdSe/ZnS QDs

Lee et al. (2009) reported that exposure to CdSe/ZnS QDs increased the production of ROS and mitogen-activated protein kinases and mediated the production of tumor necrosis factor-α and CXC-chemokine ligand 8 in human primary monocytes. The results showed that core-, shell-, and surface-coatings and the shape, size, and charge of QDs affect their toxicity behavior. Therefore, these are the parameters that need to be tested for toxicity. The use of cyclic tensile strain with QDs increased their cellular uptake in human epidermal keratinocytes. The inclusion of this strain induced cytokine production and caused skin irritation in humans. The use of higher concentrations of the QDs increased uptake into exposed cells, which may have been the factor responsible for their cytotoxicity (Rouse et al. 2008).

Zhang et al. (2006) reported that coatings can suppress the toxicity caused by CdSe/ZnS QDs. PEG-coated silanized QDs (PEG-silane-QDs) were nontoxic to human fibroblasts. However, CdSe/ZnS induced toxicity by releasing Cd^{2+} ions into the cellular environment. Apoptosis/necrosis occurs in human skin fibroblasts after high- and low-dosage exposures to PEG-silane-QDs (Zhang et al. 2006). QDs, capped and stabilized against Cd^{2+} diffusion with an external ZnS shell, also produced toxicity (Tarantola et al. 2009). Hence, QDs coatings do not inhibit toxicity.

Interestingly, surface chemistry plays an important role in the cytotoxicity of CdSe/ZnS QDs. QDs that are coated with different agents had differential toxic responses; 3-mercaptopropionic acid (MPA)-coated CdSe/ZnS QDs was the least toxic and was followed by polymer-coated particles and MPA-coated CdSe QDs.

A polymer shell coating acts as a better diffusion barrier against the release of Cd^{2+} ions from CdSe surfaces than does an MPA shell. At the same time, coating of CdSe with a ZnS shell drastically reduced the release of Cd^{2+}. With this polymer coating, a higher cytotoxicity was induced because it leads to a higher precipitation of QDs on the cell surface than does a comparable MPA-coating (Kirchner et al. 2005). CdSe/ZnS QDs were reported to be nontoxic to human breast cancer MCF-7 cells (Kirchner et al. 2005).

4.1.3 CdTe QDs

The cytotoxic effects observed for cadmium telluride (CdTe) QDs were induced via mechanisms involving both Cd^{2+} and ROS, and the effects were accompanied by lysosomal enlargement (Cho et al. 2007). CdTe QDs induced dosage-, and time-dependent apoptotic and other toxic responses to NG108-15 murine neuroblastoma and human hepatocellular carcinoma cells (Jan et al. 2008). Koeneman et al. (2009) revealed that these QDs caused disruption of the epithelium monolayer and cell death of the intestinal lining and mimicked what occurred with the Caco-2 human intestinal cell line. Unlike metallic oxide and metallic NPs, this toxicity resulted from the nano nature of the QDs, rather than from cadmium ions or sodium thioglycolate capping ligands. Aggregated QDs are nontoxic to these same cell lines (Koeneman et al. 2009). Surface charges on QDs have detrimental effects on their toxicity behavior. Clift et al. (2008) treated murine macrophage-like J774 A1 cells with organic,

–COOH and $–NH_2$ (polyethylene glycol) surface-coated QDs (40 nM) and observed differential toxic responses. The organic QDs were the most cytotoxic, whereas the QDs with a –COOH coating were rapidly taken up by the macrophages; the $NH_{2–}$ (polyethylene glycol) coated QDs were not taken up or internalized within cells. None of these QDs induced detectable cell death (Clift et al. 2008).

4.2 The In Vivo Toxicity of QDs

4.2.1 CdSe/ZnS QDs

The renal filtration threshold for metal-based nanometer-sized objects is not yet known. Moreover, the type of organic coatings that are compatible with renal clearance to ensure nontoxicity of particular QDs is also unknown. If the intact form is not cleared, the potential toxicity is amplified and radiological imaging is hindered. Choi et al. (2007), using fluorescent ODs, determined the hydrodynamic diameter (HD) and surface charge for NPs. Such studies help design NPs that can be rapidly eliminated from the body. The size and charge of most QDs/NPs are critical factors that determine their intact clearance rates from the body. The coating charge is responsible for their adsorption on serum proteins, and this charge influences HD. Purely anionic or cationic QDs cause a 15-nm increase in the HD. Zwitterionic

coatings, like the amino acid cysteine, tend to prevent serum protein adsorption on QDs and thus possessed the highest solubility and smallest possible HD. Neutral PEGylated QDs do not bind to serum proteins and result in a >13.2 nm HD. A QD of HD <5.5 nm can be efficiently excreted through the urine and eliminated from the body (Choi et al. 2007). Interestingly, repeated intravenous injections of CdSe/ZnS QDs increased neutrophil infiltration in lung tissues of mice (Lee et al. 2009). However, ZnS capped CdSe QDs were nontoxic to Balb/c and nude mice. A blue shift in QDs fluorescence was reported after 2 years of administration in these animals. Either degradation of the ZnS surface, and/or a slow loss of core, a cationic exchange mechanism, and change in the size–shape aspect ratio of the QDs was the probable reason for the blue shift. Hence, it was confirmed that the ZnS-capped CdSe QDs have lower toxicity (Fitzpatrick et al. 2009).

4.2.2 CdTe QDs

Gagne et al. (2008) found that CdTe QDs were toxic to *Elliption complanata*. LPO increased in the gills of *E. complanata* at >5.6 μg ml^{-1} CdTe QDs treatment levels. In this same species only low rates of LPO occurred in digestive glands, even at <1.6 μg ml^{-1} CdTe. Interestingly, the incidence of DNA strand breaks was significantly reduced in gills at exposures <1.6 μg ml^{-1} of CdTe. In digestive glands, however, transient DNA strand breaks were induced in a concentration-dependent manner. Oddly, lower CdTe QD treatment concentrations produced more damage than did higher concentrations (Gagne et al. 2008).

5 Toxicity of SiO_2 NMs

Nonmetal oxide SiO_2 NPs have extensive applications in the chemical industry, and in mechanical polishing activities, in addition to serving as additives to drugs, cosmetics, printer toners, varnishes, food, biomedical and biosensors (Lin et al. 2006). As has occurred with other NPs, SiO_2 NP toxicity testing has revealed mixed results. Among the factors that influence toxicity of these NPs are shape, size, differential dosage levels, levels of impurities, route of administration, assay or test used for toxicity evaluation, and variations resulting from handling errors (Akhtar et al. 2010). A survey of the in vitro and in vivo toxicity studies that have been conducted on SiO_2 nanomaterials are presented in Table 5.

5.1 The In Vitro Toxicity of SiO_2 NMs

Lin et al. (2006) studied the toxicity of SiO_2 NPs in cultured human bronchoalveolar carcinoma-derived cells. Toxicity responses increased as the dose and exposure

Table 5 A survey of the in vitro and in vivo toxicity studies that have been performed on the Si NPs

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
SiO_2 (10 and 80 nm)	–	In vitro	Human lung epithelial (A549) cells	Cytotoxicity tested by MTT and LDH release assays; induction of ROS and membrane LPO measured	Toxicity exerted through ROS and LPO generation	Akhtar et al. (2010)
SiO_2 (15 nm, 46 nm)	10–100 μg ml^{-1}	In vitro	Human bronchoalveolar carcinoma-derived (A549) cell line	Cytotoxicity assessed using sulforhodamine B assay, LDH release activity measured; ROS measured using DCFH-DA and LPO level	Dose-dependent toxicity	Lin et al. (2006)
SiO_2 (21.58 and 80.21 nm); SiO_2–chitosan (10–14 nm)	667 μg ml^{-1}	In vitro	Normal skin adherent fibroblast cell lines; Human normal (MRC-5) and carcinoma (A549) lung cell line; Human adenocarcinoma (MKN-28) and colorectal adenocarcinoma (HT-29) cell lines	Cell viability assayed by MTT; cell damage assayed by LDH	Low toxicity; high dosages more toxic to normal fibroblast than cancer cells; cytotoxicity significantly reduced by involving chitosan in NPs synthesis	Chang et al. (2007)
SiO_2 (7 nm) and porous SiO_2 (5–15 nm)	1 μg ml^{-1}	In vitro	Human bronchial epithelial (Beas-2B) cell	Analyzed cell viability using PI staining and flow cytometry; ROS generation assessed using DCFH-DA and antioxidant enzymes induction by Western blotting	Toxic	Eom and Choi (2009b)

SiO_2 (20 or 50 nm)	0–1,000 μg ml^{-1}	In vitro	Human embryonic kidney cells	Cytotoxicity assayed by MTT; morphological characterization performed by contrast inverted microscopy; intracellular GSH measured by GSH- 400 colorimetric assay; ROS determined using DCFH–DA; apoptosis measured using flow cytometry	Dose-dependent toxicity	Wang et al. (2009a)
SiO_2 (50 nm)	0.1–500 μg ml^{-1}	In vitro	Lung epithelial (A549) cells	DNA single-strand breaks measured by comet assay; DNA base modification analyzed using Western blotting; cell viability assayed by MTT; apoptosis evaluated by vibrant assay, and	Low toxicity	Jin et al. (2007)
SiO_2 (30, 48, 118, and 535 nm)	50–200 μg ml^{-1}	In vitro	Mouse keratinocytes (HEL-30)	Toxicity by MTT and LDH leakage assays	NPs size <100 nm induced cytotoxicity	Yu et al. (2009)
SiO_2 (~14 nm)	0.5–500 μg ml^{-1}	In vitro	Human lung epithelial cells, carcinoma A549 cells and normal L-132 cells, human cervical adenocarcinoma cells (HeLa) and osteosarcoma cells (HOS)	Analyzed apoptosis by annexin V-FITC; ROS tested using H_2DCFDA; LDH leakage monitored by CytoTox 96 nonradioactive cytotoxicity assay; proinflammatory cytokine interleukine-8 (IL-8) production determined by ELISA	Iron oxide caused cell death; SWCNTs induced oxidative stress followed by apoptosis; Si NPs triggered inflammation response; LMH nontoxic at <250 μg ml^{-1}	Choi et al. (2009)

(continued)

Table 5 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
SiO_2	0.05–5 μg ml^{-1}	In vivo	Hemocytes, digestive gland, and gills of *Mytilus galloprovincialis*	Toxicity evaluated by using catalase and GST activity	Lysosomal membrane destabilization induced; change in lysosomal and oxidative stress biomarkers in the digestive gland	Canesi et al. (2010)
SiO_2 (10 nm)	1,000 μg per 500 cm^2	In vivo	Rats	Exposure via intratracheal instillation and BAL assessment; BALF evaluated for biochemical content (LDH release and protein)	Low cytotoxicity	Lu et al. (2009)
SiO_2 (80 nm)	4–400 μg cm^{-2}	In vitro	Human laryngeal epithelial cells (hep-2 cells)	Tested cell viability using alamar blue assay; ROS measured by H_2DCFDA, and SOD, catalase, GR and GPx enzyme activities analyzed; LPO detected by competitive ELISA	Nontoxic, induced reversible ROS generation	Fahmy and Cormier (2009)
SiO_2 (10–20, 40–50, and 90–110 nm)	0.24–2,400 ppb; 2,500,000 μg per mouse (2.5 g)	In vitro and in vivo	Human liver (Huh-7), brain (A-172), stomach (MKN-1), lung (A-549), and kidney (HEK293) cell lines; mice	Mitochondrial activity monitored using MTT; DNA content and membrane permeability tested; glutathione production determined; histopathology conducted on heart, liver, spleen, stomach, and intestinal tissues	Low cytotoxicity in vitro; in vivo nonspecific hemorrhage, lymphocytic infiltration and medullary congestion induced in heart, spleen, and liver	Cha and Heejoon (2007)

time increased. Zhang et al. (2010a) performed toxicity and uptake studies with SiO_2 NPs, and concluded that 80- and 500-nm sized SiO_2 NPs are quickly absorbed by human dermal fibroblasts, and this NP also affected cell adhesion and migration. These latter two factors were affected by uptake of the SiO_2 NPs regardless of their size. SiO_2 (80 nm) also weakened the mitochondrial membrane potential and produced a loss of cell viability (Zhang et al. 2010a). Chang et al. (2007) reported the dose-dependent toxicity of SiO_2 NPs as well. A high dose of SiO_2 was slightly toxic to normal human pulmonary and dermal fibroblast cells and to colon, gastric, and lung-tumor cells. Exposure to higher doses of SiO_2 NPs induced retarded cell proliferation, damaged cell membranes, and induced cell apoptosis/necrosis; lower doses were nontoxic to these tested cells. Furthermore, use of chitosan in the synthesis procedure of such NPs has been suggested to be an efficient way to minimize their toxic effects (Chang et al. 2007).

Formation of ROS, and induction of antioxidant enzymes, like superoxide dismutase (SOD) and heme oxygenase-1 (HO-1) are markers for oxidative stress. SiO_2 NPs induced oxidative stress-mediated toxicity in Beas-2B cells. These NPs induced an Nrf-2 (nuclear factor-E2-related factor-2)-ERK MAP (mitogen-activated protein) kinase signaling pathway that mediates production of oxidative stress responsive transcription factor HO-1 (Eom and Choi 2009). Exposure of 20- and 50-nm sized SiO_2 NPs induced oxidative stress and decreased cell viability of human embryonic kidney (HEK293) cells in a dose-dependent manner. The effects observed after SiO_2 NP exposure included cell shrinkage, nuclear condensation, and an increase in intracellular ROS levels, elevation in LPO levels and reduction in GSH content (Wang et al. 2009b). Notably, these NPs induced oxidative stress and caused dysfunction of human umbilical vein endothelial cells (HUVECs) via c-Jun N-terminal kinase (JNK), p53, and NF-κB pathways. It has been speculated that these NPs also induce cardiovascular diseases, e.g., atherosclerosis and thrombus (Liu and Sun 2010). Pure SiO_2 was reported to induce oxidative stress-mediated toxicity to human lung epithelial cells (A549 cells) (Akhtar et al. 2010). These NPs induced ROS and membrane LPO, whereas the GSH level and glutathione reductase (GR) activity remained unaffected. Interestingly, and by contrast, dye-doped luminescent SiO_2 induced low genotoxicity and low cytotoxicity to human lung epithelial A549 cells. Jin et al. (2007) suggested that the possible reason for the reduced cytotoxicity was the induction of DNA repair proteins in response to the NP exposure. Wang et al. (2010) reported that fluorescein-isothiocyanate (FITC)–SiO_2 NPs caused toxicity in a dose-, and time-dependent manner in mouse neural stem cells. A significant increase in toxicity was observed as dose and exposure time increased (Wang et al. 2010).

Amorphous SiO_2 has shown size-dependent toxicity in mouse keratinocytes (HEL-30). Yu et al. (2009) documented that, at a specific dose, small-sized (i.e., 30 and 48 nm) SiO_2 NPs induce significantly more toxicity than did the large-sized (i.e., 118 and 535 nm) NPs. Small-sized NPs also induced lactate dehydrogenase (LDH) leakage in a dose-dependent manner. However, a decrease in GSH level was induced only by the NPs of 30 nm size (Yu et al. 2009). Recently, Huh et al. (2010) designed a microsystem for assessing the pulmonary nanotoxicology of SiO_2 NPs.

They reported that physiological and mechanical stresses from breathing act synergistically with the NPs to exert early toxic effects on the lungs. The breathing motion accentuated the SiO_2 NP-induced proinflammatory activities in a manner that contributed to ROS generation and to development of acute lung inflammation. Exposure of the alveolar epithelium to the small (12 nm size) SiO_2 NPs, in the absence of mechanical distortion, caused little or no ROS production. When the cells are subjected to physiological levels of cyclic strain (10% at 0.2 Hz), the same NPs induced a steady increase in ROS production (Huh et al. 2010).

5.2 The In Vivo Toxicity of SiO_2 NMs

SiO_2 is widely used in biomedical applications (Nelson et al. 2010). Amino, and carboxyl modified MCM-41 mesoporous SiO_2 (MSN) NPs are reported to deliver plasmid DNA (pDNA) that encodes for the luciferase reporter gene, in vivo, in rat Achilles tendons. Weekly injections of the rats with pDNA/MSN alone did not induce toxicity. No inflammation or necrosis occurred in tendon, kidney, heart, and liver up to 1.5 months after initial administration of pDNA/MSN alone. Rather, tendon healing occurred more quickly with this formulation (Suwalski et al. 2010). In a long-term study, these NPs show toxic effects. Intravenous injection of SiO_2 NPs induced mouse liver injury. These NPs are taken up by macrophages and accumulated mainly in lungs, liver, and spleen. SiO_2 NPs are retained in these tissues for more than 30 days (Guangping et al. 2010). Nelson et al. (2010) found that Si nanowires that had an aspect ratio of 1 are nontoxic to embryonic zebrafish. Similarly, nanowires with aspect ratios >1 show higher toxicity and caused embryo deformities (Nelson et al. 2010). Canesi et al. (2010) revealed that the induction of lysosomal and oxidative stress biomarkers occurred in digestive glands of *Mytilus galloprovincialis* after suspending the feeding of SiO_2 NPs.

6 Toxicity of CNTs

CNTs possess several unique electrical, mechanical, and thermal properties. As a result of these properties, CNTs find wide applications in the electronics, aerospace, and computer industries (Shvedova et al. 2003). Unfortunately, however, chronic occupational inhalation of CNTs can induce health hazards. The genotoxic, and associated potential risks associated with the CNTs, and the long, straight MWCNTs are still inconclusive. To the degree that the CNTs are absorbed and distributed in organs of sensitive organisms they may produce toxic effects (Aschberger et al. 2010). A survey of the in vitro and in vivo toxicity studies that have been performed on CNTs are presented in Table 6.

