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David M. Whitacre *Editor*

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Foreword

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

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

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

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

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

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

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

Coordinating Board of Editors

Preface

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

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

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

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

The ultimate role of publishing scientific research is to enhance understanding of the environment in ways that allow the public to be better informed. The term "informed public" as used by Thomas Jefferson in the age of enlightenment conveyed the thought of soundness and good judgment. In the modern sense, being "well informed" has the narrower meaning of having access to sufficient information. Because the public still gets most of its information on science and technology from TV news and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is the newest global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic.Will the new politics of the twenty-first century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

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

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

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

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

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

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Contents

Guiana Dolphins (*Sotalia guianensis*) as Marine Ecosystem Sentinels: Ecotoxicology and Emerging Diseases

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1 Introduction

Historically, human activities have impacted cetacean populations; such activities have included commercial fishing operations, overexploitation of marine species, habitat degradation, and commercial harvesting. As a result, many species are now classified as being threatened (Gulland and Hall 2007; Sherman 2000). Additionally, most species are exposed to anthropogenic contaminants, even those inhabiting areas far from human activities (Aono et al. 1997; Bard 1999). Such exposure may lead to health problems, such as impairment of immunological resistance, increased susceptibility to infectious diseases, and reduced reproductive fitness (Ross and Birnbaum 2003; Tanabe 2002).

In the last few decades, many pollutants have been detected in the marine environment, and these pose a potential threat to environmental integrity, biodiversity, and human health (Fleming et al. 2006; Hacon et al. 2005; Sherman 2000). Marine mammals have been proposed as sentinels of marine environmental health, because these organisms are top predators and may accumulate great concentrations of pollutants in their tissues through bioaccumulation and biomagnification processes (Ross and Birnbaum 2003; Siciliano et al. 2005). Since the 1960s, the number of chemical contaminants detected in cetaceans has increased dramatically (O'Shea and Tanabe 1999). Despite the development of more sensitive laboratory equipment and analytical techniques, this trend probably also reflects the increasing contamination released to the marine environment (Tanabe 2002).

Many marine mammals, as top-predators, regulate marine populations (i.e., by ingesting prey and controlling populations of certain species that, if unchecked, would dramatically increase and negatively affect ecosystems), and thereby, promote the integrity of their ecosystem. Their decrease or disappearance in some areas could drastically alter community structures (Tanabe et al. 1997).

Some cetaceans, particularly odontocetes, harbor high levels of substances in their tissues that are known or suspected to be endocrine disruptors, such as the organochlorine compounds (OCs), brominated flame retardants, tributyltin, and heavy metals (e.g., mercury and cadmium). Marine scientists have reported a range of effects of these contaminants on marine mammals including immunosuppression, cancer, skin lesions, secondary infections and diseases, sporadic die-offs, and reduced reproductive success (Fossi and Marsili 2003; Ylitalo 2005). For instance, the endangered beluga whales of the St. Lawrence estuary, now amongst the most contaminated animals of the world, have shown many negative health effects that include tumors and reproductive impairments, in association with high concentrations of a complex mixture of ubiquitous pollutants present in the marine environment (De Guise et al. 1995; Martineau et al. 2002). Pathologic studies of marine mammals further indicate that emerging or resurging infectious and neoplastic diseases may reflect environmental distress, and that these diseases have direct and/or indirect implications for human health (Bossart 2011; Sandifer et al. 2004). By determining the health status of marine mammals, it is possible to identify anthropogenic influences (such as exposure to pollutants and terrestrial pathogens)

on marine environmental health itself, and on the well-being of these sensitive animals (Gulland and Hall 2007; Harwood 2001; Kennedy-Stoskopf 2001; Wells et al. 2004).

The aim of this paper is to critically examine the available information on the contaminants, diseases and pathogens that exist in Guiana dolphins (*Sotalia guianensis*), and to evaluate the vulnerability of this coastal dolphin to such environmental stressors.

2 Guiana Dolphins as Sentinels

The Guiana dolphin (*S. guianensis*) is a coastal dolphin with an apparently continuous distribution from Santa Catarina, Southern Brazil, to Honduras, in the Caribbean coast (Figs. 1 and 2) (Flores and da Silva 2009). This species inhabits coastal waters associated with shallow and protected estuarine areas, generally at depths of less than 30 m, where individuals feeds on pelagic and semipelagic prey in the near shore ecosystem (Di Beneditto and Ramos 2004; Di Beneditto and Siciliano 2007; Flores and da Silva 2009).

Fig. 1 Guiana dolphins (*Sotalia guianensis*) group photographed in Sepetiba Bay southeastern Brazil showing some morphology characteristic of the species: Slightly triangular and wide-based dorsal fin; rounded melon, which is not separated from the beak by a distinct crease; color *brownish–gray* on the upper surface, fading to *light gray* (often with a pinkish tinge) on the belly (Flores and da Silva, 2009). Photo: Leonardo Flach—Projeto Boto-cinza

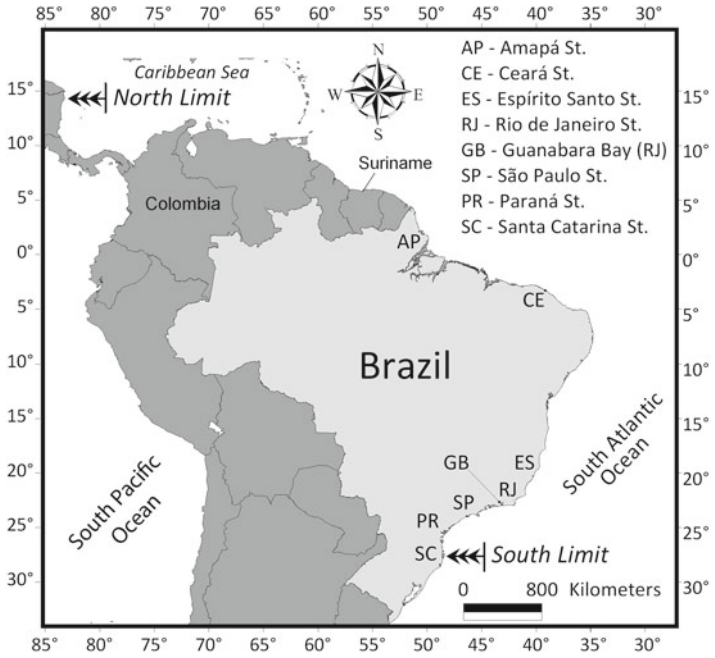

Fig. 2 Distribution of Guiana dolphins (*Sotalia guianensis*) from Nicaragua (Caribbean Sea; North Limit) to Santa Catarina (Southern Brazil; South Limit). The acronyms refer to the states mentioned in the text as other sites cited. GB = Guanabara Bay (RJ)

The most important threats to conserving this species along its distribution are fishery interactions. Mortality of Guiana dolphins from fishery activities has been reported from their entanglement in fishing gear, such as gillnets, driftnets, beach seines, trawling nets, longlines, and fixed traps (Siciliano 1994). However, most mortalities (>90%) have been suffered from use of gillnets (Crespo et al. 2010; Moura et al. 2009b). Nonlethal injuries caused by fishing artifacts and ingestion of plastic debris have also been described for Guiana dolphins (Azevedo et al. 2009; Geise and Gomes 1996; Nery et al. 2008). Habitat loss is increasingly a threat to *S. guianensis* populations, especially those located near coastal populated cities, areas of intense tourism, coastal waters with aquaculture activities and around harbors. The human population density around such habitats, and associated human activities, may produce cumulative, additive, or synergistic effects (e.g., noise, chemical pollution, eutrophication, rising oxygen demand, among others) that enhance the risk to dolphin populations (Crespo et al. 2010).

As a result of the coastal distribution, trophic composition, and biological characteristics *of S. guianensis,* this species is highly exposed to environmental contaminants that are released from industrial and agricultural sites and from cities (Crespo et al. 2010; Kajiwara et al. 2004; Moura et al. 2009a; Van Bressem et al. 2009a, b; Yogui et al. 2003).

Unlike most cetaceans and certain other marine mammals, *S. guianensis* shows a strong pattern of site fidelity (Azevedo et al. 2007; Flores and Bazzalo 2004), i.e., the home ranges for *S. guianensis* have been calculated to be 15 and 135 km^2 for Baía Norte (Southern Brazil) and Guanabara Bay, respectively (Azevedo et al. 2007; Flores and Bazzalo 2004). Thus, the chemical and biological contaminants that they accumulate reflect a more local than regional character. Guiana dolphins have a thick layer of blubber that is important to their thermoregulation, energy storage, and buoyancy (Geraci and Lounsbury 2005). Unfortunately, this blubber layer has the ability to accumulate high levels of the lipophilic contaminants present in their prey species, such as organochlorine compounds (Siciliano et al. 2005).

Monitoring programs have been carried out throughout the distribution area inhabited by *S. guianensis* to better understand the biology and natural ecology of this dolphin, to identify potential threats, and to develop conservation measures for this endangered species. In addition, biological data collected and stored from stranded and caught dolphins can be used to evaluate the vulnerability of this species to environmental contamination.

By evaluating the health status of dolphins found ashore or captured by gillnets, both natural and anthropogenic stressors on the marine environment can be identified, and, moreover, these dolphins can serve as sentinels of the environmental health and can reflect the health status of lower trophic levels in the marine ecosystem (Bossart 2011; Siciliano et al. 2005; Wells et al. 2004). Guiana dolphins bioaccumulate the metals and organochlorine substances that they are exposed to in their prey (Di Beneditto and Ramos 2004). Because they are known to live about 30 years, exposures to such persistent contaminants (e.g., mercury, cadmium, and lead, and the organochlorines) are chronic.

Additionally, Guiana dolphins are very charismatic marine mammal species that evoke strong human emotions. As an example, Filla et al. (2012) recently conducted a study to evaluate how tourists value Guiana dolphin-watching activities in Cananéia Bay, on the southern coast of São Paulo state, Brazil. According to these authors, tourist satisfaction and the economic value (US$ 556,734 over two years of activities) in observing dolphins is relatively important, and exceeds the importance of certain other tourist activities in Cananéia. The use of this dolphin as a sentinel species may, thus, enhance human attention to the deterioration of oceanic health and to conservation problems (Bossart 2011).

3 Hazardous and Persistent Chemical Contaminants

3.1 Persistent Organic Contaminants

Few studies have been conducted on the contaminants present in marine mammals along the South Atlantic Ocean. Globally, most studies have been carried out in the North Atlantic and North Pacific oceans (Aguilar et al. 2002). The scientific studies

carried out in Brazilian waters have been concentrated in the southeast region, which is the most populated and consequently, most polluted region (e.g., Rio de Janeiro and São Paulo states), and is home to several universities and ecotoxicology specialists.

The concentrations of organochlorine (OC) residues found in the blubber of Guiana dolphins (*S. guianensis*), collected at different points in its distribution area, are presented in Table 1.

The earliest analytical monitoring study performed with Guiana dolphins was conducted by Koeman et al. (1972). These authors detected low levels of the polychlorinated biphenyls (PCBs) (<0.4 μg g^{-1}) in a specimen collected on the coast of Suriname. Later, in 1977 Duinker et al. (1989) analyzed PCB concentrations in a male and female *S. guianensis* sampled from the Colombia coast, and found 7.26 and 9.14 μg g^{-1} of these compounds, respectively. After these studies, 10 years elapsed before additional cetacean data from Brazilian waters surfaced. Recent analyses have identified the presence of novel toxicants and purport to show a variety of impacts to exposed organisms (Kajiwara et al. 2004; Yogui et al. 2003). Some researchers have shown that high levels of persistent organic pollutants exist in this species. High mean PCBs levels were observed in mature males sampled from the coast of the São Paulo and Paraná states (79 μg g^{-1}), whereas mature females were 1/3 less contaminated (Kajiwara et al. 2004). At Guanabara Bay, Lailson-Brito et al. (2010) also found high levels (39.35 ± 31.41 μg g^{-1}) in males, varying from 6.66 to 99.17 μg g^{-1}. In females, the highest level of PCB detected was 60.09 μg g^{-1}, with an average value of 31.63 μg g^{-1}. Guiana dolphins accidentally caught at the coast of Cananéia and Baixada Santista, São Paulo State, presented average concentrations of 10.8 μg g^{-1} in males and 16.4 μg g^{-1} in a sexually mature female (Alonso et al. 2010). Further, in the estuary of Cananéia, Yogui et al. (2003) analyzed nine samples of *S. guianensis* and found relatively low PCBs concentrations (viz., 5.7 in males and 3.74 μg g^{-1} in females), with the highest concentration found in a male (9 μg g^{-1}). These PCB levels in the blubber of Guiana dolphins are probably related to the industrial activities conducted in São Paulo and Rio de Janeiro State, the two most industrialized states of Brazil. The mean concentrations of PCBs in studies carried out with this species in Brazilian waters are quite similar and sometimes higher than those found in studies conducted with small cetaceans in other regions of the world (Fig. 3).

High DDTs levels were observed in male *S. guianensis* from Cananéia, São Paulo (72 μg g^{-1}), and the highest concentration recorded was 125 μg g^{-1} (Yogui et al. 2003). These authors found considerably lower mean concentrations in females (6.81 μg g^{-1}, with maximum concentrations of 9 μg g^{-1}), when compared to males. Similarly, Kajiwara et al. (2004) detected a mean concentration of 52 μg g^{-1} of DDT in males sampled at the coast of São Paulo and Paraná, with 150 μg g^{-1} being the highest level detected. In females, the average and highest concentrations found were 7.6 and 29 μg g^{-1}, respectively. A recent study carried out on the northern coast of Brazil (Amazon coast) showed extremely low levels of ∑DDT in blubber of Guiana dolphins (mean: 0.201 μg g^{-1}), which varied from 0.014 to 0.438 μg g^{-1} (Emin-Lima 2012). Although DDT was extensively used in Pará state, these results

Table 1 Concentrations of organochlorine residues (μg g^{-1} lipid wt) in the blubber of *S. guianensis* collected at localities along its distribution

N	TL (cm)	ΣPCB	ΣDDT	ΣHCH	ΣCHL	HCB	Mirex	Locality	References
9	163–197	4.61 ± 3.31 (0.2–9.22)	35.9 ± 46.8 (0.541–125)	0.016 ± 0.017 (<0.003–0.044)	0.024 ± 0.013 (0.001–0.047)	0.015 ± 0.009 (ND–0.024)	0.151 ± 0.085 (0.014–0.312)	Cananéia, São Paulo, São Paulo State, BR	Yogui et al. (2003)
3	163–186	47.78 (25.87–66.03)	34.03 (16.91–48.04)	0.07 (0.06–0.07)	0.33 (0.3–0.39)	0.11 (0.08–0.14)	1.26 (0.57–1.87)	Ubatuba, São Paulo State, BR	Alonso et al. (2010)
3	122–173	39.69 (27.86–61.34)	36.98 (24.57–55.91)	0.09 (0.03–0.21)	0.30 (0.11–0.49)	0.12 (0.07–0.17)	0.76 (0.24–1.04)	Baixada Santista, São Paulo State, BR	Alonso et al. (2010)
1	196	1.97	5.87	0.011	0.014	0.067	0.046	São Paulo State, BR	Yogui et al. (2010)
26	89–198	(1.3–79)	(1–150)	(<0.001–0.061)	(0.0063–1.1)	(0.0016–0.40)	–	Northern Paraná State and São Paulo State, BR	Kajiwara et al. (2004)
12	122–191	34.8 ± 26.3 (6.7–99.2)	7.9 ± 6.9 (2.1–21.5)	–	–	0.046 ± 0.037 (<0.004–0.109)	–	Guanabara Bay, Rio de Janeiro State, BR	Lailson-Brito et al. (2010)
15	147–198	4.6 ± 4 (0.76–14.3)	5.7 ± 5.8 (0.98–23.5)	–	–	0.041 ± 0.040 (<0.004–0.156)	–	Paranaguá Bay, Paraná State, BR	Lailson-Brito et al. (2010)
5	150–195	12.3 ± 11.7 (1.7–25.5)	3.9 ± 3.9 (0.65–9.99)	–	–	0.029 ± 0.028 (0.013–0.078)	–	Sepetiba Bay, Rio de Janeiro State, BR	Lailson-Brito et al. (2010)
2	–	(7.26–9.14)	(51.18–63.33)	–	–	–	–	Colombia	Duinker et al. (1989)
1	–	<0.4	2.77	–	–	–	–	Suriname	Koeman et al. (1972)
8	150–195	21.6 (2.1–44.9)	8.37 (1.11–21.19)	–	–	–	–	Sepetiba and Guanabra Bays, Rio de Janeiro State, BR	Torres et al. (2006)
5	73–198	2.3 ± 2.6 (0.11–7.65)	0.6 ± 0.42 (0.05–5.42)	–	–	–	–	Rio de Janeiro State	Lailson-Brito et al. (2004)
36	158.7 (mean)	–	0.201 (0.014–0.438)	–	–	–	–	Amazon coast (Northern Brazil)	Emin-Lima (2012)

TL = Total length
ND = Non detected

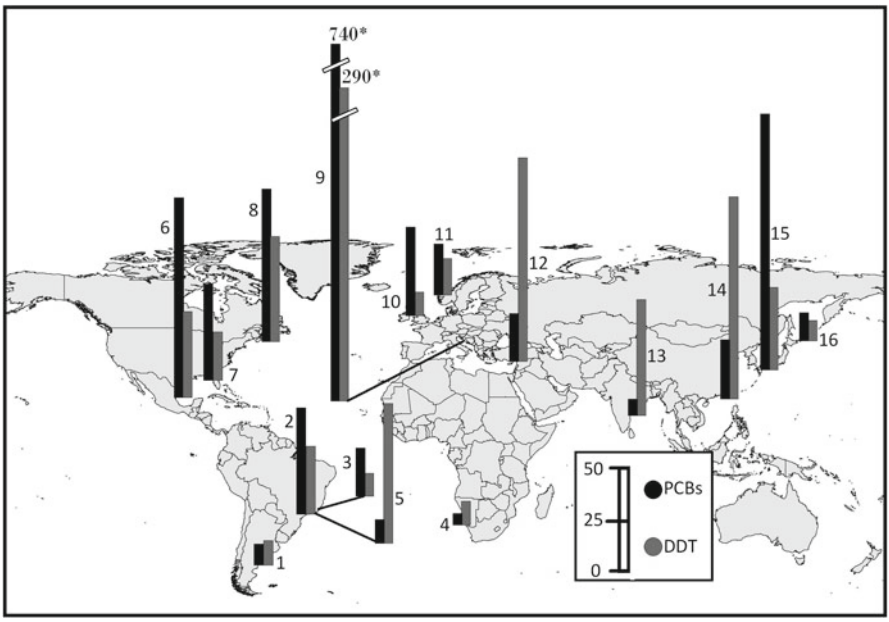

Fig. 3 Comparison of mean concentrations of PCBs and DDTs in the blubber of small cetaceans (males) from various regions (levels in ppm). 1, *Phocoena spinipinnis* (Corcuera et al. 1995); 2, *S. guianensis* (Kajiwara et al. 2004); 3, *S. guianensis* (Lailson-Brito et al. 2010); 4, *Lagenorhynchus obscurus* (Kock et al. 1994); 5, *S. guianensis* (Yogui et al. 2003); 6, *Tursiops truncatus* (Kuehl and Haebler 1995); 7, *Steno bredanensis* (Struntz et al. 2004); 8, *Delphinapterus leucas* (Muir et al. 1996); 9, *T. truncatus* (Corsolini et al. 1995); 10, *Phocoena phocoena* (Kuiken et al. 1993); 11, *P. phocoena* (Kleivane et al. 1995); 12, 16, *P. phocoena* (Tanabe et al. 1997); 13, *Sousa chinensis* (Prudente et al. 1997); 14, *Neophocaena phocaenoides* (Minh et al. 1999); 15, *N. phocaenoides* (JEA 1999)

indicate that the Amazon coastal waters present low levels of the pollutant and may not be a problem for the conservation of those population of dolphins.

At the Guanabara and Sepetiba Bays, located in the Rio de Janeiro State, Torres et al. (2006) reported mean concentrations of 8.37 μg g^{-1} for males, and a maximum of 21 μg g^{-1}. In Guanabara Bay, Lailson-Brito and collaborators (2010) detected mean DDT concentrations of 5.06 μg g^{-1}, and a maximum concentration of 8.05 μg g^{-1} in females. In males, these authors found average levels of 31.63 μg g^{-1} of DDT, reaching 60.09 μg g^{-1} as the highest level. These results demonstrate that the southern and southeastern coasts of Brazil are severely contaminated by the DDT chemical complex. This probably resulted from the intensity of agricultural activities in these regions, wherein DDT was extensively used in the past for agricultural development and for controlling of vector-borne diseases. Because of DDT's high environmental persistence and bioaccumulative capability throughout the food web, this compound was detected as early as the 1970s in human tissue samples (Grisolia 2005). Among all analyses performed on this compound in the

blubber of Guiana dolphins, the metabolite present at the highest level was always p′p-DDE, indicating that DDT has not recently been released to the environment. However, Moura et al. (2009a) analyzed organochlorine pesticides in a milk sample and found a low proportion of p-p′-DDE (9%) in comparison to the p-p′-DDT concentration, which was high, indicating ongoing DDT contamination of the marine environment in the north coast of the Rio de Janeiro state, where this study was carried out.

The PCB- and DDT-residue concentrations, therefore, suggest that the southern and southeastern Brazilian coastal areas are contaminated by these chemicals, which may be enhanced by industrial activities in these coastal regions (Kajiwara et al. 2004; Torres et al. 2006).

Figure 3 shows a geographic comparison of the average levels of PCBs and DDT that exist in the blubber of small cetaceans worldwide. The residues found in females were excluded to avoid the differences that result from the transference of contaminants through gestation and lactation. However, other differences, such as prey consumed by different species of small dolphins (or even in the same species in different localities), age, and excretion and assimilation of contaminants may affect the variability of these contaminants worldwide (Aguilar et al. 2002; Kajiwara et al. 2004). Aguilar et al. (2002) reported that the concentrations of organochlorine compounds frequently found in cetacean tissues tend to be more intense in the northern hemisphere than in the south. However, the concentrations of OCs found in *S. guianensis* blubber are similar to those found in small cetaceans of North America, Europe, and Asia (Fig. 3).

Low levels of other organochlorine compounds such as HCH (hexachlorocyclohexane) and HCB (hexachlorobenzene) in Guiana dolphins were also reported in the studies we reviewed (Table 1). The lower levels detected in the tissue of the Guiana dolphins found along the Brazilian coast may be attributed to the volatile nature of these chemical compounds, which tend to be carried by the atmospheric circulation from low to high latitudes. Therefore, it is expected to find lower levels of these compounds in tropical regions (Tanabe et al. 1994; Yogui et al. 2003).

Among HCH isomers that were found as tissue contaminants, the β-HCH was predominant, whereas only low concentrations of α-HCH existed. This may indicate that cetaceans have the capability to metabolize α-HCH. Or perhaps this is simply a reflection of the high environmental persistency of β-HCH (Minh et al. 1999). The concentrations of the commercial organochlorine insecticides chlordane, mirex, and dieldrin reflect the use of these contaminants in Brazil (Kajiwara et al. 2004; Yogui et al. 2003).

