Volume 229

David M. Whitacre *Editor*

Reviews of Environmental Contamination and Toxicology

Springer

Reviews of Environmental Contamination and Toxicology

VOLUME 229

For further volumes:
http://www.springer.com/series/398

Reviews of Environmental Contamination and Toxicology

Editor
David M. Whitacre

VOLUME 229

Springer

Coordinating Board of Editors

Dr. David M. Whitacre, *Editor*
Reviews of Environmental Contamination and Toxicology

5115 Bunch Road
Summerfield, North Carolina 27358, USA
(336) 634-2131 (PHONE and FAX)
E-mail: dmwhitacre@triad.rr.com

Dr. Erin R. Bennett, *Editor*
Bulletin of Environmental Contamination and Toxicology
Great Lakes Institute for Environmental Research

University of Windsor
Windsor, Ontario, Canada
E-mail: ebennett@uwindsor.ca

Peter S. Ross, *Editor*
Archives of Environmental Contamination and Toxicology

Fisheries and Oceans Canada
Institute of Ocean Sciences
Sidney, British Colombia, Canada
E-mail: peter.s.ross@dfo-mpo.gc.ca

ISSN 0179-5953 ISSN 2197-6554 (electronic)
ISBN 978-3-319-03776-9 ISBN 978-3-319-03777-6 (eBook)
DOI 10.1007/978-3-319-03777-6
Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

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

Foreword

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

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

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

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with

any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

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

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

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

Coordinating Board of Editors

Preface

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

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

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

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

Contents

Mercury Toxicity and Neurodegenerative Effects 1
Alessia Carocci, Nicola Rovito, Maria Stefania Sinicropi, and Giuseppe Genchi

E-Waste Disposal Effects on the Aquatic Environment: Accra, Ghana 19
Jingyu Huang, Philip Nti Nkrumah, Desmond Ofosu Anim, and Ebenezer Mensah

Environmental Fate and Toxicology of Clomazone 35
April R. Van Scoy and Ronald S. Tjeerdema

Modulation of Plant Growth and Metabolism in Cadmium-Enriched Environments 51
Shaista Qadir, Sumiya Jamshieed, Saiema Rasool, Muhammad Ashraf, Nudrat Aisha Akram, and Parvaiz Ahmad

Effect of Fruit and Vegetable Processing on Reduction of Synthetic Pyrethroid Residues 89
Reena Chauhan, Beena Kumari, and M.K. Rana

Toxicity Reference Values for Polybrominated Diphenyl Ethers: Risk Assessment for Predatory Birds and Mammals from Two Chinese Lakes 111
Ruiqing Zhang, Jianyang Guo, Fengchang Wu, Yunsong Mu, John P. Giesy, Hong Chang, Xiaoli Zhao, and Chenglian Feng

Index 139

Mercury Toxicity and Neurodegenerative Effects

Alessia Carocci, Nicola Rovito, Maria Stefania Sinicropi, and Giuseppe Genchi

Contents

1 Introduction 1
2 Chemical Forms and Properties of Mercury 2
3 Sources of Human Exposure to Mercury 3
4 Absorption, Distribution, and Toxicity of Mercury 5
5 Mercury Toxicity: Molecular Mechanism 7
6 Mercury and Neurodegenerative Diseases 10
7 Summary 12
References 13

1 Introduction

Neurodegeneration is a broadly defined term that describes the loss of neuronal structure and function, and produces disorders known as neurodegenerative diseases. A common feature of neurodegeneration is the progressive cell loss in specific neuronal populations of the central nervous system (CNS), often associated with cytoskeletal protein changes that led to intracytoplasmic and/or intranuclear inclusions in neurons and/or glia. The neurological consequences of neurodegeneration in patients are often devastating and result in severe mental and physical effects, accounting for a large number of hospitalizations and disabilities.

A. Carocci
Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari "A. Moro", Bari 70125, Italia

N. Rovito • M.S. Sinicropi (✉) • G. Genchi (✉)
Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Arcavacata di Rende (Cosenza) 87036, Italia
e-mail: s.sinicropi@unical.it; genchi@unical.it

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229*, Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6_1,

Although the causes of the majority of neurodegenerative diseases are still unknown, it has become increasingly clear that the major basic processes that induce neurodegeneration are multifactorial ones that are caused by genetic, endogenous, and environmental factors. Protein misfolding and aggregation, oxidative stress, mitochondrial dysfunction, and phosphorylation impairment are the major shared neurodegenerative pathogenic processes (Jellinger 2003).

Although still debatable, evidence has pointed to a potential link between exposures to chemical contaminants and neurodegenerative disorders such as Alzheimer's disease (Coppede and Migliore 2010), and amyotrophic lateral sclerosis (Johnson and Atchison 2009). Chemical contaminants reported to cause neurotoxic effects in humans at environmentally relevant exposure levels include arsenic (Wasserman et al. 2004), lead (Lanphear et al. 2005), manganese (Takser et al. 2003), some pesticides (Rauh et al. 2006), mercury and mercury compounds (Farina et al. 2011a).

In this review, we attempt to present an understanding of the role that exposures to mercury compounds play in neurodegeneration and neurodegenerative diseases.

2 Chemical Forms and Properties of Mercury

Mercury (hydrargyrium, Hg) is classified as a heavy metal (atomic weight 200.59) and is well known as being among the most toxic of metals (Clarkson et al. 2003; World Health Organization 2007). Mercury is a non-transition metal and is an extremely rare element in the Earth's crust, having an average mass abundance of only 0.08 ppm (Sinicropi et al. 2010). Hg has three valence states (0, I and II), and exists in three main forms, each of which have different toxicities, implications for health and measures to prevent exposure (IPCS 2000). These three forms of mercury are: elemental mercury or quicksilver (Hg^0, metallic mercury and mercury vapor), inorganic mercury (Hg^+ and Hg^{2+}), and organic mercury such as methylmercury (CH_3Hg, MeHg) and ethylmercury (C_2H_5Hg, EtHg).

Elemental mercury (Hg^0) has a peculiar behavior, in that it is monoatomic in the vapor phase, and has a relatively high vapor pressure at 20 °C ($1.3\ 10^{-3}$ mm). It uniquely exists in liquid form at room temperature and quickly turns to vapor when heated above room temperature. The high volatility of Hg^0 prolongs the effects of anthropogenic releases through repeated atmospheric recycling to and from the land and the sea (Mason et al. 1994). Hg^0 can remain suspended in the atmosphere for up to 1 year, where it can be transported and deposited globally.

Hg^0 is oxidized in air to its inorganic forms (Hg^+ and Hg^{++}) and is released during rain events to be deposited in soil, or into the waters of rivers, lakes and oceans. Inorganic mercury, derived from industrial release and from contaminated water, is biomethylated (in the aqueous environment and by phytoplankton in the ocean) to methylmercury (MeHg), primarily by sulfate-reducing bacteria (Compeau and Bartha 1985; Morel et al. 1998). MeHg is accumulated to high concentrations in shellfish, predatory fish (i.e., swordfish, shark and king mackerel) and sea mammals. It is bioaccumulated especially by the liver, brain, kidney and muscle

(Compeau and Bartha 1985). The rapid interconversion of the inorganic forms into the organic ones, including the possibility of disproportionation reactions, means that the environmental behavior of Hg is complex (Jonasson and Boyle 1972; Rasmussen 1994).

Mercury has no known physiological role in humans, and is among the most harmful heavy metals to which humans and wildlife can be exposed. Furthermore, the human body lacks effective mechanisms to excrete it. The organometallic compounds of mercury have a higher solubility in lipids than its inorganic species. Organometallic mercury compounds diffuse more easily through the lipid bilayer of biological membranes, which increases their potential toxicity. Both Hg^0 and MeHg are neurotoxic, whereas inorganic mercury salts are nephrotoxic (Magos et al. 1980).

3 Sources of Human Exposure to Mercury

Hg is released to the environment from both anthropogenic and natural sources. Annually, volcanic and geothermal activities (for example the Phlegrean fields, Pozzuoli, Italy) release an estimated 1,500 t of mercury to the environment. Anthropogenic release occurs from manifold industrial point sources, and is estimated to constitute 2,320 t of mercury emitted annually into the atmosphere (Pirrone et al. 2010). Sources of Hg^0 exposure resulting from human enterprise include industrial consumption of fossil fuels, cement production and incineration of solid wastes, contact with topical medicines, use of cathartics, thermometers, sphygmomanometers, barometers, incandescent lights, and batteries, in addition to medical waste incineration, Hg-based substances used in ritualistic practices and dental amalgams (Pirrone et al. 2001). In fact, autopsy studies have shown that dental amalgams are the main source of mercury in human tissues. Amalgam bearers have about 2–12 fold more mercury in their tissues, including the brain, than individuals without amalgams (Drasch et al. 1994; Guzzi et al. 2006; Björkman et al. 2007; Mutter et al. 2007; Mutter 2011).

In some countries, consumption of inorganic mercury preparations (mercury salts, Hg^+ and Hg^{++}) are a significant source of human intoxication. The reason for this is that such preparations have long been used as medications, germicidal soaps and skin creams (Guzzi and La Porta 2008). Some skin creams contain as much as 6–10 % mercurial chloride or calomel (Hg_2Cl_2). For many years, calomel was used in infant teething powders, worm drugs, and as an analgesic.

MeHg is an organo-mercurial compound primarily found as a pollutant in the aquatic environment. When MeHg is present in nature, its source is usually from biomethylation of inorganic mercury that is carried out by aquatic anaerobic sulfate-reducing bacteria (Compeau and Bartha 1985; Morel et al. 1998). MeHg ultimately derives from anthropogenic sources, and when formed will be released into rivers, lakes and oceans. Consequently, people, whose diet consists mainly of fish and shellfish, may be exposed to high levels of MeHg. Such exposure is

unfortunate, because humans are highly vulnerable to the toxicity of this compound. Approximately 85% of MeHg ingested by fish is absorbed in the gastrointestinal tract, while about 5% is present in blood and 10% in brain.

There are two epidemics that have occurred from MeHg poisoning events that are worthy of mention: the first occurred in the Japanese villages of Minamata Bay (1953); the second along the Agano river in Niigata (1964) (Tsubaki and Irukajama 1997). Minamata disease is the term used to describe the poisoning that occurred among Japanese residents of Minamata Bay from ingesting methylmercury-containing fish and shellfish. Over a period of 36 years (1932–1968), the Chisso Corporation's chemical factory dumped about 27 t of methylmercury-associated waste into Minamata Bay. MeHg is bioaccumulated within the food chain from plankton, microorganisms up to fish and shellfish. More than 10,000 Japanese living in the bay, who ate fish and shellfish contaminated with methylmercury, were afflicted by Minamata disease (Tsubaki and Irukajama 1997).

In the early 1950s, the people of Minamata Bay began to exhibit symptoms of neurological illness, i.e., uncontrollable trembling, loss of motor control, and partial paralysis. Newborn babies also exhibited symptoms of Minamata disease (Tsubaki and Irukajama 1997).

The second epidemic of severe methylmercury intoxication resulted in the hospitalization of about 7,000 people and the death of 460 individuals occurred in rural Iraq in 1971–1972 (Bakir et al. 1973). This incident occurred as a result of bread being prepared and eaten from wheat seed that had been treated with a mercury-based fungicide. The wheat seed was supposed to be planted, but labeling problems and other errors resulted in the treated wheat seed being used to bake bread. Both the Japanese and Iraq methylmercury poisoning incidents produced not only deaths, but multiple and long-lasting intoxication symptoms that included blindness, deafness, mental retardation, cerebral palsy, and dysarthria, especially in children exposed in utero (Guzzi and La Porta 2008).

In the 1970s, research performed in the Faroe Islands revealed a significant increase in mercury contamination of meat, blubber, liver and kidney of pilot whales (*Globicephalus meleanus*) (Andersen et al. 1987; Simmonds et al. 1994). These studies demonstrated that MeHg taken in from consuming pilot whale meat adversely affected human fetal development of the nervous system, and that the effects of exposure to mercury were still detectable during adolescence. Weihe and Joensen (2012) reported that mercury residues in pilot whale meat eaten by women of the Faroe Islands affected brain function and blood pressure of their breast milk-fed children. In addition, this study revealed decrements in attention, language and verbal memory from MeHg exposure.

Ethylmercury (EtHg) is another organic mercury compound that, in the form of thimerosal (sodium ethylmercury thiosalicylate; Dórea 2011b), has been used as a topical antiseptic and preservative in vaccines. It is important to emphasize that about 90% of vaccines used worldwide contain thimerosal (multidose vials). In addition, thimerosal has been used to preserve topical medications, contact lens cleaners, and cosmetics. Thimerosal contains 50% mercury and is metabolized in the human body to EtHg and

thiosalicylate. In some cases, local hypersensitivity reactions have been noted when vaccines containing thimerosal were administered. Although thimerosal is considered to be stable, it can decompose in vivo at high fever temperatures, or in the presence of bacteria, to produce more toxic molecules like MeEtHg or Et_2Hg (Drum 2009). Thimerosal is also subject to a slow photodecomposition in the presence of sunlight (Drum 2009).

One other significant source of human exposure to MeHg and other organic mercury compounds is from its use in dental amalgams (Leistevuo et al. 2002).

4 Absorption, Distribution, and Toxicity of Mercury

The toxicity of metals and metal compounds largely depends on the degree to which they are bioavailable, i.e. the degree to which they are absorbed through cell membranes, are distributed within the cell and bind to cellular macromolecules (Beyersmann and Hartwig 2008). When Hg^0 from dental amalgams (Fig. 1) is inhaled as a vapor into the lungs, about 80% is absorbed (Schafer et al. 1999; Mutter et al. 2010). Due to its uncharged monoatomic form, Hg^0 is highly diffusible and lipid soluble, and easily crosses the blood–brain barrier and lipid bilayers of cells

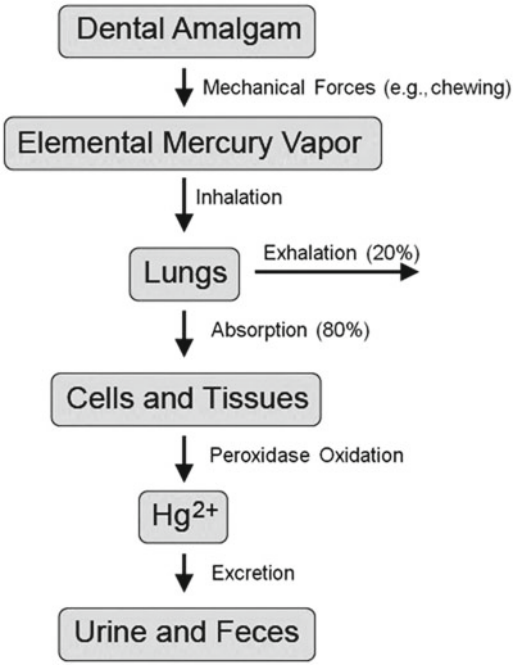


Fig. 1 Fate in the body of mercury vapor released from dental amalgams. Mercury adsorbed from amalgams ranges from 9 to 17 μg/day. Inhaled mercury vapor is easily absorbed by the lungs, deposited in different tissues and cells and oxidized to Hg^{++}. Mercury ions within cells are not excreted at all, and 60–70% accumulate or remains in body tissues and in cells. The half life of Hg in the brain is 18–30 years (Opitz et al. 1996). However, inorganic mercury is excreted largely via urine and feces, and partially through sweat, tears and breast milk

and cell organelles, such as mitochondria. Mercury vapor also penetrates the mucosa and connective tissue of the oral and nasal cavities and may be transported into nerve cells (Mutter et al. 2010).

Exposure to toxic Hg^0 vapors may be either acute or chronic. Both acute and chronic Hg^0 exposures may result in human poisoning. In particular, such exposures can cause coughing, dyspnea, fever, tremors, malaise, axonal sensor motor polyneuropathy, gingivitis, hallucinations and mercurial erythrism, a syndrome that includes excitability, loss of memory, insomnia and neurocognitive disorders (Guzzi and La Porta 2008). Case–control studies have demonstrated an association between exposure to Hg^0 and the potential to develop amyotrophic lateral sclerosis (ALS). Schwarz et al. (1996) described a case of ALS that culminated from 3 years of periodic accidental injection of mercury. This metal infiltrated soft tissue and caused weakness of the musculature, cerebellar ataxia, and fasciculation, syndromes similar to those observed in ALS patients. Callaghan and coauthors (2011) addressed the role of heavy metals (e.g., lead, mercury and selenium) as potential epigenetic factors in the development of ALS. These authors, investigated the interaction of these heavy metals with epigenetic phenomena that govern DNA modifications (i.e., DNA-bound histones, DNA methylation, and chromatin remodeling), and observed that these modifications produced certain toxic actions that played a role in the etiology of ALS.

In the inorganic form, mercury is absorbed from the gastrointestinal tract and acts to produce inflammatory reactions in the kidneys and gastrointestinal apparatus. Intracellularly, Hg^{++} is produced from metabolic oxidation of Hg^0. This mercuric ion immediately reacts with intracellular molecules, such as enzymes, GSH (glutathione), tubulin, ion channels, and transporters. These interactions inhibit the activities of such molecules and interfere with normal cellular functions; in addition, even very low concentrations of Hg^{++} decrease GSH levels and increase oxidative and nitrosative stress (Mutter et al. 2010).

Research suggests that mercury induces autoimmune processes (Schiraldi and Monestier 2009), and may be mutagenic at low concentrations (Schurz et al. 2000). Immunotoxic effects could potentially enhance susceptibility to infections, to malaria (Silbergeld et al. 1998) or immunologically-mediated diseases (McCabe and Lawrence 1994). Ben-Ozer et al. (2000) proposed that mercury exposure may alter membrane potassium conductance, modulate chlorine channels, destroy sodium-potassium-ATPase activity, inhibit phospholipid turnover, cause DNA strand breakage, and activate phospholipase C.

Inorganic mercury accumulates in the human breast and is secreted in breast milk, which can damage the developing infant's CNS, pulmonary and nephrotic systems (Counter and Buchanan 2004). Inorganic mercury exposure can also induce Kawasaki disease (Mutter and Yeter 2008), which results from impairment of the immune system. The symptoms of children affected by Kawasaki disease include fever, photophobia, pharyngitis, oral lesions, skin rashes, and tachycardia, among others (Walsh 1982; Goyer and Clarkson 2001).

Xu and coworkers (2012) investigated the effect of $HgCl_2$ (25 nM–25 μM) on neurite initiation and network formation during outgrowth in rat cortical neuron cultures.

The results indicate that low levels of mercury (25–100 nM) induced neuronal degeneration and perturbed neuronal excitability interacting with *N*-methyl-D-aspartate (NMDA) receptors. Moreover, pre-incubation of cells in the presence of MK801 (a non-competitive antagonist of NMDA receptor) inhibited the $HgCl_2$ effect (Xu et al. 2012). It is noteworthy that, in previous studies, other authors (Drasch et al. 1992; Guzzi et al. 2006) reported this same range of mercury concentration to exist in individuals having dental amalgams. Drasch et al. (1992) found mercury levels in the kidney of Germans to be 2.5 μM, whereas Guzzi et al. (2006) reported that 1.5 μM of mercury is present in the brain of Italians.

The rate at which MeHg is absorbed in the gastrointestinal tract is high (about 90–95%), and contributes to its high toxicity and degree of bioavailability (Nielsen and Andersen 1992). The CNS is the region in which MeHg preferentially accumulates; this is unfortunate for the unborn, because MeHg absorption takes place during the prenatal brain development (Johansson et al. 2007; Zareba et al. 2007; Grandjean and Herz 2011). Organic mercury (e.g., methylmercury) can be adsorbed at rates up to 90% of that taken in. As alkyl chain length of organic mercury compounds increases, toxicity decreases. Once organo mercury compounds enter the body they are rapidly distributed to tissues, mainly liver (approximately 50%), CNS and kidney. Because organo mercury compounds are lipophilic, they readily cross the placental barrier and have toxic consequences for the developing cerebellum of the fetus. In addition, their levels in cord blood tend to be higher than in maternal blood (Vather et al. 2000). Cernichiari et al. (1995) reported that Hg levels in the fetal brain are about 5–7 times higher than in maternal blood. MeHg is slowly metabolized to inorganic form by intestinal micro flora, and in this form is predominantly accumulated for long periods in the CNS. At some level of accumulation paresthesia appears as the first symptom of organo mercury poisoning, and may progress to ataxia, dysarthria, and constriction of visual field and loss of hearing (Bakir et al. 1973).

Studies on the neurotoxicity induced by MeHg have described the following phenomena: depletion of intracellular antioxidants (Franco et al. 2007; Johansson et al. 2007), inhibition of specific enzymes (Rocha et al. 1993; Franco et al. 2009; Wagner et al. 2010) and modulation of transporter or neuromodulator activities of receptors (Fitsanakis and Aschner 2005; Yin et al. 2007).

5 Mercury Toxicity: Molecular Mechanism

The processes that mediate MeHg toxicity are related to this molecule's electrophilic properties; MeHg reacts preferentially with soft nucleophiles, and therefore is known as a soft electrophile. In particular, MeHg reacts with -SH and with -SeH (sulfhydryl or thiol and selenohydryl or seleno groups, respectively), which are the two types of soft nucleophiles found in animal proteins. The interaction between MeHg and the soft nucleophilic groups of biomolecules (e.g., proteins and amino acids) to form stable complexes (e.g., $RSHgCH_3$ or $RSeHgCH_3$; Farina et al. 2011a)

partially decreases the antioxidant capacity of such biomolecules. The resulting increased production of reactive oxygen species (ROS) produces oxidative damage to lipids, enzymes, (e.g., glutathione peroxidase (GPX)) and thioredoxin reductase (TrXR)), and nucleic acids; such damage leads to cell death (Farina et al. 2011b).

In addition, the interaction of MeHg with nucleophilic groups of proteins damages their catalytic (Rocha et al. 1993; Farina et al. 2009), binding (Soares et al. 2003) and transport functions (Aschner et al. 2000). The formation of S-Hg or Se-Hg bonds in proteins and enzymes creates protein deposits rich in cysteine (Dórea 2011a). For this reason, it is difficult to find free MeHg in the cells of living organism, because of the high affinity it has for thiol and selenol groups (Sugiura et al. 1976; Onyido et al. 2004). Seleno-cysteine (–SeH) differs by one atom from cysteine (-SH), i.e., selenium vs. sulfur, and although they have similar chemical properties, the lower pKa value and stronger nucleophilicity of seleno-cysteines make them more reactive (Sugiura et al. 1976; Khan et al. 2009).

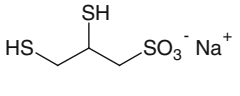
Selenium is an essential trace element and it is absolutely necessary to achieve the activity of the 25–30 selenoenzymes that are required to protect CNS and brain from oxidative damage. Homeostatic mechanisms normally replace optimal selenoenzymes activities in the brain, but high MeHg levels bind selenium by irreversibly inhibiting selenoenzymes activity. However, a diet rich in selenium (present in many food, including fish), may replace MeHg-bound selenium, and thereby protect the activity of brain cells (Raymond and Ralston 2004; Ralston et al. 2008; Ralston and Raymond 2010).

Thus, selenoenzymes and selenoproteins are important targets for MeHg (Farina et al. 2009; Carvalho et al. 2010; Branco et al. 2011), although such -SeH groups are less abundant than -SH ones. In fact, although thiols are present in cysteine, lipoic acid, reduced glutathione and in proteins, selenols are found only in a small group of selenoproteins (Lobanov et al. 2009; Lu and Holmgren 2009).

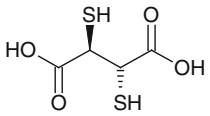
Metallothioneins, proteins rich in -SH groups, play an important protective role in the kidney against mercury toxicity (Satoh et al. 1997). Indeed, among compounds that chelate and immobilize mercury, there are several molecules that contain sulfhydryl groups, such as 2,3-dimercapto-1-propanesulfonic acid (sodium salt, DMPS), meso-2,3-dimercaptosuccinic acid (DMSA), D-penicillamine (β,β-dimethylcysteine), *N*-acetyl-DL-penicillamine, and 2,3-dimercaptopropanol (British anti Lewisite, BAL) (Fig. 2) (Baum 1999). Any selenium that exists in seleno-cysteine amino acids or in enzymes as -SeH groups antagonizes mercury-induced adverse effects by forming seleno-mercury complexes that reduce toxicity (Stoewsand et al. 1974; Singhal et al. 1987; Seppanen et al. 2000; Yoshizawa et al. 2002).

Although the molecular mechanisms mediating MeHg-induced neurotoxicity are not completely understood, evidence indicates that oxidative stress is important to actions that generate MeHg neurotoxic effects (Farina et al. 2011a).

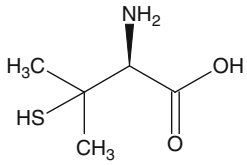
The most important mechanism by which mercury causes toxicity appears to be damage to mitochondria via GSH depletion. Such damage results in the generation of free radicals. Mercury compounds, bound to thiol groups, deplete sulfhydryl proteins (Valko et al. 2005) and GSH (Nicole et al. 1998), which are essential to mitigate oxidative damage.



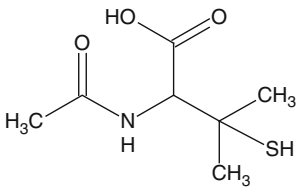
2,3-dimercapto-1-propanesulfonic acid (sodium salt, DMPS)



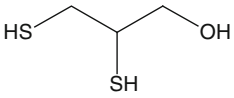
meso-2,3-dimercaptosuccinic acid (DMSA)



D-penicillamine (dimethyl-cysteine)



N-acetyl-DL-penicillamine



2,3-dimercaptopropanol (British anti Lewisite, BAL)

Fig. 2 Structures of several common agents that can chelate mercury

Mitochondria are cellular powerhouses, and are responsible for generating energy and heat, in the form of adenosine triphosphate (ATP). In addition, mitochondria are involved in the apoptosis-signaling pathway. Both nucleus and mitochondria contain DNA (mtDNA); however, unlike nuclear DNA, mitochondrial DNA has no histones to protect against the action of free radicals. Mitochondria are damaged primarily by ROS generated by the mitochondria themselves (Wei et al. 1998).

It is thought that the majority of ROS are generated by the complexes I and II (Harper et al. 2004), because of electrons released by NADH and $FADH_2$ into the mitochondrial respiratory chain. This results in depolarization and autoxidation of the inner mitochondrial membrane, which severely disrupts mitochondrial functions from phosphatidylserine being translocated to the outer mitochondrial membrane, thereby producing cell death by apoptosis (Sutton and Tchounwou 2007). MeHg accumulates in mitochondria, decreases oxygen consumption, alters electron transport and induces loss of mitochondrial membrane potential and apoptosis.

About 85% of the oxygen is utilized by mitochondria to produce ATP. During oxidative phosphorylation about 0.4–4.0% of all oxygen consumed is converted to free radicals (Shigenaga et al. 1994). Thereafter, mitochondrial lipids, proteins, oxidative phosphorylation enzymes and mtDNA are particularly vulnerable to free radical action (Shigenaga et al. 1994; Tanaka et al. 1996). Damage caused by MeHg to mitochondrial enzymes and proteins decreases their substrate and coenzyme affinity, thereby disrupting their biochemical and physiological functions (Liu et al. 2002).

Mercury and thimerosal inhibit in vitro methionine synthase by 50% (Waly et al. 2004); this enzyme is necessary for the synthesis of GSH, and is crucial for biological mercury detoxification. Delayed detoxification of mercury prevents methylation of DNA, RNA, histones, and prevents synthesis of methylcobalamin, phosphatidylcholine and neurotransmitters, resulting in diminished brain development and promoting attention deficit hyperactivity disorders (Deth 2004). Magos (2001) suggested that EtHg can induce neuro-developmental problems, despite recent reports suggesting that EtHg is neurologically less toxic than MeHg.

6 Mercury and Neurodegenerative Diseases

Experimental evidence has demonstrated that exposures to environmental toxicants may increase the risk of developing neuronal damage in many neurodegenerative disorders, including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (Rivera-Mancia et al. 2010). It has been observed that patients who have degenerative diseases accumulate metals in their nervous systems. If so, metals may have a role in neurobiological processes, such as in the regulation of synaptic transmission (Barnham and Bush 2008). It is known that very low levels of mercury induce neurodegenerative disease and nerve damage through NMDA receptors (Xu et al. 2012).

AD is the most common neurodegenerative disease, and currently affects about 24 million people worldwide (Mount and Downtown 2006; Qiu et al. 2007). The most identifiable symptom of AD is dementia, with the majority of diagnosis occurring in men 65 years old, or older; however, early-onset cases do occur and typically arise from genetic causes.

The central pathogenic factors that causes AD is neurodegeneration and inflammatory processes, which, in turn produce oxidative stress that accelerates neuronal damage. The key features of AD are the loss of neurons and synapses in the brain, the formation of extracellular plaques composed of amyloid β peptide (Aβ) and the formation of intraneuronal neurofibrillary tangles composed of helical filaments of hyperphosphorylated tau protein (Tiraboschi et al. 2004). Aβ oligomers and plaques are potent synaptotoxins that block proteasome function, inhibit mitochondrial activity, and stimulate inflammatory processes. Aβ interacts with signaling pathways that regulate the phosphorylation of tau protein.

Furthermore, degradation of hyperphosphorylated tau protein by the proteasome is inhibited by the action of Aβ. Currently, compelling evidence suggests that Aβ secretion is the triggering event in the pathogenesis of AD and that tau aggregation is a downstream event, but could reinforce neuronal dysfunction and cognitive impairment (Kennedy et al. 2007). The etiology of most cases of AD is yet unknown. Several genetic factors contributing to AD have been revealed (Blennow et al. 2006); however, epidemiological studies suggest that environmental factors may also be involved and may augment existing genetic risk factors. Circumstantial evidence suggests a potential pathogenic role for inorganic mercury in causing AD (Mutter et al. 2010). Some studies have shown a higher mercury concentration than in control groups, in

the brains of deceased AD patients, and in the blood of living patients. As we have already reported, the level of toxic mercury in experimental and animal studies is in the range of 0.1–1 μM, and residues in this range have been found in tissues of deceased individuals who possess dental amalgams. The arithmetic mean concentration in Italian human brains of amalgam bearers was reported to be 1.5 μM (Guzzi et al. 2006). The arithmetic mean of Hg levels in the kidneys of German amalgam bearers was 2.5 μM (Drasch et al. 1992). Mutter et al. (2004) revealed that even small amounts of mercury results in nerve cell changes that are typical for AD. They indicated that exposure to both organic and inorganic forms of mercury can induce the biochemical changes in tubulin structures that are found in the brains of AD patients. In healthy human brain tissue cultures, even at low concentrations, mercury inhibits guanosine triphosphate binding, which is necessary for tubulin synthesis and proper neuronal function (Duhr et al. 1993).

The inheritance of the ε4 allele of apolipoprotein, an important protein in the CNS lipid homeostasis, is the best known genetic risk factor for AD in many populations (Farrer et al. 1997). Some 40–80% of patients that suffer from AD express at least one allele of apolipoprotein E4 (ApoEε4) (Mahley et al. 2006). Having the ε2 genotype (Apo Eε2) reduces the risk of developing Alzheimer's disease (Strittmatter 1996), whereas, the presence of the ApoEε4 allele increases this risk (Strittmatter 1996).

The two ApoE isoforms consist of 299 aminoacids. At position 112 and 158, ApoEε2 contains two cysteines, and ApoEε4 two arginines (Mahley 1988). In contrast to arginine, cysteine has one sulfhydryl group, at which chemically bivalent metals (such as lead, copper, zinc and mercury) may bind. Therefore, ApoEε2, showing two cysteines and two -SH groups, could better bind and detoxify mercury (Mutter et al. 2004) and other heavy metals, while ApoEε4 cannot. This means that ApoEε4 has a reduced ability to bind heavy metals like mercury, and may explain the corresponding increased risk of developing AD.

Amyotrophic lateral sclerosis (ALS) is a progressive muscle loss and paralysis disease. It is another of progressive and lethal neurodegenerative diseases that are characterized by the degeneration of corticospinal tract neurons and α-motor neurons in the brainstem and ventral horn of the spinal cord (Bento-Abreu et al. 2010). Once ALS is diagnosed, survival time is generally 2–5 years, making it one of the most rapidly progressive and fatal neurological disorder. ALS occurs either as a sporadic (90%) or familial form (10%); both forms present indistinguishable clinical symptoms. The cause of sporadic ALS remains unknown and only a small percentage of familial cases have an identified genetic basis. Approximately 20% of the familial cases are linked to mutations in Cu/Zn-superoxide dismutase 1 gene (SOD1) (Rosen et al. 1994). Since SOD1 is an enzyme that is involved in the reduction of superoxide free radical, which is a byproduct of oxidative phosphorylation in the mitochondria, it is plausible that environmental agents eliciting oxidative stress may have a role in ALS. However, the connection between SOD1 function and ALS is much more complicated than an increase in oxidative stress. More than 125 SOD1 mutations have been identified in ALS patients, but it is not yet clear how different mutants gain a common toxic property relevant to the disease pathogenesis (Deng et al. 2006).

The role of environmental exposures in ALS is poorly understood. Although many studies have been published on links between specific exposures and ALS, few have been confirmed (Cannon and Greenamyre 2011). Exposure to lead, mercury and pesticides have all been cited as potential risk factors in the development of ALS (Johnson and Atchison 2009). Mercury toxicity can cause syndromes very similar to those observed in ALS, including tremor, extremity weakness, spasticity, hyperreflexia, fasciculation and ataxia. In fact, epidemiological and case–control studies have shown an association between exposure to mercury and the potential to develop ALS (Praline et al. 2007). In a mouse model for ALS, when the mutant human SOD1 gene (TgN SOD1G93A) was overexpressed, chronic MeHg exposure induced symptoms similar to those observed in ALS such as the early onset of hind limb weakness (Johnson and Atchison 2009). This suggests that, if an individual has an underlying genetic polymorphism for ALS, exposure to MeHg may hasten the onset of ALS. However a cause and effect relationship between exposure to MeHg and ALS has never been specifically demonstrated. Thus, MeHg exposures may or may not superimpose effects on an underlying genetic predisposition for initiation of ALS.

7 Summary

Mercury is among the most toxic heavy metals and has no known physiological role in humans. Three forms of mercury exist: elemental, inorganic and organic. Mercury has been used by man since ancient times. Among the earliest were the Chinese and Romans, who employed cinnabar (mercury sulfide) as a red dye in ink (Clarkson et al. 2007). Mercury has also been used to purify gold and silver minerals by forming amalgams. This is a hazardous practice, but is still widespread in Brazil's Amazon basin, in Laos and in Venezuela, where tens of thousands of miners are engaged in local mining activities to find and purify gold or silver. Mercury compounds were long used to treat syphilis and the element is still used as an antiseptic, as a medicinal preservative and as a fungicide. Dental amalgams, which contain about 50% mercury, have been used to repair dental caries in the U.S. since 1856. Mercury still exists in many common household products around the world. Examples are: thermometers, barometers, batteries, and light bulbs (Swain et al. 2007). In small amounts, some organo mercury-compounds (e.g., ethylmercury tiosalicylate (thimerosal) and phenylmercury nitrate) are used as preservatives in some medicines and vaccines (Ball et al. 2001).

Each mercury form has its own toxicity profile. Exposure to Hg^0 vapor and MeHg produce symptoms in CNS, whereas, the kidney is the target organ when exposures to the mono- and di-valent salts of mercury (Hg^+ and Hg^{++}, respectively) occur. Chronic exposure to inorganic mercury produces stomatitis, erethism and tremors. Chronic MeHg exposure induced symptoms similar to those observed in ALS, such as the early onset of hind limb weakness (Johnson and Atchison 2009). Among the organic mercury compounds, MeHg is the most biologically available

and toxic (Scheuhammer et al. 2007). MeHg is neurotoxic, reaching high levels of accumulation in the CNS; it can impair physiological function by disrupting endocrine glands (Tan et al. 2009).

The most important mechanism by which mercury causes toxicity appears to be mitochondrial damage via depletion of GSH (Nicole et al. 1998), coupled with binding to thiol groups (-SH), which generates free radicals. Mercury has a high affinity for thiol groups (-SH) and seleno groups (-SeH) that are present in amino acids as cysteine and *N*-acetyl cysteine, lipoic acid, proteins, and enzymes. *N*-acetyl cysteine and cysteine are precursors for the biosynthesis of GSH, which is among the most powerful intracellular antioxidants available to protect against oxidative stress and inflammation.

Mercury and methylmercury induce mitochondrial dysfunction, which reduces ATP synthesis and increases lipid, protein and DNA peroxidation. The content of metallothioneines, GSH, selenium and fish high in omega-3 fatty acids appear to be strongly related with degree of inorganic and organic mercury toxicity, and with the protective detoxifying mechanisms in humans. In conclusion, depletion of GSH, breakage of mitochondria, increased lipid peroxidation, and oxidation of proteins and DNA in the brain, induced by mercury and his salts, appear to be important factors in conditions such as ALS and AD (Bains and Shaw 1997; Nicole et al. 1998; Spencer et al. 1998; Alberti et al. 1999).

Acknowledgements This work was supported by grants from Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, Italy).

References

Alberti A, Pirrone P, Elia M, Waring RH, Romano C (1999) Sulphation deficit in "low-functioning" autistic children. Biol Psychiatry 46:420–424

Andersen A, Julshamn K, Ringdal O, Mørkøre J (1987) Trace elements intake in the Faroe Islands. II. Intake of mercury and other elements by consumption of pilot whales (*Globicephalus meleanus*). Sci Total Environ 65:63–68

Aschner M, Yao CP, Allen JW, Tan KH (2000) Methylmercury alters glutamate transport in astrocytes. Neurochem Int 37:199–206

Bains JS, Shaw CA (1997) Neurodegenerative disorders in humans and role of glutathione in oxidative stress mediated neuronal death. Brain Res Rev 25:335–358

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, Al-Rawi NY, Tikriti S, Dahahir HI, Clarkson TW, Smith JC, Doherty RA (1973) Methylmercury poisoning in Iraq. Science 181:230–241

Ball LK, Ball R, Pratt RD (2001) An assessment of thimerosal use in childhood vaccines. Pediatrics 107:1147–1154

Barnham KJ, Bush A (2008) Metals in Alzheimer's and Parkinson's diseases. Curr Opin Chem Biol 12:222–228

Baum CR (1999) Treatment of mercury intoxication. Curr Opin Pediatr 11:265–268

Ben-Ozer EY, Rosenspire AJ, McCabe MJ, Worth RG, Kindzelskii AL, Warra NS, Petty HR (2000) Mercury chloride damages cellular DNA by a non-apoptotic mechanism. Mutat Res 470:19–27

Bento-Abreu A, Van Damme P, Van Den Bosch L, Robberecht W (2010) The neurobiology of amyotrophic lateral sclerosis. Eur J Neurosci 31:2247–2265
Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanism. Arch Toxicol 82:493–512
Björkman L, Lundekvam BF, Laegreid T, Bertelsen BI, Morild I, Lilleng P, Lind B, Palm B, Vahter M (2007) Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study. Environ Health 6:30–44
Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. Lancet 368:387–403
Branco V, Canário J, Holmgren A, Carvalho C (2011) Inhibition of the thioredoxin system in the brain and liver of zebra–seabreams exposed to waterborne methylmercury. Toxicol Appl Pharmacol 251:95–103
Callaghan B, Feldman D, Gruis K, Feldman E (2011) The association of exposure to lead, mercury, and selenium and the development of amyotrophic lateral sclerosis and the epigenetic implications. Neurodegener Dis 8:1–8
Cannon JR, Greenamyre JT (2011) The role of environmental exposures in neurodegeneration and neurodegenerative diseases. Toxicol Sci 124:225–250
Carvalho CM, Lu J, Zhang X, Arner ES, Holmgren A (2010) Effects of Selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning. FASEB J 25:370–381
Cernichiari E, Brewer R, Myers GJ, Marsh DO, Lapham LW, Cox C, Shamlaye CF, Berlin M, Davidson PW, Clarkson TW (1995) Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicology 16:705–710
Clarkson TW, Magos L, Myers GJ (2003) The toxicology of mercury–current exposures and clinical manifestations. New Engl J Med 349:1731–1737
Clarkson TW, Vyas JB, Ballatori N (2007) Mechanisms of mercury disposition in the body. Am J Ind Med 50:757–764
Compeau GC, Bartha R (1985) Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. Appl Environ Microbiol 50:498–502
Coppede F, Migliore L (2010) Evidence linking genetics, environment, and epigenetics to impaired DNA repair in Alzheimer's disease. J Alzheimer's Dis 20:953–966
Counter SA, Buchanan LH (2004) Mercury exposure in children: a review. Toxicol Appl Pharmacol 198:209–230
Deng HX, Shi Y, Furukawa Y, Zhai H, Fu R, Liu E, Gorrie GH, Khan MS, Hung WY, Bigio EH, Lukas T, Dal Canto MC, O'Halloran TV, Siddique T (2006) Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. Proc Natl Acad Sci U S A 103:7142–7147
Deth RC (2004) Truth revealed: new scientific discoveries regarding mercury in medicine and autism. Congressional Testimony before the US House of Representatives. Subcommittee on human rights and wellness. http://reform.house.gov/WHR/Hearings/EventSingle.aspx?EventID=18156 Accessed 20 Sept 2005
Dórea JG (2011a) Environmental contaminants as biomarkers of fish intake: a case for hair mercury concentrations. Eur J Clin Nutr 65:419–420
Dórea JG (2011b) Integrating experimental (in vitro and in vivo) neurotoxicity studies of low-dose thimerosal relevant to vaccines. Neurochem Res 36:927–938
Drasch G, Schupp I, Riedel G, Gunther G (1992) Einfluß von Amalgamfüllungen auf die Quecksilber-konzentration in menschlichen Organen. Dtsch Zahnärztl Z 47:490–496
Drasch G, Schupp I, Hofl H, Reinke R, Roider G (1994) Mercury burden of human fetal and infant tissues. Eur J Pediatr 153:607–610
Drum DA (2009) Are toxic biometals destroying your children future? Biometals 22:697–700
Duhr EF, Pendergrass JC, Slevin JT, Haley BE (1993) HgEDTA complex inhibits GTP interactions with the E-site of brain beta-tubulin. Toxicol Appl Pharmacol 122:273–280
Farina M, Campos F, Vendrell I, Berenguer J, Barzi M, Pons S, Suñol C (2009) Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells. Toxicol Sci 112:416–426

Farina M, Rocha JBT, Aschner M (2011a) Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. Life Sci 89:555–563
Farina M, Aschner M, Rocha JBT (2011b) Oxidative stress in MeHg-induced toxicity. Toxicol Appl Pharmacol 256:405–417
Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278:1349–1356
Fitsanakis VA, Aschner M (2005) The importance of glutamate, glycine, and gammaaminobutyric acid transport and regulation in manganese, mercury and lead neurotoxicity. Toxicol Appl Pharmacol 204:343–354
Franco JL, Braga HC, Stringari J, Missau FC, Posser T, Mendes BG, Leal RB, Santos AR, Dafre AL, Pizzolatti MG, Farina M (2007) Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. Chem Res Toxicol 20:1919–1926
Franco JL, Posser T, Dunkley PR, Dickson PW, Mattos JJ, Martins R, Bainy AC, Marques MR, Dafre AL, Farina M (2009) Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. Free Radic Biol Med 47:449–457
Goyer RA, Clarkson TW (2001) Toxic effects of metals. In: Klaassen CD (ed) Casarett and Doull's toxicology: the basic science of poisons, 6th edn. McGraw-Hill, New York, pp 811–867
Grandjean P, Herz KT (2011) Methylmercury and brain development: imprecision and underestimation of developmental neurotoxicity in humans. Mt Sinai J Med 78:107–118
Guzzi G, La Porta CA (2008) Molecular mechanism triggered by mercury. Toxicology 244:1–12
Guzzi G, Grandi M, Cattaneo C, Calza S, Minoia C, Ronchi A, Gatti A, Severi G (2006) Dental amalgam and mercury levels in autopsy tissues: food for thought. Am J Forensic Med Pathol 27:42–45
Harper ME, Bevilacqua L, Hagopian K, Weindruch R, Ramsey JJ (2004) Ageing, oxidative stress, and mitochondrial uncoupling. Acta Physiol Scand 182:321–331
International Programme on Chemical Safety IPCS (2000) International Chemical Safety Cards 0056, 0978, 0979, 0980, 0981, 0982 and 0984. World Health Organization, International Programme on Chemical Safety, Geneva
Jellinger KA (2003) General aspects of neurodegeneration. J Neural Transm Suppl 65:101–144
Johansson C, Castoldi AF, Onishchenko N, Manzo L, Vahter M, Ceccatelli S (2007) Neurobehavioral and molecular changes induced by methylmercury exposure during development. Neurotox Res 11:241–260
Johnson FO, Atchison WD (2009) The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis. Neurotoxicology 30:761–765
Jonasson IR, Boyle RW (1972) Geochemistry of mercury and origins of natural contamination of the environment. Can Min Metall Bull 65:32–39
Kennedy GJ, Golde TE, Tariot PN, Cummings JL (2007) Amyloid based interventions in Alzheimer's disease. CNS Spectr 12:1–14
Khan MA, Asaduzzaman AM, Schreckenbach G, Wang F (2009) Synthesis, characterization and structures of methylmercury complexes with selenoamino acids. Dalton Trans 29:5766–5772
Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R (2005) Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 11:894–899
Leistevuo J, Leistevuo T, Helenius H, Pyy L, Huovinen P, Tenovuo J (2002) Mercury in saliva and the risk of exceeding limits for sewage in relation to exposure to amalgam fillings. Arch Environ Health 57:366–370
Liu J, Killilea DW, Ames BN (2002) Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substrate binding affinity and activity in brain by feeding old rats acetyl-L-carnitine and/or R-alpha-lipoic acid. Proc Natl Acad Sci U S A 99:1876–1881
Lobanov AV, Hatfield DL, Gladyshev VN (2009) Eukaryotic selenoproteins and selenoproteomes. Biochim Biophys Acta 1790:1424–1428

Lu J, Holmgren A (2009) Selenoproteins. J Biol Chem 284:723–727
Magos L (2001) Review on the toxicity of ethylmercury, including its presence as a preservative in biological and pharmaceutical products. J Appl Toxicol 21:1–5
Magos L, Webb M, Clarkson TW (1980) The interactions of selenium with cadmium and mercury. CRC Crit Rev Toxicol 8:1–42
Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630
Mahley RW, Weisgraber KH, Huang Y (2006) Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. Proc Natl Acad Sci U S A 103: 5644–5651
Mason RP, Fitzgerald WF, Morel FMM (1994) The biogeochemical cycling of elemental mercury. Anthropogenic influences. Geochim Cosmochim Acta 58:3191–3198
McCabe MJ, Lawrence DA (1994) The effects of metals of the development of the immune system. In: Schook LB, Laskin DL (eds) Xenobiotics and inflammation. Academic Press, New York, pp 193–216
Morel FMM, Kraepiel AMI, Amyot M (1998) The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst 29:543–566
Mount C, Downtown C (2006) Alzheimer disease: progress or profit. Nat Med 12:780–784
Mutter J (2011) Is dental amalgam safe for humans? The opinion of the scientific committee of the European Commission. J Occup Med Toxicol 6:2–19
Mutter J, Yeter D (2008) Kawasaki's disease, acrodynia, and mercury. Curr Med Chem 15: 3000–3010
Mutter J, Naumann J, Sadaghiani C, Schneider R, Walach H (2004) Alzheimer disease: mercury as pathogenetic factor and apolipoprotein E as a moderator. Neuroendocrinol Lett 25:331–339
Mutter J, Naumann J, Guethlin C (2007) Comments on the article "The toxicology of mercury and its chemical compounds" by Clarkson and Magos (2006). Crit Rev Toxicol 47:537–549
Mutter J, Curth A, Naumann J, Deth R, Walach H (2010) Does inorganic mercury play a role in Alzheimer's disease? A systematic review and a integrated molecular mechanism. J Alzheimer Dis 22:357–374
Nicole A, Santiard-Baron D, Ceballos-Picot I (1998) Direct evidence for glutathione as mediator of apoptosis in neuronal cells. Biomed Pharmacother 52:349–355
Nielsen JB, Andersen O (1992) The toxicokinetics of mercury in mice offspring after maternal exposure to methylmercury-effect of selenomethionine. Toxicology 74:233–241
Onyido I, Norris AR, Buncel E (2004) Biomolecule–mercury interactions: modalities of DNA base-mercury binding mechanisms. Remediation strategies. Chem Rev 104:5911–5929
Opitz H, Schweinsberg F, Grossmann T, Wendt-Gallitelli MF, Meyermann R (1996) Demonstration of mercury in the human brain and other organs 17 years after metallic mercury exposure. Clin Neuropath 15:139–144
Pirrone N, Costa P, Pacyna JM, Ferrara R (2001) Mercury emissions to the atmosphere from natural and anthropogenic sources in the Mediterranean region. Atmos Environ 35:2997–3006
Pirrone N, Cinnirella S, Feng X, Finkelman RB, Friedli HR, Leaner J (2010) Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmos Chem Phys 10:5951–5964
Praline J, Guennoc AM, Limousin N, Hallak H, de Toffol B, Corcia P (2007) ALS and mercury intoxication: a relationship? Clin Neurol Neurosurg 109:880–883
Qiu C, De Ronchi D, Fratiglioni L (2007) The epidemiology of the dementias: an update. Curr Opin Psychiatry 20:380–385
Ralston NVC, Raymond LJ (2010) Dietary selenium's protective effects against methylmercury toxicity. Toxicology 278:112–123
Ralston NVC, Ralston CR, Blackwell JL 3rd, Raymond LJ (2008) Dietary and tissue selenium in relation to methylmercury toxicity. Neurotoxicology 29:802–811
Rasmussen PE (1994) Current methods of estimating atmospheric mercury fluxes in remote areas. Environ Sci Technol 28:2233–2241

Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW (2006) Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118:1845–1859

Raymond LJ, Ralston NVC (2004) Mercury: selenium interaction and health implication. SMDJ Seychelles Medical Dent J 7:72–77

Rivera-Mancia S, Pérez-Neri I, Rìos C, Tristàn-Lopez L, Rivera-Espinosa L, Montes S (2010) The transition metals copper and iron in neurodegenerative diseases. Chem Biol Interact 186: 184–199

Rocha JB, Freitas AJ, Marques MB, Pereira ME, Emanuelli T, Souza DO (1993) Effects of methylmercury exposure during the second stage of rapid postnatal brain growth on negative geotaxis and on delta-aminolevulinate dehydratase of suckling rats. Braz J Med Biol Res 26:1077–1083

Rosen DR, Bowling AC, Patterson D, Usdin TB, Sapp P, Mezey E, McKenna-Yasek D, O'Regan J, Rahmani Z, Ferrante RJ (1994) A frequent ala 4 to val superoxide dismutase-1 mutation is associated with a rapidly progressive familial amyotrophic lateral sclerosis. Hum Mol Genet 3:981–987

Satoh M, Nishimura N, Kanayama Y, Naganuma A, Suzuki T, Tohyama C (1997) Enhanced renal toxicity by inorganic mercury in metallothionein-null mice. J Pharmacol Exp Ther 283: 1529–1533

Schafer SG, Dawes RLF, Elsenhaus B, Forth W, Schumann K (1999) Metals. In: Marquardt H, Schafer SG, McClellan RO, Welsch F (eds) Toxicology. Academic Press, San Diego, p 782

Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW (2007) Effects of environmental methylmercury on the health of wild birds, mammals, and fish. AMBIO: J Human Environ 36:12–19

Schiraldi M, Monestier M (2009) How can a chemical element elicit complex immunopathology? Lessons from mercury-induced autoimmunity. Trends Immunol 30:502–509

Schurz F, Sabater-Vilar M, Fink-Gremmels J (2000) Mutagenicity of mercury chloride and mechanisms of cellular defence: the role of metal-binding proteins. Mutagenesis 15:525–530

Schwarz S, Husstedt IW, Bertram HP, Kuchelmeister K (1996) Amyotrophic lateral sclerosis after accidental injection of mercury. J Neurol Neurosurg Psychiatry 60:698

Seppanen K, Kantola M, Laatikainen R, Nyyssönen K, Valkonen VP, Kaarlöpp V, Salonen JT (2000) Effect of supplementation with organic selenium on mercury status as measured by mercury in pubic hair. J Trace Elem Med Biol 14:84–89

Shigenaga M, Hagen T, Ames B (1994) Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 91:10771–10778

Silbergeld EK, Woodruff S, Gutirrez P, McKenna K, Azad A, Sacci J (1998) Effects of mercury (HG) on immune function in male and female mice (Abstr. 1012). Toxicol Sci

Simmonds MP, Johnston PA, French MC, Reeve R, Hutchinson JD (1994) Organochlorines and mercury in pilot whale blubber consumed by Faroe islanders. Sci Total Environ 149:97–111

Singhal RK, Anderson ME, Meister A (1987) Glutathione, a first line of defense against cadmium toxicity. FASEB J 1:220–223

Sinicropi MS, Amantea D, Caruso A, Saturnino C (2010) Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning. Arch Toxicol 84:501–520

Soares FA, Farina M, Santos FW, Souza D, Rocha JB, Nogueira CW (2003) Interaction between metals and chelating agents affects glutamate binding on brain synaptic membranes. Neurochem Res 28:1859–1865

Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B (1998) Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. J Neurochem 71:2112–2122

Stoewsand GS, Bache CA, Lisk DJ (1974) Dietary selenium protection of methylmercury intoxication of Japanese quail. Bull Environ Contam Toxicol 11:152–156

Strittmatter WJ (1996) Roses Alzheimer's disease. Apoliprotein E and Alzheimer's disease. Annu Rev Neurosci 19:53–77

Sugiura Y, Hojo Y, Tamai Y, Tanaka H (1976) Letter: selenium protection against mercury toxicity. Binding of methylmercury by the selenohydryl-containing ligand. J Am Chem Soc 98: 2339–2341

Sutton DJ, Tchounwou PB (2007) Mercury induces the externalization of phosphatidyl-serine in human renal proximal tubule (HK-2) cells. Int J Environ Res Public Health 4:138–144

Swain EB, Jakus PM, Rice G, Lupi F, Maxson PA, Pacyna JM, Penn A, Spiegel SJ, Veiga MM (2007) Socioeconomic consequences of mercury use and pollution. Ambio 36:45–61

Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G (2003) Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 24:667–674

Tan SW, Meiller JC, Mahaffey KR (2009) The endocrine effects of mercury in humans and wildlife. Crit Rev Toxicol 39(3):228–269

Tanaka M, Kovalenko SA, Gong JS, Borgeld HJ, Katsumata K, Hayakawa M, Yoneda M, Ozawa T (1996) Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. Ann N Y Acad Sci 786:102–111

Tiraboschi P, Hansen LA, Thal LJ, Corey-Bloom J (2004) The importance of neuritic plaques and tangles to the development and evolution of AD. Neurology 62:1984–1989

Tsubaki T, Irukajama K (1997) Minamata disease. Methylmercury poisoning in Minamata and Niigata, Japan. In: Tsubaki T, Irukajama K (eds) Minamata disease. Methylmercury poisoning in Minamata and Niigata, Japan. Kodansha, Tokyo, pp 317. ISBN 0-444-99816-0

Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12:1161–1208

Vather M, Akesson A, Lind B, Bjors U, Schutz A, Berglung M (2000) Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ Res 84:186–194

Wagner C, Sudati JH, Nogueira CW, Rocha JB (2010) In vivo and in vitro inhibition of mice thioredoxin reductase by methylmercury. Biometals 23:1171–1177

Walsh CT (1982) The influence of age on the gastrointestinal absorption of mercury chloride and methylmercury chloride in the rat. Environ Res 27:412–420

Waly M, Olteanu H, Banerjee R, Choi SW, Mason JB, Parker BS, Sukumar S, Shim S, Sharma A, Benzecry JM, Power-Charnitsky VA, Deth RC (2004) Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal. Mol Psychiatry 9:358–370

Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, Slavkovich V, Loiacono NJ, Levy D, Cheng Z, Graziano JH et al (2004) Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 112:1329–1333

Wei YH, Lu CY, Lee HC, Pang CY, Ma YS (1998) Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. Ann N Y Acad Sci 854:155–170

Weihe P, Joensen HD (2012) Dietary recommendation regarding pilot whale meat and blubber in the Faroe Islands. Int J Circumpolar Health 71:18594–18598

World Health Organization (2007) Exposure to mercury: a major public health concern, Preventing disease through healthy environment. World Health Organization, Geneva

Xu F, Farkas S, Kortbeek S, Zhang F-X, Chen L, Zamponi GW, Syed NI (2012) Mercury-induced toxicity of rat cortical neurons is mediated through N-methyl-D-aspartate receptors. Mol Brain 5:30–57

Yin Z, Milatovic D, Aschner JL, Syversen T, Rocha JBT, Souza DO, Sidoryk M, Albrecht J, Aschner M (2007) Methylmercury induces oxidative injury, alterations in permeability and glutamine transport in cultured astrocytes. Brain Res 1131:1–10

Yoshizawa K, Rimm EB, Morris JS, Spate VL, Hsieh CC, Spiegelman D (2002) Mercury and the risk of coronary heart disease in men. N Engl J Med 347:1755–1760

Zareba G, Cernichiari E, Hojo R, Nitt SM, Weiss B, Mumtaz MM, Jones DE, Clarkson TW (2007) Thimerosal distribution and metabolism in neonatal mice: comparison with methylmercury. J Appl Toxicol 27:511–518

E-Waste Disposal Effects on the Aquatic Environment: Accra, Ghana

Jingyu Huang, Philip Nti Nkrumah, Desmond Ofosu Anim, and Ebenezer Mensah

Contents

1 Introduction 20
2 Management of E-Waste in Ghana 21
2.1 E-Waste Recycling in Ghana 22
2.2 Proximity of the Agbogbloshie E-Waste Disposal Site to Water Bodies 22
3 E-Waste Contaminants 24
4 Effects of E-Waste Contaminants on Aquatic Organisms 25
4.1 Effects of Heavy Metals on Aquatic Life 26
4.2 Effects of Organic Pollutants on Aquatic Life 28
5 Summary 29
References 30

J. Huang
Ministry of Education Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Hohai University, Nanjing, China

P.N. Nkrumah (✉)
Ministry of Education Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Hohai University, Nanjing, China

Department of Agricultural Engineering, Kwame Nkrumah University of Science and Technology, PMB, Kumasi, Ghana

College of Environment, Hohai University, No. 1 Xikang Road, Nanjing 210098, China

D.O. Anim
College of Environment, Hohai University, No. 1 Xikang Road, Nanjing 210098, China

E. Mensah
Department of Agricultural Engineering, Kwame Nkrumah University of Science and Technology, PMB, Kumasi, Ghana

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229*, Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6_2,

1 Introduction

In most developing countries, the rapid pace of urbanization is a challenge to urban environmental management. One major challenge of waste management facing some urban areas is electronic waste (e-waste). The increasingly rapid evolution of electronic technology, coupled with rapid product obsolescence, has compounded the e-waste problem (Otsuka et al. 2012).