Table 6 A survey of the in vitro and in vivo toxicity studies that have been performed on the CNTs NPs

NPs types (size)	Doses	Assays	Cell/tissue or animal	Methods	Results	References
HiPco® SWCNT (0.8–1.2×800 nm), Arc discharge tubes (1.2–1.5 nm×2,000–5,000 nm), CB (14 nm)	0–400 μg ml^{-1}	In vitro	Human alveolar carcinoma epithelial (A549), normal bronchial epithelial (BEAS-2B), and keratinocyte (HaCaT) cell lines	Toxicity tested by clonogenic assay	Toxic	Herzog et al. (2007)
SWCNTs	0.78125–200 μg ml^{-1}	In vitro	Human HEK293 cells	Cell viability and proliferation determined by MTT assay; Biochip analysis of up-regulated expression in cell cycle-associated genes conducted; Western blot analysis conducted of down-regulated expression of adhesion-associated proteins; DNA fragmentation assayed using proteinase K; cells analyzed by flow cytometry	SWCNTs inhibit cell growth; induces cell apoptosis and decreased cellular adhesion ability	Cui et al. (2005)
SWCNTs (1.5 nm×1,000 nm)	0–240 μg ml^{-1}	In vitro	Human keratinocyte (HaCaT) cell line	Electron spin resonance (ESR) spin trapping used to measure free radicals; Alamar blue used to assay cell viability; oxidative stress and associated morphological changes determined by SEM, TEM, and confocal microscopy	Toxic	Shvedova et al. (2003)

(continued)

Table 6 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or animal	Methods	Results	References
SWCNTs	15–60 μg ml^{-1}	In vitro	Murine macrophage cells (J 774 cell line) and human macrophage cells	Uptake measured using CLSM; cytotoxicity assayed using PI stain and cell counter; morphological changes analyzed using SEM	Very low cytotoxicity potential	Fiorito et al. (2006)
SWCNTs (dia ~20 nm)	7.5–30 μg ml^{-1}	In vitro	Human MSTO-211H cells	Analyzed cell proliferation by Hoechst 33258 assay; cell viability assayed via MTT and morphology by light microscopy	Agglomerated SWCNTs more toxic than well dispersed NTs	Wick et al. (2007)
SWCNT (2 nm × 500 nm)	0.8–100 μg ml^{-1}	In vitro	Human dermis fibroblasts	Assessed cell death using DNA fragmentation ELISA kit used; treated cells analyzed for immunocytochemicals; SEM used to determine cell morphology and Western blot assay used for cell–cell adhesion and cell cycle-related protein testing	Toxic; refined NTs more toxic than unrefined counterpart	Tian et al. (2006)
SWCNTs (1–2 nm × 100,000 nm) and MWCNTs (dia 10–30 nm)	5–100 μg ml^{-1}	In vitro	Rat alveolar macrophage (NR8383) and human alveolar epithelial (A549) cell lines	Cell viability assayed by MTT and WST; apoptosis and necrosis detected by annexin V binding and PI incorporation analysis; intracellular ROS generation measured by DCF assay; morphology analyzed using TEM	Toxic as such; purified SWCNT nontoxic	Pulskamp et al. (2007)

SWCNTs (1–4 nm dia)	120–500 μg ml^{-1}	In vitro	RAW 264.7 macrophages	Analyzed superoxide by flow-cytometry, and DHE and NO generation were analyzed by DAF-2DA oxidation assays; GSH was estimated by fluorescence assay	Nontoxic	Kagan et al. (2006)
SWNTs (1.4 nm × 1,000 nm), MWNTs (10–20 nm × 500–40,000 nm) Fullerenes (C60)	0–226 μg cm^{-2}	In vitro	Alveolar macrophages	MTT assay performed; cell morphology analyzed by optical light microscopy and TEM	Toxic	Jia et al. (2005)
MWCNTs (20–40 nm × 1,000–5,000 nm)	40–400 μg ml^{-1}	In vitro	Human T cells	Tested cell viability using trypan blue exclusion assay; programmed cell death assessed by annexin V-FITC binding assay; cell proliferation measured by Neubauer hemocytometer	Oxidized MWCNTs more toxic than Pristine CNTs; induced loss of cell viability through programmed cell death	Bottini et al. (2006)
MWCNTs (dia. 20 nm, aspect ratios 80–90)	0.002–0.2 μg ml^{-1}	In vitro	Human lung-tumor cell lines (H596, H446 and Calu-1)	MTT assay performed; morphology examined using light microscopy	Size-dependent toxicity; functionalized MWCNTs also toxic	Magrez et al. (2006)
MWCNTs (15 nm × 12,000 nm)	0–400 μg ml^{-1}	In vitro	Human dermal fibroblast cells	Analyzed cell viability via MTT and LDH release assays; DNA damage determined by Comet assay; and apoptosis by annexin-V assay	Toxic	Patlolla et al. (2010)

(continued)

Table 6 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or animal	Methods	Results	References
MWCNTs	0–100 μg ml^{-1}	In vitro	Mouse embryonic stem cells	AP and annexin-V stained and analyzed by scanning confocal microscopy; cell cycle checkpoint protein p53 expression analyzed by Western blot	Toxic	Zhu et al. (2007)
MWCNTs (88 ± 5 × 5,000 ± 4.5 nm)	0–400 μg ml^{-1}	In vitro	Chinese hamster lung cells (CHL/IU)	Tested cytotoxicity using colony formation and LDH assays; genotoxicity examined using chromosome aberration test, micronucleus induction and HGPR mutation assays	Cytotoxic and genotoxic; cytotoxicity depends on solvent used for suspension and ultrasonication duration	Asakura et al. (2010)
SWCNTs	0.25–50 μg ml^{-1}	In vitro	Neonatal rat ventricular cardiomyocytes	Measured changes in cellular electrophysiology and intercellular gap junctional coupling; ROS generation measured; myofibrillar structure investigated using LM and TEM	Nontoxic; slight alteration in cell functioning	Helfenstein et al. (2008)
MWCNTs (100–200 × few thousand nm)	0–80 μg ml^{-1}	In vitro	Human lung epithelial cell line (A549)	Evaluated cytotoxicity using trypan blue; oxidative DNA lesions measured by comet assay, and ROS production by DCFH-DA	Toxic; induced DNA damage	Karlsson et al. (2008)

SWCNTs (1.2–1.5 nm×2,500 nm)	0.5–500 μg ml^{-1}	In vitro	Human cervical adenocarcinoma epithelial cells (HeLa), human lung epithelial normal (L-132) and carcinoma (A549) cells	Cell viability tested by MTT or WST-1 assay and trypan blue exclusion method; apoptosis measured using annexin V-FITC and PI and ROS generation by H_2DCFDA; and, LDH leakage measured using CytoTox 96 nonradioactive cytotoxicity assay	Toxic	Choi et al. (2009)
MWCNTs (10–160×100–12,000 nm)	10–100 μg ml^{-1}	In vitro	Type II lung epithelium cell line (A549)	Tested cytotoxicity by LDH, WST-1, and XTT assays; intracellular NP accumulation and distribution determined using TEM	Comparatively more toxic than metal oxide NPs	Simon-Deckers et al. (2008)
SWCNTs (~1×300–1,000 nm), MWCNTs (20–30×500–2,000 nm)	60 μg per injection	In vivo	Mice	Exposed via intravenous administration; urine analyzed by TEM; pharmacokinetic profile of NTs studied by radioactive tracer techniques	Nontoxic	Singh et al. (2006)
SWCNTs (~100 nm)	~100 μg ml^{-1}	In vivo	Mice	Exposed via intravenous administration; necropsy, histology, and blood chemistry analyzed; used Raman spectroscopy to conduct a biodistribution study	Nontoxic	Liu et al. (2008)

(continued)

Table 6 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or animal	Methods	Results	References
SWCNTs (1–4 nm dia.)	0–40 μg per mouse	In vivo	Mice	Evaluated pulmonary toxicity by LDH and γ-glutamyl transferase activities in BALF; histopathology examined in lung tissue; assessed apoptosis by Hoechst 33342 dye and fluorescent microscopy; pulmonary function measured by whole body plethysmography	Toxic; changes in pulmonary functions	Shvedova et al. (2005)
SWCNTs (dia. 1 nm)	0–500 μg kg^{-1} bw	In vivo	Mice	Exposed via intratracheal instillation; lung tissue examined using light microscopy	Toxic	Lam et al. (2004)
SWCNTs (1.4 × 1,000 nm)	1,000 or 5,000 μg kg^{-1} bw	In vivo	Mice and rabbits	Exposed via intratracheal instillation; histopathology of lower respiratory tract examined; biochemical assays of BALF used clinical chemistry analyzer; chemotaxis of lung macrophage analyzed using light microscopy	Toxic; mortality at high dose; nonirritating to skin and eyes	Warheit et al. (2004)

SWCNT (11 nm × 500–100,000 nm)	0–360 μg ml^{-1}	In vivo	Zebrafish (*Danio rerio*) embryos	Measured head–trunk angle and body length; analyzed cell death by acridine orange staining of live embryos and blood vessel formation by staining; investigated developmental regulatory factors by whole-mount in situ hybridization and whole-mount immunostaining	Catalysts contamination in SWCNTs induced delay in hatching; no effect on embryonic development	Cheng et al. (2007)
Taurine-MWCNTs (10–20 × 10–600 nm)	10 μg per mouse	In vivo	Mice	Exposed via intravenous instillation; used [^{14}C-taurine]-MWCNTs and liquid scintillation for studying biodistribution and translocation; uptake analyzed using TEM	Low liver acute toxicity	Deng et al. (2007)
MWCNTs (5–15 × 100–10,000 nm)	0–2,500 μg m^{-3}	In vivo	Rats	Exposed via inhalation (head/nose); hematology and differential cell counts performed; also conducted were gross necropsy, functional observational battery, and histopathology of tissues	Very less toxicity induced	Ma-Hock et al. (2009)

(continued)

Table 6 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or animal	Methods	Results	References
MWCNT (10×200–300 nm)	100–6,000 μg m^{-3}	In vivo	Rats	Exposed via nasal inhalation; conducted gross pathology of tissues, general hematology, clinical chemistry, and urine analysis	Very low toxicity induced	Pauluhn (2010)
MWCNTs		In vivo	Mice	Exposed via intravenous administration; testis histology, sperm integrity, pregnancy rate, and delivery success evaluated	Oxidative stress-mediated reversible damage to testis; no effect on sperm quantity, quality, integrity and the levels of major sex hormones unaffected	Bai et al. (2010)
MWNTs (<50 ×~450,000 nm) and N-doped MWCNTs (CNx) 30–40 ×~300,000 nm	1,000–5,000 μg kg^{-1}	In vivo	Mice	Different doses administered to mice by various routes (nasal, oral, intratracheal, and intraperitoneal); tissues then histologically examined	CNx not toxic and more compatible compared to MWNTs; only extremely high concentrations of CNx nanotubes into trachea induced inflammation; Other routes of administration safe	Carrero-Sanchez et al. (2006)

6.1 The In Vitro Toxicity of CNTs

Many types of drug delivery systems have been developed. Among these are the CNTs, which show great promise for drug delivery because they can easily be functionalized by different molecules. Such ease of functionalization opens many new prospects for designing, transporting, and translocating therapeutic molecules. A classical review has been published on this topic, and describes the nonimmunogenic and mild toxicity that can be produced by functionalized CNTs (Bianco et al. 2005a). Also, cationic-functionalized CNTs were found to be nontoxic to mitogen-activated Conconavalin A and to nonactivated mouse splenocytes (Bianco et al. 2005b). Lindberg et al. (2009) reported the genotoxic effect on BEAS 2B cells resulting from exposure to CNTs and to graphite nanofibres. The fibrous nature and presence of catalyst metals (Co and Mo in CNTs, and Fe in graphite nanofibres) was thought to be responsible for the genotoxic effects produced (Lindberg et al. 2009).

6.1.1 SWCNTs

The degree of toxicity produced by single-walled carbon nanotubes (SWCNTs) is dependent upon the cell lines involved and on the production methods used. HiPco® SWCNTs produced higher toxicity, because they were more reactive as compared to SWCNTs produced by arc discharges. However, both of these NTs induced stronger cytotoxic responses than did the CB NPs. NTs inhibited cell proliferation in human alveolar carcinoma epithelial cell lines, normal BEAS-2B, and the human keratinocyte (HaCaT) cell lines (Herzog et al. 2007). However, viability decreased for only the HaCaT and BEAS-2B cell lines. Shvedova et al. (2003) reported that oxidative stress and cellular toxicity are responsible for inhibiting human epidermal keratinocytes (HaCaT). Generation of free radicals, accumulation of peroxide products, loss of cell viability, and ultrastructural and morphological modifications were suggested to be the primary changes induced by the SWCNTs to this cell line (Shvedova et al. 2003). SWCNTs are also reported to affect the cell cycle and induce cell apoptosis. Cui et al. (2005) reported that exposure to SWCNTs decreased cell adhesion and inhibited the proliferation of human embryo kidney (HEK293) cells in a dose- and time-dependent manner. However, highly purified SWCNTs were found to cause very low toxicity to human macrophage cells (Fiorito et al. 2006).

Wick et al. (2007) revealed that the agglomeration level affects the toxicity of SWCNTs towards human MSTO-211H cells. More toxicity was induced by agglomerated SWCNTs than to well-dispersed nanotubes. Further, Tian et al. (2006) reported that acid-refined SWCNTs induced more toxicity to human dermis fibroblasts cells than did unrefined nanotubes. However, unrefined SWCNTs were found to induce a dose- and time-dependent increase in the level of intracellular ROS, and to decrease the potential across the mitochondrial membrane. In contrast, acid refined SWCNTs did not induce such effects, as a result of having lower metal impurities (Pulskamp et al. 2007). SWCNTs that have been purified are also reported

to be nontoxic. Kagan et al. (2006) found that both purified and nonpurified SWCNTs have no effect on generation of intracellular superoxide radicals or on nitric oxide in RAW 264.7 macrophages. Jia et al. (2005) found that SWCNTs induced cytotoxicity in alveolar macrophages of the guinea pig in a dose-dependent manner. The toxicity of the SWCNTs increases as dose increases, and higher doses induce apoptotic cell death (Jia et al. 2005).

6.1.2 MWCNTs

Bellucci et al. (2009) reported that MWCNT (as buckypaper) induced selective an inhibitory effect on proliferation of human colorectal, breast, and leukemic cancer cell lines. However, proliferation and viability of normal human arterial smooth muscle cells and human dermal fibroblasts were unaffected (Bellucci et al. 2009). Bottini et al. (2006) reported that MWCNTs produced toxic effects on human T cells in a concentration-dependent manner. Oxidized MWCNTs produced higher toxicity than did pristine MWCNTs, and induced a massive loss of cell viability from programmed cell death at higher dose. Such toxic effects by oxidized MWCNTs are produced at dose >40 μg ml^{-1}. The toxicity of these agents is also affected by physical form, diameter, length, and the nature of any attached molecules (Bottini et al. 2006). MWCNT exposure was reported to inhibit cell proliferation and induce death to human lung-tumor cell lines (Magrez et al. 2006). Interestingly, surface functionalization of MWCNTs with carbonyl, carboxyl, and hydroxyl groups increased their toxicity (Magrez et al. 2006). Also, functionalized pure MWCNTs were toxic to human dermal fibroblast cells, and induced DNA damage and programmed cell death-mediated loss of cellular viability (Patlolla et al. 2010). Pulskamp et al. (2007) reported that MWCNTs induced intracellular ROS generation in rat macrophages (NR8383) and in human A549 lung cells. But these NT exposures did not affect cell viability (Pulskamp et al. 2007). However, long-term exposure of MWCNTs to mouse embryonic stem cells induced apoptosis, and a twofold increase in mutation frequency (Zhu et al. 2007). At higher doses, MWCNTs also induced necrosis and degeneration in the alveolar macrophages of guinea pigs, in a dose-dependent manner (Jia et al. 2005). Recently, Asakura et al. (2010) also documented the cytotoxic, and genotoxic responses of MWCNTs on Chinese hamsters lungs cells.

6.2 The In Vivo Toxicity of CNTs

6.2.1 SWCNTs

The SWCNTs are similar to other NPs in that their surface chemistry plays an important role in determining their in vivo fate. SWCNTs that were functionalized with diethylentriaminepentaacetic (DTPA) were reported to be safe to mice. Singh et al. (2006) revealed that intravenous administration of indium (^{111}In)-labeled NTs

resulted in a rapid excretion from the body in an unchanged form. Similarly, Liu et al. (2008) found that SWCNTs, coated with PEG, were nontoxic to mice. Most of the NTs administered intravenously are excreted in feces via the biliary pathway and a smaller fraction via the renal pathway. The surface coating of NTs with PEG rendered them biologically inert and that helped in their fast clearance from organs and finally from the body (Liu et al. 2008).

Shvedova et al. (2005) reported that pharyngeal aspiration of SWCNTs induced acute inflammation and formed granulomas in mice. In these granulomas, dense micrometer-scale SWCNTs aggregates were observed. Such aggregates induced a persistent change in pulmonary functions and reduced bacterial clearance (Shvedova et al. 2005). Intratracheal instillation of SWCNTs was reported to induce lung toxicity. In addition, inflammation of epithelioid granulomas, peribronchial regions, cellular injury, and necrosis were induced by the NTs in a dose-dependent manner (Lam et al. 2004). Warheit et al. (2004) revealed multifocal granuloma formation in pulmonary tissues of rats after exposure to SWCNTs. The effect was dose-independent. Higher SWCNT doses (5 mg kg^{-1}) made via instillation caused ~15% mortality of rats from mechanical blockage of upper airways (Warheit et al. 2004). However, SWCNTs did not produce skin irritation or allergic reactions when applied to rabbits (Huczko and Lange 2001).

Use of SWCNTs at concentrations >120 μg ml^{-1} induced a significant delay in the hatching of zebrafish embryos. The presence of Co and Ni catalysts in these NTs are major factors responsible for producing this toxic effect (Cheng et al. 2007). These SWCNTs also produce toxic effects in rainbow trout. Although these fish can overcome the effects of oxidative stress and the osmoregulatory disturbances caused by NT exposure, mortality was produced as a result of gill irritation and brain injury from the exposure. Furthermore, the SWCNTs also produced neurotoxic defects and cell cycle effects resulting in a rise in ventilation rate and pathologies in the gill, liver, brain, gut lumen, and intestine (Smith et al. 2007).

6.2.2 MWCNTs

Deng et al. (2007) found that intravenous administration of MWCNTs to mice resulted in their long-term accumulation, predominantly in liver. MWCNTs appear to be entrapped in hepatic macrophages (Kupffer cells), and cause an acute low-level liver toxicity (Deng et al. 2007). Ma-Hock et al. (2009) concluded that the MWCNTs did not produce systemic toxicity in Wistar rats that were exposed for 13 weeks by the inhalation route (head–nose exposure, 6 h per day for 5 days per week). Only limited numbers of neutrophiles were produced at higher-level MWCNTs exposures. Additionally, nonspecific adaptive responses were noted to exist in the nasal cavity and larynx, as well as lesion formations in the lungs and associated lymph nodes (Ma-Hock et al. 2009). Pauluhn (2010) demonstrated that higher doses of the MWCNTs induced goblet cell hyper/or metaplasia, eosinophilic globules, and focal turbinate remodeling in the upper respiratory tract. Inflammatory changes in the bronchio-alveolar region of lower respiratory tract were also observed

with the higher doses of MWCNTs. The clearance of NTs from cellular bodies were delayed at low doses and inhibited at high doses (Pauluhn 2010). Bai et al. (2010) found that repeated intravenous injections of water-soluble MWCNTs produced oxidative stress and resulted in their accumulation in mice testis and decreased the thickness of their seminiferous epithelium. The conclusion was that MWCNTs exposure induced reversible testis damage. Interestingly, levels of sex hormones, fertility, and sperm were unaffected (Bai et al. 2010).

Carrero-Sanchez et al. (2006) documented that mouse exposure at several doses to pure- and nitrogen-doped MWCNTs via the nasal, oral, intratracheal, and intraperitoneal routes induced cytotoxic effects. Injection of the MWCNTs into the trachea of mice caused death from dyspnea, in a dose-dependent manner. Interestingly, pure- and nitrogen-doped MWCNTs were far less harmful than the MWCNTs were (Carrero-Sanchez et al. 2006). Intravenous administration of acid-oxidized MWCNTs and Tween-80-dispersed MWCNTs produced different levels of toxicity (Ji et al. 2009). Both types of NTs induced spotty necrosis, inflammatory cell infiltration in the portal region, hepatocyte mitochondrial swelling and lysis. In addition the animals treated with these two agents showed a decrease in body-weight gain and a dose-dependent increase in the total bilirubin and aspartate aminotransferase levels. Ji et al. (2009) also found that a high dose of MWCNTs induced hepatic toxicity in mice. However, acid-oxidized MWCNTs showed comparatively lower toxicity. Moreover, Tween-80-dispersed MWCNTs induced a more severe liver damage than did the acid-oxidized MWCNTs (Ji et al. 2009).

7 Toxicity of Other Carbon NMs

Finely divided carbon particles, such as charcoal, lampblack and diamond particles, have been used for inscribing skin ornamentation and tattoos since ancient times. Because of their unique physical and chemical properties, carbon-based NMs, such as fullerenes, nanodiamonds (NDs), C-dots, and carbon black (CB) NPs, have been put to a wide range of applications in various fields (Schrand et al. 2007a). A survey of the in vitro and in vivo toxicity studies that have been conducted on these other carbon NMs are presented in Table 7.