Most studies show differences of organochlorine concentrations between sexes. This reflects the transference of OCs from the mother to the newborns through placental transfer and via lactation. The amount and diversity of organochlorine pesticide residues in the milk of a lactating *S. guianensis* found by Moura et al. (2009a) confirms this trend. Usually, OC concentrations are similar in immature males and females, and increase with age until sexual maturity, after which they continue to increase in males but either plateau or slightly decrease in females through transference of the burden to the calf. Residue levels may also increase in senescent females (Wells et al. 2005).

Other persistent organic toxicants such as perfluorinated compounds (PFCs) have been recently assessed in liver samples of Guiana dolphins from the Brazilian coast. Among the PFCs, perfluorooctane sulfonate (PFOS) has shown more capacity to bioaccumulate at high levels in dolphin tissue (Dorneles et al. 2010; Quinete et al. 2011). Dorneles et al. (2010) detected high levels of PFOS in 23 liver samples of *S. guianensis* from Guanabara Bay (Rio de Janeiro State), and six samples distributed among other localities in the Rio de Janeiro state. The individuals that were sampled from Guanabara Bay, where there is history of contamination, showed concentrations varying from 43 to 2,431 ng g^{-1}, whereas dolphins from other localities showed concentrations varying from 76 to 427 ng g^{-1} (dry wt). Three samples from fetuses showed high levels of PFOS (666–1590 ng g^{-1}), indicating that maternal transfer does indeed occur. The concentrations of this pollutant in the Guiana dolphins from Guanabara Bay are among the highest detected to date in any marine mammal species. In addition, Quinete et al. (2009) analyzed drinking water and tissue samples from fish and Guiana dolphins ($n = 10$) sampled from the northern coast of the Rio de Janeiro state. The authors found PFOS levels in dolphin liver that ranged from 25.9 to 149 ng g^{-1} (wet wt; 90.5 ± 42.4 ng g^{-1}). The concentrations observed in dolphins were higher than those in water and fish.

Analyses of the polybrominated diphenylethers (PBDEs) have been conducted on *S. guianensis* samples collected from the Brazilian coast (Dorneles et al. 2010; Quinete et al. 2011). Dorneles et al. (2010) analyzed PBDEs in several species of cetaceans, including *S. guianensis* from the southeastern coast of Brazil. The concentration of PBDEs in liver tissue of Guiana dolphins varied from 260 to 1620 ng g^{-1} (lipid wt, "lwt") in males ($n = 13$; 670 ± 430) and from 13 to 450 ng g^{-1} lwt in females ($n = 06$; 160 ± 150 ng g^{-1}). Quinete et al. (2011) detected PBDEs in *S. guianensis* individuals accidentally killed in fishing nets in the northern coast of the Rio de Janeiro state. They found mean levels of 53 ng g^{-1} wet wt. There was also evidence of placental transference in this species. PBDEs were detected in organisms from several remote areas, highlighting the environmental persistence and global distribution of these compounds (Dorneles et al. 2010). For example, Dorneles et al. (2010) found high levels, not only in dolphins that inhabit the Continental Shelf (e.g., genus *Stenella*), but also in oceanic species such as the false killer whale (*Pseudorca crassidens*). PBDEs are structurally similar to the PCBs and DDT, and pose potential toxic risks to exposed organisms (Tanabe 2004).

The concentrations of the organic toxicants reported in Guiana dolphin tissues reflect their environmental persistence and their distribution along the marine trophic systems of the South Atlantic Oceanic coasts. For example, persistent organic pollutants (e.g., PCBS, and organochlorine pesticides) have been detected in water (Pereira and Tommasi 1985), sediment (Muniz et al. 2006), and many marine species from the Brazilian coast, including: mussels and sponges (Liebezeit et al. 2011), shrimp (Gorni and Weber 2004), crabs (Gorni and Weber 2004; Magalhaes et al. 2012), fish/sharks (Lavandier et al. 2013; Quinete et al. 2011; Silva et al. 2009), seabirds (Colabuono et al. 2012), and sea turtles (Liebezeit et al. 2011). Further studies are required to infer how these widespread and harmful synthetic compounds act to compromise the survivorship of this vulnerable small cetacean.

3.2 Contamination by Metals

Metal accumulation is thought to occur in all species of cetaceans, and some bioavailable elements enter the food web and bioaccumulate in marine top predator species (Kunito et al. 2004; Moura et al. 2011a, b). Although metals occur naturally (e.g., soil erosion, volcanic activity), human activities have also released them into the environment (Moura et al. 2011a, b). The degree to which metals are absorbed and accumulate depends on the biological, ecological, and physiological mechanisms of each predator species, and is a function of the exposition, absorption, and elimination of the metals present in lower trophic level organisms. Guiana dolphins, have a small coastal home range, and are vulnerable to the effects of metals, like mercury, that are released by human activities in coastal zones. Relatively high levels of metals, especially mercury, have been detected in Guiana dolphin samples from the Brazilian coast. In studies carried out in northern coast of the Rio de Janeiro State and the Verificar, southern Brazil, mercury concentrations in dolphin samples were detected at levels of 22 and 190 μg g^{-1} wet wt respectively (in liver) (Castelo et al. 1996, 2008). Average cadmium concentrations were 0.34 μg g^{-1} wet wt (in liver) in Northern Rio de Janeiro, 0.8 μg g^{-1} wet wt (in kidney) in the Ceará State, 0.11 μg g^{-1} wet wt (in liver) in Guanabara and Sepetiba Bays, Rio de Janeiro and 1.18 μg g^{-1} wet wt (in kidney) from other places located in Rio de Janeiro (Carvalho et al. 2008; Dorneles et al. 2007; Lailson-Brito et al. 2000; Monteiro-Neto et al. 2003). Moura et al. (2011a, b) detected low levels of total Hg (0.2–1.66 μg g^{-1} wet wt) in muscle tissue of 20 Guiana dolphins stranded at the central-north coast of Rio de Janeiro state, Brazil. Lopes and collaborators (2008) detected mercury levels up to 55.6 μg g^{-1} dry wt in liver samples from the Espírito Santo coast. Carvalho et al. (2008) also found zinc concentrations ranging from 66 to 107 μg g^{-1} wet wt (in liver) in *S. guianensis* samples from the northern coast of Rio de Janeiro State. These results reflect the existence of extensive environmental contamination by these elements. Cadmium levels, however, were very low in all the reviewed studies. According to Lailson-Brito et al. (2000), the low cadmium levels that do exist in Guiana dolphin tissues do not accurately reflect environmental concentrations, since, for example, the Sepetiba Bay is extensively polluted by this contaminant. Mercury levels were relatively low in the Rio de Janeiro State, as described in other studies; however, the levels measured by Castelo et al. (1996) in the Paraná State indicated that the marine environment is highly polluted by this element. A study carried out by Moura et al. (2012) showed low levels (i.e., 0.07–0.79 μg g^{-1} wet wt) of total Hg in muscle tissues of accidentally captured Guiana dolphins from the Amazon coast. Recently, Emin-Lima (2012) analyzed total Hg in Guiana dolphins stranded at three locations of the north coast of Brazil, including Pará and Amapá states. Low levels were found, varying from 0.003 to 1.276 μg g^{-1} wet wt. These results suggest that the Amazon coast contains low levels of Hg. In addition, the results also suggest that the seaward transport of water and sediment by the Amazon River is not an important contributor to coastal contamination by Hg. Figure 4 compares mean concentrations of mercury (Hg) in liver of small cetaceans collected from various regions (levels in ppm, wet wt).

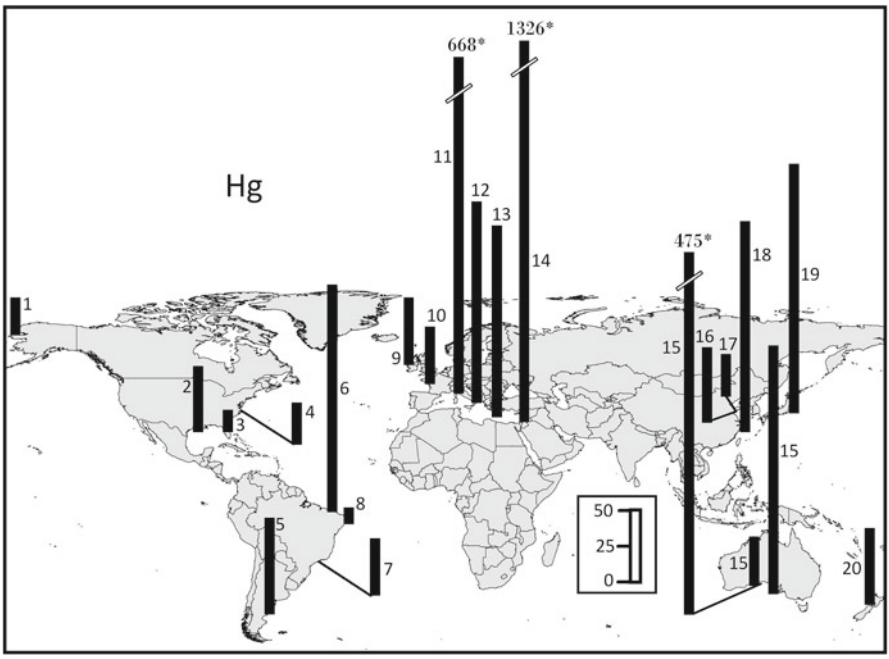

Fig. 4 Comparison of mean concentrations mercury (Hg) in liver of small cetaceans from various regions (levels in ppm, wet weight). Some levels presented in dry weight were converted to wet weight according to Yang and Miyazaki (2003). 1, *Delphinapterus leucas* (Becker et al. 1995); 2, *Tursiops truncatus* (Rawson et al. 1993); 3, *T. truncatus* (Beck et al. 1997); 4, *T. truncatus* (Kuehl and Haebler 1995); 5, *T. gephyreus* (Marcovecchio et al. 1990); 6, *S. guianensis* (Monteiro-Neto et al. 2003); 7, *S. guianensis* (Kunito et al. 2004); 8, *S. guianensis* (Carvalho et al. 2008); 9, *S. guianensis* (Lopes et al. 2008); 10, *T. truncatus* (Carvalho et al. 2002); 11, *Delphinus delphis* (Holsbeek et al. 1998); 12, *Stenella coeruleoalba* (Andre et al. 1991); 13, 14, *S. coeruleoalba* (Roditi-Elasar et al. 2003); 15, *Grampus griseus* (Shoham-Frider et al. 2010); 16, *T. truncatus*, *T. aduncus*, *D. delphis* (Lavery et al. 2008); 17, *Souza chinensis* (Parsons 1998); 18, *S. chinensis* (Hung et al. 2007); 19, *S. coeruleoalba* (Itano et al. 1984); 20, *G. griseus* (Endo et al. 2004); 21, *D. delphis* (Stockin et al. 2007)

Seixas et al. (2009) studied the accumulation of trace elements (viz., As, Cd, Cu, Pb, Se, and Ag) in the livers of Guiana dolphins, Atlantic spotted dolphins (*S. frontalis*) and Franciscana dolphins (*P. blainvillei*) along the northern coast of the Rio de Janeiro state. The authors found relatively high mean concentrations of Se (20.70 μg g^{-1} dry wt) and lower levels of As, Cd, Pb, Ag, and Cu, in comparison with previous studies conducted with small cetaceans along the Southwest Atlantic coast. These authors concluded that not only were environmental conditions different, but also the total length of the dolphins, and, (mainly) their feeding habits influenced the accumulation of trace elements in the liver of these cetacean species.

Kunito et al. (2004) analyzed 22 trace elements in liver samples of several small cetaceans (including 20 Guiana dolphins) incidentally caught on the coast of São Paulo State, Brazil. The highest concentrations of trace elements were observed for

Fe (794 μg g^{-1} dry wt), Cu (157 μg g^{-1} dry wt), Zn (192 μg g^{-1} dry wt), and Hg (77 μg g^{-1} dry wt). However, very high concentrations of Cu (262–1970 μg g^{-1} dry wt) and Zn (242–369 μg g^{-1} dry wt) were detected in the liver of sucklings. Although anthropogenic emissions of trace elements are much lower in the Southern vs. the Northern Hemisphere, the hepatic concentrations found in this study were comparable to those in marine mammals of the Northern Hemisphere. The concentrations of Hg in samples collected from the coasts of Paraná, São Paulo and Espírito Santo state, indicate that Guiana dolphins may be exposed to high levels of this element, and these levels could be detrimental to their health status (Table 2). As an example, Rawson et al (1993) detected liver abnormalities in bottlenose dolphins (*Tursiops truncatus*) that were associated with total Hg-liver levels up to 61 μg g^{-1} wet wt.

In a recent study, high concentrations of tin was identified in the liver of five Guiana dolphins sampled from the Guanabara Bay; tin levels ranged from 1,249 to 5,191 ng g^{-1} wet wt. These results contrasted with levels found in *S. guianensis* collected from the Espírito Santo State, where sampled animals retained low levels of tin (i.e., from 346 ng g^{-1} to below the detection limit of 150 ng g^{-1}) (Dorneles et al. 2008). The high concentrations of tin in Guiana dolphins from the Guanabara Bay reflect the current state of this estuary, in that this Bay is highly contaminated by organotins. Despite several negative effects that can be caused by the organotins on marine organisms, it continues to be used legally as an antifouling agent in Brazilian waters.

The presence of the radio isotope Polonium-210 (^{210}Po) has been recently assessed in samples of small cetaceans that were found stranded along the coast of Rio de Janeiro state (Godoy et al. 2012). Few studies have addressed the toxicity of ^{210}Po to cetaceans. Godoy et al. (2012) analyzed nine muscle and four liver samples for ^{210}Po in Franciscana (*Pontoporia blainvillei*) and Guiana dolphins (*Sotalia guianensis*). The mean concentrations in muscle and liver were respectively 25.3 ± 5.7 and 292 ± 106 Bq kg (wet wt). Levels reported in this study for muscle tissue were lower than those reported in studies from other regions around the world, while liver concentrations were more than two times those detected in other studies.

4 Emerging Diseases and Their Possible Link to Environmental Stressors

South American researchers are still in the early phase of studying the epizootiology of infectious diseases in marine mammals (Van Bressem et al. 2007a, b; Van Bressem et al. 2009a, b). However, studies dedicated to skin and skeletal diseases have increased from the large number of bone samples now available and some long-term studies with stranded free-ranging cetaceans (Flach 2006; Fragoso 2001; Laeta et al. 2010; Van Bressem et al. 2007a, b). Small dolphins from South American waters are affected by a variety of congenital, traumatic, infectious, and parasitic diseases (Van Bressem et al. 2007a, b). The sources of some of these pathogens may be from release of industrial and agricultural waste and/or treated or untreated waste

Table 2 Concentrations of cadmium (Cd), mercury (Hg), and lead (Pb) in samples of kidney and liver of *S. guianensis* collected at localities along its distribution

			Cadmium		Mercury		Lead		
Locality	*N*	Sex	kidney	Liver	Muscle	Liver	kidney	Liver	References
Ceará State, BR[a]	11	M+F	0.234 (0.003–1.23)	0.066 (0.003–0.4)		1.39 (0.03–8.85)	0.03 (0.03–0.38)	0.03 (0.03–0.04)	Monteiro-Neto et al. (2003)
São Paulo and Paraná State, BR[a]	20	M+F	–	0.2 (0.003–0.66)	–	23.1 (0.45–114)	–	0.021 (0.005–0.06)	Kunito et al. (2004)
North coast of Rio de Janeiro State, BR	6	M+F	–	0.33 (0.18–0.56)	0.73 0.34–1.42	9.98 (1.10–21.7)	–	–	Carvalho et al. (2008)
Rio de Janeiro State, BR	8	M	0.23 (<0.019–0.43)	0.05 (<0.028–0.23)	–	–	–	–	Lailson-Brito et al. (2000)
Amapá State (Amazon coast)	27	M+F	–	–	0.4±0.16 (0.07–0.79)	–	–	–	Moura et al. (2012)
North coast of Rio de Janeiro State, BR[a]	29	M+F	–	–	–	2.66 (0.25–26.37)	–	–	Kehrig et al. (2008)
North coast of Rio de Janeiro State, BR	21	M+F	–	0.39±0.37 (0.001–1.48)	–	–	–	1.55±0.75 (0.74–2.73)	Seixas et al. (2009)
Guanabara Bay, Rio de Janeiro State, BR	15	M+F	–	–	0.7 (0.2–2.5)	1.62 (1.1–132.6)	–	–	Kehrig et al. (2004)
Amazon coast (Northern Brazil)	27	M+F	–	–	0.003–1.27	–	–	–	Emin-Lima (2012)
Guanabara Bay, Rio de Janeiro State, BR	24	M+F	–	–	–	(0.53–132.62)	–	–	Lailson-Brito et al. (2008)
Espírito Santo State, BR	5	M+F	–	–	1.8±0.46	55.6±106	–	–	Lopes et al. (2008)

Concentrations in ng g^{-1} wet wt

[a]Converted from dry weights assuming 70% moisture content (Yang and Miyazaki 2003)

from cities; some of these pathogens may cause the infectious diseases found in Guiana dolphins (Higgins 2000).

Toxoplasmosis is caused by *Toxoplasma gondii,* a single-celled protozoal parasite. It is a potentially fatal human and animal disease (Bossart 2011). Bandoli and Oliveira (1977) reported the first case of toxoplasmosis for marine mammals in a individual *S. guianensis* collected in Guanabara Bay, Rio de Janeiro State, Brazil. Wild and domestic felids are the only known definitive hosts for *T. gondii*, but many mammals, including humans and marine mammals, can be infected. Infection occurs through the ingestion of contaminated food or water, and may be transferred transplacentally. The toxoplasmosis case that occurred in the Guanabara Bay was correlated with the release of untreated waste into the Bay. Recently, Gonzales-Viera et al. (2013) reported another case of toxoplasmosis for a Guiana dolphin stranded in Cananéia Bay, at the southern coast of São Paulo state, Brazil. According to these authors, the sewage run-off from the main urban areas and the presence of domestic and wild felids in areas surrounding the bay could be a source of *T. gondii* oocysts from land to sea. The specimen from Cananéia was negative for morbillivirus and did not present high levels of organochlorine pollutants in its tissues. Such contamination was expected, because clinical toxoplasmoses cases have generally been associated with immune suppression in marine mammals, as has exposure to persistent contaminants, such as polychlorinated biphenyls (PCBs) and infection by morbilliviruses (Di Guardo et al. 2005; Mikaelian et al. 2000).

Altieri et al. (2007) recently reported pathologic findings in tissues of an emaciated Guiana dolphin that were associated with infection caused by *Giardia* sp. *Giardia* is flagellated protozoan parasites that infests the small intestine of several vertebrate species, including pinnipeds, cetaceans, and humans, as well as domestic animals. The infestation of *Giardia* causes giardiasis, an infection characterized in humans with symptoms such as diarrhea, abdominal cramps, bloating, malabsorption, and weight loss (Feng and Xiao 2011). The infected Guiana dolphin was found in the Ceará State, northeastern Brazil. Cysts of this protozoan can survive in water for weeks and can be transmitted by the fecal–oral route between humans and animals (Fayer et al. 2004). This intestinal parasite is one of the most common in humans, causing about 200 million symptomatic infections annually in people from Africa, Asia, and Latin America (Feng and Xiao 2011). *Giardia* can be found in coastal waters and may contaminate a variety of shellfish and infect many species of marine mammals. The presence of this parasite causes concern for the health of animals in coastal waters, and for humans who eat raw shellfish, or use these waters for recreation (Altieri et al. 2007; Fayer et al. 2004). According to Measures and Olson (1999), the release to the marine environment of untreated human and domestic animal effluents that are contaminated by *Giardia* cysts may be the main source of this parasite in dolphins.

Skin lesions have been detected in cetaceans in several regions around the world. Despite the unknown etiology of these lesions, and of the absence of any clear association with environmental stressors, authors have attributed skin diseases in cetaceans to the compromised state of the marine environment (Van Bressem et al. 2009a, b; Van Bressem et al. 2007a, b). Coastal distribution and home ranges of

S. guianensis expose this small dolphin to microbial and chemical contamination, which could trigger or facilitate the occurrence of skin diseases, such as those that have afflicted dolphins.

One confirmed case of lobomycosis and other possible cases (referred to as lobomycosis-like disease; LLD) in Guiana dolphins have been reported. The confirmed case was reported in an adult female from the estuary of the Surinam River in 1971 (De Vries and Laarman 1973), and the LLD cases were recently reported after examination of free-ranging dolphins in the Paranaguá estuary (Paraná state, Southern Brazil), where the disease occurred in 3.9% of the Guiana dolphin population as estimated by photo-identification studies ($n = 103$) (De Vries and Laarman 1973; Siciliano et al. 2008; Van Bressem et al. 2009a, b). Lobomycosis is a chronic cutaneous and subcutaneous fungal granulomatous disease, caused by *Lacazia loboi*, an uncultivated fungal pathogen. LLD has evolved over the years and is associated with death in affected dolphins (Van Bressem et al. 2009a, b). In the Americas, *L. loboi* naturally affects *T. truncatus* and *S. guianensis,* as well as humans. In humans, lobomycosis acts as a chronic fungal skin infection that mainly occurs in rural areas of South and Central America, where this disease is endemic (Brito and Quaresma 2007). According to Haubold et al. (2000) the etiologic agents responsible for lobomycosis in humans and dolphins are not the same; notwithstanding, evidence from serologic data suggests that humans and dolphins are infected by the same *L. loboi* strains (Mendoza et al. 2008). In dolphins, lobomycosis is distributed from southern Brazil to the Gulf of Mexico and Atlantic coast of Florida (Reif et al. 2006). Van Bressem and collaborators (2007a, b) reported several cases of lobomycosis in coastal *T. truncatus* from different localities of South America, including Bahia Malaga (Colombia), Guayaquil Gulf (Ecuador), Callao (Peru), and Santa Catarina (Brazil). This disease has an unknown etiology and seems to be emerging in some populations of small cetaceans, especially bottlenose dolphins. Van Bressem et al. (2007a, b) has speculated that this possibly emerging disease may be associated with exposure to persistent contaminants present in the marine environment, as well as exposures to the ballast water from ships that could disseminate alien microorganisms including *L. loboi*.

Another skin disease that has recently emerged in dolphin populations worldwide is the tattoo skin disease (TSD). This disease is characterized by irregular typical stippled skin lesions that can occur in any region of the body, and has been reported to occur in *S. guianensis* from the Paraná state and Sepetiba Bay, Rio de Janeiro State (Flach et al. 2008). TSD lesions have only been reported in adults. No cases of TSD were reported for the 91 *S. guianensis* incidentally caught and examined in the northern coast of the Rio de Janeiro State, but this population could be at risk from contact with infected dolphins (Van Bressem et al. 2007a, b). Other cases of TSD have been reported in small cetaceans from other coastal regions of South America, such as in Peru (*Delphinus capensis, Tursiops truncatus*), Ecuador (*Delphinus delphis*), Chile (*Cephalorhynchus eutropia, T. truncatus, Phocoena spinipinnis*), and Argentina (*Cephalorhynchus commersonii, T. truncatus*). This disease is highly prevalent and possibly endemic in small Peruvian cetaceans, affecting mainly juveniles. It is caused by poxviruses, possibly from the genus

Chordopoxvirinae. Although TSD does not represent an elevated mortality risk when endemic, the introduction of this disease could pose a potential threat to naive populations (Van Bressem et al. 2007a, b). TSD may kill neonates and calves that lack protective immunity, and thus could interfere with the host population dynamics.