The amount of e-waste generated globally is growing at a rate nearly three times faster than the growth of overall municipal solid waste (Schluep et al. 2009). According to UNEP (2010), the annual e-waste generated worldwide is estimated to be 20–50 million tons (t). Unfortunately, between 50% and 80% of such e-waste is prospectively exported to developing countries like Ghana, China, India and Nigeria (Puckett and Smith 2002; UNEP 2005; Orisakwe and Frazzoli 2010; Environmental Investigation Agency 2011; Lundstedt 2011).

The University of Ghana's Institute for Environment and Sanitation Studies reported that Ghana has been identified as a popular dumping ground for old electronics (Koranteng and Darko 2011), making e-waste an alarming and growing menace in the country. This accumulated e-waste is poorly managed in the country, because proper systems for recycling and disposal of them are lacking (Nordbrand 2009; Darko 2010). According to Amoyaw-Osei et al. (2011), hundreds of tons of e-waste end up at the scrap yards in Ghana every month, where it is disassembled to extract valuable components and metals. Specifically, the main center for recovering and recycling e-waste materials is the Agbogbloshie Scrap Market in the Greater Accra region (Brigden et al. 2008). Prakash et al. (2010) estimated that about 8,000 metric t of e-waste is being treated annually at the Agbogbloshie metal scrap yard.

The uncontrolled dumping and inappropriate recycling of e-waste poses serious threats to human health and the environment at large (Prakash et al. 2010), because e-waste contains a multitude of hazardous substances that may be released as the waste is handled and processed (Lundstedt 2011; Tysdenova and Bengtsson 2011). The toxic chemicals that exist in e-waste include a wide range of heavy metals, such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As) and nickel (Ni), and also persistent organic compounds, such as brominated flame retardants (BFRs) and phthalates. Other chemicals that appear in e-waste include the polychlorinated biphenyls (PCBs), nonylphenol (NP), and triphenyl phosphate (TPPs), among others (Azuka 2009; Robinson 2009). A study conducted by Greenpeace International (2008) at the Agbogbloshie scrap yard showed that some samples contained Cd, Hg and Pb in quantities that are considered especially toxic to aquatic life.

Unfortunately, according to the Director of the Chemicals Control and Management Centre of the Ghana Environmental Protection Agency, data on the adverse impact of e-waste on human health and the environment in Ghana is highly limited (Pwamang 2009). It is sad to note that research conducted to date at the main Ghanaian e-waste dumpsite (Agbogbloshie) has been only on a small scale, and as a result, the true extent of e-waste chemical contamination at the site is largely unknown (Caravanos et al. 2011). Moreover, to the best of our knowledge, the environmental concerns of e-waste in particular have not yet been properly addressed in Ghana (Agyei-Mensah and Oteng-Ababio 2012; Asante et al. 2012; Otsuka et al. 2012; Caravanos et al. 2013; Riederer et al. 2013).

Notwithstanding the dearth of knowledge, there is concern for the contamination at the Agbogbloshie disposal site because of its close proximity to other water bodies, such as the Odaw River and its estuary, and the Korle lagoon. This near proximity, coupled with the incessant floods that occur in the area, enhances the probability that aquatic life will be exposed to e-waste. According to Nixon et al. (2007), Korle Lagoon, which is in the downstream estuary, has become one of the most polluted water bodies on earth, and this is clearly a very serious concern.

In the present paper, our goal is to review the potential hazards posed by e-waste contaminants on the aquatic environment at the Agbogbloshie disposal site in Ghana, which we contend typifies the situation in the majority of developing countries. To achieve our goal, we estimated the volume of e-waste disposed of in Ghana, and evaluated the available e-waste management options. We sought to know what chemical contaminants are commonly detected in the various kinds of e-waste disposed of in Ghana and assessed the significance of the Agbogbloshie disposal site's proximity to nearby water bodies. In addition, we analyzed the factors that may facilitate the contamination of the water bodies by hazardous substances that exist in e-waste. Ultimately, we assessed the possible adverse effects to aquatic life from release of e-waste-associated chemical contaminants (primarily heavy metals and organic compounds).

2 Management of E-Waste in Ghana

As mentioned in the introduction, Ghana has become a popular dumping ground for e-waste. Despite the enormous amounts of e-waste that is dumped in Ghana, there is no clear measure for how many used-computer or other electronic-equipment shops exist around the country; nor is the quantity of e-waste that now exists in the country well established. Orisakwe and Frazzoli (2010) reported that the main reason for this is that records are simply not kept on these parameters. However, limited data do suggest a rise in the quantity of e-waste that has been imported to Ghana since 2003 (Fig. 1). The main components of e-waste processed at Ghanaian scrap yards include obsolete computers and televisions (Brigden et al. 2008).

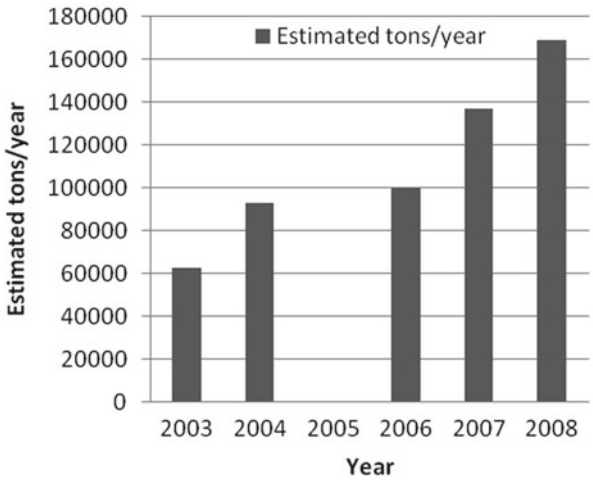


Fig. 1 Estimated quantity of E-waste imported into Ghana (Adapted from UN Comtrade 2010)

2.1 E-Waste Recycling in Ghana

The e-waste recycling activities in Ghana are mainly performed by the informal sector. This sector uses rudimentary methods to salvage copper and other metallic components that can be sold. For example, the e-waste is dismantled and sometimes burned (Amoyaw-Osei et al. 2011; Lundstedt 2011). During the recycling process, simple hand tools such as hammers, chisels, or even stones are employed to break electronic devices down to their individual components. Materials of no value are disposed of in a large area at the disposal sites. Such materials are piled up on the dump site and are periodically burned to reduce volume. It must be noted that wet chemical leaching processes, often associated with the recovery of precious metals from printed wiring boards (PWBs), have not been observed in Ghana (Prakash et al. 2010; Amoyaw-Osei et al. 2011). However, there are indications that these PWBs are exported to Asia for further processing (Grant and Oteng-Ababio 2012).

2.2 Proximity of the Agbogbloshie E-Waste Disposal Site to Water Bodies

Agbogbloshie is located geographically at 05°35′N and 00°06′W (Fig. 2). The town covers an area of approximately 16 km^2 and has a population of about 40,000. It lies within the tropics.

The Agbogbloshie scrap yard, as depicted in Fig. 2, is situated on flat ground on the left bank of the Odaw River, and in the upper reaches of the Korle Lagoon in Accra (Amoyaw-Osei et al. 2011; Caravanos et al. 2011; Oteng-Ababio 2012). These water bodies adjacent to the disposal site form part of one of the major catchments (Odaw-Korle) in the Accra metropolis, and cover an area of 250 km^2.

The mean annual rainfall in Ghana is estimated to be 1,187 mm (FAO 2005) and the average annual rainfall in the Accra Metropolitan Assembly is about 730 mm (Accra Metropolitan Assembly 2006). This region has a distinct rainy season, in which routine heavy rains fall primarily during two rainy seasons. There are two rainfall peaks, one notably in June and the other in October. Rain usually falls during intensive short storms and gives rise to local flooding. The flooding particularly affects low-lying areas such as the Agbogbloshie scrap yard. We must emphasize that the lower-lying lagoons and the Odaw river, which ultimately flow into the ocean, are just adjacent to the disposal site. Therefore, the threat that moving water will leach or wash contaminants into the local water bodies is high; once such contaminated run-off occurs, the threat to aquatic organisms in these water bodies is also high. Actually, Biney (1998) classified urban run-off as one of the main types of pollution that reaches the Odaw-Korle catchment. Brigden et al. (2008) confirmed that many chemicals present in the Korle lagoon sediment were the same as, or were similar to, those found at the contaminated sites where waste was burned or processed; site sampling suggested that pollutants migrated from the burning sites

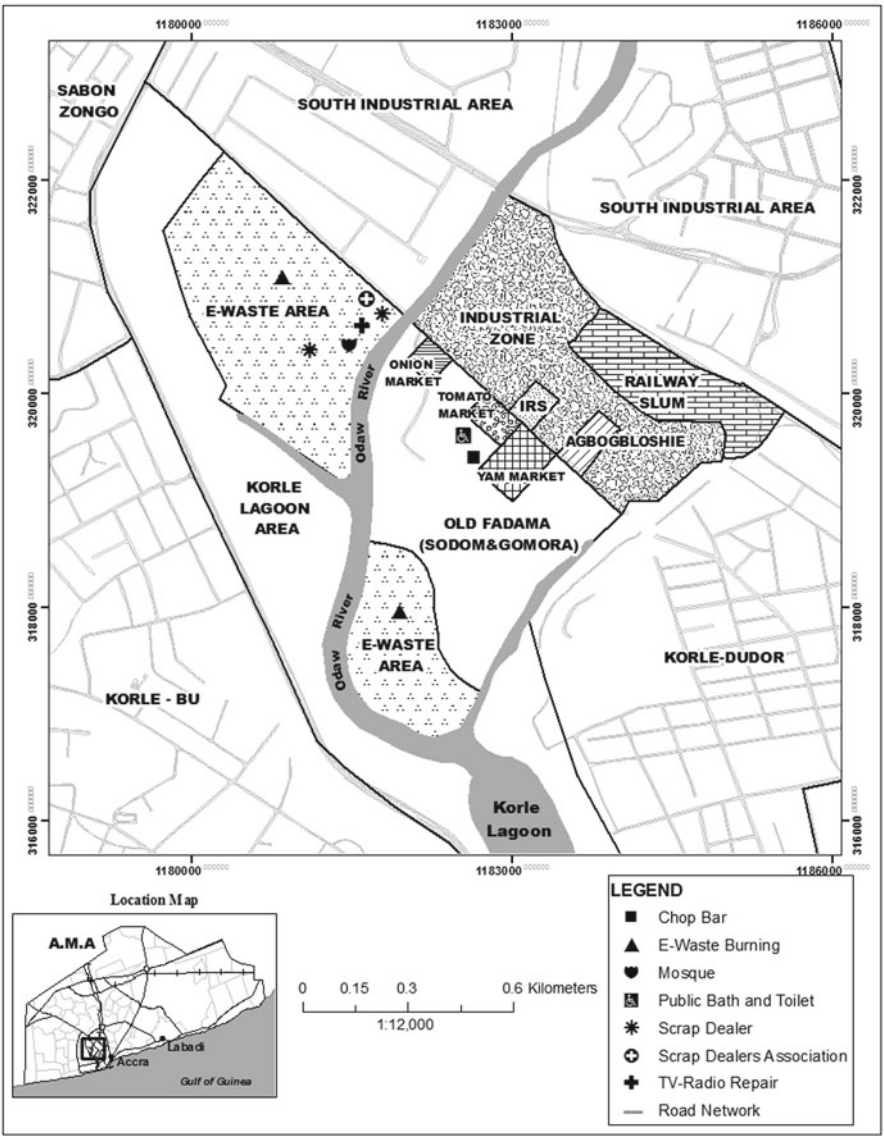


Fig. 2 Map of the Agbogbloshie E-waste recycling site in Ghana (Adapted from Oteng-Ababio 2012)

to surface waters, probably as a result of heavy rainfall and flooding. The direct disposal of e-waste into water bodies is augmented by the introduction of these same contaminants into the same water bodies via leaching.

Another mechanism that is likely to introduce e-waste contaminants to the local water bodies is atmospheric deposition. The region lies within the dry coastal

equatorial climatic zone and is therefore rather dry (Ghana Districts 2006). This fact, coupled with the open burning practices that take place during e-waste recycling, results in the formation of thick fumes. The fume particles may be introduced to the nearby surface waters through wet or dry atmospheric deposition. We describe the contaminants that are introduced to the local water bodies in Sect. 3.

3 E-Waste Contaminants

E-waste contains numerous hazardous chemicals and materials (e.g., heavy metals such as lead and cadmium, and many chlorinated or brominated organic compounds). Some of the types of chemicals found in e-waste are identified in Table 1.

The occurrence of these contaminants within the local environment of the e-waste recycling site in Ghana cannot be disputed. Brigden et al. (2008), Caravanos et al. (2011) and Otsuka et al. (2012) have tested soil and ash samples at the Agbogbloshie site, and have identified high concentrations of toxic metals (in quantities that were as much as 20-times above background levels), such as zinc, lead and copper, and organic chemicals, such as the phthalates and

Table 1 The nature of chemical contaminants that exist in E-waste

Chemical	Source of these components
Lead	Glass of cathode ray tubes (CRT) in televisions and monitors, lead-acid batteries, polyvinyl chloride (PVC) cables
Arsenic	Integrated circuit boards
Beryllium	Connectors; Mother boards and finger clips
Polychlorinated biphenyls (PCBs)	Electrical transformers, capacitors, PVC
Cadmium	Switches, solder joints, Housing, PVC cables, cathode ray tubes, rechargeable Batteries
Polybrominated diphenyl ethers (PBDEs)	Casings
Polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs)	Formation during thermal processes
Nonylphenol (NP)	Insulators, Housing, Casing
Triphenyl phosphate (TPP)	Casings of computer monitors
polychlorinated naphthalenes (PCNs)	Capacitors, insulated wires
Mercury (Hg)	Batteries, flat screen electronic displays, switches, relays, Housing
Phthalates (e.g., di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP))	PVC
Polycyclic aromatic hydrocarbons (PAHs)	Formation during thermal processes
Chromium	Steel housing
Barium	CRT, Vacuum tubes

Source: Adapted from Nordic Council of Ministers (1995), Matthews (1996), Microelectronics and Computer Technology Corporation (1996), OECD (2003), Brigden et al. (2005), Azuka (2009), Environmental Investigation Agency (2011) and Lundstedt (2011)

polybrominated diphenyl ethers (PBDEs). However, we also note that studies on the detection of harmful chemicals in the sediments of the nearby water bodies are extremely limited.

4 Effects of E-Waste Contaminants on Aquatic Organisms

Obviously, the introduction of the e-waste contaminants to the local water bodies poses hazards to the local aquatic organisms. A study conducted by the Institute for Applied Ecology (the Öko-Institut) indicated that local residents living near the lagoon, where uncontrolled dumping and e-waste recycling activities occur, lamented the adverse impacts of the site on the aquatic life of nearby water bodies (Prakash et al. 2010). This study revealed that the lagoon, which used to be a common fishing ground for the residents of the local communities until a few years ago, is now heavily polluted. As a result, many aquatic species in the lagoon have been eliminated. Amoyaw-Osei et al. (2011) also observed that the Odaw River, which was formerly an important fishing ground, has become dead because of the extensive pollution caused by uncontrolled dumping and the crude processing of e-waste in the area.

According to the US EPA (1998), an ecological risk assessment method can be applied to predict the potential adverse effects of bioaccumulative pollutants to organisms by comparing the exposure concentrations to an effect concentration. In Table 2, we compare sediment concentrations of contaminants from Korle Lagoon with sediment toxicity thresholds for saltwater benthos proposed by Australia, Canada, and by Long et al. (1995) working in the United States. The metals posing the highest potential risk in Korle Lagoon sediments appear to be copper, lead and zinc, whose concentrations ranged from 20-times higher than the sediment

Table 2 A comparison of high-level contaminants in Korle Lagoon, with sediment quality guideline values for saltwater benthos of Australia and New Zealand (ANZECC) and Canada. In columns 2–4, two values are given for each sediment quality guideline; the lower one is the one below which no effects are expected; the higher one is associated with an increased probability of adverse effects (concentrations are given in mg kg^{-1} dry wt)

Contaminant	Korle Lagoon[a]	Long et al. (1995)	ANZECC 2012[b]	Canada 2012[c]
Cadmium	6	1.2/9.6	1.5/10	0.7/4.2
Chromium	34	81/370	80/370	52.3/160
Copper	2,260	34/270	65/270	18.7/108
Lead	1,685	47/218	50/220	30.2/112
Mercury	<0.5	1.0/3.7	0.15/1	0.13/0.7
Zinc	2,425	150/410	200/410	124/271

[a] Source: Brigden et al. (2008)
[b] ANZECC (2012)
[c] CCME (2012)

concentration most associated with adverse effects to benthos (Cu), 15-times higher (Pb), and 5.6-times higher (Zn).

The sediment toxicity thresholds shown in Table 2 do not account for site-specific influences on the bioavailability of contaminants, and pertain mainly to toxicity arising from short-term (10–30 day) sediment exposures, rather than chronic toxicity, and effects arising from the concentration of contaminants in the aquatic food chains. Thus, Table 2 incompletely characterizes potential risks associated with Korle Lagoon sediments and surface waters. Nevertheless, concentrations of copper, lead and zinc are high enough in the sediments to warrant further investigations into the effects of these and other contaminants, and implementation of controls on their release into the water bodies. It must be pointed out that we have predicted pollutant effects on aquatic life, based on their release from sediments into the water column, which is expected from both diffusion and advection, and depends on the pH and Eh of the sediments (Reuber et al. 1987; Simpson et al. 1998, 2000, 2002).

4.1 Effects of Heavy Metals on Aquatic Life

The adverse impacts that heavy metals impose on aquatic life have been well established. Heavy metals are highly persistent, toxic in trace amounts, and can potentially induce severe oxidative stress in aquatic organisms (Frazier 1979; Nammalwar 1983; Forster and Whittmann 1983; Meria 1991; Al-Masri et al. 2002; Karbassi et al. 2006; Guo et al. 2009; Woo et al. 2009; Jakimska et al. 2011). Aquatic organisms may absorb heavy-metal pollutants directly from water or indirectly via uptake from the food chain.

Metals may typically act together to potentiate toxicity (Corrill and Huff 1976). Nammalwar (1983) addressed both the indirect and direct effects by which heavy metals affect aquatic organisms. The author noted that indirect effects are produced on food chain organisms and via ecological stress, whereas direct effects are observed on behavior, migration, physiology, metabolism, reproduction, development and growth of aquatic animals. In this review, we considered and addressed the direct effects caused by these pollutants on aquatic organisms.

Contamination of a river with heavy metals may cause effects on the ecological balance of the aquatic environment, and may narrow the diversity of aquatic organisms as the extent of contamination increases (Ayandiran et al. 2009). Khayatzadeh and Abbasi (2010) reported that heavy metals in polluted reservoirs may also affect fish species at the population level.

Stohs and Bagchi (1995) and Leonard et al. (2004) reported that molecular mechanisms of heavy metal cytotoxicity include the following: damage to plasma membranes, binding to proteins and phospholipids, inhibition of Na- &, K-dependent ATPases, inhibition of transmembrane amino acid transport; enzyme inhibition; lipid peroxidation and oxidative DNA damage, along with depletion of antioxidant enzymes via generation of Reactive Oxygen Species (ROS).

Toxicity of Lead to Aquatic Organisms

Lundstedt (2011) reported that lead, a particularly problematic metal, is highly abundant in e-waste. Pb accumulates in the environment and produces both high acute and chronic effects on biological systems (i.e., plants, animals and micro-organisms), even at low concentrations (Biesinger et al. 1972; LeBlanc 1982). Lead causes behavioral disturbances, affects survival, growth, learning and metabolism, and inorganic compounds of lead may be carcinogenic (Weber and Dingel 1997; Ribeiro et al. 2009; Huang et al. 2010). Moreover, Pb causes scoliosis in fish (Stomiñska and Jezierska 2000).

Chronic toxicity occurs when lead is bioconcentrated in aquatic species over a period of time, and when it accumulates in internal organs. However, biomagnification has not been observed to occur in the aquatic environment (Prosi 1989). The author noted that the dissolved chemical forms of lead are extremely toxic in the aquatic environment, when present at high concentrations.

When lead concentrations in algae exceed 500 ppb, enzymes needed for photosynthesis are inhibited (Nagpal 1987; Rioboo et al. 2009). When photosynthesis is reduced, algal growth is adversely affected. Decreased algal growth means less food for animals, which has repercussions for the entire ecosystem. Lead also has dire effects on fish. The primary mode of uptake of aqueous Pb^{2+} in freshwater fishes is through their gills into the blood stream (Seymore et al. 1995). Once absorbed, Pb^{2+} is distributed particularly to the liver, kidney, heart and male gonads (ATSDR 2005). When lead concentrations exceed 100 ppb, gill function is affected. Fishes exposed to high levels of lead exhibit a wide-range of effects including muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems, and paralysis (US EPA 1976; Eisler 1988; Rademacher et al. 2003). Acute effects of Pb on freshwater invertebrates (for water exposures and food chain exposures) are normally reported at concentrations of 100–100,000 µg/L (Nagpal 1987; Boyle et al. 2010; Mager et al. 2010, 2011).

Toxicity of Cadmium to Aquatic Organisms

Cd is a nonessential heavy metal and is considered to be one of the most toxic of aquatic contaminants. Cd can cause toxicity to organisms at each biological level, from populations and communities to cellular elements (Rashed 2001). Even at sub-lethal concentrations, Cd has a cumulative effect and may cause serious disturbances to fish metabolism that produces abnormal behavior, locomotor anomalies or anorexia (Bryan et al. 1995; Cicik and Engin 2005). Hayat et al. (2007) noted that long-term exposure (20 days or more) of juvenile and adult rainbow trout, *Oncorhynchus mykiss,* to waterborne cadmium at sub-lethal concentrations resulted in decreased growth. It is reported that Cd primarily accumulates in fish in the liver, stomach and gills (Abu Hilal and Ismail 2008). Solomon (2008) stated that Cd impairs aquatic plant growth and thus, adversely affects the entire aquatic ecosystem, since green plants are at the base of all food chains.

4.2 Effects of Organic Pollutants on Aquatic Life

Toxicity of PBDEs to Aquatic Organisms

PBDEs are of significant environmental concern because they are toxic, bio-accumulative and persistent (Michigan Department of Environmental Quality 2007). In particular, it was observed in OECD testing that these compounds are not naturally biodegradable (EU draft RAR 2000). They easily bio-accumulate in fatty tissues and bio-magnify throughout food chains (Law et al. 2006). Wollenberger et al. (2005) reported the PBDEs to be very toxic to aquatic organisms. However, current knowledge concerning their effects on aquatic organisms is limited (Breitholtz and Wollenberger 2003; Tam et al. 2012).

Nevertheless, we note that adverse effects on neurobehavioral development (Branchi et al. 2005) and endocrine disruption (Muirhead et al. 2006) have been reported in the literature for PBDE compounds. PBDEs are known to cause many deformities in aquatic organisms, and these morphological abnormalities are more pronounced during embryogenesis (Lema et al. 2007). Specifically, Mhadhbi et al. (2010) observed abnormal skeletal formations and pericardial edema in turbot. These authors also noted that PBDEs are teratogenic to the embryo–larval stages, during which embryo development is adversely affected, perhaps leading to embryo mortality.

Toxicity of the PCDD/Fs to Aquatic Organisms

The polychlorodibenzodioxins and furans (PCDD/Fs) are persistent organic pollutants displaying high toxicity and bioaccumulation potential (US EPA 2000). They tend to biomagnify in higher trophic levels of the aquatic food web. In a study conducted for the Ontario Ministry of the Environment, the authors noted that because PCDD/Fs are hydrophobic, the majority of them released into aquatic systems tend to bind to the organic fraction of suspended and/or bed sediments (Dillon Consulting Limited 2007). They also have an affinity for lipid-rich tissues of aquatic organisms. According to the Canadian Council of Ministers of the Environment (2001), aquatic organisms may either take up PCDD/Fs from water or sediment, or by consuming contaminated prey.

In fish, PCDD/Fs are thought to elicit most, if not all, of their toxic and biochemical effects via the aryl hydrocarbon (Ah) receptor (Environment Canada 2000). The adverse consequences of PCDD/Fs on fish are primarily manifested as reproductive effects such as survival of eggs and embryos (Dillon Consulting Limited 2007). As a result, the long-term population of local species is negatively affected. The authors noted that PCDD/Fs also affect the survival, growth and reproduction of adult fish.

5 Summary

The volume of e-waste is growing around the world, and, increasingly, it is being disposed of by export from developed to developing countries. This is the situation in Ghana, and, in this paper we address the potential consequences of such e-waste disposal. Herein, we describe how e-waste is processed in Ghana, and what the fate is of e-waste-chemical contaminants during recycling and storage. Finally, to the extent it is known, we address the prospective adverse effects of e-waste-related contaminants on health and aquatic life downstream from a large e-waste disposal facility in Accra, Ghana.

In developing countries, including Ghana, e-waste is routinely disassembled by unprotected workers that utilize rudimentary methods and tools. Once disassembled, e-waste components are often stored in large piles outdoors. These processing and storage methods expose workers and local residents to several heavy metals and organic chemicals that exist in e-waste components. The amount of e-waste dumped in Ghana is increasing annually by about 20,000 t. The local aquatic environment is at a potential high risk, because the piles of e-waste components stored outside are routinely drenched or flooded by rainfall, producing run-off from storage sites to local waterways. Both water and sediment samples show that e-waste-related contaminants have entered Ghana's water ways.

The extent of pollution produced in key water bodies of Ghana (Odaw River and the Korle Lagoon) underscores the need for aquatic risk assessments of the many contaminants released during e-waste processing. Notwithstanding the fact that pollutants from other sources reach the water bodies, it is clear that these water bodies are also heavily impacted by contaminants that are found in e-waste. Our concern is that such exposures have limited and will continue to limit the diversity of aquatic organisms. There have also been changes in the abundance and biomass of surviving species and changes in food chains. Therefore, the need for actions to be taken to reduce entry of e-waste pollutants into Ghana's aquatic environment is real and is immediate.

Heavy metals (e.g., lead, cadmium, copper and zinc) and organic pollutants (e.g., PCDD/Fs and PBDEs) have been detected in the sediments of local water bodies in quantities that greatly exceed background levels. This fact alone suggests that aquatic organisms that live in the affected water bodies are highly exposed to these toxic, bio-accumulative, and persistent contaminants. These contaminants have been confirmed to result from the primitive methods used to recycle and process e-waste within the local environment.

Only limited local data exist on the threats posed by these e-waste-related contaminants on nearby natural resources, especially aquatic organisms. In this review, we have addressed the potential toxicity of selected heavy metals and organic pollutants on aquatic organisms. Since there are no data on concentrations of contaminants in the water column, we have based our predictions of effects on pollutant release rates from sediments. Pollutants that are attached to sediments are routinely

released into the water column from diffusion and advection, the rate of which depends on pH and Eh of the sediments. E-waste contaminants have the potential to produce deleterious effects on the behavior, physiology, metabolism, reproduction, development and growth of many aquatic organisms. Because it is confirmed that both heavy metal and organic contaminants are reaching the biota of Ghana's local waterways, we presume that they are producing adverse effects. Because local data on the aquatic toxicity of these contaminants are as yet unavailable, we strongly recommend that future research be undertaken to examine, on a large scale and long-term basis, both contamination levels in biota, and adverse effects on biota of the nearby water bodies.

Acknowledgement The authors are grateful for the financial support from the National Program on Key Basic Research Project of China (2012CB719804), the Natural Science Foundation of China (51208177), the Fundamental Research Funds for the Central Universities (2012B03414) and the Natural Science Foundation of Jiangsu Province (BK2012086). We are also extremely grateful to Dr. Dave Whitacre, RECT Editor, for his excellent comments and editing of the manuscript. The authors acknowledge all anonymous reviewers for their time and profound suggestions. We would also like to thank Cecilia Kwofie, Amos Tiereyagn Kabo-bah, Jennifer Abena Kwofie, Akosua Korantemaa Agadzi and Thelma Dede Baddoo for their assistance and language editing.

References

Abu Hilal AH, Ismail NS (2008) Heavy metals in eleven common species of fish from the Gulf. J Biol Sci 1(1):13–18

Accra Metropolitan Assembly (2006) Metro information. http://ama.ghanadistricts.gov.gh/?arrow=dnf&_=3&r=1&rlv=climate. Accessed Mar 2013

Agyei-Mensah S, Oteng-Ababio M (2012) Perceptions of health and environmental impacts of e-waste management in Ghana. Int J Environ Health Res 22(6):500–517

Al-Masri M, Aba S, Khalil AH, Al-Hares Z (2002) Sedimentation rates and pollution history of a dried lake. Sci Total Environ 293(1–3):177–189

Amoyaw-Osei Y, Opoku Agyekum O, Pwamang JA, Mueller E, Fasko R (2011) Ghana e-waste country assessment. United Nations Environment Programme (UNEP). http://ewasteguide.info/files/Amoyaw-Osei_2011_GreenAd-Empa.pdf. Accessed Sept 2012

Asante KA, Agusa T, Biney CA, Agyekum WA, Bello M, Otsuka M, Itai T, Takahashi S, Tanabe S (2012) Multi-trace element levels and arsenic speciation in urine of e-waste recycling workers from Agbogbloshie, Accra in Ghana. Sci Total Environ 424:63–73

ATSDR (2005) Draft toxicological profile for lead. US Department of Health and Human Services, Atlanta, pp 102–225

Ayandiran TA, Fawole OO, Adewoye SO, Ogundiran MA (2009) Bioconcentration of metals in the body muscle and gut of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluent. JCAB 3(8):113–118

Azuka AI (2009) The influx of used electronics into Africa: a perilous trend. LEAD J 5(1): 90–106

Biesinger KE, Christensen GM, Fiandt JT (1972) Effects of metal salt mixtures on *Daphnia magna* reproduction. Ecotoxicol Environ Saf 11:9–14

Biney CA (1998) The threat of pollution to the coastal zone of the Greater Accra metropolitan area, Ghana. Ghana J Sci 31(36):47–54

Boyle D, Amlund H, Lundebye A, Hogstrand C, Bury NR (2010) Bioavailability of a natural lead-contaminated invertebrate diet to zebrafish. Environ Toxicol Chem 29:708–714

Branchi I, Capone F, Vitalone A, Madia F, Santucci D, Alleva E, Costa LG (2005) Early developmental exposure to BDE 99 or aroclor 1254 affects neurobehavioural profile: interference from the administration route. Neurotoxicology 26:183–192

Breitholtz M, Wollenberger L (2003) Effects of three PBDEs on development, reproduction and population growth rate of the harpacticoid copepod *Nitocra spinipes*. Aquat Toxicol 64(1): 85–96

Brigden K, Labunska I, Santillo D, Allsopp M (2005) Recycling of electronic wastes in China and India: workplace and environmental contamination. Greenpeace Research Laboratories Technical Note 09/2005, 56 pp

Brigden K, Labunska I, Santillo D, Johnston P (2008) Chemical contamination at e-waste recycling and disposal sites in Accra and Koforidua, Ghana. Greenpeace Research Laboratories Technical Note 2008, pp 1–23

Bryan MD, Atchison GJ, Sandheinrich MB (1995) Effects of cadmium on the foraging behavior and growth of juvenile bluegill, *Lepomis macrochirus*. Can J Fish Aquat Sci 52:1630–1638

Canadian Council of Ministers of the Environment (2001) Canadian sediment quality guidelines for the protection of aquatic life: polychlorinated dioxins and furans (PCDD/Fs). In: Canadian environmental quality guidelines, 1999. Canadian Council of Ministers of the Environment, Winnipeg. http://ceqg-rcqe.ccme.ca. Accessed Sept 2012

Caravanos J, Clark E, Fuller R, Lambertson C (2011) Assessing worker and environmental chemical exposure risks at an e-waste recycling and disposal site in Accra, Ghana. J Health Pollut 1:16–25

Caravanos J, Clarke EE, Osei CS, Amoyaw-Osei Y (2013) Exploratory health assessment of chemical exposures at e-waste recycling and scrap yard facility in Ghana. J Health Pollut 3(4):11–22

CCME (2012) Canadian environmental quality guidelines. http://ceqg-rcqe.ccme.ca/. 18 Dec 2012, from http://www.ccme.ca/assets/pdf/wqg_aql_protocol.pdf. Accessed June 2013

Cicik B, Engin K (2005) The effect of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio L.* (1758). Turk J Vet Anim Sci 29:113–117

UN Comtrade (2010) United Nations Commodity Trade Statistics Database, International Trade Center (ITC) Country Profile, Trade in goods statistics. http://www.intracen.org/dbms/country/. Accessed July 2012

Corrill LS, Huff JE (1976) Occurrence, physiologic effects, and toxicity of heavy metals-arsenic, cadmium, lead, mercury, and zinc-in marine biota: an annotated literature collection. Environ Health Persp 18:181–219

Darko R (2010) Electronic waste dumping on Ghana still continues. http://www.ghanaweb.com/GhanaHomePage/features/artikel.php?ID=188950. Accessed July 2012

Dillon Consulting Limited (2007) Ecological risk assessment for the Trent River mouth sediment depositional areas. Final report. http://www.ene.gov.on.ca/stdprodconsume/groups/lr/@ene/@resources/documents/resource/std01_079840.pdf. Accessed Sept 2012

Eisler R (1988) Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. fish and wildlife service biological report 85 (1.14), Contaminant Hazard Reviews report 14, 134 pp

Environment Canada (2000) Canadian sediment quality guidelines for the protection of aquatic life and Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota: polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs). Technical supporting document, 2 vols. National Guidelines and Standards Office, Environmental Quality Branch, Ottawa

Environmental Investigation Agency (2011) System failure: The UK's harmful trade in electronic waste. http://www.greencustoms.org/docs/EIA_E-waste_report_0511_WEB.pdf. Accessed July 2012

FAO (2005) Ghana. http://www.fao.org/nr/water/aquastat/countries_regions/ghana/index.stm. Accessed March 2013

Forster U, Whittmann GTW (1983) Metal pollution in the aquatic environment. Springer, Berlin, pp 30–61

Frazier JM (1979) Bioaccumulation of cadmium in marine organisms. Environ Health Persp 28:75–79
Ghana Districts (2006) Greater Accra. http://www.ghanadistricts.com/region/?r=1&sa=25. Accessed March 2013
Grant R, Oteng-Ababio M (2012) Mapping the invisible and real African economy: urban e-waste circuitry. Urban Geogr 33:11–21
Greenpeace (2008) Poisoning the poor – electronic waste in Ghana. http://www.greenpeace.org/international/en/news/features/poisoning-the-poor-electroni/. Accessed July 2012
Guo Y, Huang C, Zhang H, Dong Q (2009) Heavy metal contamination from electronic waste recycling at Guiyu, Southeastern China. J Environ Qual 38(4):1617–1626
Hayat S, Javed M, Razzao S (2007) Growth performance of metal stressed. PVJ 27(1):8–12
Huang W, Cao L, Ye Z, Yin X, Dou SH (2010) Antioxidative responses and bioaccumulation in Japanese flounder larvae and juveniles under chronic mercury exposure. Comp Biochem Physiol 152(1):99–106
Jakimska A, Konieczka P, Skóra K, Namieńsik J (2011) Bioaccumulation of metals in tissues of marine organisms, Part I; the role of heavy metals on organisms. Pol J Environ Stud 20(5): 1117–1125
Karbassi R, Bayati I, Moattar F (2006) Origin and chemical partitioning of heavy metals in river-bed sediments. Int J Environ Sci Technol 3(1):35–42
Khayatzadeh J, Abbasi E (2010) The effects of heavy metals on aquatic animals. The 1st International Applied Geological Congress, Department of Geology, Islamic Azad University – Mashad Branch, Iran, 26–28 Apr 2010
Koranteng SS, Darko DA (2011) The e-waste menace in Ghana. Information note: 000. Institute for Environment and Sanitation Studies, University of Ghana. http://www.ug.edu.gh/iess/post/Senyo_e-waste_info-brief%5B1%5D.pdf. Accessed July 2012
Law RJ, Allchin CR, de Boer J, Covaci A, Herzke D, Lepom P, Morris S, Tronczynski J, de Wit CA (2006) Levels and trends of brominated flame retardants in the European environment. Chemosphere 64:187–208
Leblanc GA (1982) Laboratory investigation into the development of resistance of *Daphnia magna* to environmental pollutants. Environ Pollut 27:309–322
Lema SC, Schultz I, Scholz N, Incardona J, Swanson P (2007) Neural defects and cardiac arrhythmia in fish larvae following embryonic exposure to 2,2′,4,4′-tetrabromodiphenyl ether (PBDE-47). Aquat Toxicol 82:296–307
Leonard SS, Harris GK, Shi X (2004) Metal-induced oxidative stress and signal transduction. Free Radic Biol Med 37:1921–1942
Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manage 19:81–97
Lundstedt S (2011) Recycling and disposal of electronic waste: health hazards and environmental impacts. Swedish Environmental Protection Agency, report 6417. http://www.naturvardsverket.se/Documents/publikationer6400/978-91-620-6417-4.pdf. Accessed 23 July 2012
Mager EM, Brix KV, Grosell M (2010) Influence of bicarbonate and humic acid on effects of chronic waterborne lead exposure to the fathead minnow (*Pimephales promelas*). Aquat Toxicol 96:135–144
Mager EM, Brix KV, Gerdes RM, Ryan AC, Grosell M (2011) Effects of water chemistry on the chronic toxicity of lead to the cladoceran, *Ceriodaphnia dubia*. Ecotoxicol Environ Saf 74: 238–243
Matthews G (1996) PVC: production, properties and uses. The Institute of Materials, London. ISBN 0901716596
Meria E (1991) Metals and their compounds in the environment; occurrence, analysis and biological relevance. Verlag Chemie, New York
Mhadhbi L, Fumega J, Boumaiza M, Beiras R (2010) Lethal and sublethal effects of polybrominated diphenyl ethers (PBDEs) for turbot (*Psetta maxima*) early life stage (ELS). Nature Precedings http://dx.doi.org/10.1038/npre.2010.4656.2. Accessed Sept 2012

Michigan Department of Environmental Quality (2007) Polybrominated diphenyl ethers: a scientific review with risk characterization and recommendations. http://www.michigan.gov/documents/deq/deq-keytopics-tsg-pbdereview_187119_7.pdf. Accessed Sept 2012

Microelectronics and Computer Technology Corporation (1996) Electronics industry environmental roadmap. MCC, Austin

Muirhead EK, Skillman AD, Hook SE, Schultz IR (2006) Oral exposure of PBDE-47 in fish: toxicokinetics and reproductive effects in Japanese medaka (*Oryzias latipes*) and fathead minnows (*Pimephales promelas*). Environ Sci Technol 40:523–528

Nagpal NK (1987) Water quality criteria for lead. Technical Appendix. Water Management Branch. Ministry of Environment and Parks. British Columbia

Nammalwar P (1983) Heavy metals pollution in the marine environment. Sci Spectr 20(3):158–160

Nixon SW, Buckley BA, Granger SL, Entsua-Mensah M, Ansa-Asare O, White MJ, Mckinney RA, Mensah E (2007) Anthropogenic enrichment and nutrients in some tropical lagoons of Ghana, West Africa. Ecol Appl 17:144–164

Nordbrand S (2009) Out of control: E-waste trade flows from the EU to developing countries. SwedWatch. http://www.swedwatch.org/sites/default/files/SwedWatch_E-waste_Report_April_2009.pdf. Accessed 13 Sept 2012

Nordic Council of Ministers (1995) Waste from electrical and electronic products: a survey of the contents of materials and hazardous substances in electric and electronic products, TemaNord report 554. Nordic Council of Ministers, Copenhagen

OECD (2003) Technical guidance for the environmentally sound management of specific waste streams: used and scrap personal computers, no 304. Organisation for Economic Co-operation and Development (OECD) Working Group on Waste Prevention and Recycling. Document: ENV/EPOC/WGWPR(2001)3/FINAL

Orisakwe OE, Frazzoli C (2010) Electronic revolution and electronic wasteland: the West/waste Africa experience. J Nat Environ Sci 1(1):43–47

Oteng-Ababio M (2012) When necessity begets ingenuity: e-waste scavenging as a livelihood strategy in Accra, Ghana. Afr Stud Quart 13(1 & 2):1–21

Otsuka M, Itai T, Asante KA, Muto M, Tanabe S (2012) Trace element contamination around the e-waste recycling site at agbogbloshie, Accra City, Ghana. Interdisciplinary Studies on Environmental Chemistry—Environ Pollut Ecotoxicol 6:161–167

Prakash S, Manhart A, Amoyaw-Osei Y, Agyekum O (2010) Socio-economic assessment and feasibility study on sustainable e-waste management in Ghana. http://www.oeko.de/oekodoc/1057/2010-105-en.pdf. Accessed July 2012

Prosi F (1989) Factors controlling biological availability and toxic effects of lead in aquatic organisms. Sci Total Environ 79(2):157–169

Puckett J, Smith T (2002) Exporting harm: the high-tech trashing of Asia. Basel Action Network and Silicon Valley Toxics Coalition, 25 Feb 2002. http://www.ban.org/E-waste/technotrashfinalcomp.pdf. Accessed June 2012

Pwamang JA (2009) The e-waste situation in Ghana. http://inece.org/ewaste/03_pwamang.pdf. Accessed Aug 2012

Rademacher DJ, Steinpreis RE, Weber DN (2003) Effects of dietary lead or dimercapto sussinic acid exposure on regional serotonin and serotonin metabolic content in rainbow trout (*Oncorhynchus mykiss*). Neurosci Lett 339:156–160

EU draft RAR (2000) EU Risk Assessment Reports for bis (pentabromophenyl) ether (decabromodiphenyl ether) CAS 1163-19-5, EC chemical number R013, RAR draft number as stated. http://ecb.jrc.it/existing-chemicals/. Accessed July 2012

Rashed MN (2001) Cadmium and lead levels in fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. Environ Monit Assess 68:75–89

Reuber B, Mackay D, Paterson S, Stokes P (1987) A discussion of chemical equilibria and transport at the sediment-water interface. Environ Toxicol Chem 6(10):731–739

Ribeiro AR, Eira C, Torres J, Mendes P, Miquel J, Soares AMVM, Vingada J (2009) Toxic element concentrations in the razorbill *Alca torda* (Charadriiformes, Alcidae) in Portugal. Arch Environ Contam Toxicol 56:588–595

Riederer AM, Stephanie Adrian S, Kuehr R (2013) Assessing the health effects of informal e-waste processing. J Health Pollut 4:1–3

Rioboo C, O'Connor JE, Prado R, Herrero C, Cid A (2009) Cell proliferation alterations in *Chlorella cells* under stress conditions. Aquat Toxicol 94:229–237

Robinson BH (2009) E-waste: an assessment of global production and environmental impacts. Sci Total Environ 408:183–191

Schluep M, Hagelueken C, Kuehr R, Magalini F, Maurer C, Meskers C, Mueller E, Wang F (2009) Recycling – from e-waste to resources, final report. http://www.unep.org/PDF/PressReleases/E_waste-_publication_screen_FINALVERSION-sml-pdf. Accessed July 2012

Seymore T, Du Preez HH, Van Vuren JHJ (1995) Manganese, lead and strontium bioaccumulation in the tissues of the yellowfish, *Barbus marequensis* from the lower Olifants River, Eastern Transvaal. Water SA 21(2):159–172

Simpson SL, Apte SC, Batley GE (1998) Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. Environ Sci Technol 32:620–625

Simpson SL, Apte SC, Batley GE (2000) Effect of short-term resuspension events on the oxidation of cadmium, lead, and zinc sulfide phases in anoxic estuarine sediments. Environ Sci Technol 34:4533–4537

Simpson SL, Rochford L, Birch GF (2002) Geochemical influences on metal partitioning in contaminated estuarine sediments. Mar Freshw Res 53:9–17

Solomon F (2008) Impacts of metals on aquatic ecosystems and human health. Environment and Communities. http://magazine.mining.com/issues/0804/ImpactsMetalsAquaticHealth.pdf. Accessed Sept 2012

Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 18:321–336

Stomiñska I, Jezierska B (2000) The effect of heavy metals on post embryonic development of COMMON CARP, *Cyprinus carpio L.* Arch Pol Fish 8(1):119–128

Tam NFY, Wang P, Gao QT, Wong YS (2012) Toxicity of water-borne persistent organic pollutants on green microalgae. BALWOIS 2012 – Ohrid, Republic of Macedonia. http://balwois.com/2012/USB/papers/765.pdf. Accessed Sept 2012

Tysdenova O, Bengtsson M (2011) Chemical hazards associated with treatment of waste electrical and electronic equipment. Waste Manage 31(1):45–58

UNEP (2005) E-waste: the hidden side of IT equipment's manufacturing and use. Early warnings on emerging environmental threats no 5. United Nations Environment Programme. www.grid.unep.ch. Accessed July 2012

UNEP (2010) Urgent need to prepare developing countries for surge in e-wastes. Rocketing sales of cell phones, gadgets, appliances in China, India, elsewhere forecast. http://www.unep.org/Documents.Multilingual/Default.Print.asp?DocumentID=612&ArticleID=6471. Accessed July 2012

US EPA (1976) Effects of exposure to heavy metals on selected fresh water fish: toxicity of copper, cadmium, chromium, and lead to eggs and fry of seven fish species. Environmental Research Laboratory, Office of Research and Development, Duluth, 600/3-76-105

US EPA (1998) Guidelines for ecological risk assessment. EPA/630/R-95/002F, final report, Washington, DC

US EPA (2000) Bioaccumulation testing and interpretation for the purpose of sediment quality assessment: status and needs. EPA-823-R-00-001, U.S. Environmental Protection Agency, Washington, DC

Weber DN, Dingel WM (1997) Alterations in neurobehavioral responses in fishes to exposed to lead and lead-chelating agents. Am Zool 37:354–362

Wollenberger L, Dinan L, Breitholtz M (2005) Brominated flame retardants: activities in a crustacean development test and in an ecdysteroid screening assay. Environ Toxicol Chem 24(2):400–407

Woo S, Yum S, Park HS, Lee TK, Ryu JC (2009) Effects of heavy metals on antioxidants and stress-responsive gene expression in Japanese medaka (*Oryzias javanicus*). Comp Biochem Physiol Part C 149:289–299

Environmental Fate and Toxicology of Clomazone

April R. Van Scoy and Ronald S. Tjeerdema

Contents

1 Introduction 36
2 Chemistry and Physicochemical Properties 36
3 Environmental Chemodynamics 37
3.1 Soil 37
3.2 Water 38
3.3 Air and Volatilization 39
4 Environmental Degradation 39
4.1 Abiotic Processes 39
4.2 Biotic Processes 40
5 Toxicology 42
5.1 Mode of Action in Plants 42
5.2 Plant Effects 43
5.3 Aquatic Organisms 44
5.4 Non-target Fauna 45
6 Summary 46
References 47

A.R. Van Scoy (✉)
Department of Environmental Toxicology, College of Agricultural and Environmental Sciences, University of California, Davis, CA 95616-8588, USA

Department of Environmental Toxicology, University of California, One Shields Ave, Davis, CA 95616, USA
e-mail: avanscoy@ucdavis.edu

R.S. Tjeerdema
Department of Environmental Toxicology, College of Agricultural and Environmental Sciences, University of California, Davis, CA 95616-8588, USA

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229*, Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6_3, © Springer International Publishing Switzerland 2014

1 Introduction

The herbicide clomazone (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone; CAS 81777-89-1; Fig. 1) was first approved for use in 1986 (US EPA 2007). It is produced by the FMC Corporation under the trade names that include Command® and Cerano® 5 MEG (TenBrook and Tjeerdema 2006).