7.1 The In Vitro Toxicity of Other Carbon NMs

7.1.1 NDs

NDs easily enter and accumulate in cells. Importantly, surface-modified carboxylated nanometer-sized diamonds (NDs) have been found to be nontoxic to human lung epithelial cells, and to normal fibroblasts, although they show a concentra-

Table 7 A survey of the in vitro and in vivo toxicity studies that have been performed on other carbon nanomaterials

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Carboxylated NDs (5 or 100 nm)	0.1–100 μg ml^{-1}	In vitro	Human lung (A549) epithelial cells and normal fibroblasts (HFL-1)	Analyzed ND accumulation and cellular interaction by atomic force microscopy, flow cytometer and laser scanning confocal microscopy; ND internalization measured by flow cytometer	Nontoxic	Liu et al. (2007)
NDs (100 nm)	400 μg ml^{-1}		Human kidney cells 9293T line	MTT assay performed; uptake measured by confocal fluorescence microscopy	Very low toxicity	Yu et al. (2005)
NDs	–	In vitro	Mouse permanent cell line (L929) and human gingival fibroblasts	MTT assay performed and growth pattern analyzed	Nontoxic; slight enhancement in cell proliferation	Amaral et al. (2009)
NDs (2–8 nm)	25–100 μg ml^{-1}	In vitro	Murine macrophage (RAW 264.7); human colorectal adenocarcinoma (HT-29) cells	Assayed DNA fragmentation by MTT assay; inflammation and apoptotic responses measured by RT PCR	NDs nontoxic; DOX-functionalized NDs induced cell death	Huang et al. (2007)
NDs (2–10 nm)	0–100 μg ml^{-1}	In vitro	Neuroblastoma cells, macrophages, keratinocytes, and PC-12 cells	Cell viability measured by MTT and CellTiter-Glo luminescent assays; ROS measured by DCHF-DA fluorescent probe; uptake analyzed by fluorescent microscopy, SEM, and TEM	Nontoxic	Schrand et al. (2007b)

(continued)

Table 7 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
C60-fullerenes	15–60 μg ml^{-1}	In vitro	Murine macrophage (J 774 cell line) and human macrophage	Uptake studied using CLSM; cytotoxicity evaluated using SEM	Nontoxic to murine macrophages; low toxicity to human macrophages	Fiorito et al. (2006)
C dots (4–5 nm)	0–200 μg ml^{-1} and 8, 40 mg kg^{-1}	In vitro and in vivo	Human breast cancer (MCF-7) and colorectal adenocarcinoma (HT-29) cells	Exposed via intravenous route; cell mortality assayed using trypan blue and cell viability measured via the MTT assay; biochemical assays performed using a clinical chemistry analyzer; histopathology analysis performed via light microscopy; biodistribution measured by isotope-ratio mass spectroscopy	Nontoxic	Yang et al. (2009b)
CB (14, 56 and 95 nm)	0–100 μg ml^{-1}	In vitro	Rat alveolar epithelial cell line (SV40T2) and alveolar macrophages (AM)	Measured innate oxidative capacity using dithiothreitol assay; analyzed HO-1 by ELISA	Have innate oxidative capacity; can induce oxidative stress	Koike and Kobayashi (2006)
NDs (2–10 nm) and CB (20 nm)	25–100 μg ml^{-1}	In vitro	Neuroblastoma and alveolar macrophages	Performed MTT assay; ROS estimated using a DCHF-DA fluorescent probe; mitochondrial membrane permeability measured by epifluorescent microscopy; morphology examined using TEM	ND nontoxic; Biocompatibility follow order ND > CB > MWCNTs > SWCNTs	Schrand et al. (2007a)

G (3–5 nm)	0–100 μg ml^{-1}	In vitro	PC12 cell line	MTT assay and LDH release assays conducted; ROS monitored using DCFH-DA and apoptosis with Caspase 3/7 assays; morphology conducted using light microscopy	Toxic	Zhang et al. (2010a)
CB, C_{60} fullerene	0.05–5 μg ml^{-1}	In vitro and In vivo	Hemocytes; *Mytilus galloprovincialis*	Studied membrane stability, catalase and GST activities	Induced lysosomal membrane destabilization; change in oxidative stress biomarkers in the digestive gland	Canesi et al. (2010)
$C_{60}(OH)_{24}$ (7.1 ± 2.4 nm)	1–100 μg ml^{-1}	In vitro	Human umbilical vein endothelial cells (HUVECs)	Assayed cytotoxicity using CytoTox 96 nonradioactive LDH, and cell proliferation using the cell counting-8 kit; apoptosis analyzed using TEM and autophagy by Western blotting	Toxic	Yamawaki and Iwai (2006)
$C_{60}(OH)_x$ (x=22, 24) (20.0 ± 3.8 nm)	–	In vivo	Rats	Exposed via intratracheal instillation; biodistribution of 99mTc-C60(OH)x in rats imaged with SPECT and gamma-ray counting	Highest retention in the lungs; mainly distributed in liver, bone and spleen; fast clearance from blood	Xu et al. (2007)

(continued)

Table 7 (continued)

NPs types (size)	Doses	Assays	Cell/tissue or Animal	Methods	Results	References
Water–nC_{60} and THF–nC_{60} (10–200 nm)	0.5–35 ppm	In vivo	*Daphnia magna* and *Pimephales promelas*	Determined LPO using the malonaldehyde method; CYP2 isozymes utilized immunodetection (that were involved in metabolizing fullerene or in repairing LPO)	THF–nC60 toxic; water–nC_{60} nontoxic	Zhu et al. (2006)
PEGylated G (10–50 nm)	20,000 μg kg^{-1}	In vivo	Mice	Exposed via intravenous administration; imaging conducted via in vivo fluorescence; histology, blood chemistry, and complete blood panel conducted	Nontoxic	Yang et al. (2010a)
C dots (4–5 nm)	30 μg per 30 μl	In vivo	Mice	Used lumazone FA in vivo as the imaging system; visually observed mortality	Nontoxic	Yang et al. (2009a)
C_{60} and $C_{60}HxC_{70}Hx$ (10–20 nm)	0.260 μg ml^{-1}	In vivo	*Daphnia magna*	Measured changes in postabdominal claw curling, heart beats rate, and feeding	Induced increase in hopping frequency and appendage movement	Lovern et al. (2007)
CB (14 nm)	0–1,000 μg ml^{-1}	In vitro	Mouse testis Leydig (TM3) cell line	Assessed cell viability by hemocytometer and trypan blue exclusion method; studied steroidogenic acute regulatory (StAR) gene expression; investigated oxidative stress through HO-1 gene expression	Toxic at higher dose	Komatsu et al. (2008)

NDs (~120 nm)	500–1,000 μg ml^{-1}	In vivo	*C. elegans*	Imaged *C elegans* using inverted fluorescence microscopy; assayed brood size and life span; measured ROS using H_2DCFDA; assayed stress responses	Nontoxic	Mohan et al. (2010)

tion-dependent increase in florescence intensity (Liu et al. 2007). The 100-nm size fluorescent NDs induced a low level of toxicity to 293T human kidney cells. These fluorescent NDs enter into cells through endocytosis, but their entry into the nucleus is restricted (Yu et al. 2005). Small sized NDs (2–8 nm) were found to be nontoxic to HT-29 human colorectal adenocarcinoma cells, although NDs functionalized with DOX induced cell death (Huang et al. 2007). A nanocrystalline diamond coating on Si_3N_4 ceramic is used in biomedical dental applications. An in vitro cytotoxicity study of NDs on fibroblast cells (L929; a mouse permanent cell line), and human gingival fibroblasts suggest that this nanocrystalline diamond coating is safe. Proper coating of NDs is necessary to ensure that they will have appropriate cell attachment, spreading, and proliferation (Amaral et al. 2009). Schrand et al. (2007a) reported that NDs (2–10 nm size) with and without surface modification (acid or base) are nontoxic and biocompatible with neuroblastoma, macrophage, keratinocyte, and PC-12 cells.

7.1.2 Fullerene

Fullerenes (C_{60}) find a wide range of applications in the material and biomedical sciences. Highly purified C_{60} is nontoxic to murine macrophages and displays only little toxicity to human macrophages (Fiorito et al. 2006). Pristine C_{60} does not cause any acute or subacute toxicity on human leukocytes. Interestingly, chemical modifications, either covalently or noncovalently, can convert them into highly toxic nanomaterials (Kolosnjaj et al. 2007). For example, exposure of hydroxyl fullerene ($C_{60}(OH)_{24}$ dia. 7.1 ± 2.4 nm) at higher dose causes cytotoxic injury or cell death to human vascular endothelial cells (i.e., umbilical vein cells). Hence, these NPs tend to cause atherosclerosis and ischemic heart disease. Activation of the ubiquitin-autophagy pathway from C_{60} exposure is reported to be responsible for cell death (Yamawaki and Iwai 2006).

7.1.3 C Dots

Fluorescent carbon dots (C dots) are small carbon NPs and their surfaces are rendered passive by using oligomeric PEG molecules. Yang et al. (2009a) reported C dots as being nontoxic to human breast cancer MCF-7 and to human colorectal adenocarcinoma HT-29 cells.

7.1.4 CB NPs

Because the CB NPs have a larger surface area, they generally induce more inflammatory response than other similar large NPs. Koike and Kobayashi (2006) reported that various sized CB NPs (14, 56, and 95 nm) induced oxidative stress

in rat alveolar epithelial cells and in alveolar macrophages. This innate oxidative stress-generating capacity tends to be more prominent with smaller particles (Koike and Kobayashi 2006).

7.1.5 Comparative Toxicity Studies

Schrand et al. (2007b) reported that the NDs are biocompatible with and nontoxic to neuroblastoma and rat alveolar macrophages. CB NPs, MWCNTs, and SWCNTs all are known to induce toxicity. However, NP biocompatibility follows the following trend: ND > CB > MWCNTs > SWCNTs. The CNTs induced membrane leakage and generate ROS (Schrand et al. 2007b), while the SWCNTs and CB NPs induced low oxidative stress to human bronchial epithelial and alveolar epithelial carcinoma cells. Importantly, dispersion media also affect the toxic behavior of these NPs. When these NPs are dispersed in dipalmitoylphosphatidylcholine, use of them increases intracellular ROS formation in human lung epithelial cells. Fetal calf serum, however, protects these cells against the oxidative stress generated by exposure to these NPs (Herzog et al. 2009). The feeding of suspensions of CB NPs and C_{60} produced oxyradicals and released lysosomal enzymes in the hemocytes of the marine mussel *M. galloprovincialis* (Canesi et al. 2010).

Zhang et al. (2010b) reported that acid purified graphene (G) layers induced oxidative stress and apoptosis pathway-dependent cytotoxic effects on a neuronal PC12 cell line. ROS were generated in a neuronal PC12 cell line, in a concentration-, and time-dependent manner, upon exposure to G. At lower concentrations, G structures induced a more intense toxic response, as compared to acid purified SWCNTs. However, as the concentration was increased the cytotoxic effects were reversed. The shape of nanomaterials were reported to be responsible for their differential toxicity. The tubular structure of the SWCNTs facilitates their rapid membrane penetration and strong interactions with various protein systems. This behavior enhanced the toxicity of the SWCNTs at higher concentrations. The flat shape of G is regarded to result in stronger interactions with cellular membranes. However, their aggregations at higher concentration have no such toxic effect (Zhang et al. 2010b).

7.2 The In Vivo Toxicity of Other Carbon NMs

7.2.1 NDs

Yuan et al. (2009) observed that intravenous administration of 50-nm sized NDs in mice resulted in their accumulation in liver (60%) and lungs (8%). Such long-term entrapment (28 days post injection) of NDs in vital organs warrants more detailed future toxicity study. However, Mohan et al. (2010) reported that feeding of suspensions and microinjection of fluorescent NDs into the gonads of *C. elegans* causes no harm. Evaluations have been made of longevity and reproductive potential and stress response measurements with the help of reporter genes, and these evaluations have been used to document the stable and nontoxic nature of NDs (Mohan et al. 2010).

7.2.2 Fullerene

Intratracheal instillation of 20.0 ± 3.8-nm sized fullerene $C_{60}(OH)_x$ ($x = 22, 24$) that was derivatized with polyhydroxylation and labeled with ^{99}mTc produced the highest retention rate in lungs of the test organism. Few fullerene particles are observed to rapidly penetrate through the alveolar-capillary barrier to enter the circulation. Fullerene is mainly distributed in liver, bones, and spleen tissues, and there is no evidence of accumulation in the brain. The labeled fullerene complex shows in vivo stability and rapid clearance from the blood (Xu et al. 2007).

According to Kolosnjaj et al. (2007), pristine C_{60} is neither acutely nor subacutely toxic in drosophila, mice, rats, or guinea pigs. At times, chemically modified (covalently or noncovalently modified) fullerenes can be highly toxic. Furthermore, under light exposure, C_{60} behaves as an efficient singlet oxygen sensitizer. As a result, the presence of oxygen and irradiation with UV–visible light converts the nontoxic pristine C_{60} into a highly toxic form (Kolosnjaj et al. 2007). One study revealed that exposure of THF-solubilized and water-stirred nC_{60} (nC_{60}) to *D. magna* and adult male fathead minnows (*Pimephales promelas*) produced a toxic response (Zhu et al. 2006). Furthermore, THF solubilized nC_{60} was more toxic to daphnia than was water-stirred nC_{60}. The 0.5-ppm dose of the THF-solubilized nC_{60} product to male fathead minnows induced 100% mortality within 6 h. In contrast, water-stirred nC_{60} exposure did not induce any such effect even after a 48-h exposure (Zhu et al. 2006). Exposure to C_{60} induced a significant lysosomal membrane destabilization in the digestive gland of *M. galloprovincialis* (Canesi et al. 2010). Upon such exposure, lysosomal lipofuscin accumulated in the digestive gland. C_{60} exposure also enhanced catalase, and glutathione transferase (GST) activity in digestive glands and gills of *M. galloprovincialis* (Canesi et al. 2010).

7.2.3 Graphene NPs

Yang et al. (2010b) found PEGylated G sheets to be nontoxic to mice. These sheets show high tumor cell uptake, efficient tumor passive targeting, and relatively low retention in the reticuloendothelial systems of xenograft tumor mouse models. The G sheet-PEG injection to mice was safe even after 40 days of exposure. Also, no abnormality was noticed in the histology or chemistry from the blood panel (Yang et al. 2010b).

7.2.4 C Dots

When present in solutions, C dots gave a bright fluorescent appearance. They have been tested as in vivo contrast agents in mice. Intravenous injection of C dot solutions to mice did not induce an acute toxicological response. These NPs were excreted primarily via urine within ~3 h of injection (Yang et al. 2009b). Therefore, C dots are considered to be nontoxic. After 4 h of intravenous injection, florescence

of C dots can be observed in liver and in kidneys. Generally, low florescence is observed in liver because C dots are not as highly accumulated in them as in the kidney. The kidney tends to show higher florescence, because urine is the main C dot excretion pathway (Yang et al. 2009b). In addition to low toxicity from other routes of exposure, intravenous administration of C dots to male CD-1 mice did not produce any toxicity. Exposure of C dots, even for a period of 28 days was found to be safe (Yang et al. 2009a).

7.2.5 CB NPs

A suspension of CB NPs produced toxicity in mussels (Canesi et al. 2010). In this same study, catalase and GST activities were enhanced in the digestive gland and gills. Feeding a CB NPs suspension to mussels also produced an accumulation of lysosomal neutral lipid (Canesi et al. 2010).

8 Summary

Nanotechnology is a fast growing field that provides for the development of materials that have new dimensions, novel properties, and a broader array of applications. Various scientific groups are keen about this technology and are devoting themselves to the development of more, new, and better nanomaterials. In the near future, expectations are that no field will be left untouched by the magical benefits available through application of nanotechnology.

Presently, there is only limited knowledge concerning the toxicological effects of NPs. However, it is now known that the toxic behavior of NPs differ from their bulk counterparts. Even NPs that have the same chemical composition differ in their toxicological properties; the differences in toxicity depend upon size, shape, and surface covering. Hence, before NPs are commercially used it is most important that they be subjected to appropriate toxicity evaluation. Among the parameters of NPs that must be evaluated for their effect on toxicity are surface charges, types of coating material, and reactivity of NPs.

In this article, we have reviewed the literature pertinent to the toxicity of metal oxide NPs, metallic NPs, quantum dots (QDs), silica (SiO_2) NPs, carbon nanotubes (CNTs), and certain other carbon nanomaterials (NMs). These NPs have already found a wide range of applications around the world. In vitro and in vivo studies on NPs have revealed that most are toxic to animals. However, their toxic behavior varies with their size, shape, surface charge, type of coating material and reactivity. Dose, route of administration, and exposure are critical factors that affect the degree of toxicity produced by any particular type of NP. It is for this reason that we believe a careful and rigorous toxicity testing is necessary before any NP is declared to be safe for broad use. We also believe that an agreed upon testing system is needed that can be used to suitably, accurately, and economically assess the toxicity of NPs.

NPs have produced an array of different toxic effects in many different types of in vivo and in vitro studies. The types of effects that NPs have produced are those on the pulmonary, cardiac, reproductive, renal and cutaneous systems, as well as on various cell lines. After exposures, significant accumulations of NPs have been found in the lungs, brain, liver, spleen, and bones of test species. It has been well established that the degree of toxicity produced by NPs is linked to their surface properties. Soluble NPs are rendered toxic because of their constituents; however, the situation is entirely different for insoluble NPs. Stable metal oxides do not show any toxicity, whereas metallic NPs that have redox potential may be cytotoxic and genotoxic. The available data on NP toxicity is unfortunately limited, and hence, does not allow scientists to yet make a significant quantitative risk assessment of the safety of synthesized NPs.

In this review, we have endeavored to illustrate the importance of having and using results from existing nanotoxicological studies and for developing new and more useful future risk assessment systems. Increased efforts of both an individual and collective nature are required to explore the future pros and cons of nanotechnology.

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Adsorption and Desorption of Chlorpyrifos to Soils and Sediments

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1 Introduction

Although the use of pesticides is as old as agriculture, the advent of synthetic pesticides in the 1940s was one of the most important achievements that spawned the "Green Revolution" (Tilman 1998). Synthetic pesticides, along with the introduction of chemical fertilizers, enabled dramatic increases in agricultural productivity and quality without the need to increase farmland and labor (Seiber and Ragsdale 1999; Cooper and Dobson 2007). Pesticide use reduces the impact of pests on agricultural productivity by about half (Oerke and Dehne 2004; Oerke 2006), and many argue that reduction or cessation of pesticide use would lead to significant crop loss, increased food prices, and lack of food for the world's growing human population (Fernandez-Cornejo et al. 1998; Ragsdale 1999; Oerke and Dehne 2004; Cooper and Dobson 2007). Moreover, the use of pesticides alleviates food shortages in developing countries, allowing them to grow crops multiple times a year and export produce to developed nations (Ecobichon 2000, 2001; Oerke and Dehne 2004).

However, the growing use of pesticides to produce food, fiber, and fuel to meet the need of the growing global population is dramatically affecting both ecosystem and public health. Acute pesticide poisoning is already a global health problem. One million unintentional and two million intentional poisonings occur annually (Jeyaratnam 1990; WHO 1990; Eddleston et al. 2002). In some regions of developing nations, these poisonings cause more deaths than do infectious diseases (Ecobichon 2001; Eddleston et al. 2002; Wesseling et al. 2005). In addition, while a definite relationship between adverse public health effects and pesticide residues have not been conclusively established, a substantial number of studies have linked pesticide exposure to reproductive abnormalities and birth defects (Garry et al. 2002), cancer (Richter and Chlamtac 2002), neurodegenerative diseases (Kanthasamy et al. 2005), and developmental neurotoxicity, including attention-deficit and hyperactivity disorders in children (Ruckart et al. 2004; Rohlman et al. 2005; Grandjean et al. 2006; Eskenazi et al. 2007; Bouchard et al. 2010). Similarly, the adverse consequences of pesticides on nontarget species and ecosystem biological processes has been reported in several studies (Stevens et al. 1985; Finlayson et al. 1993; Bailey et al. 1994; Matern et al. 2002; Schwarzenbach et al. 2006; Ostrach et al. 2008). However, while ecological and health risks related to pesticide use were recognized as early as in the 1960s, major policy changes in pesticide use have never been achieved, and global production and use of pesticides have not abated (WHO 1990; Tilman et al. 2001).

Often, only a small amount of an applied pesticide reaches the target species (~0.1%; Pimentel 1995), thereby leaving a large portion of the pesticide to migrate off-site. Concerns for such events has already motivated considerable research on various physical, chemical, and biological processes that mediate off-site pesticide transport

and environmental impact. Of these processes, adsorption and desorption are critically important. Knowledge of these two processes is key to evaluate the environmental risk of agricultural chemicals (Dabrowski et al. 2002), to conduct remediation of pesticide contamination (El Bakouri et al. 2007; Memon et al. 2009), and to develop pesticide disposal technologies (Mullins et al. 1992, 1993). Moreover, with the continuing unabated use of pesticides, there is an urgent need to improve the ability to predict the environmental fate of pesticides and to develop management strategies that reduce pesticide mobility and toxicity; neither of these needs can be accomplished without a clearer understanding of pesticide adsorption and desorption processes.