Flach et al. (2008) observed whitish lesions having a velvety appearance that were associated with unrelated wounds, scars and tooth rakes on the back, dorsal fin and flukes of seven adult Guiana dolphins (4.2%). These seven afflicted dolphins were among 168 specimens that inhabited Sepetiba Bay from 2003 to 2005, and one additional dolphin from the Paraná State, Southern Brazil. These authors also reported a case of vesicular skin disease (VSD), possibly caused by a member of the Caliciviridae family in a Guiana dolphin incidentally captured along the coast of Amapá state, northern Brazil.

Infections caused by Papillomaviruses (family Papillomaviridae) have been detected in high prevalence among several marine mammals in South American waters, especially along the Peruvian coast (Van Bressem et al. 1996). One case of genital papillomatosis was detected in *S. guianensis* collected from Brazilian water (Van Bressem et al. 2009a, b). Papillomaviruses can cause papillomas and condylomas in marine mammals and in other vertebrates and can produce lesions by proliferation of the stratified squamous epithelia of the skin and mucosa. It is believed that environmental factors, such as immunosuppressive contaminants, as well as genetic factors and nutritional conditions, may also be involved in the development of tumors (Howley and Lowy 2001).

Cetaceans are definitive hosts of several helminth species, and most cetaceans are infected through the food chain, with fish, mollusks, and crustaceans acting as the intermediate hosts (Geraci and Lounsbury 2005). Only a few helminth parasites have been described in Guiana dolphins. *Nasitrema attenuata* has been reported to infest the respiratory tract of *S. guianensis*, *Halocercus brasiliensis* the lungs of animals from Brazilian and Colombian coastal waters, and *Braunina cordiformis*, *Nasistrema* sp., and *Anisakis typica* have also been reported in this species (Da Silva and Best 1996; Di Beneditto and Ramos 2004; Melo et al. 2006; Motta 2006; Ruopollo 2003; Santos et al. 1996). The genus *Anisakis*, in particular, is a public health problem, since it causes anisakidosis. Anisakidosis promotes infections in humans, when the larvae are taken in as either raw or lightly cooked fish or squid are eaten. The larvae cannot develop to the adult stage in the human digestive tract, but can damage the gastric mucosa or the intestinal wall (Raga et al. 2002). According to Melo et al. (2006) the Atlantic cutlassfish, *Trichiurus lepturus*, could be the intermediate host of this parasite. Some studies have addressed the pathology of this parasite to Guiana dolphins, but most of these were conducted in dolphins from Southeast region of the Brazil.

At points along the Brazilian coast, researchers have demonstrated the presence in the aquatic ecosystem of *Vibrio* spp., which may be the cause of cutaneous lesions in fishermen and gastrointestinal impairment in humans and animals via seafood ingestion (Pereira et al. 2008). In a recent study, strains of *V. parahaemolyticus*, *V. fluvialis*, *V. alginolyticus*, and *V. vulnificus* were isolated from the superficial

lesions of marine mammals taken from Brazilian waters (Pereira et al. 2007). These pathogens are considered to be dangerous microorganisms to public health and have been recognized as major causative agents of foodborne illness. In *S. guianensis,* the authors observed the following strains: *V. alginolyticus, V. vulnificus, V. parahaemolyticus, V. damsela, V. mimicus, V. harveyi, V. aestuarinus, V. pelagicus, and V. campbelli* (Pereira et al. 2008).

Guiana dolphins also suffer from skeleton lesions. In northern Rio de Janeiro, congenital malformation has been reported in 7.6% of the skulls and 9.4% of the axial skeleton of 53 dolphins incidentally captured, and most of the affected dolphins were immature (Van Bressem et al. 2007a, b). The most common congenital malformation was the incomplete closure of the vertebral arch of the seventh cervical present in 48.4% of 31 analyzed dolphins. The high incidence of congenital bone malformations in *S. guianensis* from northern Rio de Janeiro is of great concern, and may indicate a genetic bottleneck for that population. These malformations may facilitate the occurrence of fractures seen in the vertebrae of this species, and may be caused by social or fishery interactions (Flach 2006; Fragoso 2001). Osteomyelitis and/or osteolysis have also been observed to occur in Guiana dolphins from Brazillian and Venezuela waters (Ferigolo et al. 1996; Laeta et al. 2010; Van Bressem et al. 2007a, b). A possible case of crassicaudiasis was seen in the pterygoids of a mature *S. guianensis* specimen collected from northern Rio de Janeiro State. There is strong evidence that parasitism by crassicaudiasis may constitute the major factor that affects the natural mortality of small cetaceans (Pascual et al. 2000).

5 Human-Induced Injuries and Marine Debris

Although accidental mortality in fishing nets have been recognized as the major threat for the conservation of the Guiana dolphins throughout their range, non-acute injuries caused by fishing apparatus also represent an important cause of concern (Azevedo et al. 2009; Nery et al. 2008; Ramos et al. 2001; Van Bressem et al. 2007a, b). These negative interactions with fisheries have been described in some locations of the distribution of *S. guianensis*. Nery et al. (2008) examined the population of Guiana dolphins from Sepetiba Bay, western coast of Rio de Janeiro state, Brazil. According to these authors, 40.3% of the 382 catalogued specimens exhibited injuries on dorsal fin as result of gillnet or other fishing apparatus. The injuries included total or partial missing or disfiguration of the dorsal fin. Van Bressem et al. (2007a, b) reported that 25% of the 167 photo-identified Guiana dolphins from Sepetiba Bay presented with infectious skin diseases, emaciation and body deformations that could be triggered by a high level stress in the injured dolphins. In Guanabara Bay, seven (9%) out of 78 catalogued Guiana dolphins presented nonlethal injuries,

including cut-like wounds, skin ulceration, and mutilation. Parts of fishing apparatus were also observed in some dolphins affected. Ramos et al. (2001) presented a case of nonlethal fishery interaction with a Guiana dolphin that died as a result of a secondary encounter with a fishery apparatus. This 6-year-old dolphin was accidentally caught along the northern coast of Rio de Janeiro state, Brazil. The carcass displayed cicatrized lacerative lesions on the skin and on the subcutaneous tissue around the posterior extremity of the rostrum. Nylon twine encroachments were identified as having triggered these lesions.

Although these negative events may not generally result in acute-lethal interactions, the damage of the dorsal fin can trigger animal health impairments that disturb vital mechanisms (Hammer et al. 2012). Such interactions may make catching prey more difficult, leading to starvation or enhanced energy loss while searching for prey. Additionally, the injuries caused by fishing apparatus may lead to infectious diseases or a severe inflammation process that could produce death.

Marine debris has been identified as a modern and increasing problem to marine biodiversity from even coastal and offshore environments (Hammer et al. 2012). Macrodebris, classified as plastic objects measuring over 20 mm to several meters, is a potentially passive risk for marine life. Of particular concern are "ghost nets." Ghost nets refer to abandoned or lost fishing nets that have been released to the ocean for several reasons (Hammer et al. 2012). Nonlethal fishing interactions of the sort described with Guiana dolphins may be associated with ghost nets or longlines that are abandoned or lost in Guiana dolphin habitats.

Ingestion of plastic debris has also been recognized as an important threat to conserving many species of marine biota. Incidents of ingestion of plastic debris have been described in some species of small dolphins that are resident along the Southwest Atlantic coast, including: Rough toothed dolphin (*Steno bredananeis*) (Meirelles and Barros 2007), Franciscana dolphin (*Pontoporia blainvillei*) (Bassoi 1997; Denuncio et al. 2011; Pinedo 1982), Blainville's beaked whale (*Mesoplodon densirostris*) (Secchi and Zarzur 1999), and Guiana dolphin (Geise and Gomes 1992). Geise and Gomes (1992) identified substantive amounts of small plastic debris in the stomach chamber of a *S. guianensis* stranded on the coast of Rio de Janeiro state. According to these authors, this dolphin may have confused the plastic ingested with their usual prey, squids. However, the ingestion of plastic debris may not be a big problem to Guiana dolphins, given that, of the many studies of stomach contents performed, only one work has been published that show plastic ingestion to be a problem. Due to its coastal habit, however, *S. guianensis* is vulnerable to several other anthropogenic activities, mainly fishery activities. Fishery is among the most important activities, and severely affects dolphin populations in many ways that include: reducing or disrupting prey composition, causing accidental acute mortality in nets, or causing nonlethal injuries.

6 Conclusions

Based on the results of a comprehensive literature review on the vulnerabilities of the Guiana dolphins, including marine pollution, pathogens, and diseases, and considering their biology and habitat characteristics, we present the following conclusions and considerations:

1. *Fishery interaction*. Fishing activities are regarded to be the main human-induced cause of acute mortality in Guiana dolphin populations that have been studied, and certainly this interaction poses a great risk for the conservation of this species.
2. *Anthropogenic-related pollution*. Human activities along the coastal areas inhabited by the Guiana dolphins have intensified over the last decades, and pollutant emissions to the ocean from multiple sources of human activity have increased. Coastal chemical contaminants have negatively affected dolphin populations, as clearly shown by members of this species having biomagnified many harmful metals and organochlorine chemicals in their tissues.
3. *Emerging pathogens and infectious diseases*. The pathogens detected on Guiana dolphins are possibly related to waste water released by industrial and coastal cities. Consequently, some pathogens may trigger infection diseases potentially exacerbated by the immunosuppression caused by environmental toxicants.
4. *Contaminants and population vulnerabilities*. Although the research that has been carried out on Guiana dolphins have not been uniform along the entire distribution, toxicological results to date do suggest that dolphin populations residing near industrial areas that are highly populated by humans (e.g., São Paulo coast) are more affected.
5. *Conservation status*. There is a general lack of basic information about this small cetacean. Despite this, because this dolphin has a confined coastal distribution in near-shore ecosystems that characteristically poses several threats (e.g., passive fisheries, habitat degradation, marine pollution, and diseases), we suggest that the overall conservation status of *S. guianensis* should be changed from "data deficient" (IUCN Red List; http://www.iucnredlist.org) to "vulnerable."
6. *Research Needs*. Data on biology, habitat use, abundance, and threats to the overall population of the Guiana dolphins are required. The most pressing need however is to perform research along Caribbean and northern Brazilian coastal areas and to enhance conservation management of this coastal cetacean.

The results on marine pollution, harmful pathogens, and diseases highlighted in this study show that the Guiana dolphins are chronically exposed to many potentially damaging human-induced pressures in their marine environment. Therefore, this sensitive species is signaling that environmental changes are occurring in their habitat. Future longitudinal monitoring programs are encouraged for Guiana dolphin populations to better evaluate what environmental changes are affecting the health status and to what degree.

7 Summary

Guiana dolphins (*Sotalia guianensis*) are small cetaceans that inhabit coastal regions down to a 50 m depth. As a coastally distributed species, they are exposed to a variety of human-induced risks that include passive fishing nets, persistent environmental pollution, and emerging diseases. As a top predator *S. guianensis* occupies an important ecological niche in marine ecosystems. However, this niche also exposes this dolphin to extensive biomagnification of marine contaminants that may accumulate and be stored throughout their life of about 30 years.

In this paper, we have compiled available data on the Guiana dolphin as regards its exposure to chemical pollutants, pathogenic microbes, infectious diseases, and injuries caused by interactions with passive fishing gears. Our analysis of the data shows that Guiana dolphins are particularly sensitive to environmental changes. Although the major mortal threat to dolphins results from contact with fishing, other human-related activities in coastal zones also pose risks and need more attention. Such human-related risks include the presence of persistent toxicants in the marine environment, such as PCBs and PBDEs. Residues of these chemicals have been detected in Guiana dolphin's tissues at similar or higher levels that exist in cetaceans from other known polluted areas. Another risk encountered by this species is the nonlethal injuries caused by fishing gear. Several incidents of this sort have occurred along the Brazilian coast with this species. When injuries are produced by interaction with fishing gear, the dorsal fin is the part of the dolphin anatomy that is more affected, commonly causing severe laceration or even total loss.

The Guiana dolphins also face risks from infectious diseases. The major ones thus far identified include giardiasis, lobomycosis, toxoplasmosis, skin and skeletal lesions. Many bacterial pathogens from the family Aeromonadaceae and Vibrionaceae have been isolated from Guiana dolphins. Several helminth species have also been observed to affect *S. guianensis*. These results suggest a vulnerability of this species to environmental disturbances. Moreover, there is some evidence that the effects of some infectious diseases may be enhanced from stress caused by habitat impairment. For example, certain diseases and pathogenic organisms in *S. guianensis* may be associated with the high levels of endocrine-disruptor contaminants (e.g., PCBs; DDTs; PBDEs) that have been detected in marine waters.

Although the data available on *S. guianensis* is growing, most of the work has been focused on a small portion of the species total area of distribution. Most studies, to date, have been carried out in the Southern region of the distribution, and in northeastern Brazil. Few studies have been conducted in the northern region of the South America or in Central America. Therefore, future studies should be conducted that address the heterogeneity of this species total distribution.

The biology and ecology of the Guiana dolphin renders this species potentially useful as a sentinel species for detecting environmental changes, such as chemical and biological pollution. Research about this dolphin is encouraged as a way to assess what coastal environmental changes have occurred and to continue evaluating the health status of this vulnerable species in a changing environment.

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Predicted No-Effect Concentration and Risk Assessment for 17-[Beta]-Estradiol in Waters of China

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1 Introduction

Endocrine-disrupting chemicals (EDCs) comprise a group of environmental contaminants that can disturb the normal functioning of the hormonal system, can cause adverse effects in wildlife and humans (Jobling et al. 2003; Arcand-Hoy and Benson 1998), and have garnered increasing concern in recent decades (Jobling and Sumpter 1993; Desbrow et al. 1998; Heberer 2002; Silva et al. 2002; Ying et al. 2008, 2009, 2002a, b). EDCs can act as mimics of natural hormones, agonists or antagonists of hormone receptors, and may cause indirect effects by modulating certain processes (e.g., synthesis, transport, metabolism) that disrupt endocrine function (Klaassen and Admur 2001).

One class of EDC chemicals that has received a great deal of attention is those that bind to estrogen receptors (ERs), stimulating the ER-dependent response, or influencing expression of estrogen-responsive genes (Byford et al. 2002; Okubo et al. 2001). These substances are classified as estrogenic chemicals. They include some synthetic estrogens, such as 17-[alpha]-ethinylestradiol (EE2), diethylstilbestrol (DES), natural estrogens, such as estrone (E1), 17-[beta]-estradiol (E2), and some xenoestrogens, such as 4-tert-octylphenol (4-t-OP), 4-nonylphenol (4-NP), bisphenol-A (BPA), and phthalic acid esters (PAEs) (Zhao et al. 2011a).

E2, which plays an essential role in the reproductive physiology of females (Choudhry and Chaudry 2008), is released into the aquatic environment of several countries mainly from livestock, sewage treatment plant (STP), and industrial effluents (Kang et al. 2002). E2 also occasionally exists in some rivers (Furuichi et al. 2004; Ping 2011; Zhao et al. 2009), streams (Kolpin et al. 2002), and coastal water ecosystems (Hashimoto et al. 2005; Pojana et al. 2007) at ng/L levels, and has been detected in some sediments at ng/g levels (Hashimoto et al. 2005; Labadie and Hill 2007; Schlenk et al. 2005; Wang et al. 2011). E2 as a potent endocrine-disrupting compound, binds to organs, activates a hormone response at relatively small concentrations (Melnick 1999), and disrupts normal sex differentiation and gametogenesis (Thompson et al. 2009). Multiple potential endocrine-disrupting effects of E2 have been observed in aquatic species; such effects include reduced egg production, altered sexual characteristics, and aberrant expression of mRNA for vitellogenin (Vtg), estrogen (ER), and androgen (AR) receptor isoforms (Jobling et al. 1998).

Derivation of a predicted no-effect concentration (PNEC) is a key step in assessing the ecological risk of chemicals. In recent decades, the approach for deriving PNECs for a limited number of chemicals has been well defined. However, deriving PNECs for EDCs in the aquatic environment is a more recent challenge that is not as well developed. In 2008, a draft document entitled “aquatic life criteria for contaminants of emerging concern” was submitted by the American OW/ORD Emerging Contaminants Workgroup; this group proposed a PNEC for EE2 in this report (Caldwell et al. 2008). In 2012, Caldwell and his colleagues, after reviewing the reproductive effect of E2 on fishes, derived a PNEC for E2 by using the species sensitivity distribution (SSD) methodology. The Caldwell group constructed an

SSD curve based on 21 NOEC values (from 21 studies), all of which relied on reproductive endpoints. In these studies, reproductive effects in nine fish species, which had been exposed to E2, were investigated (Caldwell et al. 2012). Moreover, in China, PNECs for chlorophenols have been developed by using the assessment factor (AF) and SSD methods (Jin et al. 2011a, b). Because little other data on PNECs for EDCs was found in the literature, we undertook this review to derive PNEC values for selected EDCs.

EDCs are toxic to reproductive and endocrine regulator systems and act via a special toxic mechanism. Although they are unlikely to cause lethality at concentrations observed in the environment, some EDCs (e.g., E2 and EE2) can cause adverse effects on the reproductive and endocrine systems of aquatic organisms, even at relatively small concentrations. Therefore, the procedure for deriving PNECs for the EDCs might benefit from considering parameters or endpoints that were different from certain other organic chemicals. Traditionally, both long-term protection PNEC values, called the "criterion continuous concentration" (CCC), and short-term protection values, called the "criterion maximum concentration" (CMC), have been developed for certain environmental contaminants (USEPA 1998). Deriving an acceptable PNEC values for EDCs requires focus on the critical endpoints (viz., reproductive effects and endocrine-disrupting effects), rather than on endpoints that have lower sensitivity, such as lethality.

In the present study, we chose E2 as a representative estrogen-like substance on which to derive PNECs by using the SSD approach. We constructed the SSD function from information available on the aquatic toxic effects of E2. We reviewed 31 NOECs (No observed effect concentrations) that were based on reproductive endpoints for different species (representing amphibians, crustaceans, rotifers, fishes, and algae). In addition, the reproductive effects of multigenerational exposures to E2 were analyzed to compare responses of the F_0 and F_1 generations. We also briefly evaluated the occurrence of E2 residues in surface water, sediment, and STP effluents, and assessed the potential estrogenic risk for E2, in the context of the PNEC for E2.

2 Review of E2 Toxicity to Aquatic Organisms

In previous studies, multiple biological effects resulting from exposure to E2 were described. The endpoints affected by E2 included mortality, growth rates, sexual maturity, and expression of mRNA for Vtg, ER, AR, metallothionein (MT), and cytochrome P4501A (CYP1A).

Robinson et al. (2007) exposed a marine fish species, sand goby (*Pomatoschistus minutus*), to E2 for 8-months, and traced the effects of E2 on mortality, growth rates, sexual maturation, hepatic VTG mRNA expression, and reproductive success. Results were that an exposure level of 97 ng E2/L significantly inhibited male sexual maturation, reduced egg fertility, induced male VTG mRNA expression, and delayed spawning. An exposure level of 669 ng E2/L increased mortality, adversely

affected hematological parameters and impaired reproductive activity, and delayed sexual maturation (Robinson et al. 2007).

Exposure to E2 caused testis–ova in male roche (*Rutilus rutilus*) that lived downstream of a STP (Jobling et al. 1998). Vtg, a precursor of egg yolk normally produced in females, was observed in the blood of male rainbow trout (*Oncorhynchus mykiss*) that were caged in areas downstream of the STP (Harries et al. 1996). VTG induction as an indicator of estrogenic effects differs in sensitivity among studies. For example, VTG induction was observed in male Japanese medaka that were exposed to E2 at 29.3 ng/L, whereas other reproductive effects, such as decreases in fecundity, fertility, and gonadal somatic index, occurred at an exposure of 463 ng E2/L (Kang et al. 2002). In contrast, when sheepshead minnows (*Cyprinodon variegatus*) were exposed to E2, plasma VTG induction was not as sensitive as other reproductive effects (Cripe et al. 2009). Moreover, several studies have demonstrated that VTG induction is reversible, and bears no relationship to long-term effects on health or reproductive performance (Brion et al. 2004; Mills et al. 2003; Nash et al. 2004). It has also been reported that exposure to environmentally relevant concentrations of E2 (<10 ng/L) during early life stages can alter sexual differentiation and fecundity of Japanese medaka (*Oryzias latipes*) (Nimrod and Benson 1998).

E2 inhibited expression of mRNA for CYP1A in juvenile and adult grey mullet (Cionna et al. 2006). Kim et al. (2008) reported that CYP1A was not as strongly expressed in male Japanese medaka liver, after the fish were exposed to E2. Exposure to E2 also reduced the expression of mRNA for CYP1A in cultured rainbow trout hepatocytes, but expression of mRNA for this trait was still up-regulated from exposure to other xenobiotics in the effluents (Navas and Segner 2001). Various studies have shown that E2 (and other estrogenic compounds) are capable of inhibiting the expression of MT in some fishes (Huang et al. 2012; Costa et al. 2010; Gerpe et al. 2000; Olsson et al. 1995).

Expression of mRNA for Vtg after exposure to E2 has been demonstrated to occur in several male fishes, including mosquitofish (*Gambusia holbrooki*), zebrafish (*Danio rerio*), and Japanese medaka (Leusch et al. 2005; Tong et al. 2004; Huang et al. 2012). In teleosts, expression of mRNA for hepatic ERα is significantly up-regulated by E2 exposure (Sabo-Attwood et al. 2004; Esterhuyse et al. 2010). In response to E2 exposure, the auto-regulation of ERα in liver is a common feature of teleosts that has been attributed to transcription of Vtg in the liver of mature females (Pinto et al. 2006). During an 84-day exposure, the NOEC for E2 on mosquitofish (*Gambusia affinis*), was 100 ng/L, based on maturation of the gonopod; however, this value was 20 ng/L, when based on frequency of sexual activity (Doyle and Lim 2002, 2005).

Exposure of Japanese medaka to 463 ng/L E2 for 3 weeks resulted in reduced fecundity and fertility; however, Japanese medaka (*Oryzias latipes*) were unaffected when exposed to 227 ng/L E2. By comparison, treating Japanese medaka with 817 ng E2/L for 2 weeks resulted in production of fewer eggs (Shioda and Wakabayashi 2000). The sexual behavior of male Japanese medaka was suppressed after exposure to 3 and 30 μg E2/g BW(body weight)/day in the diet for 2 weeks (Oshima et al. 2003). The phenotypic sex of Japanese medaka was reversed when eggs were microinjected with 2.1 ng E2 per egg (Edmunds et al. 2000). When

Japanese medaka were exposed to concentrations <2.86 ng E2/L, no effect on sexual differentiation, induction of Vtg, or any other reproductive impairment effect was observed. However, Vtg was induced in male Japanese medaka when they were exposed to a level of 8.94 ng E2/L (Seki et al. 2002, 2005). Alternatively, estrogen-responsive genes were abnormally expressed when Japanese medaka were exposed to 10 ng E2/L (Yamaguchi et al. 2005; Chen et al. 2008). Based on these studies, we suggest that a NOEC of 8.94 ng E2/L for Japanese medaka is appropriate.