Clomazone is the only isoxazolane herbicide registered for use within the United States (US EPA 2007). It is used for annual control of broad-leaf and grassy weeds such as barnyard grass (*Echinochloa crus-galli*), crab grass (*Digitaria spp*.), foxtails (*Setaria spp*.), and others that infest soybean, tobacco, rice and other row crops (Scott et al. 1995; Lee et al. 2004; Schocken 1997). Within the USA, approx. 503,487 kg of active ingredient is applied each year (US EPA 2007). It is formulated as an emulsifiable concentrate and microencapsulated flowable granule (5% clomazone) and is applied either pre- or post-emergence (CDPR 2003; US EPA 2007).

Clomazone is highly water soluble and weakly to moderately persistent in soils with half-lives ($t_{1/2}$s) ranging from 5 to 60 days. Because of its water solubility, the potential impact of clomazone on surface water, groundwater and aquatic organisms is of great concern. In this paper, we have reviewed the relevant literature and address clomazone's chemistry, environmental fate and toxicity.

2 Chemistry and Physicochemical Properties

Clomazone is an isoxazolane herbicide containing a chloroaromatic ring (Fig. 1). When pure, clomazone is a crystalline solid (CDPR 2003). At room temperature it is highly soluble in water and has a low-to-moderate affinity for soil. This herbicide is denser than water, and is susceptible to microbial degradation. The physiochemical properties of clomazone are presented in Table 1.

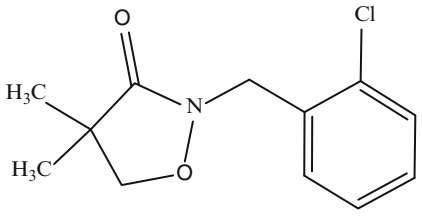


Fig. 1 Clomazone structure

Table 1 Physicochemical properties of clomazone

Physicochemical property	Value
Chemical name	2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone
Chemical Abstract Service registry number (CAS#)	81777-89-1
Molecular formula[a]	$C_{12}H_{14}ClNO_2$
Molecular weight[a]	239.7
Boiling point (°C)[a]	275.4–281.7
Water solubility at 23 °C[b]	1,100 mg/L
Vapor pressure at 25 °C (mmHg)[b]	1.44×10^{-4}
Density at 22 °C (g/mL)[b]	1.16
Octanol-water partition coefficient (log K_{ow})[b]	352 (2.55)
Adsorption coefficient (K_d)[c]	1.5–7.4
Organic carbon normalized partition coefficient (K_{oc})[c]	139–608
Henry's law constant (atm-m^3/mol)[a]	4.14×10^{-8}

[a] Data from Tomlin (2009)
[b] Data from CDPR (2003)
[c] Data from US EPA (2007)

3 Environmental Chemodynamics

3.1 Soil

Clomazone is not expected to bind to soils strongly given its relatively low K_d and its hydrophilic nature. However, sorption to various soil types (with varying temperature and moisture) has been investigated. Mervosh et al. (1995b) observed that a concentration of ca. 9 mg/kg of ^{14}C-clomazone sorbed to a silty clay loam soil; such sorption was independent of temperature, and soil moisture content had a minor sorbtive effect. Although overall soil sorption is low, the agent has a higher affinity for binding to humic acid than to whole soil (Gunasekara et al. 2009). Furthermore, it appears that the presence of black carbon or burned residues, in fire-affected locations, increase the sorption of this herbicide (Gunasekara et al. 2009). Loux et al. (1989) determined that adsorption was dictated by organic matter rather than by clay content; K_d values for clomazone ranged from 0.47 for silt loam to 5.3 for loamy sand.

Half-lives and desorption coefficients were determined for clomazone in four Tasmanian soils. A first-order half-life ($t_{1/2}$) for ferrosol (clay loam), kurosol (loamy sand), sodosol (silt loam), and vertosol (light clay) soils ranged from 79 to 124 days, respectively. Half-lives derived from the Hoerl equation ranged from 6 to 59 days, respectively; this equation provided a good fit to the measured concentrations (Cumming et al. 2002). Desorption also varied with soil type; K_d values ranged from 1.7 to 3.6, respectively.

The persistence of clomazone was examined under both conventional and no-tillage practices. Following an initial application rate of 1.4 kg/ha, measurable

amounts of clomazone were detected at a soil depth of 0–10 cm, 120-days later (Mills et al. 1989). Soil concentrations of 124 ±54 and 30 ±12 ng/g, respectively, were measured following conventional and no-till practices (Mills et al. 1989).

Quayle et al. (2006) applied clomazone to simulated flooded rice plots and measured resulting soil concentrations. Analytical results varied 7.5-fold between the 4 and 48 day post-application samplings. In addition, a measured $t_{1/2}$ of 14.6 days was attributed to anaerobic conditions. Half-lives of 32.9 and 37.4 days, respectively, in Montana loam and silty clay loam soils were noted by Gallandt et al. (1989). Field half-lives for Tennessee clay loam and loam soils ranged from 5 to 29 days and a $t_{1/2}$ of 34 days resulted under laboratory conditions (Kirksey et al. 1996); this indicates that environmental conditions affect clomazone's dissipation rate.

3.2 Water

Due to its high water solubility (1,102 mg/L) and relatively low K_{ow} value, clomazone is expected to concentrate within the aqueous phase; thus, concerns exist for potential impacts on drinking water systems. To investigate risks posed to drinking water, Byers et al. (1995) measured clomazone concentrations in vadose zone waters at depths of 0.3, 0.6 and 1.5 m using tension lysimeters. They found concentrations to decrease (ca. 3-fold) as soil depth increased. In addition, soil treatments no mulch was used, or plastic mulch was used had measurable clomazone concentrations respectively of 0.09 and 0.04 ppb (Byers et al. 1995).

Since flooded fields discharge excess water into surrounding creeks and rivers, there is potential for applied residual organics to contaminate surrounding water bodies. The dissipation of clomazone from floodwaters was studied by Quayle et al. (2006). When Quayle et al. (2006) applied clomazone to small replicated rice plots at a rate of 0.5 L/ ha (i.e., as commercially formulated Magister® containing 480 g/L a.i.), an initial measured mean water concentration of 202 ug/L was produced. However, within 4 days the concentration had decreased to 83 ug/L, and by 19 days the concentration declined to 3 ug/L (Quayle et al. 2006). The $t_{1/2}$ for chlomazone in this study was 7.2 days. Furthermore, the releasing waters contained 3 ug/L clomazone, which was assessed as having a low toxicity hazard. Two Brazilian rivers, the Vacacaí-Mirim and the Vacacaí, were monitored for residues of clomazone, particularly sourced from rice field irrigation. An average level of 4.5 ug/L was detected in 41% of samples collected from the Vacacaí River, whereas the Vacacaí-Mirim River had detectable concentrations of 3.7 ug/L in 33% of samples (Marchesan et al. 2007). The higher rate and level of detections in the Vacacaí River were attributed to its larger surrounding drainage area and plot acreage. Zanella et al. (2002) reported residual clomazone concentrations in samples collected from experimental rice fields in the central region of the Rio Grande do Sul, Brazil. During both December 1999 and 2000, samples collected 130 days post application were found to contain clomazone concentrations of 0.9 and 0.2 ug/L, respectively (Zanella et al. 2002).

3.3 Air and Volatilization

The volatility of various formulations of clomazone from Flanagan silt loam was studied under both moist soil and simulated rainfall conditions. Mervosh et al. (1995c) reported that each of the granular formulations reduced volatilization; small granules (20–30 mesh) produced greater volatilization than did those of 14–20 mesh. In addition, they found that soil-water content greatly affected volatilization flux; highly saturated soil resulted in increased flux rates. Compared to others, starch-based formulations reduced off-site movement (Mervosh et al. 1995c).

Thelen et al. (1988) observed volatilization up to 2 weeks post-application in both surface applied or soil-incorporated treatments; surface application resulted in higher volatilization. In addition, the presence of rainfall increased clomazone's overall tendency to volatilize. The off-site movement of vapors from extremely wet soil was observed by Halstead and Harvey (1988). Such vapors traveled as far as 32 m from the application site as measured by phytotoxicity to sunflower and wheat chlorosis. The application rate was a major factor in producing phytotoxic effects at this distance; however soil moisture and wind speed may have contributed to clomazone's transport (Halstead and Harvey 1988). Mervosh et al. (1995a) observed increased volatilization from increasing temperature, but not from soil moisture. Schummer et al. (2010) determined that air samples, collected from a farming site in Northeastern France, contained gas-phase concentrations of clomazone ranging from 0.14 to 0.68 ng/m^3.

4 Environmental Degradation

4.1 Abiotic Processes

In buffered solutions (pH 4.65, 7.0 and 9.25, 25 °C, 41 days) clomazone was found to be hydrolytically stable over the entire test period (Dziedzic 1982). Breakdown of the herbicide was <10% of the initial concentration at each pH, but the natures of the resulting products were not determined. CDPR (2003) reported similar observations, discovering that clomazone was stable under various pH conditions, as measured after 34–40 days. We conclude that hydrolysis is unlikely to be a major degradation route for clomazone.

Experimental studies, in which the direct and indirect photolytic degradation of clomazone was assessed under California rice field conditions, were conducted by Tomco and Tjeerdema (2012). Clomazone was found to degrade slowly when exposed for 35 days to either natural or artificial sunlight (8W UV lights, exhibited $\lambda_{max} = 300 \pm 50$ nm, 30 °C). Half-lives were determined to be 145 and 158 days, respectively, for artificial and natural light exposures (Tomco and Tjeerdema 2012).

Photolytic degradation from surface waters contributes to the dissipation of many pesticides and other xenobiotics. Zanella et al. (2008) investigated the

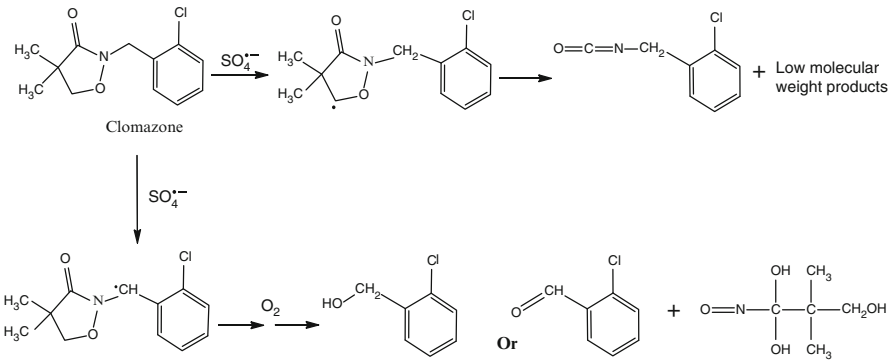


Fig. 2 Photolytic degradation of clomazone with $S_2O_8^{2-}$ at $\lambda > 200$ nm, as proposed by David Gara et al. (2009)

photodegradation rate of clomazone in both distilled and agricultural field water irradiated for up to 120 min; after 60 min, a 6.5-fold higher concentration remained in agricultural than in distilled water. However, clomazone's rate of degradation in agricultural field water was affected by pH (pH 3 caused more efficient degradation than pH 6); the difference between the two pH values was attributed to the photo-Fenton process (Zanella et al. 2008).

It is thought that humic substances act as photosensitizing agents that increase the likelihood of oxidative degradation (Gara et al. 2009). Since clomazone strongly sorbs to organic matter, photolysis would be more dominant under conditions of high humic acid content. To examine this theory, Gara et al. (2009) irradiated ($\lambda > 300$ nm) air-saturated Aldrich humic acid (20 ppm) and observed a degradation enhancement and depletion in total organic carbon (TOC); byproducts included 2-chlorobenzylic alcohol and 2-chlorobenzaldehyde. In addition, irradiated ($\lambda > 200$ nm, 125 W mercury lamp, 20 min, 27 °C) aqueous peroxydisulfate ($S_2O_8^{2-}$) solutions degraded clomazone by >90%. A proposed degradation pathway for clomazone is shown in Fig. 2. Photochemical experiments (aqueous, 25 °C, pH 4–5) conducted by Kirmser et al. (2010) used SO_4^- radicals to degrade clomazone; two products resulted – 2-chlorobenzylalcohol and 2-chlorobenzaldehyde.

4.2 Biotic Processes

Biotransformation of clomazone was studied by Helbling et al. (2010). Sludge collected from a pilot-scale wastewater treatment plant was spiked with pesticides and pharmaceuticals; clomazone was spiked at a concentration of 100 ug/L. Both mono- and di-hydroxylated clomazone transformation products were identified (Fig. 3); however the positioning of the hydroxy groups were not confirmed.

Liu et al. (1996) exposed both *Aspergillus niger* (UI-X172) and *Cunninghamella echinulata* (NRRL-3655), a common soil fungus and bacterium, respectively, to

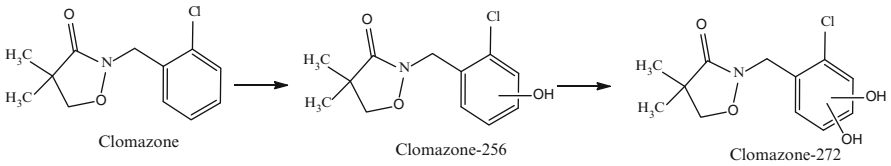


Fig. 3 Suggested microbial transformation pathway for clomazone (Adapted from Helbling et al. 2010)

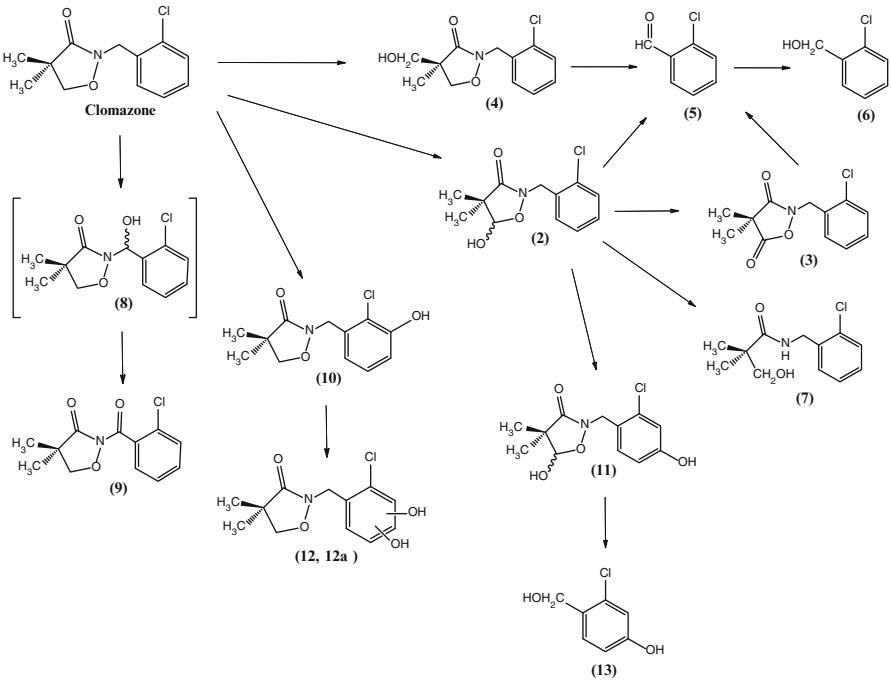


Fig. 4 Proposed microbial breakdown route for clomazone (Adapted from Liu et al. 1996). Metabolites include: (*2*) 5-hydroxyclomazone, (*4*) hydroxymethylclomazone, (*6*) 2-chlorobenzyl alcohol and (*10*) 3′-hydroxyclomazone

clomazone; 95% of the agent was metabolized by *A. niger* (X172). Transformation processes included both aromatic ring hydroxylation and benzylic hydroxylation – with subsequent dehydrogenation, and identified metabolites included 5-hydroxyclomazone, hydroxymethylclomazone, 2-chlorobenzyl alcohol and 3′-hydroxyclomazone (Fig. 4; Liu et al. 1996).

Mervosh et al. (1995a) investigated both the mineralization and microbial degradation of ^{14}C-clomazone in Flanagan silt clay loam soil and found mineralization to be dependent on microbial activity; mineralization was more active at lower temperatures. According to Mills et al. (1989), microbial degradation of clomazone is favored under neutral soil pH conditions, whereas microbial populations tend to be more abundant under no-till conditions.

In several studies, clomazone has been observed to degrade more rapidly under flooded conditions, suggesting that anaerobic bacteria play an important role in degrading it. One such study was designed to simulate aerobic and anaerobic California rice field conditions and to measure the anaerobic degradation rate for clomazone. Results were that clomazone was anaerobically degraded to produce metabolites within 3 days of its application. In contrast, under aerobic conditions, clomazone residues became soil-sorbed residues (Tomco et al. 2010). Anaerobic and aerobic half-lives of clomazone were reported to be 7.9 and 47.3 days, respectively. However, ^{13}C-labeled clomazone was found to significantly mineralize under aerobic conditions (18.6% of applied compound), whereas only 3% was mineralized under anaerobic conditions (Tomco et al. 2010).

TenBrook and Tjeerdema (2006) suggested that microbial degradation of clomazone could be subject to photolytic enhancement; however, to date this phenomenon has not been experimentally demonstrated. Lack of photolytic assistance was further confirmed by Tomco and Tjeerdema (2012). They found soil microbial degradation to be more relevant than photolysis, and thus, it appears to be the major degradative pathway for clomazone (Tomco and Tjeerdema 2012).

5 Toxicology

5.1 Mode of Action in Plants

Clomazone is designed to target broad-leaf grasses; however, it has been known to cause toxicity in other plants, where it systemically enters through the roots and shoots and translocates via the xylem (US EPA 2007). Studies have shown that this herbicide impairs the formation of photosynthetic pigments, reducing both chlorophyll and carotenoids and bleaching foliar structures (see Sect. 5.2). It is thought that the metabolite 5-ketoclomazone may be responsible for such toxicity (US EPA 2007). The mode of action, particularly on the methylerythritol-4-phosphate (MEP) pathway, resulting from clomazone, 5-hydroxyclomazone and 5-ketoclomazone, has been investigated. Ferhatoglu and Barrett (2006) reported that clomazone and 5-hydroxyclomazone did not inhibit the MEP pathway in spinach, although they are known to cause plant bleaching; however, 5-ketoclomazone did inhibit this pathway. In addition, the parent and hydroxylated metabolite did not inhibit synthesis of either chloroplastic isoprenoids or deoxyxylulose 5-phosphate (DXP) within this pathway, whereas 5-ketoclomazone did. Ferhatoglu and Barrett (2006) concluded that subsequent toxicity and plant bleaching results from the ultimate toxicant 5-ketoclomazone. However, the exact site of action is in question. Suggested sites include: (1) isopentenyl pyrophosphate isomerase, (2) prenyl transferases, and (3) enzymatic phytylation of chlorophyllide (Duke et al. 1985; Duke and Kenyon 1986; Sandmann and Boger 1987).

5.2 Plant Effects

Since clomazone is extensively used to control weeds in rice culture, many researchers have investigated its impact on rice production and plant growth. Bollich et al. (2000) applied various rates of microencapsulated clomazone (0.28–2.2 kg a.i./ha) to both water-seeded and drill-seeded fields. Minimal bleaching of rice seedlings occurred at low application rates, although increased application rates increased bleaching. Furthermore, higher rates also reduced seed head emergence and grain yield; drill-seeded production was more impacted than water-seeded (Bollich et al. 2000). Webster et al. (1999) observed injury to rice 7 days after emergence; however less injury was produced by post-emergence treatments.

The spectrum of clomazone's weed control was investigated by Westberg et al. (1989). Pre-emergence application (at 280 g a.i./ha) controlled 90–100% of weeds (e.g., barnyardgrass, foxtail, crabgrass and velvetleaf) and was more effective at equal rates than was pre-plant incorporated application. Weed tolerance to this herbicide was studied by Liebl and Norman (1991). They found soybean seedlings to be 256 times more tolerant to clomazone than velvetleaf, whereas corn and smooth pigweed were 66 and 13 times less tolerant, respectively. In addition, a reduction in shoot fresh weight and leaf chlorophyll was observed only for velvetleaf.

ElNaggar et al. (1992) applied ^{14}C-clomazone (1.1 and 2.2 kg a.i./ha) to soybean seeds planted in pots and at a depth of between 1.2 and 2.5 cm; plants were harvested after either 30 or 60 days exposure periods. Clomazone metabolites within plant tissues were identified. It appeared that the major degradation processes included dealkylation and monohydroxylation (Fig. 5); subsequent conjugation processes resulted in glycoside formation.

TenBrook and Tjeerdema (2006) identified glucose conjugation as the main route of phase II detoxification. They also identified metabolites in rice (*Oryza sativa*) and early watergrass (*Echinochia spp*.) to be conjugated glucosides. Although Norman et al. (1990) did not identify specific clomazone metabolites, they did note that up to 5.9% of recovered residues in the seeds of both soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) were β-glucosides.

Kana et al. (2004) investigated the photosynthetic capability of barley seedlings (*Hordeum vulgare*) that were cultivated on filter paper containing either 0.25 or 0.5 mM clomazone (12 days, continuous light, 10 °C). Adverse effects included a reduction in chlorophyll (a + b) and carotenoid levels. Kana et al. (2004) concluded that the photochemical processes in this species cannot operate fully due to pigment loss brought on by clomazone toxicity. Similarly, Yasuor et al. (2008) found clomazone and 5-ketoclomazone to illicit greater inhibition of chlorophyll a and carotenoids in susceptible populations of late watergrass (*Echinochloa phyllopogon*) than in resistant populations. Further studies on susceptible cotton (*Gossypium hirsutum* L.) showed a slowing of chlorophyllide to chlorophyll conversion and complete inhibition of carotenoid synthesis (Duke et al. 1991). These findings indicate that clomazone inhibits terpenoid synthesis.

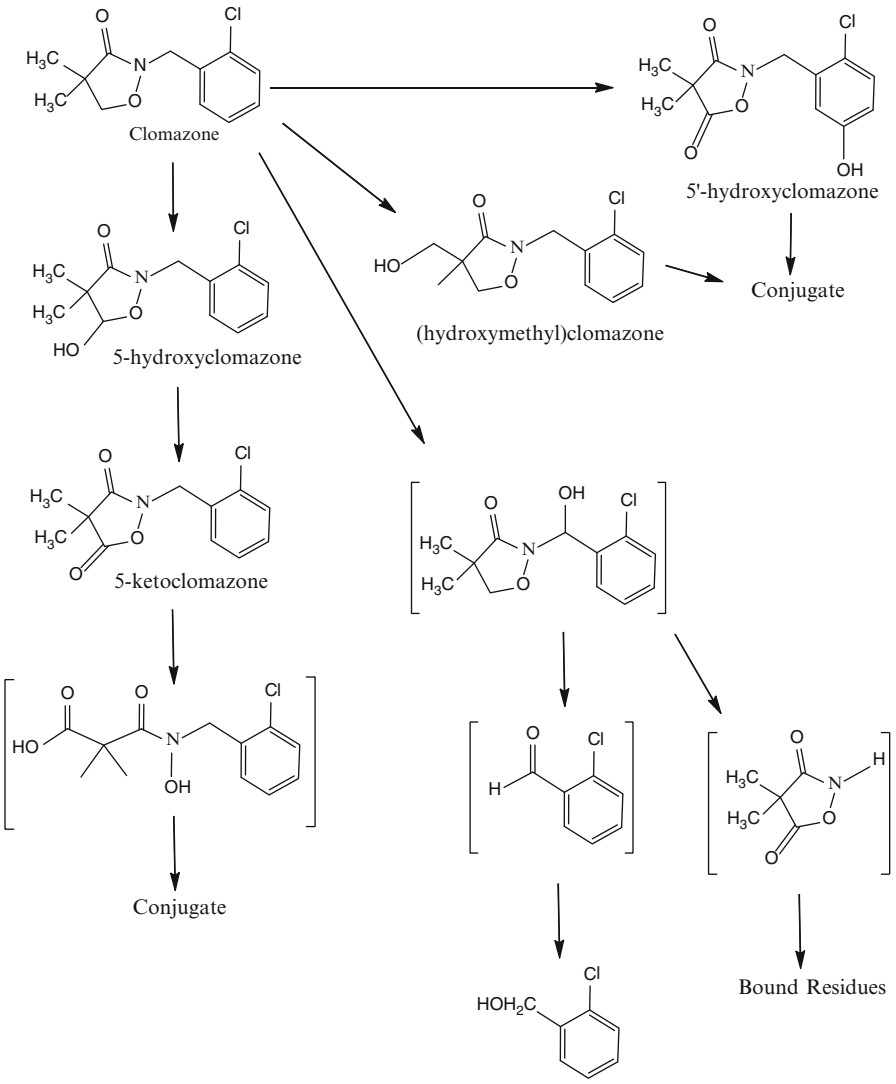


Fig. 5 Proposed metabolic pathway for clomazone in soybean plants (Adapted from ElNaggar et al. 1992)

5.3 Aquatic Organisms

Although clomazone is highly water soluble, its affinity to partition across biological membranes is minimal, as indicated by its low log K_{ow} value (Table 1). However, silver catfish (*Rhamdia quelen*) fingerlings, exposed to varying concentrations of clomazone, produced a 96-h LC_{50} value of 7.32 mg/L; thus, clomozone is more highly toxic to this species than to others, like rainbow trout (*Oncorhynchus mykiss*;

LC_{50} = 19 mg/L) and bluegill (*Lepomis macrochirus*; LC_{50} = 34 mg/L; dos Santos Miron et al. 2004).

Silver catfish exposed for 192 h resulted in brain and muscle AChE (acetylcholinesterase) inhibition of 47% (at 1.0 mg/L clomazone) and 45% (at 0.5 and 1.0 mg/L concentrations), respectively, within 12-h; hepatic vacuolation was also observed (Crestani et al. 2007). Although, biological responses resulted, fish placed into clean water for either 96- or 192-h did recover. Similar results for brain, muscle and heart tissues from piava (*Leporinus obtusidens*) were reported by dos Santos Miron et al. (2008); however, AChE recovery only occurred in heart. AChE activity was also studied in *Cyprinus carpio* exposed to clomazone for 7, 30 and 90 days under laboratory and field conditions. Although Cattaneo et al. (2012) observed no alteration in AChE activity in field-exposed fish, laboratory-treated fish showed decreased muscle activity after 7 days.

A teleost species (*Leporinus obtusidens*) was exposed to clomazone at a relevant rice field concentration (0.5 mg/L, 30 days), and both tissue AChE and catalase activities were examined. Moraes et al. (2007) reported differential results for clomazone's activity in this species, to wit, a decrease in brain AChE activity, but a significant increase in muscle tissue. Another effect observed was an increase in liver catalase activity. Moraes et al. (2009) also studied the effects of exposed to commercially formulated clomazone in a rice field (0.376 mg/L a.i., 90 days). They reported results similar to those reported in their 2007 study; however liver catalase activity decreased from the latter exposure.

Clomazone's toxicity to aquatic invertebrates has also been investigated. Mysid shrimp (*Americamysis bahia*) are very susceptible, having a 96-h LC_{50} value of 556 ug/L (CDPR 2003). The water flea (*D. magna*; 48-h EC_{50} = 5,400 ug/L) and Eastern oyster (*Crassostrea virginica*; 96-h EC_{50} = 5,300 ug/L; US EPA 2009) were considerably more tolerant to clomazone's toxicity. Although clomazone is designed to target plants, it is also moderately-to-highly toxic to aquatic organisms.

5.4 Non-target Fauna

The effects of clomazone on non-target aquatic species were investigated by Burdett et al. (2001). Midge (*Chironomus tepperi*) first instar larvae, exposed at levels up to 0.288 mg/L or to the anticipated field concentration (0.576 mg/L), displayed no significant adverse effects (changes in emergence, development time or wing length) when compared to thiobencarb. Furthermore, clomazone-treated ponds were found to significantly reduce macrophyte biomass and contain higher populations of both ostracods and corixids, when compared to control ponds (Burdett et al. 2001). Pershbacher et al. (2002) exposed pond phytoplankton and zooplankton to clomazone (0.6 kg a.i./ha), in addition to other rice herbicides; no measurable effects were observed.

Early life-stage effects have also been studied. The soybean cyst nematode, *Heterodera glycines* (females, cysts and eggs) was exposed to 50 and 500 ug/mL

clomazone (dark, 25 °C, 24 days) to assess hatching effects (Wong et al. 1993). Hatching of eggs was not impacted by the herbicide and results did not differ significantly from those of the controls.

Mortality and behavioral responses resulting from clomazone exposures were examined for spiders. Four species (*Dictyna uncinata, Pardosa palustris, Philodromus cespitum and Theridion impressum)* were exposed to fresh (2 h; approx. 0.4 g of a.i. in 100 ml of distilled water) and aged (5–20 days) herbicide residues (concentrations unknown). For all four species mortality induced by clomazone exposure was minimal (<20%; herbicide activity declined with aging); however, increased movement was observed in *P. palustris* (Pekar and Benes 2008). Pekar (2002) also noted that this herbicide was harmless to *T. impressum*, thus recommending it for plant protection.

According to the California Department of Pesticide Regulation (CDPR 2003), toxicity studies on rats, various birds and honeybees have shown clomazone to be relatively non-toxic to moderately toxic to these species. Generally, study results have shown that clomazone poses little to no risk to non-target species.

Human adverse effects from exposure to clomazone have also been observed. For example, human erythrocytes have been exposed *in vitro* to a range of clomazone concentrations (0, 100, 250 and 500 ug/L) for 1 h to investigate effects on oxidative stress biomarkers and on AChE activity (Santi et al. 2011); both AChE and catalase activities decreased with each concentration, whereas glutathione (GSH) was not affected.

6 Summary

Clomazone, an isoxazolane herbicide, was first registered for use in 1986 for pest grasses and broadleaf weeds. Although the exact mode of action is still unclear, it is well documented that clomazone causes bleaching of foliar structures; the clomazone metabolite 5-ketoclomazone is regarded to cause the bleaching and to be the ultimate plant toxicant. Although clomazone exhibits low mammalian toxicity and is selective towards certain plant species, studies have shown that it does inhibit AChE and catalase activities. In addition, it has been found to be highly toxic to aquatic invertebrates, in particular mysid shrimp.

Clomazone has a low Henry's law constant and moderate vapor pressure, and thus may volatilize from dry soils. Photolysis represents a minor dissipation pathway; however, clomazone can be photolytically degraded under both direct and indirect conditions. Clomazone has high water solubility, and it is often assumed to undergo hydrolysis easily; unfortunately, this is not the case. Clomazone is stable over a wide pH range and does not hydrolyze. Clomazone has a weak to moderate soil adsorption coefficient; therefore, its affinity to sorb to soil is minimal, rendering it a potential threat to groundwater supplies.

Microbial metabolism is the major degradation pathway, resulting in products such as 5-hydroxyclomazone, hydroxymethylclomazone, 2-chlorobenzyl alcohol

and 3′-hydroxyclomazone. Although clomazone has not been shown to degrade via hydrolysis, it nonetheless represents a potential threat to aquatic organisms. With this in mind, caution should be taken when applying clomazone or when draining fields that have detectable clomazone residues.

References

Bollich PK, Jordan DL, Walker DM, Burns AB (2000) Rice (*Oryza sativa*) response to the micro-encapsulated formulation of clomazone. Weed Technol 14:89–93

Burdett AS, Stevens MM, Macmillan DL (2001) Laboratory and field studies on the effect of molinate, clomazone and thiobencarbb on nontarget aquatic invertebrates. Environ Toxicol Chem 20(10):2229–2236

Byers ME, Tyess D, Antonious GF, Hilborn D, Jarret L (1995) Monitoring herbicide leaching in sustainable vegetable culture using tension lysimeters. Bull Environ Contam Toxicol 54: 848–854

Cattaneo R, Moraes BS, Loro VL, Pretto A, Menezes C, Sarzi Sartori GM, Clasen B, de Avila LA, Marchesan E, Zanella R (2012) Tissue biochemical alterations of *Cyprinus carpio* exposed to commercial herbicide containing clomazone under rice-field conditions. Arch Environ Contam Toxicol 62:97–106

CDPR, California Department of Pesticide Regulation, Sacramento (2003) Public report 2003–01, Clomazone

Crestani CM, Menezes C, Glusczak L, dos Santos Miron D, Spanevello R, Silveira A, Goncalves FF, Zanella R, Loro VL (2007) Effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*) and recovery pattern. Chemosphere 67:2305–2311

Cumming JP, Doyle RB, Brown PH (2002) Clomazone dissipation in four Tasmanian topsoils. Weed Sci 50:405–409

David Gara PM, Bosio GN, Arce VB, Poulsen L, Ogilby PR, Giudici R, Gonzalez MC, Mártire DO (2009) Photoinduced degradation of the herbicide clomazone model reactions for natural and technical systems. Photochem Photobiol 85(3):686–692

Diaz Kirmser EM, Martire DO, Gonzalez MC, Rosso JA (2010) Degradation of the herbicide clomazone, paraquat and glyphosate by thermally activated peroxydisulfate. J Agric Food Chem 58:12858–12862

dos Santos Miron D, da Silva LVF, Golombieski JI, de Oliveira Machado SL, Marchezan E, Baldisserotto B (2004) Lethal concentration of clomazone, metsulfuron-metil, and quinclorac for silver catfish, *Rhamdia quelen*, fingerlings. Ciênc Rural 34(5):1465–1469

dos Santos Miron D, Pretto A, Crestani M, Glusczak L, Schetinger MR, Loro VL, Morsch VM (2008) Biochemical effects of clomazone herbicide on piava (*Leporinus obtusidens*). Chemosphere 74:1–5

Duke SO, Kenyon WH (1986) Effects of dimethazone (FMC 57020) on chloroplast development. II. Pigment synthesis and photosynthetic function in cowpea (*Vigna unguiculata* L.) primary leaves. Pestic Biochem Physiol 25:11–18

Duke SO, Kenyon WH, Paul RN (1985) FMC 57020 effects on chloroplast development in pitted morning glory (*Ipomoea lacunosa*) cotyledons. Weed Sci 33:786–794

Duke SO, Paul RN, Becerril JM, Schmidt JH (1991) Clomazone causes accumulation of sesquiterpenoids in cotton (*Gossypium hirsutum* L.). Weed Sci 39:339–346

Dziedzic JE (1982) FMC 57020 hydrolysis study; FMC Corporation, Princeton, unpublished report no.: P-0465 (FMC no.: CL 1.1.2/1). Final addendum to the Draft Assessment Report (DAR) – initial risk assessment provided by the Rapporteur Member State Denmark for the Existing Active Substance Clomazone

ElNaggar SF, Creekmore RW, Schocken MJ, Rosen RT, Robinson RA (1992) Metabolism of clomazone herbicide in soybean. J Agric Food Chem 40:880–883
Ferhatoglu Y, Barrett M (2006) Studies of clomazone mode of action. Pest Biochem Physiol 85(1):7–14
Gallandt ER, Fay PK, Inskeep WP (1989) Clomazone dissipation in two Montana soils. Weed Technol 3(1):146–150
Gunasekara AS, de la Cruz IDP, Curtis MJ, Claassen VP, Tjeerdema RS (2009) The behavior of clomazone in the soil environment. Pest Manag Sci 65:711–716
Halstead SJ, Harvey RG (1988) Effect of rate and carrier on clomazone movement off-site. Weed Technol 2(2):179–182
Helbling DE, Hollender J, Kohler HPE, Singer H, Fenner K (2010) High-throughput identification of microbial transformation products of organic micropollutants. Environ Sci Technol 44:6621–6627
Kana R, Spundova M, Ilik P, Lazar D, Klem K, Tomek P, Naus J, Prasil O (2004) Effect of herbicide clomazone on photosynthetic processes in primary barley (*Hordeum vulgare* L.) leaves. Pest Biochem Physiol 78(3):161–170
Kirksey KB, Hayes RM, Krueger WA, Mullins CA, Mueller TC (1996) Clomazone dissipation in two Tennessee soils. Weed Sci 44(4):959–963
Lee DJ, Senseman SA, O'Barr JH, Chandler JM, Krutz LJ, McCauley GN, Kuk YI (2004) Soil characteristics and water potential effects on plant-available clomazone in rice. Weed Sci 52:310–318
Liebl RA, Norman MA (1991) Mechanism of clomazone selectivity in corn (*Zea mays*), soybean (*Glycine max*) smooth pigweed (*Amaranthus hybridus*), and velvetleaf (*Abutilon theophrasti*). Weed Sci 39(3):329–332
Liu SY, Shocken M, Rosazza JPN (1996) Microbial transformations of clomazone. J Agric Food Chem 44:313–319
Loux MM, Liebl RA, Slife FW (1989) Adsorption of clomazone on soils, sediments and clays. Weed Sci 37(3):440–444
Marchesan E, Zanella R, de Avila LA, Camargo ER, de Oliveria Machado SL, Macedo VRM (2007) Rice herbicide monitoring in two Brazilian rivers during the rice growing season. Sci Agric 64(2):131–137
Mervosh TL, Sims GK, Ellsworth TR (1995a) Clomazone fate in soil as affected by microbial activity, temperature, and soil moisture. J Agric Food Chem 43:537–543
Mervosh TL, Sims GK, Ellsworth TR (1995b) Clomazone sorption in soils: incubation time, temperature, and soil moisture effects. J Agric Food Chem 43(8):2295–2300
Mervosh TL, Stoller EW, Simmons FW, Ellsworth TR, Sims GK (1995c) Effects of starch encapsulation on clomazone and atrazine movement in soil and clomazone volatilization. Weed Sci 43:445–453
Mills JA, Witt WW, Barrett M (1989) Effects of tillage on the efficacy and persistence of clomazone in soybean (*Glycine max*). Weed Sci 37(2):217–222
Moraes BS, Loro VL, Glusczak L, Pretto A, Menezes C, Marchesan E, de Oliveria Machado S (2007) Effects of four rice herbicides on some metabolic and toxicology parameters of teleost fish (*Leporinus obtusidens*). Chemosphere 68:1597–1601
Moraes BS, Loro VL, Pretto A, Braga da Fonseca M, Menezes C, Marchesan E, Boschmann Reimche G, de Avila LA (2009) Toxicological and metabolic parameters of the teleost fish (*Leporinus obtusidens*) in response to commercial herbicides containing clomazone and propanil. Pest Biochem Physiol 95:57–62
Norman MA, Liebl RA, Widholm JM (1990) Uptake and metabolism of clomazone in tolerant-soybean and susceptible-cotton photomixotrophic cell suspension cultures. Plant Physiol 92:777–784
Pekar S (2002) Susceptibility of the spider *Theridion impressum* to 17 pesticides. J Pest Sci 75:51–55
Pekar S, Benes J (2008) Aged pesticide residues are detrimental to agrobiont spiders (Araneae). J Appl Entomol 132:614–622
Pershbacher PW, Ludwig GM, Slaton N (2002) Effects of common aerially applied rice herbicides on the plankton communities of aquaculture ponds. Aquaculture 214:241–246

Quayle WC, Oliver DP, Zrna S (2006) Field dissipation and environmental hazard assessment of clomazone, molinate, and thiobencarb in Australian rice culture. J Agric Food Chem 54:7213–7220

Sandmann G, Boger P (1987) Interconversion of prenyl pyrophosphates and subsequent reactions in the presence of FMC-57020. Z Naturforschung 42c:803–807

Santi A, Menezes C, Duarte MMF, Leitemperger J, Lopes T, Loro VL (2011) Oxidative stress biomarkers and acetylcholinesterase activity in human erythrocytes exposed to clomazone (in witro). Interdiscip Toxicol 4(3):149–153

Schocken MJ (1997) Review: microbial synthesis of agrochemical metabolites. J Ind Microbiol Biotechnol 19:392–400

Schummer C, Mothiron E, Appenzeller BMR, Rizet A, Wennig R, Millet M (2010) Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg. France Environ Pollut 158:576–584

Scott JE, Weston LA, Jones RT (1995) Clomazone for weed control in transplanted cole crops (*Brassica oleracea*). Weed Sci 43:121–127

TenBrook PL, Tjeerdema RS (2006) Biotransformation of clomazone in rice (*Oryza sativa*) and early watergrass (*Echinochloa oryzoides*). Pestic Biochem Phys 85(1):38–45

Thelen KD, Kells JJ, Penner D (1988) Comparison of application methods and tillage practices on volatilization of clomazone. Weed Technol 2(3):323–326

Tomco PL, Tjeerdema R (2012) Photolytic versus microbial degradation of clomazone in a flooded California rice field soil. Pest Manag Sci 68(8):1141–1147

Tomco PL, Holstege DM, Zou W, Tjeerdema RS (2010) Microbial degradation of clomazone under simulated California rice field conditions. J Agric Food Chem 25:3674–3680

Tomlin CDS (2009) The pesticide manual: a world compendium, 15th edn. The British Crop Protection Council, Surrey, pp 220–221

US EPA (2007) Clomazone summary document: registration review. http://www.epa.gov/oppsrrd1/registration_review/clomazone/clomazone_summary.pdf. Accessed 26 May 2013

US EPA (2009) Clomazone: registration review. Ecological risk assessment and effects determination. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0081-0124. Accessed 20 Aug 2013

Webster EP, Baldwin FL, Dillon TL (1999) The potential for clomazone use in rice (*Oryza sativa*). Weed Technol 13:390–393

Westberg DE, Oliver LR, Frans RE (1989) Weed control with clomazone alone and with other herbicides. Weed Technol 3:678–685

Wong ATS, Tylka GL, Hartzler RG (1993) Effects of eight herbicides on in vitro hatching of *Heterodera glycines*. J Nematol 25(4):578–584

Yasuor H, TenBrook PL, Tjeerdema RS, Fischer AJ (2008) Responses to clomazone and 5-ketoclomazone by *Echinochloa phyllopogon* resistant to multiple herbicides in Californian rice fields. Pest Manag Sci 64(10):1031–1039

Zanella R, Primel EG, Machado SLO, Gonçalves FF, Marchesan E (2002) Monitoring of the herbicide clomazone in environmental water samples by solid-phase extraction and high-performance liquid chromatography with ultraviolet detection. J Cromatogr 55(9):257–262

Zanella R, Primel EG, Gonçalves FF, Martins ML, Adaime MB, Marchesan E, Machado SLO (2008) Study of the degradation of the herbicide clomazone in distilled and in irrigated rice field waters using HPLC_DAD and GC-MS. J Braz Chem Soc 19(5):987–995

Modulation of Plant Growth and Metabolism in Cadmium-Enriched Environments

Shaista Qadir, Sumiya Jamshieed, Saiema Rasool, Muhammad Ashraf, Nudrat Aisha Akram, and Parvaiz Ahmad

Contents

1 Introduction 52
2 Sources of Cadmium 53
2.1 Natural Sources 53
2.2 Anthropogenic Sources 53
3 The Behavior of Cd in Soil and Plants 54
4 Cadmium Accumulation in Plants 54
5 Effects of Cd on Plant Morphological Traits 55
6 Cadmium-Induced Physiochemical Alterations 59
6.1 Pigment Concentration 59
6.2 Protein Levels 61
6.3 Protease Activity 61
6.4 Free Amino Acids and Proline 62
6.5 Effect on Genetic Material 63
6.6 Fatty Acid Composition 63
6.7 Antagonistic and Synergistic Effects of Cd on Uptake of Metals 64
6.8 Effect of Cd on Synthesis/Accumulation of Key Organic Osmotica 65
6.9 Effect of Cd on the Oxidative Defense System 66

S. Qadir
Department of Botany, Womens Degree College,
Moulana Azad Road, Srinagar, Jammu and Kashmir 190001, India

S. Jamshieed • S. Rasool
Department of Botany, Jamia Hamdard, New Delhi 110062, India

M. Ashraf
University College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan

N.A. Akram
Department of Botany, GC University, Faisalabad 38000, Pakistan

P. Ahmad (✉)
Department of Botany, A.S. College, University of Kashmir, Srinagar 190008, India
e-mail: parvaizbot@yahoo.com

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229*, Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6_4,

6.10 Cd and the Effect of Plant Growth Regulators 67
6.11 Effect of Cd on Photosynthesis 69
6.12 Effect of Cd on Water Relations 70
7 Conclusions and Future Prospects 71
8 Summary 73
References 73

1 Introduction

The term "heavy metal" refers to an element that usually has an atomic number greater than 20 and a density higher than 5.0 g cm^{-3}. Anthropogenic activities are the main source of heavy metal release, and such releases may cause considerable damage to ecosystems (Tyler et al. 1989; Zhang et al. 2009; Olubunmi and Olorunsola 2010; Lin et al. 2011).

Some heavy metals are toxic at very low concentrations (Ne et al. 2005; Kokkali et al. 2011; Khan et al. 2011b, c) and exposure to them may pose hazards to many biological systems including humans (Rauser and Muwly 1995; Zhang et al. 2009; Ashraf et al. 2010; Olubunmi and Olorunsola 2010; Lin et al. 2011). Enzymes are adversely affected by the toxic actions of heavy metal ions. Such toxic actions include alteration of catalytically active groups or protein denaturation (Sottnikova et al. 2003; Bavi et al. 2011), which impairs metabolism and leads to poor plant growth and development. Among the heavy metals, Cd is increasingly recognized by the scientific community as a toxic threat to the environment. The water solubility of Cd is considerably higher than that of other heavy metal pollutants, and it is this attribute of Cd that most contributes to its high toxicity.

Cadmium is known to accumulate in plants, and such accumulation alters metabolic processes. For example, Cd disturbs enzyme activities (Hasan et al. 2008), generates reactive oxygen species (ROS) (Singh et al. 2006), alters the uptake, accumulation, translocation and consumption of essential nutrients (Liu et al. 2006), and impairs photosynthesis (Dong et al. 2005). All of these effects ultimately result in symptomatic browning of root tips, necrosis, chlorosis and, ultimately, tissue death (Hasan et al. 2011).

Plants address elevated levels of heavy metals by triggering a wide range of cellular responses. Such responses include synthesis of metal-detoxifying peptides (e.g., phytochelatins or metallothioneins), changes in gene expression, and establishing metal ion homeostasis and compartmentalization of ligand-metal complexes (Clemens 2001; Cobbett and Goldsbrough 2002; Hall and Williams 2003; Jonak et al. 2004). For example, plants avoid excessive Cd ions from entering the cytosol by compartmentalizing them in vacuoles, which is regarded to be one of the best strategies for its removal (Toppi and Gabrielli 1999). Several studies have indicated that the vacuole is the storehouse for several toxic metals, including Cd (Hall 2002; Abbas et al. 2010).

In the present review, we address the accumulation of Cd in plants that derives from different sources, the effect of Cd on physio-biochemical attributes of plants, and remedial measures that are applied for removing it as an environmental contaminant.

2 Sources of Cadmium

2.1 Natural Sources

Cadmium naturally exists primarily in zinc minerals as a sulfide (CdS), but chlorides, oxides and sulfates also occur. The regular erosion of rocks releases cadmium into the environment. It is estimated that about 15,000 metric tons (mt) of Cd are released per annum (OECD 1994). The natural Cd concentration in the atmosphere is 0.1–0.5 ng m^{-3}, and in the Earth's crust 0.1–0.5 μg g^{-1}, although sedimentary rocks and marine phosphates can retain considerably higher levels, e.g., phosphorites contain Cd levels as high as 500 μg g^{-1} (Cook and Morrow 1995; Kumar et al. 2000). Air emissions from forest fires also add about 1–70 mt annually to the atmosphere (Nriagu 1980; Pope et al. 2009).

2.2 Anthropogenic Sources

The main source of Cd release into the environment, however, is via industrial applications that have utilized Cd. Industrial uses of Cd emerged during the first half of the twentieth century. Cadmium became widely used as a result of its unique physico-chemical characteristics, which include its considerable resistance to high temperatures, chemicals, and ultraviolet radiation (Morrow and Keatings 1997; Seuntjens et al. 2002; Laurent et al. 2009). Some pigments are referred to as cadmium pigments and contain high amounts of Cd. Sufficient exposure to Cd pigments causes Cd toxicity. Although the majority of Cd produced is now used in Ni-Cd rechargeable batteries (Morrow 1996), considerable amounts are also employed to produce yellow-to-red pigments, primarily for paints and plastics that have high temperature applications (http://www.jufapigment.com/Cadmium/). Cadmium is also emitted into the atmosphere from the burning of coal, litter, and from metal mining and refining activities. Moreover, cadmium is released to water courses and to soils from household or industrial wastewater disposal. All such releases elevate the environmental soil level of Cd, the burden of which now varies from 100 to 600 mg kg^{-1} dry weight (Lombi et al. 2000; Arora and Sharma 2009). In general, industrial/urban areas suffer the highest metal contamination, followed by rural and more remote areas.

Cadmium enters the human body via inhalation and dietary intake; food intake is more significant than intake from water (Clemens et al. 2013). The Cd concentration in food commodities is an important parameter for determining the quality of food derived from those commodities (van Assche 1998). In some occupations, exposure to Cd poses a hazard, although many countries have established safe worker exposure limits for this metal.

3 The Behavior of Cd in Soil and Plants

Cadmium is absorbed by plants through passive transport (Weis and Weis 2004; Ueki and Citovsky 2005; Liu et al. 2011) and is translocated freely to plant tissues. The uptake of Cd from soil to above-ground plant parts depends upon many factors such as total amount of Cd in the rhizosphere and the soil pH. Soil pH controls the available exchange sites on clay micelles and thus the cation exchange capacity (CEC) of soil. At low pH, any metal bound to soil particles will be removed, since, under such conditions, H^+ binds to soil particles more tightly than to other cations (Garcia Miragaya and Page 1978; Dube et al. 2001; McCauley 2009). In contrast, high pH limits the bioavailability of cations because it results in reduced competition with H^+ for available binding sites. Decreased soil pH promotes increased cadmium bioavailability and increases uptake of cadmium until the concentration reaches a toxic level in plants. A pH range of 4.5–5.5 is regarded to be optimal for cadmium soil or plant mobility (Bingham et al. 1980; Joner and Leyval 1997; Horsfall and Abia 2003; Januškaitiene et al. 2008). It has been estimated that the adsorption of cadmium is almost doubled for each increase of 0.5 pH units from 4 to 7 (Andersen and Andersen 1988; Prelota et al. 2002). High concentrations of Cu and Zn in soil renders Cd more easily removed from soil and therefore more bioavailable. Since Zn and Cd have structural similarity, they compete with each other in plant uptake processes. In contrast, adding calcium (Ca^{2+}) to soil increases soil pH, which, in turn, reduces Cd bioavailability. Manganese (Mn) also competes with Cd during plant utilization processes (Cailliatte et al. 2010).

4 Cadmium Accumulation in Plants

Generally, most of the Cd entering a plant accumulates in the roots and only a small amount is transferred to the upper plant parts (i.e., stems, leaves, fruit and seeds; Vitoria et al. 2001; Malkowski et al. 2002). The amount of Cd that accumulates in a plant depends on the amount applied to the growth medium and the plant growth stage. Furthermore, the accumulation rate of Cd depends on the plant species involved, and on the soil characteristics (Dong et al. 2005; John et al. 2009; Faizan et al. 2011; He et al. 2013). Vassilev et al. (1998) found a 10-fold greater Cd accumulation in barley roots than in shoots. Similarly, Shah and Dubey (1998) reported a two- and four-fold greater accumulation of Cd in roots than in above-ground plant parts in two different rice cultivars.

While observing time-course accumulation of Cd in durum wheat plants, using ^{109}Cd-labelled nutrient solutions, Harris and Taylor (2004) found that in the short-term, i.e., less than 3 h, no significant change was observed among different lines in concentration or time-dependent ^{109}Cd uptake through roots. However, after a longer exposure (48–60 h) a 1.8-fold higher accumulation was found in the high Cd-accumulating line. Leita et al. (1991) treated *Phaseolus vulgaris* plants with 1,

2 and 2.5 mM of $Cd(NO_3)_2$ and found that Cd accumulated at a considerably lower level in stems than in roots. In another study, *Cichorium intybus* roots accumulated 10-fold more Cd than shoots, whereas in *C. roseus,* the relative accumulation was five-fold, indicating that these species are shoot Cd excluders (Lozano-Rodriguez et al. 1997). Other species (e.g., *Alyssum* spp., *Thlaspi caerulescens*) accumulated more Cd in shoots than in roots and have been called Cd hyper-accumulators (Kramer et al. 1996). Nonetheless, high-accumulation of Cd in the roots may occur from bonding between Cd and either carboxylate or thiol groups of cell wall proteins (Andelkovic et al. 2010). This is probably the mechanism by which roots can tolerate high levels of Cd (Chaoui et al. 1997; Kil et al. 2006; Hynek et al. 2012).