Although regulatory agencies require adsorption and soil mobility data prior to registering pesticides, such data are not necessarily adequate to accurately predict the environmental fate or mobility of any particular chemical. The unexpected detection of hydrophobic pesticides in remote ecosystems and ground waters, compounds deemed immobile based on their partition coefficients, is indicative of an incomplete understanding of pesticide adsorption and desorption processes in natural environments (McCall et al. 1980; Corsolini et al. 2002; Montone et al. 2005). One key element needed to fill this knowledge gap, in addition to continued basic research, is to comprehensively synthesize existing research findings pertinent to the adsorption and desorption of pesticides. Therefore, we have analyzed an extensive number of peer-reviewed journal articles, and herewith present a critical examination of the environmental presence, adsorption, and desorption of chlorpyrifos (CPF), one of the most widely used organophosphorus pesticides worldwide. This review complements past reviews that have addressed the environmental fate and ecotoxicology of CPF (Racke 1993; Barron and Woodburn 1995; Giesy et al. 1999), and the general process of soil adsorption for multiple pesticides (Delle Site 2001; Wauchope et al. 2002). We first review the environmental presence of CPF and then address CPF adsorption data for a range of solid matrices, including soils, sediments, organic matter, and minerals. Our review was performed using the framework of the common methods employed to quantify pesticide adsorption: batch equilibrium, chromatography, and use of ancillary pesticide characteristics such as water solubility, the octanol–water partition coefficient, and topological structure. Thereafter, we address peer-reviewed data that documents CPF desorption, a key process that affects the long-term fate and impact of CPF in the environment, but has heretofore been inadequately addressed. We conclude the review by providing key recommendations for future research.

2 Environmental Behavior and Presence of Chlorpyrifos

2.1 Chemical Properties

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is an insecticide commonly known as Dursban® or Lorsban®, trademarks of Dow AgroScience, LLC (IN, the USA). It is one of several compounds designed to replace

persistent and ecologically toxic organochlorine pesticides banned in the USA in the 1970s (USEPA 1986). Chlorpyrifos is an organophosphorus insecticide that has low water solubility and an intermediate vapor pressure. The compound exhibits a moderate level of hydrophobicity and a strong tendency to sorb to organic matter and soil. When compared to most other organophosphorus pesticides, CPF exhibits lower water solubility and a higher log K_{ow}, approaching that of DDT (Readman et al. 1992). Major chemical properties relating to its environmental fate are summarized in Table 1.

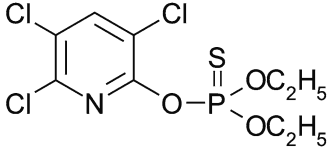
2.2 *Persistence and Ecotoxicity*

Degradation of CPF in the natural environment is the result of abiotic and biotic processes that often work in tandem. A key process that results in CPF degradation involves enzymatic or clay-/metal-catalyzed hydrolysis leading to cleavage of the phosphorothioate ester bond to form the by-product 3,5,6-trichloro-2-pyridinol (TCP) (Racke 1993). A rapid increase in the rate of hydrolysis was reported with increasing pH (Chapman and Cole 1982; Macalady and Wolfe 1985), temperature (Meikle and Youngson 1978; Getzin 1985), and catalytically by dissolved Cu(II) (Mortland and Raman 1967). Similarly, CPF will undergo photolytic degradation in sunlight, resulting in partial dechlorination of the pyridine ring and demethylation of the phosphorothioate ester (Attila and Diana 2009).

Environmental dissipation half-lives of CPF range from a few days to more than 4 years, depending on application rate, ecosystem type, and pertinent environmental factors (Racke 1993; Liu et al. 2001). Higher application rates, such as termiticidal applications, resulted in considerably increased persistence (Racke et al. 1994; Murray et al. 2001). Wright and coworkers studied dissipation of CPF over a long time following a residential termiticidal application. CPF was detected in indoor air and soil at respective concentrations of 6 μg/m^3 and 499 mg/kg 4 years after application (Wright et al. 1991), and at levels of 0.7 μg/m^3 and 295 mg/kg 8 years after application (Wright et al. 1994). Chlorpyrifos can be completely mineralized, but the process is slow. Only 5% of the compound was mineralized to CO_2 after a 13-month incubation in soils (Racke et al. 1994), and only 2.5% was mineralized when incubated for almost 2 months in wetland sediments (Gebremariam and Beutel 2010).

The toxicity of CPF generally arises from its inhibition of the neuroenzyme acetylcholinesterase (AChE) in exposed organisms, though the level of toxicity is variable across organisms of different species and orders. Acute toxic concentrations of CPF for the most sensitive species range from as low as 1.0 ng/L for insect larvae to 10 μg/L for freshwater crustaceans. Chlorpyrifos displayed acute toxicity to fish in ponds contaminated by runoff from treated soils and in laboratory experiments at doses equivalent to recommended agricultural application rates (Davey et al. 1976; Carr et al. 1997). Similar acute toxicity has been reported for soil nematodes (Roh and Choi 2008). Chlorpyrifos changed the composition of the plankton community

Table 1 Physical and chemical properties of chlorpyrifos

Property	Method/media		Reference
Chemical name (C.A.)		O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	
Chemical formula		$C_9H_{11}Cl_3NO_3PS$	
Molecular wt		350.6 a.m.u.	
CAS number		2921-88-2	
Molecular structure			
Trade names		Dursban®, Lorsban®	
Appearance		White crystalline solid	
Melting point		42–43.5°C	Brust (1966)
Vapor pressure		1.87×10^5 mmHg, 25°C	Brust (1966)
Solubility	Distilled water	0.4 mg/L, 23°C	Brust (1966)
		0.73 mg/L, 24°C	Felsot and Dahm (1979)
		0.44 mg/L, 25°C	Swann et al. (1983)
		0.3 mg/L, 25°C	Briggs (1981)
		1.12 mg/L, 20°C	Bowman and Sans (1983)
	$CaCl_2$ solution	0.78 mg/L, 24°C	Felsot and Dahm (1979)
	Soil solution	0.84–0.92 mg/L, 24°C (Mean = 0.883 mg/L)	Felsot and Dahm (1979)
	Sea water	0.073 mg/L, 22°C	Schimmel et al. (1983)
$\log K_{ow}$	Shake-flask method	5.11, 20°C	Chiou et al. (1977)
	Slow-stirring method	5.267, 25°C	Bruijn et al. (1989)
	Shake-flask method	5.2, 25°C	Schimmel et al. (1983)
	Shake-flask method	4.96, 20°C	Bowman and Sans (1983)

(continued)

Table 1 (continued)

Property	Method/media		Reference
Henry's law constant (air/water)	Wetted-wall column method	1.7×10^4, 25°C, distilled water	Fendinger and Glotfelty (1990)
	Wetted-wall column method	1.3×10^4, 20°C, distilled water	Rice et al. (1997)
	Wetted-wall column method	2.02×10^4, 20°C, salt water (33.3‰)	Rice et al. (1997)
	Calculated from solubility and pressure	5.0×10^4	Glotfelty et al. (1987)
	Calculated from solubility and pressure	7.3×10^4, 20°C	Suntio et al. (1988)

at a concentration of 100 ng/L in sea water (Tagatz et al. 1982), and had an acute toxicity against mysid shrimp at 35 ng/L (96-h LC50) (Schimmel et al. 1983). The environmental toxicity of CPF to a broad range of biota has been extensively reviewed by Barron and Woodburn (1995) and Giesy et al. (1999).

2.3 Environmental Presence

Stability and effectiveness against a wide range of insect pests are major factors that have made CPF one of the most used pesticides worldwide. It has been formulated into over 400 registered products that are marketed for a very broad range of agricultural, industrial, and residential pest control (USEPA 2002b). In the USA alone, approximately 10 million kg active ingredient (AI) of CPF was used annually from 1987 to 1999 (USEPA 2002a; Donaldson et al. 2002). The annual use of CPF in the USA decreased to 7 million kg AI in 2001, and to 5 and 4.1 million kg AI in 2003 and 2005, respectively (Grube et al. 2011). In 2007, CPF was ranked as the most used organophosphorus pesticide in the USA, and its total use for the year was approximately 5 million kg AI (Grube et al. 2011). The annual average global use of CPF between 2002 and 2006 was 25 million kg AI, of which 98.5% was used for agricultural purposes (Eaton et al. 2008).

Continuous and excessive use of CPF has already led to widespread environmental contamination in many countries. This insecticide has been detected in marine sediments, streams, sumps, sloughs, rivers, urban storm drains, freshwater lakes, groundwater, fog, rain, and air (Glotfelty et al. 1987; Readman et al. 1992; Coupe et al. 2000; Hoffman et al. 2000; Kolpin et al. 2000; Kuang et al. 2003; Zamora et al. 2003; Gilliom et al. 2006; Wightwick and Allinson 2007). It has also been detected in solid and liquid food samples from both urban and rural areas, raising significant health concerns. A study conducted in six North Carolina counties to assess exposure of preschool children to CPF and its by-product TCP in 129 homes and 13 day-care centers that received CPF applications 2–17 months before sampling indicated detection of residues of CPF and TCP in soils, outdoor and indoor air, indoor floor dust, indoor surfaces, and solid and liquid food samples at a frequency of 10–100% (Morgan et al. 2005). Chlorpyrifos was detected in indoor floor dust and indoor air at concentrations reaching 15.1 μg/kg and 0.4 μg/m^3, respectively. Monitoring of children in this study indicated a median daily TCP urine level of 117 ng/kg and inhalation, dermal absorption and dietary ingestion were considered major exposure routes to CPF. Chlorpyrifos was also detected in house dust in central Washington State regardless of whether the house was occupied by a pesticide applicator, a farm-worker, or a nonagricultural worker at concentrations ranging from 100 to 400 μg/kg (Fenske et al. 2002). In a similar monitoring study conducted in Japan, CPF was detected in indoor air of 41 out of 43 treated houses and TCP was detected in urinary samples of the residents (Dai et al. 2003). A Minnesota children's pesticide exposure study also indicated occurrence of CPF in various media collected from urban and rural houses and identified solid food as a major exposure

route representing a median CPF intake of up to 263 ng/day for children (Clayton et al. 2003). Although the USEPA canceled residential use of CPF in 2000 (USEPA 2002b), the insecticide is still heavily used in the agricultural and industrial sectors, leading to an increasing frequency of detection of CPF and its metabolites in humans and the environment. The frequency of detection of TCP in urine samples, collected from individuals living in the USA, has risen from 82% ($n = 1{,}000$) in 1995 (Hill et al. 1995) to 96% ($n = 2{,}000$) in 2005 (Barr et al. 2005).

The occurrence of CPF in surface waters away from application sites has also been extensively reported (Larson et al. 1995; Bailey et al. 2000; Dubrovsky et al. 2000). According to a 10-year water quality assessment study performed by the United States Geological Survey, CPF was the most heavily used and frequently detected insecticide; it was found at concentrations exceeding an aquatic-life benchmark of 0.04 μg/L for water in 37% samples collected from water bodies with diverse land-use settings throughout the USA (Gilliom et al. 2006). Chlorpyrifos was detected frequently in both urban and rural streams and major rivers in the USA, but less frequently in groundwater samples (Kolpin et al. 2000). However, in several recent studies, CPF has been detected in groundwater. CPF was detected in the majority of ground water and surface water samples collected along the Mediterranean coast of Turkey (Tuncel et al. 2008). The detection frequency of CPF in drinking water well samples from the state of Rio Grande do Sul, Brazil, at times, exceeded that of surface water samples (Bortoluzzi et al. 2007). Chlorpyrifos was also detected in many samples taken from Australian water wells (Wightwick and Allinson 2007). However, the concentration of CPF detected in groundwater samples is generally low when compared to levels that appear in surface water samples. Selected other studies that provide data on the occurrence of CPF in various surface water bodies are summarized in Table 2.

Although spray-drift may play a role in off-site migration of CPF to aquatic ecosystems, the transport of CPF to waterways is often exacerbated by storm runoff. The concentration of dissolved CPF detected after a rain event in the Lourens River, South Africa, increased from nondetectable to 0.19 μg/L (Dabrowski et al. 2002). Similarly, CPF concentrations in the Selangor River, Malaysia, almost doubled in samples collected in the wet season, compared to those collected in the dry season (Leong et al. 2007). A spike in storm-related transport of CPF to rivers was reported by Kratzer et al. (2000). They found that peak concentrations of dissolved CPF in the San Joaquin River, CA and its tributaries corresponded with peak flows.

Although CPF exhibits low volatility (Table 1), it has been widely detected in air and rain, apparently through the combined effects of spray-drift and volatilization from plant and soil surfaces. The fraction of CPF volatilized from conventional till and no-till plots 4 days after application was estimated at 7 and 23%, respectively (Whang et al. 1993). Chlorpyrifos was also one of the few insecticides detected in all air samples over the Mississippi River from New Orleans, LA, to St. Paul, MN (Majewski et al. 1998). Similarly, McConnell et al. (1997) reported that CPF was detected in all air samples collected over Chesapeake Bay and CPF was the most frequently detected insecticide in wet deposition over the Midwestern USA (Majewski et al. 2000). Zamora et al. (2003) monitored pesticide loads in

Table 2 Occurrence of chlorpyrifos in surface water bodies

Location	Water body	Catch area[a]	Pollution source	Sampling sites	Sampling frequency	Sampling duration	Dissolved concen[b]	DF[c]	LOD[d]	Reference
Patuxent Watershed, MD	Patuxent river	2.3	Agricultural runoff	3 Shorelines and 8 transects	Twice for transects, every other day at shorelines	1995	0.0–190 (shoreline) and 0.8–14.0 (transect)	(–)[e]	0.005(–)	Harman-Fetcho et al. (1999)
Columbia Basin Irrigation Project Central, WA	Royal lake	6,400	Irrigation runoff	2 Lake sites	1-5 time per month	1993–1995	120 (max)	52	–(4)	Gruber and Munn (1998)
Sacramento-San Joaquin Delta, CA	Sacramento and San Joaquin rivers and tributaies	3,000	Agricultural drainage	44 River sites	Monthly	1993–1995	58–444	–	–	Werner et al. (2000)
San Joaquin River Basin, CA	San Joaquin river and tributaies	19,023	Agricultural drainage	7 River, 8 precipitation and 1 urban storm drain	19–64 times weekly/after storm events	2001	<2.0–68.0 (water samples); 2.0–148 (precipitation)	–	–(3)	Zamora et al. (2003)
State of Selangor, Malaysia	Selangor river	1,743	Urban and agricultural drainage	9 Shorelines	Seasonal	2002–2003	20.8–195.2	35	–(10)[f]	Leong et al. (2007)

(continued)

Table 2 (continued)

Location	Water body	Catch area[a]	Pollution source	Sampling sites	Sampling frequency	Sampling duration	Dissolved concen[b]	DF[c]	LOD[d]	Reference
Nova Boemia-Candido Brum-Passo do Meio basin, Brazil	Creeks and drinking water wells	17.4	Agricultural drainage	12 wells and 12 creek/channel	5 times	2001–2002	0.06–0.19	–	50(–)	Bortoluzzi et al. (2007)
Salinas River watershed, CA	Salinas river and tributaries	11,500	Agricultural drainage	8 river sites	15 times	1998–2000	0.032–0.18	–	50(–)	Hunt et al. (2003)
Various urban streams, The USA	Streams in CT, CO, DC, FL, GA, NV, NY, and OR	5,777	Urban drainage	14 urban stream lines	1–8 times per month	1993–1994	0.3 (maximum)	17.7	10(–)	Hoffman et al. (2000)
Lourens River catchment, South Africa	Lourens river and tributaries	44	Agricultural drainage	6 tributaries and edge-of-field ditches	Before and after rainfall event	April–May 1999	0.19–0.47		–	Dabrowski et al. (2002)

[a]Catchment area in km^2
[b]Detected concentrations in ng/L
[c]*DF* detection frequency
[d]Limit of detection (LOD) in ng/L. Values in parenthesis are method detection limits in ng/L
[e] "–" values not reported
[f]Limit of quantification in ng/L

precipitation and storm runoff in the San Joaquin River basin, CA. They found high concentrations of CPF in precipitation, with the average concentrations exceeding storm runoff concentrations by a factor of 2.5.

3 Adsorption by Batch Equilibrium

Owing to diverse factors affecting pesticide–soil interactions, it has been widely recognized that development of a general mechanistic model of pesticide adsorption/desorption is exceedingly difficult (Wauchope et al. 2002). Commonly, researchers default to an empirical method specific to each soil/pesticide combination. These methods involve experimental techniques such as the batch equilibrium and chromatography techniques.

The batch equilibrium technique is one of the most widely followed experimental approaches for evaluating pesticide adsorption (OECD 1981; USEPA 1998c). The method involves addition of a range of solute concentrations to a known amount of soil at a constant temperature, and agitating the mixture until equilibrium conditions are achieved. Then, the equilibrium concentration in the liquid phase (C_e) is measured, and the equilibrium concentration of the solute in the solid phase (Q_e) is usually calculated by mass balance considerations. The plot of Q_e versus C_e, the so-called adsorption isotherm, is then commonly modeled as a Langmuir, Freundlich, or linear isotherm:

$$Q_e = \frac{Q_{max} K_L C_e}{[1 + K_L C_e]} \tag{1}$$

$$Q_e = K_f C_e^n \tag{2}$$

where Q_{max} is the maximum adsorption capacity, K_L is a parameter specific to the Langmuir isotherm, and K_f and n are parameters specific to the Freundlich isotherm. The linear isotherm is obtained when the exponential term n in the Freundlich isotherm equals 1:

$$Q_e = K_d C_e \tag{3}$$

where K_d is the slope of the linear isotherm, also known as the partition coefficient. Sometimes, the exponent n in (2) is written as $1/n$. In this review, we use the notation in (2), and have converted n accordingly, if needed. The K_d has been used as a key parameter to quantify pesticide risk and transport potential. It has been demonstrated in several studies that the simple linear isotherm model applies at environmentally relevant concentrations, and that pesticide movement in soils and K_d were inversely correlated (McCall et al. 1980, 1981; Bockting et al. 1993). A higher K_d value is indicative of strong affinity of the pesticide for soil, while a lower K_d indicates an increased risk of pesticide migration through soils.

The wide variability of K_d values for a given pesticide in different soils prompted the development of a single pesticide-specific soil-independent parameter to quantify adsorption potential. Since there is a strong correlation between K_d and the fraction of soil organic matter for many organic chemicals (Upchurch and Pierce 1958; Bailey and White 1964), the organic matter–water partition constant (K_{om}) has been used as a soil-independent partition constant to describe pesticide adsorption in soils (Lambert et al. 1965; Lambert 1968). Because of a similar positive correlation between pesticide adsorption and organic carbon content, and partly to avoid inconsistencies in usage of the factor for converting percent organic matter to percent organic carbon, the soil organic carbon–water partition constant (K_{oc}) is the preferred pesticide–soil adsorption constant (Hamaker and Thomson 1972). The K_{oc} is generally assumed to be constant for a given nonionic pesticide (Karickhoff et al. 1979; Chiou et al. 1979; McCall et al. 1980; Briggs 1981). The K_{oc} has also been used to calculate an index for pesticide leachability to groundwater (Gustafson 1989), and is widely used to determine K_d for soils in which the fraction of soil organic carbon (f_{oc}) is known:

$$K_d = K_{oc} f_{oc} \tag{4}$$

Application of the batch equilibrium technique to adsorption of CPF has generated a large adsorption database for a number of geosorbents since the 1970s, which are discussed in detail in the subsection below. Adsorption of CPF has been investigated in a range of agricultural soils, aquatic sediments, agricultural by-products and various organic substances, and clay minerals.