When early life stages of the zebrafish (*Danio rerio*) were exposed to 54 ng E2/L, the sex ratio was significantly altered and Vtg was significantly induced (Holbech et al. 2006). Brion et al. (2004) recommended a NOEC ranging from 5 to 25 ng/L, based on gonadal development and induction of Vtg endpoints. The EC_{10} (concentration expected to cause a 10% effect) was 15.4 ng E2/L, based on a logistic regression of Vtg induction (Rose et al. 2002). Van der Ven et al. (2007) exposed adult zebrafish for 21 days to E2 at levels of 27.2, 87, and 272 ng/L, and their offspring were exposed to the same concentration for another 42 days. In males of the parental generation, Vtg production increased significantly at the dose of 87 ng/L. Results for the F1 progeny included decreased survival, increased body length and weight, Vtg-related edema and kidney lesions, and inhibited spermatogenesis at the 272 ng/L exposure level (van der Ven et al. 2007). Based on these results, the NOEC for zebrafish was determined to be 15.4 ng E2/L.

Multiple effects of exposure to E2 on male fathead minnow (*Pimephales promelas*) have been reported (Brian et al. 2007; Parks et al. 1999; Seki et al. 2006). When fathead minnows were exposed to E2 for 19 day, the calculated EC_{10} values, based on egg production, hematocrit of males and females, and plasma alkaline-labile phosphorous, were 6.6, 52.5, 562, 36.3 ng E2/L, respectively. The EC_{50} (concentration expected to cause a 50% effect) for inducing Vtg in males was 251 ng E2/L, whereas no Vtg induction plateau was observed in females (Kramer et al. 1998). The lowest-observed effect concentration (LOEC) of E2, based on induction of Vtg in male fathead minnows, was 28.6 ng E2/L (Seki et al. 2006). From evaluating these results, we suggest a NOEC of 6.6 ng E2/L for fathead minnow, based on reproductive endpoints.

When tadpoles of African clawed frogs (*Xenopus laevis*) were exposed to 27.2 ng E2/L for 4 weeks, slight abnormalities in the histology of the gonad were observed, although the sex of the frogs was not reversed (Kramer et al. 1998). However, when African clawed frogs were exposed to 74 ng E2/L, adverse effects were observed, such as fewer sperm cells, inhibition of meiotic division of germ cells, more lipid droplets (i.e., storage compartments for the sex steroid hormone precursor cholesterol), and lower plasma T concentrations (Hecker et al. 2005). Based on these results, a NOEC of 27.2 ng E2/L is suggested for tadpoles of the African clawed frog.

When juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to E2 for 14 days, a dose of 14 ng E2/L was the threshold for inducing Vtg protein, Vtg mRNA, vitelline envelope protein (VEP)β and VEPγ, whereas, VEPα was induced at a dose of 4.8 ngE2/L (Thomas-Jones et al. 2003). When female rainbow trout were exposed to E2 for 14 days, a dose–response for inducing plasma Vtg was observed; the LOEC for inducing plasma Vtg was 8.9 ng E2/L (Thorpe

et al. 2000). After being exposed to E2 at concentrations of ≥1 ng/L for 35 days, the semen volume obtained per male rainbow trout was significantly reduced, and after 50 days, sperm density and fertility were also reduced (Lahnsteiner et al. 2006). Based on this information, we recommend a NOEC of 1 ng E2/L for rainbow trout.

3 Data Selection, Evaluation, and Analysis

3.1 Data Selection and Evaluation

Information on effects of E2 on freshwater organisms was collected from the ECOTOX database (http://cfpub.epa.gov/ecotox/), individual research papers, and government reports. Toxicity threshold values for E2, expressed as NOEC, were derived for several effect endpoints, including sexual differentiation, gonad development, and sex ratio (Table 1). The accuracy, reliability, and relevance of these data were evaluated by standard methods (Klimisch et al. 1997; Bureau 2003). We classified data from the literature into four levels of reliability (Classes 1–4):

1. Reliable without restrictions
   Data were obtained from studies that were performed according to valid and/or internationally accepted testing guidelines (i.e., adherence to Good Laboratory Practice (GLP) was preferred), or in which test parameters adhered to specific (national) testing guidelines (GLP preferred) or met a comparable standard.

Table 1 List of toxicity study data for 17-[beta]-estradiol (E2) on aquatic species from the literature

Class	Test species	NOEC[a] (ng/L)	Endpoint	Klimisch code	References
Amphibian	*Xenopus laevis*	27.24	Sex differentiation	1	Oka et al. (2006)
Crustacean	*Macrobrachium rosenbergii*	1,000,000	Gonad development	1	Wu et al. (2007)
	Eurytemora affinis	4,000	Reproductive output	1	Forget-Leray et al. (2005)
	Tigriopus japonicus	100	Sex ratio	1	Marcial et al. (2003)
	Neocaridina denticulata	10,000	Egg production	2	Huang et al. (2006)
	Marsupenaeus japonicus	980.64	Vtg induction	1	Yano and Hoshino (2006)
	Daphnia magna	100,000	Sex differentiation	1	Brennan et al. (2006)
Alga	*Melosira varians*	80,000	Enhancing cell growth	1	Julius et al. (2007)

(continued)

Table 1 (continued)

Class	Test species	NOEC[a] (ng/L)	Endpoint	Klimisch code	References
Rotifer	*Brachionus calyciflorus*	10	Mictic rate	1	Ke et al. (2008)
Fish	*Danio rerio*	15.4	Vtg[b]	1	Rose et al. (2002)
	Leuciscus idus	100	Vtg	2	Allner et al. (1999)
	Poecilia reticulata	30	GSI[c]	1	Nielsen and Baatrup (2006)
	Ambystoma macrodactylum	272.4	Day of hatch	1	Ingermann et al. (1999)
	Oncorhynchus tshawytscha	250	Gonadal feminization	1	Nakamura (1984)
	Oncorhynchus KETA	500	Sex differentiation	2	Nakamura (1984)
	Salmo trutta	100	Vtg	1	Sherry et al. (1999)
	Pimephales promelas	6.6	Egg production	1	Kramer et al. (1998)
	Oncorhynchus mykiss	1	Semen volume	1	Lahnsteiner et al. (2006), Routledge et al. (1998)
	Thymallus thymallus	1	Number of males give semen	1	Lahnsteiner et al. (2006)
	Rutilus rutilus	10	Plasma levels of Vtg	1	Routledge et al. (1998)
	Carassius carassius	30	Vtg induction	1	Zhang et al. (2008a)
	Carassius auratus	5	Vtg induction	1	Zhang et al. (2008b)
	Cyprinus carpio	100	Quality of milt	2	Gimeno et al. (1998)
	Gobiocypris rarus	5	Vtg induction	1	Liao et al. (2009)
	Misgurnus anguillicaudatus	500	Vtg	1	Lv et al. (2006)
	Acanthogobius flavimanus	20	UCH[d]-mRNA expression	2	Mochida et al. (2003)
	Gambusia holbrooki	20	Sexual activity	1	Doyle and Lim (2002)
	Tanichthys albonubes	500	Vtg	1	Yang et al. (2011)
	Oryzias javanicus	16	Reproduction	1	Imai et al. (2005)
	Oryzias latipes	2.86	Vtg in male	1	Doyle and Lim (2005)
	Cyprinodon variegatus	40	Reproductive rate	1	Cripe et al. (2009)

[a] *NOEC* no observed effect concentrations
[b] *Vtg* vitellogenin
[c] *GSI* gonadosomal index
[d] *UCH* ubiquitin C-terminal hydrolase

2. Reliable with restrictions
   Data were obtained from studies (most not performed according to GLP), in which the test parameters documented were not wholly consistent with a specific testing guideline. However, the study data were scientifically acceptable and were well documented, even if not performed according to standard guidelines.
3. Not reliable
   Data were obtained from the literature or from reports in which conflicting information existed in the measuring system, or in which organisms/test systems, or exposure routes were nonrelevant, or unacceptable methods or documentation were used.
4. Not assignable
   Data were obtained from studies that did not give sufficient experimental details, or were found only in short abstracts or in secondary literature (books, reviews, etc.).

Only data classified as "1" or "2" were used in constructing the SSD or to determine a PNEC for E_2. If there were more than one datum for multiple reproductive endpoints for a species, the most sensitive value was selected as the final one for that species. If there were more than one datum for the same reproductive endpoint for a species, the geometric mean of the combined values were used as the final toxicity value for that species.

3.2 Derivation of a PNEC

The SSD approach, which represents the variation in sensitivity among species to a contaminant, was used as a statistical extrapolation method to derive a PNEC of E2 in the study. The basic assumption of the SSD approach is that species are randomly selected for analysis and are representative of the entire range of species sensitivities in a given ecosystem. The SSD for a pollutant is constructed from available NOEC values for all species, and then the threshold, which is usually denoted as the HC_5 (pollutant concentration hazardous to 5% of the species), is determined by comparing the pollutant concentration with a predetermined cumulative probability (Van Straalen and van Rijn 1998). The HC_5 value was selected because the concentration of E2 associated with the HC_5 would protect 95% of the species tested (Caldwell et al. 2008). The median value determined by the HC_5 was selected as the PNEC (Europea Commission 1996).

The qualified NOEC values were assigned correlative orders from 1 to N after being ranked, and the cumulative probability for each species is calculated from (1):

$$P = \frac{R}{(N+1)} \tag{1}$$

where: R is the rank of a species in the data series, and N is the total number of examined species (Hall et al. 1998; Schuler et al. 2008).

Given that the SSD method assumes that the selection of the species is random and is representative of the entire range of possible sensitivities among species, the NOEC values (or their logarithmic values) for the examined species should be first checked for normality, and then transformed to approximate a normal probability density function, when necessary (CCME 2007).

Several models are available for fitting distributions of toxicological data, such as the log-normal, log-logistic, Gaussian, and Burr Type III distributions (Wagner and Løkke 1991; Shao 2000). None of these models, however, allow all types of toxicological data to be perfectly fitted. Therefore, parameters (e.g., the adjusted coefficient of determination (r^2) residual sum of squares (RSS) and F value) can be used to compare the suitability of models for a given data set that will determine the optimum model.

4 Selecting NOECs and Establishing PNEC Values

4.1 NOECs of E2 Based on Reproductive Endpoints

Information on E2 reproductive effects of 31 species, which had been evaluated according to the Klimisch Criteria (Table 1), was chosen for constructing a SSD from which the HC_5 was derived. Theoretically, with a total of 31 species the resolution of the SSD would be 3.1%. Since this is less than the 5% chosen to estimate the threshold for effects on species, the HC_5 is an appropriate parametric estimator. The NOEC values for E2 were based on reproductive endpoints, and ranged from 1 ng E2/L (rainbow trout, and grayling, *Thymallus thymallus*) to 1,000,000 ng E2/L (*Decapoda palaemonidae*, *Macrobrachium rosenbergii*). In addition, although 6 NOEC values were selected to represent multigeneration studies, these were insufficient for deriving an HC_5. Therefore, only the first generation (F_0) and the second generation (F_1) results were compared to assess the relative E2 response sensitivities of F_0 and F_1 generations. The two sets of NOECs obtained from F_0 and F_1 generations were both log-normally distributed, so the values were log-transformed (Kolmogorov–Smirnov statistic, F_0: 0.418; F_1: 0.503). Below, we compare the multiple endpoints that existed for some species, and indicate what data were ultimately selected.

Some trans-generational effects were observed in multigenerational exposures to E2. Lifecycle exposure of fish to E2 significantly decreased the production of embryos in the F_1 and F_2 generations; this occurred at concentrations lower than those affecting the F_0 generation, which emphasizes the importance of evaluating estrogenic chemical effects on reproduction through at least two (F_0 and F_1) generations (Cripe et al. 2009). Adverse effects were observed on F_0 individuals, but not F_1 individuals, when they were exposed to comparable concentrations (Marcial et al. 2003; Brennan et al. 2006; Cripe et al. 2009; Ke et al. 2008). A summary of available data on multigenerational toxic effects is given in Table 2. Data were insufficient to meet the requirements for deriving a PNEC. Therefore, when more data become available in the future, E2's potential to produce trans-generational effects should be reevaluated.

Table 2 List of available NOEC values for E2 on aquatic species exposed during the F_0 and F_1 generations

Test species	NOEC for F_0 (ng/L)	Endpoint for F_0	NOEC for F_1 (ng/L)	Endpoint for F_1	Klimisch code	References
Tigriopus japonicus	100	Sex maturity	10	Sex ratio	1	Marcial et al. (2003)
Danio rerio	25	Reproductive performance	100	Sex differentiation	1	Brion et al. (2004)
Oryzias latipes	2.86	Vtg in male	8.66	Vtg in male	1	Seki et al. (2005)
Daphnia magna	400,000	Survival	200,000	Survival	1	Brennan et al. (2006)
Cyprinodon variegatus	40	Reproductive rate	10	Infertile eggs	1	Cripe et al. (2009)
Brachionus calyciflorus	10	Mictic rate	1	Survival	1	Ke et al. (2008)

4.2 PNEC Obtained from SSD

After reviewing, classifying and assessing the data available from various studies, reproductive effects were determined to be the most sensitive and critical assessment endpoints for E2. Subsequently, an SSD was constructed by using NOEC values, based on reproductive endpoints that were reported in 31 studies (Table 1). This information was used to derive a PNEC to protect F_0 individuals from the effects of E2. There are several frequency distributions that could be used to describe the data, and subsequently to interpolate and extrapolate the data. These distributions include the Allometric (Power Law model), Exponential Decay, Gaussian, and Sigmoidal models. Several parameters, such as residual sum of squares and r^2, can be used to judge the goodness of fit of a function to the actual data (Table 3). After all possible functions were evaluated, the logistic function was chosen to construct an SSD for fitting the NOEC values of E2 (Fig. 1). The selection of the logistic function was based on the fact that it resulted in the least residual sum of squares and greatest r^2 for the 31 NOEC values. After the SSD was fitted by the logistic function, an HC_5 value of 1.46 ng E2/L for aquatic organisms was derived. The HC_5 values, derived by using other functions, were similar to those derived from the logistic function. Thus, the choice of a theoretical function did not introduce a significant error into the assessment. The PNEC was generally calculated from $HC_5/2$, which was suggested by Stephan and his colleagues (Stephan et al. 1985). Thus, we recommended a PNEC of 0.73 ng E2/L (a half of HC_5 derived in the study) for protecting aquatic organisms from chronic and full-life cycle exposures to E2.

Moreover, the 6 NOEC values for E2 obtained from multigeneration exposure studies (specifically from F_1 and F_0) were also fitted by the logistic function (Table 4). Although the F_1 generation of zebrafish and Japanese medaka were less

Table 3 PNEC values for 17-[beta]-Estradiol(E_2) as calculated by using different models

Model	Formula	Parameters	R^2	Residual sum of squares	$Y=0.05$	PNEC (ng/L)
Allometric model	$y=ax^b$	$a=0.3262$ $b=0.6942$	0.9011	0.0070	$X=0.0671$	0.58
Exponential Decay model	$y=y_0+Ae^{-x/t}$	$y_0=1.1899$ $A=-1.2488$ $t=2.9318$	0.9623	0.0033	$X=0.2677$	0.93
Gaussian model	$y = y_0 + Ae^{-\frac{(x-x_c)^2}{2w^2}}$	$y_0=-1.3425$ $X_C=4.9122$ $W=4.6579$ $A=2.3104$	0.9767	0.0021	$X=0.2251$	0.84
Sigmoidal model	$y = \frac{A_1 - A_2}{1+(x/x_0)^p} + A_2$	$A_1=0.0461$ $A_2=1.0108$ $X_0=1.7907$ $P=2.3217$	0.9923	0.0006	$X=0.1668$	0.73

PNEC predicted no-effect concentration

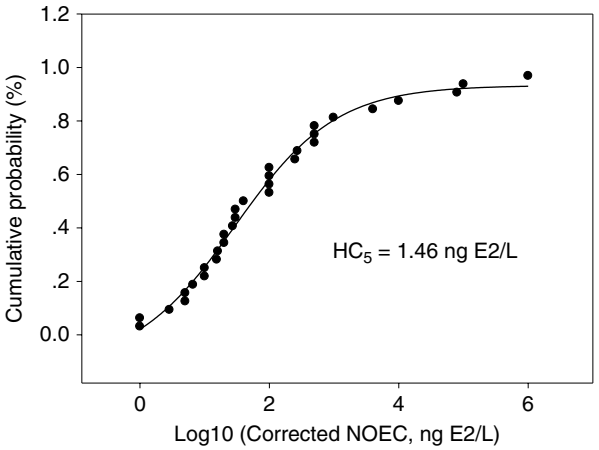

Fig. 1 The distribution of species sensitivity data for the toxicity of E2. Fifth centile of species sensitivity distribution (HC_5, ng E2/L) is presented with two-sided 90% confidence limits that are given in *parenthesis*. The goodness of fit test by using the Anderson–Darling and Kolmogorov–Smirnov test was accepted. *NOEC* no observed effects concentration

sensitive to E2 than was the F_0 generation, we observed a trend that individuals of the F_1 generation were more sensitive than those of the F_0 generation (Fig. 2). The two fitted curves for the F_1 and F_0 generation intersected at $X=2$, $Y=0.714$, and after that they were both stabilized. Therefore, for nearly 71.4% of aquatic species, F_1 individuals were more sensitive than F_0 individuals when exposed to E2 at concentrations less than 100 ng E2/L. Individuals of both the F_0 and F_1 generations would be at reproductive risk when exposed to a concentration greater than 100 ng E2/L. Because these F_1 aquatic organisms were so sensitive to the effects of E2, regulators should consider additional measures or standards to protect them.

Table 4 Comparison of fitted data results for F_0 and F_1 generation organisms

	F_0	F_1
Model	Logistic	
Formula	$y = \frac{A_1 - A_2}{1+(x/x_0)^p} + A_2$	
Parameters	$A_1 = 0.1475$	$A_1 = 0.1384$
	$A_2 = 0.8648$	$A_2 = 0.8578$
	$X_0 = 1.4883$	$X_0 = 1.1328$
	$P = 4.1855$	$P = 2.7759$
Residual sum of squares	0.0009	0.0332
R^2	0.9869	0.7676

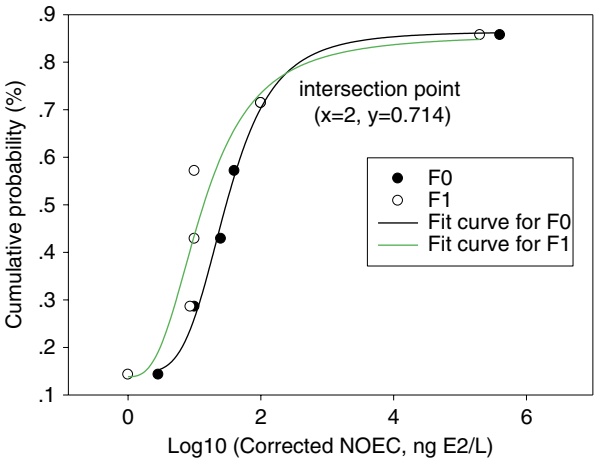

Fig. 2 The distribution of species sensitivity data for E2 to F_0 generation and F_1 generation organisms. The filled and unfilled dots represent the toxicity data of F_0 generation and F_1 generation, respectively. The *black curve* and the *green curve* represent the fitted curve for F_0 and F_1 generation, respectively

5 Discussion

5.1 Reasonableness of PNECs

The SSD method for statistically deriving water quality criteria (WQC) was first proposed to bridge the gap between dose–response data of single-species toxicity and risk assessment for populations, communities, and ecosystems (Kooijman 1987). Thereafter, this method was improved (Aldenberg and Slob 1993; Newman et al. 2000; Wagner and Løkke 1991), and was finally adapted as a standard guideline for ecological risk assessment by the US Environmental Protection Agency (USEPA 1998). The SSD approach assumes that the sensitivity among species can be adequately described by using a specified statistical distribution, such as the normal (Wagner and Løkke 1991; Aldenberg and Jaworska 2000), logistic

(Kooijman 1987; Aldenberg and Slob 1993), triangular (Stephan et al. 1985), or Weibull (Caldwell et al. 2008) probability functions, or by using distribution-free, nonparametric methods (Ling 2004; Newman et al. 2000). The advantage of the SSD approach is that it allows researchers to determine which species are most likely to be affected by an agent by estimating the HC_5. When the HC_5 was compared with other estimates of thresholds and PNEC values, it was found that the HC_5 corresponded to the concentration of chemicals that did not have any statistically significant effects on population or communities.

As an effective method to characterize variation in sensitivity to chemicals among species, the SSD method has been used not only to assess risk or develop WQC for aquatic species (Caldwell et al. 2008; Schuler et al. 2008), but also to confirm quality criteria to protect top predators from residues in soils (Jongbloed et al. 1996; Traas et al. 1996). Because data for toxicity of contaminants to wildlife are generally insufficient for constructing an SSD, using the SSD approach to assess wildlife risks has not been widely accepted. However, SSDs have been constructed for predicting the toxicity threshold value of 23 chemicals to wildlife, by incorporating interspecies toxicity correlation models (Awkerman et al. 2008). A specified effect level, such as the proportion of species expected to respond to a particular exposure for a specific measurement endpoint, can be determined to protect most of species by constructing an SSD curve. In summary, the SSD is considered to be an appropriate approach for deriving a PNEC value for E2.

The accuracy or reasonableness of a PNEC derived from a SSD is likely to depend upon the quantity of data and the particular data selected. In the present study, the Klimisch classification system was applied to evaluate the quality of the data used in to construct the SSD curve. A steady SSD can be constructed at a sample size of 10–15 data points (Wheeler et al. 2002), and an accurate PNEC value can be obtained from a SSD created for 15 or more species (Awkerman et al. 2008). Hence, the quantity of data was unlikely to affect the stabilization of the SSD or accuracy of the PNEC, as long as the quantity of stringent data encompasses at least 15 species. In this study, 31 species from 3 phyla and 8 families met the requirements proposed by USEPA for constructing an SSD. The number and diversity of taxa for which data are available ensure the stabilization of the SSD curve and allowed us to obtain an accurate and reasonable PNEC value. Even if one of the NOEC values is changed, the PNEC value will not be significantly influenced.

Several mathematical models including the Probabilistic, Allometric (Power Law model), Exponential Decay, Gaussian, and Sigmoidal could be used to describe the data and could subsequently be used for interpolation and extrapolation. After evaluating the fitness of all the possible functions by several parameters, such as residual sum of squares, and r^2, the logistic model was determined to be the best one for fitting the 31 NOECs addressed in this paper. The logistic model has been deemed by some authors to be a more appropriate model for deriving the PNEC because of its shape and curvature (Knoben et al. 1998). The advantages of the logistic model are not only its fitting goodness but also its mathematical simplicity. The simple least squares regression can be applied to probit and log-transformed data, and confidence intervals can be calculated from assumptions of the normal distribution (Wheeler et al. 2002).