5 Effects of Cd on Plant Morphological Traits

Both deficient and excessive levels of a metal can exert adverse effects on plant morphology (Table 1). The relationship between exposure to an element and plant growth can be deciphered from a dose-response curve. Under similar conditions, different species respond differently to Cd exposure; thus some plants are indicators, some excluders and others are accumulators (Ghosh and Singh 2005; Rotkittikhun et al. 2006). The pattern of Cd uptake and its effects on plants is governed by several factors, and the interactions of Cd with other metals at the root-soil surface may be independent, antagonistic or synergistic (Luan et al. 2008; Kalavrouziotis et al. (2009a, b); Eshghi et al. 2010; Khan et al. 2011b). However, there is a general consensus that symptoms of excess environmental exposure to most non-essential metals, including Cd, occur first in roots then in leaves (Di Cagno et al. 1999; Page and Feller 2005). Cadmium toxicity generally results in a reduction in root elongation, a decline in root metabolic activities and root biomass production, alterations in root architecture, reduced hair formation and lateral root initiation, and development of a relatively compact and dense root system (Kahle 1993; Archambault et al. 2001; Peralta et al. 2000; Shamsi et al. 2007).

Shoot growth is also adversely affected by Cd in a concentration-dependent manner. Usually, low levels of Cd have no effect; high doses of Cd, however, may be very injurious. Setia et al. (1993) found that Cd at 8 mM caused a 23% reduction in the diameter of new wheat stems. Carlson and Bazzaz (1977) examined new stem and leaf growth in American sycamore (*Plantanus occidentalis*) exposed to Cd concentrations ranging from 10 to 100 μg g^{-1}. They showed that Cd significantly reduced shoot growth. Generally, when shoot growth is adversely affected, overall plant biomass also declines. For example, the root dry weight of *Plantanus occidentalis* was reduced by 10–30% when seedlings were exposed to 10–100 mg g^{-1} Cd in the growth medium (Kahle 1993). Similarly, while working with *Vigna catjang* seedlings, Bhattacharyya and Choudhuri (1994) reported a considerable decrease (33.6%) in seedling biomass at a Cd exposure level of 10^{-5} M. Similarly, a considerable reduction in seed mass (16%) and number of pods (83%) in soybean

Table 1 Effects of different concentrations of cadmium (Cd) on growth and physio-chemical attributes of various plant species

Plant species	Cd level	Effect on growth and physiological attributes	Reference
Cowpea (*Vigna unguiculata* L.)	10, 30, and 50 mg kg^{-1}	Different concentrations of Cd decreased the root and shoot length, fresh and dry weight of root and shoot, total leaf area, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of cowpea plants compared to those of no metal treated cowpea plants	Vijayaragavan et al. (2011)
Tomato (*Lycopersicon esculentum* L.)	100 mg kg^{-1}	Exogenous application of Cd decreased shoot growth, chlorophyll content, whereas carotenoid and ascorbic acid contents increased in all tomato plants	Unyayar et al. (2010)
Maize (*Zea mays* L.)	0.1–0.5 mmol L^{-1} $CdCl_2$	Cd suppressed the activity of δ-aminolevulinic acid dehydratase (ALAD), as well as the contents of aminolevulinic acid (ALA), total chlorophyll and protein content in excised etiolated segments of maize leaves during greening. It was suggested that Cd adversely affected ALAD activity by affecting ALA binding to the enzyme and/or disrupting thiol interaction	Sarangthem et al. (2011)
Soybean (*Glycine max*)	0, 50, 100, 200, 400, 800 and 1,600 mg kg^{-1}	Seed germination decreased significantly and maximum decrease observed at 1,600 mg kg^{-1} Cd	Luan et al. (2008)
Chickpea (*Cicer arietinum* L.)	0, 25, 50 and 100 mg Cd kg^{-1} soil	Cd treatment increased the seedling mortality, chlorosis, necrosis, stunting of shoot growth, and proline contents, while decreased plant dry matter, leaf area and nitrate reductase activity	Faizan et al. (2011)
Bean (*Phaseolus vulgaris* L.)	3 µM	Cd-treated plants showed decreased leaf relative water contents, root and leaf expansion growth and higher stomatal resistance as compared to non-treated plants. About 400% increase in the leaf ABA concentration was observed after 120 h exposure to Cd	Poschenreider et al. (1989)
Pakchoi (*Brassica campestris*) and Mustard (*Brassica juncea*)	0^{-}24 mg kg^{-1}	A significant reduction at 24 mg Cd kg^{-1} soil in shoot and root weight, chlorophyll *a* and *b* contents in both pakchoi and mustard plants were observed. The increase in Cd concentration also caused decrease in rate of photosynthesis and stomatal conductance	Chen et al. (2011)
Hybrid poplar (*Populusnigra* × *maximowitzii* × P. nigra var. Italica)	$10^{-5}$and 10^{-4} M	Cd stress induced stunted growth (plant height and biomass), decreased stem and leaf growth, root length as well as caused chlorosis of young leaves. In addition, the decrease in photosynthetic rate of Cd-treated plants attributed to decrease in chlorophyll contents. The activities of SOD (superoxide dismutase), CAT (catalase), GST and GSH-Px (glutathione peroxidise) and proline contents were also adversely affected	Nikolić et al. (2008)

Barley (*Hordeum vulgare* L.)	3.0 and 6.0 mg L^{-1}	In Cd-treated plants, a decreased photosynthetic rate was observed from disturbances in the chloroplast ultrastructural organization	Vassilev et al. (1995)
Barley (*Hordeum vulgare* L.)	54 µM	Decreased plant growth from reduced chlorophyll and carotenoid content, net photosynthetic and transpiration rates and increased rate of dark respiration	Vassilev et al. (1998)
Pea (*Pisum sativum* L.)	25 µM	Cd stress caused severe damage to PS-II, inducing a drastic decline in the amplitude of flash-induced oxygen yields	Yordanova et al. (2009)
Cauliflower (*Brassica oleracea*)	50, 100, 250, 500, 750 and 1,000 µM	55–66% inhibition in seed germination was observed at a concentration of 1,000 µM $CdCl_2$	Theriappan et al. (2011)
Bean (*Phaseolus vulgaris* L.)	0.05, 0.06 and 0.08 mM	Total chlorophyll content decreased while a significant increase of retinol, α-tocopherol and ascorbic acid content was observed after exposure to cadmium stress	Zengin and Munzuroglu (2005)
Wheat (*Triticum aestivum* L.)	100, 200 and 300 µM	On Cd exposure, free proline, soluble phenolics and total soluble protein contents were increased in wheat seedlings	Ergün and Öncel (2012)
Tomato (*Solanum lycopersicum*)	3, 6, 9, 12 mg kg^{-1}	Cd stress caused reduced plant growth and biological yield by adversely affecting the antioxidant system of tomato fruit	Hayat et al. (2012)
Bean (*Phaseolus vulgaris* L.)	1 mM Cd^{2+}	Application of $CdCl_2$ considerably reduced plant growth, pigment amount, green pod yield and pod protein. In addition, Cd stress also increased electrolyte leakage, lipid peroxidation and plant Cd^{2+} content, whereas relative water content and the membrane stability index were decreased	Rady (2011)
Mungbean (*Vigna radiata* L.)	3, 6, 9 and 12 mg kg^{-1}	Cadmium stress decreased seed yield in terms of reduced number of seeds per pod and number of seeds per plant.	Ghani (2010)
Rice (*Oryza sativa* L.)	0, 2, 4 and 6 mmol L^{-1}	Cd stress induced high oxidative damage and reduced the activities of GST and CAT	Zhao et al. (2009)
Peanut (*Arachis hypogaea* L.)	0, 10, 100 and 500 µM $CdCl_2$	Seedling growth and the maximal photochemical efficiency of photosystem II were suppressed from Cd treatments, particularly at 500 µM of Cd^{2+}. High concentrations of H_2O_2 and malondialdehyde (MDA) indicated that Cd stress caused high accumulation of reactive oxygen species and induced oxidative stress. Moreover, Cd stress affected shoots less than roots	Shan et al. (2012)

(continued)

Table 1 (continued)

Plant species	Cd level	Effect on growth and physiological attributes	Reference
Rice (*Oryza sativa* L.)	0.0, 0.1, 1.0 and 5.0 µmol L^{-1}	Cd stress reduced plant height and chlorophyll content, altered malondialdehyde (MDA) content and activities of SOD, CAT and peroxidase (POD) in rice plants. Roots and shoots of rice plants responded differently to Cd stress in terms of antioxidant enzyme activity. Generally, the activities of SOD, POD and CAT in shoots and roots declined with increased Cd level, while MDA content increased with increase in external Cd level	Guo-sheng et al. (2004)
Tomato (*Lycopersicon esculentum* L.)	0, 0.1, 1, 5 and 10 µmol L^{-1}	At 1.0 and 10 µmol L^{-1} Cd levels, root length, plant height, photosynthetic rate and internal CO_2 concentration all decreased markedly	Dong et al. (2005)
Powder Suma (*Pfaffia glomerata*)	0, 20, 40, 60 and 80 µM	Exogenous application of Cd increased Cd levels in both shoots and roots; Cd level was 12-fold greater in root than in shoot tissues. Shoot and root dry wts decreased in plants treated with 80 µM Cd. Zinc and Cu concentrations in both shoot and roots did not change with Cd treatment, although Mn uptake decreased significantly	Skrebsky et al. (2008)
Onion (*Allium cepa*)	0, 1, 10 and 100 µM	High Cd accumulation induced chromosomal aberrations including C-mitoses, chromosome bridges, chromosome fragments and chromosome stickiness. Cadmium treatment affected mineral nutrient metabolism e.g., at 100 µM Cd, levels of Mn, Cu and Zn in roots, bulbs and leaves decreased, while MDA content in roots and leaves increased with increasing Cd concentration	Zhou et al. (2012)
Pea (*Pisum sativum* L.) and Barley (*Hordeum vulgare* L.)	1 mM	Under Cd stress the photosynthetic rate of pea and barley plants decreased by 16.7% and 12.8%, respectively. Intercellular CO_2 concentration decreased by 27.4% in pea leaves, but it increased by 33.5% in barley leaves	Januskaitiene (2010)
Pole bean (*Phaseolus coccineus*)	25 µM	In the leaves of Cd-treated plants, the content of anthocyanins and free fatty acids, lipoxygenase activity as well as non-enzymatic lipid peroxidation increased while, glutathione-*S*-transferase level decreased significantly	Skórzyńska-Polit and Krupa (2006)
Barley (*Hordeum vulgare* L.)	25 µM	Plant size, biomass, photosynthetic carboxylation efficiency, and stomatal conductance decreased significantly with Cd exposure. In addition, plants exposed to Cd showed an increase in F_o and F_q, and a decrease in F_v/F_m indicating Cd-induced photoinhibitory damage to PSII	Chena and Huerta (1997)
Maize (*Zea mays* L.)	100 and 250 µM	Cd applied decreased root and shoot length, caused changes in ultrastructure of chloroplast and reduced rate photosynthetic rate	Rascio et al. (1993)

was observed at a concentration of 0.05 mg/L Cd (Malan and Farrant 1998). Guo-Sheng et al. (2004), working with rice plants, found that stress from Cd exposure (0.1, 1.0 and 5.0 μmol L^{-1}) reduced the height of rice plants, which was attributed to Cd-induced alterations in malondialdehyde (MDA) content in rice leaves. Recently, Gill et al. (2011) examined the role of Cd in different mustard (*Brassica juncea*) lines using $CdCl_2$ (25, 50, 100 and 150 mg Cd kg^{-1} soil), resulting in adverse effects on plant growth and yield, particularly at 150 mg Cd kg^{-1} soil in all lines tested. The reduced growth may have resulted from suppression in the elongation phase of the cells, and from an irreversible Cd-induced inhibition of different proton pumps (Aidid and Okamoto 1992, 1993; John et al. 2009; Gill et al. 2011; Khan et al. 2011a).

6 Cadmium-Induced Physiochemical Alterations

6.1 Pigment Concentration

Photosynthetic pigments, particularly the chlorophylls and carotenoids, are essential for plant photosynthesis, and levels of both are modulated in response to ambient light and the soil environment to achieve vegetative and reproductive growth (Hall and Rao 1999; Akram and Ashraf 2011a, b; Akram et al. 2012; Saleem et al. 2011, 2012). The quantities of these two pigments indicate the nutritional status and predict crop productivity (Seyyedi et al. 1999; Akram et al. 2012).

Chlorophyll

Cadmium toxicity results in leaf chlorosis and necrosis, which affects photosynthesis. Chlorophyll (chl) is a tetrapyrrole molecule containing Mg^{2+} in its center (Kannangara 1991; Tanaka and Tanaka 2007; Molins et al. 2013). The chloroplast organelle contains chlorophylls in the thylakoid membranes, within the antenna light-harvesting arrays and the photosynthetic reaction centers. The vascular plants contain chl '*a*' and chl '*b*' in the ratio of 2:1 or 3:1 (Lidia et al. 2011). Many researchers have reported cadmium-induced decreases in chlorophyll content (Schlegel et al. 1987; Rascio et al. 1993; Ouzounidou et al. 1997; Skorzynska-Polit and Baszynski 1997; Larsson et al. 1998; Erdei et al. 2002; Zengin and Munzuroglu 2005; Zhang and Huang 2007; Tantrey and Agnihotri 2010; Vijayaragavan et al. 2011) and this phenomenon can be used as a marker of Cd toxicity. Decreased chlorophyll content from Cd exposure has several causes, including effects on several enzymes as well as on sub-cellular organization. During necrosis, Cd in the plant is mobilized to other plant parts (Skorzynska-Polit and Baszynski 1997; Yang et al. 2004). Bhattacharya and Chaudhuri (1995) observed a decline in the total chlorophyll and carotenoid contents in seedlings of *Vigna catjang* at a Cd concentration of 10^{-5} M.

Similarly, Ralph and Burchett (1998) reported decreased chlorophyll in *Halophila ovalis* after 5 h of Cd exposure at 1, 5 and 10 kg L^{-1}. A considerable reduction in chlorophyll content was observed in barley upon exposure to a high amount (1 mM) of exogenous Cd (Horvath et al. 1996). The reduction in chlorophyll was caused by a perturbance in the integration of chlorophyll molecules into stable complexes, rather than from impaired biosynthesis of chlorophyll molecules.

Cadmium is known to affect the biosynthesis of δ-aminolevulinic acid (ALA), a precursor in chlorophyll biosynthesis. Two molecules of ALA are condensed to porphobilinogen (PBG) by Mg^{2+} or Zn^{2+}-dependent ALA dehydratase (Sasa and Sugahara 1976; Toneva et al. 2002; Sarangthem et al. 2011), and this is the limiting step for chlorophyll production (Dahlin et al. 2000; Tanaka and Tanaka 2007; Tanaka et al. 2011; Akram et al. 2012). Disorganization of lamellar structures, mainly in the stroma, also produces inhibition of chlorophyll biosynthesis (Halloin et al. 1970; Simidjiev et al. 2000; Kovacs and Keresztes 2002). In heavy-metal-treated plants, enzymatic degradation of chlorophyll occurs and is ascribed to increased chlorophyllase activity (Bhattacharjee and Mukherjee 2003; Kambhampati et al. 2005).

Carotenoids

Carotenoids are accessory pigments that absorb light and transfer it to key chl'*a*' molecules taking part in photosynthetic light reactions. Like chlorophyll, the carotenoids are also present on the thylakoid membrane. As part of their role in light harvesting, carotenoids are active in preventing the formation of reactive oxygen species (ROS) (Young and Britton 1990; Müller-Moulé et al. 2004). The carotenoid content has been reported to increase in plants exposed to heavy metals (Ralph and Burchett 1998; Tiwari et al. 2001; Dar et al. 2010), although less of an effect has been observed in some cases (Clijsters and Van-Assche 1985). Ying et al. (2010) studied the effects of Cd on carbon assimilation and chloroplast ultrastructure of *Picris divaricata*, which is considered to be a Cd/Zn hyperaccumulator. Exogenous application of 75 μM Cd did not alter the content of chlorophyll '*a*' or '*b*', chlorophyll *a/b* ratio or carotenoid content. In contrast, Unyayar et al. (2010) observed a more typical response, with a significant increase in carotenoid content of tomato (*Lycopersicon esculentum* Mill.) plants after applying Cd. The authors attributed this Cd-induced increase in carotenoids to their protective role against oxidative damage. However, in mustard (*Brassica juncea* L.), it was found that treating seeds prior to planting with 1.0 mM $CdCl_2$ reduced the concentration of carotenoids and chlorophylls (Bauddh and Singh 2011). Recently, on exposure to 50 or 200 μM cadmium, Mohamed et al. (2012) reported a considerable decrease in both chlorophyll and carotenoid content of *B. juncea* plants and stimulation in the xanthophyll cycle, suggesting the need to protect the photosynthetic apparatus from photoinhibition.

6.2 Protein Levels

Proteins play a key role in maintaining the structure and function of cells. They function as enzymes, and transport regulatory proteins. However, heavy metal stress changes protein metabolism in plants. Heavy-metal exposed plants may accelerate protein synthesis (Brune et al. 1995; Chakravarty and Srivastava 1997) or decrease it (Costa and Spitz 1997).

Plants contain small metal binding proteins known as metallothioneins (MTs), which have a role in regulating the metabolism of metal ions (Krezel and Maret 2008; Nordberg and Nordberg 2009; Palacios et al. 2011). For example, Lue-Kim and Rauser (1986) observed that a high concentration of Cd induced a 31 kDa Cd-binding protein, and at a low Cd concentration induced a 21.5 kDa protein. An increase in soluble proteins occurred in tobacco leaves at a concentration of 20 μM $CdCl_2$ (Vogeli-Lange and Wagner 1990), and in pea roots and shoots at 0.05 mM Cd; in contrast, no effect of this treatment occurred at 0.05 mM on maize (Lozano-Rodriguez et al. 1997). At high Cd levels in in vitro studies, protein content was increased; e.g., *Bacopa monniera* plantlets showed increased protein content under cadmium stress (Ali et al. 1998). Hirt et al. (1989) observed that Cd (100 μM) stimulates protein and RNA synthesis in tobacco cell suspensions. The increased protein content may be attributed to *de novo* protein synthesis that is stimulated to reduce the endogenous level of Cd through chelation (Hirt et al. 1989).

Rubisco, a key enzyme of the C_3 carbon fixation pathway, constitutes more than 50% of the soluble leaf protein in plants (Woolhouse 1974). Cadmium caused a marked suppression of rubisco activity, ultimately reducing the rate of photosynthesis (Mishra and Dubey 2005; Hussain et al. 2012). In an earlier study with tomato, total soluble proteins and rubisco activity were decreased at exposure levels of 15 and 30 mg L^{-1} Cd (Gill et al. 1995).

6.3 Protease Activity

Protein molecules are synthesized and degraded continuously in each cell (Stimpson et al. 2006). Proteases play a vital role in degrading proteins, with some performing slight post-translational modification, whereas others completely degrade proteins, and convert precursor proteins into functional enzymes (Rao et al. 1998). Plant stress caused by heavy metals such as Cd induce leaf senescence through programmed senescence pathways (Heise et al. 2007; Lim et al. 2007; Xiao and Chye 2011; Ahmad et al. 2012).

One event common in plants during senescence is the development of active oxygen species (AOS). Under any unfavorable environmental condition, the AOS concentration rises to toxic levels causing cellular injuries, denaturation of enzymes and DNA damage (Jonak et al. 2004; Ai-jun et al. 2007). Protease activity is reported to increase in the leaves of terrestrial (*Vigna* spp.) and aquatic (*Hydrilla* spp.) plants

treated with Cd at a concentration of 1.124 mg L^{-1} (Bhattacharyya and Choudhuri 1994). Shah and Dubey (1997) reported that Cd^{2+} at 500 μM $Cd(NO_3)_2$ reduced protease activity in roots and shoots of rice seedlings, as well as in embryo axes and endosperm. However, under in vitro conditions protease activity was enhanced at lower Cd levels (50–100 μM), although activity was inhibited at a higher concentration (Shah et al. 1998). Nagoor (1999) treated maize seeds with $CdCl_2$ at 50–300 μg mL^{-1} and found that cadmium generally decreased protease activity. While studying the effects of Cd (100, 200 and 300 μM $CdCl_2$) on the balance between protein synthesis and degradation in sunflower leaves, Pena et al. (2006) reported that untreated and Cd-treated plants had similar soluble protein contents, despite the fact that protease activity increased with Cd treatment. In another study, Mohan and Hosetti (2006) treated *Salvinia natans* plants with 0.5, 1.0, 5.0 and 10 μmol Cd dm^{-3} and observed that protein content and protease activity decreased under Cd stress.

These study results indicate that Cd exposure may decrease or increase the activity of proteases. The data showed that these changes depend on the Cd concentration, the degree of its accumulation or uptake, type of cultivar or crop, and protein content, indicating that proteases involved in several processes are affected.

6.4 Free Amino Acids and Proline

The regulation of amino acid synthesis in plant tissues is precisely controlled to meet the requirements for biosynthesis of proteins and secondary metabolites to support growth. For example, molecules such as glutathione, have a variety of vital biological roles (Ashraf and Foolad 2007; Cameron and Pakrasi 2010). Any amino acid surplus that exceeds requirements for proteins or other biomolecule synthesis cannot be stored, and therefore are usually used as metabolic fuels (Staswick 1994; Galili and Höfgen 2002). The cells of all organisms always retain a free amino acid pool. Costa and Morel (1994) reported that at low cadmium concentration in roots and leaves of lettuce seedlings both total amino acid levels and the rate of incorporation of ^{14}C into amino acid increased; however, at a high Cd concentration (10 μM) in the medium both the amount of amino acids and ^{14}C incorporation into amino acids declined, suggesting reduced plant metabolism. Heavy-metal-induced stress in germinating maize seeds increased the amino acid pool (Nagoor 1999). Heavy metal (Pb^{2+} and Cd^{2+}) stress in *Hydrila* and *Vigna* sp. increased the free amino acid content (Bhattacharya and Chaudhuri 1995). Shah and Dubey (1997) studied rice seedlings after treating them with 500 μM $Cd(NO_3)_2$. The result was an increase of about 20–40% over control values in free amino acid content of roots, and a 40–80% increase over control values in shoots.

Of the 20 key amino acids, proline differs from the others by having a side chain covalently bonded to both the nitrogen and the ∝ carbon atoms. Proline is an imino acid with an aliphatic side chain (Berger et al. 2000). This cyclic structure influences the three dimensional structure of proteins. Glutamate is a precursor of

proline and it is the only imino acid that accumulates rapidly under unfavorable conditions in plants. It plays a significant role in mitigating salinity, water and heavy-metal stresses in plants (Kavi Kishor et al. 1995; Ashraf and Foolad 2007; Perez-Arellano et al. 2010). Chakravarty and Srivastava (1997) observed high proline accumulation in linseed under Cd stress. Bhattacharyya and Choudhuri (1994) reported a marked increase in proline content in *Hydrilla* and *Vigna,* following application of 10^{-5} M cadmium. Proline accumulation in algal cells, in response to Cd stress, was confirmed in a study that addressed the impact of Cu^{2+} and Cd^{2+} on the endogenous concentration of proline in several algal species (Wu et al. 1995).

6.5 *Effect on Genetic Material*

Cadmium is classified as a carcinogen by IARC (1993). Moreover, Cd induces DNA damage in living organisms including plants (Mouron et al. 2001; Stohs et al. 2000; Bagchi et al. 1996; Koppen and Verschaeve 1996; Badisa et al. 2007; Bork et al. 2010). The toxic effect of Cd on genetic material is observed only at high cellular concentrations. However, at lower concentrations, Cd exposure indirectly affects the genetic material by increasing the effect of other DNA damaging agents (Hengstler et al. 2003; Yen et al. 2005; Zhang et al. 2010). Cadmium inhibits DNA damage recognition and incision steps by interfering with nucleotide excision repair (NER) (Hartwig et al. 1996). Several adverse effects of Cd stress at the genetic level have been studied, such as nuclear apoptotic alterations, chromosomal aberrations and abnormalities in chromatin structure (Pulido and Parrish 2003; Deckert 2005; Banfalvi et al. 2007). It has been observed in *Vicia faba* that Cd stress causes oxidative stress that produces DNA damage (Lin et al. 2007). Cd toxicity to genetic material may be caused via three processes: non-genotoxic, epigenetic and/or signaling pathways disruption. However, disruption of signaling pathways in plants has, thus far, not been identified.

6.6 *Fatty Acid Composition*

Carboxylic acids with hydrocarbon chains of 4–36 carbon atoms are called fatty acids. Biological systems commonly contain 16–18 carbon fatty acids. In some fatty acids, the carbon chain is saturated and unbranched, others are unsaturated and contain one or more double bonds, whereas a few contain 3-carbon rings. Fatty acids are important because they serve as components of phospholipids and glycolipids for membranes, as signaling molecules, and are precursors of hormones and intracellular messengers (Piotrowska-Seget and Mrozik 2003; Mrozik et al. 2004; Xiao 2010). Fatty acids are synthesized in the cytosol. In plants, NADPH is required for the synthesis of fatty acids via the pentose phosphate pathway (Berg et al. 2002; Hutchings et al. 2005).

Unfavorable environmental conditions, including exposure to heavy metals (Krupa and Baszynski 1989; Frostegard et al. 1993; Fodor et al. 1995; Jemal et al. 2000; Xu et al. 2011; Zhong et al. 2011; Zhang et al. 2012), alter the fatty acid composition of plants. Krupa and Baszynski (1989) observed that 4-week-old tomato seedlings grown for 14 days in a medium containing Cd, displayed an approximately 75% reduction in glyco- and phospho-lipids in thylakoids. The phosphatidylcholine content decreased most and the acyl lipids extracted from the thylakoids showed a characteristic decrease in the *trans*-δ-3 hexadecanoic acid component of the phodphatidylglycerol (Gichner et al. 2008; Bork et al. 2010). These studies clearly demonstrated that Cd stress induced a reduction in fatty acid levels in plants by adversely affecting fatty acid metabolism.

6.7 *Antagonistic and Synergistic Effects of Cd on Uptake of Metals*

Environmental pollution from industrial activities that allow metals to accumulate can pose a major threat to human and plant health (Zhao 2011; Zhang et al. 2012). Moreover, exposure to a mixture of heavy metal pollutants may accentuate environmental effects over those resulting from individual exposure (Zhou et al. 2006). For example, Spurgeon et al. (1994) reported that the toxicity of pollutant mixtures may be higher to ecosystems than individual toxicants. Guo and Zhou (2003) reported antagonistic effects of joint exposure of Cd and Pb in watermelon. Sunda and Huntsman (1998) confirmed that Cd enters *Thalassiosira pseudonana* cells through either Mn or Zn ion channels, and the presence of high free Zn or high free Mn decreases the Cd uptake. Wei et al. (2003) also reported antagonism from high joint exposure of Cu and Cd in *T. pseudonana* cells. The pooled effect of the Cd, Cu and Zn on *Chlorella* species was either antagonistic or synergistic when the three were applied as mixtures (Franklin et al. 2002).

Some researchers have reported that Cd and Cu reduce the assimilation of CO_2 by affecting Calvin cycle enzymes (Burzynski and Żurek 2007; Ying et al. 2010). Copper (Cu) and Cd decreased the activities of 3-phosphoglyceric acid kinase (PGK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and also affected the synthesis of proteins in cucumber cotyledons (Burzynski and Żurek 2007). In another study, both Cu and Cd affected the PGK titer in maize leaves (Stiborová et al. 1986). Sharif and Suzelle (2006) demonstrated that the application of Cd and Zn to young wheat plants negatively affected the transpiration rate and yield. High amounts of Cd, Zn, Cu, Pb and Ni added to soil decreased plant yield and adversely affected the food quality or fiber quality in several plant species (Sutapa and Bhattacharyya 2008). A number of nutrients (Ca, Mg, Cu and K) are known to affect both Cd uptake by plants, and its interaction with other elements (e.g., P, Mg, Zn, Cu, Fe, Ca and Se) in plants and soil (Zhou et al. 2006; Sutapa and Bhattacharyya 2008; Ying et al. 2010).

The uptake mechanism of Cd by plant roots involves competition for absorption sites with other heavy metals and mineral nutrients (Przedpełska-Wąsowicz et al. 2012; Yasar et al. 2012). For example, high Cd levels in the tissues of cucumber, tomato and maize led to a reduction in K, Ca and Mg (Walker et al. 1977). Costa and Morel (1994), using lettuce roots, observed antagonism in active absorption between Cd and Zn.

Certain mineral nutrients having chemical characteristics dissimilar to Cd, such as nitrate, are also affected by its presence. Interactions have been documented to occur for Cd with Cu and Zn at the root surface and for Zn with Cd within the plant (Weltje 1998; Trakal et al. 2012). The interaction of Zn with Cd within the plant is antagonistic, whereas Cd with Zn at the root surface is synergistic (Aravind and Prasad 2005). However, Cd was antagonistic to the uptake of Zn in barley (Akay and Koleli 2007) and maize (Root et al. 1975). Jalil et al. (1994) found that the application of Cd reduced Zn and Mn contents in shoots and roots of wheat, but the Cu concentration remained unchanged. It is known that the concentration of Zn in soil influences Cd accumulation in crop plants (Sarwar et al. 2010). Some researchers have reported an antagonistic behavior between Cd and Zn, although other interactions between these two metals have documented synergistic outcomes (Root et al. 1975; Aravind and Prasad 2005; Akay and Koleli 2007).

6.8 *Effect of Cd on Synthesis/Accumulation of Key Organic Osmotica*

Like other abiotic stresses, heavy-metal exposure in plants trigger the accumulation of osmolytes, particularly of glycinebetaine (GB), sucrose, mannitol, trehalose and proline (Dhir et al. 2009). Of the different osmoprotectants, GB and proline have most often been reported to respond to heavy metal stress (Alia and Saradhi 1991; Talanova et al. 2000; Zengin and Munzuroglu 2005). Costa and Morel (1994) reported that the proline concentration in plants under Cd stress is linked to decreased plant water potential. High proline accumulation in certain Cd-stressed plants (e.g., *Brassica juncea, Phaseolus vulgaris* and *Silene vulgaris*) has been recorded (Schat et al. 1997; Talanova et al. 2000; Zengin and Munzuroglu 2005). How proline affects plant water potential under stress conditions was studied by Kastori et al. (1992), who observed high proline accumulation in metal-exposed isolated leaf discs; they suggested that this was attributed to metal uptake rather than to water deficiency (Farago and Mullen 1979). Proline-induced formation of phytochelatins, which chelate heavy metals, particularly Cd, reduces toxic effects to plants (de Knecht et al. 1994). Notwithstanding this, there is no clear evidence that proline accumulation contributes to metal stress tolerance.

Duman et al. (2011) examined the comparative effect of Cd, GB and trehalose (TR) on duckweed (*Lemna gibba*). These authors exogenously applied Cd, GB or TR individually or/and in combination, and recorded increased Cd accumulation in this plant.

Furthermore, a high Cd concentration adversely affected antioxidant metabolism, oxidative systems and photosynthesis, as well as proline content. Although cadmium (100 μM) stress also caused a considerable reduction in growth of tobacco BY-2 cells, proline and GB substantively suppressed this inhibition.

Metabolism is affected by Cd stress throughout the plant life cycle. For example, heavy metals such as Cd, Co and Zn inhibit seed germination and seedling growth in pigeon pea, black gram and wheat, and proline content increased markedly in the germinated seeds and seedlings of all three species when they were under heavy metal stress (Alia and Saradhi 1991). Similarly, Pb, Hg and Cd induced differential accumulation of proline in lemongrass treated at 50–500 mg kg^{-1} (Handique and Handique 2009). The proline content was considerably higher in young tender leaves than in old leaves of lemongrass (*Cymbopogon flexuosus*), irrespective of the metal tested or duration of exposure (Handique and Handique 2009). However, the results of another study demonstrated that proline did not accumulate from heavy metal stress induced by a low Cd exposure level (Schat et al. 1997). In addition, researchers have suggested that proline protects plants from heavy metal toxicity (Kavi Kishor et al. 1995).

Exogenous application of GB is also known to enhance stress tolerance by reducing heavy metal uptake, preventing photoinhibition, minimizing lipid peroxidation and up-regulating the antioxidative defense system (Ma et al. 2006; Banu et al. 2009; Islam et al. (2009a, b); Abbas et al. 2010). TR is a non-reducing disaccharide and plant exposure to it has been linked to enhanced plant metal tolerance (Mahmud et al. 2009; Fernandez et al. 2010). TR has been reported in plants, yeasts and fungi (Wang et al. 2005; Luo et al. 2008) and can stabilize membranes and biological macromolecules such as proteins (Luo et al. 2008). However, there is little information available in the literature on the role of TR in plant Cd tolerance.

In summary, a high accumulation of osmoprotectants, particularly GB and proline, protect plant cellular activities by improving water potential, reducing lipid peroxidation, stabilizing membranes and macromolecules, and chelating metals; in addition, they protect vital cellular activities. However, research is needed to define the mode of action by which proline and GB act in plants that are exposed to Cd stress.

6.9 Effect of Cd on the Oxidative Defense System

Cadmium is the most toxic metal to plants due to its high water solubility (Lockwood 1976). It is known to induce a burst of reactive oxygen species (ROS) in plant tissues that produce oxidative stress (Chien et al. 2001; Gouia et al. 2003; Cosio et al. 2006; Solis-Dominguez et al. 2007; Shahbaz et al. 2008; Perveen et al. 2011, 2012). To overcome the effect of ROS, plants have evolved enzymatic and non-enzymatic defense systems (Stroinski 1999; Ashraf 2009; Noreen et al. 2010; Batool et al. 2013). Among these defense systems, antioxidative enzymes play a vital role in detoxifying ROS via a series of complex reactions (Smeets et al. 2005; Pal et al. 2006;

Shahbaz et al. 2012; Shahbaz and Ashraf 2013). Such reactions include the dismutation of O_2^- to H_2O_2, and detoxification of H_2O_2 by peroxidases, catalases and ascorbate peroxidase (Salin 1988; Asada 1992; Mishra et al. 2006; Akram and Ashraf 2013). The first enzyme in the detoxifying process is superoxide dismutase (SOD) that rapidly converts O^{2-} to H_2O_2 (Polle and Rennenberg 1994). Cadmium-induced changes in the activities of these enzymes have been reported in many important plant species including rice (Chien et al. 2001), willow (Cosio et al. 2006), pea (Chaoui and El Ferjani 2005), bean (Chaoui et al. 1997), soybean (Cataldo et al. 1981), sunflower (Di Cagno et al. 1999) and wheat (Luo et al. 1998). Oxidative stress is induced by Cd (Hendy et al. 1992) either via oxygen free-radical production (Balaknina et al. 2005) or by reducing the concentrations of both non-enzymatic and enzymatic antioxidants (Mohan and Hosetti 2006). These defense systems consist of metabolites such as glutathione, ascorbate and tocopherols, and enzymatic scavengers such as peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) (Demiral and Turkan 2005; Mandhania et al. 2006). The presence of Cd ions can inhibit or stimulate the activity of several antioxidant enzymes. For example, in sunflower leaves, Cd increased the lipid peroxidation and lipoxygenase activity, but reduced the activity of SOD, GR, catalase and ascorbate peroxidase (APX) (Gallego et al. 1996). Cadmium stimulated the activity of peroxidase (POX) in soybean (Fuhrer 1982), in the roots and/or leaves of rice (Reddy and Prasad 1993), brassicas (Singh and Tewari 2003; Hayat et al. 2007), chickpea (Hasan et al. 2007), *Bacopa monniera* (Mishra et al. 2006) and *Calamus tenuis* (Khan and Patra 2007).

In addition to enhanced production of enzymatic antioxidants under Cd stress, it has been documented that non-enzymatic compounds, including non-protein thiols (phytochelatins and glutathione) and ascorbic acid are also produced by and accumulate in plants (Mishra et al. 2006; Tiryakioglu et al. 2006; Srivastava et al. 2011). Mishra et al. (2006) observed phytochelatins in bacopa plants at 10 μM Cd in roots and at 50 μM Cd in leaves. Therefore, the capacity of plants to tolerate a high concentration of Cd through increased level of phytochelatins and different antioxidants is considered to be potentially important in phytoremediation (Mishra et al. 2006). Similarly, the content of ascorbic acid and non-protein thiol groups, especially glutathione, increased at the 400 and 1,000 μM Cd L^{-1} concentrations in cucumber plants (Goncalves et al. 2007). In another study with soybean, Srivastava et al. (2011) found that although Cd exposure (0.05, 0.10, 0.50, 1.00 and 2.00 mM) adversely affected plant growth, Cd tolerance could be attributed to increased levels of non-enzymatic antioxidants such as the total ascorbate and glutathione content.

6.10 Cd and the Effect of Plant Growth Regulators

Plant hormones are involved in many physio-biochemical and developmental processes, and play an important role in plant adaptation to stresses as indicated by the changes in hormone biosynthesis that occur when plants are exposed to heavy metals (Ashraf et al. 2010; Perveen et al. 2010; Peleg and Blumwald 2011; Hayat et al. 2012).

Several plant hormones, particularly ethylene, polyamines, cytokinins, brassinosteroids, abscisic acid and gibberellins, are implicated in oxidative and heavy metal stress responses. In recent years, plant growth regulators have been used to treat Cd stressed plants, and most of them have been effective in mitigating damage. Meng et al. (2009) applied jasmonic acid (JA) (12.5 μM), abscisic acid (ABA) (10 μM), gibberellin (GA_3) (50 μM) and salicylic acid (SA) (50 μM), and three levels of Cd (0, 50 and 100 μM) to oilseed rape (*Brassica napus*) plants. Plants thus treated with any of these PGRs showed reduced Cd toxicity symptoms, in that they had higher fresh weights, less malondialdehyde in leaves and lower activities of antioxidant enzymes (Meng et al. 2009).

Ethylene

Plants exposed to toxic levels of Cd, Cu, Fe and Zn produced higher levels of ethylene, a regulator associated with plant ageing/senescence (Maksymiec 2007). Cd and Cu stimulate ethylene synthesis by up-regulating expression and activity of ACC synthase, the key enzyme in ethylene biosynthesis (Pell et al. 1997). Copper and Cd also induced the rapid accumulation of JA in *Phaseolus coccineus* (Saniewski et al. 2003; Maksymiec et al. 2005). Heavy metals can induce lipoxygenase-mediated synthesis of ROS, as well as trigger the JA pathway (Kruzmane et al. 2002).

Polyamines

Compared to controls, cadmium stress in sunflower plants reduced the endogenous level of polyamines, including putrescine (by 52%) and spermidine (by 21%), and enzymatic antioxidants (viz., GR, SOD). In addition, exogenously applied spermine (1 mM) reduced the influence of Cd on lipid peroxidation. GR activity was completely unchanged by spermine, in line with the well-known protection against oxidative stress offered by polyamines (Groppa et al. 2001).

Brassinosteroids

Hayat et al. (2012) evaluated the interactive effect of Cd and two analogs of brassinosteroids (BRs) on antioxidant systems and photosynthetic assimilation efficiency in tomato. Exogenously-applied BRs (10^{-8} M) counteracted the damaging effects of Cd on plant growth and yield by improving the antioxidant system in the fruits. In an earlier study (Sharma et al. 2010), the effect of BR on photosynthetic and antioxidant systems was examined in two tomato lines subjected to Cd at 3–12 mg kg^{-1} soil. Leaf water potential, photosynthetic parameters and activity of several enzymes was reduced considerably in tomato plants with increasing Cd level in soil. However, foliar application 10^{-8} M 28-homobrassinolide/24-epibrassinolide (HBL/EBL) at 59 days after germination improved the antioxidant defense system and photosynthetic machinery in both tomato lines (Hasan et al. 2011). A significant reduction in biomass, protein content, length and activities of antioxidant enzymes was observed

in radish seedlings grown under varying levels of Cd (0.5–1.5 mM). However, brassinosteroid (28-HBL) treatments reduced the Cd toxicity by increasing seedling length, biomass and antioxidant enzymes activities (Sharma et al. 2010).

Abscisic Acid and Gibberellins

While investigating the effect of external applications of ABA and GA_3 on carbohydrate content and growth, and on net photosynthesis of heavy metal-stressed rice plants, Moya et al. (1995) found that treatment with Cd (0.1 mM) and Ni (0.5 mM) suppressed growth and enhanced carbohydrate accumulation. However, the addition of GA_3 (14 μM) to the rice culture solution, together with Cd or Ni partially reversed the toxic effects of the metals (Moya et al. 1995). In wheat plants, Ergün and Öncel (2012) studied the effects of Pb, Zn and Cd alone, and investigated their interactions with ABA and GA_3 hormones on soluble proteins, soluble phenolics and the amount of free proline. Of all the metals, Cd was most toxic; however, application of these metals in combination with ABA or GA_3 decreased the soluble protein content and increased proline and phenolic content.

Cytokinins

Cytokinins have been tested for their effectiveness in mitigating the adverse effects of Cd. While investigating the effect of foliar application of kinetin on pea plants growing in soil containing 25 or 50 μM Cd, Al-Hakimi (2007) reported that Cd application suppressed growth rate, free amino acids, soluble sugars, chlorophyll content and net photosynthesis. However, the application of kinetin partially restored the growth rate, chlorophyll content, photosynthetic rate, soluble sugars and free amino acids in the shoots and roots of the pea plants. Salicylic acid was also reported to be involved in heavy metal stress responses, as shown by the increase in SA levels in barley roots in the presence of Cd and the ability of exogenous SA to protect roots from lipid peroxidation caused by Cd toxicity (Metwally et al. 2003; Maksymiec et al. 2005; Manara 2012).

From the above reports it is evident that plant growth regulators play a vital role in mitigating the adverse effects of Cd on plant growth and metabolism. However, there is still a substantial gap in knowledge on this topic, and more research is needed to elucidate how different hormones affect specific metabolic processes in plants to achieve their mitigation.

6.11 Effect of Cd on Photosynthesis

Heavy metals perturb a variety of vital physiological processes including photosynthesis, which is the key metabolic energy producing process for plant growth (Perfus-Barbeoch et al. 2002; Heckathorn et al. 2004; Jhanji et al. 2012). A heavy-metal

stress-induced reduction in photosynthesis has been observed in many plants, e.g., rice, bean, pea, pakchoi and mustard (Moya et al. 1993; Skorzynska and Baszynski 1995; Hattab et al. 2009; Chen et al. 2011). It is apparent that the effects of any specific metal on photosynthesis differ among plant species, making it difficult to identify a general mode of action (Heckathorn et al. 2004; Jhanji et al. 2012). The effect of Cd on photosynthesis is relatively well studied (Vrettos et al. 2001). Previous studies have shown that Cd-induced inhibition in photosynthesis is indirectly linked to the effects of Cd on plant water status, stomatal conductance and CO_2 availability (Vrettos et al. 2001), and directly to the inhibitory effects of Cd on chloroplast structural organization, chlorophyll biosynthesis (Stobart et al. 1985; Padmaja et al. 1990), the PSII electron transport system, functioning of photochemical reactions (Skorzynska et al. 1995) and the activities of enzymes involved in photosynthetic carbon metabolism (Atal et al. 1993; Horvath et al. 1996; Krupa 1999; Vrettos et al. 2001). Faller et al. (2005) reported that Cd induced inhibition in photoactivation of PSII is due to its competitive binding to the essential Ca^{2+} site. Earlier in was demonstrated that the net rate of photosynthesis generally decreases in plants with increasing concentrations of Cd in the habitat (Vrettos et al. 2001; Jhanji et al. 2012). Recently, Jhanji et al. (2012) reported a significant reduction in photosynthesis and photosynthesis-related attributes, such as leaf area per plant, photosynthetic pigment content and Hill reaction activity, in Cd-treated *Brassica* plants.

Any comprehensive insight into the effects of Cd toxicity on photosynthesis is still a matter of speculation, owing to variations in parameters among studies (e.g., metal dose applied, differences in the involvement of Cd with electron transport in light reactions, and enzyme activities of the dark reactions). Therefore, more research is needed to elucidate the effect and nature of these interactions and parameters.

6.12 Effect of Cd on Water Relations

Heavy metals, including Cd, are known to adversely affect plant water status; however, the method by which tolerant plants maintain their water relations under such conditions is not clear (Arduini et al. 2004; Wojcik et al. 2005; Kholodova et al. 2011). Generally, the presence of heavy metals in the soil results in reduced plant growth from disturbance of cellular metabolism and water transport, thereby disturbing the water balance (Kirkham 1978; Barceló and Poschenrieder 1990; Gratao et al. 2005). For example, in bean plants (*Phaseolus vulgaris*), Poschenreider et al. (1989) observed that Cd (3 μM) reduced relative leaf water content (RWC) compared with that in non Cd-treated plants. However, the leaf turgor potential of Cd-treated plants was unchanged, suggesting that the Cd-induced decrease in RWC could not be attributed to changes in turgor potential. While assessing leaf water relations in bean plants that were subjected to well-watered and water-deficit conditions supplemented with Cd, Barceló et al. (1986) found that water stress tolerance of plants were reduced from loss in cell turgor, RWC and water potential (Ψ_w).

In addition, Cd increased the bulk elastic modulus and decreased the cell wall elasticity, suggesting that low cell wall elasticity from enzymatic degradation is an important cause of low water stress tolerance in Cd-treated plants (Barceló et al. 1986). In another study, the effect of exposure to excessive concentrations of various metals (Pb, Cd, Cu and Zn) on water relations of sunflower (*Helianthus annuus*) plants was assessed (Kastori et al. 1992). The authors found that transpiration and RWC were decreased significantly by all the heavy metals and suggested that high levels of heavy metals significantly disturbed the plant water status resulting in water deficiency, thereby causing adverse changes in plants. However, little work has been done to reveal the underlying mechanisms that regulate water relations in plants that are exposed to Cd stress.

7 Conclusions and Future Prospects

The following summarizes the key conclusions we have drawn from performing this review, and our suggestions for areas that are ripe for future research on Cd:

1. Cadmium-induced injury to plant growth and human health is greater than that caused by most other metals. This metal is highly toxic even at the very low concentrations (viz., 0.005 mg L^{-1}) (EPA 2007) and poses a hazard to most biological systems including humans.
2. In plants, patterns of distribution and accumulation of Cd are variable. Generally, most of the Cd entering the plant accumulates in the roots and only a small amount is transferred to upper plant parts such as stems, leaves, fruit and seeds. The ability of plant species to accumulate high metal concentrations in their roots could be a prospective indicator for developing metal tolerant plants via classical breeding or genetic engineering.
3. The degree to which Cd accumulates in plants depends on the amount present in the growth medium, type of growth medium, soil/water pH, plant growth stage, plant species and soil characteristics. However, excessive amounts of Cd in soil elicit stress symptoms that includes reduced growth (especially root growth), and disturbances in mineral nutrition and carbohydrate metabolism. These stress effects strongly reduce plant biomass production and ultimately plant yield.
4. Many authors have reported effects from plant exposure to toxic metal mixtures. Such combinations of heavy metals may impose different and more serious environmental effects than do those of individual metals. However, very little information is available in the literature on the specific cellular processes regulated by metal mixtures. Thus, this is a vital area for future research.
5. Cadmium exposure in plants is known to generate reactive oxygen species, interfere with utilization, uptake and transport of essential nutrients and water, and to alter the machinery of photosynthesis, thereby resulting in symptoms such as chlorosis, necrosis and ultimately tissue death. These effects of Cd at

the cellular level affect enzyme activity, alter macromolecules, denature proteins, and cause photoinhibition. All of these Cd-induced interactive cellular processes need to be fully explained if the basis of resistance/tolerance of plants against Cd-enriched environment is to be understood.

6. In response to excessive heavy metal contact, plants defensively respond by synthesizing antioxidants, the phytochelatin peptides or metallothioneins, which maintain metal ion homeostasis and subsequently compartmentalize the ligand-metal complexes to protect physiologically vital processes. These protective processes may result from changes in gene expression and signaling processes, in response to the metabolic disturbances caused by Cd. A detailed elucidation of all such processes is a viable area for future research, because a complete understanding of such processes could facilitate developing new Cd-tolerant plants.
7. Plants achieve tolerance to Cd by excluding entry of its ions into the cytosol, via the action of plasma membrane transport processes. Vacuolar compartmentalization prevents free circulation of Cd ions in the cytosol. This attribute could be used as a potential selection criterion in breeding programs designed to develop plants that minimize the adverse effects of Cd.
8. Phytoremediation or phytoextraction is an important strategy that utilizes plants to detoxify or/and sequester pollutants, including metals, in the environment. This approach is considered to be an in situ cost-effective technology that is a good alternative to expensive physical and chemical decontamination techniques. Phytoremediation has received increasing attention over the last 15 years, because of its large-scale applicability, environment-friendly nature, and relative ease of use. Phytoremediation is increasingly recognized to be a cost-effective alternative for cleaning contaminated soil that can achieve optimum plant growth and yield productivity in contaminated areas. Although much is known about Cd accumulation in plants and its effects on plant processes, gaps exist in our understanding of certain aspects of Cd's plant effects, and these are barriers to developing improved remedial measures and plant breeding strategies. In particular, more research is needed to delineate Cd's toxic action at the genetic level and the nature of the important signaling pathways involved in Cd toxicity.
9. When osmoprotectants (e.g., GB and proline) accumulate in metal-stressed plants, plant cellular activities are protected via improved water potential, reduced lipid peroxidation, membrane and macromolecule stabilization, and metal chelation. However, research is needed to define the mode of action of by which proline and GB protects plants exposed to Cd stress. In addition, insight is needed into the effects of Cd toxicity on nutrient metabolism, the antioxidant system, photosynthesis and related pigments.
10. Research has revealed that there are four major plant transport and accumulation processes for Cd. These are root uptake, root-to-shoot translocation via xylem flow, redirection at nodes and recycling from leaves. Research is needed to better define Cd transport mechanisms in plants. With such definition it may be possible to better influence how Cd transport and accumulation can be regulated through genetic engineering, biotechnology, and marker-assisted breeding techniques.

11. Future research is needed to better elucidate the biochemistry of metal homeostasis factors in plants. The physical interaction of transporters, chelators, and chaperones is likely to play an important role in improving our understanding of plant-metal interactions. This would provide a better background for developing strategies to manipulate plants in ways that decrease Cd content. In turn, this would help in developing crops capable of tolerating environmental contaminant changes.

8 Summary

Cadmium (Cd) is a water soluble metal pollutant that is not essential to plant growth. It has attracted attention from soil scientists and plant nutritionists in recent years because of its toxicity and mobility in the soil–plant continuum. Even low levels of Cd (0.1–1 μM) cause adverse effects on plant growth and metabolism. Cadmium is known to trigger the synthesis of reactive oxygen species, hinder utilization, uptake and transport of essential nutrients and water, and modify photosynthetic machinery, thereby resulting in plant tissue death. Although the effects of Cd are dose- as well as plant species-dependent, some plants show Cd tolerance through a wide range of cellular responses. Such tolerance results from synthesis of osmolytes, generation of enzymatic and non-enzymatic antioxidants and metal-detoxifying peptides, changes in gene expression, and metal ion homeostasis and compartmentalization of ligand-metal complexes. Cd toxicity in plants produces effects on chlorophyll biosynthesis, reduces photosynthesis, and upsets plant water relations and hormonal and/or nutritional balances. All of these effects on plants and on plant metabolism ultimately reduce growth and productivity.

In this review, we describe the extent to which Cd affects underlying metabolic processes in plants and how such altered processes affect plant growth. We review the sources of Cd contamination, its uptake, transportation and bioavailability and accumulation in plants, and its antagonistic and synergistic effects with other metals and compounds. We further address the effects of Cd on plant genetics and metabolism, and how plants respond to mitigate the adverse effects of Cd exposure, as well as strategies (e.g., plant breeding) that can reduce the impact of Cd contamination on plants.