3.1 Adsorption to Soils

Owing to its nonpolar behavior, CPF exhibits strong partitioning from aqueous solutions to soils. In the reviewed studies, the adsorptive partitioning of CPF was experimentally determined for about 60 different soils from throughout the world and having a range of land uses (Table 3). The pH of the soils varied from strongly acidic (Laabs and Amelung 2005) to moderately alkaline (Valverde et al. 1992). Soil organic-matter content varied from very low in agricultural soils from China (Yu et al. 2006) to 75% in an organic soil from Canada (Sharom et al. 1980). The experiments were mostly conducted at temperatures in the range of 20–25°C and with a calcium chloride solution to enhance flocculation and separation by centrifugation. Reported times at which no significant change was observed in the liquid-phase concentration of the batch systems for soils ranged from 2 h (Felsot and Dahm 1979) to 72 h (Li et al. 2005), with most experiments being conducted at 24 h, which is consistent with regulatory recommendations (USEPA 1998c; OECD 2000). Some experiments lasted much longer to minimize the chances of nonequilibrium adsorption.

Experimental data was commonly modeled in terms of either a Freundlich isotherm and/or a linear isotherm. Consistent with the theory of adsorption of hydrophobic organic chemicals, CPF adsorption closely followed the linear

Table 3 Sorption of chlorpyrifos to soils

Soil/source	pH[a]	Sand (%wt)	Silt (%wt)	Clay (%wt)	OM[b] (%wt)	Temp.[c] (°C)	L:S[d] (mL:g)	C[e] (mg/L)	Model isotherm	K_f	n	K_d (L/kg)	K_{oc} (L/kg)	Reference
Sarpy fine sandy loam, IA	7.3 (5.7)	77	15	8	0.88 (0.51)	24 (2)	5:1	0.1–0.4	Freundlich	28.4	0.86	–[f]	–	Felsot and Dahm (1979)
Thurman loamy fine sand, IA	6.8 (6.1)	83	9	8	1.84 (1.07)	24 (2)	5:1	0.1–0.4	Freundlich	47	0.77	–	–	Felsot and Dahm (1979)
Clarion soil, IA	5.0 (21.0)	37	42	21	4.56 (2.64)	24 (2)	5:1	0.1–0.4	Freundlich	162	0.91	–	–	Felsot and Dahm (1979)
Harps soil, IA	7.3 (37.8)	21	55	24	6.55 (3.8)	24 (2)	5:1	0.1–0.4	Freundlich	397	0.98	–	–	Felsot and Dahm (1979)
Peat soil, IA	7.0 (77.3)	42	39	19	31.7 (18.4)	24 (2)	5:1	0.1–0.4	All sorbed	–	–	–	–	Felsot and Dahm (1979)
Organic soil	6.1 (–)	52	34	14	75.3 (–)	−(8)	200:1	<0.7	Freundlich	1,862	1.09	–	–	Sharom et al. (1980)
Beverly soil	6.8 (–)	56	30	14	2.5 (–)	−(4)	200:1	<0.7	Freundlich	118	0.99	118[g]	–	Sharom et al. (1980)
Plainfield sand	7.0 (–)	91.5	1.5	7	0.7 (–)	−(4)	200:1	<0.7	Freundlich	18	0.99	18[g]	–	Sharom et al. (1980)
Commerce soil, MS	6.7 (–)	38	48	14	0.68 (–)	−(12)	15:4	1.0	Linear	–	–	49.5	7,300	Swann et al. (1981)
Tracy soil, IN	6.2 (–)	56	30	14	1.12 (–)	−(12)	15:4	1.0	Linear	–	–	65.6	5,900	Swann et al. (1981)
Catlin soil, IL	6.2 (–)	12	56	32	2.01 (–)	−(12)	15:4	1.0	Linear	–	–	99.7	5,000	Swann et al. (1981)
EPA-14 reference soil, WV	4.3 (18.9)	2	64	34	−(0.48)	−(–)	5:1	–	Linear	–	–	83	17,292	Macalady and Wolfe (1985)
Tsukuba soil, Japan	6.5 (–)	18.9	26.3	54.8	−(4.24)	22 (24)	5:1	0.01–1.0	Linear	–	–	116.2	2,740	Kanazawa (1989)
Kanuma soil, Japan	5.7 (–)	23.1	15.4	61.5	−(1.35)	22 (24)	5:1	0.01–1.0	Linear	–	–	13.4	995	Kanazawa (1989)
Clermont soil, IN	–	22	66	12	−(0.7)	25 (24)	2:1	0–7.0	Linear	–	–	198	28,286	Kladivko et al. (1991)
Xerorthents soil, Spain	8.5 (13.1)	–	–	13.1	2.02 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	1,723	1.58	687	–	Valverde et al. (1992)
Xerocherpts soil, Spain	8.9 (11.9)	–	–	14.3	2.55 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	1,423	1.45	685	–	Valverde et al. (1992)
Rendolls soil, Spain	8.1 (6.3)	–	–	7.8	1.12 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	650	1.27	435	–	Valverde et al. (1992)
Fluvaquents soil, Spain	8.2 (10.0)	–		4.1	2.84 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	1,868	1.42	961	–	Valverde et al. (1992)
Haplardgids soil, Spain	8.7 (3.8)	–	–	5.9	0.64 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	1,488	1.83	471	–	Valverde et al. (1992)

(continued)

Table 3 (continued)

Soil/source	pH[a]	Sand (%wt)	Silt (%wt)	Clay (%wt)	OM[b] (%wt)	Temp.[c] (°C)	L:S[d] (mL:g)	C[e] (mg/L)	Model isotherm	K_f	n	K_d (L/kg)	K_{oc} (L/kg)	Reference
Camborthids soil, Spain	7.9 (4.4)	–	–	12.2	0.57 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	598	1.08	550	–	Valverde et al. (1992)
Xerochrepts soil, Spain	8.1 (25.6)	–	–	14.1	3.35 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	771	1.18	583	–	Valverde et al. (1992)
Xerofrluvents soil, Spain	8.0 (9.4)	–	–	11.7	1.57 (–)	30 (24)	400:1	0.125–0.5	Freundlich; linear	813	1.36	506	–	Valverde et al. (1992)
Sand, OH	7.4 (9.2)	–	–	–	–(0.29)	22 (24)	40:1	0.1–0.4	Freundlich linear	19	0.82	22.6	7,931	Spieszalski et al. (1994)
Wooster silt loam, OH	7.2 (9.4)	–	–	–	–(1.1)	22 (24)	200:1	0.1–0.4	Freundlich; linear	178	0.93	190.2	17,272	Spieszalski et al. (1994)
Wooster sandy loam, OH	7.0 (21.5)	–	–	–	–(3.41)	22 (24)	500:1	0.1–0.4	Freundlich; linear	832	0.84	1,036	30,381	Spieszalski et al. (1994)
Peat-amended clay loam (9:1(v/v)), OH	5.5 (25.5)	–	–	–	–(17.3)	22 (24)	800:1	0.1–0.4	Freundlich; linear	1,514	0.91	1,813	10,479	Spieszalski et al. (1994)
Typic Haplustox soil, Brazil	4.3 (12.5)	45.9	7.1	47	–(2.63)	21 (48)	5:1	0.1	Linear			191	7,247	Laabs et al. (2000)
Organic soil, The Netherlands	6.5 (–)	–	–	–	–(11.4)	25 (24)	147:1	0.36	Linear	–	–	130	1,100	Ramos et al. (2000)
Sandy soil, The Netherlands	8.3 (–)	–	–	–	–(1.3)	25 (24)	147:1	0.36	Linear	–	–	110	8,200	Ramos et al. (2000)
Typic haplustoxs soil, Brazil	4.3 (12.5)	45.9	7.1	47	–(2.63)	21 (48)	5:1	0.1	Linear	–	–	190.6	7,247	Laabs et al. (2000)
Toronto soil, IN	4.4 (11.2)	12	68	20	–(1.3)	23 (24)	200:1	0–0.35	Linear	–	–	90.5	696	Huang and Lee (2001)
Raub soil, IN	6.7 (23.0)	10	66	24	–(1.35)	23 (24)	200:1	0–0.35	Linear	–	–	88	652	Huang and Lee (2001)
Toronto soil + DOM (poultry waste), IN	–	12	68	20	–	23 (24)	200:1	0–0.35	Linear	–	–	76	–	Huang and Lee (2001)

Raub soil + DOM (poultry waste), IN		–	10	66	24	–	23 (24)	200:1	0.125–0.5	Linear	–	–	73	–	Huang and Lee (2001)
Toronto soil + swine waste, IN		–	12	68	20	–	23 (24)	200:1	0–0.35	Linear	–	–	68.7	–	Huang and Lee (2001)
Raub soil + swine waste, IN		–	10	66	24	–	23 (24)	200:1	0.125–0.5	Linear	–	–	57.3	–	Huang and Lee (2001)
Toronto soil + cow waste, IN		–	12	68	20	–	23 (24)	200:1	0–0.35	Linear	–	–	63	–	Huang and Lee (2001)
Raub soil + cow waste, IN		–	10	66	24	–	23 (24)	200:1	0.125–0.5	Linear	–	–	48	–	Huang and Lee (2001)
Toronto soil + humic acid, IN		–	12	68	20	–	23 (24)	200:1	0–0.35	Linear	–		51	–	Huang and Lee (2001)
Raub soil + humic acid, IN		–	10	66	24	–	23 (24)	200:1	0.125–0.5	Linear	–	–	42	–	Huang and Lee (2001)
Urrbrae soil (0–15 cm), Australia	6.5 (20.1)	–	–	18	−(2.3)	25 (16)	5:1	1–16	Linear	–	–	144.2	6,269	Baskaran et al. (2003)	
Urrbrae soil (15–30 cm), Australia	6.0 (23.6)	–	–	26.8	−(1.8)	25 (16)	5:1	1–16	Linear	–	–	96.1	5,338	Baskaran et al. (2003)	
Urrbrae soil (30–45 cm), Australia	6.6 (27.0)	–	–	32.6	−(1.2)	25 (16)	5:1	1–16	Linear	–	–	72.6	6,050	Baskaran et al. (2003)	
Urrbrae soil (45–60 cm), Australia	7.2 (26.5)	–	–	36.8	−(0.9)	25 (16)	5:1	1–16	Linear	–	–	43.1	4,788	Baskaran et al. (2003)	
Urrbrae soil (60–90 cm), Australia	7.7 (30.4)	–	–	41.3	−(0.6)	25 (16)	5:1	1–16	Linear	–	–	30.8	5,133	Baskaran et al. (2003)	

(continued)

Table 3 (continued)

Soil/source	pH[a]	Sand (%wt)	Silt (%wt)	Clay (%wt)	OM[b] (%wt)	Temp.[c] (°C)	L:S[d] (mL:g)	C[e] (mg/L)	Model isotherm	K_f	n	K_d (L/kg)	K_{oc} (L/kg)	Reference
Wiesenboden soil (0–15 cm), Australia	6.6 (19.4)	–	–	24.6	–(2.9)	25 (16)	5:1	1–16	Linear	–	–	209.6	7,227	Baskaran et al. (2003)
Wiesenboden soil (15–30 cm), Australia	6.3 (32.5)	–	–	29.5	–(2.0)	25 (16)	5:1	1–16	Linear	–	–	136.2	6,810	Baskaran et al. (2003)
Wiesenboden soil (30–45 cm), Australia	7.0 (36.4)	–	–	33.6	–(1.4)	25 (16)	5:1	1–16	Linear	–	–	101.3	7,235	Baskaran et al. (2003)
Wiesenboden soil (45–60 cm), Australia	7.8 (39.6)	–	–	36.8	–(1.0)	25 (16)	5:1	1–16	Linear	–	–	68.9	6,890	Baskaran et al. (2003)
Wiesenboden soil (60–90 cm), Australia	7.8 (42.5)	–	–	45.6	–(0.5)	25 (16)	5:1	1–16	Linear	–	–	40.3	8,060	Baskaran et al. (2003)
Golf sand, MA	–	–	–	–	–(0.48)	23 (72)	–	0.008–1.0	Freundlich	25	0.96	–	–	Li et al. (2005)
Golf sand + 60 mg/L Milorganite fertilizer extract, MA	–	–	–	–	–	23 (72)	–	0.008–1.0	Freundlich	11	0.98	–	–	Li et al. (2005)
Golf sand + 120 mg/L Milorganite fertilizer extract, MA	–	–	–	–	–	23 (72)	–	0.008–1.0	Freundlich	9	1.0	9	–	Li et al. (2005)
Golf sand + 240 mg/L Milorganite fertilizer extract, MA	–	–	–	–	–	23 (72)	–	0.008–1.0	Freundlich	8	0.99	8[g]	–	Li et al. (2005)

Golf sand + 60 mg/L NatureSafe fertilizer extract, MA		–	–	–	–	–	23 (72)	–	0.008–1.0	Freundlich	11	0.92	–	–	Li et al. (2005)
Golf sand + 120 mg/L NatureSafe fertilizer extract, MA		–	–	–	–	–	23 (72)	–	0.008–1.0	Freundlich	14	0.97	–	–	Li et al. (2005)
Ustox soil, Brazil	4.3 (12.5)	45.5	6.8	47.7	–(2.64)		22 (48)	5:1	0.04–2.0	Freundlich	98.2	0.86	189.6	7,180	Laabs and Amelung (2005)
Psamments soil, Brazil	4.0 (3.2)	94.4	0.8	4.8	–(0.73)		22 (48)	5:1	0.04–2.0	Freundlich	46.7	0.81	77.4	10,600	Laabs and Amelung (2005)
Zhejiang soil, China	6.8 (10.6)	21.5	57.9	20.6	3.05(–)		20 (24)	5:1	1–12	Freundlich	1,028	1.08	–	–	Yu et al. (2006)
Zhejiang soil, China	7.3 (11.7)	26.8	53	20.2	2.78(–)		20 (24)	5:1	1–12	Freundlich	960	1.12	–	–	Yu et al. (2006)
Zhejiang soil, China	4.6 (11.2)	16.7	20.5	62.8	0.81(–)		20 (24)	5:1	1–12	Freundlich	704	1.15	–	–	Yu et al. (2006)
Zhejiang soil, China	4.3 (10.6)	13.1	24.5	62.4	0.69(–)		20 (24)	5:1	1–12	Freundlich	508	1.1	–	–	Yu et al. (2006)
Zhejiang soil, China	7.5 (3.35)	22.4	71.3	6.3	0.31(–)		20 (24)	5:1	1–12	Freundlich	332	1.05	–	–	Yu et al. (2006)
Orchard soil, Thailand	4.4 (–)		–	–	–	2.22(1.29)	–(48)	2:1	0.07–0.35	Linear	–	–	108	8,364	Romyen et al. (2007)
San Joaquin Std soil, CA		–	–	–	–	1.37(0.79)	25 (16)	25:1	<1	Freundlich; linear	68	0.94	71	9,000	Rogers and Stringfellow (2009)
Sandy loam soil, Greece	8.5 (–)		–	–	–	–(0.9)	25 (4)	2:1	1–20	Linear	–	–	17	1,888	Kravvariti et al. (2010)
Soil + compost (50%), Greece	7.6 (–)		–	–	–	–(6.7)	25 (4)	2:1	1–20	Linear	–	–	746	9,816	Kravvariti et al. (2010)

[a]Numbers in parenthesis are cation exchange capacity of the soils in cmol/kg
[b]Organic matter content; numbers in parenthesis represent fraction of organic carbon in % wt
[c]Temperature; numbers in parenthesis represent time to reach equilibrium in hours
[d]Liquid to solid ratio
[e]Initial liquid phase concentration ranges of CPF applied
[f] "–" represents values not reported
[g]K_d was estimated from K_f when not reported and $n = 0.99$

isotherm for low concentrations typically found in the environment. Soil adsorption parameters reported for CPF varied even when soil properties were approximately similar. For studies in which data was fitted with the Freundlich model, adsorption isotherms showed both convex ($n<1$) and concave ($n>1$) shapes with values of n varying from 0.77 to 1.83. Excluding reports investigating the effect of dissolved organic matter (DOM), the Freundlich constant, K_f, spanned two orders of magnitude from 18 to 1,868 with a mean and median value of 653 and 553 ($n=28$), respectively. Similarly, when data was fitted to the linear isotherm, calculated K_d values varied from 13.4 L/kg in Japanese soil with little organic carbon (Kanazawa 1989) to 1,813 L/kg in a peat-blended clay loam from Ohio (Spieszalski et al. 1994). The mean and median K_d were 271 and 116 L/kg ($n=43$), respectively.

The K_d showed little correlation with soil pH, cation exchange capacity (CEC) and clay fraction, factors that are often reported to control pesticide adsorption (Fig. 1a–c). A similar lack of correlation between CPF adsorption and soil pH, CEC, clay content, and specific surface area has been reported in several studies (Macalady and Wolfe 1985; Valverde et al. 1992; Baskaran et al. 2003). However, K_d exhibited a strong linear positive correlation with organic carbon fraction ($p<0.0001$, $R^2=0.61$, $n=30$) (Fig. 1d). This observation confirms that partitioning of hydrophobic organic chemicals to soils is primarily mediated via physical partitioning to hydrophobic sites of soil organic matter (Seger and Maciel 2006). Other observations also highlighted the role of organic carbon in controlling CPF adsorption. Authors have commonly reported that K_d increased with increasing organic matter content (Baskaran et al. 2003; Yu et al. 2006). The K_d was higher in unoxidized soils versus oxidized soils (Moreale and van Bladel 1976; Wahid and Sethunathan 1978; Felsot and Dahm 1979; Moorman et al. 2001), and extreme organic matter content in soils sometimes led to complete removal of CPF from solution (Felsot and Dahm 1979). A similar strong correlation with soil organic carbon was observed for K_f ($R^2=0.68$; $p<0.0001$, $n=12$), but, like K_d, it showed no significant correlation with any of other reported soil properties.

A surprisingly high variation was observed for the soil organic carbon–water partition coefficient K_{oc}, although it is often assumed to be invariant with soil type. Reported K_{oc} values varied from 652 L/kg in a moderately organic (1.35% organic carbon) Indiana soil (Huang and Lee 2001) to 30,381 L/kg in a relatively high organic (3.41% organic carbon) Wooster sandy loam soil from Ohio (Spieszalski et al. 1994). The mean and median K_{oc} value from the reviewed data set were 8,163 and 7,227 L/kg ($n=33$), respectively.