Toxicity data representing different endpoints exhibit different potencies for E2. For example, when Japanese medaka were exposed to E2 and body length was used as the endpoint, the LOEC value was 1,000 ng E2/L (Metcalfe et al. 2001); when the total number of eggs from F_0 was used as the endpoint, the LOEC value was 463 ng E2/L (Kang et al. 2002); when the sex ratio of F_1 generation was used as the endpoint, the LOEC value was 8.66 ng E2/L (Ke et al. 2008). However, when induction of Vtg was used as the endpoint, the LOEC was 0.94 ng E2/L (Seki et al. 2006). The greatest LOEC value for Japanese medaka responding to E2 was 1,000-fold greater than the least LOEC. Some other effects, such as lethality to crustaceans and fish, growth of amphibian tadpoles, and development of copepod, were reported in the literature, and the NOEC or LOEC values associated with them were generally at mg/L level (Forget-Leray et al. 2005; Hogan et al. 2006; Rang et al. 2003; Hirano et al. 2004; Kashiwada et al. 2002). Thus, it can be concluded that the reproductive endpoint is most sensitive for assessing the effect of E2 on aquatic organisms.

5.2 Comparison to Other PNECs for E2

The HC_5 value derived in the present study was 1.46 ng E2/L, which is close to the HC_5 of 1.5 ng E2/L derived by using the SSD that was constructed from 77 in vivo NOECs by Zhao et al. (2011a). In Zhao's study, the selected NOECs were based on reproductive and many other endpoints (i.e., body length, body weight, and survival ratio), while in the present study, only reproductive endpoints proved to be more sensitive were selected for constructing the SSD curve.

The PNEC value was 0.73 ng E2/L (1/2 of the HC_5), which is consistent with other E2 PNEC values derived by the European Union (0.4 ng/L) for protecting aquatic life (European Union 2011). Caldwell and his colleagues recommended a slightly higher PNEC for E2 (2 ng E2/L), which was derived from an SSD curve constructed from 21 in vivo NOECs (Caldwell et al. 2012). The difference may be attributed to the species taxa selection in constructing the SSD curve. In Caldwell's study, only 21 NOECs of fishes were selected to construct the SSD curve, while in the present study, 31 NOECs from five taxa, including amphibians, crustaceans, rotifers, fishes, and algae, were chosen for constructing the SSD curve. The quality and quantity of the available data in the present study not only meet the requirement of representing 8 families from 3 different phyla as recommended by USEPA (USEPA 1986), but also satisfy the requirement of 30–50 data points for a stable SSD curve (Wheeler et al. 2002). From the perspective of protecting aquatic species rather than fishes only, 0.75 ng E2/L may be more reasonable than 2 ng E2/L, and 0.75 ng E2/L may be more protective for aquatics organisms. The PNEC of 17-[alpha]-Ethinylestradiol (EE2), a synthetic estrogen with high estrogenic potency, whose estrogenic equivalent factor was about two times that of E2 in some in vitro studies (Johnson and Sumpter 2001; Zhao et al. 2011a), was reported to be 0.35 ng E2/L to protect 95% of species; the PNEC for EE2 was derived from NOEC values based on reproductive effects from 39 papers in 26 species (Versteeg et al.

1999; Caldwell et al. 2008). The PNEC value for EE2 was consistent with its estrogenic potency being higher than that of E2 (Versteeg et al. 1999). The PNEC value for E2, derived in the present study, was consistent with the PNEC for EE2 recommended by Caldwell, when their estrogenic equivalent factors (EEFs) were incorporated. Using such comparative data could generate a new method for deriving PNECs for other estrogenic substances. In further study, EEFs or toxicity equivalent factors (TEFs) might be used as a modification factor in confirming PNECs for some toxicity data-poor chemicals. For example, if we need to derive a PNEC value for a chemical, whose toxicity data do not meet the requirement of PNEC derivation, we can refer to a PNEC value of another chemical with a similar toxicity model or toxicity mechanism or consider their EEFs or TEFs to confirm the PNECs.

5.3 Limitations of the SSD Approach

Although the SSD method is useful for assessing the range of sensitivities among species for deriving WQC and chemical risk assessment, this method has limitations. Some of these limitations are related to the properties of probability distributions. Since there is no zero (0) or 100% on a probability scale, it is impossible to establish a PNEC that would affect 0% of species, or provide 100% protection of all species. When the SSD approach is used, two sources of uncertainty need to be considered: The first is the relationship between the LOEC and NOEC. There is never a discrete boundary between LOEC and NOEC for biological phenomena. NOEC values are dependent on both author judgment and the experimental study design used to generate the NOEC. Specifically, both the range between designed doses and the number of designed doses determine the achievable resolution in establishing LOEC and NOEC values. Uncertainty that is inherent in establishing the NOEC is transmuted to estimations of the EC_5, which results in uncertainties associated with deriving the PNEC. Considering the LOEC to NOEC ratios of species, a value of 2.0 was assigned to the LOEC to NOEC correction factor (Jongbloed et al. 1996).

The second source of uncertainty, when using the SSD approach, lies in the gap between extrapolations from laboratory tests to field conditions (Forbes and Forbes 1993; Smith and Cairns 1993). Under field conditions, the actual exposure dose is generally less than the theoretical exposure dose, as a result of faster dissipation and reduced bioavailability of the test chemical. Alternatively, under laboratory conditions, exposures are kept constant and the bioavailable fraction is nearly 100%. These differences have been demonstrated in various studies done under field, or laboratory conditions or in a mesocosm system (Giesy et al. 1999). Furthermore, the adverse effects of chemicals can be mitigated by adaptive responses of organisms, not only at the individual and population levels, but also at the community level. In ecosystems, there is functional redundancy among species. Theoretically, each species cannot occupy exactly the same ecological niche. That is, they cannot have overlaps in feeding guilds; for example, if one species is affected or even removed from the ecosystem, another species can accomplish the function of the lost species.

By using the SSD approach, researchers can identify the most sensitive species, which can then be compared to economically important species, endangered species, or ecological keystone species. Thus, the use of the HC_5 value should not be blind, but rather should be used as part of a risk assessment and risk management strategy conducted by experienced professionals. Subsequently the results need to be communicated to the public by experienced professional communicators of risk. Uncertainty factors have been used to correct toxicity data from laboratory tests, and have been used to account for differences in metabolic rate, caloric content of food, and food assimilation efficiency between laboratory and wild species (Traas et al. 1996). Moreover, some authors have used a statistical procedure, which is more scientifically defensible, to estimate uncertainty factors so as to obtain more precise uncertainty factors and criteria (Calabrese and Baldwin 1994; Dourson and Parker 2007; Gaylor and Kodell 2000). Therefore, when the SSD approach is used to derive PNECs for chemicals to protect wildlife, much attention should be focused on how to bridge the extrapolation gap between laboratory and field testing.

5.4 Risk Assessment of Ambient Concentrations in China

Generally, E2 concentrations in the environment are quite small (generally less than 10 ng/L) (Table 5). The greatest concentration of E2 reported for surface water was 200 ng/L, which was reported in the USA. In China, E2 was detected in the Pearl River, Yangtze River, and Liao River at concentrations ranging from ND-7.5, 6-24 and ND-7.4 ng/L, respectively. In Great Britain, the greatest reported concentration of E2 in surface water was 17 ng E2/L, while in Italy, the greatest reported concentration was 36 ng E2/L. E2 was also detected in sediment samples from several countries or regions, including Japan, the USA, and Great Britain at ng/g concentrations. The greatest E2 concentration detected in sediments was 4.8 ng E2/g, which was reported in Japan. The greatest concentration of E2 (64 ng E2/L) occurred in Canada in effluents of domestic STPs, whereas domestic effluent levels from STPs in Great Britain ranged from 2.7 to 48.0 ng E2/L.

Potential estrogenic risk for E2 in surface waters was assessed by ranking risk quotients (RQ), which is the ratio of ambient E2 concentration to E2 PNEC value. Risk was ranked by utilizing common risk ranking criteria: $RQ < 0.1$ represents minimal risk, $0.1 \leq RQ < 1$ represents median risk, and $RQ \geq 1$ represents the greatest risk class (Hernando et al. 2006). According to these criteria, risks posed by estrogenic substances occurring in surface water of many countries including Japan, the USA, Great Britain, and Italy would be classified in the greatest risk class. If other estrogenic chemicals are considered, the risks posed in these countries would be greater.

In China, risks posed by estrogenic compounds could occur in some regions of the Pearl River, the Liao River, the Yangtze River, and the Yellow River. The E2 equivalent concentration (EEQ) (i.e., the sum of all individual compound concentration values multiplied by the corresponding estradiol equivalency factors (EEFs)), was used when we assessed the risk of other estrogenic chemicals in the aquatic

Table 5 Occurrence of E2 residues in surface water, sediment, and sewage treatment plant (STPs) effluents as reported in the cited studies

	Surface water (ng/L)		Sediment (ng/g)		STPs effluents (ng/L)	
Location	Concentration	References	Concentration	References	Concentration	References
Asia						
China					ND to 4.8 ng/L	Liu et al. (2012)
Pearl River	ND[a] to 7.5	Zhao et al. (2009)				
Yellow River	ND	Wang et al. (2012)				
Yangtze River	6–24 ng/L	Ping (2011)				
Liao River	ND to 7.4 (1.0)	Wang et al. (2011)	<LOQ[b]	Wang et al. (2011)		
Japan	ND to 12.3	Furuichi et al. (2004)	<0.1–4.8	Hashimoto et al. (2005)	0.05–2.63	Nakada et al. (2007)
	0.4–1.7	Hashimoto et al. (2005)				
Oceania						
Australia	ND to 1.2 (0.19)	Hohenblum et al. (2004)			1–4.2	Ying et al. (2008)
	0.54–3.77 (1.54)	Ying et al. (2009)				
America						
USA	0–4.5	Zhang et al. (2007)	0.16–0.45 (0.3)	Schlenk et al. (2005)	ND to 1.0	Esperanza et al. (2007)
	ND to 200 (160)	Kolpin et al. (2002)			<LOD to 3.7 (0.9)	Snyder et al. (1999)
Canada					<LOD to 64 (6)	Ternes et al. (1999)
					<1–7.4	Lee et al. (2004, 2005)
Europe						
Britain	ND to 7.1 (3.0)	Xiao et al. (2001)	<0.03–1.20	Labadie and Hill (2007)		
	<0.1	Boyd et al. (2004)	<LOQ to 4	Liu et al. (2004)		
	<LOD to 17	Liu et al. (2004)				
Italy	<1.0–36	Pojana et al. (2007)			0.35–3.5 (1.0)[a]	Baronti et al. (2000)
Germany	0.15–3.6 (0.6)	Kuch and Ballschmiter (2001)			<LOD to 3	Ternes et al. (1999)
The Netherlands					<0.1–5.0	Belfroid et al. (1999)

[a]*ND* not detected
[b]*LOQ* limit of quantification

environment. By comparing E2 residues to the PNEC (0.73 ng/L) value, 12 sites (wet season) and 21 sites (dry season) of 21 sample sites have the potential to cause estrogenic effects on some aquatic organisms; high risks existed at three sites (the EEQs were >10 ng/L) in the Liao River (Wang et al. 2011). At more than 50% of sample sites in the Pearl River system the EEQs were greater than 0.73 ng/L; this means that adverse effects from estrogenic compounds in these regions could occur (Zhao et al. 2011a). In comparison to the Liao and the Pearl River systems, the Yellow River poses a lower risk from the presence of the EDCs. Of 15 sites sampled in the Yellow River, only one site had an EEQ higher than 0.73 ng/L (Wang et al. 2012). Estrogenic effects of surface water and sediments from the Liao, Yellow, and Pearl Rivers were also assessed by using an in vitro bioassay (YES: yeast estrogen screen). Results were highly consistent with those based on chemical analysis (Zhao et al. 2011a, b; Wang et al. 2011, 2012).

6 Summary

Contamination of the aquatic environment by EDCs has received considerable attention from scientists, government officials, and the public. E2, one of the EDCs with high estrogenic effect, has the potential to cause multiple endocrine-disrupting effects, even at small concentrations. In the present review, the toxicity of E2 to aquatic organisms was reviewed. Results of published studies show that, for aquatic species, reproductive effects were the most sensitive endpoint for E2 exposure.

Although the risks posed by EDCs have caused much attention, the research on the WQC for EDCs is still at the initial stage. It has been suggested in several reports that the PNEC can be regarded as the most appropriate reference value for developing WQC for the EDCs. The SSD method was applied to derive PNECs that were based on reproductive effects endpoints. In the present review, 31 NOECs, based on reproductive effect endpoints for different species, were selected to construct the curve. The PNEC value was determined to be 0.73 ng E2/L, which could protect the biodiversity of aquatic ecosystems. Moreover, 6 NOECs for multigeneration species were also analyzed in anticipation of sensitivity comparison between the F_0 and the F_1 generations. When multiple generations of aquatic species were exposed to concentrations no greater than 100 ng E2/L, nearly 71.4% of the F_1 generation individuals were more sensitive to the effects of E2 than those of the F_0 generation. This result indicated that different generations of the same species may respond differently to EDCs exposure. Individuals of the F_1 generation were slightly more sensitive than those of the F_0 generation, in general. Therefore, protecting the F_1 generation of aquatic organisms is particularly important when WQC values for the EDCs are established.

Considering the toxic effects of EDCs on reproduction, long-term toxic effects (viz., full-life cycle study and the most sensitive life stage) should be used in setting WQC. Unfortunately, the NOECs of E2 for multigeneration species did not meet the requirement of PNEC derivation for protecting the F1 generation. Therefore, further research results are needed on the F1 generation of aquatic species to provide more insight into what constitutes adequate protection for aquatics lives.

In the present review, the PNEC values derived in the study were compared to the PNEC values developed by others, and the results showed that they were highly consistent. In addition, we also compared the PNEC value for E2 to the PNEC value for EE2, a similar estrogen, and the result was also highly consistent when their EEFs were considered. These comparisons affirmed that the method we used for deriving the PNEC value of E2 was reasonable and the PNEC values we derived were acceptable for protecting aquatic organisms. By comparing the PNEC values we calculated to actual E2 concentrations in the natural water environment, we found that E2 in surface waters may pose high risks in many countries, especially China, Japan, the USA, Great Britain, and Italy.

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Adverse Effects of Bisphenol A on Male Reproductive Function

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1 Introduction

Infertility is one of the major health problems that affect human sociocultural life. It affects 8–15% of couples around the world, and about 50% of infertility cases are attributed to male factors (WHO 1991; Cui 2010; Hamada et al. 2011). Among the factors that cause male infertility are exposures to environmental toxicants (Akingbemi et al. 2004). Bisphenol A ([2,2-bis(4-hydroxyphenyl)propane]: BPA) is among the most prominent of toxic environmental contaminants worldwide. The original synthesis of BPA is attributed to two scientists: Dianin, who is thought to have been the first to design the molecule in 1891, and Zincke, who reportedly synthesized the molecule in 1905 (Huang et al. 2011). Since then, BPA has been widely produced and used as a common ingredient in the manufacture of plastics. Plastics are broadly integrated into today's lifestyle and make a major contribution to almost all product areas (Olea et al. 1996; Völkel et al. 2011; Hammer et al. 2012). The wide and heavy use of plastics has contributed to BPA having been spread throughout the environment.

Humans are mainly exposed to BPA through food ingestion (Yamada et al. 2002), and increasing evidence supports its association with impairment of male reproductive function, as well as other health problems and diseases; such diseases include diabetes, obesity, cardiovascular diseases, and cancer (Li et al. 2010a, 2011; Salian et al. 2011; Batista et al. 2012; Shankar and Teppala 2012; Lee et al. 2012; Wang et al. 2012a). BPA acts primarily by mimicking the effect of estrogen hormones, modifying DNA methylation, and modulating enzyme activities in utero and in vivo, resulting in metabolic diseases, spermatogenesis defects, and/or infertility in males. The deleterious effect of BPA on male reproductive function may occur during embryonic, pubertal, and/or adult life. The fact that BPA is a causal agent in such effects is supported by its repeated detection in human biological samples (Sun et al. 2004; He et al. 2009a; Li et al. 2010a).

In this review, we address the topic of BPA effects on male reproductive function and emphasize its effects on testicular steroidogenesis, spermatogenesis, and sperm function.

2 Sources and Routes of Bisphenol A Exposure

BPA is a man-made industrial chemical that is used as a component of plastics. The USA, Japan, and Europe are the areas in which the majority of BPA is produced. BPA's annual production capacity now exceeds six billion pounds, and this high production level is sustained and encouraged by the widespread use of plastics to manufacture food containers, water bottles, medical devices, and other objects that must be made of materials that are both flexible and durable. More than 100 t of the annual BPA production volume is released into the atmosphere (Vandenberg et al. 2009; Cao et al. 2011). This volume of release has made BPA environmentally ubiquitous; BPA residues are found in air, drinking water, lakes, the seas, sewage sludge, soil, house

dust, foodstuffs, paper currency, among other objects and media (Ignatius et al. 2010; Liao and Kannan 2011; Geens et al. 2012; Hammer et al. 2012; Liao et al. 2012; Molina-García et al. 2012; Rudel et al. 2011; Wang et al. 2012b; Rocha et al. 2013).

BPA enters the human body mainly via consuming contaminated food and drinking water, although exposure via environmental (from polluted air and water), domestic (household products, cosmetics), medical (from contaminated equipment and devices), and occupational sources (inhalation, dermal contact, and ingestion during manufacturing processes or industrial use) also occurs (Toppari et al. 1996; Demierre et al. 2012; Cho et al. 2012). Intake by the oral route may be enhanced when certain food preparation practices are used, such as wrapping food in plastic bags prior to thermal treatment or cooking—processes that enhance leakage of BPA from the bag into the food. BPA is known to be used in the manufacture of microwave oven ware, from which it may be released to food (EFSA 2006; Geens et al. 2012). Oral exposure to BPA may also be enhanced from the presence in the mouth of dental composites (Olea et al. 1996; Doerge et al. 2012), or from using epoxy resin-based food cans, water bottles, or plastic baby bottles (Cao et al. 2011; Kang et al. 2011; Völkel et al. 2011; Cho et al. 2012).

Absorption of BPA may also be indirect, as occurs when the fetus absorbs the chemical from maternal blood plasma (after maternal exposure). Indeed, BPA is transported across the human placenta (Mørck et al. 2010), and BPA's effect on the fetus may be exacerbated, because the production of toxic effects is inversely correlated with age (Kline and Ruhter 2012). Transdermal BPA exposure and inhalation are of greater concern because such exposure routes avoid the first-pass metabolic effect that occurs with oral intake (Welshons et al. 2006; Mørck et al. 2010; Demierre et al. 2012).

Occupational exposure to BPA occurs in those countries where the compound is manufactured. Although most intake occurs from ingestion, people who are engaged in the manufacturing or use of BPA (or related chemicals) can easily absorb it through the skin, or take it up via inhalation (He et al. 2009b; Kaddar et al. 2009; Geens et al. 2012).

Absorption or uptake of BPA into the human body is ascertained by analyzing for the presence of the chemical or its metabolites in biological fluids such as blood, breast milk, urine, etc. (Zhang et al. 2011; Geens et al. 2012). For instance, a study conducted in a reference human population of the United States showed the presence of BPA in ≥95% of urinary samples collected from urban and rural residents (Calafat et al. 2005). Similarly, BPA was detected in spot samples of urine collected from both urban and rural girls in Egypt (Nahar et al. 2012). Urinary levels of BPA, detected in children and adolescents, were reported to be higher than in adults (Calafat et al. 2009; Zhang et al. 2011).

High levels of BPA have been measured in human placental tissue, in maternal urine, and in maternal and fetal plasma. Maternal BPA levels were positively correlated with BPA concentrations detected in the fetal umbilical cord (Schönfelder et al. 2002; Lee et al. 2008; Callan et al. 2012; Ünüvar and Büyükgebiz 2012). In utero exposure appears to be more harmful in humans, since BPA accumulates in amniotic fluid of pregnant women (Schönfelder et al. 2002; Sun et al. 2004).

BPA concentrations in maternal plasma were found to be fivefold lower than levels measured in amniotic fluid (Ikezuki et al. 2002), indicating that the fetus is more highly exposed than is the mother. These observations suggest that BPA exposure is higher during fetal life, and diminishes as age increases.

Urinary BPA levels detected in workers were also consistent with degree of occupational exposure to the chemical. He et al. (2009b) reported high levels of BPA in urine and blood of people working in epoxy-resin and BPA manufacturing factories in China.

Several researchers have noted that BPA produces estrogenic and antiandronenic activity, thus emphasizing the importance of its potential harmful effects on human health upon entry into the organism (Olea et al. 1996; Lee et al. 2003; Wetherill et al. 2002, 2007; Alonso-Magdalena et al. 2012). In males, such endocrine disruption may also affect the regulation of the hypothalamic–pituitary–gonadal axis, resulting in reproductive disorders and infertility.

3 Effects of In Utero Exposure to Bisphenol A on Male Reproductive Function

The harmful effects of BPA on male reproductive function, following in utero exposure, have been widely studied in laboratory animals such as rodents. vom Saal et al. (1998) studied prenatal BPA exposure on male mice and found increased size for preputial glands and reduced epididymides size, as well as decreased efficiency of sperm production (daily sperm production). A decrease in fertility, daily sperm production, sperm count and motility in BPA-exposed male offspring during adulthood was also reported (Salian et al. 2009a, 2011). Oral administration of 2–20 ng BPA/g body weight (bwt) to female mice on gestational day (GD) 11–17 resulted in a significant decrease of relative testis weight of male pups at 8 and 12 weeks of age (Kawai et al. 2003). When female mice were concomitantly administered BPA and di(2-ethylhexyl) phthalate (another plastic component), the expression level of Anti-Müllerian hormone (AMH) and Steroidogenic Acute Regulatory Protein (StAR) was reduced in the testes of the exposed male pups, and the pups' testicular size was reduced. Importantly, the adverse effects were persistent in the sexually mature pups at postnatal day (PND) 42, and were consistent with significant reductions of epididymal sperm counts (Xi et al. 2012). Analysis of RNA samples from the hypothalamus, testes, and epididymides of rat fetuses, exposed to BPA in utero from GD 11–20, or GD 6–21, revealed modification of the gene expression profile, including hypothalamic estrogen receptors (ERs), testicular luteinizing hormone receptor (LHR), cholesterol side chain cleavage enzyme (Cyp11a1), and StAR (Naciff et al. 2005; Cao et al. 2013). BPA exposure affects hypothalamic development in the embryo. This was evidenced by enhanced dendritic and synaptic development in cultured hypothalamic cells from fetal rats, as manifested by increases in the area of dot-like staining of synapsin I and MAP2-positive area (Iwakura et al. 2010). Treatment of Ishikawa cell cultures with BPA also modulated the expression of the INSIG1 and FOS genes, which are implicated in regulating transcription and steroid metabolic processes, respectively (Naciff et al. 2010).