References

Abbas W, Ashraf M, Akram NA (2010) Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugarbeet extracts. Sci Hort 125:188–195

Ahmad P, Ashraf M, Younis M, Hu X, Kumar A, Akram NA, Al-Qurainy F (2012) Role of transgenic plants in agriculture and biopharming. Biotechnol Adv 30:524–540

Aidid SB, Okamoto H (1992) Effects of lead, cadmium and zinc on the electric membrane potential at the xylem: symplast interface and cell elongation of *Impatiens balsamina*. Environ Exp Bot 32:439–448

Aidid SB, Okamoto H (1993) Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium and zinc. Biometals 6:245–249

Ai-jun L, Xu-hong Z, Mei-mei C, Qing C (2007) Oxidative stress and DNA damages induced by cadmium accumulation. J Environ Sci 19:596–602

Akay A, Koleli N (2007) Interaction between cadmium and zinc in barley (*Hordeum vulgare* L.) grown under field conditions. Bangladesh J Bot 36:13–19

Akram NA, Ashraf M (2011a) Pattern of accumulation of inorganic elements in sunflower (*Helianthus annuus* L.) plants subjected to salt stress and exogenous application of 5-aminolevulinic acid. Pak J Bot 43:521–530

Akram NA, Ashraf M (2011b) Improvement in growth, chlorophyll pigments and photosynthetic performance in salt-stressed plants of sunflower (*Helianthus annuus* L.) by foliar application of 5-aminolevulinic acid. Agrochimica 55:94–104

Akram NA, Ashraf M (2013) Regulation in plant stress tolerance by a potential plant growth regulator, 5-aminolevulinic acid (ALA). J Plant Growth Regul 32:663–679

Akram NA, Ashraf M, Al-Qurainy F (2012) Aminolevulinic acid-induced regulation in some key physiological attributes and activities of antioxidant enzymes in sunflower (*Helianthus annuus* L.) under saline regimes. Sci Hort 142:143–148

Al-Hakimi AMA (2007) Modification of cadmium toxicity in pea seedlings by kinetin. Plant Soil Environ 53:129–135

Ali G, Srivastava PS, Iqbal M (1998) Effect of cadmium and copper on growth of *Bacopa monnieri*. Biol Plant 41:635–639

Alia P, Saradhi PP (1991) Proline accumulation under heavy metal stress. J Plant Physiol 138:554–558

Andelkovic T, Nikolic R, Bojic A, Andelkovic D, Nikolic G (2010) Binding of cadmium to soil humic acid as a function of carboxyl group content. Maced J Chem Chem Eng 29:215–224

Andersen HR, Andersen O (1988) Effect of cadmium chloride on hepatic lipid peroxidation in mice. Pharmacol Toxicol 63:173–177

Aravind P, Prasad MNV (2005) Modulation of cadmium-induced oxidative stress in *Ceratophyllum demersum* by zinc involves ascorbate-glutathione cycle and glutathione metabolism. Plant Physiol Biochem 43:107–116

Archambault DJ, Marentes E, Buckley W, Clarke J, Taylor GJ (2001) A rapid, seedling-based bioassay for identifying low cadmium-accumulating individuals of durum wheat (*Triticum turgidum* L.). Euphytica 117:175–182

Arduini I, Masoni A, Mariotti M, Ercoli L (2004) Low cadmium application increase miscanthus growth and cadmium translocation. Environ Exp Bot 52:89–100

Arora K, Sharma S (2009) Toxic metal (Cd) removal from soil by AM fungi inoculated sorghum. Asian J Exp Sci 23:341–348

Asada K (1992) Ascorbate peroxidase-hydrogen peroxide scavenging enzyme in plants. Physiol Plant 85:235–241

Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 27:84–93

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216

Ashraf M, Akram NA, Arteca RN, Foolad MR (2010) The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit Rev Plant Sci 29:162–190

Atal N, Saradhi PP, Mohanty P (1993) Effect of iron on photosystem II mediated photochemical activities and proline levels in wheat seedlings during Cd^{2+} stress. In: Proceedings of the DAE symposium on photosynthesis and plant molecular biology, Bhabha Atomic Research Centre, Bombay, pp 1–5

Badisa VLD, Latinwo LMO, Caroline O, Ikediobi CO, Badisa RB, Ayuk-Takem LT, Nwoga J, West J (2007) Mechanism of DNA damage by cadmium and interplay of antioxidant enzymes and agents. Environ Toxicol 22:144–215

Bagchi D, Bagchi M, Hassoun EA, Stohs SJ (1996) Cadmium-induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in sprague – Dawley rats. Biol Trace Elem Res 52:143–154

Balaknina T, Kosobryukhov A, Ivanov A, Kreslauskii V (2005) The effect of cadmium on CO_2 exchange, variable fluorescence of chlorophyll and the level of antioxidant enzymes in pea leaves. Russ J Plant Physiol 52:15–20

Banfalvi G, Ujvarosi K, Trencsenyi G, Somogyi C, Nagy G, Basnakian A (2007) Cell culture density dependent toxicity and chromatin changes upon cadmium treatment in murine pre-B-cells. Apoptosis 12:1219–1228

Banu MNA, Hoque MA, Watanabe-Sugimoto M, Matsuoka K, Nakamura Y, Shimoishi Y (2009) Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. J Plant Physiol 166:146–156

Barceló J, Poschenrieder C (1990) Plant water relations as affected by heavy metal stress: a review. J Plant Nutr 13:1–37

Barceló J, Poschenrieder C, Andreu I, Gunsé B (1986) Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. J Plant Physiol 125:17–25

Batool S, Ashraf M, Akram NA, Al-Qurainy F (2013) Salt-induced changes in growth, some key physio-biochemical attributes, activities of enzymatic and levels of non-enzymatic antioxidants in cauliflower (*Brassica oleracea* L.). J Hort Sci Biotechnol 88:231–241

Bauddh K, Singh RP (2011) Differential toxicity of cadmium to mustard (*Brassica juncia* L.) genotypes under higher metal levels. J Environ Biol 32:355–362

Bavi K, Kholdebarin B, Moradshahi A (2011) Effect of cadmium on growth protein content and peroxidase activity in pea plants. Pak J Bot 43:1467–1470

Berg AH, Combs TP, Scherer PE (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13:84–89

Berger V, Bremaeker ND, Larondelle Y, Trouet A, Schneider Y (2000) Transport Mechanisms of the imino acid l-proline in the human intestinal epithelial caco-2 cell line. J Nutr 130:2772–2779

Bhattacharjee S, Mukherjee AK (2003) Heavy metals alter photosynthetic pigment profiles as well as activities of chlorophyllase and 5-aminolevulinic acid dehydratase (ALAD) in *Amaranthus lividus* seedlings. J Environ Biol 24:395–399

Bhattacharya M, Chaudhuri MA (1995) Heavy metal (Pb^{2+} and Cd^{2+}) stress-induced damages in Vigna seedlings and possible involvement of phytochelation-like substances in mitigation of heavy metal stress. Indian J Environ Bull 33:236–238

Bhattacharyya M, Choudhuri MA (1994) Effect of lead and cadmium on the biochemical changes in the leaves of terrestrial Vigna and aquatic Hydrilla plants under solution culture. J Plant Physiol 37:99–103

Bingham FT, Page AL, Strong JE (1980) Yield and cadmium content of rice grain in relation to addition rates of cadmium, copper, nickel with sewage sludge and liming. Soil Sci 130:32

Bork U, Lee WK, Kuchler A, Dittmar T, Thevenod F (2010) Cadmium-induced DNA damage triggers G2/M arrest via chk1/2 and cdc2 in p53-deficient kidney proximal tubule cells. Am J Physiol Renal Physiol 298:F255–F265

Brune A, Urbach W, Dietz KJ (1995) Differential toxicity of heavy metals is partly related to a loss of preferential extraplasmic compartmentation: a comparison of Cd-, Mo-, Ni- and Zn-stress. New Phytol 129:404–409

Burzynski M, Zurek A (2007) Effects of copper and cadmium on photosynthesis in cucumber cotyledons. Photosynthetica 45:239–244

Cailliatte R, Schikora A, Briat J, Mari S, Curie C (2010) High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. Plant Cell 22:904–917

Cameron JC, Pakrasi HB (2010) Glutathione in Synechocystis 6803: a closer look into the physiology of a Δ*gshB* mutant. Plant Signal Behav 6:89–92

Carlson RW, Bazzaz FA (1977) Growth reduction in American sycamore (*Plantanus occidentalis* L.) caused by Pb-Cd interaction. Environ Pollut 12:243–253

Cataldo DA, Garland TR, Wildung RE (1981) Cadmium distribution and chemical fate in soybean plants. Plant Physiol 68:835–839
Chakravarty B, Srivastava S (1997) Effect of cadmium and zinc interaction on metal uptake and regeneration of tolerant plants in linseed. Agric Ecosyst Environ 61:45–50
Chaoui A, El Ferjani E (2005) Effects of cadmium and copper on antioxidant capacities, lignification and auxin degradation in leaves of pea (*Pisum sativum* L.) seedlings. Crit Rev Biol 328:23–31
Chaoui A, Mazhoudi S, Ghorbal MH, El Ferjani E (1997) Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Sci 127:139–147
Chen Y, Huerta AJ (1997) Effects of sulfur nutrition on photosynthesis in cadmium-treated barley seedlings. J Plant Nutr 20:845–856
Chen X, Wang J, Shi Y, Zhao MQ, Chi GY (2011) Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard. Bot Stud 52:41–46
Chien HF, Wang JW, Lin CC, Kao CH (2001) Cadmium toxicity of rice leaves is mediated through lipid peroxidation. Plant Growth Regul 33:205–213
Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475–486
Clemens S, Aarts MJM, Thomine S, Verbruggen N (2013) Plant science: the key to preventing slow cadmium poisoning. Trends Plant Sci 18:92–99
Clijsters H, Van-Assche F (1985) Inhibition of photosynthesis by heavy metals. Photosynth Res 7:31–40
Cobbett CS, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182
Cook M, Morrow H (1995) Anthropogenic sources of cadmium to the Canadian environment. In: Workshop proceedings, National workshop on cadmium transport into plants, Canadian Network of Toxicology Centres, Ottawa
Cosio C, Vollenweider P, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.) I. Macrolocalization and phytotoxic effects of cadmium. Environ Exp Bot 58:64–74
Costa G, Morel JL (1994) Water relations gas exchange and amino acid content in Cd treated lettuca. Plant Physiol Biochem 32:561–570
Costa G, Spitz E (1997) Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. Plant Sci 128:131–140
Dahlin C, Aronsson H, Almkvist J, Sundqvist C (2000) Protochlorophyllide-independent import of two NADPH: Pchlide oxidoreductase proteins (PORA and PORB) from barley into isolated plastids. Physiol Plant 109:298–303
Dar SH, Agnihotri RK, Sharma R, Ahmad S (2010) Nickel and lead induced variations in pigment composition of *Triticum aestivum* L. Res J Agric Sci 1:128–131
de Knecht JA, van Dillen M, Koevoets PLM, Schat H, Verkleij JAC, Ernst WHO (1994) Phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*. Chain length distribution and sulphide incorporation. Plant Physiol 104:255–261
Deckert J (2005) Cadmium toxicity in plants: is there any analogy to its carcinogenic effect in mammalian cells? BioMetals 18:475–481
Demiral T, Turkan I (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ Exp Bot 53:247–257
Dhir B, Sharmila P, Pardha Saradhi P, Nasim SA (2009) Physiological and antioxidant responses of *Salvinia natans* exposed to chromium-rich wastewater. Ecotoxicol Environ Saf 72: 1790–1797
Di Cagno R, Guidi L, Stefani A, Soldatini GF (1999) Effects of cadmium on growth of *Helianthus annuus* seedlings: physiological aspects. New Phytol 144:65–71
Dong J, Wu F, Zhang G (2005) Effect of cadmium on growth and photosynthesis of tomato seedlings. J Zhejiang Univ Sci B 6:974–980

Dube A, Zbytniewski R, Kowalkowski T, Cukrowska E, Buszewski B (2001) Adsorption and migration of heavy metals in soil. Pol J Environ Stud 10:1–10

Duman F, Aksoy A, Aydin Z, Temizgul R (2011) Effects of exogenous glycinebetaine and trehalose on cadmium accumulation and biological responses of an aquatic plant (*Lemna gibba* L.). Water Air Soil Pollut 217:545–556

Environmental Protection Agency (2007) (eds) Monitored natural attenuation of inorganic contaminants in ground water. In: Ford RG, Wilkin RT, Puls RW (eds). U.S. Office of Research and Development, National Risk Management Research Laboratory, Ada, Oklahoma 74820

Erdei L, Tari I, Csiszár J, Pécsváradi A, Horváth F, Szabó M, Ördög M, Cseuz L, Zhiponova M, Szilák L, Györgyey J (2002) Osmotic stress responses of wheat species and cultivars differring in drought tolerance: some interesting genes (advices for gene hunting). Acta Biol Szeged 46:63–65

Ergün N, Öncel I (2012) Effects of some heavy metals and heavy metal hormone interactions on wheat (*Triticum aestivum* L. cv. Gun 91) seedlings. Afr J Agric Res 7:1518–1523

Eshghi S, Mahmoodabadi MR, Abdi GR, Jamali B (2010) Zeolite ameliorates the adverse effect of cadmium contamination on growth and nodulation of soybean plant (*Glycine max* L.). J Biol Environ Sci 4:43–50

Faizan S, Kausar S, Perveen R (2011) Varietal differences for cadmium-induced seedling mortality, foliar toxicity symptoms, plant growth, proline and nitrate reductase activity in chickpea (*Cicer arietinum* L.). Biol Med 3:196–206

Faller P, Kienzler K, Krieger-Liszkay A (2005) Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of photosystem II by competitive binding to the essential Ca^{2+} site. Biochim Biophys Acta 1706:158–164

Farago ME, Mullen WA (1979) Plants which accumulate metals. Part IV. A possible copper-proline complex from the roots of *Armeria maritima*. Inorg Chim Acta 32:L93–L94

Fernandez O, Béthencourt L, Quero A, Sangwan RS, Clément C (2010) Trehalose and plant stress responses: friend or foe? Trends Plant Sci 15:409–417

Fodor E, Szabo-Nagy A, Erdei L (1995) The effects of cadmium on the fluidity and H^+-ATPase activity of plasma membrane from sunflower and wheat roots. J Plant Physiol 147:87–92

Franklin NM, Stauber JL, Lim RP, Petocz P (2002) Toxicity of metal mixtures to a tropical freshwater alga (*Chlorella* sp.): the effect of interactions between copper, cadmium, and zinc onmetal cell binding and uptake. Environ Toxicol Chem 21:2412–2422

Frostegard A, Tunlid A, Baath E (1993) Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl Environ Microbiol 59:3605–3617

Fuhrer J (1982) Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans *Phaseolus vulgaris* L. Plant Physiol 70:162–167

Galili G, Höfgen R (2002) Metabolic engineering of amino acids and storage proteins in plants. Metab Eng 4:3–11

Gallego SM, Benavides MP, Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves: evidence of involvement of oxidative stress. Plant Sci 121:151–159

Ghani A (2010) Effect of cadmium toxicity on the growth and yield components of mungbean [*Vigna radiata* (L.) Wilczek]. World Appl Sci J 8:26–29

Garcia MJ, Page A (1978) Sorption of trace quantities of cadmium by soils with different chemical and mineralogical composition. Water Air Soil Pollut 9:289–299

Ghosh M, Singh SP (2005) A comparative study of cadmium phytoextraction by accumulator and weed species. Environ Pollut 133:365–371

Gichner T, Znidar I, Száková J (2008) Evaluation of DNA damage and mutagenicity induced by lead in tobacco plants. Mutat Res Genet Toxicol Environ Mutagen 652:186–190

Gill R, Malik KA, Sanago MHM, Saxena PK (1995) Somatic embryogenesis and plant regeneration from seedling cultures of tomato (*Lycopersicon esculentum* Mill.). J Plant Physiol 147:273–276

Gill SS, Khan NA, Tuteja N (2011) Differential cadmium stress tolerance in five Indian mustard (*Brassica juncea* L.) cultivars: an evaluation of the role of antioxidant machinery. Plant Signal Behav 6:293–300

Goncalves JF, Becker AG, Cargnelutti D, Tabaldi LA, Pereira LB, Battisti V, Spanevello RM, Morsch VM, Nicoloso FT, Schetinger MRC (2007) Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings. Braz J Plant Physiol 19:24–26

Gouia H, Suzuki A, Brulfert J, Ghorbal MH (2003) Effects of cadmium on the co-ordination of nitrogen and carbon metabolism in bean seedlings. J Plant Physiol 160:367–376

Gratao PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494

Groppa MD, Tomaro ML, Benavides MP (2001) Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. Plant Sci 161:481–488

Guo GL, Zhou QX (2003) Advances of research on combined pollution in soil-plant systems. Chin J Appl Ecol 14:823–828

Guo-sheng S, Hassan MJ, Xiu-fu Z, Guo-ping Z (2004) Effects of cadmium stress on plant growth and antioxidative enzyme system in different rice genotypes. Chin J Rice Sci 18:239–244

Hall JL (2002) Cellular mechanism for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Hall DO, Rao KK (1999) Photosynthesis, 6th edn. Cambridge University Press, Cambridge, pp 1–5

Hall JL, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54:2601–2613

Halloin JM, De Zpeten GA, Guard G (1970) The effects of tentoxin on chlorophyll synthesis and plastid structure in cucumber and cabbage. Plant Physiol 45:310–314

Handique GK, Handique AK (2009) Proline accumulation in lemongrass (*Cymbopogon flexuosus* Stapf.) due to heavy metal stress. J Environ Biol 30:299–302

Harris NS, Taylor GJ (2004) Cadmium uptake and translocation in seedlings of near isogenic lines of durum wheat that differ in grain cadmium accumulation. BMC Plant Biol 4:1–12

Hartwig A, Schlepegrell R, Dally H, Hartmann M (1996) Interaction of carcinogenic metal compounds with deoxyribonucleic acid repair processes. Ann Clin Laborat Sci 26:31–38

Hasan SA, Ali B, Hayat S, Ahmad A (2007) Cadmium induced changes in the growth and carbonic anhydrase activity of chickpea. Turk J Biol 31:137–140

Hasan SA, Hayat S, Ali B, Ahmad A (2008) 28-Homobrassinolide protect chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidant. Environ Pollut 151:60–66

Hasan SA, Hayat S, Ahmad A (2011) Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. Chemosphere 84:1446–1451

Hattab S, Dridi B, Chouba L, Kheder MB, Bousetta H (2009) Photosynthesis and growth responses of pea *Pisum sativum* L. under heavy metals stress. J Environ Sci 21:1552–1556

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Expt Bot 60:33–41

Hayat S, Alyemeni MN, Hasan SA (2012) Foliar spray of brassinosteroid enhances yield and quality of Solanum lycopersicum under cadmium stress. Saudi J Biol Sci 19:325–335

He J, Li H, Luo J, Ma C, Li S, Qu L, Gai Y, Jiang X, Janz D, Polle A, Tyree M, Luo Z (2013) A transcriptomic network underlies microstructural and physiological responses to cadmium in *Populus x canescens*. Plant Physiol 162:424–439

Heckathorn SA, Muller JK, Laguidice S, Zhu B, Berrett T, Blair B, Dong J (2004) Chloroplast small heatshock proteins protect photosynthesis during heavy metal stress. Am J Bot 91:1312–1318

Heise J, Krejci S, Miersch J, Krauss GJ, Humbeck K (2007) Gene expression of metallothioneins in barley during senescence and heavy metal treatment. Crop Sci 47:1111–1118

Hendy GAF, Baker AJM, Evart CF (1992) Cadmium tolerance and toxicity, oxygen radical processes and molecular damage in cadmium tolerant and cadmium-sensitive clones of *Holcus lanatus*. Acta Bot Neerl 41:271–281

Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Gotte W, Jung D, Mayer-Popken O, Fuchs J, Gebhard S (2003) Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis 24:63–73

Hirt H, Casari G, Barta A (1989) Cadmium-enhanced gene expression in suspension-culture cells of tobacco. Planta 179:414

Horsfall M Jr, Abia AA (2003) Sorption of cadmium (II) and zinc (II) ions from aqueous solutions by cassava waste biomass (*Manihot esculenta* Vanz). Water Res 37:4913–4923

Horvath G, Droppa M, Oraveez A, Raskin VI, Marder JB (1996) Formation of the photosynthetic apparatus during greening of cadmium-poisoned barley leaves. Planta 199:238–243

Hussain I, Iqbal M, Qurat-ul-ain S, Rasheed R, Mahmood S, Perveen A, Wahid A (2012) Cadmium dose and exposure-time dependent alterations in growth and physiology of maize (*Zea mays*). Int J Agric Biol 14:959–964

Hutchings D, Rawsthorne S, Emes MJ (2005) Fatty acid synthesis and the oxidative pentose phosphate pathway in developing embryos of oilseed rape (*Brassica napus* L.). J Exp Bot 56:577–585

Hynek D, Krejcova L, Sochor J, Cernei N, Kynicky J, Adam V, Trnkova L, Hubalek J, Vrba R, Kizek R (2012) Study of interactions between cysteine and cadmium (II) ions using automatic pipetting system off-line coupled with electrochemical analyser. Int J Electrochem Sci 7:1802–1819

IARC (1993) Cadmium and cadmium compounds. In: Beryllium, cadmium, mercury and exposures in the glass manufacturing industry, vol 58, IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. International Agency for Research on Cancer, Lyon, pp 119–239

Islam MM, Hoque MA, Okuma E, Banu MNA, Shimoishi Y, Nakamura Y et al (2009a) Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. J Plant Physiol 166:1587–1597

Islam MM, Hoque MA, Okuma E, Jannat R, Banu MNA, Jahan S et al (2009b) Proline and glycinebetaine confer cadmium tolerance on tobacco bright yellow-2 cells by increasing ascorbate-glutathione cycle enzyme activities. Biosci Biotechnol Biochem 73:2320–2323

Jalil A, Selles F, Clarke JM (1994) Effect of cadmium on growth and the uptake of cadmium and other elements by durum wheat. J Plant Nutr 17:1839–1858

Januskaitiene I (2010) Impact of low concentration of cadmium on photosynthesis and growth of pea and barley. Environ Res Eng Manage 3:24–29

Januškaitiene I, Juknys R, Pipiraitė A (2008) Spring barley photosynthetic rate and changes in stress phases under different cadmium impact. Agriculture 95:73–85

Jemal F, Zarrouk M, Ghorbal MH (2000) Effect of cadmium on lipid composition of pepper. Biochem Soc Trans 28:907–910

Jhanji S, Setia RC, Kaur N, Kaur P, Setia N (2012) Role of nitric oxide in cadmium-induced stress on growth, photosynthetic components and yield of *Brassica napus* L. J Environ Biol 33:1027–1032

John R, Ahmad P, Gadgil K, Sharma S (2009) Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. Int J Plant Prod 3:65–76

Jonak C, Nakagami H, Hirt H (2004) Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. Plant Physiol 136:3276–3283

Joner E, Leyval C (1997) Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol 135:353–360

Kahle H (1993) Response of roots of trees to heavy metals. Environ Exp Bot 33:99–119

Kalavrouziotis IK, Koukoulakis PH, Papadopoulos AH (2009a) Heavy metal interrelationships in soil in the presence of treated waste water. Global NEST J 11:497–509

Kalavrouziotis IK, Koukoulakis PH, Sakellarkou-Makrantonaki M, Papanikolaou C (2009b) Effect of treated municipal wastewater on the essentaia nutrient interactions in the plant of *Brassica oleracea* var. Italica. Desalination 242:297–312

Kambhampati MS, Begonia GB, Begonia MFT, Bufford V (2005) Morphological and physiological responses of morning glory (*Ipomoea lacunosa* L.) grown in a lead- and chelateamended soil. Int J Environ Res Public Health 2:299–303

Kannangara CG (1991) The photosynthetic apparatus. In: Bogorad L, Vasil IK (eds) Cell culture and somatic cell genetics of plants. Academic Press, San Diego, pp 301–329

Kastori R, Petrovic M, Petrovic N (1992) Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower. J Plant Nutr 15:2427–2429

Kavi Kishor PB, Hong Z, Miao GH, Hu CAA, Verma DPS (1995) Overexpression of Δ^1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol 108:1387–1394

Khan MH, Patra HK (2007) Sodium chloride and cadmium induced oxidative stress and antioxidant response in *Calamus tenuis* leaves. Ind J Plant Physiol 12:34–40

Khan MJ, Jan MT, Mohammad D (2011a) Heavy metal content of alfalfa irrigated with waste and tubewell water. Soil Environ 30:104–109

Khan ZI, Ashraf M, Ahmad K, Akram NA (2011b) A study on the transfer of cadmium from soil to pasture under semi-arid conditions in Sargodha, Pakistan. Biol Trace Element Res 142:143–147

Khan ZI, Mukhtar MK, Raza N, Ashraf M, Ahmad K, Akram NA (2011c) A study on the transfer of iron in soil-plant-animal continuum under semi-arid environmental conditions in Sargodha Pakistan. Biol Trace Elements Res 142:890–895

Kholodova V, Volkov K, Abdeyeva A, Kuznetsov V (2011) Water status in *Mesembryanthemum crystallinum* under heavy metal stress. Environ Exp Bot 71:382–389

Kil SI, Shin SW, Yeo HS, Lee YS, Park JW (2006) Mitochondril NADP-dependent isocitarte dehydrogenase protects cadmium-induced apoptosis. Mol Pharmacol 70:1053–1061

Kirkham MB (1978) Water relations of cadmium-treated plants. J Environ Qual 7:334–336

Kokkali V, Katramados I, Newman JD (2011) Monitoring the effect of metal ions on the mobility of *Artemia salina* Nauplii. Biosensors 1:36–45

Koppen G, Verschaeve L (1996) The alkaline comet test on plant cells: a new genotoxicity test for DNA strand breaks in *Vicia faba* root cells. Mutat Res 360:193–200

Kovacs E, Keresztes A (2002) Effect of gamma and UV-B/C radiation on plant cell. Micron 33:199–210

Kramer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638

Krezel A, Maret W (2008) Thionein/metallothionein control Zn(II) availability and the activity of enzymes. J Biol Inorg Chem 13:401–409

Krupa Z (1999) Cadmium against higher plant photosynthesis – a variety of effects and where do they possibly come from? Zeitschrift für Naturforschung 54:723–729

Krupa Z, Baszynski T (1989) Acyl lipid composition of thylakoid membranes of cadmium treated tomato plants. Acta Physiol Plant 11:111–116

Kruzmane D, Jankevica L, Ievinsh G (2002) Effect of regurgitant from *Leptinotarsa decemlineata* on wound responses in *Solanum tuberosum* and *Phaseolus vulgaris*. Physiol Plant 115: 577–584

Kumar R, Pant N, Srivasta SP (2000) Chlorinated pesticides and heavy metals in human semen. Int J Androl 23:145–149

Larsson EH, Bornman JF, Asp H (1998) Influence of UV-B radiation and Cd^{2+} on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. J Exp Bot 49:1031–1039

Laurent J, Pierra M, Casellas M, Dagot C (2009) The fate of heavy metals during thermal and ultrasound treatment of activated sludge. Environ Prot Eng 35:5–15

Leita L, Contin M, Maggioni A (1991) Distribution of cadmium and induced Cd-binding proteins in roots, stems and leaves of *Phaseolus vulgaris*. Plant Sci 77:139–147

Lidia A, Magdalena ZM, Zenovia O, Irina B (2011) Observations on the foliar assimilating pigments content for wild and garden roses. J Plant Dev 18:47–54

Lim PO, Kim HJ, Nam HG (2007) Leaf senescence. Annu Rev Plant Biol 58:115–136

Lin R, Wang X, Luo Y, Du W, Guo H, Yin D (2007) Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). Chemosphere 69:89–98

Lin YP, Cheng BY, Chu HJ, Chang TK, Yu HL (2011) Assessing how heavy metal pollution and human activity are related by using logistic regression and kriging methods. Geoderma 163:275–282

Liu DH, Wang M, Zou JH, Jiang WJ (2006) Uptake and accumulation of cadmium and some nutrient ions by roots and shoots of maize (*Zea mays* L.). Pak J Bot 38:701–709

Liu Y, Chen G, Zhang J, Shi X, Wang R (2011) Uptake of cadmium from hydroponic solutions by willows (*Salix spp.*) seedlings. Afr J Biotechnol 10:16209–16218

Lockwood MP (1976) Effects of pollutants on aquatic organisms. Cambridge University Press, New York

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2000) Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. New Phytol 145:11–20

Lozano-Rodriguez E, Hernandez LE, Bonay P, Carpena-Ruiz RO (1997) Distribution of cadmium in shoot and root tissues of maize and pea plants: physiological disturbances. J Exp Bot 306:123–128

Luan ZQ, Cao HC, Yan BX (2008) Individual and combined phytotoxic effects of cadmium, lead and arsenic on soybean in Phaeozem. Plant Soil Environ 54:403–411

Lue-Kim H, Rauser WE (1986) Partial characterization of cadmium-binding protein from roots of tomato. Plant Physiol 81:896–900

Luo LX, Sun TH, Jin YH (1998) Accumulation of superoxide radical in wheat leaves under cadmium stress. Acta Sci Circumtant 18:495–499

Luo Y, Li WM, Wang W (2008) Trehalose: protector of antioxidant enzymes or reactive oxygen species scavenger under heat stress? Environ Exp Bot 63:378–384

Ma QQ, Wang W, Li YH, Li DQ, Zou Q (2006) Alleviation of photoinhibition in drought stressed wheat (*Triticum aestivum*) by foliar applied glycinebetaine. J Plant Physiol 163:165–175

Mahmud SA, Nagahisa K, Hirasawa T, Yoshikawa K, Ashitani K, Shimizu H (2009) Effect of trehalose accumulation on response to saline stress in *Sacccharomyces cerevisiae*. Yeast 26:17–30

Maksymiec W (2007) Signaling responses in plants to heavy metal stress. Acta Physiol Plant 29: 177–187

Maksymiec W, Wianowska D, Dawidowicz AL, Radkiewicz S, Mardarowicz M, Krupa Z (2005) The level of jasmonic acid in *Arabidopsis thaliana* and *Phaseolus coccineus* plants under heavy metal stress. J Plant Physiol 162:1338–1346

Malan HL, Farrant JM (1998) Effects of the metal pollutants cadmium and nickel on soybean seed development. Seed Sci Res 8:445–453

Malkowski E, Kita A, Galas W, Karcz W, Kuperberg M (2002) Lead distribution in corn seedling (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. Plant Soil 37:69–76

Manara A (2012) Plant responses to heavy metal toxicity. In: Furini A (ed) Plants and heavy metals, Springer briefs in biometals. Springer, New York, pp 27–53

Mandhania S, Madan S, Sawhney V (2006) Antioxidant defense mechanism under salt stress in wheat seedlings. Biol Plant 227:227–231

McCauley A (2009) Soil pH and organic matter. Nutr Manage Module 8:1–12

Meng H, Hua S, Shamsi IH, Jilani G, Li Y, Jiang L (2009) Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L., and its alleviation through exogenous plant growth regulators. Plant Growth Regul 58:47–59

Metwally A, Finkemeier I, Georgi M, Dietz KJ (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol 132:272–281

Mishra S, Dubey RS (2005) Heavy metal toxicity induced alterations in photosynthetic metabolism in plants. In: Pessarakli M (ed) Photosynthesis, 2nd edn. CRC Press/Taylor and Francis Group, New York, pp 845–863

Mishra S, Srivastava S, Tripathi RD, Govindarajan R, Kuriakose SV, Prasad MNV (2006) Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol Biochem 44:25–37

Mohamed AA, Castagna A, Ranieri A, Sanità di Toppi L (2012) Cadmium tolerance in Brassica juncea roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. Plant Physiol Biochem 12:15–22

Mohan BS, Hosetti BB (2006) Phytotoxicity of cadmium on the physiological dynamics of *Salvinia natans* L. grown in macrophyte ponds. J Environ Biol 27:701–704

Molins H, Michelet L, Lanquar V, Agorio A, Giraudat J, Roach T, Krieger-Liszkay A, Thomine S (2013) Mutants impaired in vacuolar metal mobilization identify chloroplasts as a target for cadmium hypersensitivity in *Arabidopsis thaliana*. Plant Cell Environ 36:804–817

Morrow H (1996) The environmental and engineering advantages of cadmium coatings. Sources of Cadmium in the Environment, Inter-Organisation Programme for the Sound Management of Chemicals (IOMC), Organisation for Economic Co-Operation and Development (OECD), Paris

Morrow H, Keating J (1997) Overview paper for OECD workshop on the effective collection and recycling of nickel-cadmium batteries. OECD workshop on the effective collection and recycling of nickel-cadmium batteries, Lyon. Proceedings to be published by OECD, Paris

Mouron SA, Golijow CD, Dulout FN (2001) DNA damage by cadmium and arsenic salts assessed by the single cell gel electrophoresis assay. Mutation Res 498:47–55

Moya JL, Ros R, Picazo I (1993) Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynth Res 36:75–80

Moya JL, Ros R, Picazo I (1995) Heavy metal hormone interactions in rice plants: effects on growth, net photosynthesis, and carbohydrate distribution. J Plant Growth Regul 14:61–67

Mrozik A, Piotrowska-Seget Z, ŁabuŻek S (2004) Cytoplasmatic bacterial membrane responses to environmental perturbations. Pol J Environ Stud 13:487–494

Müller-Moulé P, Golan T, Niyogi KK (2004) Ascorbate-deficient mutants of *Arabidopsis grow* in high light despite chronic photooxidative stress. Plant Physiol 134:1163–1172

Nagoor S (1999) Physiological and biochemical responses of cereal seedlings to graded levels of heavy metals. II. Effects on protein metabolism in maize seedlings. Adv Plant Sci 12: 425–433

Ne O, Igwe JC, Onwuchekwa EC (2005) Risk and health implications of polluted soils for crop production. Afr J Biotechnol 4:1521–1524

Nikolić N, Kojić D, Pilipović A, Pajević S, Krstić B, Borišev B, Orlović S (2008) Responses of hybrid poplar to cadmium stress: photosynthetic characteristics, cadmium and proline accumulation, and antioxidant enzyme activity. Acta Biol Cracov Ser Bot 50:95–103

Nordberg M, Nordberg GF (2009) Metallothioneins: historical development and overview. Met Ions Life Sci 5:1–29

Noreen Z, Ashraf M, Akram NA (2010) Salt-induced regulation of some key physio-biochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). J Agron Crop Sci 196:273–285

Nriagu JO (1980) Production uses and properties of cadmium. In: Nriagu JO (ed) Cadmium in the environment, Part 1 ecological cycling. Wiley, Toronto

OECD (1994) Organisation for Economic Co-operation and Development (OECD), Risk reduction monograph no. 5: cadmium. OECD Environment Directorate, Paris

Olubunmi FE, Olorunsola OE (2010) Evaluation of the status of heavy metal pollution of sediment of *Agbabu bitumen* deposit area, Nigeria. Eur J Sci Res 41:373–382

Ouzounidou G, Moustakas M, Eleftheriou EP (1997) Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. Arch Environ Contam Toxicol 32:154–160

Padmaja K, Prasad DDK, Prasad ARK (1990) Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* seedlings by cadmium acetate. Photosynthetica 24:399–405

Page V, Feller U (2005) Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves in young wheat plants. Ann Bot 96:425–434

Pal M, Horvath E, Janda T, Paldi E, Szalai G (2006) Physiological changes and defence mechanisms induce by cadmium stress in maize. J Plant Nutr Soil Sci 169:239–246

Palacios Ò, Atrian S, Capdevila M (2011) Zn- and Cu-thioneins: a functional classification for metallothioneins? J Biol Inorg Chem 16:991–1009

Peleg Z, Blumwald E (2011) Hormone balance and abiotic stress tolerance in crop plants. Curr Opin Plant Biol 14:290–295

Pell EJ, Schlagnhaufer CD, Arteca RN (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol Plant 100:264–273

Pena LB, Pasquini LA, Tomaro ML, Gallego SM (2006) Proteolytic system in sunflower (*Helianthus annuus* L.) leaves under cadmium stress. Plant Sci 171:531–537

Peralta JR, Gardea-Torresdey L, Tiemann KJ, Gomez S, Arteaga E, Rascon J, Parsons G (2000) Study of the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. Environ Sci Eng 15:135–140

Perez-Arellano I, Carmona-Alvarez F, Martinez AI, Rodriguez-Diaz J, Cervera J (2010) Pyrroline-5-carboxylate synthase and proline biosynthesis: from osmotolerance to rare metabolic disease. Protein Sci 19:372–382

Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548

Perveen S, Shahbaz M, Ashraf M (2010) Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. Pak J Bot 42:3073–3081

Perveen S, Shahbaz M, Ashraf M (2011) Modulation in activities of antioxidant enzymes in salt stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. Pak J Bot 43:2463–2468

Perveen S, Shahbaz M, Ashraf M (2012) Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under salt stress? J Appl Bot Food Qual 85:41–48

Piotrowska-Seget Z, Mrozik A (2003) Signature lipid biomarker (SLB) analysis in determining changes in community structure of soil microorganisms. Pol J Environ Stud 12:669–675

Polle A, Rennenberg H (1994) Photooxidative stress in trees. In: Foyer CH, Mullineauv PM (eds) Causes of photoxidative stress and amelioration of defense systems in plants. CRC Press, Boca Raton, pp 199–218

Pope CA, Burnett RT, Krewski D, Jerrett M, Shi Y, Calle EE et al (2009) Cardiovascular mortality and exposure to airborne fine particulate matter and cigarette smoke: shape of the exposure-response relationship. Circulation 120:941–948

Poschenreider CR, Gunse B, Barcelo L (1989) Influence of cadmium on water relations, stomatal resistance and abscisic acid content in expanding bean leaves. Plant Physiol 90:1365–1371

Prelota B, Janusz W, Thomas F, Villieras F, Charmas R, Piasecki W, Rudzinski W (2002) Adsorption of cadmium ions at the electrolyte/silica interface I. Experimental study of surface properties. Appl Surf Sci 196:322–330

Przedpełska-Wąsowicz E, Polatajko A, Wierzbicka M (2012) The influence of cadmium stress on the content of mineral nutrients and metal-binding proteins in *Arabidopsis halleri*. Water Air Soil Pollut 223:5445–5458

Pulido MP, Parrish AR (2003) Metal induced apoptosis: mechanisms. Mutation Res 533:227–241

Rady MM (2011) Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. Sci Hort 129:232–237

Ralph PJ, Burchett MD (1998) Photosynthetic response of *Halophila ovalis* to heavy metal stress. Environ Pollut 103:91–101

RAO MB, Tanksale AM, Ghatge MS, Deshpande VV (1998) Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev 62:597–635

Rascio N, Vecchia FD, Ferretti M, Merlo L, Ghisi R (1993) Some effects of cadmium on maize plants. Arch Environ Contam Toxicol 25:244–249

Rauser WE, Muwly P (1995) Retention of cadmium in roots of maize seedlings. Role of complexation by phytochelatins and related thiol peptides. Plant Physiol 109:195–202

Reddy GN, Prasad MNV (1993) Tyrosine is not phosphorylated in cadmium induced hsp70 cognate in maize (*Zea mays* L.) seedlings: role in chaperone function. Biochem Arch 9:25–32

Root RA, Miller RJ, Koeppe DE (1975) Uptake of cadmium its toxicity, and effect on the iron ratio in hydroponocally grown corn. J Environ Qual 4:473–476

Rotkittikhun R, Kruatrachue M, Chaiyarat R, Ngernsansaruay C, Pokethitiyook P, Paijitprapaporn A, Baker AJM (2006) Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand. Environ Pollut 144:681–688

Saleem A, Ashraf M, Akram NA (2011) Salt (NaCl)-induced modulation in some key physio-biochemical attributes in okra (*Abelmoschus esculentus* L.). J Agron Crop Sci 197:202–213

Saleem A, Ashraf M, Akram NA, Al-Qurainy F (2012) Salinity-induced changes in the composition of some key enzymatic and non-enzymatic antioxidants, osmoprotectants, chlorophyll pigments and some inorganic elements in okra (*Abelmoschus esculentus* L.) fruit. J Hort Sci Biotechnol 87:271–277

Salin ML (1988) Toxic oxygen species and protective systems of the chloroplast. Physiol Plant 72:681–689

Saniewski M, Ueda J, Miyamoto K, Urbanek H (2003) Interaction between ethylene and other plant hormones in regulation of plant growth and development in natural conditions and under abiotic and biotic stresses. In: Vendrell M, Klee H, Pech JC, Romojaro F (eds) Biology and biotechnology of the plant hormone ethylene III. IOS Press, Washington, DC, pp 263–270

Sarangthem J, Jain M, Gadre R (2011) Inhibition of δ-aminolevulinic acid dehydratase activity by cadmium in excised etiolated maize leaf segments during greening. Plant Soil Environ 57: 332–337

Sarwar N, Saifullah SSM, Munir HZ, Asif N, Sadia B, Ghulam F (2010) Role of mineral nutrition in minimizing cadmium accumulation by plants. J Sci Food Agric 90:925–937

Sasa T, Sugahara K (1976) Photoconversion of protochlorophyll to chlorophyll a in a mutant of *Chlorella regularis*. Plant Cell Physiol 17:273–279

Schat H, Sharma SS, Vooijs R (1997) Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. Physiol Plant 101:477–482

Schlegel H, Godbold DL, Hüttermann A (1987) Whole plant aspects of heavy metal induced changes in CO_2 uptake and water relation of spruce (*Picea abies*) seedlings. Physiol Plant 69:265–270

Setia RC, Bala R, Setia N, Anuradha G (1993) Photosynthetic characteristics of heavy metal treated wheat plant (*Triticum aestivum* L.). Plant Sci Res 9:47–49

Seuntjens R, Mallants D, Simunek J, Patyn J, Jacques D (2002) Sensitivity analysis of physical and chemical properties affecting field-scale cadmium transport in a heterogeneous soil profile. J Hydrol 264:185–200

Seyyedi M, Timko MP, Sundqvist C (1999) Protochlorophyllide, NADPH-protochlorophyllide oxidoreductase, and chlorophyll formation in the lip1 mutant of pea. Physiol Plant 106: 344–354

Shah K, Dubey RS (1997) Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings. Role of proline as a possible enzyme protectant. Biol Plant 40:121–130

Shah K, Dubey RS (1998) A 18 kDa cadmium inducible protein complex from rice: its purification and characterization from rice (*Oryza sativa* L.) roots tissues. J Plant Physiol 152:448–454

Shah S, Tugendreich S, Forbes D (1998) Major binding sites for the nuclear import receptor are the integral nucleoporin Nup153 and the adjacent nuclear filament protein Tpr. J Cell Biol 141:31–49

Shahbaz M, Ashraf M (2013) Improving salinity tolerance in cereals. Crit Rev Plant Sci 32:237–249

Shahbaz M, Ashraf M, Athar HR (2008) Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.). Plant Growth Regul 55:51–64

Shahbaz M, Ashraf M, Al-Qurainy F, Harris PJC (2012) Salt tolerance in selected vegetable crops. Crit Rev Plant Sci 31:303–320

Shamsi IH, Wei K, Jilani G, Zhang G (2007) Interactions of cadmium and aluminum toxicity in their effect on growth and physiological parameters in soybean. J Zhejiang Univ Sci B 8:181–188

Shan S, Liu F, Li CC, Wan S (2012) Effects of cadmium on growth, oxidative stress and antioxidant enzyme activities in peanut (*Arachis hypogaea* L.) seedlings. J Agric Sci 4:142–151

Sharif AS, Suzelle FB (2006) Effect of soil fertility and transpiration rate on young wheat plants (*Triticum aestivum*) Cd/Zn uptake and yield. Agric Water Manage 82:177–192

Sharma I, Pati PK, Bherdwaj N (2010) Regulation of growth and antioxidant enzyme activities by 28-homobrassinolide in seedlings of *Raphanus sativus* L. under cadmium stress. Indian J Biochem Biophys 47:172–177

Simidjiev I, Stoylova S, Amenitsch H, Jávorfi T, Mustárdy L, Laggner P, Holzenburg A, Garab G (2000) Self-assembly of large, ordered lamellae from non-bilayer lipids and integral membrane proteins in vitro. Proc Natl Acad Sci 97:1473–1476

Singh PK, Tewari RK (2003) Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. J Environ Biol 24:107–112

Singh S, Eapen S, Souza SFD (2006) Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. Chemosphere 62: 233–246

Skorzynska E, Baszynski T (1995) Photochemical activities of primary leaves in cadmium stressed *Phaseolus coccineus* depends on their growth stages. Acta Soc Bot Pol 64:273–279

Skórzyńska-Polit E, Krupa Z (2006) Lipid peroxidation in cadmium-treated *Phaseolus coccineus* plants. Arch Environ Toxicol 50:482–487

Skorzynska E, Bednara J, Baszynski T (1995) Some aspects of runner bean plant response to cadmium at different stages of the primary leaf growth. Acta Soc Bot Pol 64:165–170

Skorzynska-Polit E, Baszynski T (1997) Differences in sensitivity of photosynthetic apparatus in Cd-stressed runners bean plants in relation to their age. Plant Sci 128:11–21

Skrebsky ET, Tabaldi LA, Pereira LB, Rauber R, Maldaner J, Cargnelutti D, Gonçalves JF, Castro GY, Shetinger MRC, Nicoloso FT (2008) Effect of cadmium on growth, micronutrient concentration, and d-aminolevulinic acid dehydratase and acid phosphatase activities in plant of *Pfaffia glomerata*. Braz J Plant Physiol 20:285–294

Smeets K, Cypers A, Lamrechts A, Semane B, Hoet P, Laere AV, Vangronsveld J (2005) Induction of oxidative stress and antioxidative mechanism in *Phaseolus vulgaris* after Cd application. Plant Physiol Biochem 43:437–444

Solis-Dominguez FA, Gonzalez-Chavez MC, Carrillo-Gonzalez R, Rodriguez-Vazquez R (2007) Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system. J Hazard Mater 141:630–636

Sottnikova A, Lunackova L, Masarovicova E, Lux A, Stresko V (2003) Changes in the rooting and growth of willows and poplars induced by cadmium. Biol Plant 46:129–134

Spurgeon DJ, Hopkin SP, Jones DT (1994) Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetid*a (Savigny): assessing the environmental impact of point source metal contamination in terrestrial ecosystems. Environ Pollut 84: 123–130

Srivastava P, Pandey A, Sinha DP (2011) Genetic diversity analysis in different varieties of black gram using RAPD markers. J Plant Breed Crop Sci 3:53–59

Staswick PE (1994) Storage proteins of vegetative plant tissues. Annu Rev Plant Physiol Plant Mol Biol 45:303–322

Stiborová M, Doubravová M, Leblová S (1986) A comparative study of the effect of heavy metal ions on ribulose-1,5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase. Biochem Physiol Pflanz 181:373–379

Stimpson HE, Lewis MJ, Pelham HR (2006) Transferrin receptor-like proteins control the degradation of a yeast metal transporter. EMBO J 25:662–672

Stobart AK, Griths WT, Ameen-Bukhari I, Sherwood RP (1985) The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Plant Physiol 63:293–298

Stohs SJ, Bagchi D, Hassoun E, Bagchi M (2000) Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 19:201–213

Stroinski A (1999) Some physiological and biochemical aspects of plant resistance to cadmium effect. I. antioxidative system. Acta Physiol Plant 21:175–188

Sunda WG, Huntsman SA (1998) Interactions among Cu^{2+}, Zn^{2+}, and Mn^{2+} in controlling cellular Mn, Zn, and growth rate in the coastal alga Chlamydomonas. Limnol Oceanogr 43:1055–1064

Sutapa B, Bhattacharyya AK (2008) Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. Chemosphere 70:1264–1272

Talanova VV, Titov AF, Boeva NP (2000) Effect of increasing concentrations of lead and cadmium on cucumber seedlings. Biol Plant 43:441–444

Tanaka R, Tanaka A (2007) Tetrapyrrole biosynthesis in higher plants. Annu Rev Plant Biol 58:321–346

Tanaka R, Kobayashi K, Masuda T (2011) Tetrapyrrole metabolism in *Arabidopsis thaliana*. Arabidopsis Book 9:e0145

Tantrey MS, Agnihotri RK (2010) Chlorophyll and proline content of gram (*Cicer arietinum* L.) under cadmium and mercury treatments. Res J Agric Sci 1:119–122

Theriappan P, Gupta A, Dhasarathan P (2011) Accumulation of proline under salinity and heavy metal stress in cauliflower seedlings. J Appl Sci Environ Manage 15:251–255

Tiryakioglu M, Eker S, Ozkutlu F, Husted S, Cakmak I (2006) Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. J Trace Elem Med Biol 20:181–189

Tiwari SB, Wang XJ, Hagen G, Guilfoyle TJ (2001) AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell 13:2809–2822

Toneva TV, Dimitrova SD, Pavlova BI, Minkov IN (2002) Influence of nitropyrine on the early stages of chlorophyll synthesis in wheat. Bulg J Plant Physiol 28:92–98

Toppi SL, Gabrielli R (1999) Responses to cadmium in higher plants. Environ Exp Bot 41: 105–130

Trakal L, Komárek M, Száková J, Tlustoš P, Tejnecký V, Drábek O (2012) Sorption behavior of Cd, Cu, Pb, and Zn and their interactions in phytoremediated soil. Int J Phytoremed 14:806–819

Tyler G, Pahlsson AM, Bengtsson G, Baath E, Tranvik L (1989) Heavy metal ecology and terrestrial plants, microorganisms and invertebrates: a review. Water Air Soil Pollut 47:189–215

Ueki S, Citovsky V (2005) Control improves with age: systemic transport in plant embryos and adults. Proc Natl Acad Sci USA 102:1817–1818

Unyayar S, Değer AG, Celik A, Cekic FO, Cevik S (2010) Cadmium-induced antioxidant status and sister-chromatid exchanges in *Vicia faba* L. Turk J Biol 34:413–422

van Assche FJ (1998) A stepwise model to quantify the relative contribution of different environmental sources to human cadmium exposure. Paper was presented at NiCad '98, Prague, 21–22 Sept 1998

Vassilev A, Iordanov I, Chakalova E, Kerin V (1995) Effect of cadmium stress on growth and photosynthesis of young barley (*H. vulgare* L.) plants. 2. Structural and functional changes in the photosynthetic apparatus. Bulg J Plant Physiol 21:12–21

Vassilev A, Berova M, Zlatev Z (1998) Influence of Cd^{2+} on growth, chlorophyll content and water relations in young barley plants. Biol Plant 41:601–606

Vijayaragavan M, Prabhahar C, Sureshkumar J, Natarajan A, Vijayarengan P, Sharavanan S (2011) Toxic effect of cadmium on seed germination, growth and biochemical contents of cowpea (*Vigna unguiculata* L.) plants. Int Multidiscip Res J 1:1–6

Vitoria AP, Lea PJ, Azevedo RA (2001) Antioxidant enzymes responses to cadmium in radish tissues. Phytochemistry 57:701–710

Vogeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Implication of a transport function for cadmium-binding peptides. Plant Physiol 92:1086–1093

Vrettos JS, Stone DA, Brudvig GW (2001) Quantifying the ion selectivity of the Ca^{2+} site in photosystem II: evidence for direct involvement of Ca^{2+} in O_2 formation. Biochemistry 40:7937–7945

Walker WM, Miller JE, Hassett JJ (1977) Effect of lead and cadmium upon calcium magnesium, potassium and phosphorus, concentration in young corn plants. Soil Sci 124:145–151

Wang YJ, Hao YJ, Zhang ZG, Chen T, Zhang JS, Chen SY (2005) Isolation of trehalose-6-phosphate phosphatase gene from tobacco and its functional analysis in yeast cells. J Plant Physiol 162:215–223

Wei L, Donat JR, Fones G, Ahner BA (2003) Interactions between Cd, Cu, and Zn influence particulate phytochelatin concentrations in marine phytoplankton: laboratory results and preliminary field data. Environ Sci Technol 37:3609–3618

Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ Int 30:685–700

Weltje L (1998) Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. Chemosphere 36: 2643–2660

Wojcik M, Vangronsveld J, Tukiendorf A (2005) Cadmium tolerance in *Thlaspi caerulescens* I. Growth parameters, metal accumulation and phytochelatin synthesis in response to cadmium. Environ Exp Bot 53:151–161

Woolhouse HW (1974) Longevity and senescence in plants. Sei Prog 61:123–147

Wu JT, Chang SJ, Chou TL (1995) Intracellular proline accumulation in some algae exposed to copper and cadmium. Bot Bull Acad Sin 36:89–93

Xiao L (2010) Evaluation of extraction methods for recovery of fatty acids from marine products. Bjørn Grung, University of Bergen, Bergen

Xiao S, Chye ML (2011) New roles for acyl-CoA-binding proteins (ACBPs) in plant development, stress responses and lipid metabolism. Prog Lipid Res 50:141–151

Xu Y, Jin J, Liu T, Zhou H, Hu T, Wang Q, Long M (2011) Regulation function of nitric oxide (NO) in leaves of plant under environmental stress. Afr J Biotechnol 10:15673–15677

Yang XE, Long XX, Ye HB, He ZL, Calvert DV, Stoffella PJ (2004) Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* H.). Plant Soil 259:181–189

Yasar U, Ozyigit II, Yalcin IE, Dogan I, Demir G (2012) Determination of some heavy metals and mineral nutrients of bay tree (*Laurus nobilis* L.) in bartin city, Turkey. Pak J Bot 44:81–89

Yen JL, Su N, Kaiser P (2005) The yeast ubiquitin ligase SCFMet30 regulates heavy metal response. Mol Biol Cell 16:1872–1882

Ying R, Qiu R, Tang Y, Hu P, Qiu H, Chen H, Shi T, Morel J (2010) Cadmium tolerance of carbon assimilation enzymes and chloroplast in Zn/Cd hyper accumulator *Picris divaricata*. J Plant Physiol 167:81–87

Yordanova R, Maslenkova L, Paunova S, Popova L (2009) Sensitivity of photosynthetic apparatus of pea plants to heavy metal stress. Biotechnol Biotechnol Equip 23:347–350

Young A, Britton G (1990) Carotenoids and stress. In: Alscher RG, Cumming JR (eds) Stress responses in plants: adaptation and acclimation mechanisms. Wiley-Liss, New York, pp 87–112

Zengin FK, Munzuroglu O (2005) Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. Acta Biol Cracov Ser Bot 47:157–164

Zhang JB, Huang WN (2007) Effects of cadmium stress on photosynthetic functions of strawberry. Ying Yong Sheng Tai Xue Bao 18:1673–1676

Zhang XY, Lin FF, Wong MTF, Feng XL, Wang K (2009) Identification of soil heavy metal sources from anthropogenic activities and pollution assessment of Fuyang County, China. Environ Monit Assess 154:439–449

Zhang X, Zhang S, Xu X, Li T, Gong G, Jia Y, Li Y, Deng L (2010) Tolerance and accumulation characteristics of cadmium in *Amaranthus hybridus* L. J Hazard Mater 180:303–308

Zhang C, Qiao Q, Appel E, Huang B (2012) Discriminating sources of anthropogenic heavy metals in urban street dusts using magnetic and chemical methods. J Geochem Exp 119–120: 60–75

Zhao Y (2011) Cadmium accumulation and antioxidative defenses in leaves of *Triticum aestivum* L. and *Zea mays* L. Afr J Biotechnol 10:2936–2943

Zhao FY, Liu W, Zhang SY (2009) Different responses of plant growth and antioxidant system to the combination of cadmium and heat stress in transgenic and non-transgenic rice. J Integr Plant Biol 51:942–950

Zhong L, Hu C, Tan Q, Liu J, Sun X (2011) Effects of sulfur application on sulfur and arsenic absorption by rapeseed in arsenic-contaminated soil. Plant Soil Environ 57:429–434

Zhou QX, Zhang QR, Liang J (2006) Toxic effects of acetochlor and methamidops on earthworm *Eisenia fetida* in Phaeozem, Northeast China. J Environ Sci 18:741–745

Zhou ZS, Song JB, Yang ZM (2012) Genome-wide identification of *Brassica napus* microRNAs and their targets in response to cadmium. J Exp Bot 63:4597–4613

Effect of Fruit and Vegetable Processing on Reduction of Synthetic Pyrethroid Residues

Reena Chauhan, Beena Kumari, and M.K. Rana

Contents

1 Introduction 89
2 Food Processing 91
2.1 Washing with Tap Water 91
2.2 Washing with Salt Solutions 101
2.3 Washing with Chemical Solutions or Detergents 101
2.4 Peeling 102
2.5 Juicing 102
2.6 Boiling/Cooking 103
2.7 Blanching 104
2.8 Freezing 104
3 Summary 105
References 106

1 Introduction

Pesticides prevent crop loss, increase crop productivity, reduce production costs, improve quality, and generally help farmers increase their income. Presently, food residues of pesticides seldom exceed the Maximum Residue Limits (MRLs) set by

R. Chauhan (✉)
Department of Soil Sciences, CCS Haryana Agricultural University,
Hisar, Haryana 125 004, India
e-mail: reenavansh82@gmail.com

B. Kumari
Department of Entomology, CCS Haryana Agricultural University,
Hisar, Haryana 125 004, India
e-mail: beena@hau.ernet.in

M.K. Rana
Department of Vegetable Science, CCS Haryana Agricultural University,
Hisar, Haryana 125 004, India
e-mail: mkrlotus@gmail.com

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229*, Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6_5,

the WHO/FAO (1989) and Prevention of Food Adulteration act (PFA) (1954). Some scientists believe that pesticide residues on fruits and vegetables that exceed their respective MRL limits (Taneja 2005) may cause health hazards to consumers (Elliion et al. 2000; Mukherjee and Gopal 2003). However, in a conference held in New Delhi on February 1–3, Sharma (2013) reported that out of 4,000 samples analyzed each year in India, only a small number (1.5–3%) exceeded the MRL value for pesticide residues. Therefore, the magnitude of any safety problem is unclear. What is clear, however, is that strict monitoring of pesticide residues in fruits and vegetables by Governmental Agencies is indispensable if any health hazard to consumers that may exist are to be curtailed.