3.2 *Adsorption to Aquatic Sediments*

Adsorption of CPF to aquatic sediments is of interest for predicting its fate in surface and groundwater, as well as for designing structural management strategies for pesticide capture, such as agricultural vegetated ditches, detention ponds, and constructed treatment wetlands. It also has implication for some unregulated agricultural activities, such as field inundation and the resulting drainage of irrigation

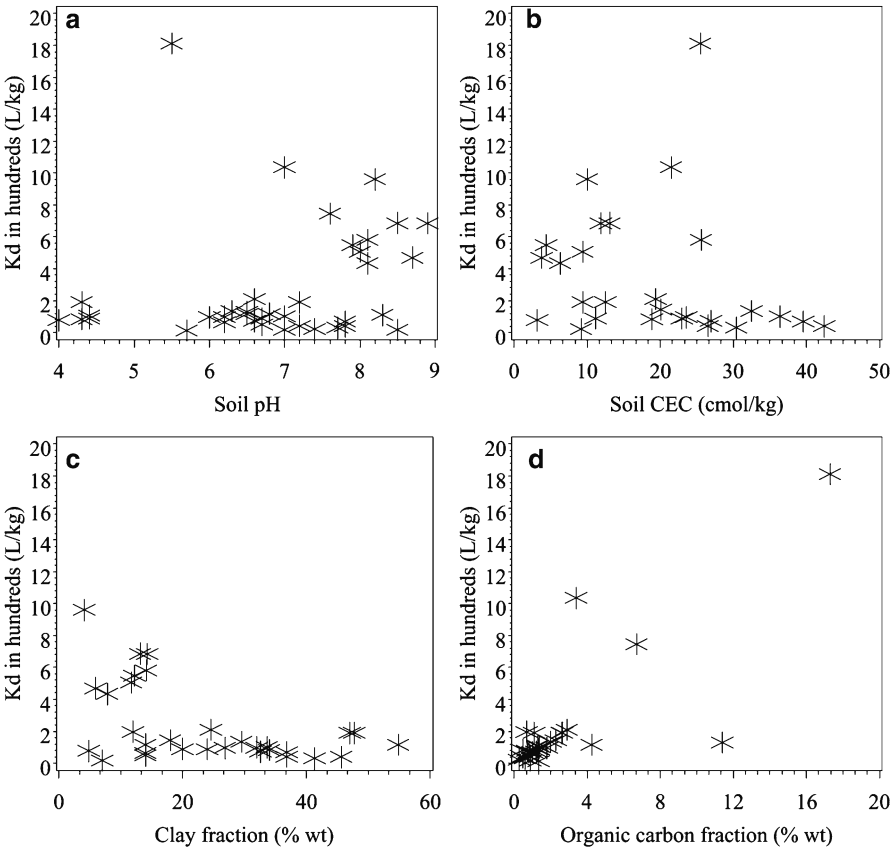


Fig. 1 Variation of linear partition coefficient (K_d) of chlorpyrifos with soil properties: (**a**) pH ($n=40$), (**b**) cation exchange capacity ($n=29$), (**c**) clay fraction ($n=33$), and (**d**) organic carbon fraction ($n=30$, $R^2=0.61$, $p<0.0001$). See Table 3 for data set

water to natural waterways. Cranberry bogs, in particular, receive CPF applications during cultivation when plants are intermittently inundated by water on a regular basis (Anderson and Davis 2000; Coots 2003). However, adsorptive retention of CPF by aquatic sediments has not been extensively studied. The few available studies indicated that CPF shows a relatively higher affinity for aquatic sediments than soils. The K_d values varied from 40 L/kg for a vegetated ditch sediment (Rogers and Stringfellow 2009) to 767 L/kg for nursery recycling pond sediment (Lu et al. 2006) (Table 4). As was the case for soils, adsorption generally followed a linear model yielding mean and median K_d of 385 and 403 L/kg. The corresponding mean and median K_{oc} were 13,439 and 15,500 L/kg, respectively. From among various aquatic sorbents tested, the highest K_{oc} value of 25,565 L/kg was reported for CPF adsorption to suspended sediment from a river system (Wu and Laird 2004). The strong association of CPF with suspended sediments presents a potential CPF migration route unique to aquatic environments and may explain reported detections of CPF in water wells and marine sediments (Readman et al. 1992; Tuncel et al. 2008).

Table 4 Sorption of chlorpyrifos to aquatic sediments

Sediment/source	pH[a]	Sand (%wt)	Silt (%wt)	Clay (%wt)	OM[b] (%wt)	Temp.[c] (°C)	L:S[d] (mL:g)	C[e] (mg/L)	Model isotherm	K_f	n	K_d (L/kg)	K_{oc} (L/kg)	Reference
Big Creek sediment	6.6 (–)	71	22	7	2.8 (–)	–(4)	200:1	<0.7	Freundlich	139	0.98	–	–[f]	Sharom et al. (1980)
Illinois River slough sediment (EPA-23)	7.1 (31.15)	17	69	14	–2.38	–	–	–	Linear	–	–	403	16,933	Macalady and Wolfe (1985)
Mississippi River sediment (EPA-26)	8.1 (20.86)	2	43	55	–1.48	–	–	–	Linear	–	–	307	20,743	Macalady and Wolfe (1985)
Haringvliet River sediment, The Netherlands	7.2 (–)	–	–	–	–5.4	25 (24)	147:1	0.36	Linear	–	–	160	3,000	Ramos et al. (2000)
Cedar River suspended sediment, IA	–	1	73	26	–1.84	20 (24)	100:1	0–0.1	Linear	–	–	470.4	25,565	Wu and Laird (2004)
Nursery recycling pond sediment, CA	6.9 (36.9)	15	50	35	6.08 (–)	–(6)	4:1	<0.04	Linear	–	–	546	15,500	Lu et al. (2006)
Nursery recycling pond sediment, CA	7.2 (56.3)	41	44	15	17.79 (–)	–(6)	4:1	<0.04	Linear	–	–	767	7,430	Lu et al. (2006)
Vegetated ditch sediment, CA	–	21	38	41	1.42 (0.82)	25 (16)	25:1	<1.0	Freundlich; Linear	40	0.95	40	4,900	Rogers and Stringfellow (2009)

[a]Numbers in parenthesis are cation exchange capacity of the soils in cmol/kg
[b]Organic matter content; numbers in parenthesis represent fraction of organic carbon in % wt.
[c]Temperature; numbers in parenthesis represent time to equilibrium in hours
[d]Liquid to solid ratio
[e]Initial liquid phase concentration ranges of CPF applied
[f] "–" values not reported

Storm events that cause soil or sediment erosion are major factors driving transport of CPF adsorbed to surface soils or suspended particles. In a study on CPF entering the Lourens River, South Africa, a mass flow rate of 1.8 g/h CPF from agricultural fields in a single rainfall event 2 months after pesticide application was measured, and all of the CPF was associated with suspended sediment (344 μg/kg) (Schulz et al. 2001). Similarly, Dabrowski et al. (2002) noted that CPF concentrations in suspended sediment were as high as 245 μg/kg following a rainfall event. The frequent occurrence of CPF in sediments from coastal areas of Mexico, Costa Rica, Nicaragua, and Panama was explained in terms of riverine discharge laden with CPF-rich suspended sediment from agricultural drainage (Readman et al. 1992). Chlorpyrifos was also detected in suspended particles of agricultural streams in Argentina at concentrations four times higher than concentrations that led to 100% mortality in freshwater shrimp (Jergentz et al. 2005).

Nevertheless, little is known about the fraction of CPF that is bound to suspended particles and enters surface waters, because water quality studies commonly only measure dissolved CPF in filtered water samples (Gilliom et al. 2006). Measurement of filtered samples has led to estimations that less than 1% of applied CPF reaches rivers (Larson et al. 1995; Kratzer et al. 2000). However, Fuhrer et al. (2004) noted that the fraction of strongly sorbing pesticides transported in runoff increased as the percentage of agricultural land irrigated with techniques known to cause soil erosion increased. Authors of previous studies also identified relationships between adsorption of hydrophobic pesticides and sediment particle-size distribution (Kay and Elrick 1967; Richardson and Epstein 1971). These observations suggest that the flux of CPF entering surface waters, along with suspended particles, is higher than commonly estimated and can adversely affect some key aquatic ecosystem compartments (Pionke and Chesters 1973; Karickhoff et al. 1979). For example, because sediment deposition is controlled by particle-size fractionation, suspended sediment and associated pesticides can accumulate in areas of intense biological activity and productivity, such as littoral zones or deltas, and thereby pose a long-term threat to aquatic ecosystems.

Because aquatic sediments are generally anaerobic and have a reduced redox potential, the organic matter they contain may have a stronger affinity for CPF than does oxidized terrestrial soils. This conclusion is supported by aquatic sediments having consistently higher K_d values with smaller variation (median, 403 L/kg; CV, 63%) than do soils (median, 116 L/kg; CV, 132%). However, because so few studies have addressed the adsorption of CPF to aquatic sediments, the characterization and distribution of sediment-associated CPF in aquatic environment remains speculative.

3.3 Adsorption to Organic Matter

Adsorption of CPF to various organic sorbents has been investigated by many researchers, and these sorbents have proven to be very efficient in removing the insecticide from aqueous solutions (Table 5). The environmental sorbents studied

Table 5 Sorption of chlorpyrifos to biomass and humic acids

Sorbent/source	OM[a] (%wt)	Temp.[b] (°C)	L:S[c] (mL:g)	C[d] (mg/L)	Model isotherm	K_f	n	K_d (L/kg)	K_{oc} (L/kg)	Reference
Turfgrass thatch	–(34.5)	22 (24)	2000:1	0.1–0.4	Freundlich; Linear	3,467	0.87	4,341	12,456	Spieszalski et al. (1994)
Aquatic macrophytes (*Chara globularis*)	36 (–)	15 (24)	12:1	–	Linear	–	–	2,126	–[e]	Crum et al. (1999)
Aquatic macrophytes (*Elodea nuttallii*)	86 (–)	15 (24)	12:1	–	Linear	–	–	1,660	–	Crum et al. (1999)
Aquatic macrophytes (*Lemna gibba*)	87 (–)	15 (24)	15:1	–	Linear	–	–	2,150	–	Crum et al. (1999)
Lagoon effluent (Poultry waste), IN	–	23 (24)	–	0.125–0.5	Linear	–	–	9,333	–	Huang and Lee (2001)
Lagoon effluent (Swine waste), IN	–(24.7)	23 (24)	–	0.125–0.5	Linear	–	–	11,000	–	Huang and Lee (2001)
Lagoon effluent (Cow waste), IN	–(22.7)	23 (24)	–	0.125–0.5	Linear	–	–	12,250	–	Huang and Lee (2001)
Humic acid	–(40)	23 (24)	–	0.125–0.5	Linear	–	–	19,250	–	Huang and Lee (2001)
Ca-humate	–(40)	20 (24)	100:1	0–0.1	Linear	–	–	–	–	Wu and Laird (2004)
Holm sawdust *Quercus rotundifolia*	–(32.2)	–	100:1	0.1–0.8	Freundlich	4,002	1.11	–	17,707[f]	Rodriguez-Cruz et al. (2007)
Oak sawdust *Quercus rubra*	–(38.5)	–	100:1	0.1–0.8	Freundlich	3,176	1.15	–	17,450[f]	Rodriguez-Cruz et al. (2007)
Beech sawdust *Fagus sylvatica*	–(38.1)	–	100:1	0.1–0.8	Freundlich	4,794	1.71	–	21,594[f]	Rodriguez-Cruz et al. (2007)
Walnut sawdust *Juglans nigra*	–(37.9)	–	100:1	0.1–0.8	Freundlich	3,702	1.67	–	16,600[f]	Rodriguez-Cruz et al. (2007)
Elm sawdust *Ulmus minor*	–(40.4)	–	100:1	0.1–0.8	Freundlich	5,911	1.33	–	21,973[f]	Rodriguez-Cruz et al. (2007)
Pine sawdust *Pinus sylvestris*	–(41.5)	–	100:1	0.1–0.8	Freundlich	6,559	1.18	–	26,881[f]	Rodriguez-Cruz et al. (2007)

Poplar sawdust *Populus sp*	–(38.4)	–	100:1	0.1–0.8	Freundlich	3,926	0.84	–	17,764[f]	Rodriguez-Cruz et al. (2007)
Plane sawdust *Platanus hibrida*	–(48)	–	100:1	0.1–0.8	Freundlich	3,297	0.98	–	17,171[f]	Rodriguez-Cruz et al. (2007)
Maple sawdust *Acer negundo*	–(46)	–	100:1	0.1–0.8	Freundlich	3,389	0.82	–	5,689[f]	Rodriguez-Cruz et al. (2007)
Coconut husk	78.62 (45.4)	- (48)	20:1	0.07–0.35	Linear	–	–	1,475	3,250	Romyen et al. (2007)
Peat moss	61.1 (35.44)	RT[g] (48)	20:1	0.07–0.35	Linear	–	–	3,371	9,513	Romyen et al. (2007)
Rice husk	85.2 (49.4)	RT (48)	20:1	0.07–0.35	Linear	–	–	1,317	2,665	Romyen et al. (2007)
Peanut shell	70.73 (41.61)	RT (48)	20:1	0.07–0.35	Linear	–	–	1,072	2,577	Romyen et al. (2007)
Cattail *Typhus sp.*	–	25 (16)	250:3	<1.0	Freundlich; Linear	2,600	1.02	2,600	–	Rogers and Stringfellow (2009)
Gray rush *Juncus patens*	–	25 (16)	250:3	<1.0	Freundlich; Linear	1,400	0.97	1,500	–	Rogers and Stringfellow (2009)
Rye grass *Lolium sp.*	–	25 (16)	250:3	<1.0	Freundlich; Linear	730	0.81	1,100	–	Rogers and Stringfellow (2009)
Wheat *Triticum aestivum*	–	25 (16)	250:3	<1.0	Freundlich; Linear	480	0.82	680	–	Rogers and Stringfellow (2009)
Alfalfa *Medicago sativa*	–	25 (16)	250:3	<1.0	Freundlich; Linear	470	0.84	640	–	Rogers and Stringfellow (2009)
Wastewater sludge	–	22 (24)	9:1	0–100	Linear	–	–	27,786	–	Thomas et al. (2009)

[a]Organic matter content; numbers in parenthesis represent organic carbon fraction in % weight
[b]Temperature; numbers in parenthesis represent time to equilibrium in hours
[c]Liquid to solid ratio
[d]Initial liquid phase concentration ranges of CPF applied
[e] "-" values not reported
[f]Partition coefficients normalized by fraction of lignin content
[g]RT: Room temperature

include aquatic plant biomass, sawdust of various woods, crop by-products, animal waste, and DOM. Because of their high organic carbon content, K_d values for CPF adsorption to these sorbents were higher than values for most organic soils, by an order of magnitude. In the case of aquatic plants, adsorption appeared to increase with increasing internal surface area and porosity of plant tissue (Rogers and Stringfellow 2009). Aquatic plants showed stronger affinity for CPF than did terrestrial plants, suggesting the potential of constructed treatment wetlands for removing CPF from contaminated agricultural runoff. In the case of sawdust, adsorption correlated significantly with soluble carbon and lignin content, but not with total organic carbon, suggesting that carbon quality plays a role in controlling CPF adsorption (Rodriguez-Cruz et al. 2007). Wastewater sludge resulted in extremely high K_d value of 27,786 L/kg (Thomas et al. 2009). Chlorpyrifos also adsorbed substantially to DOM with K_d values of 12,250 L/kg for DOM derived from cow waste and 19,250 L/kg for humic acid (Huang and Lee 2001).

The enhanced pesticide-sorbing capacity of biomass has led to the investigation of amending agricultural soils with organic matter to reduce pesticide leaching to groundwater. The results of several studies indicated that pesticide mobility was reduced and K_d values increased by 2–3 order of magnitude, when soils were amended with organic wastes; such wastes included municipal sewage sludge, animal manure, straw, composted domestic solid waste, and humic substances (Dao 1991; Guo et al. 1993; Arienzo et al. 1994; Johnson et al. 1997; Sánchez-Camazano et al. 1997). Nevertheless, because DOM substantially enhances solubility of organic solutes that resist solubilization in water (Chiou et al. 1986, 1987; Guetzloff and Rice 1996; Cho et al. 2002), there is concern that the dissolved component of organic matter used for amending soils could enhance pesticide mobility. Several studies have addressed this subject and the majority indicated that DOM significantly decreased adsorption and increased leaching potential of various pesticides via complexation and cotransport (Graber et al. 1995; Celis et al. 1998; Nelson et al. 1998; Cox et al. 2000), or by competing with pesticides for adsorption sites on the soil surfaces (Flores-Cespedes et al. 2002).

Dissolved organic matter also exhibited a negative impact on CPF sequestration by soil. When adsorption of CPF to soils was investigated in the presence of 70 mg/L of DOM derived from animal waste, K_d values decreased by 20–60%, with the largest reduction occurring in the presence of humic acid (Huang and Lee 2001). Also, the sorbing capacity of sand, treated with 60–120 mg/L DOM derived from organic fertilizer, decreased by 40–70% relative to untreated sand (Li et al. 2005). Column studies also indicated that more CPF leached when soil columns were treated with DOM. These results suggest that DOM from agricultural applications of organic fertilizers, animal wastes, and wastewater effluents may enhance CPF mobility, particularly in the subsurface environment. By contrast, the CPF sorbing capacity of clay colloids increased in the presence of DOM. The amount of CPF sorbed onto gibbsite and kaolinite increased three- to fourfold in the presence of 50 mg/L humic acid relative to adsorption without humic acid (van Emmerik et al. 2007). This observation was apparently due to formation of an organic coating on mineral surfaces by humic acid that provided hydrophobic sites to which CPF partitioned

from aqueous solution. Wu and Laird (2004) also reported that virtually all CPF sorbed when it was equilibrated with calcium-saturated humic acid. These observations suggest increased CPF migration potential when suspended clay colloids are present in concert with DOM.

3.4 Adsorption to Clay Minerals

Retention of CPF by inorganic mineral sorbents has also been investigated, but compared to soils and aquatic sediments, studies are limited (Table 6). Studies were generally conducted to identify sorbents suitable for removing CPF from contaminated waters (Suciu and Capri 2009), or to determine the effect of clay minerals on CPF hydrolysis (Wu and Laird 2002, 2004). Wu and Laird (2004) studied adsorption of CPF on six reference calcium-saturated smectites in aqueous suspension and reported that adsorption did not significantly correlate with cation exchange capacity, surface area, or surface charge density. They concluded that CPF adsorption was not governed by surface chemistry of inorganic minerals. Adsorption was, however, partly correlated with trace levels of organic carbon associated with the minerals. The K_d values were relatively higher (473–1,315 L/kg) in the Wu and Laird (2004) study, when compared to clayey soils and sediments having similar organic carbon content (Baskaran et al. 2003; Rogers and Stringfellow 2009). These results suggest that CPF may undergo preferential adsorption to the clay fractions in soils. It is notable that not all clay minerals have a strong affinity for CPF. Van Emmerik et al. (2007) studied the adsorption of CPF to Wyoming montmorillonite (SWy-2), Georgia kaolinite (KGa-2), and gibbsite. They reported that uptake by kaolinite and gibbsite was one-fifth of that of montmorillonite. Moreover, adsorption was independent of pH for all three minerals except for montmorillonite, where adsorption decreased with increasing pH. Modifying montmorillonite clay with cationic surfactant micelles did not significantly enhance adsorption (Suciu and Capri 2009). Diatomaceous earth reached rapid adsorption equilibrium (5 min) with CPF, yielding the Freundlich K_f value of $\mu g^{1-1/n} L^{1/n} kg^{-1}$ (Agdi et al. 2000). In general, the capacity of CPF to bind to mobile colloidal clay minerals may constitute an additional migration route from application sites to aquatic and subsurface environments (Flury 1996; Grolimund et al. 1996; Flury et al. 2002).

4 Adsorption by Modified Batch Equilibrium

Difficulties in resolving the temporal dynamics of adsorption processes, in particular the phenomenon known as aging, has necessitated modifications of the conventional batch equilibrium technique (Wauchope et al. 2002). Aging is a widely demonstrated but poorly understood occurrence in which adsorption increases when the pesticide resides in soils for longer durations (Pignatello et al.

Table 6 Sorption of chlorpyrifos to inorganic minerals

Minerals/source	OC[a] (%wt)	Temp.[b] (°C)	L:S[c] (mL:g)	C[d] (mg/L)	Model isotherm	K_f	n	K_d (L/kg)	Reference
Diatomaceous earth	–	4 (0.08)	200:1	10	Freundlich	227.1	0.35	–[e]	Agdi et al. (2000)
Floridin hectorite	0.9	20 (24)	100:1	0–0.1	Linear	–	–	1,315	Wu and Laird (2004)
Panther Creek beidellite	0.8	20 (24)	100:1	0–0.1	Linear	–	–	1,069	Wu and Laird (2004)
IMV saponite	0.4	20 (24)	100:1	0–0.1	Linear	–	–	1,132	Wu and Laird (2004)
Amory montmorillonite	0.5	20 (24)	100:1	0–0.1	Linear	–	–	473	Wu and Laird (2004)
Polkville montmorillonite	0.4	20 (24)	100:1	0–0.1	Linear	–	–	89	Wu and Laird (2004)
Otay white montmorillonite	0.3	20 (24)	100:1	0–0.1	Linear	–	–	45	Wu and Laird (2004)
Cloisite 20A Micelle-clay	–	20 (24)	200:1	5–35	Freundlich	5.91	0.03	–	Suciu and Capri (2009)
Cloisite 30B Micelle-clay	–	20 (24)	200:1	5–35	Freundlich	7.76	0.05	–	Suciu and Capri (2009)

[a]Organic carbon
[b]Temperature; numbers in parenthesis represent time to equilibrium in hours
[c]Liquid to solid ratio
[d]Initial liquid phase concentration ranges of CPF applied
[e] "–" values not reported

1993; Pignatello and Xing 1996; Xing and Pignatello 1996; Regitano et al. 2006). The primary modification to the method for studying aging is holding the water–pesticides–soil batch system for a longer time before conducting shaking, centrifugation and phase separation (Sharer et al. 2003). Others have applied pesticide to the soil weeks or months before conducting the batch equilibrium technique in which treated soils were agitated with a solute-free solution (Regitano et al. 2006; Cabrera et al. 2008).