In pregnant female rats exposed to BPA from GD 1 throughout parturition, serum testosterone levels were decreased in male fetuses and pups (Tanaka et al. 2006). Exposures of the dams from GD 12 to PND 21 also resulted in decreased testosterone levels in the testicular interstitial fluid of male pups in adulthood (Akingbemi et al. 2004). The testosterone inhibition is probably induced by the BPA-suppressive effect on testicular Leydig cell steroidogenic proteins. In fact, BPA inhibits expression of the StAR protein, and the 17-β-hydroxysteroid dehydrogenase enzyme (17β-HSD) (Horstman et al. 2012; Nanjappa et al. 2012). Protein expression of the LHR is also compromised following BPA exposure, and may lead to decreasing androgen secretion by testicular Leydig cells (Nanjappa et al. 2012). The testosterone concentration increased in 9-week-old male pups exposed to BPA in utero and through lactation (Watanabe et al. 2003), and this could be attributed to in utero BPA-induced proliferative activity (mitogenic effect) on testosterone-producing Leydig cells (Nanjappa et al. 2012). In addition to modulating the Leydig cells, BPA also induced down-regulation of several genes associated with Sertoli cell function (Msi1h, Ncoa1, Nid1, Hspb2, and Gata6) in 6-week-old male mice after prenatal exposure (Tainaka et al. 2012), thereby disrupting the blood–testis barrier (BTB) and impairing spermatogenesis (Cheng et al. 2011; Su et al. 2011). Perturbation of BTB (reduction in the expression of Connexin 43 and increases in the expression of N-cadherin and Zona Occludin-1) and spermatogenesis were also observed in 45/90-day-old rats neonatally exposed to BPA (≥400 μg/kg bwt/day, during PND 1–5) (Salian et al. 2009b).

The biological effect of thyroid hormones that act on male reproductive function by modulating germ cell development (Krassas and Pontikides 2004) can also be compromised by BPA exposure during fetal life. BPA affects the expression of thyroid specific genes that have been implicated in thyroid development, as well as control of gene expression in rat thyroid cells and zebra fish embryos in vitro (Gentilcore et al. 2013). BPA antagonizes triiodothyronine (T3) action at the transcriptional level in human TSA201 cells, through displacement of T3 from the hormone receptor (TSH) and recruitment of a transcriptional repressor (a T3- negatively regulated TSHalpha promoter), resulting in gene suppression (Moriyama et al. 2002). A BPA antithyroid effect in rats was also observed in vivo. In fact, feeding pregnant Sprague-Dawley rats a BPA-containing diet during pregnancy and lactation caused an increase in serum total thyroxin in male pups on PND 15, with up-regulation of the expression of the thyroid hormone-responsive gene RC3/neurogranin in the dentate gyrus. This suggests a thyroid-hormone antagonist effect of BPA on the beta-thyroid receptor, which mediates the negative feedback effect of the hormone on the pituitary gland (Zoeller et al. 2005). In utero exposure of CD-1 pregnant mice to BPA (50 μg BPA/kg/day, during GD 16–18) also resulted in increasing the anogenital distance (AGD: distance from the center of the anus to the anterior base of the penis, an androgen-dependent variable, used as a sensitive marker of androgenic and antiandrogenic effects of in utero chemical exposure) in male pups (Gupta 2000; Foster and McIntyre 2002). This contrasted with studies of Talsness et al. (2000), who reported shortening of the AGD, following prenatal BPA exposure. Notwithstanding, these two studies indicated that BPA has the ability to modulate AGD during prenatal life.

These in utero effects are likely to also occur in BPA exposed human males. Such evidence has been derived from a recent epidemiological study conducted by Braun et al. (2012), who detected BPA in urine samples of pregnant women, suggesting gestational exposure. Moreover, Miao et al. (2011a) demonstrated an association between parental occupational BPA exposure during pregnancy and shortened AGD in male offspring. The latter association was stronger for maternal exposure, and the authors found a dose–response relationship between increased BPA levels in pregnancy and greater magnitude of shortened AGD. In Table 1, we summarize data from selected studies that addressed BPA's male reproductive effects.

Because of its accumulation in amniotic fluid of pregnant women, BPA exposure appears to be more harmful in utero (Schönfelder et al. 2002; Sun et al. 2004), a critical hormonally dependent step in development of the individual. BPA acts as an endocrine disruptor (estrogenic, antiandrogenic, or antithyroid). It has been shown to reduce total blood T4 levels in pregnant women, with associated decreased TSH in their respective male neonates (Olea et al. 1996; Moriyama et al. 2002; Lee et al. 2003; Zoeller et al. 2005; Wetherill et al. 2007; Chevrier et al. 2013). BPA binds to ERs, inhibits androgen-induced androgen receptor (AR) transcriptional activity and androgen (dihydroxytestosterone) binding to AR (Lee et al. 2003; Alonso-Magdalena et al. 2012). However, recent findings support an additional BPA action mechanism, through a non-genomic pathway, initiated at membrane receptors, including classical ERs and/or G protein-coupled receptor 30 (reviewed by Iwakura et al. 2010). The estrogenicity of BPA can also prevent AMH action on the Müllerian ducts in the male (Pryor et al. 2000), leading to the feminization of male fetus (Hutson et al. 1994). Such feminization may be triggered by up-regulation of genes required for ovary development (*Foxl2* and *Wnt4*), with concomitant repression of genes responsible for testis development (*Sox9* and *Fgf9*) in the embryo, as reported elsewhere (Aoki and Takada 2012). By disrupting hormone levels or receptor activity, the detrimental effect of BPA may be to alter male reproductive-organ development during fetal life (Miao et al. 2011a, b). Moreover, the BPA effect may be more pronounced and irreversible during this development stage, unlike in adults, who have a matured and functional sex-specific physiology, in which the deleterious effect is potentially reversible once exposure ends (Kline and Ruhter 2012).

4 Effects of Bisphenol A on Spermatogenesis and Sperm Function Following Postnatal Exposure

4.1 Effects on the Hypothalamic–Pituitary–Testicular Axis

The spermatogenesis process in mammals is coordinated by the hypothalamic–pituitary–testicular axis and the thyroid gland (Zoeller et al. 2005; Moriyama et al. 2002). Dysfunction of the axis, triggered by endocrine disruptors such as BPA, may result in arrest or alteration of spermatogenesis (Table 1).

Table 1 Investigations that have addressed the effects of BPA on male reproductive function

Experimental model	BPA dose or concentration	Major findings	Reference(s)
In vitro (rat and human testis microsomes)	10^{-8}–10^{-4} M, 3 h incubation	Decrease of 3β-HSD, and 17β-HSD activities	Ye et al. (2011)
In vitro exposure (Leydig cells from adult rats)	0.01 nM, 18 h incubation	Inhibition of CYP19 expression, and estradiol release	Akingbemi et al. (2004)
In vitro exposure (human spermatozoa)	1 μM, 2 h incubation	Absence of effect on calcium fluxes, and acrosome reaction	Luconi et al. (2001)
Ex vivo (Leydig cells from BPA-treated male rats)	2.4 μg/kg/day, PND 21–35	Inhibition of testosterone release	Akingbemi et al. (2004)
In utero exposure (Male rats)	2 and 20 μg/kg dam/day, GD 11–17	Enlargement of the prostate	Nagel et al. (1997)
In utero exposure (Male mice)	2–20 ng/g dam, GD 11–17	Decrease of testis weight	Kawai et al. (2003)
In utero exposure (CD-1 mice)	50 μg/kg dam/day, GD16–18	Enlargement of the prostate, increase of AGD, and reduction of epididymal weight	Gupta (2000)
In utero (male rat fetuses)	0.002–400 mg/kg dam/day, GD11–20	Modulation of LHR, Cyp11a1, and StAR	Naciff et al. (2005)
In utero (male rat fetuses), and postnatal exposures	2.4 μg/kg dam/day, GD12–PND35; 20 and 200 μg/kg dam/day, GD 1–PND 0	Decrease in serum testosterone	Akingbemi et al. (2004); Tanaka et al. (2006)
In utero and postnatal exposures	1.2–2.4 μg/kg dam/day, G6–PND21	Spermatogenesis inhibition, reduction of fertility of male offspring	Salian et al. (2009a)
In vivo (male mice and rats) and in utero exposures	6.25–125 ng/mouse; 12.5–500 ng/rat; 1.2–2.4 μg/kg/day dam from GD 6–21	Disruption of BTB	Toyama and Yuasa (2004); Salian et al. (2009b)
In vivo (adult male rats)	1 mg/rat, 14 days 10 mg/kg/day (14 days) 0.005–500 μg/kg/day, 45 days	Decrease in serum testosterone, and testicular antioxidant enzymes	Tohei et al. (2001); El-Beshbishy et al. (2012); D'Cruz et al. (2012a)
In vivo (adult male rats)	1 mg/rat/day, 14 days	Increase in plasma LH	Tohei et al. (2001)
In vivo (3-week-old male rats)	200 mg/kg/day, 5 days/week, 6 weeks	Decrease in serum LH	Nakamura et al. (2010)
	2.1 μg/kg/day, 70 days (PND 21–90)	Decrease in seminal vesicles size	Akingbemi et al. (2004)

(continued)

Table 1 (continued)

Experimental model	BPA dose or concentration	Major findings	Reference(s)
In vivo (young mice)	50 µg/mL (in drinking water), 8 weeks	Decrease in serum testosterone	Takao et al. (1999)
In vivo (male mice)	10–40 mg/kg/day, 2 weeks	Induction of DNA standard breaks in germ cells, decrease of sperm count and motility	Dobrzyńska and Radzikowska (2013)
In vivo (adult male rats)	20 µg–2 mg/kg/day, 6 days	Decrease in sperm production	Sakaue et al. (2001)
In vivo (male rats)	0.2–20 µg BPA/kg/day, 45–60 days	Increase of ventral prostate weight, decrease of epididymal and testicular weights	Chitra et al. (2003a, b)
In vivo (male chicks)	2 µg–200 mg/kg/day, 3–23 weeks	Decreased testis size	Furuya et al. (2006)
In vivo (brown trout, prespawning and spawning exposures, in aquarium)	1.75–2.40 µg/L, late prespawning and spawning periods	Decrease of semen quality	Lahnsteiner et al. (2005)
In vivo (male rats, oral; and male goldfish, in aquarium)	200 mg/kg, 10 days; 0.2 and 20 µg/L, 20-90 days	Alteration of sperm motility and velocity, increase of sperm DNA damage	De Flora et al. (2011); Hatef et al. (2012a, b)
In vivo (adult male guppies, in aquarium)	274–549 µg/L, 21 days	Decline in total sperm counts	Haubruge et al. (2000)
Male workers (humans) exposed to high levels of BPA at work	ND (exposure ascertained by higher BPA levels in blood, urine, or personal air)	Erectile and ejaculatory difficulties, reduction of sexual desire and sperm morphology and density	Xiao et al. (2009); Li et al. (2010b)
Men occupationally exposed to BPA diglycidyl ether	ND (exposure ascertained by higher urinary BPA levels)	Decrease of plasma FSH	Hanaoka et al. (2002)

Available data relating to the deleterious effect of BPA on male reproductive function were gathered and tabularly summarized from studies on rodents (rats and mice). Fish and chicks have also been included as experimental (animal) models; results gathered for all examined species support the view that BPA causes an endocrine-disrupting effect, i.e., decreased testosterone levels, altered sperm quality, etc. Moreover, altered sperm quality effects were confirmed in men who were occupationally exposed to BPA

3β-HSD 3-β-hydroxysteroid dehydrogenase, *17β-HSD* 17-β-hydroxysteroid dehydrogenase enzyme, *AGD* anogenital distance, *BTB* blood–testis barrier, *CYP19* aromatase, *Cyp11a* Cytochrome P450, subfamily 11A, *GD* gestational day, *FSH* follicular-stimulating hormone, *LHR* luteinizing hormone receptor, *ND* not determined, *PND* postnatal day, *StAR* steroidogenic acute regulatory protein

One adverse effect attributable to BPA is atrophy of the testes, as reported to have occurred in adult male Swiss mice treated with BPA glycerolate dimethacrylate (BISGMA, 100 μg/kg/day) for 60 days (Al-Hiyasat and Darmani 2006). White Leghorn male chicks treated with BPA (2 μg-200 m/kg/day) from 2 to 25 weeks of age also showed decreased testes size, and growth inhibition of androgen-dependent organs such as comb and wattle. The chicks receiving a higher BPA dose (≥20 μg/kg) showed inhibition of seminiferous tubuli development and spermatogenesis (Furuya et al. 2006), conditions more likely attributed to inhibition of testosterone synthesis. BPA's modulatory effect on testosterone synthesis has been reported in several studies (Table 2).

Oral administration of BPA to young mice for 4–8 weeks resulted in a dramatic decrease of plasma free-testosterone levels (Takao et al. 1999). BPA exposure also decreased the serum testosterone level in adult male rats, and suppressed human chorionic gonadotropin (hCG)-induced testosterone release by the testis (Tohei et al. 2001; El-Beshbishy et al. 2012; D'Cruz et al. 2012a). This antiandrogenic activity results from the BPA inhibitory effect on testicular Leydig cell StAR protein and steroidogenic enzymes, such as 3-β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-HSD (D'Cruz et al. 2012a; Hatef et al. 2012a).

Decreased activity of 3β-HSD and 17β-HSD, following BPA exposure, was also observed in both rat and human testis microsomes, together with inhibition of 17α-hydroxylase/17,20-lyase (CYP17A1) (Ye et al. 2011). Similarly, Akingbemi et al. (2004) reported that BPA inhibited Leydig cell CYP17A1. The inhibitory effect of BPA on CYP17 is likely a competitive-type inhibition, as demonstrated in *Escherichia coli* that expressed steroidogenic CYP17 (Niwa et al. 2001). Aromatase (CYP19) catalyzes conversion of androgens (testosterone) to estrogens (Carreau and Hess 2010; Carreau et al. 2010), and BPA exposure increased its expression in testes of male chicks, or in rat testicular Leydig cells (Furuya et al. 2006; Kim et al. 2010). This induction of testicular aromatase by BPA may thus contribute to decreased serum levels of androgens. However, Akingbemi et al. (2004) reported that postnatal BPA exposure of rats resulted in inhibited Leydig cell CYP19 expression and decreased serum 17β-estradiol levels. The latter authors exposed Long-Evans rats to BPA during perinatal period (PND 21–35) and noticed that serum estradiol was inhibited in the animals treated with the lower doses of BPA (0.2 μg–100 mg/kg bwt/day). The inhibitory effect was not observed at the highest dose (100 mg BPA/kg bwt/day), suggesting a dose-dependent effect of BPA on aromatase.

The fact that BPA causes an antisteroidogenic effect is further sustained by its ability to inhibit cAMP formation by preventing adenylate cyclase coupling to the luteinizing hormone (LH) receptor in vitro in mLTC-1 Leydig tumor cells (Nikula et al. 1999). Secretion of LH was also compromised in male animals exposed to BPA (Nakamura et al. 2010). The suppressed serum LH was associated with decreased LHbeta, decreased hypothalamic KiSS1 mRNA levels, and increased pituitary and testicular estrogen receptor (ER) mRNA levels (Akingbemi et al. 2004; Furuya et al. 2006; Navarro et al. 2009; Bai et al. 2011; Hatef et al. 2012a). An increased excretion (vs. matched controls) of BPA and decreased plasma

Table 2 Selected data relating to BPA effects on androgen/estrogen synthesis and action

Experimental model	Doses of BPA	Effect (estrogenic/antiandrogenic)	Mechanism of action/endpoint	Reference(s)
Perinatal exposure (male rat fetuses and pups)	2.4 μg/kg dam/day, GD12–PND35	Antiandrogenic	Decrease of testosterone levels	Akingbemi et al. (2004); Tanaka et al. (2006)
Perinatal exposure, male rat fetuses and pups	400 mg/kg dam/day GD6–PND20	Androgenic	Increase of testosterone levels Induction of AR in Leydig cells	Watanabe et al. (2003); Nanjappa et al. (2012)
In utero, and ex-vivo (Leydig cell cultures, from perinatally BPA-exposed rats) exposures	2.5 and 25 μg/kg dam/day, GD12–PND21; 10 μg/kg dam/day, GD11–20	Antiandrogenic/estrogenic	Inhibition of testosterone release, LH receptor, StAR, and 17β-HSD expression in Leydig cells Induction of ERs (ER1) in Leydig cells	Horstman et al. (2012); Nanjappa et al. (2012)
In utero exposure (CD-1 male mice fetuses)	50 μg BPA/kg dam/d, GD16–18	Antiandrogenic	Increase of AGD and prostate, reduction of epididymal weight	Gupta (2000)
Perinatal exposure (male rat pups)	1.2–2.4 μg/kg dam from GD6–PND21	Antiandrogenic	Decrease in testicular steroid receptors	Salian et al. (2009a)
In vivo exposure (male chicks)	2 μg–200 mg/kg/day, from 2 to 25 weeks of age	Antiandrogenic	Decrease of testis size, inhibition of spermatogenesis	Furuya et al. (2006)
In vivo exposure (male mice)	50 μg/mL (in drinking water), 8 weeks	Antiandrogenic	Decrease of plasma testosterone	Takao et al. (1999)
In vivo exposure (adult male rats)	1 mg/rat (≈3 mg/kg/day), 14 days; 10 mg/kg/day, 14 days	Antiandrogenic	Decrease of serum testosterone	Tohei et al. (2001); El-Beshbishy et al. (2012)
In vivo exposure (adult male rats)	0.005–500 μg/kg/day, 45 days	Antiandrogenic	Decrease in testicular activities of 3β-HSD, 17β-HSD, and serum testosterone	D'Cruz et al. (2012a)

In vivo exposure (male goldfish)	0.2 and 20 µg/L, 30–90 days	Antiandrogenic and estrogenic	Reduction of StAR, increase of testicular ERβ2 mRNA transcript at 0.2 µg BPA/L; Increase of AR, ERβ1 and CYP19 mRNA transcript in testis at 20 µg BPA/L	Hatef et al. (2012a)
In vitro (rat Leydig cells, rat and human testis microsomes)	0.01 nM; 10^{-8}–10^{-4} M	Antiandrogenic	Decreased activity of 3β-HSD, and 17β-HSD	Akingbemi et al. (2004); Ye et al. (2011)
In vivo (male chick), and in vitro (rat Leydig cells)	2 µg/kg–200 mg/kg; 0.1–10 nM	Estrogenic	Increases of CYP19 activity	Furuya et al. (2006); Kim et al. (2010)
In vivo (pubertal and adult rats)	≥0.2 µg BPA/kg/day, 45–60 days	Antiandrogenic	Decreased epididymal and testicular weights Increased ventral prostate weight	Chitra et al. (2003a, b)

17β-HSD 17-β-hydroxysteroid dehydrogenase, *3β-HSD* 3-β-hydroxysteroid dehydrogenase, *AGD* anogenital distance, *AR* androgen receptor, *CYP19* aromatase, *ERs* estrogen receptors (e.g., ERβ2, ERβ1), *GD* gestational day, *LHR* LH receptor, *PND* postnatal day, *StAR* steroidogenic acute regulatory protein

follicle-stimulating hormone (FSH) was reported in men occupationally exposed to epoxy-resin hardening agents containing BPA diglycidyl ether (Hanaoka et al. 2002), suggesting inhibition of FSH release by the chemical (Salian et al. 2011). In contrast, Tohei et al. (2001) reported increased plasma LH, following treatment of adult male rats of the Wistar-Imamichi strain (300–350 g) with BPA (1 mg/rat/day for 2 weeks). The LH stimulatory effect was associated with decreased plasma concentrations of testosterone and prolactin, as well as testicular contents of inhibin, suggesting that BPA directly inhibits testicular functions. The increased level of plasma LH was probably due to a reduction in the negative feedback regulation of the hypothalamic–pituitary axis by testosterone. Discrepancies in modulation of LH secretion by BPA (stimulation, or inhibition) as demonstrated by Tohei et al. (2001) and Nakamura et al. (2010), emphasize that the BPA endocrine-disrupting effect may be affected by the age of the animal at the onset of exposure.

Inhibin is synthesized in adult rat testes by Sertoli cells. Any reduction of inhibin's concentration in plasma, or in the testis (Tohei et al. 2001), therefore, suggests a dysfunction of the Sertoli cells. BPA-induced apoptosis of rat Sertoli cell was reported by Iida et al. (2003), and may result from an induction of caspase-3 by BPA (Mørck et al. 2010). BPA also promotes contact between harmful substances and developing sperm cells, by inducing inter-Sertoli cell BTB impairment (Toyama and Yuasa 2004; Salian et al. 2009b; Cheng et al. 2011). Sertoli cell function is pivotal in spermatogenesis, because it coordinates the differentiation of spermatogonia to mature spermatozoa, under stimulation of the FSH. Modulation of the Sertoli cells by BPA, directly or indirectly via inhibition of FSH synthesis (Hanaoka et al. 2002), may impair reproductive function in exposed males.

4.2 Effects of Bisphenol A on Spermatogenesis

The antiandrogenic and estrogenic effects of BPA that have been described in male goldfish (*Carassius auratus*) were associated with impairment of their spermatogenesis, as illustrated by the altered sperm parameters that were observed (viz., reduction in total sperm number, volume, density, motility, and velocity) (Hatef et al. 2012a, b). Lower semen quality was also observed in brown trout exposed to 1.75–2.40 μg/L BPA during the late prespawning and spawning periods (Lahnsteiner et al. 2005). Similarly, Haubruge et al. (2000) demonstrated declines in total sperm counts in adult male guppies exposed to BPA (274–549 μg/L) for 21 days. Such adverse effects of BPA on fish spermatogenesis have been documented to occur in rodents following the postnatal and pubertal periods, and in adulthood.

In mice, BPA induced the formation of morphologically multinucleated giant cells in testicular seminiferous tubules, having greater than three nuclei each (Takao et al. 1999). Similarly, a decrease of sperm count and motility was observed, and an increase of sperm morphological abnormalities, following 2 weeks of BPA administration (10–40 mg/kg bwt) (Dobrzyńska and Radzikowska 2013). The latter sperm parameters were also affected by a BPA derivative, BPA glycerolate dimethacrylate;

this derivative induced decreased male mouse fertility (Al-Hiyasat and Darmani 2006). Administration of BPA (≥20 μg/kg bwt/day) to adult rats for 6 days decreased daily sperm production (Sakaue et al. 2001). Similarly, Chitra et al. (2003a) reported a reduction in epididymal sperm motility in adult rats exposed to BPA for 60 days.

The antispermatogenic effect of BPA demonstrated in experimental animals has been confirmed by several epidemiological studies conducted among groups of BPA-exposed human males. Examples include a study carried out in China in 2008, in which it was revealed that male factory workers exposed to high levels of BPA at work experienced a sexual dysfunction, characterized by reduced sexual desire, and greater erectile and ejaculatory difficulties (Li et al. 2010b). During a cross-sectional pilot study, Xiao et al. (2009) analyzed blood BPA and semen quantity in workers exposed to BPA, and compared results to a control group. The sperm density of exposed workers was significantly lower than that of the control group, which had a lower blood BPA concentration. Furthermore, there was a negative correlation between blood BPA concentration and the percentage of normal sperm, indicating the negative influence of BPA on the semen quality.