Currently, the synthetic pyrethroid and organophosphate insecticide groups enjoy wide use in the world, having become alternatives to the formerly used persistent organochlorine products (Lyton et al. 1996; Subhani et al. 2001; Toan et al. 2007). Because of the widespread use of pesticides, their residues (Torres et al. 2004) appear on or in various environmental media and on agricultural commodities (Cox et al. 1999; Kumari et al. 2002, 2003a, b, 2004, 2005, 2006; Kumari and Kathpal 2009; Srivastava et al. 2000, 2001, 2006; Wang et al. 2008). Both, the indiscriminate use of pesticides (particularly at the fruiting stage), and failure to adopt a safe waiting period after they are applied accentuates pesticide residue accumulation in consumable vegetables. Because people consume pesticide residues daily, simple and cost effective strategies are needed to enhance food safety from potentially harmful pesticide residues, particularly in developing countries like India.

Many scientists and food processors have long been interested in the effect that commercial processing has on the persistence of pesticide residues in food. Several processing operations, such as canning, are known to reduce the levels of pesticide residues in different vegetables (Elkins 1989; Chin 1991). The extent to which pesticide residues are removed by commercial processing depends on several factors, such as the chemical properties of pesticide, the nature of the food commodity, the processing steps adopted and the length of time the compound has been in contact with food (Farris et al. 1992; Holland et al. 1994). The synthetic pyrethroid insecticides have increasingly been used on many crops, because the newer ones are photostable, are effective at low concentrations, have low environmental persistence and they breakdown more easily than do the organochlorine and organophosphorus insecticides (Pang et al. 1994a, b). The organochlorine and organophosphorus pesticides, which have been used for more than 50 years, recently constituted approximately 25% of the total insecticides used world over for plant protection (Shafer et al. 2005).

Little information exists in the recent literature on what effect commercial and common household processing has on reducing pyrethroid insecticide residues in vegetables. Hence, there is a need to develop such data to provide insights to consumers about the fate of potentially harmful pesticide residues during food preparation and processing. Such information may also offer means to mitigate the consumption of residues that could cause harm. The purpose of this review was to compile and summarize information that has been published in recent years on the effects that different food processing techniques have on reducing pesticide residues, with an emphasis on pyrethroid insecticides.

2 Food Processing

When food commodities are processed, it is implied that an effort has been made to transform the original perishable raw commodity to a value-added product that has greater shelf life and is closer to being table ready (Chin 1997). When food is processed, either for commercial or home use, the first step is often a mechanical one, washing, peeling, cutting, juicing, blanching, etc., in which the food is made suitable for cooking. Such treatment often reduces the amount of pesticide residues in the final edible portion (Petersen et al. 1996; Celik et al. 1995; Schattenberg et al. 1996). If there are subsequent processing steps, then each of them will have a cumulative effect to reduce the amount of the pesticides present (Geisman et al. 1975). Below, we review the effect that different food processing steps have on removing pesticide residues, and tabulate key processing study results in Table 1.

2.1 Washing with Tap Water

In most households, fruits and vegetables are washed before being consumed, and vegetables are often peeled prior to being cooked. When vegetables are commercially prepared for consumption they are virtually always washed. The effect on reducing pesticide residues form washing agricultural food commodities has been widely studied and recently reviewed (Kaushik et al. 2009; Zabik et al. 2000; Krol et al. 2000; Holland et al. 1994). The most attention-grabbing conclusion of these studies is that the capacity for pesticides to be rinsed from commodities does not correlate well with their water solubility (Cengiz et al. 2007; Boulaid et al. 2005; Angioni et al. 2004; Krol et al. 2000). In addition, different pesticides may be rinsed with different degrees of success from food commodities when different washing procedures are employed (Angioni et al. 2004; Pugliese et al. 2004; Lentza-Rizos and Kokkinaki 2002; Cabras et al. 1998). Pesticide residues that loosely adhere to raw foods are removed with reasonable efficiency by various washing procedures (Street 1969). Washing procedures carried out in homes normally involve use of standing water that has moderate temperature. However, the effectiveness of a washing procedure can be increased by adding chlorine or ozone to the wash water (Ong et al. 1996). If necessary, multiple washings can be used to remove soil particles from vegetable surfaces that carry pesticide residues (Guardia et al. 2007, 2006; Angioni et al. 2004; Cabras et al. 1997).

Many studies reveal that successfully washing (with water) pesticide residues from raw foods correlates better with a pesticide's Kow value (octanol–water partition coefficient) than with its water solubility. In fact, how a pesticide partitions between the peel of a fruit and the washing with water correlates well with the pesticide Kow value (Baur et al. 1997). Therefore, using an appropriate detergent solubilizes waxes in ways that may dissipate residues present in the epicuticular wax layer of fruits (Angioni et al. 2004). Treatments that involve dipping may also

Table 1 Effects of different food processing methods on the reduction of pyrethroid residue levels in fruits and vegetables

Sr. no.	Processing: Washing	Commodity	Insecticide dose	Initial residues (ppm)	Final residues (ppm)	Percent reduction	Reason	Reference
1.	Tap water (30 s)	Tomato	λ-cyhalothrin				–	Chauhan et al. (2012a)
			15 g a.i. ha^{-1}	0.144	0.090	37.50		
			30 g a.i. ha^{-1}	0.354	0.212	40.11		
2.	Tap water (30 s)	Tomato	Bifenthrin				The rinsability of a pesticide is not always correlated with its water solubility	Chauhan et al. (2012b)
			25 g a.i. ha^{-1}	0.107	0.073	31.77		
			50 g a.i. ha^{-1}	0.234	0.152	35.04		
3.	Tap water (30 s)	Okra	Bifenthrin				This shows that with the passage of time, the residues penetrate into fruit and were less accessible for removal by washing	Kumari (2012)
			25 g a.i. ha^{-1}	0.365	0.231	36.71		
			50 g a.i. ha^{-1}	0.584	0.348	40.00		
4.	Tap water (30 s)	Brinjal	Cypermethrin				–	Walia et al. (2010)
			0.001%	1.570	1.170	25.47		
5.	Tap water	Brinjal	Cypermethrin				The maximum reduction was observed on 0 day in both the treatments, which might be due to the less availability of residues on surface as with the passage of time, the surface residues become less available due to the penetration into fruit	Kaur (2011)
			43.75 g a.i. ha^{-1}	0.60	0.39	35.00		
			87.50 g a.i. ha^{-1}	1.095	0.729	33.42		
			Decamethrin					
			11.20 g a.i. ha^{-1}	0.430	0.310	27.90		
			22.40 g a.i. ha^{-1}	0.90	0.675	25.00		
6.	Tap water (2 min)	Okra	λ-cyhalothrin				These results indicate the extent of decontamination of residues varied with dose and time interval	Singh et al. (2007)
			15 g a.i. ha^{-1}	0.120	0.072	40.00		
			30 g a.i. ha^{-1}	0.164	0.094	42.70		

7.	Tap water (30 s)	Okra	Cypermethrin				The results indicate that as the age of the residues increased, the extent of reduction due to washing decreased because of penetration of insecticide into tissues	Singh et al. (2004)
			43.75 g a.i. ha^{-1}	0.274	0.161	41.00		
			87.50 g a.i. ha^{-1}	0.382	0.197	48.30		
8.	Tap water (1 min)	Okra	Cypermethrin				The decontamination processes become effective and could lower down the residues to non-toxic levels	Deen et al. (2009)
			60 g a.i. ha^{-1}	0.53	0.33	37.40		
			λ-cyhalothrin					
			15 g a.i. ha^{-1}	0.64	0.45	35.60		
9.	Tap water (1 min)	Brinjal	Synthetic pyrethroids	0.027	0.020	26	It is found effective in dislodging the residues as it depends on a number of factors like location of residues, age of residues, water solubility, temperature and type of washing	Kumari (2008)
		Cauliflower		0.688	0.490	29		
		Okra		0.019	0.013	31		
10.	Tap water (5 min)	Tomato	λ-cyhalothrin				–	Jayakrishanan et al. (2005)
			15 g a.i. ha^{-1}	0.526	0.321	39		
			30 g a.i. ha^{-1}	0.950	0.553	42		
11.	Tap water (1 min)	Brinjal	α-cypermethrin				–	Samanta et al. (2006)
			30 g a.i. ha^{-1}	0.78	0.34	54.8		
			60 g a.i. ha^{-1}	1.02	0.50	51.2		
12.	Tap water (30 s)	Cauliflower	α-methrin				–	Malik et al. (1998)
			0.005%	0.732	0.452	38.52		
13.	Tap water (30 s)	Spinach	Deltamethrin	0.421	0.378	10	Washing with water is advisable to remove most of the surface residues from vegetables	Randhawa et al. (2008)
		Cauliflower	1 ml L^{-1}	0.306	0.208	32		
		Potato		0.023	0.017	26		
		Brinjal		0.174	0.139	20.12		
		Tomato		0.275	0.178	35		
		Okra		0.285	0.242	15.08		

(continued)

Table 1 (continued)

Sr. no.	Processing: Washing	Commodity	Insecticide dose	Initial residues (ppm)	Final residues (ppm)	Percent reduction	Reason	Reference
14.	Tap water (30 s)	Okra	Cypermethrin	–	–	18–100	–	Anonymous (2002)
		Brinjal	Fenvalerate	–	–	24–41		
15.	Washing	Brinjal	β-cyfluthrin	0.42–1.20	–	44–48	–	Dikshit et al. (2001)
			λ-cyhalothrin	0.98–1.00	–	46–48		
16.		Lettuce	Permethrin	–	–	64	–	JMPR (1992)
17.		Green gram	Permethrin	–	–	31–36	–	Sukul and Handa (1986)
18.		Green bean	Permethrin	–	–	20	–	Celino and Magallona (1985)
19.	Washing	Tomato	λ-cyhalothrin				Washing of samples with citrus solution will not be economical and meaningful	Jayakrishanan et al. (2005)
			15 g a.i. ha^{-1}	0.526	0.309	41		
			30 g a.i. ha^{-1}	0.950	0.542	43		
20.	Tap water (30 s)	Okra	Cypermethrin				–	Samriti (2010)
			50 g a.i. ha^{-1}	–	0.017	15		
			100 g a.i. ha^{-1}	–	0.013	7.14		
21.	Tap water (1 min)	Hot pepper leaves	λ-cyhalothrin 1.0 mg kg^{-1}	–	0.092	70 (Avg.)	–	Lee and Jung (2009)
22.	Tap water (30 s)	Cauliflower	α-methrin 0.005%	0.732	0.450	38.52	–	Malik et al. (1998)
23.	Tap water (30 s)	Brinjal	α-methrin	0.322	0.028	28.26	–	Gill et al. (2001)
		Tomato	0.005%	0.606	0.026	30.36		
24.	Tap water (30 s)	Tomato	Cypermethrin				–	Rani (2012)
			40 g a.i. ha^{-1}	0.058	0.039	32.75		
			80 g a.i. ha^{-1}	0.133	0.085	36.09		

25.	Tap water	Brinjal	Cypermethrin	1.13	0.45	60.18	Washing had a significant effect on the removal of residues in eggplant and okra fruit because of less/no penetration of the chemical into cuticle layer of the plant surface	Zafar et al. (2012)
			Deltamethrin	0.65	0.39	40		
			Cyhalothrin	1.40	0.97	30.71		
		Okra	Cypermethrin	1.17	0.59	49.57		
			Deltamethrin	1.08	0.72	33.33		
			Cyhalothrin	0.48	0.37	22.92		
26.	Washing in saline solution	Tomato	λ-cyhalothrin				Brine water did not remove residues	Jayakrishanan et al. (2005)
			15 g a.i. ha^{-1}	0.526	0.294	44.00		
			30 g a.i. ha^{-1}	0.956	0.516	46.00		
27.	Washing in detergent	Eggplant	Cypermethrin	–	–	50–60	–	Awasthi (1986)
			Fenvalerate	–	–	50–60		
			Permethrin	–	–	50–60		
28.	Washing in detergent	Green gram	Cypermethrin	–	–	44–45	–	Sukul and Handa (1986)
			Fenvalerate	–	–	34–47		
29.	Peeling	Mango	Cypermethrin	0.60	–	Complete removal	Peeling off fruit skin removed the residues at all stages, reflecting that residues accumulated in fruit pericarp only, with no movement to fruit pulp	Awasthi (1993)
			Fenvalarate	0.68	–			
30.	Peeling	Potato	Deltamethrin	0.023	0.004	76	The peeling process had a significant effect on the removal of residues	Randhawa et al. (2008)
		Brinjal	1 ml L^{-1}	0.174	0.027	80		
		Tomato	Cypermethrin	0.058	0.010	60	Peeling had a significant effect on removing cypermethrin residues due to the non-systemic nature of the insecticide on this food crop	Rani (2012)
				0.133	0.036	72.93		
31.	Peeling	Brinjal	Cypermethrin	1.13	0.23	79.65	These three are non-systemic insecticides; so, when peel was removed, most residues were also removed	Zafar et al. (2012)
			Deltamethrin	0.65	0.18	72.31		
			Cyhalothrin	1.40	0.55	60.71		

(continued)

Table 1 (continued)

Sr. no.	Processing: Washing	Commodity	Insecticide dose	Initial residues (ppm)	Final residues (ppm)	Percent reduction	Reason	Reference
32.	Washing followed boiling	Brinjal	Cypermethrin 0.001%	1.570	0.920	41.40	The rate at which residues are dislodged from cooking depends on temperature, duration of the process, amount of water, food additives and type of system (open/closed)	Walia et al. (2010)
		Okra	Cypermethrin				–	Samriti (2010)
			50 g a.i. ha^{-1}	–	0.019	71.64		
			100 g a.i. ha^{-1}	–	0.015	78.87		
		Tomato	Cypermethrin				–	Rani (2012)
			40 g a.i. ha^{-1}	0.058	0.015	40		
			80 g a.i. ha^{-1}	0.133	0.030	43.39		
		Okra	Bifenthrin				Reduction was higher than washing only, for both doses	Kumari (2012)
			25 g a.i. ha^{-1}	0.365	0.017	64.58		
			50 g a.i. ha^{-1}	0.0580	0.068	56.41		
33.	Washing followed boiling	Brinjal	Cypermethrin				–	Kaur (2011)
			43.75 g a.i. ha^{-1}	0.600	0.3500	41.66		
			87.50 g a.i. ha^{-1}	1.095	0.690	36.98		
			Decamethrin					
			11.20 g a.i. ha^{-1}	0.430	0.270	37.20		
			22.40 g a.i. ha^{-1}	0.900	0.607	32.55		
34.	Washing followed boiling	Tomato	λ-cyhalothrin				Washing + boiling is more effective than washing only, in reducing the residues	Chauhan et al. (2012b)
			15 g a.i. ha^{-1}	0.144	0.022	74.41		
			30 g a.i. ha^{-1}	0.354	0.020	83.87		

35.	Washing followed boiling	Tomato	Bifenthrin 25 g a.i. ha^{-1} 50 g a.i. ha^{-1}	 0.107 0.234	 0.022 0.023	 42.10 45.23	–	Chauhan et al. (2012a)
36.	Washing followed boiling	Brinjal Cauliflower Okra	Synthetic pyrethroid	0.027 0.68 0.019	0.017 0.412 0.011	37.00 40.00 42.00	Processing substantially lowers the pesticide residues in vegetables	Kumari (2008)
37.	Washing followed boiling	Tomato	λ-cyhalothrin 15 g a.i. ha^{-1} 30 g a.i. ha^{-1}	 0.526 0.950	 0.035 0.068	 76.00 79.00	Washing followed by steaming gave the best results	Jayakrishanan et al. (2005)
38.	Washing followed boiling	Brinjal	α-cypermethrin 30 g a.i. ha^{-1} 60 g a.i. ha^{-1}	 0.78 1.02	 0.23 0.35	 70.20 66.10	–	Samanta et al. (2006)
39.	Washing followed boiling (20 min)	Cauliflower	A-methrin 0.005%	 0.676	 0.413	 16.73	Less reduction can be attributed to its thermal stability	Malik et al. (1998)
	(Boil 10–15 min)	Brinjal Tomato	α-methrin 0.005%	0.322 0.606	0.122 0.127	29.06 18.06	Boiling was not very effective in reducing the α-methrin residues, probably because of the thermal stability of the insecticide	Gill et al. (2001)
40.	Washing followed boiling	Brinjal	β-cyfluthrin λ-cyhalothrin	0.42–1.20 0.98–1.00	– –	62–64% 62–63	Reduction of residues from washing followed by boiling was high	Dikshit et al. (2001)
41.	Washing followed cooking	Spinach Cauliflower Potato Brinjal Tomato Okra	Deltamethrin 1 ml l^{-1}	0.421 0.306 0.023 0.174 0.275 0.285	0.302 0.124 0.003 0.022 0.124 0.174	28.26 59.47 86.95 87.35 54.90 38.94	–	Randhawa et al. (2008)

(continued)

Table 1 (continued)

Sr. no.	Processing: Washing	Commodity	Insecticide dose	Initial residues (ppm)	Final residues (ppm)	Percent reduction	Reason	Reference
42.	Cooking	Brinjal	Cypermethrin	1.13	0.060	94.69	Cooking/boiling was the most effective among all processing operations. Processes involving heat can enhance volatilization of the chemicals and their hydrolysis thus reducing the residue levels	Zafar et al. (2012)
			Deltamethrin	0.65	0.10	98.46		
			Cyhalothrin	1.40	0.30	75		
		Okra	Cypermethrin	1.17	0.12	89.74		
			Deltamethrin	1.08	0.19	82.41		
			Cyhalothrin	0.48	0.08	83.33		
43.	(Boil 30 min)	Plums	Cypermethrin	–	–	10	–	JMPR (1992)
	(Boil 45 min)	Cabbage		–	–	25		
44.	(Boil 45 min)	Apple	Permethrin	–	–	0	–	JMPR (1992)
	(Boil 2–3 min)	Tomato		–	–	0		
45.	Boil	Spinach	Deltamethrin	–	–	20	–	Mestres and Mestres (1992)
46.	Boil	Green bean		–	–	50		
47.	Blanching	Green gram	Fenvalerate	–	–	50–51	–	Sukul and Handa (1986)
			Permethrin	–	–	38–39		
48.	Blanching	Green bean	Permethrin	–	–	62	–	Celino and Magallona (1985)
49.	Blanching	Hot pepper leaves	λ-cyhalothrin 1.0 mg kg^{-1}	0.296	< 0.1	95 (Avg.)	–	Lee and Jung (2009)
50.	Blanching	Brinjal	Cypermethrin	1.13	0.20	82.30	–	Zafar et al.(2012)
			Deltamethrin	0.65	0.19	70.77		
			Cyhalothrin	1.40	0.49	65		
		Okra	Cypermethrin	1.17	0.23	80.34		
			Deltamethrin	1.08	0.54	50		
			Cyhalothrin	0.48	0.19	60.42		

51.	Cooking in oil (frying)	Brinjal	Cypermethrin 0.001%	1.570	0.860	45.22	–	Walia et al. (2010)
52.	Grilling	Brinjal	Cypermethrin 0.001%	1.570	0.783	50.12	–	Walia et al. (2010)
53.	Freezing (4–5 °C)	Cauliflower	α-methrin 0.005%	0. 732	0.558	23.77	Under refrigerated conditions, the reduction was comparatively less than at room temp	Malik et al. (1998)
54.	Freezing (5 °C)	Brinjal	α-methrin 0.005%	0.322	0.230	28.57	The reduction was almost 50% under cold conditions, which might be considered logical since low temperature decreased the enzymatic as well as chemical degradation of the insecticides	Gill et al. (2001)
		Tomato		0.606	0.155	24.75		
55.	Freezing (4 °C)	Tomato	Bifenthrin	0.107	0.007	93.45	–	Chauhan et al. (2012a, b)
				0.234	0.013	94.44		
			λ-cyhalothrin	0.144	0. 015	89.58	–	
				0.354	0.025	92.93		

selectively remove pesticide residues that have systemic action via similar mechanisms (Cabras et al. 1998; Femenia et al. 1998). Pesticide residues that remain in fruits after washing can be ascribed to amounts that have penetrated into the cuticle. Several authors (Ou-Yang et al. 2004; Pugliese et al. 2004; Cabrera et al. 2000; Zhang and Pehkonen 1999) have also studied and reported on the fact that the washing process may convert the parent pesticide compound into byproducts that may also be toxic.

The food washing process reduces the amount of hydrophilic pesticide residues that are located on the fruit surface. In addition, the temperature of the washing water and type of washing action both influence the level of residue that remains. Holland et al. (1994) reported that washing with hot water, to which detergent was added, was more effective than washing with cold water alone. Washing fruit under tap water, coupled with gentle rubbing it by hand for 1 min significantly dislodged pesticide residues (Barooh and Yein 1996). Washing okra-contaminated fruit (*viz.*, with the insecticides cypermethrin, λ–cyhalothrin and endosulfan at 0.53, 0.64 and 9.25 μg g^{-1}, respectively) under tap water, respectively removed 16–17%, 14–36% and 16–24% for cypermethrin, λ-cyhalothrin and endosulfan (Deen et al. 2009). Washing brinjals in water for 30 s removed 25.47% of residues, which originally were 1.57 μg g^{-1} (Walia et al. 2010). Cauliflower curds that initially showed residues of α-methrin (*viz.*, 0.676 μg g^{-1}), displayed 38% lower residues after 30 s of washing with tap water (Malik et al. 1998). Washing of brinjal and tomato reduced α–methrin residues by 17–28 and 12–30%, respectively (Gill et al. 2001). Okra that had respective cypermethrin and fluvalinate residues of 0.274–0.382 and 0.091–0.126 mg kg^{-1}, showed reduced residue levels of 37–48% and 32.4–44%, after washing (Singh et al. 2004).

Kaur (2011) studied the reduction of cypermethrin and decamethrin residues in brinjal after using different processing methods. The authors observed that washing brinjal with tap water reduced cypermethrin and decamethrin to 25–42% and 20–37% of original levels, respectively, although tap water was least effective in reducing the residues of these insecticides. Jayakrishanan et al. (2005) studied the effect of washing procedure on pesticides residues in tomato. A tomato crop was treated with λ–cyhalothrin at a rate of 15 or 30 g a.i. ha^{-1}. At harvest, washing the fruit with either a 1.5% saline solution or a 5% citrus solution was found to be ineffective for removing residues, although washing with tap water dislodged 39–42% of the original residues. Kumari (2008) estimated the reduction of synthetic pyrethroids in brinjal, cauliflower and okra, after applying washing procedures; this author found that washing reduced pyrethroid residues in the three vegetables by 26%, 29% and 31%, respectively. Kwon et al. (2009) also studied the decline of pesticide residues after washing of spinach, chard and mellow. These authors reported that washing reduced bifenthrin residues by 58–64%. Lee and Jung (2009) studied the effects of washing hot pepper fruit that had been treated with λ–cyhalothrin at a rate of 1.0 mg kg^{-1}, and found that washing reduced residues from 0.296 to 0.092 mg kg^{-1}. Various pesticide decontamination processes, such as washing the fruits with water, were reported to dislodge the residues to varying degrees that depended on constitution of the fruit, chemical nature of the pesticide and environmental conditions.

In fruits and fruit-type vegetables, the concentration of pesticide residue was higher in the fruit stalk and near the epidermis (exocarp and fruit receptacle), than in the sarcocarp or pericarp. In leafy vegetables, pesticide residue concentrations were higher in the outer leaves than in inner ones (Yoshida et al. 1992).

The washing of treated mango fruit was reported to reduce surface residues by 21–27% for pyrethroids at the initial stages of spray treatment. The effectiveness of washing, however, decreased (Elliot 1980; Briggs 1985) if the insecticide was sprayed at a later plant stage (which resulted from the strong bonding that occurred between the insecticide molecules and the waxy layer of fruit skin). Oliveira (1995) reviewed the effects of removing pesticide residues from raw foods by washing with water. The author compared the response of different pesticides, removal conditions and food type. Foods types covered included: apples, plums, red currants, tomatoes, oranges, pears, strawberries, grapes, papayas, spinach, lettuce, beans, rice, mustard, onions, celery and aubergine.

Various methods were tested for removing pathogens, pesticides and other contaminants from the surfaces of fresh fruits and vegetables (Michaels et al. 2003). A range of fresh produce was subjected to different combinations of cleaning processes, such as water rinsing steps, use of produce-cleaning brushes, disinfectants, air drying and paper towels. Tests on waxed and unwaxed apples that were contaminated with a cocktail of pesticides showed that certain treatments (e.g., wiping with paper towels) were more effective than other procedures for removing pesticides.

2.2 Washing with Salt Solutions

Washing with a dilute salt solution is a convenient method to lower the contaminant load from food surfaces, particularly from fruits and vegetables. This method may be equally effective for reducing pesticide residues in other food commodities too. This procedure is recommended as being practical for household use, as well. Washing tomato samples with a salt solution reduced λ-cyhalothrin residues by 44–46% (Jayakrishanan et al. 2005). Dipping of green chilies in a 2% salt solution for 10 min, followed by washing in water removed 90.56% and 66.93% of residues of cypermethrin from chilies at 0 and 5 days after final spraying, respectively (Phani Kumar et al. 2000).

2.3 Washing with Chemical Solutions or Detergents

Solutions of NaOH, acetic acid, potassium dichromate and detergents have been used as food decontaminating agents. Several studies indicate that these chemicals also play an effective role in removing pesticide residues. Fanchun et al. (2003) reported that ozone can be used to prolong the shelf life of fruits and vegetables. Moreover, ozone treatment removes ethylene, inhibits respiration activity and

inactivates microorganisms (Fanchun et al. 2003). They also reported that ozone has the potential to reduce pesticide levels in fruits and vegetables, although this treatment imposes possible negative effects on fruit and vegetable quality.

Washing fruits alone with NaOH solution reduced the surface residues by 40–50%, while washing with detergent solution reduced surface pesticides residues by 50–60% (Bajwa and Sandhu 2011). Because of its powerful oxidizing property, ozone is used to effectively convert waste water to drinking water. Ozonation is also a safe and promising process for removing pesticides from aqueous solution or from surface of vegetables, in domestic settings. First, washing with tap water and then with ozonated water significantly reduced the pesticide residues present on vegetables surface (Fanchun et al. 2003).

2.4 Peeling

Insecticides applied directly to crops undergo very limited movement from, or penetration into the fruit's peel. Therefore, the majority of residues of these insecticides are normally confined to the outer surface, where they are subject to removal by peeling, hulling, or trimming operations. Peeling of fruit, *viz.* avocado, bananas, citrus, kiwifruit, mango, pineapple or vegetables, such as roots, tubers and bulbs, with a peeler or knife is a common practice. Many studies show that the majority of residues are located in or on the peel. It has been documented in numerous reports that peeling eliminates 70–100% of the pesticide residues present on different fruits (Cengiz et al. 2007; Boulaid et al. 2005; Fernández-Cruz et al. 2004; Rasmusssen et al. 2003; Burchat et al. 1998; Clavijo et al. 1996; Celik et al. 1995; Holland et al. 1994; Rouchaud et al. 1991). Peeling off the skin of mangoes completely removes residues of dimethoate, fenthion, cypermethrin and fenvalerate, reflecting that the accumulated residues resided only in or on fruit skin, and not in fruit pulp (Awasthi 1993). Moreover, peeling of the fruit skin was reported to dislodge residues to a varying degree, depending on the constitution of the fruit, chemical nature of the pesticide and environmental conditions (Nath et al. 1975; Awasthi 1986).

2.5 Juicing

Depending on how a pesticide partitions between the fruit skin, pulp and juice, pesticide residues that penetrate the surface to reach fruit solids and vegetable juices are generally reduced by 70–100% (Rasmusssen et al. 2003; Zabik et al. 2000; Abou-Arab 1999; Will and Kruger 1999; Burchat et al. 1998; Holland et al. 1994). After processing, pulp byproducts, which often include skin, retain a substantial proportion of lipophilic residues. Rasmusssen et al. (2003) reported that only 2–9% of different insecticide residues (e.g., cypermethrin, deltamethrin, fenitrothion, fenpropathrin and λ–cyhalothrin) on fortified apples were transferred to apple juice. In

contrast, the residues in apple pulp were detected at levels 2–3.5 mg kg^{-1} higher than in unprocessed apples, as a result of mass concentration. In other studies, pesticides with the highest water solubility were present at higher levels in the juices of carrot, tomato and strawberry (Will and Kruger 1999; Burchat et al. 1998). When juices are processed, they are clarified by filtration or centrifugation, which actions may further eliminate pesticide residues that are retained in suspended particles (Liapis et al. 1995; Miliadis et al. 1995). Juice concentration via vacuum procedures may also occur and may concentrate any pesticides that are transferred to the juice (Zabik et al. 2000).

2.6 Boiling/Cooking

Cooking is the most effective treatment for reducing the residues of synthetic pyrethroids in different vegetables. The degree to which cooking removes pesticide residue depends on the process used. The details of time, temperature, degree of moisture loss and whether the system is open or closed are important as regards quantitative effects on residue concentration. The application of heat in cooking reduces residue levels and can enhance volatilization and hydrolysis of any chemicals present (Holland et al. 1994). Furthermore, Nagayama (1996) reported that during cooking, some residual pesticides were translocated from raw materials to cooking water, according to their water solubility, and the pesticide remained in processed food according to its Kow value.

Although washing with water was usually effective in reducing initial pesticides residue levels, removal efficiency declined when residues were aged (samples collected long after spraying), rather than having been collected immediately after spraying, where boiling or cooking is effective in removing the aged residues. For low volatile synthetic pyrethroid insecticides that are relatively stable to hydrolysis, residue losses through cooking may be lower, and insecticide levels may actually increase from moisture loss (Holland et al. 1994).

Washing, followed by boiling did not further reduce bifenthrin residues from spinach, mellow, or chard (Kwon et al. 2009). Interestingly, boiling did not reduce cypermethrin, deltamethrin, fenpropathrin and λ–cyhalothrin residues on apples either (Rasmusssen et al. 2003). In contrast, brinjals that had residues of 1.570 mg/kg^{-1} in fruit were washed then boiled; the reduction of residues achieved from such treatment was 41%; (Walia et al. 2010). Washing and boiling of cauliflower reduced α–methrin residues from an initial level of 0.732 to a post-treatment level of 0.413 mg kg^{-1} (17% reduction); however, boiling alone was ineffective in reducing residues (Malik et al. 1998). Gill et al. (2001) reported that α–methrin residues in brinjal and tomato (initially 0.322 and 0.606 mg kg^{-1}, respectively) declined to 0.122 and 0.127 mg kg^{-1} (29% and 18% reduction), respectively after washing, followed by boiling. Kadian et al. (2001) reported that cypermethrin residues declined in tomato, okra, bottlegourd and ridgegourd after being cleaned through several processing steps, i.e., about 5–14% by washing, 6–26% by blanching, 6–19% by

washing in brine solution and 15–33% by cooking. They suggested that cooking was more effective in reducing the cypermethrin residues in vegetables. Boulaid et al. (2005) evaluated the effect of household processing (*viz*., washing, peeling and cooking) and unit-to-unit variability as regards residues of pyrifenox, pyridaben and tralomethrin in tomatoes. Levels of pyrifenox, pyridaben and tralomethrin residues were determined in tomatoes, both before and after processing. Processing factor results for washing were 0.9 ± 0.3 for pyridaben, 1.1 ± 0.3 for pyrifenox and 1.2 ± 0.5 for tralomethrin, whereas the processing factors for peeling were 0.3 ± 0.2 for pyridaben and 0.0 ± 0.0 for both pyrifenox and tralomethrin. The average loss of water in the tomato samples during cooking was approximately 50%; the processing factors from cooking were 2.1 ± 0.8 for pyridaben, 3.0 ± 1.1 for pyrifenox and 1.9 ± 0.8 for tralomethrin. The unit-to-unit variability factor results were within the range of 1.3–2.2. Washing and steaming removed 40–60% of deltamethrin residues that remained on chick peas after storage (Kumar and Dikshit 2000).

2.7 Blanching

Blanching is a heating process, in which vegetables or fruits are immersed into hot water, removed after a short time, and are finally plunged into cool water to halt the heating process. Blanching typically involves only fruits and vegetables, rather than other types of food (like meat), because vegetables are susceptible to enzyme-triggered changes that can result in lost flavor, lost color, or lost texture during freezing (Anonymous 2011). Preventing unwanted changes in flavor, color, and texture is not the only purpose of blanching, however. Other purposes are to thoroughly cleanse the vegetable surface, and to increase the safety of frozen vegetables by potentially removing unwanted microorganisms or pesticide residues. Sukul and Handa (1986) reported that blanching of green gram lowered the residues of fenvelrate and permethrin by 50–51% and 38–39%, respectively. Similarly, permethrin residues in green beans were reduced by 62% (Celino and Magallona 1985). Hot pepper leaves treated with λ-cyhalothrin showed a 95% reduction (Lee and Jung 2009) from blanching. Zafar et al. (2012) reported residues of cypermethrin, deltamethrin and cyhalothrin dissipated to 0.20, 0.19 and 0.49, after brinjal was blanched. This equates to residue declines of 82.30%, 70.77% and 65%, respectively.

2.8 Freezing

Freezing is the most common method for preserving food. Freezing considerably delays both food decay and most chemical reactions. Chauhan et al. (2012b) observed that when tomatoes treated with λ–cyhalothrin (15 and 30 g a.i. ha^{-1}) were frozen, residues were reduced from 0.107 to 0.015 and 0.234 to 0.025 mg kg^{-1}; this equates to a 89.58% and 92.93% reduction at the recommended and double the

recommended doses, respectively on the 7th day after pesticide application. Under refrigerated conditions, the dissipation was slightly lower than at room temperature. The average initial residues from recommended (25 g a.i ha^{-1}) and double the recommended dose (50 g a.i. ha^{-1}) for bifenthrin on tomatoes were 0.107 and 0.234 mg kg^{-1}, respectively (Chauhan et al. 2012a). Under refrigerated conditions, the above mentioned initial residues were reduced to 0.007 and 0.013 mg kg^{-1}; this represented a respective reduction of 93.45% and 94.44% on 10th day after application. Gill et al. (2001) studied brinjals and tomatoes sprayed with the recommended dose (0.005%) of alphamethrin up to the drenching level; samples were then stored at either ambient (40 °C) or under a refrigerated condition (5 °C). Alphamethrin residues were observed to dissipate faster at room temperature than under cold storage conditions for both brinjals and tomato fruits; the recorded reduction amounts were 28.57% and 24.75%, respectively. The reductions in residues of α–methrin (0.005%) for cauliflower were recorded at 23.77% (Malik et al. 1998).

3 Summary

In this review, we emphasize that the advantages associated with applying pesticides to enhance agricultural productivity must be weighed against the possible health hazards arising from the appearance of toxic pesticide residues in food. First and foremost, pesticides should be handled and applied in compliance with good agricultural practices to minimize environmental or food commodity contamination. In developing countries, good agricultural practices are not fully abided by. When vegetables are produced in such countries, pesticides are applied or prospectively applied at each growth stage of the crop. Hence, contamination of vegetables and other food commodities occur. It is well known that processing of food derived from pesticide treated crop commodities can serve to reduce residues that reach consumers. Food safety can therefore partially be enhanced by employing suitable food processing techniques and appropriate storage periods, even in developing countries. Even common and simple household processing techniques for certain foods acquire significance as means to reduce the intake of harmful pesticide food residues.

Pesticide residue levels in post-harvest raw agricultural commodities (RAC) are affected by the storage, handling and the processing steps they pass through, while being prepared for human consumption. The review of cogent literature presented in this article demonstrated differences among the pyrethroid insecticide residues present on or in foods, depending on how the RAC from which they came were processed for consumption. Peeling vegetables or fruit reduced pyrethroid residues the most (60–100%), and juicing was nearly as effective in reducing residues (70–100%). The least reduction occurred for foodstuffs that were only washed with tap water (10–70%). Washing RACs with saline water and detergent was more effective (34–60%) in reducing residues than was simple washing under tap water. Freezing is also effective in reducing residue levels and achieved reductions between 24%

and 94%. Cooking of food products eliminated 75–98% of the pesticide residues present, so was also relatively effective. When foods were cooked in oils, however, reductions in pesticide residues were less (45%).

References

Abou-Arab AAK (1999) Behavior of pesticides in tomatoes during commercial and home preparation. Food Chemistry 65:509–514

Angioni A, Schirra M, Garau VL, Melis M, Tuberoso CIG, Cabras P (2004) Residues of azoxystrobin, fenhexamid and pyrimethanil in strawberry following field treatments and the effect of domestic washing. Food Addit Contam 21:1065–1070

Anonymous (2002) Annual progress report and proceedings of AICRP on pesticide residues. Project Coordinating Cell, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, p 1

Anonymous (2011) www.fruitvegetable.com

Awasthi MD (1986) Chemical treatments for the decontamination of brinjal fruit from residues of synthetic pyrethroids. Pestic Sci 17(2):89–92

Awasthi MD (1993) Decontamination of insecticide residues on mango by washing and peeling. J Food Sci Technol 30(2):132–133

Bajwa Usha, Sandhu KS (2011) Effect of handling and processing on pesticide residues in food – a review. J Food Sci Technol. doi:10.1007/s13197-011-0499-5

Barooh AK, Yein BR (1996) Residues of quinalphos in/on brinjal (*Solanum melongona*) fruits. Pestic Res J 8:56–60

Baur P, Buchholz A, Schönherr J (1997) Diffusion in plant cuticles as affected by temperature and size of organic solutes: similarity and diversity among species. Plant Cell Environ 20:982–994

Boulaid M, Aguilera A, Camacho F, Soussi M, Valverde A (2005) Effect of household processing and unit-to-unit variability of pyrifenox, pyridaben and tralomethrin residues in tomatoes. J Agric Food Chem 53:4054–4058

Briggs GC (1985) Physical properties and environmental behaviour of pyrethroids. Pestic Sci 16:193–194

Burchat CS, Ripley BD, Leishmann PD, Ritcey GM, Kakuda Y, Stephenson GR (1998) The distribution of nine pesticides between the juice and pulp of carrots and tomatoes after home processing. Food Addit Contam 15:61–71

Cabras P, Angioni A, Garau VL, Melis M, Pirisi FM, Karim M, Minelli EV (1997) Persistence of insecticide residues in olives and olive oil. J Agric Food Chem 45:2244–2247

Cabras P, Angioni A, Garau VL, Melis M, Pirisi FM, Cabitza F, Cubeddu M (1998) Pesticide residues on field-sprayed apricots and in apricot drying processes. J Agric Food Chem 46: 2306–2308

Cabrera HAP, Menezes HC, Oliveira JV, Batista RFS (2000) Evaluation of residual levels of benomyl, methyl parathion, diuron, and vamidothion in pineapple pulp and Bagasse (*Smooth Cayenne*). J Agric Food Chem 48:5750–5753

Celik S, Kunc S, Asan T (1995) Degradation of some pesticides in the field and effect of processing. Analyst 120:1739–1743

Celino LP, Magallona ED (1985) Philipp Agric 68:525

Cengiz MF, Certel M, Karakas B, Goçmen H (2007) Residue contents of captan and procymidone applied on tomatoes grown in greenhouses and their reduction by duration of a pre-harvest interval and post-harvest culinary applications. Food Chem 100:1611–1619

Chauhan R, Monga S, Kumari B (2012a) Dissipation and decontamination behavior of bifenthrin residues in tomato. Bull Environ Contam Toxicol 189(1):181–186

Chauhan R, Monga S, Kumari B (2012b) Dissipation of λ–cyhalothrin on tomato (*Lycopersicon esculentum* Mill.) and effect of processing on removal of residues. Bull Environ Contam Toxicol 88(3):352–357

Chin HB (1991) The effect of processing on residues in foods: the food processing industry's database. In: Tweedy BG (ed) Pesticide residues and food safety: a harvest of viewpoints. American Chemical Society, Washington, DC, pp 175–181

Chin HB (1997) The effect of processing on pesticide residues in processed fruits and vegetables. Book of abstracts, 213th ACS national meeting, San Francisco, 13–17 Apr, AGFD-189. American Chemical Society, Washington, DC

Clavijo MP, Medina MP, Asensio JS, Bernal JG (1996) Decay study of pesticide residues in apple samples. J Chromatogr 740:146–150

Cox I, Karakaya AE, Afkham BL, Burgaz S (1999) Organochlorine pesticide contaminants in human milk samples collected in Tebriz (Iran). Bull Environ Contam Toxicol 63:444–450

Deen MK, Kumari B, Sharma SS (2009) Dissipation and decontamination of residues of 3 pesticides in okra fruits. Pestic Res J 21(1):80–82

Dikshit AK, Srivastava YN, Lal OP (2001) Residue studies and bioefficacy of β–cyfluthrin and λ–cyhalothrin in brinjal (*Solanum melongena* L.) fruits. Pestology 25(10):27–35

Elkins ER (1989) Effect of commercial processing on pesticide residues in selected fruits and vegetables. J Assoc Anal Chem 72:533–535

Elliion J, Sauve F, Selwyn J (2000) Multiresidues method for determination of residues of 251 pesticide in fruits and vegetables by gas liquid chromatography/mass spectrometry and liquid chromatography with fluorescence detector. J AOAC Int 83:698–713

Elliot M (1980) Established pyrethroids. Pestic Sci 11:119–128

Fanchun K, Shengmin L, Qun W (2003) Application of ozone for pesticide degradation and preservation of fruits and vegetables. Food Mach 5:24–26

Farris GA, Cabras P, Spanedda L (1992) Pesticide residues in food processing. Italian J Food Sci 3:149–169

Femenia A, Sanchez ES, Simal S, Rossello C (1998) Effects of drying pretreatments on the cell wall composition of grape tissues. J Agric Food Chem 46:271–276

Fernández-Cruz ML, Villarroya M, Llanos S, Alonso-Prados JL, García-Baudín JM (2004) Field incurred fenitrothion residues in kakis: comparison of individual fruits, composite samples, peeled and cooked fruits. J Agric Food Chem 52:860–863

Geisman JR, Gunther FA, Gunther JD (eds) (1975) Reduction of pesticide residues in food crops by processing. Residue Rev Residues Pestic Other Contam Total Environ 54:43–54

Gill K, Kumari B, Kathpal T (2001) Dissipation of alphamethrin residues in/on brinjal and tomato during storage and processing conditions. J Food Sci Technol 38:3–16

Guardia RM, Medina RA, Molina DA, Canada AMJ (2006) Influence of harvesting method and washing on the presence of pesticide residues in olives and olive oil. J Agric Food Chem 54:8538–8544

Guardia RM, Medina RA, Molina DA, Canada AMJ (2007) Multiresidue analysis of three groups of pesticides in washing waters from olive processing by solid-phase extraction-gas chromatography with electron capture and thermionic specific detection. Micro Chem J 85:257–264

Holland PT, Hamilton D, Ohlin B, Skidmore MW (1994) Effects of storage andprocessing on pesticide residues in plant products. Pure and Applied Chemistry 66(2):335–356

Jayakrishanan S, Dikshit AK, Singh JP, Pachauri DC (2005) Dissipation of λ–cyhalothrin on tomato (*Lycopersicon esculentum* Mill.) and removal of its residues by different washing processes and steaming. Bull Environ Contam Toxicol 75:324–328

JMPR (1992) FAO plant production and production paper series "pesticide residues in food–evaluations part 1" published annually by FAO, Rome

Kadian S, Kumar R, Grewal RB, Srivastava SP (2001) Effect of household processing on cypermethrin residues in commonly used vegetables. Pestology 25:10–13

Kaur P (2011) Bioefficacy of cypermethrin and decamethrin against *Leucinodes orbonalis* Guenee and their residues in brinjal fruits. M.Sc. thesis CCS Haryana Agricultural University, Hisar

Kaushik G, Satya S, Naik SN (2009) Food processing a tool to pesticide residue dissipation– a review. Food Res Intern 42:26–40

Krol WJ, Arsenault TL, Pylypiw HM Jr, Mattina MJI (2000) Reduction of pesticide residues on produce by rinsing. J Agric Food Chem 48:4666–4670

Kumar Lal A, Dikshit AK (2000) Persistence of deltamethrin on chickpea and its decontamination. Pestic Res J 12(1):74–79

Kumari B (2008) Effects of household processing on reduction of pesticide residues in vegetables. ARPN J Agric Biol Sci 3(4):46–48

Kumari S (2012) Persistence of bifenthrin in okra (*Abelmoschus esculentus* L.) and its leaching behaviour in soil. Ph.D. thesis, CCS Haryana Agricultural University, Hisar

Kumari B, Kathpal TS (2009) Monitoring of pesticide residues in vegetarian diet. Environ Monit Assess 151(1–4):19–26

Kumari B, Madan VK, Kumar R, Kathpal TS (2002) Monitoring of seasonal vegetables for pesticide residues. Environ Monit Assess 74:263–270

Kumari B, Gulati R, Kathpal TS (2003a) Monitoring of pesticidal contamination in honey. Korean J API 18(2):155–160

Kumari B, Kumar R, Madan VK, Singh R, Singh J, Kathpal TS (2003b) Magnitude of pesticidal contamination in winter vegetables from Hisar, Haryana. Environ Monit Assess 87:311–318

Kumari B, Madan VK, Singh J, Singh S, Kathpal TS (2004) Monitoring of pesticidal contamination of farm gate vegetables from Hisar. Environ Monit Assess 90:65–77

Kumari B, Singh J, Singh S, Kathpal TS (2005) Monitoring of butter and ghee (clarified butterfat) for pesticidal contamination from cotton belt of Haryana, India. Environ Monit Assess 105:111–120

Kumari B, Madan VK, Kathpal TS (2006) Monitoring of pesticide residues in fruits. Environ Monit Assess 123:407–441

Kwon HY, Lee HD, Kim JB, Jin YD, Moon BC, Park BC, Son KA, Kwon OK, Hong MK (2009) Reduction of pesticide residues in field sprayed leafy vegetables by washing and boiling. J Food Hyg Saf 24(2):182–187

Lee GM, Jung DI (2009) Processing factors and removal ratios of select pesticides in hot pepper leaves by a successive process of washing, blanching and drying. Food Sci Biotech 18(5): 1076–1082

Lentza-Rizos C, Kokkinaki K (2002) Residues of cypermethrin in field–treated grapes and raisins produced after various treatments. Food Add Contam 19:1162–1168

Liapis KS, Miliadis GE, Aplada-Sarlis P (1995) Dicofol residues on field sprayed apricots and in apricot juice. Bull Environ Contam Toxicol 54:579–583

Lyton WB, Donald LF, Seiber NJ, Thomas RP (1996) Ambient air concentration of pesticide in California. Environ Sci Tech 30:1365–1368

Malik K, Kumari B, Kathpal TS (1998) Persistence and decontamination of α–methrin residues in/on cauliflower at two different temperatures. Pestic Res J 10(2):246–250

Mestres R, Mestres G (1992) Rev Environ Contam Toxicol 124:1–25

Michaels B, Gangar V, Schattenberg H, Blevins M, Ayers T (2003) Effectiveness of cleaning methodologies used for removal of physical, chemical and microbiological residues from produce. Food Serv Technol 3(1):9–15

Miliadis GE, Aplada-Sarlis P, Liapis KS (1995) Disappearance of tetradifon from field sprayed apricots and the apricot juice produced from them. J Agric Food Chem 43:1698–1700

Mukherjee I, Gopal M (2003) Pesticide residues in vegetable. In: Proceedings of the symposium on risk assessment of pesticide residues in water and food. ITRC/ICMR, Lucknow/New Delhi, pp 28–29

Nagayama T (1996) Behavior of residual organophosphorus pesticides in foodstuffs during leaching or cooking. J Agric Food Chem 44:2388–2393

Nath G, Jat RN, Srivastava BP (1975) Effect of washing, cooking and dehydration on the removal of some insecticides from okra (*Abelmoschus esculentus* Moench.). J Food Sci Technol 12:27–130

Oliveira JJDV (1995) The effect of water washing foods for removal of pesticide residues. Coletanea do Instituto de Tecnologia de Alimentos 25(2):115–122
Ong KC, Cash JN, Zabik MJ, Saddiq M, Jones AL (1996) Chlorine and ozone washes for pesticide removal from apples and processed applesauce. Food Chem 55(2):153–160
Ou-Yang XK, Liu SM, Ying M (2004) Study on the mechanism of ozone reaction with parathion–methyl. Saf Environ Eng 11:38–41
Pang GF, Fan CL, Chao YZ (1994a) Packed column gas chromatographic method for the simultaneous determination of 10 pyrethroid insecticide residues in fruits. J Assoc Anal Chem Intern 77(3):738–747
Pang GF, Fan CL, Chao YZ, Zhao TS (1994b) Rapid method for the determination of multiple pyrethroid residues in fruits and vegetables by capillary column gas chromatography. J Chromatogr 667(1–2):348–353
Petersen B, Tomerlin JR, Barraj L (1996) Pesticide degradation: exceptions to the rule. Food Technol 50(5):221–223
PFA (1954) Department of Health, Ministry of Health and Family welfare, Government of India, New Delhi. S.R.451 (E) dt.15-7-2004
Phani Kumar K, Jagdishwar Reddy D, Narasimha Reddy K, Ramesh Babu T, Narendranath VV (2000) Dissipation of cypermethrin residues in chilli. Pestic Res J 12(1):130–132
Pugliese P, Molto JC, Damiani P, Marin R, Cossignani L, Manes J (2004) Gas chromatographic evaluation of pesticide residue contents in nectarines after nontoxic washing treatments. J Chromtogr 1050:185–191
Randhawa MT, Anjum FM, Randhawa MQ, Ahmed A, Farooq U, Abrar M, Randhawa AM (2008) Dissipation of deltamethrin on supervised vegetables and removal of its residues by household processing. J Chem Soc Pak 30(2):227–231
Rani M (2012) Persistence of ready pre–mix formulation (chlorpyriphos+cypermethrin) in/on tomato (*Lycopersicon esculentum* Mill.). Ph.D. thesis CCS Haryana Agricultural University, Hisar
Rasmusssen RR, Poulsen ME, Hansen HCB (2003) Distribution of multiple pesticide residues in apple segments after home processing. Food Add Contam 20:1044–1063
Rouchaud J, Gustin F, Creemers P, Goffings G, Herrgods M (1991) Fate of the fungicide tolylfluanid in pear cold stored in controlled or non controlled atmosphere. Bull Environ Contam Toxicol 46:499–506
Samanta A, Chowdhury A, Somchoudhry AK (2006) Residues of different insecticides in/on brinjal and their effect on *Tricogramma* spp. Pestic Res J 18(1):35–39
Samriti (2010) Persistence behaviour of a ready-mix insecticide chlorpyriphos+cypermethrin in soil and okra (*Abelmoschus esculentus* L.). Ph.D. thesis CCS Haryana Agricultural University, Hisar
Schattenberg HJ, Geno PW, Hsu JP, Fry WG, Parker RP (1996) Effect of household preparation on levels of pesticide residues in produce. J Assoc Anal Chem Intern 76:1447–1453
Shafer TJ, Meyer DA, Crofton KM (2005) Developmental neurotoxicity of pyrethroid insecticides: critical review and future. Environ Health Perspect 113(2):123–136
Sharma KK (2013) Pesticide residues in food commodities in India: an overview. In: Proceedings of the conference on doubling food production in five years, Crop Care Foundation of India, New Delhi, 1–3 Feb 2013.
Singh SP, Kiran Kumar S, Tanwar RS (2004) Dissipation and decontamination of cypermethrin and fluvalinate residues in okra. Pestic Res J 16(2):65–67
Singh SP, Lal MK, Kiran (2007) Residues of λ–cyhaloyhrin in/on okra and the effect of processing. Pestic Res J 19(1):98–100
Srivastava LP, Gupta KP, Raizada RB (2000) Organochlorine pesticide residues in herbal preparation. Bull Environ Contam Toxicol 64:502–507
Srivastava LP, Buuhar R, Raizada RB (2001) Organochlorine pesticide residues in Indian spices. Bull Environ Contam Toxicol 67:856–862

Srivastava LP, Kumar N, Gupta KP, Raizada RB (2006) Status of HCH residues in Indian medicinal plant materials. Bull Environ Contam Toxicol 76:782–790

Street JC (1969) Methods of removal of pesticide residues. Canadian Med Assoc J 100:154–160

Subhani A, Liano M, Huang CY, Xie ZM (2001) Impact of some agronomics practices on paddy field soil health under varied ecological condition: influence of soil moisture. Pedosphere 11:38–48

Sukul P, Handa SK (1986) Persistence of permethrin on chickpea (*Cicer arietinum*). Indian J Agric Sci 56(8):605–608

Taneja A (2005) Monitoring of organochlorine pesticide residues in vegetables from Agra, India–a case study. Environ Monit Assess 110:341–346

Toan VD, Thao VD, Walde J, Schmutz HR, Ha CT (2007) Contamination by selected organochlorine pesticide (OCPs) in surface soil in Hanoi, Vietnam. Bull Environ Contam Toxicol 78:195–200

Torres RL, Torres ILS, Gamaro GD, Fontella FU, Silveira PP, Moreira JSR (2004) Lipid peroxidation and total radical–trapping potential of the lungs of rats submitted to chronic and subchronic stress. Braz J Med Biol Res 37:185–192

Walia S, Boora P, Kumari B (2010) Effect of processing of dislodging of cypermethrin residues on brinjal. Bull Environ Contam Toxicol 84:465–468

Wang L, Yongchao L, Xin J (2008) Analysis of eight organphosphorus pesticide residues in fresh vegetables retailed in agricultural product market of Nanjing, China. Bull Environ Contam Toxicol 81:377–382

WHO/FAO (1989) Guide to Codex recommendations concerning pesticide residues Part 4: Codex Classification of foods and animal feeds CAC/PR4-1986, Rome

Will F, Kruger E (1999) Fungicide residues in strawberry processing. J Agric Food Chem 47:858–861

Yoshida S, Murata H, Imaida M (1992) Distribution of pesticide residues in vegetables and fruits and removal by washing. Nippon Nogeikagaku Kaishi; J Agric Chem Soc Japan 66(6): 1007–1011

Zabik MJ, El-Hadidi MFAJ, Cash N, Zabik ME, Jones AL (2000) Reduction of azinphos–methyl, chlorpyrifos, esfenvalerate and methomyl residues in processed apples. J Agric Food Chem 48:4199–4203

Zafar S, Ahmed A, Ahmed R, Randhawa MA, Gulfraz M, Ahmad A, Siddique F (2012) Chemical residues of some pyrethroid insecticides in eggplant and okra fruits: effect of processing and chemical solutions. J Chem Soc Pak 34(5):1169

Zhang Q, Pehkonen SO (1999) Oxidation of diazinon by aqueous chlorine: kinetics, mechanisms, and product studies. J Agric Food Chem 47:1760–1766

Toxicity Reference Values for Polybrominated Diphenyl Ethers: Risk Assessment for Predatory Birds and Mammals from Two Chinese Lakes

Ruiqing Zhang, Jianyang Guo, Fengchang Wu, Yunsong Mu, John P. Giesy, Hong Chang, Xiaoli Zhao, and Chenglian Feng

Contents

1 Introduction 112
2 Data Collection and Analysis 113
2.1 Selection of Toxicity Data 113
2.2 Approaches to Develop TRVs 114
2.3 Sample Collection and Quantification of PBDEs 115
3 Derivation of TRVs of PBDEs for Aquatic Mammals 115
3.1 Toxicity of DE-71 to Mink (*Mustela vison*) 115
3.2 Recommended TRVs of PBDEs for Aquatic Mammals 119
4 Derivation of TRVs of PBDEs for Aquatic Birds 121
4.1 Toxicity of DE-71 to Birds 121
4.2 Recommended TRVs of PBDEs for Aquatic Birds 125

R. Zhang
College of Environment and Resources, Inner Mongolia University, Huhhot 010021, Inner Mongolia, China

State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

J. Guo
State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China

F. Wu (✉) • Y. Mu • H. Chang • X. Zhao • C. Feng
State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
e-mail: wufengchang@vip.skleg.cn

J.P. Giesy
Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada

Zoology Department and Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229*, Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6_6,

5 Comparison to Ambient Tissue Concentrations 128
6 Ecological Risk Assessment of PBDEs in DCL and TL 130
7 Summary 132
References 133

1 Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFRs), that are widely used in products such as polymer resins, furniture and plastics (Environment Canada 2006). However, PBDEs have been recognized as persistent and bioaccumulative pollutants that can undergo long-range atmospheric transport (De Wit et al. 2006). Based on thresholds for effects of PBDEs on animals under laboratory conditions, PBDEs could accumulate to sufficient concentrations so as to pose a dietary risk to wildlife (Environment Canada 2006; Bureau 2001). Some PBDE congeners can bind to the arylhydrocarbon receptor (AhR), but the binding affinities are very weak or negligible (ATSDR 2004). PBDEs can affect neurobehavioral development, thyroid hormone concentrations in blood plasma, fetal development, reproductive performance, intracellular signaling processes, and also have estrogenic potency (Fernie et al. 2009; Darnerud 2003; ATSDR 2004). Therefore, production and use of some PBDEs technical mixtures have been banned or phased out in most countries (Gao et al. 2009a).