There are few studies in which aging effects on adsorption of CPF were investigated. Bondarenko and Gan (2004) incubated sediments from San Diego Creek and Bonita Creek, CA, spiked with CPF at concentration of 10 mg/kg for varying durations. Adsorption K_d increased by 1–2 orders of magnitude between day 0 and 28. The K_{oc} on day 28 was 300,000 L/kg for San Diego Creek sediment and 100,000 L/kg for Bonita Creek sediment, both of which are much higher than values reported from conventional batch system for organic soils and sediments. This finding suggests that potential transport of CPF would be over predicted if partition coefficients from conventional batch equilibrium methods are used to predict off-site mobility in systems that receive water long after the pesticide is applied, as is often the case in field applications. However, in contrast to the Bondarenko and Gan (2004) study, others have observed only a minimal effect of aging on adsorption of nonpolar chemicals (Xing and Pignatello 1996; Sharer et al. 2003). Cryer (2005) used a modified batch system, designed to accommodate a small column, to evaluate the effect of different formulation additives on adsorption kinetic of CPF. Chlorpyrifos was dominantly sorbed during the first 6 h. However, additives reduced sorptivity and caused a delay in time to equilibrium, suggesting that adsorption data derived from pure AI may under predict mobility of formulated CPF products manufactured for field applications and that additives may enhance the aging effect. In general, the wide range of time to equilibrium reported for CPF (5 min to 72 h) suggests that adsorption kinetics and the effect of aging on adsorption are less dependent on the chemical behavior of pure organic compounds than the physicochemical properties of the sorbents and formulation types.

5 Adsorption by Chromatography

The painstaking nature of the batch equilibrium method has led investigators to develop other experimental alternatives for estimating pesticide adsorption parameters, among which chromatography methods are most notable. These methods include soil column chromatography, soil thin-layer chromatography, and reverse-phase high-performance liquid chromatography. These methods primarily depend on the determination of relative mobility (distance) or retardation (time) of the solute with respect to that of a conservative tracer or solvent front within the solid phase of the chromatography column. A review of CPF adsorption data, based on these chromatography methods from peer-reviewed sources, is presented below.

5.1 Soil Column Chromatography

The use of flow-through columns for studying adsorption behavior of pesticides in water/soil systems dates back to the introduction of synthetic pesticides in the 1950s (Ogle and Warren 1954; Harris 1964, 1966, 1967). Early efforts focused on assessing leaching capacity of pesticides. Lambert (1967) was among the first to suggest estimating adsorptive property of pesticides by treating movement of herbicides through soils in a manner analogous to the movement of materials through chromatographic columns. Soil column chromatography is now commonly used to assess adsorption properties of various agrochemicals under variable drainage regimes and water saturation conditions (Relyea 1982; van Genuchten and Wierenga 1986; Pignatello et al. 1993; USEPA 1999; Pignatello 2000). To conduct soil column chromatography measurements, a known concentration of a solute in water is applied along with a nonreactive tracer either as a step or pulse input to a column packed with soil of known bulk density and porosity. Effluent is collected and evaluated for the solute and tracer concentration as a function of time (Relyea 1982; USEPA 1999; Levenspiel 1999). The adsorption coefficient, K_d (L/kg), can then be calculated as:

$$K_d = \left(\frac{n}{\rho_b}\right)\left(\frac{v_t}{v_s} - 1\right) \tag{5}$$

where v_t and v_s are the mean velocities (m/s) of the tracer and solute, respectively, n is soil porosity, and ρ_b (kg/m^3) is soil bulk density. The ratio v_t and v_s is also known as the retardation coefficient. Tracer and solute velocities are calculated from breakthrough curves by plotting column effluent concentrations versus pore volume. A key advantage of soil column chromatography over the batch equilibrium technique is its ability to simulate natural systems. If minimally disturbed soil cores are used, column-derived adsorption data can account for such factors as the effects of colloids, hydrodynamic dispersion, and natural heterogeneity of soils. However, we could not find any data in which this method was applied to determinate the adsorption parameters for CPF.

5.2 Soil Thin-Layer Chromatography

Soil thin-layer chromatography, in which soil replaces silica-gel or other sorbents used in conventional thin-layer chromatography, was developed by Helling and Turner (1968). They employed this method to qualitatively evaluate adsorption properties of pesticides using the mobility factor (R_f), the ratio of solute migration distance in soil thin-layer plates to that of the solvent front. Guidelines for the method have been published by SETAC (Lynch 1995) and the US EPA (USEPA 1998b). Soil thin-layer chromatography is prepared from thin-layer chromatography glass plates and a soil slurry upon which the solute is spotted. The chromatogram is

then developed by ascending chromatography in a conventional thin layer chromatography apparatus. The distance traveled by the test chemical can be determined by radioautography (radiolabeled chemicals) or by sectioning the soil plates into small segments, which are then separately scrapped off to extract and analyze for the compound of interest using applicable methods. R_f is calculated as:

$$R_f = \frac{\sum d_n C_n}{d_w \sum C_n} \tag{6}$$

where C_n (μg/L) is sorbate concentration on the nth segment of the plate, d_n (m) and d_w (m) are the distance of the nth segment and the migration distance of the water front, respectively, both measured from a reference line. Soil thin-layer chromatography is not recommended for use with water repelling organic soils, especially when the test substance solubility is lower than <50 mg/L (Agriculture Canada et al. 1987).

Application of soil thin-layer chromatography to the evaluation of CPF adsorption has produced conflicting results. R_f values ranging from 0.15 to 0.28 were obtained in three different soils from Zhejiang Province, China (Li et al. 2007). R_f values negatively correlated with fraction of organic matter, which varied from 0.415 to 2.56%, pH, CEC, and clay content. However, an investigation of CPF movement in three soils from U.P. Province, India, yielded R_f values ranging from 0.25 to 0.325 that did not correlate with soil properties (Singh and Kumar 2000). Measurement of CPF adsorption with soil thin-layer chromatography by Somasundaram et al. (1991) noted extremely low R_f values of ranging from 0 to 0.02 in six different soils from Iowa with organic matter content varying from 0.7 to 6.4%. Evidence from other studies also indicated that soil thin-layer chromatography may not be suitable for studying adsorption of CPF in soils. Mohammad et al. (2001) investigated mobility of pesticides using soil thin-layer chromatography in which the solid phase was soil or soil amended with sorbents such as silica gel and cellulose. Either single or binary mobile phases were used and were composed of pure water, aqueous salt solutions or nonaqueous solvents. R_f values for CPF were zero for all amended and unamended soils, except when 0.1 M of aqueous sodium chloride (R_f=0.92) or 0.5 M of aqueous ammonium sulfate (R_f=0.95) was used as a solvent. The CPF migrated further in soil-free silica gel (R_f=0.71–0.95), soil-free cellulose (R_f=0.95–0.97), alumina (R_f=0.95), and diatomaceous earth (R_f=0.62–0.90) with aqueous salt solutions.

5.3 *Reverse-Phase Chromatography*

The possibility of estimating adsorption parameters by using reverse-phase high-performance liquid chromatography (RP-HPLC) was first suggested by McCall (1975) and Carlson et al. (1975). They demonstrated correlations between octanol–water partition coefficients and the chromatography retention time for organic compounds, assuming that the partitioning of a test substance on column packing material is

Table 7 Application of an RP-HPLC method for determination of chlorpyrifos K_{oc} (L/kg) using C_{18} packed columns

R_t (min)	a[a]	b[a]	n[2]	R^2	K_{oc}	Reference
15.01	3.076	0.392	9	0.89	10,258	Swann et al. (1981)
10.86	3.446	0.447	9	0.96	10,362	Swann et al. (1983)
42.66	2.352	0.705	15	0.69	34,578	Kanazawa (1989)

[a]"a" and "b" are regression coefficients based on the Collander equation
[b]Number of compounds used to develop the regression equation

proportional to its partitioning to soil organic carbon. This was confirmed later by Veith et al. (1979) for various organic chemicals including pesticides. Suggested packing materials for RP-HPLC columns included long-chain hydrocarbons (Veith et al. 1979) and octadecyl or cyanopropyl silica-gel containing lipophilic and polar moieties (Vowles and Mantourab 1987; Hodson and Williams 1988). Soil components such as humic acid and clay have also been used for column packing (Kördel et al. 1995).

Some authors have reported a correlation between K_{oc} (L/kg) values obtained from the batch techniques and HPLC retention times (t_r, min) that follow the Collander equation (Collander 1951):

$$\log K_{oc} = a \log t_r + b \tag{7}$$

where a and b fitting parameters. Swann et al. (1981) used both the batch technique and RP-HPLC to determine the adsorption parameters for nine pesticides, including CPF. The predicted K_{oc} for CPF was 10,258 L/kg. The authors of other studies that used the RP-HPLC methods reported variable K_{oc} values for CPF ranging from around 10,300 to 34,600 L/kg (Table 7). The large range of reported retention times and predicted K_{oc} values for CPF likely result from variations in chromatography operational conditions, including the selection of the mobile phase. The dependency of the RP-HPLC method upon column type and chromatography analytical conditions also makes comparison between experimental results difficult.

6 Adsorption by Nonexperimental Approaches

With an increasing need to prescreen numerous chemicals for potential environmental impact, the necessity of having rapid, reproducible, and simple techniques for estimating pesticide adsorption coefficients, without using laborious and expensive experimental approaches, is well recognized (Sabljic 1984; Briggs et al. 1990). One approach that has gained considerable research attention is the indirect estimation of adsorption parameters that are based on the chemical properties of pesticides. Several investigators have developed linear regression equations, commonly known as quantitative structural activity–property relationship models, which can predict K_{oc} values of test chemicals from their chemical properties. This section describes peer-reviewed data related to the estimation of K_{oc} for CPF, based on key properties such as water solubility, octanol–water partition coefficient, and topological structures.

6.1 Estimation from Solubility and K_{ow}

Recognition of the correlations between K_{ow}, bioconcentration factor and water solubility of hydrophobic chemicals in the 1970s led to the hypothesis that similar correlations exist between K_{oc}, K_{ow}, and water solubility for pesticides (Briggs 1973; Carringer et al. 1975). The physical explanation provided was that adsorption of nonionic organic compounds is a simple partitioning of the solute between the aqueous phase and the hydrophobic component of the soil. This logic led to development of a range of regression equations that predict K_{oc} values of various organic pesticides from their K_{ow} or water solubility, according to the log–log Collander relationship (Weber 1972; Briggs 1973; Carringer et al. 1975; Chiou et al. 1977; Karickhoff et al. 1979; Chiou et al. 1979; Briggs 1981; Swann et al. 1983). Class-specific regression equations were developed in later studies to improve their predictive capability (Mingelgrin and Gerstl 1983; Gerstl 1990).

Although predicting adsorption properties of pesticides from K_{ow} and water solubility is a compelling approach because of its simplicity, the approach is unreliable because identifying the appropriate equation and obtaining accurate measurements of solubility and K_{ow} are difficult. The literature is replete with regression equations relating K_{oc} values to water solubility or K_{ow} values (Table 8). Hence, identification of an appropriate equation for predicting the K_{oc} value of a specific compound is often subject to speculation (Gawlik et al. 1997). Many K_{ow} equations are developed using databases obtained from dominantly hydrophobic pesticides and thus can be candidates for predicting CPF adsorption. Reliable prediction depends on accurate measurement of K_{ow} and water solubility, and it is common to find different values for the same compound (Sabljic 1987). When these equations are used to estimate K_{oc} values that are based on reported ranges for water solubility (0.3–1.2 mg/L) and log of K_{ow} (4.96–5.267) values for CPF (see Table 1), estimates of K_{oc} range over orders of magnitude, even when the equations were developed for organophosphorus pesticides (Table 8, column 6). Equations that used K_{ow} as a dependent variable predicted K_{oc} values that bracketed those measured experimentally (by the batch-equilibrium technique) for soils, aquatic sediments, organic matter and clay minerals (see Tables 6). Regression equations that depended on water solubility generally did not capture higher experimental K_{oc} values. Gerstl (1990) also noted that K_{ow} was a better predictor than was water solubility.

6.2 Estimation from Topological Structures

With water solubility or K_{ow} values being insufficient to accurately estimate adsorption, investigators have developed a group of regression equations that predict adsorption properties based on the topological structure of a given chemical (Randic 1975; Murray et al. 1975). Topological indices that depend on branching, cyclization, unsaturation, and heteroatom content have also been used as predictors of K_{oc}. Of particular interest is the first-order simple molecular connectivity index that is

Table 8 Estimation of chlorpyrifos K_{oc} (L/kg) from water solubility (WS) and K_{ow}

Regression equation	n[a]	R^2	WS unit[b]	Compounds[c]	K_{oc}[d]	Reference
$\log K_{oc} = -0.550 \log WS + 3.64$	106	0.71	mg/L	Pesticides	4,101–8,464	Kenaga (1980)
$\log K_{oc} = -0.561 \log WS + 3.67$	7	0.87	μmol/L	Pesticides	2,438–5,105	Gerstl and Mingelgrin (1984)
$\log K_{oc} = -0.460 \log WS + 3.79$	10	0.8	μmol/L	Pesticides	3,614–6,624	Kawamoto and Urano (1989)
$\log K_{oc} = -0.356 \log WS + 3.01$	15	0.79	μg/mL	Pesticides	983–1,571	Kanazawa (1989)
$\log K_{oc} = -0.508 \log WS + 0.953$	419	0.76	mol/L	Mostly pesticides	5,556–10,849	Gerstl (1990)
$\log K_{oc} = -0.609 \log WS + 0.564$	35	0.79	mol/L	Organophosphates	8,144–18,165	Gerstl (1990)
$\log K_{oc} = 1.00 \log K_{ow} - 0.21$	10	1	–	Pesticides	56,234–114,025	Karickhoff et al. (1979)
$\log K_{oc} = 0.544 \log K_{ow} + 1.377$	45	0.74	–	Pesticides	11,892–17,468	Kenaga (1980)
$\log K_{oc} = 0.52 \log K_{ow} + 0.88$	105	0.95	–	Pesticides	2,879–4,158	Briggs (1981)
$\log K_{oc} = 0.58 \log K_{ow} + 0.457$	7	0.94	–	Organophosphates	2,157–3,250	Briggs (1981)
$\log K_{oc} = 0.87 \log K_{ow} - 0.43$	17	0.73	–	Pesticides	7,677–14,200	Mingelgrin and Gerstl (1983)
$\log K_{oc} = 0.72 \log K_{ow} + 0.27$	17	0.73	–	Pesticides	6,937–11,541	Gerstl and Mingelgrin (1984)
$\log K_{oc} = 0.64 \log K_{ow} + 1.14$	10	0.87	–	Pesticides	20,625–32,423	Kawamoto and Urano (1989)
$\log K_{oc} = 0.689 \log K_{ow} + 0.53$	–	0.67	–	Organophosphates	8,860–14,420	Gerstl (1990)
$\log K_{oc} = 0.679 \log K_{ow} + 0.663$	–	0.83	–	Heterogeneous	10,736–17,350	Gerstl (1990)
$\log K_{oc} = 0.47 \log K_{ow} + 1.09$	216	0.681	–	Pesticides	2,638–3,677	Sabljic et al. (1995)
$\log K_{oc} = 0.49 \log K_{ow} + 1.17$	41	0.73	–	Organophosphates	3,985–5,634	Sabljic et al. (1995)
$\log K_{oc} = 0.621 \log K_{ow} + 0.827$	21	0.77	–	Pesticides	8,075–12,526	Szabó et al. (1999)

[a]No. of compounds used to develop the regression equation
[b]Solubility unit
[c]Chemical type used to develop the regression equation
[d]Predicted CPF K_{oc} (L/kg) for the reported solubility and K_{ow} range in Table 1

calculated from the number of adjacent nonhydrogen atoms to which each atom is bonded. Molecular connectivity index equations have been reported as better predictors of K_{oc} for hydrophobic pesticides, compared to equations based on K_{ow} and/or water solubility (Sabljic 1984, 1987; Meylan et al. 1992). Further development of this approach has resulted in formulation of various forms of regression equations that rely on diverse chemical descriptors as reviewed by Gawlik et al. (1997).

Application of this method for predicting the K_{oc} of CPF yielded a fairly tight range of values from ~2,500 to 5,000 L/kg (Tao et al. 1999; Schuurmann et al. 2006; Gonzalez et al. 2006). We calculated K_{oc} of 7,283 L/kg from the recent version of PCKOCWIN (USEPA 2011), a computer program previously developed by Meylan et al. (1992) that uses the molecular connectivity index approach. These values are in good agreement with K_{oc} values determined experimentally for soils having low organic carbon content. Unlike predictor equations developed from water solubility and K_{ow}, topological indices are relatively invariant, and the use of large databases and more molecular descriptors can reduce prediction uncertainty (Nguyen and Do 2000; Schuurmann et al. 2006). The observed variation in predicted K_{oc} values that rely on a topological approach is likely due to the variable number of molecular descriptors used in each model. However, the large variation in experimental K_{oc} data used for developing topological models will undoubtedly limit the use of these indices as accurate predictors of pesticide adsorption coefficients, such as those for CPF. The approach also falls short of predicting higher K_{oc} values that were experimentally measured in organic soils.

7 Desorption

The fate and environmental impact of soil- and sediment-bound pesticides, to a large extent, are dependent upon the rate at which the pesticide returns to the aqueous phase (Gerstl 1990; Doucette 2003). Thus, the capacity to predict their transport and environmental impact relies heavily on understanding desorption characteristics. Desorption processes are also useful in determining remediation criteria for contaminated sediments (Chen et al. 2002). With 10% of aquatic sediments being contaminated in the USA alone (USEPA 1998a), selection of sediment remediation strategy, assessment of exposure risk, and development of quality criteria for waters overlying contaminated sediments are primarily dependent upon an understanding of the desorption process. Unfortunately, compared to adsorption, desorption of CPF has not been extensively studied.

Two approaches are generally followed when a batch equilibrium technique is used to determine desorption parameters of a pesticide from contaminated sediments or soils: consecutive desorption and serial dilution. In consecutive desorption, a known volume of solute-free percolation solution is added to a known amount of contaminated soil of known sorbate concentration in which adsorption had previously occurred. After agitation to reach equilibrium, the liquid phase is removed and replaced by an equal volume of solute-free solution. The process is repeated

several times. After measuring the liquid phase concentration, the amount of desorbed solute is determined by mass balance. In serial dilution, a known amount of soil or sediment to which a known concentration of sorbate partitioned receives a range of volumes of solute-free percolation solutions to cause various levels of dilution in contaminant concentration. After agitation to reach equilibrium followed by phase separation, the liquid concentration is measured to determine solute desorption (Bowman and Sans 1985a).

Regardless of the desorption technique used, once the phase concentrations are determined, isotherms, commonly known as single-point desorption isotherms, can be developed for each desorption step by plotting liquid-phase equilibrium concentration versus solid phase equilibrium concentration. Presentation of desorption data using single-point desorption isotherms has been criticized for not properly reflecting the amount of desorbed compound. Instead, plotting a family of curves that join successive desorption steps for each concentration level has been suggested (Bowman and Sans 1985b). Unless adsorption is completely reversible, partition coefficients obtained from single-point desorption isotherms are greater than partition coefficients from adsorption.