Meeker et al. (2010) found a positive association, though not statistically significant, between BPA exposure (urinary BPA concentration) and altered sperm parameters (viz., decreased sperm count, altered morphology and motility, and increased sperm DNA damage) among infertile men. In a study carried out in fertile men, the correlation of urinary BPA concentration with semen quality was suggestive of an inverse association with sperm count and sperm motility (Mendiola et al. 2010). The urinary BPA concentration of the subjects was inversely associated with the free androgen index (FAI) and the FAI/LH ratio, and positively correlated to sex hormone-binding globulin (SHBG), indicating a deregulation of spermatogenesis. Similarly, Wang et al. (2012c) reported an association between higher urinary BPA concentrations and clinically abnormal thyroid hormones (elevated serum free T3 levels) that also influence spermatogenesis.

Although a three-generation reproductive toxicity study of BPA exposure in CD Sprague-Dawley rats showed no treatment-related effects from BPA exposure on reproductive organs/parameters (Tyl et al. 2002), other adverse effects appeared to be consistent with those caused by BPA in previous studies. Furthermore, Tyl et al. (2002) investigated physiological parameters, but did not assess biochemical changes. Other authors have studied BPA and have reported that it caused various effects, including genotoxicity and clastogenicity in blood cells (Ulutaş et al. 2010; Dobrzyńska and Radzikowska 2013; Tiwari et al. 2012), increased susceptibility to chemically induced mammary carcinogenesis (Jenkins et al. 2012), and induced meiotic aneuploidy in oocytes (Hunt et al. 2003), suggesting possible modification of the DNA in male germ cells that may be transmitted to the next generation (Salian et al. 2011).

Further evidence that BPA causes adverse effects is provided by the results of an in vitro binding assay involving proteins that transport sex hormones (Déchaud et al. 1999). BPA is a xenoestrogen that binds to SHBG (a steroid transporter in human plasma), with a reversible and competitive binding activity for both testosterone and estradiol, and produces a dose-dependent increase in concentrations of

hSHBG-unbound testosterone and/or estradiol. BPA may thus displace endogenous sex steroid hormones from hSHBG binding sites and disrupt the androgen-to-estrogen balance that is required for normal spermatogenesis (Déchaud et al. 1999; Carreau and Hess 2010).

4.3 *Effects of Bisphenol A on the Testicular and Epididymal Antioxidant System*

Another important mechanism by which environmental toxicants exert their adverse effects on male reproductive function is to disturb the pro-oxidant–antioxidant balance of the testis, resulting in impairment of testicular function (Mathur et al. 2008). Testicular function is associated with production of reactive oxygen species (ROS) that are regulated by an antioxidant system, under normal physiologic conditions. Exposure to environmental toxicants such as BPA aggravates the production of ROS, leading to testicular oxidative stress (see Fig. 1). El-Beshbishy et al. (2012), orally administered BPA to male rats at a dose of 10 mg/kg bwt for 14 days, and observed a decrease of testicular antioxidant enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase. The levels of hydrogen peroxide (H_2O_2) and lipid peroxidation were also increased in testes and spermatozoa of BPA-treated animals. In another study, testicular antioxidant enzymes were impaired by a very low-dose (viz., 0.005 mg/kg bwt/day) of BPA following 45 days of exposure (De Flora et al. 2011; D'Cruz et al. 2012a, b). BPA also decreased antioxidant enzyme activities and induced lipid peroxidation in both epididymides and sperm cells (Chitra et al. 2003a) (Fig. 1). The latter antioxidant enzymes were negatively affected in liver (Bindhumol et al. 2003), the organ that synthesizes steroid transport proteins such as hSHBG (Pugeat et al. 2010).

BPA acts to significantly disturb the pro-oxidant–antioxidant balance; therefore, reinforcing the ROS scavenging activity in the reproductive organs may represent a promising strategy to mitigate the BPA-related disturbances. To illustrate, in a recent study, Fang et al. (2013) reported that adolescent male mice, whose diet was supplemented with vitamin E during BPA exposure, showed an enhanced antioxidant response (i.e., increased SOD activity), and vitamin E protected against the reproductive inhibition normally caused by BPA.

4.4 *Effects of Bisphenol A on Sperm Function*

Whether BPA directly affects spermatozoa is still unclear. Luconi et al. (2001) incubated human spermatozoa in the presence of 1 μM BPA, and the results revealed no significant modification in calcium influxes and acrosome reaction in the spermatozoa. Similarly, the DNA integrity of sperm cells, as assessed using the Comet and TUNEL assays, and redox activity were not affected by BPA treatment in vitro

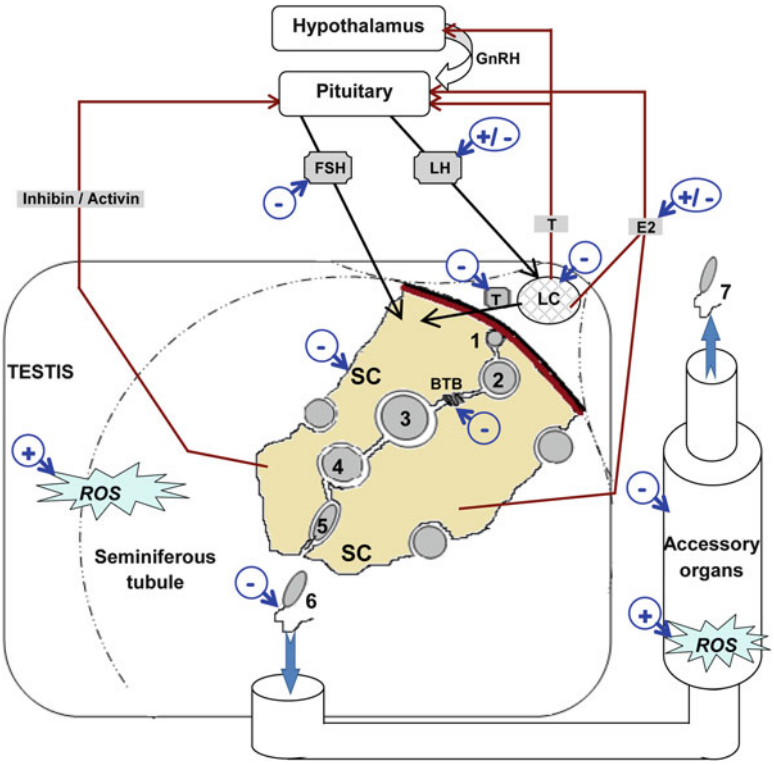

Fig. 1 A schematic diagram showing the main effects and action sites of BPA on male reproductive function: This schematic drawing depicts the hypothalamic–pituitary–testicular axis, and accessory organs. It also summarizes the spermatogenesis and sperm maturation processes. The main action sites where BPA modulates male reproductive function are indicated in the chart. The positive and negative signs indicate the following: (−): BPA disrupts cell function, reduces the weight of accessory organs, or inhibits hormone levels; (+): BPA induces increased hormone levels or reactive oxygen species (ROS), or stimulates enlargement of the prostate; (+/−): BPA exposure results in either induction (+) or inhibition (−) of hormone production. The numbers 1 to 7 refer to germ cells, at different developmental/maturation stages, viz. 1: spermatogonium; 2: preleptotene spermatocyte; 3: pachytene spermatocyte; 4: round spermatid; 5: elongated spermatid; 6 and 7: spermatozoa, before and after capacitation, respectively. Abbreviations: *BTB* blood–testis barrier, *E2* estradiol, *LC* Leydig cell, *FSH* follicle-stimulating hormone, *GnRH* gonadotropin-releasing hormone, *SC* Sertoli cell, *T* testosterone

(Bennetts et al. 2008). This suggests that the adverse effects on male reproductive function caused by BPA are mediated in vivo by other mechanisms, such as alteration of the hypothalamic–pituitary–gonadal axis and thyroid function (Akingbemi et al. 2004; Zoeller et al. 2005). Other examples of the effects caused by in vivo BPA exposure include altered sperm motility and velocity in goldfish (i.e., following 20–90 days exposure to 0.2 and 20 μg BPA/L) (Hatef et al. 2012a, b), and enhanced fragility of spermatozoa (i.e., as revealed by DNA fragmentation or sperm chromatin dispersion) in rats, following 10 days of administration of 200 mg BPA/kg bwt

(De Flora et al. 2011). Meeker et al. (2010) also studied human male partners of subfertile couples seeking treatment from the Vincent Andrology Lab at Massachusetts General Hospital, and observed increased DNA damage in sperm, and reduced semen quality that were associated with BPA exposure (Fig. 1).

5 Effects of Bisphenol A on Accessory Reproductive Organs

Secretions of male reproductive accessory organs have been implicated in the maturation, motility, and vitality of spermatozoa in the female tract. These organs represent potential targets for antifertility compounds, such as BPA. When female rats were orally dosed to 2 and 20 μg BPA/kg bwt/day on GD 11–17, their male offspring showed enlargement of the prostate in adulthood (Nagel et al. 1997). The latter effect was also reported by Gupta (2000) in CD-1 mice, whose female progenitors received 50 μg BPA/kg/day, during GD 16–18. In addition, the pups showed reduced AGD and decreased epididymal weight (Gupta 2000; Talsness et al. 2000). Exposure of rats to low doses of BPA (≥0.2 μg BPA/kg/day for 45 days) during pubertal and adult life also induced decreased epididymal and testicular weights, as well as increased weight of ventral prostate (Chitra et al. 2003a, b). BPA also affected the prostate epigenome during development, and thereby promoted prostate cancer (Ho et al. 2006). Mitogenic effects in the prostatic gland have been further affirmed by in vitro studies on human prostatic adenocarcinoma (LNCaP). In fact, BPA initiated both an androgen-independent (inappropriate proliferation, through activation of the tumor-derived androgen receptor, AR-T877A) and an estrogen/androgen-dependent proliferation signalling pathway in LNCaP (Wetherill et al. 2002; Lee et al. 2012). Moreover, treatment of Ishikawa cell cultures with BPA modulated several genes implicated in regulation of transcription (SUZ12, HES2, FST, ATF3) (Naciff et al. 2010). The increased size of the preputial glands and atrophy of epididymides were also reported in mice prenatally exposed to BPA (vom Saal et al. 1998).

6 Effects of Bisphenol A Derivatives on Male Reproduction

Because of concerns for the effects of BPA, Canada was the first country to regulate the products in which BPA could be used. Canada banned the use of BPA in baby bottles in 2008. Since this occurred, other developed countries (e.g., Japan and the USA) have also acted to restrict the use of BPA in baby bottles, and to some degree, the manufacture or production of BPA. These countries have also promoted the development of alternative BPA isomers, such as Bisphenol S ((BPS; 4,4′-sulfonyldiphenol), Bisphenol F (BPF; 4,4′-dihydrox-ydiphenylmethane), Bisphenol AF (BPAF; 4,4′-(hexa fluoroisopropylidene)diphenol), and Bisphenol B (BPB; 2,2-bis(4-hydroxyphenyl)butane), all of which are thought to be safer (Health

Canada 2008; Liao et al. 2012). Unfortunately, the biological activity (genotoxicity and estrogenicity) of these derivatives appears to be similar to that of BPA (Liao et al. 2012). BPAF acts as an agonist or antagonist to estrogen receptor alpha (ERα) or estrogen receptor beta (ERβ) (Matsushima et al 2010), and its administration to adult male rats for 14 days induced dysregulation of the hypothalamic–pituitary–testicular axis, characterized by increased LH and FSH levels, reduced serum testosterone, and a decline in testicular mRNA levels of inhibin B, ERα, and LHR (Feng et al. 2012).

7 Implications of Updated Data for Bisphenol A Exposure in Risk Assessment Studies

Human exposure to BPA is now of great concern, because of how widespread the chemical has become in the environment and because it is detected in human biological fluids. The harmful effects of BPA on male reproductive function have been clearly illustrated in animal-based studies, and the effects it produces appear to be more pronounced during fetal life (Lagos-Cabré and Moreno 2012). The reported data were essentially gathered from investigations that used rodents (rats and mice) as the animal model. However, BPA's kinetics within rodent and primate (chimpanzee and monkey) systems are similar (Taylor et al. 2011), and support a conclusion that BPA effects observed in rodents may well be expected to occur in humans. Moreover, although glucuronidation of BPA (prior to urinary elimination) occurs both in rat and human liver microsomes, human liver microsomes do this less efficiently than do those of rats (Elsby et al. 2001), and this can lead to BPA bioaccumulation in humans, as recently suggested by Stahlhut et al. (2009). It is also of concern that human fetal testes are more sensitive to the deleterious effects (inhibition of testosterone secretion and insulin-like 3 mRNA levels in Leydig cells) of BPA than are testes of rodents (N'Tumba-Byn et al. 2012).

Therefore, we believe that regulators should reinforce actions to prevent exposures to BPA and BPA-related products, with particular emphasis on reducing exposures during fetal life (pregnancy) and babyhood, since these are the stages that are most sensitive to toxicity by this chemical. BPA also preferentially attacks the developing (rather than adult) testis during the puberty stage.

The current BPA tolerable daily intake values (TDI) proposed by the European Food Safety Authority and by Health Canada are 0.025 and 0.05 mg/kg bwt/day, respectively (EFSA 2010; Cao et al. 2011; Hengstler et al. 2011; Geens et al. 2012). These values are respectively based on animal studies, in which the lowest-observed-effect-level (LOEL) for BPA is 0.025 μg/kg bwt and 0.05 μg/kg bwt. These TDIs were derived from LOEL values using an uncertainty factor of 1,000 (10 for interspecies differences, 10 for interindividual differences, and 10 for LOEL to no-observed-effect-level or TDI). However, recent studies included in this review have provided evidence that harmful or adverse effects result from administering very low doses of BPA. D'Cruz et al. (2012a, b) showed that

administering 0.005 μg BPA/kg bwt/day for 45 days inhibited rat testicular steroidogenic and antioxidant enzymes. This indicates that a more appropriate LOEL for BPA may be 0.005 μg/kg bwt in rats. Hence, we suggest a proper TDI to be 5 μg/kg bwt/day, instead of 25 or 50 μg/kg bwt/day (with an uncertainty factor of 1,000). Establishing a lower TDI that is more appropriate will assist in guiding authorities to limit human BPA exposure, and reduce the risk burden it places on health, and particularly on male reproductive function.

8 Summary

BPA is a ubiquitous environmental contaminant, resulting mainly from manufacturing, use or disposal of plastics of which it is a component, and the degradation of industrial plastic-related wastes. Growing evidence from research on laboratory animals, wildlife, and humans supports the view that BPA produces an endocrine-disrupting effect and adversely affects male reproductive function. To better understand the adverse effects caused by exposure to BPA, we performed an up-to-date literature review on the topic, with particular emphasis on in utero exposure, and associated effects on spermatogenesis, steroidogenesis, and accessory organs.

BPA studies on experimental animals show that effects are generally more detrimental during in utero exposure, a critical developmental stage for the embryo. BPA has been found to produce several defects in the embryo, such as feminization of male fetuses, atrophy of the testes and epididymides, increased prostate size, shortening of AGD, disruption of BTB, and alteration of adult sperm parameters (e.g., sperm count, motility, and density). BPA also affects embryo thyroid development.

During the postnatal and pubertal periods and adulthood, BPA affects the hypothalamic–pituitary–testicular axis by modulating hormone (e.g., LH and FSH, androgen and estrogen) synthesis, expression and function of respective receptors (ER, AR). These effects alter sperm parameters. BPA also induces oxidative stress in the testis and epididymis, by inhibiting antioxidant enzymes and stimulating lipid peroxidation. This suggests that employing antioxidants may be a promising strategy to relieve BPA-induced disturbances.

Epidemiological studies have also provided data indicating that BPA alters male reproductive function in humans. These investigations revealed that men occupationally exposed to BPA had high blood/urinary BPA levels, and abnormal semen parameters. BPA-exposed men also showed reduced libido and erectile ejaculatory difficulties; moreover, the overall BPA effects on male reproduction appear to be more harmful if exposure occurs in utero.

The regulation of BPA and BPA-related products should be reinforced, particularly where exposure during the fetal period can occur. The current TDI for BPA is proposed as 25 and 50 μg/kg bwt/day (European Food Safety Authority and Health Canada, respectively). Based on the evidence available, we believe that a TDI value of 5 μg/kg bwt/day is more appropriate (the endpoint is modulation of rat testicular function). Certain BPA derivatives are being considered as alternatives to BPA.

However, certain of these related products display adverse effects that are similar to those of BPA. These effects should be carefully considered before using them as final alternatives to BPA in plastic production.

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Biochar: An Effective Amendment for Remediating Contaminated Soil

Lu-Lu Kong, Wei-Tao Liu, and Qi-Xing Zhou

Contents

1 Introduction

Soil is one of the most fundamental resources for agricultural production systems. Besides serving as the main medium for crop growth, soils sustain the productivity of plants and animals, maintain or enhance the quality of water and air, and support human health and habitation, within both natural and managed ecosystem boundaries (Sun et al. 2001; Zhou and Song 2004). However, soil quality is now seriously threatened by anthropogenic contamination, which may pose unacceptable ecological risks to biota and human beings. In China, more than 2×10^7 ha of farmland have

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been contaminated with heavy metals (Wei and Yang 2010), and this has led to a sharp decrease in crop production and food quality in recent decades (Gu et al. 2003; Zhong and Wu 2007). Therefore, remediating soils to reduce soil contamination and to minimize downstream damage is essential (Powlson et al. 2011). A range of remedial techniques have been developed to address soil contamination. These include soil washing, soil vapor extraction, land-farming, soil flushing, ion exchanges, phytoremediation, bioremediation, and ecological remediation (Zhou and Song 2004). However, such traditional methods, when applied in situ are usually expensive and may create new problems, such as fertility loss and soil erosion (Khan et al. 2004; Kumpiene et al. 2008). Therefore, new alternatives are being sought (Sun et al. 2001). One new technique worthy of attention is soil stabilization/solidification (Zhou and Song 2004). This method offers an approach that may be less environmentally disruptive and less expensive, and hence potentially attractive as a future option.

Biochar is produced by thermal decomposition of biomass (e.g., wood, grass, dairy manure, broiler litter, and crop residues) in the partial or total absence of oxygen (Cao and Harris 2010). It is receiving increasing attention as a promising functional material in environmental remediation to stabilize soil contaminants, such as soluble heavy metals or organic molecules. The stabilization effects of biochar have been demonstrated to be longer lasting (Cheng et al. 2008; Liang et al. 2008; Cheng and Lehmann 2009) than liming applications, the effects of which gradually diminish over time because of dissolution and leaching of the liming agent, particularly under acidic conditions (Ruttens et al. 2010). In addition to biochar, other carbon-rich amendments are available. A key one is activated carbon (AC), which has physiochemical properties similar to biochar, and has been deployed to remedy contaminated soils and sediments (Cho et al. 2009; Song et al. 2012). In comparison to carbon-rich alternatives, biochar requires less energy and has a lower cost to produce, since it is generally obtained at low temperature and does not require further processing to be activated (Shinogi et al. 2003; Lehmann 2007).

The unique behavior of biochar was originally recognized from studying high charcoal-content soil (viz., *Terra Preta*) in central Amazonia. The high and sustainable fertility characteristic of *Terra Preta* was ascribed to carbon enrichment (i.e., up to 9%), which compares with 0.5% carbon content for plain soils from nearby places (Marris 2006; Glaser 2007; Gell et al. 2011). Similarly, rice husk charcoal and various ashes have been used in Asia since ancient times to enhance soil fertility (Ogawa and Okimori 2010). Applying biochar has several benefits, especially in vulnerable soils that need excessive amounts of composts and synthetic fertilizers. Specifically, application of biochar may effectively mitigate eutrophication of nearby streams and lakes, and reduce contamination of underground waters by reducing leaching of nitrogen (N) and phosphorus (P) (Laird et al. 2010; Kookana et al. 2011).

In recent years, there has been growing interest in utilizing biochar to mitigate the effect of anthropogenic-induced climate changes by permanently locking carbon into soils through a carbon-negative process (Glaser et al. 2009). Researchers have reported that >90% of biochar applied remains in terrestrial ecosystems

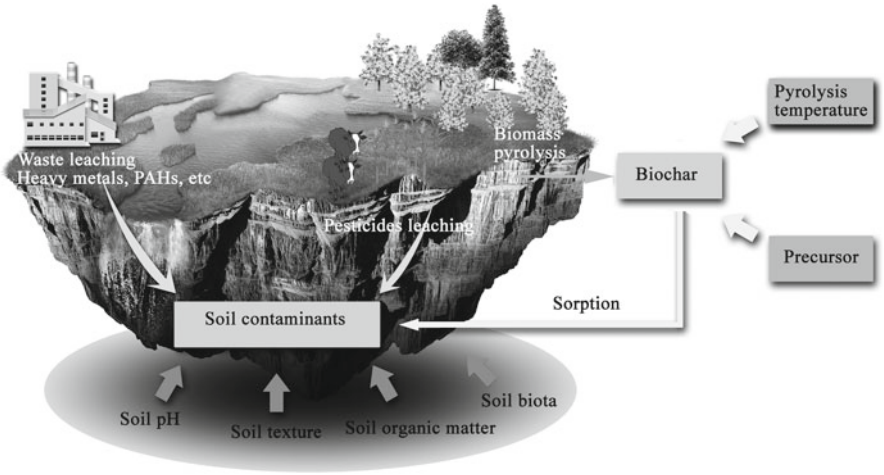

Fig. 1 The remediation of contaminated soil by biochar derived from varied biomass sources. *PAHs* polycyclic aromatic hydrocarbons

(Kuhlbusch et al. 1996; Masiello 2004). Moreover, fewer emissions of greenhouse gases, such as carbon dioxide, methane, and nitrous oxide, occurred after applying biochar, compared with applying its feedstocks (Yuan et al. 2011). Another biochar advantage is that bio-oil and syngas can be synthesized simultaneously as biochar is generated. This is accomplished through a distributed network of both slow and fast pyrolyzers (Laird 2008), in which the relative amounts and characteristics of each carbon form were controlled by pyrolysis processing conditions such as temperature, residence time, pressure, and feedstock types (Roberts et al. 2010). Thus, applying biochar to soil can be a win-win strategy for simultaneous production of bio-energy, permanent sequestration of carbon, and improvement of soil quality (Sun et al. 2001).

Despite its potential advantages, applying biochar to remediate contaminated soils has yet to be carried out on a large scale. The reason is mainly attributed to differences in the material properties of biochar (viz., O-containing functional groups, surface area (SA), aromaticity, cation exchange capacity (CEC), surface pH, nutrient contents, and porosity) that are produced from different biomass sources (Spokas 2010). In addition, the interaction mechanisms between biochar and target toxins, and the environmental constraints that affect biochar application are poorly understood. In particular, a better understanding of the limiting factors and characteristics of biochar and its soil-environmental interactions are needed before it can reach its optimum as an agent for remediation.

In the present review, it is our aim to critically examine the following: (1) the use of biochar to remediate contaminated soils, (2) the inherent and environmental aspects that affect biochar that is applied (simplified in Fig. 1), and (3) our perspectives on future research needs as regards biochar.