Because PBDEs undergo long-range transport, they have been detected in biota all over the world (Chen and Hale 2010). Their residues have especially appeared in China, where production and use of the PBDEs has occurred in textiles, plastics, and electronics, and where recycling of imported e-waste has distributed them widely in the environment (Wu et al. 2012). Concentrations of PBDEs in Chinese wildlife have generally been greater than those from wildlife from other parts of Asia, comparable to wildlife from Europe, but less than recorded for North American wildlife (Wu et al. 2012). The greatest concentrations of PBDEs in wildlife have been reported at e-waste recycling sites in China, which were greater than those from other regions around the world (Luo et al. 2009). PBDEs are biomagnified in the food web (Wan et al. 2008), where piscivorous species at higher trophic levels in aquatic systems can accumulate concentrations sufficient to be at considerable risk. Unfortunately, no specific criterion has been established against which concentrations of PBDEs in piscivorous species can be assessed for risk.

Most aquatic organisms accumulate greater proportions of the lesser-brominated congeners, with BDE-47 being the dominant congener, followed by BDE-99, BDE-100, and BDE-153 (Meng et al. 2008; Wu et al. 2012; Gao et al. 2009a). Profiles of relative concentrations of individual PBDEs congeners in aquatic organisms were similar to those in the penta-BDE mixture (DE-71), which is a commercial mixture of PBDEs. In DE-71, the percentage content of BDE-47, BDE-99, BDE-100, and BDE-153 are 38%, 49%, 13%, and 5.5%, respectively (La Guardia et al. 2006). DE-71 was extensively used as a flame retardant until its global production and use

was voluntarily discontinued in 2006 (Marteinson et al. 2010). The toxic potency of DE-71 has only been studied in a few top predators, such as mink and American kestrel (Fernie et al. 2009; Zhang et al. 2009).

For this review, the published data that address effects of PBDEs on birds and mammals were assessed. Relevant literature was reviewed and analyzed, and data were selected to determine toxicity reference values (TRVs) for both birds and mammals. These TRVs are based on threshold concentrations of the PBDEs in diet, on allowable daily intake (ADI) values, and on the concentrations in liver of mammals and eggs of birds.

In the present study, Dianchi Lake (DCL) and Tai Lake (TL) are being used as a case assessment of risks posed by PBDEs in fish to wildlife. DCL is the largest plateau lake in Yunnan Province, and TL is the second largest freshwater lake in China (Guo et al. 2012). These lakes are important breeding and wintering areas for migratory waterfowl. However, both areas are polluted with persistent contaminants (Nakata et al. 2005), which pose potential risks of adverse effects to the local fish-eating wildlife. By using TRVs that were estimated to be appropriate from our review, and the actual concentrations at which the PBDEs occur in fish from DCL and TL, a screening-level risk assessment was conducted for fish-eating wildlife.

2 Data Collection and Analysis

2.1 Selection of Toxicity Data

Toxicity threshold values for the PBDEs, expressed as no observed adverse effect levels (NOAEL), or lowest observed adverse effect levels (LOAEL), and based on several endpoints, were derived from toxicity studies that were performed on birds and mammals. These threshold values were determined for wildlife and were based on concentrations of PBDEs in wildlife feed and tissues (liver or eggs). Dietary-based data were converted to average daily intake (ADI) values and were expressed as units of mg ΣPBDE/kg body mass (bm)/day (mg ΣPBDE/kg (bm)/day). ADI values were calculated from body masses and rates of ingestion by the selected surrogate species.

The principles used as the basis for selecting utilizable NOAEL or LOAEL values were as follows (CCME 1998): (1) the study retained suitable control conditions; (2) the study was designed to consider ecologically-relevant endpoints, such as reproduction, embryonic development, offspring or survival of adults (F_0), growth and other responses; (3) a clear dose-response relationship was demonstrated in the study; (4) the form and dosage of test chemical was reported; (5) the tested chemical was administered via the oral, rather than by other routes (i.e., only the oral route is natural for wildlife in the field); (6) studies that included only acute exposures were not accepted, because they provided no data on chronic, or sub-lethal effects on wildlife.

2.2 Approaches to Develop TRVs

Several approaches can be used for deriving TRVs, such as critical study approach (CSA), averaging method, meta-analyses, or species sensitivity distribution approaches. CSA is based on selecting a critical study for deriving recommended TRVs, which involves finding a technically defensible and definitive study from which a toxicity threshold is bracketed by experimental doses (Blankenship et al. 2008; USEPA 2003). CSA is the primary method for assessing risk to wildlife and for deriving criteria for protection of wildlife (CCME 1998; USEPA 1995a, b, 2003, 2005; Sample and Suter 1993). In the present study, CSA was used to derive TRVs for wildlife that is exposed to PBDEs. The TRVs were derived by using uncertainty factors (UFs), applied either to the LOAEL or to the NOAEL that had been derived in the critical studies. UFs were assigned using the guidance given in Technical Support Document (TSD) for Wildlife Criteria for the Great Lakes Water Quality Initiative (GLWQI) (USEPA 1995c), and GLWQI Criteria Documents for the Protection of Wildlife (USEPA 1995b). Three sources of uncertainty were considered in assigning UF values: (1) interspecies differences in toxicological sensitivity (UF_A), (2) sub-chronic to chronic extrapolations (UF_S), and (3) LOAEL to NOAEL extrapolations (UF_L). Application factors for each source of uncertainty were assigned values between 1 and 10, based on available information and professional judgment (USEPA 1995c; Newsted et al. 2005). Because of the limit on this method and the quantity of data, some other sources of uncertainty were not considered in the present study, such as sensitivity differences between adult and embryo stages, and the differences in metabolic rate, caloric content of food, and food assimilation efficiency between laboratory and wild species.

Three other methods have been used to develop TRVs, but none of these were applied to derive a TRV for PBDEs in this study. In applying the average method, it must be assumed that each evaluated study is of equal quality and should be weighted equally to calculate an average of the most representative studies. Meta-analysis is a statistical procedure that integrates the results of several independent studies that have compatible data (Egger et al. 1997). Well-conducted meta-analysis can enhance accuracy when estimating levels at which treatments will produce an effect. However, in this approach it is required that the studies utilized were conducted by using standard toxicity protocols (i.e., methodology, exposure routes, exposure duration, etc.). Because the quality of different studies are generally different, the utility of the meta-analysis approach is limited. In the present study, the meta-analysis approach was not used because the toxicity data utilized different effective endpoints. Species sensitivity analysis (SSA) is another approach that has been used to assess ecological risks and to derive environmental quality criteria (Caldwell et al. 2008; Hall et al. 1998; Solomon et al. 1996; Stephan et al. 1985; Wu and Li 2012; Zhang et al. 2012, 2013). A statistical distribution can be developed with SSA, which represents the variation in sensitivity that species display to a contaminant (Posthuma et al. 2002). Because there were too few studies that have been performed on PBDE toxicity on wildlife, none of these three aforementioned methods were used in the present study.

2.3 Sample Collection and Quantification of PBDEs

Samples of fish muscle (n = 116) from seven species (viz., crucian carp (*Carassius cuvieri*), topmouth culter (*Erythroculter ilishaeformis*), mongolian culter (*Erythroculter mongolicus*), common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), yellow catfish (*Pelteobagrus fulvidraco*), and grass carp (*Ctenopharyngodon idella*)) were collected from TL in 2009. In DCL, samples of fish muscle (n = 76) from five species (viz., crucian carp (*Carassius cuvieri*), sharpbelly (*Hemiculter leucisculus*), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), and bighead carp (*Aristichthys nobilis*)) were collected in 2010. After collection, samples were stored in polyethylene bags, were kept on ice, and then transported immediately to the laboratory, where they were stored at −20 °C until analysis.

PBDE residues in collected fishes were analyzed by using previously described methods (Guo et al. 2007). Briefly, samples were homogenized and Soxhlet extracted with 200 mL of 50% acetone in n-hexane (v/v). Lipid mass was measured for each sample, and then lipids were removed by gel permeation chromatography. The subsequent cleanup and fractionation were performed on multilayer alumina/silica columns. Analysis of PBDEs were performed with a Shimadzu Model 2010 gas chromatograph (GC), coupled with a Model QP 2010 mass spectrometer (MS) (Shimadzu, Japan). MS detections were achieved by using negative chemical ionization set to a selective ion-monitoring mode. Separation for quantification was achieved by using a DB-XLB (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column for the lesser-brominated congeners (BDE-28 to BDE-183) and a CP-Sil 13 CB (12.5 m × 0.25 mm i.d., 0.2 μm film thickness) capillary column for the higher brominated congeners (including BDE 203, 206, 207, 208, and 209). Quantification of PBDEs was based on an internal calibration procedure (internal standard ^{13}C-PCB208).

Because wildlife consume whole fish and concentrations in muscle do not accurately represent whole-body concentrations of the PBDEs, whole-body concentrations were estimated for the samples for which only muscle analyses were conducted. According to the results of previous studies, ratios of concentrations in muscle to those in other tissues were 1:1–1:6 (Guo et al. 2008), and the muscle to whole-body concentration ratio was 1:5 (Xian et al. 2008). In our present assessment, the muscle concentration of PBDEs was multiplied by a conversion factor of 5.0, and was converted to concentration in whole-body fish.

3 Derivation of TRVs of PBDEs for Aquatic Mammals

3.1 Toxicity of DE-71 to Mink (*Mustela vison*)

The toxicity of DE-71 to mink was evaluated with emphasis on the following toxic endpoints: reproductive performance and development (Zhang et al. 2009), immunotoxicity (Martin et al. 2007), neurochemistry (Bull et al. 2007), bioaccumulation

and maternal transfer (Zhang et al. 2008). When reproduction and development were evaluated (Zhang et al. 2009), first-year female mink were exposed to DE-71 at four dietary doses (viz., 0, 0.1, 0.5 or 2.5 mg DE-71/kg (wet mass, wm)). Test females were mated to untreated males, and the 3-week old progeny from each group were maintained on their respective treatment diets with the females until they reached approximately 33 weeks of age. Reproductive parameters investigated in this study included the number of females bred, the number that whelped, litter size, and survivability of kits from birth to 6 weeks of age. In addition, circulating thyroid hormone levels and hepatic enzyme activity were investigated. Dams exposed to 2.5 mg DE-71/kg (wm) failed to whelp, while whelping rates were unaffected at doses of 0.1 or 0.5 mg DE-71/kg (wm) in the diet. Implantation sites were observed in 70% of dams fed 2.5 mg DE-71/kg (wm), and one dam in this group was in the latter stages of fetal resorption at the time of examination. It was concluded that failure to whelp largely resulted from toxicity to the fetus (Zhang et al. 2009).

Growth of kits at 6 weeks of age was not affected by any dose of DE-71, and no effects were observed on organ somatic index (Zhang et al. 2009). For juveniles, there were no statistically significant differences in growth between treated and control mink. Liver mass and the liver somatic index of juveniles was significantly greater for mink fed 0.5 mg DE-71/kg (wm) in the diet. Juveniles were more sensitive to the effects of DE-71 on the liver than were their dams, until a dose of 2.5 mg DE-71/kg (wm) was reached. Although treatment-related reproductive and developmental effects were observed, no clear dose-response relationship was evident for either endpoint monitored in this study (Zhang et al. 2009).

A dose-dependent negative relationship was observed between the concentration of DE-71 in the diet and concentration of total triiodothyroxine (TT3) in blood plasma of all age groups, and the effect was statistically significant at 2.5 mg DE-71/kg (wm) in dams and at 0.5 mg DE-71/kg (wm) in juveniles (Zhang et al. 2009). The effect on thyroid hormone level in mink was more sensitive to DE-71 than was reproduction. Therefore, dietary NOAEL and LOAEL values were respectively based on reduced circulating levels of T3 of the 0.1 and 0.5 mg DE-71/kg (wm) dose levels. Corresponding threshold concentrations in liver of exposed juvenile mink were 1.2 and 6.4 mg DE-71/kg (lipid mass, lm), respectively. Treated dams had similar concentrations of PBDEs in the livers of the corresponding treated juveniles, although concentrations in kits were less than those of dams (Zhang et al. 2009).

Moreover, ethoxyresorufin *O*-deethylase (EROD) activity was also determined in this reproductive toxicity study (Zhang et al. 2009). EROD activity was significantly induced in a dose-dependent manner in the livers of juveniles fed 0.1 and 0.5 mg DE-71/kg (wm) (Zhang et al. 2009). In juvenile mink, EROD activity was positively associated with the liver somatic index. EROD activity was more sensitive endpoint. Therefore, 0.1 mg DE-71/kg (wm) dose was considered to be the dietary LOAEL.

The dietary NOAEL and LOAEL mentioned above needs to be converted to ADI-based concentrations. Daily dietary intake of DE-71 in adult females prior to breeding was estimated to be 0, 0.01, 0.05, and 0.25 mg DE-71/kg body mass (bm)/day for the dietary doses of 0, 0.1, 0.5 and 2.5 mg DE-71/kg (wm), respectively

(Bull et al. 2007). Thus, dietary-based NOAEL and LOAEL were 0.01 and 0.05 mg DE-71/kg bm/day for decreased circulating TT3, and dietary-based LOAEL was 0.01 mg DE-71/kg bm/day for induction of EROD activity.

The immunotoxicity of DE-71 to ranch-grown sub-adult (20 weeks old) mink was studied, in which mink were exposed to one of four doses of 0, 1, 10 or 100 mg DE-71/kg (wm) for 9 weeks (Martin et al. 2007). Because unexpected growth effects were encountered in mink exposed to 100 mg DE-71/kg (wm), this group was switched to a diet containing 5 mg DE-71/kg (wm) for the duration of their exposure. Immune function was monitored by measuring the response of antibodies to T lymphocyte-dependent antigen, levels of keyhole limpet hemocyanin (KLH) conjugated to dinitrophenol (DNP), running phytohemagglutinin (PHA)-induced skin test and measuring hematological parameters. No significant differences were observed in the PHA-induced skin response for any of the groups; however, mink fed 5 or 10 mg DE-71/kg (wm) exhibited significantly greater anti-DNP-KLH antibody production relative to the control mink. A moderate degree of periarteriolar lymphatic sheath development and occasional germinal centers were scattered throughout the spleens from control animals and mink exposed to 1 mg DE-71/kg (wm). Significantly, greater development of germinal center and B-cell hyperplasia were observed in spleens of mink exposed to 10 mg DE-71/kg (wm). The number of germinal centers was positively associated with the liver concentration of ∑PBDEs. The hematocrit in mink from the two greatest exposure groups (5 and 10 mg DE-71/kg, (wm)) was significantly less than that of the control and 1 mg DE-71/kg (wm) mink groups, and was negatively correlated with ∑PBDE levels in the liver. The percentage of neutrophils were significantly greater, while percentages of lymphocytes were significantly less in mink fed 5 or 10 mg of DE-71/kg (wm). EROD activity in liver microsomes was significantly induced in livers of mink fed DE-71. EROD was induced approximately 20-fold greater in liver microsomes from mink fed 1 mg DE-71/kg (wm) than in the controls, 22-fold greater in the 5 mg DE-71/kg (wm) group, and least in the livers of mink fed 10 mg DE-71/kg (wm). For all treatments, there was a significant and positive association between EROD activity and the ∑PBDE concentration in livers. Based on these results, the lowest dose (viz., 1 mg DE-71/kg (wm)) was selected as the dietary LOAEL for effects on immune function, and the estimated daily intake dose was 0.079 mg DE-71/kg bm/day. The corresponding tissue-based LOAEL was 5.067 mg DE-71/kg (lm).

Effects of DE-71 on the nervous system, focusing on cholinergic parameters in the cerebral cortex of ranch mink have been studied (Bull et al. 2007). Adult female mink were exposed via the diet during and exposure extended to *in utero*, lactational, and subsequent dietary exposure of the offspring. Dietary doses to adult females were 0, 0.1, 0.5 or 2.5 mg DE-71/kg (wm). No significant effects of exposure to DE-71 were observed on the following: muscarinic acetylcholine receptor (mAChR) or nicotinic acetylcholine receptor (nAChR) binding activity, cholinesterase (ChE) activity, or acetylcholine (ACh) concentration in the cerebral cortex of adult females, 6-week-old kits, or 27-week-old juveniles. The ChE activity in blood plasma of adult females fed 2.5 mg DE-71/kg (wm) was threefold greater than for all other treatments. However, because ChE in blood plasma is synthesized in liver,

the increased ChE activity in blood plasma may have resulted from the effects of DE-71 on liver function, rather than on neurochemistry, a conclusion that is supported by other evidence (Bull et al. 2007). A NOAEL of 2.5 mg DE-71/kg (wm) was inferred for neurotoxicity in mink. The corresponding concentration of PBDEs in cerebral cortex of adult female mink was 88 ng PBDEs/g (wm).

Several measurement endpoints for effects of DE-71 on mink were examined in the studies reviewed above, and we needed to identify the most appropriate endpoint among these for deriving the TRV. EROD activity in liver is a common biomarker of exposure to environmental inducers of CYP1A1, such as those for which effects are modulated through the AhR. However, the PBDEs have been confirmed to be non-dioxin-like compounds and are incapable of inducing EROD activity (Sanders et al. 2005; Peters et al. 2004; Talsness 2008). Dioxin-like toxicity observed in studies of commercial mixtures of PBDEs under laboratory conditions were attributed to potential contaminants such as brominated biphenyls, dioxins or dibenzofurans in mixtures of PBDEs (Brown et al. 2004; Sanders et al. 2005). Effects of the polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) are similar to those of their chlorinated analogues (polychlorinated dibenzo-*p*-dioxins and dibenzofurans, PCDD/Fs) (Birnbaum et al. 2003; Behnisch et al. 2003). Thus, EROD activity in liver cannot be used as an appropriate critical effect endpoint for exposure to the PBDEs. Reduced concentrations of circulating TT3 was the second most sensitive endpoint for mink exposed to the PBDEs (Table 1). Similar findings were also observed in studies, in which thyroid hormone concentrations were correlated with the PBDE levels of at least some congeners (Leijs et al. 2012; Tomy et al. 2004; Hallgren and Darnerud 2002). However, AhR-active compounds, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have also been shown to influence thyroid hormone metabolism (Leijs et al. 2012; Boas et al. 2006). Before toxicity values, based on thyroid effects, can be used for deriving TRVs, it is necessary to eliminate effects from any AhR-active contaminants or impurities in commercial mixtures of PBDEs. Seo et al. (1995) exposed weanling rats to TCDD by gestational and lactational pathways, and reported that changes in thyroid hormone status at weaning are not among the more sensitive effects of perinatal exposure to coplanar PCB congeners or TCDD. Similar results were observed in a toxicity study of ranch mink exposed to dietary TCDD (Martin et al. 2006); no effect was observed in the TT3 and free triiodothyronime (T3) concentrations in blood plasma of the kits. Results of mink acute toxicity studies indicated that the concentrations of bound and free T3 and thyroxine (T4) were lower in mink exposed to 2.5 μg TCDD/kg bm than those in controls (Hochstein et al. 1988). In another study, T4 concentrations in mink fed dietary 0.01 or 0.1 ng TCDD/g (and survived 125 days) were not significantly different from those of controls (Hochstein et al. 1998). Based on the feed consumption and body weight of mink after 1 week exposure (Hochstein et al. 1998), the two doses, 0.01 and 0.1 ng TCDD/g feed, can be converted to values of 0.534 and 4.91 ng TCDD/kg bm/day, respectively. To preclude immunological effects of brominated dioxins and furans as co-contaminants in diet, Martin et al. (2007) analyzed a sample of the DE-71 technical material, and none of the dioxin and furan isomers were detected (detection limits of most congeners, <0.03 ppb). In addition, Sanders et al. (2005) analyzed concentrations of PBDDs and PBDFs in commercial DE-71 and

Table 1 NOAEL and LOAEL values for DE-71 in laboratory studies with mink

Effective endpoint	PBDEs concentration	Reference
Decreased TT3 concentration in plasma		
Dietary NOAEL	0.1 mg/kg (wm)	Zhang et al. (2009)
Dietary LOAEL	0.5 mg/kg (wm)	
Daily dose NOAEL	0.01 mg/kg (bm)/day	
Daily dose LOAEL	0.05 mg/kg (bm)/day	
Liver NOAEL	1.2 mg/kg (lm)	
Liver LOAEL	6.4 mg/kg (lm)	
EROD activity in liver		
Dietary LOAEL	0.1 mg/kg (wm)	Zhang et al. (2009)
Daily dose LOAEL	0.01 mg/kg (bm)/day	
Liver LOAEL	1.2 mg/kg (lm)	
Immunotoxicity effect		
Dietary LOAEL	1 mg/kg (wm)	Martin et al. (2007)
Daily dose LOAEL	0.079 mg/kg (bm)/day	
Liver LOAEL	5.067 mg/kg (lm)	
Neurotoxicity parameters in cerebral cortex		
Dietary NOAEL	2.5 mg/kg (wm)	Bull et al. (2007)
Daily dose NOAEL	0.25 mg/kg (bm)/day	
Cerebral cortex NOAEL	88 ng/g (wm)	

NOAEL no observed adverse effects level, *LOAEL* lowest observed adverse effects level, *bm* body mass, *PBDEs* polybrominated diphenyl ethers, *TT3* total triiodothyroxine, *wm* wet mass, *lm* lipid mass

determined the total concentrations of them to be 72.13 ng/g. Hanari et al. (2006) also measured PBDF levels in DE-71, and found 257 ng/g, while PBDD congeners were not detected (<100–200 ng/g). Assuming the total concentration of PBDD/Fs in DE-71 to be 257 ng/g, the concentration of PBDD/Fs in feed for 0.1 and 0.5 mg DE-71/kg (wm) treatments would be 7.213 and 36.065 pg PBDD/Fs/kg feed, respectively. The corresponding average daily dietary intake of PBDD/Fs would be 0.7213 and 3.6065 pg PBDD/Fs/kg (bm)/day. These doses of PBDD/Fs in the feed were several orders of magnitude less than the toxicity threshold values reported above (Hochstein et al. 1998). Thus, it can be assumed that the observed effects on thyroid hormone in mink (Zhang et al. 2009) were attributed primarily to the effects of PBDE exposure. Thyroid hormones in mammals play critical roles in reproductive physiology, cellular differentiation, growth and metabolic regulation. It is appropriate to derive TRVs for PBDEs by using the toxicity threshold values that are based on effects on thyroid hormone.

3.2 Recommended TRVs of PBDEs for Aquatic Mammals

Effects of PBDE exposure on mink from several toxicity studies were compared in Table 1. With a reasonable assumption that the observed effects on thyroid hormone in mink were attributed primarily to PBDEs, a NOAEL of 0.01 mg DE-71/kg (bm)/day,

based on effects of thyroid hormone in mink (Zhang et al. 2009), was used to derive the dietary-based TRV. According to the TSD for Wildlife Criteria for the GLWQI (USEPA 1995c) and GLWQI Criteria Documents for the Protection of Wildlife (USEPA 1995b), uncertainty factors from three sources were applied to derive the TRVs for effects of PBDEs on mink. The first source of uncertainty was associated with interspecies extrapolation. Mink were the only piscivorous mammals for which PBDE effects were reported, which prevented determining interspecies sensitivity to PBDEs. However, as fish-eating wildlife species, mink are capable of great trophic magnification, thereby rendering themselves more susceptible to accumulating persistent organic pollutants (Heaton et al. 1995; Tillitt et al. 1995). Mink is often used as a representative wildlife criteria species, and as a relevant animal model for environmental risk assessment of aquatic wildlife (Blankenship et al. 2008; USEPA 1995c; Giesy and Kannan 1998; Millsap et al. 2004). Thus, using mink as a surrogate species to derive TRV values is reasonable for protecting other aquatic mammals against potentially hazardous PBDEs exposure. In addition, comparing the threshold doses given for the present study to results from rodent studies shows that DE-71 caused effects on reproduction and thyroid in mink at concentrations lower than those that caused similar effects in rodents (Zhang et al. 2009; Talsness et al. 2008; Zhou et al. 2002). This suggests that mink are more sensitive than rodents. Therefore, the UF_A was set to 1.0. The second source of uncertainty was associated with LOAEL to NOAEL extrapolation. Because the NOAEL was identified in the critical study, a UF_L greater than 1.0 is not necessary. The third source of uncertainty was associated with extrapolation from results of sub-chronic exposure to chronic exposure. In the study of effects on reproduction and development of mink (Zhang et al. 2009), adult females were exposed to DE-71 following dietary exposure through pregnancy and nursing, and the offspring were then exposed to DE-71 following *in utero*, lactational, and dietary exposure until 33 weeks of age. This exposure scenario is environmentally relevant, and covers the sensitive life stages of mink. The accumulation, disposition and metabolism behavior of DE-71 in mink (Zhang et al. 2008) indicated that the study of toxicity to mink could be accepted as a chronic exposure study. Thus, an UF of >1.0 was deemed to be unnecessary. An overall uncertainty factor of 1.0 was assigned to account for data gaps in deriving TRVs, by using the results of thyroid effects in mink (Zhang et al. 2009). The TRVs, based on dietary and ADI PBDEs concentrations, were 0.1 mg DE-71/kg feed (wm) and 0.01 mg DE-71/kg (bm)/day, respectively.

Threshold values from PBDE concentrations in liver, were used to derive a tissue-based TRV. Using the cumulative ingested dose of a chemical from consuming contaminated food (e.g., tissues) provides a better assessment of exposure, since it accounts for bioaccumulation and bioavailability. Moreover, monitoring studies usually detect concentrations of contaminants in specific body tissue of animals. Therefore, TRVs that are based on concentrations of toxicants in tissues of wildlife are effective for protecting wildlife from the hazards of pollutants exposure. On the basis of results in thyroid hormone of mink (Zhang et al. 2009) and the overall UF value presented above, a tissue-based TRV for liver PBDEs concentration was derived to be 1.2 mg/kg (lm).

4 Derivation of TRVs of PBDEs for Aquatic Birds

4.1 Toxicity of DE-71 to Birds

American Kestrel (*Falco sparverius*)

A series of toxicity studies were conducted in which the American kestrel (*Falco sparverius*) was fed DE-71, after which reproduction and development endpoints were measured (Fernie et al. 2008, 2009; Marteinson et al. 2010, 2011; Sullivan et al. 2010). Adult American kestrels were exposed through their diet to control, 0.3, or 1.6 mg DE-71/kg (wm) for 75 days each year (Fernie et al. 2008). Exposure began 21 days prior to pairing and continued through courtship, egg laying, and incubation periods in each year of 2005 and 2006, or until the first chick hatched. Kestrel nestlings were exposed only *in ovo* by direct maternal transfer to DE-71. Mean concentrations of ΣPBDE in eggs of kestrel were 3.01, 288.60, and 1,130.59 ng/g (wm), respectively, for the control, lesser and greater doses, and seven major congeners (BDE-28, -47, -100, -99, -154, -153, and -183) accounted for 69%, 96%, and 94% of ΣPBDE, respectively (Fernie et al. 2008).

During the experiment, the reproductive behavior of pairs (e.g., mate choice, pair-bonding, copulation and food transfer) were assessed (Fernie et al. 2008). Compared to controls, timing, duration and frequency of courtship behavior of kestrels were altered by DE-71 in both exposure groups. For the same exposure scenarios, reproductive success and egg quality of kestrels exposed to DE-71 were evaluated (Fernie et al. 2009). Compared to controls, egg laying was significantly delayed for kestrels from the two exposure groups, and was increasingly delayed with increasing concentrations of BDE-153, BDE-154, BDE-28 and BDE-17. Eggs laid by kestrels from the greater exposure group were significantly smaller and had lower mass than did those laid by kestrels from controls and the lower exposure groups. Moreover, adverse effects on eggshell qualities occurred that were attributed to DE-71 exposure (Fernie et al. 2009). Compared to controls, the eggshells from the greater exposure were significantly thinner, and those from the lesser exposure had significantly lower mass. Eggshell thickness was significantly and negatively associated with the measured PBDE concentration. Poorer fertility, hatching, and fledging success were observed for pairs of kestrels fed the greater dose compared to control pairs, and fledging success was modestly reduced as concentrations of BDE-153 increased.

Retinol is involved in the reproduction and development of birds. Thus, concentrations of retinol in blood plasma were measured in adult American kestrels and nestlings exposed to DE-71 (Sullivan et al. 2010). Concentrations of retinol in plasma of adult females fed the lesser dose were significantly less than those in the controls, and were negatively correlated with *in ovo* BDE-153 concentrations. No significant effect on the plasma retinol level of the adult males was observed, but the retinol concentrations of the males exposed to greater dose were negatively correlated with the ΣPBDE and BDE-100 concentrations. For nestlings exposed to lesser

in ovo concentrations of DE-71, concentrations of retinol in blood plasma were significantly less than those from control nestlings, but were not correlated with the *in ovo* PBDE concentrations.

In a further study, the reproductive success and behavior of male American kestrels exposed to DE-71 as embryos were assessed (Marteinson et al. 2010). The F1 progeny of the F0 kestrels exposed via the diet (Fernie et al. 2008) were never exposed directly via the diet, but only during the 28-days embryonic period via direct maternal transfer to the egg. At 1 year of age, male kestrels that had been exposed *in ovo* to three exposures were paired with unexposed females. Throughout courtship, there were fewer copulations by all *in ovo* exposed males and fewer male-calls made by greater-exposure males, and which were negatively associated with the males' embryonic exposure to concentrations of ΣPBDEs as well as individual congeners. Compared to controls, the greater-exposure males spent less time in their nest boxes, and the amount of time decreased significantly with increasing embryonic exposure to BDE-99 and -100. Moreover, 43% of female kestrels from greater -exposure pairs failed to lay eggs, while all other pairs laid complete clutches. The female kestrels paired with males exposed *in ovo* to either of two doses of DE-71 laid significantly smaller clutches and smaller eggs, and these eggs were less fertile, compared to controls. The fertility and number of eggs in clutches were both strongly and negatively correlated with *in ovo* exposure concentrations of ΣPBDEs, as was the individual congeners. All of these reproductive parameters were influenced by the frequencies of courtship behaviors from males.

When F1 progeny of dietary exposed F0 individuals (Fernie et al. 2008) were used, effects of embryonic exposure to DE-71 on the male kestrel reproductive tract and associated endocrinology were assessed (Marteinson et al. 2011). At 1 year of age, the *in ovo*-exposed male kestrels in the three exposure groups were paired with unexposed females and allowed to complete one reproductive cycle (Marteinson et al. 2011). One year later, males in the unpaired state were euthanized at 2 years of age during the fertile period, and concentrations of thyroid hormone and testosterone in blood plasma, sperm numbers and testis mass and histology were assessed. Lower testosterone concentrations appeared in blood plasma of males exposed to the greater concentration of DE-71 at the time the first egg was laid by females. However, there were no alterations observed for thyroid function in these adult kestrels during the breeding period, and concentrations of testosterone and thyroid hormone were not associated with *in ovo* exposure concentration of PBDEs. Compared to controls, numbers of sperm on the perivitelline layer of the first egg were greater for the two treatment groups. Males fed the greater dose had a greater gonadosomatic index and a heavier right testis than controls. Testis mass was positively associated with *in ovo* concentrations of ΣPBDEs and congeners, BDE-100, -47, -85, and -183. Males exposed to the greater concentration had more seminiferous tubules containing lumen than did controls, and in proportion to the total number of tubules, males exposed to the lesser concentration had more tubules within the lumen than did controls. Mean percent of tubules containing final spermatids were 43% for males exposed to the greater dose, while they were 53% and 59% in controls and those exposed to the lesser dose, respectively. The proportion of tubules containing

final spermatids positively correlated with *in ovo* exposure to BDE-47, -85, -49, and -28 (Marteinson et al. 2011). These changes in reproductive physiology of males American kestrels might reduce the reproductive success of these birds.

Based on reproduction, a LOAEL of 0.3 mg/kg (wm), as DE-71 was inferred. No food consumption or body mass data were reported for the adult birds in these dietary exposure studies. By assuming a body mass of 0.119 kg and a food ingestion rate of 0.022 kg/day (geometric means) (Dunning 1993; Yáñez et al. 1980; USEPA 1993), a dietary-based LOAEL value of 0.055 mg DE-71/kg (bm)/day was calculated. The corresponding concentration of ΣPBDEs in kestrel eggs from the lesser-exposure group was considered to be the tissue-based LOAEL in eggs; this LOAEL value was 288.6 ng ΣPBDEs/g (wm).

Because DE-71 is a commercial PBDE mixture with the presence of some impurities (Hanari et al. 2006), it is important to preclude effects caused by co-contaminants in the toxicity studies to ensure that the TRV accurately and reasonably reflects only the effect of PBDEs on animals. It should be noted that none of the 11 brominated dioxins and furans was detected in the DE-71 mixture that was used in these toxicity studies (Fernie et al. 2008). Thus, the effect of dioxin-like compounds as impurities in the DE-71 mixture was precluded for these studies. Unfortunately, total-α-hexabromocyclododecane (HBCD) was detected in the eggs of kestrels exposed to DE-71 by diet, and its presence in the diet of the birds occurred unintentionally (Fernie et al. 2009; Sullivan et al. 2010). Concentrations of HBCD in kestrel eggs from the controls and two treatment groups of 0.3 or 1.6 mg DE-71/kg (wm) were 0.002, 3.27, and 15.61 ng/g (wm), respectively (Fernie et al. 2009). In those toxicity studies reviewed above, thickness of eggshells of kestrels was inversely proportional to dose of HBCD in the diet (Fernie et al. 2009). Concentrations of retinol in blood plasma of adult males (Sullivan et al. 2010), and frequency of courtship behaviors, clutch size and fertility in kestrels exposed *in ovo* by direct maternal transfer (Marteinson et al. 2010) were negatively correlated with *in ovo* concentrations of HBCD. However, in the study with only HBCD exposure, eggshell thickness, egg fertility and reproductive success (Fernie et al. 2011), and copulation frequency (Marteinson et al. 2012) were not affected and not correlated with *in ovo* concentrations of HBCD. The *in ovo* concentration of HBCD in kestrels exposed to the lesser concentration of DE-71 in previous studies was approximately 50 times less than the HBCD concentration (163.5 ng/g (wm)) in the study, in which eggs were exposed to HBCD only. Thus, in the present assessment, the effects from HBCD can be precluded, and it is assumed that the reproductive effects in kestrels were attributed primarily to PBDEs.

Egg Injection Studies with Birds

There are several possible routes of exposure when performing toxicity studies. These include oral, subcutaneous injection, dermal, inhalation, and egg injection. When screening toxicity data for deriving wildlife TRVs, results from oral-administration exposures are preferred (Stanton et al. 2010; USEPA 2005).

For the study of embryo development and nestling growth, the common method of exposure is via the diet of adult females with maternal transfer to eggs. However, dietary exposure of adult female birds may induce changes in their food intake and incubation behaviors, and further affect growth of both embryo and nestling unintentionally. Direct injection of a contaminant into an egg avoids many of these issues and allows one to directly assess the contaminant's effect on embryo development (Winter et al. 2013). When there is sufficient information to show that the maternally transferred concentration elicits comparable toxicity to that from egg injection, then the results from egg injection studies can be used to derive TRVs (USEPA 2005).

The effects of PBDE injected into eggs were tested in several toxicity studies. A series of egg injection studies were conducted to evaluate effects on growth (Fernie et al. 2006), immunomodulation (Fernie et al. 2005a), thyroid, retinol and oxidative stress (Fernie et al. 2005b) in American kestrels. In these studies, the chemical was injected into the air cell of eggs with safflower oil (control group) or BDE-47, -99, -100, and -153 dissolved in safflower oil (18.7 μg ΣPBDEs/egg (wm), or 1.5 μg ΣPBDEs/g egg (wm)) after the eggs had been incubated for 19 days. Nestlings were dosed daily via oral gavage with the same mixture of PBDEs (15.6 ng/g bm/day) for 29 days. The relative concentrations of BDE congeners measured in PBDE mixtures were as follows: 56.4% (BDE-47); 27.2% (BDE-99); 24.8% (BDE-100); and 0.6% (BDE-153); these proportions approximated current concentrations in Great Lakes herring gulls. Nestlings exposed to PBDEs gained weight more quickly and ate more food than did the controls, and food consumption was positively associated with concentrations of ΣPBDEs in their bodies, especially for BDE-100 (Fernie et al. 2006). A 14% greater response to PHA was observed that might be biologically important in PBDE-exposed birds (Fernie et al. 2005a). A lower antibody-mediated response was observed and was positively associated with concentrations of BDE-183. There were also histological changes in spleen, bursa and thymus, and negative associations between the spleen somatic index and ΣPBDEs, and the bursa somatic index and BDE-47 (Fernie et al. 2005a). Concentrations of plasma T4, plasma retinol, and hepatic retinol and retinyl palmitate concentrations were less in birds exposed to the PBDEs, and the first three parameters were negatively correlated with concentrations of the following individual PBDE congeners: BDE-47, BDE-99, and BDE-100 (Fernie et al. 2005b). Hepatic oxidative stress was also induced in PBDEs-exposed birds. No difference was observed in T3 concentrations between groups. However, only one dose among these three studies was the same, and a threshold of toxicity could not be obtained for derivation of TRVs.

Chickens (*Gallus gallus*), mallards (*Anas platyrhynchos*) and American kestrels were exposed to DE-71 (0.01, 0.1, 1, 10, or 20 μg DE-71/g egg (wm)) by air cell injection, and embryonic survival, piping, hatching success, and sublethal biochemical, endocrine, and histological endpoints were measured (McKernan et al. 2009). Dose-dependent decreases in piping and hatching success of kestrels were observed at doses of 1, 10, and 20 μg DE-71/g egg (wm), with significant differences occurring at 10 and 20 μg DE-71/g egg (wm), while there were no effects on survival,

Table 2 NOAEL and LOAEL values for DE-71 in laboratory studies with American kestrels

Effective endpoint	PBDEs concentration	Reference
Reproductive effects in multi-generations		
Dietary LOAEL	0.3 mg/kg (wm)	Fernie et al. (2008, 2009), Marteinson et al. (2010, 2011), Sullivan et al. (2010)
Daily dose LOAEL	0.055 mg DE-71/kg (bm)/day	
Egg LOAEL	288.6 ng ΣPBDEs/g (wm)	
Piping and hatching success		
Egg-injection LOAEL	1,800 ng ΣPBDEs/g (wm)	McKernan et al. (2009)
Egg-injection NOAEL	180 ng ΣPBDEs/g (wm)	

piping, and hatching success of chickens or mallards at any PBDEs dose. American kestrels were more sensitive to effects of DE-71 on piping and hatching success than were mallards and chickens. Thus, tissue-based NOAEL and LOAEL in egg were 1 and 10 μg DE-71/g egg (wm), respectively.

However, not all the PBDEs in these injected doses were responsible for the effects on piping and hatching success (McKernan et al. 2009). Uptake tests with PBDEs into kestrel eggs indicated that 18% of the administered dose was absorbed into kestrel embryos (McKernan et al. 2009, 2010). Based on this information, the tissue-based NOAEL and LOAEL were converted to 0.18 and 1.8 μg DE-71/g egg (wm), respectively. The NOAEL of 0.18 μg DE-71/g egg (wm), based on an egg injection study, is comparable to the LOAEL value of 0.29 μg ΣPBDEs/g (wm) in embryonic exposure of PBDEs transferred from mother birds. The hatching success of kestrel eggs dosed with 0.18 μg DE-71/g via injection is also comparable to that of lesser doses in eggs in a maternally-transferred exposure study (i.e., 60% vs. 56%) (Fernie et al. 2009). In McKernan et al. (2009) study, EROD activity was also assayed, and the EROD activity was induced only in chicken hatchlings at doses of 1, 10, or 20 μg DE-71/g egg (wm). However, there wasn't sufficient information from maternally-transferred exposure study to identify this results. Thus, the egg injection NOAEL value from the kestrel study, based on piping and hatching success rather than the EROD activity, was used as the basis for deriving the tissue-based TRV for PBDE.

4.2 Recommended TRVs of PBDEs for Aquatic Birds

Several studies, in which DE-71 produced effects on birds, were systematically reviewed and analyzed (Table 2). The LOAEL, based on measurement endpoints involving reproduction, was 0.3 mg/kg (wm) (0.055 mg DE-71/kg (bm)/day) from a series of studies on American kestrels (Fernie et al. 2008, 2009; Marteinson et al. 2010, 2011; Sullivan et al. 2010), and was selected as the basis for calculating a dietary-based avian TRV. Three uncertainty factors were considered for use with this LOAEL: interspecies differences in sensitivity (UF_A), subchronic to chronic extrapolations (UF_S), and LOAEL to NOAEL extrapolations (UF_L). The American kestrel is a terrestrial ecosystem predator that has been used as a model species for

investigating the effects of methylmercury (Albers et al. 2007; Fallacara et al. 2011) and several organic pollutants on various measurement endpoints (Marteinson 2011).

According to results of an egg injection study, American kestrel was more sensitive to PBDEs injected into eggs than were chicken or mallard (McKernan et al. 2009). Therefore, a UF_A of 1.0 was selected. In the series of studies on kestrels, multi-generational effects of reproduction were evaluated, and the effects from maternal transferred exposure were also investigated. Duration of exposure of adult birds was 75 days in each year of 2005 and 2006, and the period throughout pairing, courtship, egg laying, and incubation, was a relative sensitive life stage. An uncertainty factor of 1.0 was assigned to account for subchronic to chronic extrapolations. Because a LOAEL value was identified rather than a NOAEL value was identified from these studies, a UF_L of greater than 1.0 was applied. According to the results of these studies, several reproductive parameters were affected, but compared to controls the decrease of eggshell thickness in the lesser-exposure kestrels was only approximately 1%, which was still far from the magnitude of eggshell thinning that would render kestrels incapable of maintaining a stable population (Fernie et al. 2009). The reduced hatching success in lesser-exposed kestrels was approximately 13% of that in controls. Thus, a UF_L of 3.0 was a reasonable value for the extrapolation. An overall UF of 3.0 was assigned to account for data gaps in deriving TRVs by using the results in kestrels. Thus, TRVs, based on diet and ADI, were 0.1 mg DE-71/kg (wm) and 0.018 mg DE-71/kg (bm)/day, respectively.

Using this overall UF of 3.0, the tissue-based LOAEL of 288.6 ng ΣPBDEs/g (wm) in eggs was converted to 96.2 ng ΣPBDEs/g (wm) as the corresponding dietary exposure NOAEL value in eggs. This dietary exposure NOAEL of 96.2 ng ΣPBDEs/g (wm), and the egg-injection NOAEL of 180 ng ΣPBDEs/g (wm) from the McKernan et al. study (2009) (Table 2) were both used to derive the tissue-based TRV in the egg for aquatic species in the present study, and the tissue-based TRV in the egg were estimated as the geometric mean of these two values. Therefore, the tissue-based TRV in the egg is 131.6 ng ΣPBDEs/g (wm). The lipid content in egg of American kestrel was not reported in the toxicity studies reviewed above. The concentrations of PBDEs based on wet weight and lipid weight of egg were reported in McKernan et al. study (2009), and a lipid content of 5.6% was estimated for kestrel egg. On this lipid weight basis, the tissue-based TRV was converted to 2.35 μg ΣPBDEs/g (lm).

To assess the possible risk posed by PBDEs to fish-eating wildlife in China, the dietary- and tissue-based TRVs for DE-71 were derived for protecting birds and mammals that might eat fish (Table 3). Information on the toxicity of DE-71 to mink and American kestrel was available. Therefore, these two species were used as the surrogates for other birds and mammals, respectively. These TRVs provide points of reference for concentrations of PBDEs measured in fish and fish-eating wildlife, and can be used in the tissue residue approach to ecological risk assessment. Threshold values, based on concentrations of PBDEs in diets were compared between mink and American kestrel (Fig. 1). The LOAEL, based on reproduction of American kestrel was comparable to the LOAEL value that was based on thyroid effects in mink. The ADI-based TRV, from effects on mink thyroid, is one fold less

Table 3 PBDE toxicity reference values (TRVs) for aquatic mammals and birds based on dietary, ADI, liver, and egg toxic doses

	Mammal			Bird		
	NOAEL	UF	TRV	LOAEL	UF	TRV
Dietary, mg DE-71/kg (wm)	0.1	1	0.1	0.3	3	0.1
ADI, mg DE-71/kg (bm)/day	0.01	1	0.01	0.055	3	0.018
Tissue, μg ΣPBDEs/g (lm)[a]	1.2	1	1.2	5.15[b]	3	2.35[c]

ADI average daily intake, *UF* uncertainty factor

[a] For mammals the tissue is liver, and the tissue for birds is egg

[b] This value was derived by using the LOAEL of 288.6 ng ΣPBDEs/g (wm) in eggs and the estimated lipid weight of 5.6%

[c] This TRV was estimated as the geometric mean of the egg-injection NOAEL of 180 ng ΣPBDEs/g (wm) and the dietary exposure NOAEL of 96.2 ng ΣPBDEs/g (wm), and the NOAEL of 96.2 ng ΣPBDEs/g (wm) was derived by LOAEL of 288.6 ng ΣPBDEs/g (wm) in eggs and the UF of 3.0

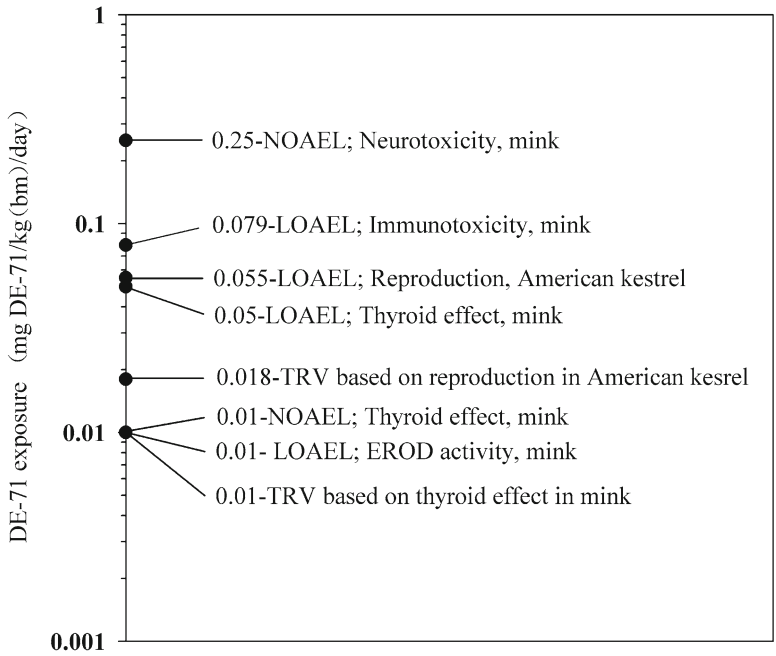


Fig. 1 Toxicity thresholds for wildlife exposed to DE-71 via diet; expressed as average daily intake (ADI). *NOAEL* no observed adverse effects level, *LOAEL* lowest observed adverse effects level, *bm* body mass. See Tables 1, 2, and 3 for data set

than the TRV that was based on reproduction in the American kestrel, which incorporated a uncertainty factor of 3.0. Therefore, the dietary-based TRV of 0.01 mg/kg (bm)/day (or 0.1 mg DE-71/kg (wm)) is appropriate to protect fish-eating wildlife.