Only a handful of studies were found that were related to CPF desorption (Table 9). In a study of clay colloids, CPF desorption was independent of surface chemistry and the desorption K_d generally exceeded adsorption K_d and increased with each subsequent desorption equilibration (Wu and Laird 2004). The amount of CPF that desorbed in five desorption steps from a range of minerals varied from 5 to 78% and did not correlate with organic carbon content. By contrast, desorption of CPF from soils is substantially less, usually <7% (Laabs and Amelung 2005; Wu and Laird 2004). Yu et al. (2006) studied CPF desorption from soils and the single-point first-step desorption isotherms were fitted with the Freundlich isotherm yielding K_f values that varied from 476 to 1,262 L/kg. Compared to the adsorption Freundlich K_f values, single-point first-step desorption K_f values increased by 8–45%. The K_f values correlated negatively with soil organic matter content, pointing out the importance of organic carbon as a retardant against desorption. Felsot and Dahm (1979) reported a similar result; they noted that pesticide adsorption to low-carbon oxidized soils was more reversible than from carbon-rich unoxidized soils. Similarly, Gebremariam (2011) noted that CPF showed strong adsorption hysteresis. Desorption K_d values were twice those of adsorption K_d values, after four desorption steps for eight different soils and aquatic sediments tested by the batch equilibrium technique. Desorption was higher in samples that had lower organic matter content.

In a number of studies, pesticide desorption was also strongly influenced by aging (McCall and Agin 1985; Scribner et al. 1992; Johnson et al. 2001; Sharer et al. 2003; Walker et al. 2005). Aging effects on desorption are commonly studied via sequential extraction methods, whereby contaminated soils or sediments are extracted first with aqueous salt solution and then with organic solvents. This approach has demonstrated that the proportion of chemicals that are water-extractable from aged treatments are considerably lower than from fresh treatments. The amount of a pesticide that could be leached from freshly treated laboratory soil

Table 9 Desorption of chlorpyrifos

Sorbent/source	OM[a] (%wt)	Temp.[b] (°C)	Model isotherm	K_{des}[c]	n[d]	Reference
Zhejiang soil (0–10 cm), China	3.05 (–)	20(24)	Freundlich	1,262	1.081	Yu et al. (2006)
Zhejiang soil (0–10 cm), China	2.78 (–)	20(24)	Freundlich	1,045	1.123	Yu et al. (2006)
Zhejiang soil (0–10 cm), China	0.81 (–)	20(24)	Freundlich	894	1.134	Yu et al. (2006)
Zhejiang soil (0–10 cm), China	0.69 (–)	20(24)	Freundlich	689	1.107	Yu et al. (2006)
Zhejiang soil (0–10 cm), China	0.31 (–)	20(24)	Freundlich	476	1.063	Yu et al. (2006)
Hectorite montmorillonite	–(0.9)	23(24)	Linear	1,224–6,846	–[e]	Wu and Laird (2004)
Panther montmorillonite	–(0.8)	23(24)	Linear	1,046–1,796	–	Wu and Laird (2004)
Saponite montmorillonite	–(0.4)	23(24)	Linear	1,099–2,857	–	Wu and Laird (2004)
Amory montmorillonite	–(0.5)	23(24)	Linear	476–1,245	–	Wu and Laird (2004)
Polkville montmorillonite	–(0.4)	23(24)	Linear	80–105	–	Wu and Laird (2004)
Otay montmorillonite	–(0.3)	23(24)	Linear	45–162	–	Wu and Laird (2004)
Ustox agricultural soil, Brazil	–(2.64)	22(24)	Linear	228.6		Laabs and Amelung (2005)
Psamments agricultural soil, Brazil	–(0.73)	22(24)	Linear	92.7		Laabs and Amelung (2005)
Grayland cranberry field soil, WA	–(2.9)	21(2)	Linear	123.3	–	Gebremariam et al. (2011)
Long Beach cranberry field soil, WA	–(2.4)	21(2)	Linear	88	–	Gebremariam et al. (2011)
Moscow (bulrush wetland) sediment, ID	–(2.1)	21(2)	Linear	84.3	–	Gebremariam et al. (2011)
Moscow (cattail wetland) sediment, ID	–(1.8)	21(2)	Linear	63.6	–	Gebremariam et al. (2011)
Paradise Creek sediment, ID	–(1.6)	21(2)	Linear	73.7	–	Gebremariam et al. (2011)
Vegetated field strip sediment, WA	–(1.2)	21(2)	Linear	45	–	Gebremariam et al. (2011)
Sunnyside (cattail wetland) sediment, WA	–(0.9)	21(2)	Linear	53.7	–	Gebremariam et al. (2011)
Spring Valley Reservoir sediment, ID	–(0.6)	21(2)	Linear	35.2	–	Gebremariam et al. (2011)

[a]Organic matter content; numbers in parenthesis represent organic carbon fraction in % weight
[b]Temperature; numbers in parenthesis represent time to equilibrium in hours
[c]Linear (K_d) or Freundlich (K_f) partition coefficient for first-step single-point desorption isotherm
[d]Freundlich exponent term
[e] "–" values not reported

columns (Pignatello et al. 1993) or intercepted in runoff from freshly treated fields (Louchart and Voltz 2007) exceeded the amounts, respectively, leached or intercepted from aged treatments. Studies in which aging effects on CPF desorption investigated, however, produced conflicting observations. Laabs and Amelung (2005) reported, based on a field study in which CPF was applied to agricultural soils, that water-extractable CPF decreased with time after application, and that K_{oc} increased two- to three-fold over 80 days. By contrast, Ciglasch et al. (2008) reported that water extractable CPF reached equilibrium within 24 h and did not exhibit aging for over 80 days, when applied to a tropical fruit orchard soils. Discrepancies may result from differences in physicochemical properties of the experimental soils as well as experimental conditions.

8 Variation in Adsorption Partition Coefficients

The impact of soil organic carbon on pesticide adsorption was well recognized early in the 1950s (Upchurch and Pierce 1958; Wade 1954), and there is now overwhelming evidence that it is a critically important parameter for controlling adsorption of hydrophobic pesticides (Boivina et al. 2005; Farenhorst 2006; Wauchope et al. 2002). In general, the adsorption of hydrophobic pesticides to soils increases with increasing organic carbon content, while desorption decreases (Chiou et al. 1983, 1985; Felsot and Dahm 1979). As noted earlier, researchers have suggested adding organic carbon to soils as a strategy for increasing pesticide retention (Barriuso et al. 1992; Iglesias-Jimenez et al. 1997; Aslam et al. 2009). Nevertheless, the relationship between pesticide adsorption and soil organic carbon is not uniform, even for the same chemical.

Based on a comprehensive review of published data (Table 3), adsorption parameters for CPF show a great deal of variability. Excluding DOM-amended experiments, the coefficient of variation associated with K_d was 132% ($n=40$). Normalizing K_d with the soil organic fraction decreased the coefficient of variation to 86% ($n=30$), which is still higher than the acceptable range of 20–50% variation of K_{oc} for any specific chemical (ASTM 1987). The fact that K_{oc} values ranged over two orders of magnitude indicates that factors other than organic content play a role in CPF adsorption to soil. In addition, values of K_{oc} for CPF were sometimes different for soils having an approximately equal fraction of organic carbon (Rogers and Stringfellow 2009), or for soils sampled from the same site (Baskaran et al. 2003). In some cases, K_d values for CPF did not correlate with organic matter content (Valverde et al. 1992). These observations contradict the long-held notion that K_{oc} is a credible soil-independent universal constant. They also undermine the common practice of using K_{oc} for predicting K_d in the absence of experimental data (Karickhoff 1981; Schwarzenbach and Westall 1981).

The practice of predicting K_d from K_{oc} is based on the unsubstantiated assumption that organic carbon fraction is the sole factor controlling adsorption of organic pesticides. But, in the reviewed data set of CPF adsorption, the organic carbon

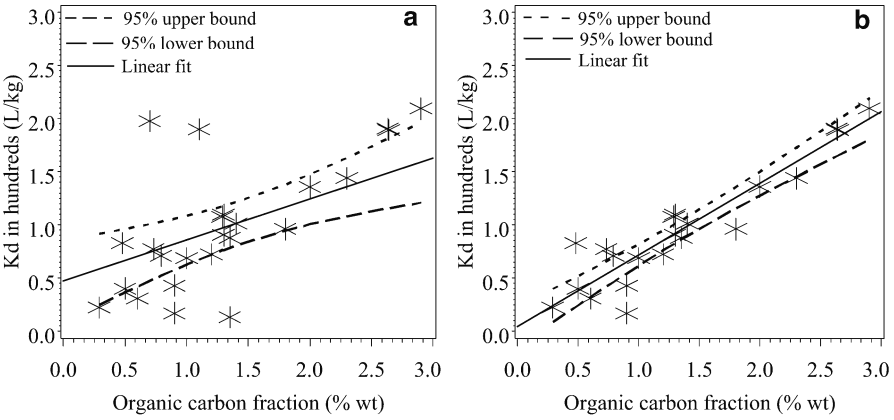


Fig. 2 Variation of chlorpyrifos K_d with soil organic carbon fraction after: (**a**) exclusion of four outliers in Fig. 1d ($n=26$, $R^2=0.34$), (**b**) exclusion of eight outliers detected with robust regression diagnostics ($n=22$, $R^2=0.88$)

fraction explained only 61% of the variance in K_d ($n=30$) (Fig. 1d). Removal of the four obvious outliers ($K_d>500$ L/kg, OC > 5%) decreased the explained variance by more than 50% (Fig. 2a), indicating that the observed predictive strength of the organic carbon fraction was, in fact, a regression bias caused by outliers. When the dataset was rigorously assessed for regression influence and outliers by using a range of SAS software regression diagnostic tools (ROBUSTREG procedure) (SAS Institute Inc., Cary, NC, the USA), eight outliers were detected that bracketed all ranges of organic carbon fraction and K_d values. Even after the exclusion of these outliers, the variance explained by organic carbon fraction was 88% ($n=22$) (Fig. 2b). Thus, about 12% of the variance in K_d for the data set excluding outliers is accounted for by other factors. These observations show that the influence of organic carbon fraction on adsorption is not uniform and that organic carbon content is not the sole factor controlling adsorption.

One explanation for the inability of organic matter content to fully predict pesticide adsorption is that the quality of soil organic carbon also matters. Recent studies have revealed that the quality of soil organic matter affects pesticide adsorption. Soil organic matter qualities linked to pesticide adsorption include soil origin and oxygen content (Garbarini and Lion 1986; Grathwohl 1990), humification (Payá-Pérez et al. 1992), structural and compositional variation of humic materials (Gauthier et al. 1987), fraction of soluble organic matter (Chiou et al. 1986; Ling et al. 2006; Spark and Swift 2002), and the degree of aromaticity and aliphatic character of the moieties (Ahmad et al. 2001; Chen et al. 1996; Salloum et al. 2002). Also, there is some evidence suggesting that diagenetically young organic matter in near-surface sediments exhibit rapid and reversible adsorption, while aged condensed organic matter exhibits slow and irreversible adsorption (Huang and Weber 1997; Rockne et al. 2002; Weber et al. 1992; Xing and Pignatello 1997).

Differences in experimental methodologies and experimental artifacts may also explain the wide variations in the estimate of partition coefficients for pesticides, including CPF. For example, the initial liquid-phase concentration and the ratio of liquid to solids used in the batch equilibrium experiments varied greatly. Although it is recommended that the initial substance concentration in batch equilibrium not exceed half its water solubility (OECD 2000), initial concentrations used in reviewed studies varied from as little as 8 μg/L to nominal concentrations that were an order of magnitude higher than the water solubility of CPF. The liquid to solid ratio for the batch systems also varied across experiments from 2:1 to 800:1. Biased partition coefficient values can also be traced to differences in mass balance calculations. Spieszalski et al. (1994) reported that partition coefficients based on a mass balance of initial and equilibrium solution-phase concentrations increased by one order of magnitude, compared to those based on both the liquid- and solid-phase concentrations. An additional experimental artifact that can result in inconsistent K_d or K_{oc} values is the strong tendency of organic compounds to sorb onto surfaces of experimental apparatus, including container walls, centrifuge glass, syringe, and other analytical equipment used during the experiment (Thomas and Mansingh 2002; Wasswa et al. 2010).

Finally, inappropriate calculation of K_{oc} values may also contribute to its variability in the literature. K_{oc} values derived from adsorption experiments, in which soil organic carbon is not primarily controlling adsorption, can be heavily skewed or even more variable than the K_d values (Elabd et al. 1986; Beck et al. 1996; Mushtaq et al. 1996). Skewed K_{oc} values can also be due to lack reproducibility of the methods for measuring soil organic carbon. While there are advanced analytical methods that can provide accurate determination of soil organic carbon, researchers often rely on simple but less accurate methods such as loss-on-ignition, the Walkley–Black method, or the use of conversion factors to estimate soil organic carbon fraction from soil organic matter content (Schumacher 2002; Bisutti et al. 2004).

9 Effect of Adsorption/Desorption on Persistence and Toxicity

Adsorption and desorption processes have a profound influence on the persistence of pesticides by controlling the rate of specific fate processes such as volatilization, biodegradation, photolysis, and hydrolysis (Ogram et al. 1985; Steinberg et al. 1987; Scribner et al. 1992; Doucette 2003; Lu et al. 2003). The dissipative half-life of CPF was significantly longer in sediments and soils than in water (Hughes et al. 1980), in soil-incorporated treatments than unincorporated surface treatments (Getzin 1985), and in organic soils versus mineral soils (Chapman and Harris 1980). Macalady and Wolfe (1985) reported that sediment-phase alkaline hydrolysis of CPF was 10 times slower than was water-phase hydrolysis. Hydrolysis of CPF was slower in water that contained clay minerals, humate, DOM, and suspended sediment (Noblet et al. 1996; Liu et al. 2001; Wu and Laird 2002, 2004). It has been reported in many studies that adsorption impedes degradation of various chemicals,

apparently from reduced access of sorbed chemicals to microorganisms (Steinberg et al. 1987; Ogram et al. 1985; Scribner et al. 1992; Guerin and Boyd 1992; Guo et al. 2000). Gebremariam and Beutel (2010) investigated mineralization of CPF in reciprocating (drain–fill) versus nonreciprocating constructed treatment wetland microcosms (constantly flooded). They found that CPF mineralization was substantially reduced in the reciprocating microcosms relative to nonreciprocating systems, apparently from enhanced adsorption of CPF to sediment by drain–fill cycles in the reciprocating microcosms. The fraction of CPF mineralized also decreased with increasing organic matter content of the wetland sediments.

Toxicity and bioaccumulation of pesticides in aquatic organisms are substantially influenced by adsorption and desorption processes, and by water chemistry that influences the degree of adsorption and/or desorption (Ankley et al. 1994; Jones and Huang 2003; Phillips et al. 2003). Studies show that adverse effects of CPF on aquatic organisms were influenced by the presence of colloids such as humic acids, although effective toxicity against particular organisms was dependent upon the route of exposure. For example, Phillips et al. (2003) found that CPF sorbed to humic acid was more toxic to fish larvae, whereas Jones and Huang (2003) reported that CPF toxicity to microorganisms decreased in the presence of humic acid. Bioaccumulation of CPF by estuarine clams was positively influenced by the concentration of colloidal matter that sorbed the pesticide (Bejarano et al. 2003). The effect of humic acid on CPF toxicity toward aquatic organisms also depended on the chemistry of the water. Mézin and Hale (2004) reported that humic acid significantly lowered toxicity of CPF against freshwater crustaceans, but had no significant effect on mortality of saltwater crustaceans, apparently because of increased CPF adsorption to humic acid in freshwater versus saltwater. Association of CPF with suspended sediment in constructed treatment wetlands was also shown to significantly reduce or eliminate CPF toxicity against water flea, fathead minnow, and midge larvae (Moore et al. 2002; Sherrard et al. 2004).

Toxicity to benthic organisms depends on the physical and chemical characteristics of sediments controlling pesticide bioavailability. Past research efforts have led to development of an equilibrium partitioning theory that takes into account the slow release of sediment contaminants based on organic carbon content, and this approach can be used to calculate organism- and chemical-specific sediment quality criteria (Di Toro et al. 1991; Ankley et al. 1994; Green et al. 1996). Jantunen et al. (2008) reported that bioaccumulation of CPF in benthic oligochaeta decreased with increasing organic content of the sediments, apparently because of slow and incomplete desorption of the pesticide from organic sediments. Similarly, Ankley et al. (1994) showed reduced bioavailability and toxicity of CPF to the midge *Chironomus tentans* from DOM in the pore-water. Reduced bioavailability and toxicity of some other pesticides in organic soils was also linked to increased microbial degradation of pesticides in the presence of organic matter (Dubey et al. 1966; Felsot and Dzantor 1995), although this may depend on the type of organic matter and property of the compound. For example, CPF showed both strongly enhanced persistence and markedly decreased bioavailability when soils were amended with biochar (Yu et al. 2009; Yang et al. 2010).

10 Summary

Chlorpyrifos, one of the most widely used insecticides, has been detected in air, rain, marine sediments, surface waters, drinking water wells, and solid and liquid dietary samples collected from urban and rural areas. Its metabolite, TCP, has also been widely detected in urinary samples collected from people of various age groups. With a goal of elucidating the factors that control the environmental contamination, impact, persistence, and ecotoxicity of chlorpyrifos, we examine, in this review, the peer-reviewed literature relating to chlorpyrifos adsorption and desorption behavior in various solid-phase matrices. Adsorption tends to reduce chlorpyrifos mobility, but adsorption to erodible particulates, dissolved organic matter, or mobile inorganic colloids enhances its mobility. Adsorption to suspended sediments and particulates constitutes a major off-site migration route for chlorpyrifos to surface waters, wherein it poses a potential danger to aquatic organisms. Adsorption increases the persistence of chlorpyrifos in the environment by reducing its availability to a wide range of dissipative and degradative forces, whereas the effect of adsorption on its ecotoxicity is dependent upon the route of exposure.

Chlorpyrifos adsorbs to soils, aquatic sediments, organic matter, and clay minerals to differing degrees. Its adsorption strongly correlates with organic carbon content of the soils and sediments. A comprehensive review of studies that relied on the batch equilibrium technique yields mean and median K_d values for chlorpyrifos of 271 and 116 L/kg for soils, and 385 and 403 L/kg for aquatic sediments. Chlorpyrifos adsorption coefficients spanned two orders of magnitude in soils. Normalizing the partition coefficient to organic content failed to substantially reduce variability to commonly acceptable level of variation. Mean and median values for chlorpyrifos partition coefficients normalized to organic carbon, K_{oc}, were 8,163 and 7,227 L/kg for soils and 13,439 and 15,500 L/kg for sediments. This variation may result from several factors, including various experimental artifacts, variation in quality of soil organic matter, and inconsistencies in experimental methodologies. Based on this review, there appears to be no definitive quantification of chlorpyrifos adsorption or desorption characteristics. Thus, it is difficult to predict its adsorptive behavior with certainty, without resorting to experimental methods specific to the soil or sediment of interest. This limitation should be recognized in the context of current efforts to predict the risk, fate, and transport of chlorpyrifos based upon published partition coefficients.

Based on a comprehensive review of the peer-reviewed literature related to adsorption and desorption of chlorpyrifos, we propose the following key areas for future research. From this review, it becomes increasingly evident that pesticide partitioning cannot be fully accounted for by the fraction of soil or solid-matrix organic matter or carbon content. Therefore, research that probes the variation in the nature and quality of soil organic matter on pesticide adsorption is highly desirable. Pesticide persistence and bioavailability depend on insights into desorption capacity. Therefore, understanding the fate and environmental impact of hydrophobic pesticides is incomplete without new research being performed to improve insights into

pesticide desorption from soils and sediments. There is also a need for greater attention and consistency in developing experimental methods aimed at estimating partition coefficients. Moreover, in such testing, choosing initial concentrations and liquid–solid ratios that are more representative of environmental conditions could improve usefulness and interpretation of data that are obtained. Future monitoring efforts should include the sampling and analysis of suspended particulates to account for suspended solid-phase CPF, a commonly underestimated fraction in surface water quality monitoring programs. Finally, management practices related to the reduction of off-site migration of CPF should be further evaluated, including alternative agricultural practices leading to reduction in soil erosion and structural best management practices, such as sedimentation ponds, treatment wetlands, and vegetated edge-of-field strips.

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