5 Comparison to Ambient Tissue Concentrations

Concentrations of PBDEs in livers of aquatic mammals found dead were collected to examine the reasonableness of the threshold values derived in the present study. The geometric mean concentration of total PBDEs in livers of Eurasian otter found dead in England and Wales in 2010 was 50.56 ng/g (wm) with a range of 3.001–717.8 ng/g (wm), and the BDE-47, -153, -100, and -99 were the predominant congeners (Walker et al. 2012). Assuming a lipid content of 5% in livers of Eurasian otter (Kannan et al. 2000), the concentration of total PBDEs in livers of Eurasian otter would be 1,011 ng/g (lm) with a range of 60.02–14,356 ng/g (lm). The mean concentration of total PBDEs, in livers of adult female sea otters found freshly dead or dying between 1992 and 2002 along the central California coast, was 2,200 ng/g (lm) with a range of 10–26,800 ng/g (lm) (Kannan et al. 2007). The mean concentration of total PBDEs in liver of harbor porpoises stranded on the North Sea coast of Belgium between 1997 and 2000 was 2,290 ng/g (lm), with a range of 410–5,810 ng/g (lm) (Covaci et al. 2002). All concentrations of PBDEs in livers of mammals mentioned above were comparable with, or greater than the tissue-based TRV of 1,200 ng/g (lm) that was derived in the present study. The toxicological evidence of exposure to PBDEs in these mammals were uncertain, and links between liver PBDE concentrations and health effects in these mammals can't be established. However, the results indicated that the PBDEs in liver still may potentially affect health, which supports this tissue-based TRV.

Concentrations of PBDEs in wild aquatic biota, including invertebrates and fishes, were compiled and compared to the dietary-based TRV values to evaluate the current risk in the Chinese environment. Concentrations of total PBDEs in wild aquatic species from the Yangzi River (Gao et al. 2009b), Baiyangdian Lake (Hu et al. 2010), Bohai Bay (Wan et al. 2008), and a reservoir in Longtang Town (Wu et al. 2008) were available. The geometric mean of concentrations of PBDEs in several species was calculated and compared for each region. Concentrations of PBDEs in aquatic species from the first three regions were less than the dietary-based TRV of 100 ng DE-71/g (wm), while the geometric mean of concentration of PBDEs in dietary items from the reservoir in Longtang Town was much greater than this TRV value (Fig. 2). These results are consistent with the known contamination status of these water bodies. The reservoir in Longtang Town is known to be polluted by crude e-waste recycling activities (Wu et al. 2008). Thus, PBDEs in these Longtang Town reservoir aquatic species may be sufficiently concentrated to pose an adverse risk to the wild fish-eating birds and mammals.

Concentrations of PBDE in eggs from wild aquatic birds in China were compared to the tissue-based TRVs for egg (Fig. 3). All concentrations of total PBDEs in bird eggs collected from Hong Kong, Xiamen, Quanzhou (Lam et al. 2007), and the Yellow River Delta (Gao et al. 2009a) were lower than the tissue-based TRV of 2.35 μg ΣPBDEs/g (lm). Concentrations of PBDEs in bird eggs might not have affected reproductive function in birds collected from these regions. This comparison suggests that the TRV values reported in this study can serve as indicators for screening-level risk assessment of piscivorous species in other Chinese aquatic systems.

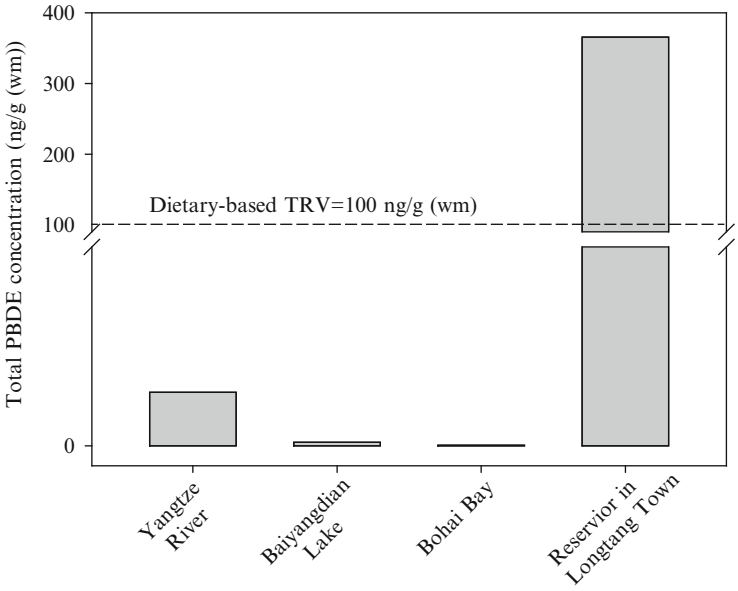


Fig. 2 Comparison of the dietary-based TRV to reported concentrations of PBDEs in wild aquatic species from different regions in China. *TRV* toxicity reference value, *wm* wet mass. Yangzi River (Gao et al. 2009b), Baiyangdian Lake (Hu et al. 2010), Bohai Bay (Wan et al. 2008), and Reservoir in Longtang Town (Wu et al. 2008)

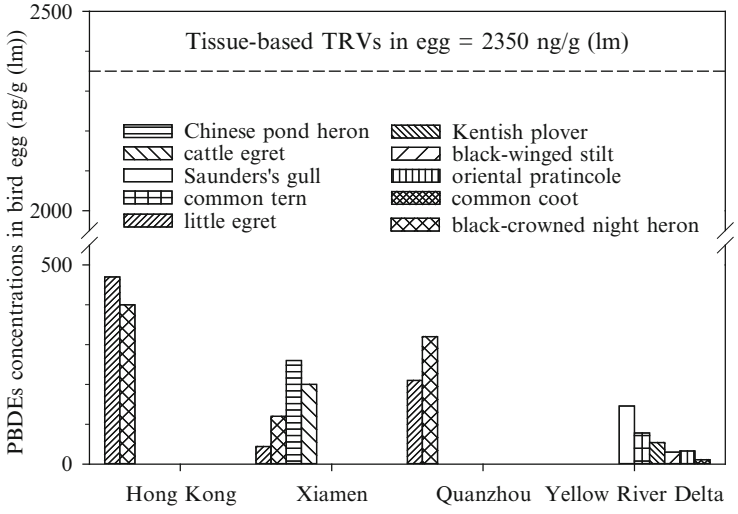


Fig. 3 Comparison of the tissue-based TRV in eggs to reported concentrations of PBDEs in wild aquatic avian species from different regions in China. *TRV* toxicity reference value, *wm* wet mass. Hong Kong, Xiamen, Quanzhou (Lam et al. 2007), and Yellow River Delta (Gao et al. 2009a)

6 Ecological Risk Assessment of PBDEs in DCL and TL

Relative congener concentration profiles of PBDEs in fishes from DCL and TL were similar to those found in DE-71; the dominant congener was BDE-47, followed by BDE-28, BDE-100, BDE-154, BDE-99, and BDE-153 (Fig. 4). The main difference in the congener profiles in fishes and DE-71 was the greater amount of BDE-17 and BDE-28 in wild fish. ΣPBDE concentrations (sum of the detected congeners) estimated in whole-body fish from DCL and TL ranged from 199 to 7,040 and 79 to 3,018 ng/kg (wm), respectively (Table 4 and Fig. 5). A screening-level risk assessment was conducted by comparing concentrations of ΣPBDE in fishes to the dietary-based TRV of 0.1 mg DE-71/kg (wm) presented in this report. Maximum concentrations of PBDEs in fishes from DCL and TL were two orders of magnitude less than the dietary-based TRV of 0.1 mg DE-71/kg (wm), which suggests that current PBDE levels would not exceed the threshold for adverse effects to piscivorous species in DCL and TL.

These criteria values for PBDEs can be used to protect wild piscivorous species and to assess ecological risk associated with increasing PBDEs concentrations in Chinese surface waters. Note that these criteria were derived from the toxicity of

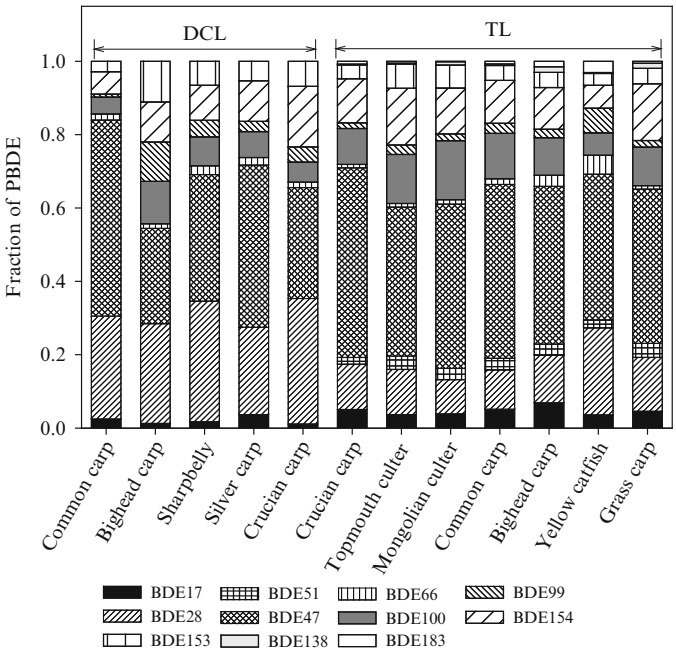


Fig. 4 Congener profile of PBDEs in wild fish from Dianchi Lake (DCL) and Tai Lake (TL)

Table 4 PBDE concentrations (ng/kg wm) in wild fish from two Chinese lakes (DCL and TL)

Species	Number of sample	PBDE concentration		
		Range	Median	Mean
TL				
Crucian carp (*Carassius cuvieri*)	26	147–1,591	554	571
Topmouth culter (*Erythroculter ilishaeformis*)	20	392–2,095	1,097	1,131
Mongolian culter (*Erythroculter mongolicus*)	4	983–3,018	1,285	1,643
Common carp (*Cyprinus carpio*)	24	128–887	405	456
Bighead carp (*Aristichthys nobilis*)	27	79–971	436	437
Yellow catfish (*Pelteobagrus fulvidraco*)	6	241–1,053	487	534
Grass carp (*Ctenopharyngodon idella*)	9	142–336	236	228
DCL				
Crucian carp (*Carassius cuvieri*)	19	199–1,042	390	423
Sharpbelly (*Hemiculter leucisculus*)	16	226–1,858	611	661
Silver carp (*Hypophthalmichthys molitrix*)	20	274–2,788	862	994
Common carp (*Cyprinus carpio*)	12	535–1,290	800	825
Bighead carp (*Aristichthys nobilis*)	9	331–7,040	643	1,691

DCL Dianchi Lake, *TL* Tai Lake

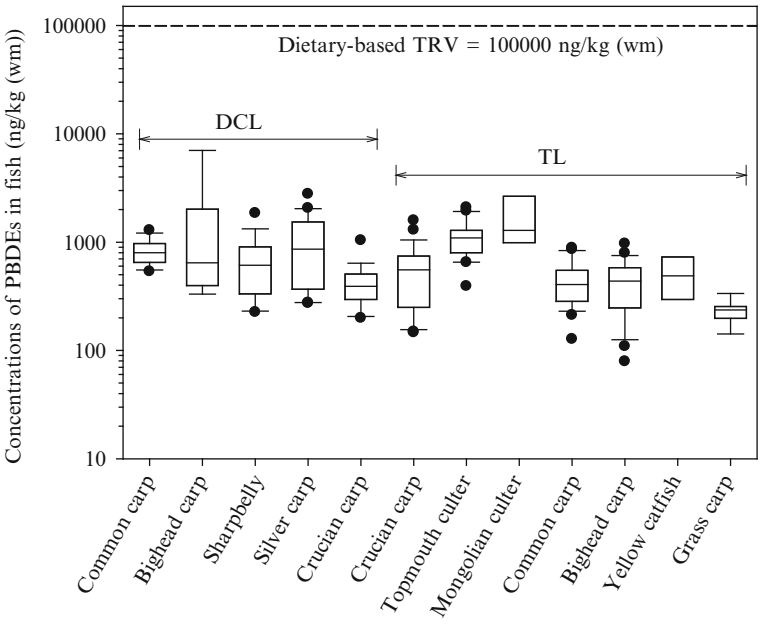


Fig. 5 Comparison of dietary-based TRV to concentrations of PBDEs in wild fish from Dianchi Lake (DCL) and Tai Lake (TL). *TRV* toxicity reference value, *wm* wet mass

DE-71, which is one of the commercial PBDE products and consists of several lesser brominated congeners. At present, though the lesser brominated diphenyl ethers were the predominant congeners in most aquatic organisms, some more highly brominated congeners, such as BDE-209, were also detected in tissues of species at higher trophic levels (Gao et al. 2009a; Lam et al. 2007). The criteria values presented in this study cannot provide information for these compounds. Concentrations of PBDEs in whole-body fish were derived by using a conversion factor and muscle concentrations, a degree of uncertainty was generated by the data gap related to the conversion of concentrations between tissues. Additionally, the diet of piscivorous birds and mammals consists of aquatic species from different trophic levels, and there are some interspecific differences in the structure of the diet. Thus, when these criteria values are used to assess ecological risk at a specific-site, information on food web structure must also be considered.

7 Summary

PBDEs are persistent organic pollutants, and have the capability to produce adverse effects on organisms. Aquatic piscivorous species at higher trophic levels have the greatest exposure risk. Information on the toxic potency of a commercial PBDE mixture, DE-71, to mink and American kestrel was reviewed, and dietary- and tissue-based TRVs were derived and evaluated for ecological risk assessment of aquatic piscivorous species inhabiting wetland areas in China. The effect on mink thyroid function was identified as the most appropriate and protective endpoint for deriving the TRVs for mammals. The TRV was based on dietary exposure, and was 0.1 mg DE-71/kg (wm) or 0.01 mg DE-71/kg (bm)/day (ADI); for liver of mammals, the TRV was 1.2 mg ΣPBDEs/kg (lm). For birds, reproductive effects on American kestrels were used to derive the TRVs, in which an overall UF of 3.0 was used. The TRV was based on dietary exposure, and was 0.1 mg DE-71/kg (wm) or 0.018 mg DE-71/kg (bm)/day (ADI); for eggs of birds, the TRV was 2.35 μg ΣPBDEs/g (lm). Reported concentrations of PBDEs in livers of aquatic mammals found dead, and in fish and bird eggs from Chinese wetland areas were compiled and compared to the corresponding criteria values. Results indicated that TRV values reported in this study can be used as indicators for screening-level risk assessment of piscivorous species in Chinese aquatic systems. Furthermore, based on monitoring concentrations of PBDEs in fishes from two lakes (DCL and TL) in China and the dietary-based TRV of 0.1 mg DE-71/kg (wm), a screening-level risk assessment of PBDEs was performed for predatory birds and mammals. The results suggest that concentrations of PBDEs in these two areas would not be expected to cause any adverse effects on the local fish-eating wild birds and mammals.

Acknowledgements This work was financially supported by National Natural Science Foundation of China (41130743, 41261140337, 41322025), and supported by Program of Higher Level talents of Innon Mongolia University (SPH-IMU).

References

Agency for Toxic Substances and Disease Registry (ATSDR) (2004) Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers. US Department of Health and Human Services, Public Health Service, Atlanta

Albers PH, Koterba MT, Rossmann R, Link WA, French JB, Bennett RS, Bauer WC (2007) Effects of methylmercury on reproduction in American kestrels. Environ Toxicol Chem 26(9):1856–1866. doi:10.1897/06-592r.1

Behnisch PA, Hosoe K, Sakai S-i (2003) Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environ Int 29(6):861–877. doi:10.1016/S0160-4120(03)00105-3

Birnbaum LS, Staskal DF, Diliberto JJ (2003) Health effects of polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs). Environ Int 29(6):855–860. doi:10.1016/S0160-4120(03)00106-5

Blankenship AL, Kay DP, Zwiernik MJ, Holem RR, Newsted JL, Hecker M, Giesy JP (2008) Toxicity reference values for mink exposed to 2,3,7,8-tetrachlodibenzo-p-dioxin (TCDD) equivalents (TEQs). Ecotoxicol Environ Saf 69(3):325–349. doi:10.1016/j.ecoenv.2007.08.017

Boas M, Feldt-Rasmussen U, Skakkebæk NE, Main KM (2006) Environmental chemicals and thyroid function. Eur J Endocrinol 154(5):599–611. doi:10.1530/eje.1.02128

Brown DJ, Van Overmeire I, Goeyens L, Denison MS, De Vito MJ, Clark GC (2004) Analysis of Ah receptor pathway activation by brominated flame retardants. Chemosphere 55(11):1509–1518. doi:10.1016/j.Chemosphere.2003.10.019

Bull K, Basu N, Zhang S, Martin JW, Bursian S, Martin P, Chan LHM (2007) Dietary and in utero exposure to a pentabrominated diphenyl ether mixture did not affect cholinergic parameters in the cerebral cortex of ranch mink (*Mustela vison*). Toxicol Sci 96(1):115–122. doi:10.1093/toxsci/kfl179

Bureau EC (2001) European union risk assessment report. Diphenyl ether, pentabromo derivative. (Pentabromodiphenyl ether): risk assessment. CAS No.: 32534-81-9. European Commission, Luxembourg

Caldwell DJ, Mastrocco F, Hutchinson TH, Länge R, Heijerick D, Janssen C, Anderson PD, Sumpter JP (2008) Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17α-ethinyl estradiol. Environ Sci Technol 42(19):7046–7054. doi:10.1021/es800633q

CCME (1998) Protocol for derivation of Canadian tissue residue guidelines for the protection of wildlife that consume aquatic biota. Canadian Council of Ministers of the Environment, Winnipeg

Chen D, Hale RC (2010) A global review of polybrominated diphenyl ether flame retardant contamination in birds. Environ Int 36(7):800–811

Covaci A, Van de Vijver K, De Coen W, Das K, Bouquegneau JM, Blust R, Schepens P (2002) Determination of organohalogenated contaminants in liver of harbour porpoises (*Phocoena phocoena*) stranded on the Belgian North Sea coast. Mar Pollut Bull 44(10):1157–1165, doi:http://dx.doi.org/10.1016/S0025-326X(02)00147-9

Darnerud PO (2003) Toxic effects of brominated flame retardants in man and in wildlife. Environ Int 29(6):841–853. doi:10.1016/S0160-4120(03)00107-7

De Wit CA, Alaee M, Muir DCG (2006) Levels and trends of brominated flame retardants in the Arctic. Chemosphere 64(2):209–233

Dunning JB Jr (1993) CRC handbook of avian body masses. CRC Press, Boca Raton

Egger M, Smith GD, Phillips AN (1997) Meta-analysis: principles and procedures. BMJ 315(7121):1533–1537. doi:10.1136/bmj.315.7121.1533

Environment Canada (2006) Canadian environmental protection act, 1999. Ecological screening assessment report on polybrominated diphenyl ethers. Environment Canada, Ottawa

Fallacara DM, Halbrook RS, French JB (2011) Toxic effects of dietary methylmercury on immune function and hematology in American kestrels (*Falco sparverius*). Environ Toxicol Chem 30(6):1320–1327. doi:10.1002/etc.494

Fernie KJ, Mayne G, Shutt JL, Pekarik C, Grasman KA, Letcher RJ, Drouillard K (2005a) Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. Environ Pollut 138(3):485–493

Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, Ritchie IJ (2005b) Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). Toxicol Sci 88(2):375–383

Fernie KJ, Shutt JL, Ritchie IJ, Letcher RJ, Drouillard K, Bird DM (2006) Changes in the growth, but not the survival, of American kestrels (*Falco sparverius*) exposed to environmentally relevant polybrominated diphenyl ethers. J Toxicol Environ Health A 69(16):1541–1554

Fernie KJ, Shutt JL, Letcher RJ, Ritchie JI, Sullivan K, Bird DM (2008) Changes in reproductive courtship behaviors of adult American kestrels (*Falco sparverius*) exposed to environmentally relevant levels of the polybrominated diphenyl ether mixture, DE-71. Toxicol Sci 102(1):171–178. doi:10.1093/toxsci/kfm295

Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Bird DM (2009) Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. Environ Sci Technol 43(6):2124–2130

Fernie KJ, Marteinson SC, Bird DM, Ritchie IJ, Letcher RJ (2011) Reproductive changes in American kestrels (*Falco sparverius*) in relation to exposure to technical hexabromocyclododecane flame retardant. Environ Toxicol Chem 30(11):2570–2575. doi:10.1002/etc.652

Gao F, Luo XJ, Yang ZF, Wang XM, Mai BX (2009a) Brominated flame retardants, polychlorinated biphenyls, and organochlorine pesticides in bird eggs from the Yellow River Delta, North China. Environ Sci Technol 43(18):6956–6962. doi:10.1021/es901177j

Gao Z, Xu J, Xian Q, Feng J, Chen X, Yu H (2009b) Polybrominated diphenyl ethers (PBDEs) in aquatic biota from the lower reach of the Yangtze River, East China. Chemosphere 75(9):1273–1279

Giesy JP, Kannan K (1998) Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. CRC Crit Rev Toxicol 28(6):511–569

Guo JY, Wu FC, Mai BX, Luo XJ, Zeng EY (2007) Polybrominated diphenyl ethers in seafood products of South China. J Agric Food Chem 55(22):9152–9158

Guo Y, Meng XZ, Tang HL, Mai BX, Zeng EY (2008) Distribution of polybrominated diphenyl ethers in fish tissues from the Pearl River Delta, China: levels, compositions, and potential sources. Environ Toxicol Chem 27(3):576–582. doi:10.1897/07-366

Guo G, Wu F, He H, Zhang R, Li H (2012) Ecological risk assessment of organochlorine pesticides in surface waters of Lake Taihu, China. Hum Ecol Risk Assess 19(4):840–856. doi:10.1080/10807039.2012.691811

Hall LW, Scott MC, Killen WD (1998) Ecological risk assessment of copper and cadmium in surface waters of Chesapeake Bay watershed. Environ Toxicol Chem 17(6):1172–1189. doi:10.1002/etc.5620170626

Hallgren S, Darnerud PO (2002) Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats—testing interactions and mechanisms for thyroid hormone effects. Toxicology 177(2–3):227–243. doi:10.1016/S0300-483X(02)00222-6

Hanari N, Kannan K, Okazawa T, Kodavanti PRS, Aldous KM, Yamashita N (2006) Occurrence of polybrominated biphenyls, polybrominated dibenzo-p-dioxins, and polybrominated dibenzofurans as impurities in commercial polybrominated diphenyl ether mixtures. Environ Sci Technol 40(14):4400–4405. doi:10.1021/es060559k

Heaton SN, Bursian SJ, Giesy JP, Tillitt DE, Render JA, Jones PD, Verbrugge DA, Kubiak TJ, Aulerich RJ (1995) Dietary exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival, and the potential risks to wild mink populations. Arch Environ Contam Toxicol 28(3):334–343. doi:10.1007/BF00213111

Hochstein JR, Aulerich RJ, Bursian SJ (1988) Acute toxicity of 2,3,7,8-tetrachlorodibenzo- p -dioxin to mink. Arch Environ Contam Toxicol 17(1):33–37. doi:10.1007/BF01055151

Hochstein JR, Bursian SJ, Aulerich RJ (1998) Effects of dietary exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in adult female mink (*Mustela vison*). Arch Environ Contam Toxicol 35(2):348–353. doi:10.1007/s002449900386

Hu G, Dai J, Xu Z, Luo X, Cao H, Wang J, Mai B, Xu M (2010) Bioaccumulation behavior of polybrominated diphenyl ethers (PBDEs) in the freshwater food chain of Baiyangdian Lake, North China. Environ Int 36(4):309–315

Kannan K, Blankenship AL, Jones PD, Giesy JP (2000) Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum Ecol Risk Assess 6(1):181–201. doi:10.1080/10807030091124491

Kannan K, Perrotta E, Thomas N, Aldous K (2007) A comparative analysis of polybrominated diphenyl ethers and polychlorinated biphenyls in southern sea otters that died of infectious diseases and noninfectious causes. Arch Environ Contam Toxicol 53(2):293–302. doi:10.1007/s00244-006-0251-8

La Guardia MJ, Hale RC, Harvey E (2006) Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. Environ Sci Technol 40(20):6247–6254. doi:10.1021/es060630m

Lam JCW, Kajiwara N, Ramu K, Tanabe S, Lam PKS (2007) Assessment of polybrominated diphenyl ethers in eggs of waterbirds from South China. Environ Pollut 148(1):258–267

Leijs M, ten Tusscher G, Olie K, van Teunenbroek T, van Aalderen W, de Voogt P, Vulsma T, Bartonova A, Krayer von Krauss M, Mosoiu C, Riojas-Rodriguez H, Calamandrei G, Koppe J (2012) Thyroid hormone metabolism and environmental chemical exposure. Environ Health 11(Suppl 1):S10. doi:10.1186/1476-069X-11-S1-S10

Luo X, Zhang X, Liu J, Wu J, Luo Y, Chen S, Mai B, Yang Z (2009) Persistent halogenated compounds in waterbirds from an e-waste recycling region in South China. Environ Sci Technol 43(2):306–311

Marteinson SC (2011) Reproductive and behavioral effects of two brominated flame retardants in captive American kestrels (*Falco sparverius*). McGill University, Montreal

Marteinson SC, Bird DM, Shutt JL, Letcher RJ, Ritchie IJ, Fernie KJ (2010) Multi-generational effects of polybrominated diphenylethers exposure: embryonic exposure of male American kestrels (*Falco sparverius*) to DE-71 alters reproductive success and behaviors. Environ Toxicol Chem 29(8):1740–1747. doi:10.1002/etc.200

Marteinson SC, Kimmins S, Bird DM, Shutt JL, Letcher RJ, Ritchie IJ, Fernie KJ (2011) Embryonic exposure to the polybrominated diphenyl ether mixture, DE-71, affects testes and circulating testosterone concentrations in adult American kestrels (*Falco sparverius*). Toxicol Sci 121(1):168–176. doi:10.1093/toxsci/kfr027

Marteinson SC, Bird DM, Letcher RJ, Sullivan KM, Ritchie IJ, Fernie KJ (2012) Dietary exposure to technical hexabromocyclododecane (HBCD) alters courtship, incubation and parental behaviors in American kestrels (*Falco sparverius*). Chemosphere 89(9):1077–1083. doi:10.1016/j.chemosphere.2012.05.073

Martin PA, Mayne GJ, Bursian S, Palace V, Kannan K (2006) Changes in thyroid and vitamin A status in mink fed polyhalogenated-aromatic-hydrocarbon-contaminated carp from the Saginaw River, Michigan, USA. Environ Res 101(1):53–67. doi:10.1016/j.envres.2005.06.002

Martin PA, Mayne GJ, Bursian SJ, Tomy G, Palace V, Pekarik C, Smits J (2007) Immunotoxicity of the commercial polybrominated diphenyl ether mixture DE-71 in ranch mink (*Mustela vison*). Environ Toxicol Chem 26(5):988–997

McKernan MA, Rattner BA, Hale RC, Ottinger MA (2009) Toxicity of polybrominated diphenyl ethers (DE-71) in chicken (*Gallus gallus*), mallard (*Anas platyrhynchos*), and American kestrel (*Falco sparverius*) embryos and hatchlings. Environ Toxicol Chem 28(5):1007–1017

McKernan MA, Rattner BA, Hatfield JS, Hale RC, Ann Ottinger M (2010) Absorption and biotransformation of polybrominated diphenyl ethers DE-71 and DE-79 in chicken (*Gallus gallus*), mallard (*Anas platyrhynchos*), American kestrel (*Falco sparverius*) and black-crowned night-heron (*Nycticorax nycticorax*) eggs. Chemosphere 79(1):100–109, doi:http://dx.doi.org/10.1016/j.chemosphere.2009.12.023

Meng XZ, Yu L, Guo Y, Mai BX, Zeng EY (2008) Congener-specific distribution of polybrominated diphenyl ethers in fish of China: implication for input sources. Environ Toxicol Chem 27(1):67–72. doi:10.1897/07-138.1

Millsap SD, Blankenship AL, Bradley PW, Jones PD, Kay D, Neigh A, Park C, Strause KD, Zwiernik MJ, Giesy JP (2004) Comparison of risk assessment methodologies for exposure of mink to PCBs on the Kalamazoo River, Michigan. Environ Sci Technol 38(24):6451–6459. doi:10.1021/es049600e

Nakata H, Hirakawa Y, Kawazoe M, Nakabo T, Arizono K, Abe S-I, Kitano T, Shimada H, Watanabe I, Li W, Ding X (2005) Concentrations and compositions of organochlorine contaminants in sediments, soils, crustaceans, fishes and birds collected from Lake Tai, Hangzhou Bay and Shanghai city region, China. Environ Pollut 133(3):415–429, doi:http://dx.doi.org/10.1016/j.envpol.2004.07.003

Newsted JL, Jones PD, Coady K, Giesy JP (2005) Avian toxicity reference values for perfluorooctane sulfonate. Environ Sci Technol 39(23):9357–9362. doi:10.1021/es050989v

Peters AK, van Londen K, Bergman Å, Bohonowych J, Denison MS, van den Berg M, Sanderson JT (2004) Effects of polybrominated diphenyl ethers on basal and TCDD-induced ethoxyresorufin activity and cytochrome P450-1A1 expression in MCF-7, HepG2, and H4IIE cells. Toxicol Sci 82(2):488–496. doi:10.1093/toxsci/kfh284

Posthuma L, Suter GW, Traas TP (2002) Species sensitivity distributions in ecotoxicology. CRC Press LLC, Boca Raton

Sample B, Suter D (1993) Toxicological benchmarks for wildlife, No. ES/ER/TM-86. ORNL Oak Ridge National Laboratory (US), Oak Ridge

Sanders JM, Burka LT, Smith CS, Black W, James R, Cunningham ML (2005) Differential expression of CYP1A, 2B, and 3A genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual components. Toxicol Sci 88(1):127–133. doi:10.1093/toxsci/kfi288

Seo BW, Li MH, Hansen LG, Moore RW, Peterson RE, Schantz SL (1995) Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. Toxicol Lett 78(3):253–262. doi:10.1016/0378-4274(95)03329-J

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW, Williams WM (1996) Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 15(1):31–76. doi:10.1002/etc.5620150105

Stanton B, de Vries S, Donohoe R, Anderson M, Eichelberger JM (2010) Recommended avian toxicity reference value for cadmium: justification and rationale for use in ecological risk assessments. Hum Ecol Risk Assess 16(6):1261–1277. doi:10.1080/10807039.2010.526499

Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA (1985) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. USEPA, Washington, DC

Sullivan KM, Bird DM, Ritchie JI, Shutt JL, Letcher RJ, Fernie KJ (2010) Changes in plasma retinol of American kestrels (*Falco sparverius*) in response to dietary or in ovo exposure to environmentally relevant concentrations of a penta-brominated diphenyl ether mixture, DE-71. J Toxicol Environ Health A 73(23):1645–1654. doi:10.1080/15287394.2010.501720

Talsness CE (2008) Overview of toxicological aspects of polybrominated diphenyl ethers: a flame-retardant additive in several consumer products. Environ Res 108(2):158–167. doi:10.1016/j.envres.2008.08.008

Talsness CE, Kuriyama SN, Sterner-Kock A, Schnitker P, Grande SW, Shakibaei M, Andrade A, Grote K, Chahoud I (2008) In utero and lactational exposures to low doses of polybrominated diphenyl ether-47 alter the reproductive system and thyroid gland of female rat offspring. Environ Health Perspect 116(3):308

Tillitt DE, Gale RW, Meadows JC, Zajicek JL, Peterman PH, Heaton SN, Jones PD, Bursian SJ, Kubiak TJ, Giesy JP, Aulerich RJ (1995) Dietary exposure of mink to carp from Saginaw bay. 3. Characterization of dietary exposure to planar halogenated hydrocarbons, dioxin equivalents, and biomagnification. Environ Sci Technol 30(1):283–291. doi:10.1021/es9503140

Tomy GT, Palace VP, Halldorson T, Braekevelt E, Danell R, Wautier K, Evans B, Brinkworth L, Fisk AT (2004) Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). Environ Sci Technol 38(5):1496–1504. doi:10.1021/es035070v

USEPA (1993) Wildlife exposure factors handbook. Volume I. EPA/600/R-93/187. USEPA, Office of Research and Development, Washington, DC

USEPA (1995a) Final water quality guidance for the Great Lakes. Fed Regist 60(56):15366–15425

USEPA (1995b) Great Lakes water quality initiative criteria documents for the protection of wildlife. EPA/820/B-95/008. USEPA, Office of Water, Washington, DC

USEPA (1995c) Great Lakes water quality initiative technical support document for wildlife criteria. EPA-820-B-95-005. USEPA, Office of Water, Washington, DC

USEPA (2003) Attachment 4–5 guidance for developing ecological soil screening levels (Eco-SSLs), Eco-SSL standard operating procedure (SOP) # 6: derivation of wildlife toxicity reference value (TRV). OWSER Directive 9285.7-55. USEPA, Washington, DC

USEPA (2005) Science advisory board consultation document. Proposed revisions to aquatic life guidelines: tissue-based criteria for "bioaccumulative" chemicals. USEPA, Office of Water, Washington, DC

Walker L, Moeckel C, Pereira M, Beith S, Potter E, Chadwick E, Shore R (2012) Polybrominated diphenyl ethers (PBDEs) in Eurasian otters (*Luta lutra*) collected from Britain in 2010: a predatory bird monitoring scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster

Wan Y, Hu J, Zhang K, An L (2008) Trophodynamics of polybrominated diphenyl ethers in the marine food web of Bohai Bay, North China. Environ Sci Technol 42(4):1078–1083

Winter V, Elliott JE, Letcher RJ, Williams TD (2013) Validation of an egg-injection method for embryotoxicity studies in a small, model songbird, the zebra finch (*Taeniopygia guttata*). Chemosphere 90(1):125–131. doi:10.1016/j.chemosphere.2012.08.017

Wu FC, Li HX (2012) Theory and methodology of water quality criteria and case study. Science Press, Beijing

Wu JP, Luo XJ, Zhang Y, Luo Y, Chen SJ, Mai BX, Yang ZY (2008) Bioaccumulation of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in wild aquatic species from an electronic waste (e-waste) recycling site in South China. Environ Int 34(8):1109

Wu J, Zhang Y, Luo X, She Y, Yu L, Chen S, Mai B (2012) A review of polybrominated diphenyl ethers and alternative brominated flame retardants in wildlife from china: levels, trends, and bioaccumulation characteristics. J Environ Sci (China) 24(2):183–194

Xian QM, Ramu K, Isobe T, Sudaryanto A, Liu X, Gao Z, Takahashi S, Yu H, Tanabe S (2008) Levels and body distribution of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in freshwater fishes from the Yangtze River, China. Chemosphere 71(2):268–276. doi:10.1016/j.chemosphere.2007.09.032

Yáñez JL, Núñez H, Schlatter RP, Jaksić FM (1980) Diet and weight of American kestrels in Central Chile. Auk 97(3):629–631

Zhang S, Bursian S, Martin PA, Chan HM, Martin JW (2008) Dietary accumulation, disposition, and metabolism of technical pentabrominated diphenyl ether (DE-71) in pregnant mink (*Mustela vison*) and their offspring. Environ Toxicol Chem 27(5):1184–1193. doi:10.1897/07-487.1

Zhang S, Bursian SJ, Martin PA, Chan HM, Tomy G, Palace VP, Mayne GJ, Martin JW (2009) Reproductive and developmental toxicity of a pentabrominated diphenyl ether mixture, DE-71, to ranch mink (*Mustela vison*) and hazard assessment for wild mink in the Great Lakes region. Toxicol Sci 110(1):107–116. doi:10.1093/toxsci/kfp095

Zhang R, Wu F, Li H, Feng C, Guo G (2012) Deriving aquatic water quality criteria for inorganic mercury in China by species sensitivity distributions. Acta Sci Circumst 32(4):440–449

Zhang R, Wu F, Li H, Guo G, Feng C, Giesy JP, Chang H (2013) Toxicity reference values and tissue residue criteria for protecting avian wildlife exposed to methylmercury in China. Rev Environ Contam Toxicol 223:53–80. doi:10.1007/978-1-4614-5577-6_3

Zhou T, Taylor MM, DeVito MJ, Crofton KM (2002) Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. Toxicol Sci 66(1):105–116

Index

INDEX FOR RECT VOL. 229

Absorption, mercury, **229**: 5
Agbogbloshie (Ghana) scrap yard, e-waste water pollution, **229**: 22
Agbogbloshie (Ghana), e-waste recycling (illus.), **229**: 23
Alzheimer's disease, main features, **229**: 11
Alzheimer's disease link, mercury, **229**: 10
American kestrels, DE-71 (PBDE isomer) NOAEL & LOAEL (table), **229**: 125
Amyotrophic lateral sclerosis, description, **229**: 11
Amyotrophic lateral sclerosis, mercury link, **229**: 6, 10
Anthropogenic sources, cadmium, **229**: 53
Aquatic avian species in China, PBDE egg TRVs (diag.), **229**: 129
Aquatic birds, PBDE TRV derivation, **229**: 121
Aquatic birds, PBDE TRV, **229**: 125
Aquatic environmental effects, e-waste disposal, **229**: 19 ff.
Aquatic life effects, heavy metals, **229**: 26
Aquatic life, organic pollutant effects, **229**: 28
Aquatic mammals, deriving PBDE TRVs, **229**: 115
Aquatic mammals, PBDE TRV derivation, **229**: 115
Aquatic mammals, TRV for PBDEs, **229**: 119
Aquatic organism toxicity, PBDEs, **229**: 28
Aquatic organisms, e-waste contaminant effects, **229**: 25
Aquatic organisms, lead & cadmium toxicity, **229**: 27
Aquatic species in China, PBDE TRVs (diag.), **229**: 129
Aquatic species, clomazone toxicity, **229**: 44
Autoimmune effects, mercury, **229**: 6

Biotic processes, chlomazone, **229**: 40
Bird toxicity, of DE-71 (PBDE isomer), **229**: 121
Birds, PBDE TRVs (table), **229**: 127

Cadmium effects, chlorophyll & carotenoids, 60, 61
Cadmium effects, on fatty acid composition, **229**: 63
Cadmium effects, on free amino acids & proline, **229**: 62
Cadmium effects, on genetic material, **229**: 63
Cadmium effects, on key organic osmotica, **229**: 65
Cadmium effects, on plant growth (table), **229**: 56
Cadmium effects, on protein levels, **229**: 61
Cadmium effects, photosynthesis, **229**: 69
Cadmium effects, plant growth regulators, **229**: 67
Cadmium effects, plant metal uptake, **229**: 64
Cadmium toxicity, to aquatic organisms, **229**: 27
Cadmium, described, **229**: 52
Cadmium, environmental sources, **229**: 52
Cadmium, natural vs. anthropogenic sources, **229**: 52
Cadmium, oxidative defense system effects, **229**: 66
Cadmium, plant accumulation, **229**: 54
Cadmium, plant effects, **229**: 59
Cadmium, plant morphology effects, **229**: 55
Cadmium, plant pigment effects, **229**: 59
Cadmium, plant water relations effect, **229**: 70
Cadmium, protein and protease effects, **229**: 61
Cadmium, soil and plant behavior, **229**: 54
Cadmium-enriched environments, plant growth effects, **229**: 51 ff.

D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 229,* Reviews of Environmental Contamination and Toxicology 229,
DOI 10.1007/978-3-319-03777-6,

Cadmium-enriched environments, plant metabolic effects, **229**: 51 ff.
Carotenoid effects, cadmium, **229**: 60
Chemical behavior, elemental mercury, **229**: 2
Chemical forms, mercury, **229**: 2
Chemical washing, during food processing, **229**: 101
Chemistry, clomazone, **229**: 36
Chemodynamics, chlomazone, **229**: 37
Chlorophyll effects, cadmium, **229**: 59
Clomazone toxicity, aquatic species, **229**: 44
Clomazone toxicity, to non-target species, **229**: 45
Clomazone, air & volatilization, **229**: 39
Clomazone, biotic processes, **229**: 40
Clomazone, chemistry & physicochemical properties, **229**: 36
Clomazone, description, **229**: 36
Clomazone, environmental chemodynamics, **229**: 37
Clomazone, environmental degradation, **229**: 39
Clomazone, environmental fate & toxicology, **229**: 35 ff.
Clomazone, metabolic pathway in soybeans (diag.), **229**: 44
Clomazone, microbial breakdown pathway (diag.), **229**: 41
Clomazone, photolytic degradation (diag.), **229**: 40
Clomazone, physicochemical properties (table), **229**: 37
Clomazone, photolysis & hydrolysis, **229**: 39
Clomazone, plant effects, **229**: 43
Clomazone, plant mode-of-action, **229**: 42
Clomazone, soil interactions, **229**: 37
Clomazone, structure (illus.), **229**: 36
Clomazone, toxicology, **229**: 42
Clomazone, water behavior, **229**: 38
Contaminants present, e-waste, **229**: 24
Contaminants present, in e-waste (table), **229**: 24
DE-71 (PBDE isomer) NOAEL & LOAEL, American kestrels (table), **229**: 125
DE-71 NOAEL & LOAEL, mink (table), **229**: 119
DE-71 toxicity, to birds, **229**: 121
DE-71 toxicity thresholds (diag.), **229**: 127
Detergent washing, during food processing, **229**: 101
Distribution, mercury, **229**: 5
Elemental mercury, chemical behavior, **229**: 2
Environmental chemodynamics, clomazone, **229**: 37
Environmental degradation, clomazone, **229**: 39
Environmental effects, e-waste disposal, **229**: 19 ff.
Environmental fate, clomazone, **229**: 35 ff.
Environmental sources, cadmium, **229**: 52
Enzyme inhibition, mercury, **229**: 10
Ethyl mercury, thimerosal in vaccines, **229**: 4
E-waste contaminant effects, on aquatic organisms, **229**: 25
E-Waste contaminants (Korle Lagoon), vs. sediment quality guideline values (table), **229**: 25
E-Waste export, to developing countries, **229**: 20
E-Waste generated, globally, **229**: 20
E-Waste in Ghana, quantity imported (diag.), **229**: 21
E-Waste management, in Ghana, **229**: 21
E-Waste pollution issue, Ghana, **229**: 20
E-Waste quantity imported, into Ghana (diag.), **229**: 21
E-waste recycling, Agbogbloshie (Ghana) (illus.), **229**: 23
E-Waste recycling, in Ghana, **229**: 22
E-Waste water pollution, Agbogbloshie (Ghana) scrap yard, **229**: 22
E-Waste, aquatic environmental effects, **229**: 19 ff.
E-waste, contaminants present (table), **229**: 24
E-waste, contaminants present, **229**: 24
E-Waste, toxic chemicals present, **229**: 20
FFate in the body, mercury (diag.), **229**: 5
Fatty acid composition, cadmium effects, **229**: 63
Food (fruit & vegetable) processing methods, pyrethroid insecticide residue effects (table), **229**: 92–99
Food blanching, pesticide residue effect, **229**: 104
Food freezing, pesticide residue effect, **229**: 104
Food juicing, pesticide residue effect, **229**: 102
Food processing & pesticides, described, **229**: 90
Food processing effects, from boiling & cooking, **229**: 103
Food processing of vegetables & fruit, peeling, **229**: 102
Food processing, described, **229**: 91
Food processing, washing with chemicals or detergents, **229**: 101
Food processing, washing with salt solutions, **229**: 101

Food processing, washing with tap water, **229**: 91
Food washing effects, hydrophilic pesticide residues, **229**:100
Free amino acid effects, cadmium, **229**: 62
Fruit processing, pyrethroid insecticide residue effect, **229**: 89 ff.
Genetic material effects, cadmium, **229**: 63
Ghana, e-waste disposal effects, **229**: 19 ff.
Ghana, e-waste management, **229**: 21
Ghana, e-waste pollution issue, **229**: 20
Ghana, e-waste recycling, **229**: 22
Heavy metal effects, on aquatic life, **229**: 26
Heavy metals, described, **229**: 52
Human exposure sources, mercury, **229**: 3
Human exposure, to mercury, **229**: 3
Human poisoning events, methyl mercury, **229**: 4
Hydrolysis, clomazone, **229**: 39
Inorganic mercury, in breast milk, **229**: 6
Insecticide residue effects, from vegetable & fruit processing, **229**: 89 ff.
Iraqi poisoning event, methyl mercury, **229**: 4
Kawasaki disease, mercury link, **229**: 6
Lead toxicity, to aquatic organisms, **229**: 27
Mammals, PBDE TRVs (table), **229**: 127
MeHg toxicity, effects described, **229**: 7
MeHg, selenium effects, **229**: 8
MeHg, thiol interactions, **229**: 7
Mercury chelators, structures (illus.), **229**: 9
Mercury exposure, humans, **229**: 3
Mercury forms, human exposure, **229**: 3
Mercury link, amyotrophic lateral sclerosis, **229**: 6, 10
Mercury link, Kawasaki disease, **229**: 6
Mercury toxicity, metallothionein effects, **229**: 8
Mercury toxicity, mitochondrial effects, **229**: 8
Mercury toxicity, molecular mechanism, **229**: 7
Mercury toxicity, neurodegenerative effects, **229**: 1 ff.
Mercury toxicity, reactive oxygen species, **229**: 8
Mercury toxicity, symptoms, **229**: 6
Mercury vapor, toxicity, **229**: 6
Mercury, absorption and distribution, **229**: 5
Mercury, Alzheimer's disease, **229**: 10
Mercury, and neurodegenerative diseases, **229**: 10
Mercury, autoimmune effects, **229**: 6
Mercury, chemical forms and properties, **229**: 2
Mercury, enzyme inhibition, **229**: 10
Mercury, fate in the body (diag.), **229**: 5
Mercury, neuronal degeneration, **229**: 7
Mercury, toxicity, **229**: 5
Metabolic pathway in soybeans, chlomazone (diag.), **229**: 44
Metal uptake in plants, cadmium effects, **229**: 64
Metallothionein effects, mercury toxicity, **229**: 8
Methyl mercury, human poisoning events, **229**: 4
Methyl mercury, nature & origin, **229**: 2
Microbial breakdown pathway, clomazone (diag.), **229**: 41
Minamata disease, mercury poisoning event, **229**: 4
Minks, DE-71 (PBDE isomer), NOAEL & LOAEL (Table), **229**: 119
Mitochondrial effects, mercury toxicity, **229**: 8
Molecular mechanism, mercury toxicity, **229**: 7
Natural sources, cadmium, **229**: 53
Neurodegenerative toxicity, defined, **229**: 1
Neurodegenerative diseases, mercury, **229**: 10
Neurodegenerative effects, mercury toxicity, **229**: 1 ff.
Neuronal degeneration, mercury, **229**: 7
Non-target species toxicity, clomazone, **229**: 45
Organic osmotica effects, cadmium, **229**: 65
Organic pollutant effects, on aquatic life, **229**: 28
Oxidative defense system effects, cadmium, **229**: 66
PBDEs (polybrominated diphenyl ethers) toxicity, to aquatic organisms, **229**: 28
PBDE congener profile, wild lake fish (diag.), **229**: 130
PBDE egg TRVs, for Chinese aquatic avian species (diag.), **229**: 129
PBDE residues, in wild fish from China (diag.), **229**: 132
PBDE residues, in wild fish from Chinese lakes (table), **229**: 131
PBDE toxicity reference values, birds & mammals, **229**: 111 ff.
PBDE TRV derivation, aquatic birds, **229**: 121
PBDE TRV derivation, for aquatic mammals, **229**: 115
PBDE TRV, for aquatic birds, **229**: 125
PBDE TRV, for aquatic mammals, **229**: 119
PBDE TRV, mammals & birds (table), **229**: 127
PBDE TRVs, Chinese aquatic species (diag.), **229**: 129
PBDEs, described, **229**: 112
PBDEs, ecological risk assessment, **229**: 130

Peeling of vegetables & fruits, pesticide residue effect, **229**: 102
Pesticide residue effect, from boiling or cooking food, **229**: 103
Pesticide residue effect, from food blanching or freezing, **229**: 103
Pesticide residue effect, from food juicing, **229**: 102
Pesticide residue effects, food washing, **229**: 100
Pesticide residue effects, from fruit & vegetable peeling, **229**: 102
Photolysis, clomazone, **229**: 39
Photolytic degradation, clomazone (diag.), **229**: 40
Photosynthesis effects, cadmium, **229**: 69
Physicochemical properties, clomazone (table), **229**: 37
Physiochemical alterations in plants, cadmium, **229**: 59
Physiochemical effects, cadmium (table), **229**: 56
Pilot whale contamination, mercury, **229**: 4
Plant accumulation, cadmium, **229**: 54
Plant alterations, cadmium, **229**: 59
Plant behavior, cadmium, **229**: 54
Plant effects, clomazone, **229**: 43
Plant growth effects, cadmium (table), **229**: 56
Plant growth regulators, cadmium effects, **229**: 67
Plant metal uptake, cadmium effects, **229**: 64
Plant mode-of-action, clomazone, **229**: 42
Plant pigment effects, cadmium, **229**: 59
Plant trait effects, cadmium, **229**: 55
Plant water relations effect, cadmium, **229**: 70
Polybrominated diphenyl ethers (PBDEs), toxicity reference values, **229**: 111 ff.
Proline effects, cadmium, **229**: 62
Properties, clomazone, **229**: 36
Protease activity effects, cadmium, **229**: 61
Protein level effects, cadmium, **229**: 61
Pyrethroid insecticides, residue changes from food processing, **229**: 89 ff.
Pyrethroid residue effects, from food processing methods (table), **229**: 92–99
Reactive oxygen species, mercury toxicity, **229**: 8
Risk assessment, PBDEs in Chinese lakes, **229**: 130
Salt solution washing, during food processing, **229**: 101
Sediment quality guideline values, vs. e-waste contaminants (table), **229**: 25
Selenium effects, MeHg, **229**: 8
Soil behavior, cadmium, **229**: 54
Soil interactions, clomazone, **229**: 37
Soybean metabolic pathway, clomazone (diag.), **229**: 44
Thimerosal in vaccines, ethyl mercury, **229**: 4
Thiol interactions, MeHg, **229**: 7
Toxic metals, in e-waste, **229**: 20
Toxicity of Hg, neurodegenerative effects, **229**: 1 ff.
Toxicity reference values (TRV), development process, **229**: 114
Toxicity reference values, for PBDEs, **229**: 111 ff.
Toxicity symptoms, mercury, **229**: 6
Toxicity thresholds for DE-71, wildlife (diag.), **229**: 127
Toxicity, mercury vapor, **229**: 6
Toxicity, mercury, **229**: 5
Toxicology, clomazone, **229**: 35 ff., 42
TRV (toxicity reference values), development process, **229**: 114
Vegetable processing, pyrethroid insecticide residue effect, **229**: 89 ff.
Volatilization, chlomazone, **229**: 39
Water behavior, clomazone, **229**: 38
Wild fish from Chinese lakes, PBDE residues (diag.), **229**: 132
Wild fish in Chinese lakes, PBDE congener profile (diag.), **229**: 130
Wild fish residues in China, PBDE (table), **229**: 131