2 Properties of Biochar That Affects Sorption Capacity of Target Pollutants

Heavy metals are persistent and are not environmentally biodegradable (Sun et al. 2008). Thus, stabilizing heavy metals to reduce their bioavailability is a useful and sensible approach for remediating contaminated soils. The type of metal ions present plays an important role in sorption by biochar. Metal ions having small ionic radii and high charges are strongly attracted or repulsed by charged biochar surfaces. Moreover, metals may be preferentially stabilized on biochar via complexation or formation of solubility-limiting substances (precipitation). Adding biochar can greatly enhance the retention of soluble heavy metals (e.g., Cu, Cd, and Ni) in soil, especially for Pb. Pb contaminant levels have been reduced to levels that are below analytical detection limits by applying biochar (Mason et al. 1999; Uchimiya et al. 2010a, b). Such great removal efficiency of Pb was attributed to its amenability to phosphatic precipitation; similar behavior to Pb^{2+} was demonstrated in a decreasing trend by Cu^{2+}, Cd^{2+}, and Ni^{2+} (Kumpiene et al. 2008). Compared with Pb^{2+}, Cu^{2+}, and Cd^{2+}, Ni^{2+} showed high soil mobility in soil interstitial waters, mainly from forming complexes with available ligands within natural organic matter (NOM) (Mason et al. 1999; Uchimiya et al. 2010a, b). The higher mobility of Ni may mean that this metal poses higher risks to the environment, due to leaching from soil into groundwater. Adding biochar can solve this problem well (Uchimiya et al. 2010a, b). Although As is regarded to be more stable than certain other metals (residual portion >60%) (Glaser 2007), it was retained on the surface of biochar (Namgay et al. 2010; Beesley and Marmiroli 2011).

Organic contaminants, such as pesticides, will remain essential components of sustainable agricultural production systems in many countries for the foreseeable future. Continuing pesticide applications will inevitably result in additional soil contamination. Recently, several researchers have reported that soil or water amended with biochar, or soil rich with char from vegetation fires, affected the transport and fate of pesticides (e.g., atrazine, simazine, carbaryl (1-naphthyl methyl carbamate), and ethion (phosphorodithioate)); an approximate 66–97% reduction in the concentration of these pesticides in soil was observed after biochar was added, although the pesticides coexisted with other pollutants (e.g., Pb) (Singh and Kookana 2009; Cao and Harris 2010; Cao et al. 2011; Jones et al. 2011). In addition to adsorbing pesticides, biochar is also a good sink for the polycyclic aromatic hydrocarbons (PAHs) and the polychlorinated biphenyls (PCBs) in water and soils (Brändli et al. 2008; Wang et al. 2013a, b). The removal efficiency of PAHs was about 1 order of magnitude higher than reported for soil/sediment organic matter, or their precursor substances alone (Nguyen et al. 2007; Sun and Zhou 2008; Beesley et al. 2010; Wang 2010). The physicochemical properties of organic contaminants are much more complex than those of heavy metals, resulting in greater influences on their sorption mechanisms. Compounds that are commonly in a solid form showed a lower affinity for biochar than those that are liquid sorbates (Nguyen et al. 2007; Sun and Zhou 2008). It was suggested that the native organic phases of biochar

might occupy space near or at pore entrances, rendering them less accessible to solid sorbates; in contrast liquid-state sorbates might effectively penetrate such occlusions and even solvate the blocking phase (Nguyen et al. 2007). Another explanation was also proposed and is known as the solid-phase condensation mechanism, i.e., chemicals existing as solid (crystalline) phases in the adsorbed state cannot be "packed" as efficiently as liquid chemicals (Xia and Ball 1998).

The critical molecular diameter seems to be more important than the physical state of sorbates in determining the sorption strength of biochar; the reason for this is well explained by pore-filling mechanisms (Nguyen et al. 2007). Small molecules are prone to penetrate into the meso- and micro-pores of biochar, whereas large molecules are less receptive to adsorption, and tend to be adsorbed on the surface in ways that may block pores. Nguyen et al. (2007) reported that the maximum sorption of biochar to the following agents increased as molecular diameter decreased in the following order: phenanthrene (PHE) < naphthalene (NAP) < 1,2-dichlorobenzene (1,2-DCB) < 1,2,4-trichlorobenzene (1,2,4-TCB) < 1,4-DCB, although 1,4-DCB is normally condensed as a solid. Sun and Zhou (2008) also reported increased adsorption to biochar in the following order: PHE > anthracene (ANT) > pyrene (PYR), with the largest PYR moieties being the least adsorbed. In addition, pollutant molecules with small molar volume and, more importantly, small calculated critical molecular diameter (the smallest cylindrical pore that can accept the molecule) may enable more pore penetration and sorption. The latter effect is known as a steric effect (Kleineidam et al. 2002; Nguyen et al. 2007). Hydrogen binding and π–π electron donor–acceptor interactions also may occur between biochar and the specific pollutants that enhance sorption (Sander and Pignatello 2005; Chen and Chen 2009).

3 Effects of Preparation Procedures on Biochar Surface Characteristics

3.1 Pyrolysis Temperature

Biochar is comprised of a continuum of charred carbon forms (i.e., char, charcoal, graphite and soot) (Nguyen et al. 2008; Spokas 2010), whose ratios vary with pyrolysis temperature. A decreased pyrolysis temperature for biochar may result in increased oxygen-containing functional groups (reflected by elemental compositions, such as O/C atomic ratio; Fig. 2) that may facilitate a stronger sorption of heavy metals via complexation (Chun et al. 2004; Liang et al. 2006; Yuan et al. 2011). Cottonseed hull chars prepared at 350 °C were most effective in removing heavy metals (Cu, Ni, Cd, and Pb) in acid-eroded Norfolk soil, and these chars prepared at the following temperatures performed in the order: 500 °C > 650 °C > 800 °C (Uchimiya et al. 2011a, b, c). These results are consistent with those from a dairy manure-produced biochar prepared at 200 (641 mmol kg^{-1}) and 350 °C

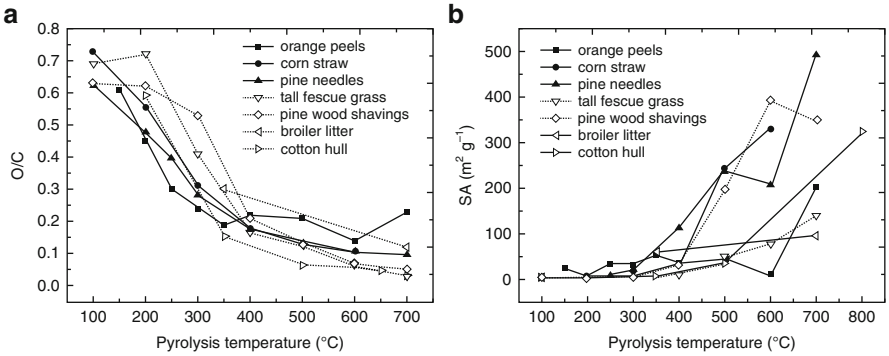

Fig. 2 The elemental composition of biochar (O/C atomic ratio, **a**) and surface area (SA, **b**) for biochars derived from various biomass sources as a function of pyrolysis temperature. Orange peel results are obtained from Chen and Chen (2009), corn straw results are obtained from Zhang et al. (2011), pine needle results are presented by Chen et al. (2008), tall fescue grass and pine wood shavings results are obtained from Keiluweit et al. (2010) and Kasozi et al. (2010), and cotton hull and broiler litter results are reported by Uchimiya et al. (2011a, b, c)

(452 mmol kg^{-1}); the former provided better stabilization capacity for Pb than the latter (Cao et al. 2009). As for organic chemicals, specific interactions with polymer aliphatic fractions of biochar may occur from partitioning at a low preparation temperature (Chen et al. 2008; Cao et al. 2009). Such aliphatic fractions can be divided into polar (e.g., -OH, -COOH, carbonyl (C=O) and/or phenolic-OH) and nonpolar types (e.g., $-CH_3$). The polar aliphatic groups are particularly important, as some organic pollutants could be immobilized by H-bonding (Cao et al. 2009, 2011; Cao and Harris 2010). However, sorption of organic chemicals by H-bonding may be overwhelmed by other forces at work, such as hydrophobic binding. The polar functional groups also may work as water binding centers (Cao and Harris 2010), thereby facilitating formation of water clusters that may reduce biochar accessibility to hydrophobic contaminants. Zhang et al. (2011) reported that corn straw biochar produced at 300 °C had fewer polar groups, and this contributed to its high sorption capacity for simazine.

When basic biochar obtained by being prepared at a higher temperature is applied, soil alkalinity may be increased (Beesley et al. 2010; Beesley and Marmiroli 2011; Gell et al. 2011). The total dissolved concentration of each metal ion present rapidly decreases above a critical pH value (Uchimiya et al. 2011a, b, c). As a result, the solubility of such metals tends to decrease after alkaline biochar is added. For example, the concentration of Cd in leachate from a column-leaching test decreased (up to 300-fold reduction), due to increased soil pH after biochar was added. Moreover, the affinity of Cd to biochar is strong. Its retention could not be reversed following subsequent leaching of the sorbed biochar with water at pH 5.5, which indicates that biochar application could decrease the potential risk of groundwater contamination (Beesley and Marmiroli 2011). Because As is more

soluble in alkaline soils, its concentrations in soil pore water increased more than 30-fold after biochar and compost were added to the soil (Beesley and Marmiroli 2011). As pyrolysis temperature increases a larger SA and a higher degree of carbonization are created. A strong π=π bond between the condensed aromatic phase (π electron acceptor) and organic compounds that are π electron donors [e.g., simazine and atrazine (Sun et al. 2010)] might subsequently be formed (Flores et al. 2009; Sun et al. 2010; Zhang et al. 2011). Such a bonding mechanism is referred to as adsorption, and means the partition medium for organic substances changes from a "rubbery" aliphatic domain to a "glassy" aromatic domain (Pignatello and Xing 1995; Chen et al. 2008). Figure 2 depicts how biochar SA increases as the heating temperature increases (Chen et al. 2008; Chen and Chen 2009; Kasozi et al. 2010; Keiluweit et al. 2010; Uchimiya et al. 2011a, b, c; Zhang et al. 2011). Biochar produced from orange peels (OP) showed an inconsistent increase in SA as pyrolytic temperature increased; this inconsistency was attributed to the fact that raw OP lacked lignin components (Chen and Chen 2009). Zhang et al. (2011) observed a positive correlation between adsorption strength and SA of corn straw biochar, when the biochar was produced at temperatures from 200 to 600 °C (CS200 to CS600). Compared to AC, which has a greater SA, biochar was less effective in reducing the concentration of organic pollutants (Yang et al. 2004; Cao et al. 2011).

3.2 Biomass Sources

The chemical composition of biochar is highly variable, and depends partly on the source of the biomass used. For example, phosphorus (P) is found more often in biochar prepared from dairy manures (Cao et al. 2009, 2011) or broiler litters (Uchimiya et al. 2011a, b, c) than in biochars prepared from plants (Singh et al. 2010). The P content of biochar contributes to a high removal rate for Pb as a phosphatic precipitate (Kumpiene et al. 2008). Compared to AC, the removal efficiency of biochar for Pb was nearly six times higher, although the biochar had a lower SA (Cao et al. 2009, 2011). However, P and As are chemically analogous (Beesley and Marmiroli 2011) and may compete for similar sorption sites. Hartley et al. (2009) previously reported increases in As mobility in biochar-treated soils, mainly from P out competing As for binding sites under alkaline conditions. Beesley et al. (2010) also found an increase of As concentrations in soil pore water after adding biochar in combination with compost (adding compost elevated P levels). Only when the concentration of soluble P is very low (Beesley and Marmiroli 2011) is As sorption unaffected and retained by hardwood biochar (Laird et al. 2010). Biochars containing Fe may promote the sorption efficiency of As (Nguyen et al. 2008). This occurs from adsorption of As onto Fe oxides by replacing surface hydroxyl groups with As ions, or by forming amorphous Fe^{3+} arsenates and/or insoluble secondary oxidation minerals (Kumpiene et al. 2008).

4 Conflicts Arising from Environmental Conditions

Soils and their ecosystems are quite complex. Many factors related to soils and their ecosystems affect the environmental behavior of biochar. Thus, understanding how soil conditions influence sorption behavior of biochar (Fig. 3) is critically important for selecting and applying biochar for sequestering contaminants in specific soil types. In addition, ecological aspects (i.e., interactions with soil microorganisms, fauna, and plants) as explicitly introduced by Beesley et al. (2011) and Lehmann et al. (2011), should be considered.

4.1 Soil pH

Most heavy metals are pH-sensitive. Some metals such as Pb, Ni, Cu, and Cd may be immobilized under basic conditions because of the formation of a solubility-limiting metal hydroxide, suggesting a reduced affinity for biochar. In addition, the interference by H^+, which competes for surface functional groups on biochar, may occur as well (Liu and Zhang 2009). Soil pH greatly influences the sorption of organic

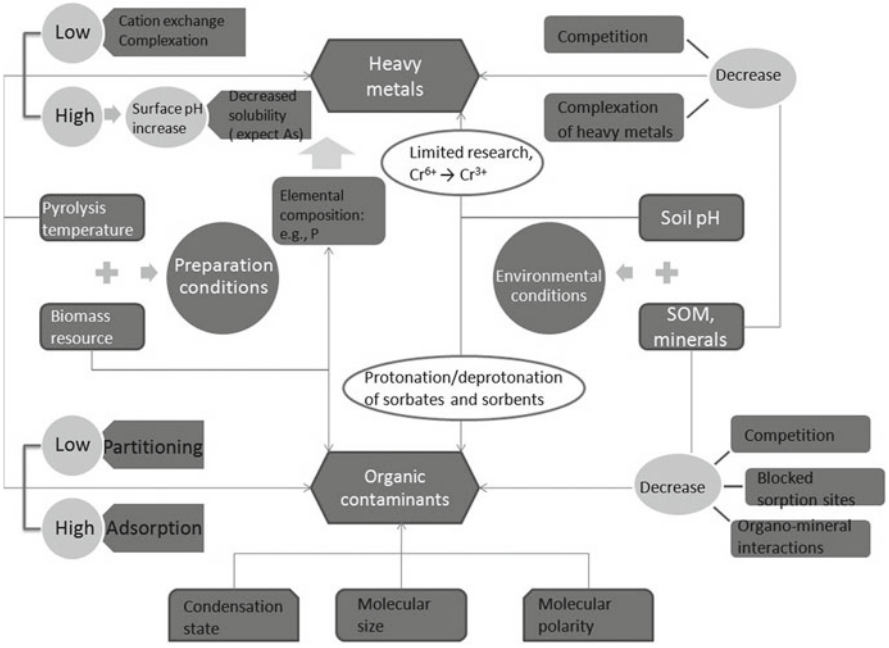

Fig. 3 Scheme depicting the main factors that affect application of biochar when used to remediate contaminated soil
SOM soil organic matter

pollutants, unless the molecules being adsorbed are electroneutral (e.g., diuron; Sheng et al. 2005). Ionizable organic contaminants may become dissociated or protonated within the pH range normally observed in soils, and thus attractive or expulsive force may exist between sorbates, soil (normally negatively charged), and biochar (Yang et al. 2004). Bromoxynil becomes dissociated at a high pH value (>pH 7) to form anionic species, resulting in reduced partitioning of the anionic species of bromoxynil into soil organic matter (SOM) and a weak interaction with the carbon surface of the char (Yang et al. 2004). Similar observations were found by Yamane and Green (1972), who reported higher ametryne adsorption, when ametryne was in the molecular form at high pH values in charcoal-amended soils. However, Sheng et al. (2005) reported significant sorption of ametryne in either its molecular or protonated form by wheat char; this suggested that other effects occurred, such as interactions with hydrated silica and surface functional groups of the char.

4.2 SOM and Minerals

Biochar is considered to be the predominant sorptive agent, as demonstrated by having been quantitatively compared to other SOM types in soil. For example, the sorption of simazine to biochar, derived from corn straw or green waste, was found to be 6.6–430 times higher than that of other forms of SOM (3.5–8.5%) (Zheng et al. 2010, 2011). Moreover, the sorption of diuron (0–6 mg L^{-1}) on soil-free chars from crop residue burning was nearly 400–2,500 times higher than when the soil itself (2.1% organic matter) was used (Yang and Sheng 2003a, b). Even when applied with 1% wheat char, the soil adsorption for diuron increased by 7–80 times (Yang et al. 2006). However, soil is a mixture of minerals and organic materials, both of which play an important role in biochar sorption. Soil remediation by using biochar is a long-standing procedure. A significant quantity of biochar or some of its components do change with time, a process commonly referred to as "aging" (Kookana et al. 2011). Researchers have proposed that biochar is aged by two distinct processes: oxidation (oxidation of biochar surface to O-functionalities) and mineralization (biochar loss via oxidation to CO_2). Biochar aging in soil may be accelerated by enhancing surface oxidation via sorption of environmental constituents (especially NOM) (Uchimiya et al. 2010a, b). Indicators of biochar oxidation include surface changes of elemental composition (Table 1), and probable surface oxidation of selected functional groups like carboxylic acids and phenols (Cheng et al. 2006; Liang et al. 2008; Cheng and Lehmann 2009; Joseph et al. 2010). Aged biochars displayed a lower adsorption capacity than did fresh biochar (Cheng and Lehmann 2009), since the binding sites for organic pollutants were blocked by NOM. After being treated to remove paramagnetic materials and organic matter, the sorption capacity for biochar increased significantly (Yang and Sheng 2003a, b; Nguyen et al. 2007; Ahangar et al. 2008; Singh and Kookana 2009). Because heavy metals are stabilized by complexation with O-functionalities or via cation exchange, this suggests that their sorption would be enhanced by biochar aging. Klasson et al.

Table 1 Elemental composition of biochar as a function of incubation time or temperature

Biochar precursor	Incubation time	Incubation temperature (°C)	C (%)	O (%)	H (%)	O/C	H/C	References
Corn stover residue	NI	NI	41.6	8.1	1.5	1.43	–	Hale et al. (2011)
	2 months	60	28.0	7.3	1.3	1.83	–	
		110	40.6	8.3	1.3	1.53	–	
Black locust	NI	NI	74.3	19.2	1.9	0.19	0.30	Cheng et al. (2006)
	4 months	30	74.1	19.9	2.1	0.20	0.33	
		70	67.7	26.3	1.9	0.29	0.33	
Wood	NI	NI	90.8	7.2	1.7	0.06	0.23	Cheng et al. (2008)
	1 year	30	88.2	9.2	2.4	–	–	
		70	85.8	10.6	3.4	–	–	
	130 years	Natural condition	70.5	24.8	4.5	0.26	0.76	
Wood	NI	Natural condition	51.1	40.9	–	–	–	Nguyen et al. (2008)
	40 years		34.2	50.6	–	–	–	
	100 years		22	58	–	–	–	

NI no incubation data available for biochar, i.e., fresh biochar

(2009) reported enhanced Cu^{2+} sorption capacity (in acidic aqueous solutions) via strengthening oxidation of AC. In contrast, labile organic matter (LOM, such as glucose) in soils may promote biochar mineralization through the action of microbes that co-metabolize biochar-C via enzymes that were produced to utilize glucose (Hamer et al. 2004; Keith et al. 2011). Thus, easily degradable portions of biochar may be depleted, leaving behind aromatic C forms (Nguyen et al. 2008). Organic pollutants may be strongly sorbed from strong π=π bonds formed with the condensed aromatic phase of biochar.

Contaminated soils often contain organic solvents (e.g., benzene derivatives), pesticides, PAHs, heavy metals, and other emerging contaminants. These soil contaminants and NOM may compete with target pollutants for similar sorption sites on biochar or on soil components (Chen et al. 2007; Uchimiya et al. 2010a, b, 2011a, b, c). Cu was better retained by acidic and eroded Norfolk soil that was amended with biochar, than by a fertile clay San Joaquin soil (Uchimiya et al. 2010a, b, 2011a, b, c). Moreover, hydration shells of dense water around adsorbed heavy metal ions may form in soils. Such hydration shells could intrude into adjacent surfaces and compete with both polar and nonpolar organic sorbates for available binding sites (Chen et al. 2007; Wang et al. 2009). The competition between SOM and organic pollutants is expected to be intense for the same sorption sites (Kwon and Pignatello 2005; Cao et al. 2011; Hale et al. 2011). However, the surface of biochar is highly heterogeneous, which is beneficial for binding contaminants simultaneously by biochar via different mechanisms (Cao et al. 2009; Wang et al. 2009). For example, biochar produced from dairy manure enhanced the sorption of Pb and atrazine simultaneously, with little competitive effect, since precipitation and partitioning were respectively responsible for sorption of Pb and atrazine (Cao et al. 2009).

4.3 Temperature

Temperature (especially under high temperatures) is perhaps the dominant factor in enhancing biochar aging (oxidation) (Cheng et al. 2006; Cheng and Lehmann 2009; Nguyen and Lehmann 2009). Biochar aging occurs even under natural conditions (Cheng et al. 2008; Liang et al. 2008) or at low temperatures (−22 °C) (Cheng and Lehmann 2009), albeit at a relatively slow rate. In addition, any resistance biochar has against high temperature greatly depends on the original materials from which it was made (Cheng et al. 2008). The oxidation of biochar starts at the surface (Cheng et al. 2006; Nguyen et al. 2008), where functional groups are selectively oxidized. Hence, aged biochars incubated at high temperatures demonstrated lower adsorption capacity for organic pollutants than did fresh biochar (Cheng and Lehmann 2009).

4.4 Soil Microorganisms

Microbial decomposition is another major mechanism by which biochar can be aged. Microbes age biochar by consuming labile C on its surface. Biological utilization of very refractory carbon sources, such as charred wood, coal (Shneour 1966; Scott et al. 1986), and graphite incubated in soils (Shneour 1966; Zimmerman 2010), has long been observed. Recently, Zimmerman (2010), by examining carbon release (i.e., CO_2 evolution), pronounced that biotic processes were consistently responsible for about half of the total biochar mineralization that took place during a 1-year incubation.

In addition, applying biochar to soils may increase microbial abundance, and change microbial community composition in ways that both reduce microbial diversity and enrich specific taxa (fungal, bacterial, and archaeal populations) to become dominant (Gell et al. 2011; Khodadad et al. 2011). These microbial community changes result from the extra supply of nutrients from the labile C of biochar, adding the fact that biochar improves microbial living conditions and affords microbes protection from grazers or competitors in biochar pores (Zimmerman 2010; Bushnaf et al. 2011; Lehmann et al. 2011). Such enhanced conditions for microbial growth may promote the biodegradation of organic pollutants. Yang and Sheng (2003a, b) reported that 1% wheat char, containing 21% potassium, 1.5% phosphorous, 0.64% nitrogen, and other "microelements," provided nutritional stimulation on benzonitrile biodegradation, when benzonitrile was not limiting. However, above ground plant biomass and worm survival rates were both increased when biochars were applied to amend industrial chemical-contaminated soil (Yang et al. 2006; Yu et al. 2009; Cao et al. 2011; Wang et al. 2013a, b). Therefore, the application of biochars may offer means to reduce pollutant uptake by soil microbes, plants, and human beings.

5 A Perspective on Future Biochar Research

Biochar derived from different precursor substances exhibits considerable potential for remediating contaminated soils. The use of biochar to improve soil quality is predicted to rise in the future. However, applying biochar to remedy contaminated soils is greatly influenced by how the biochar is prepared, the environmental conditions present when it is used, and the contaminants targeted. Below, we summarize the types of research needed to advance the use of biochars as tools in soil remediation:

1. Studies in which biochar is applied to remove new species of heavy metals for which data are now lacking. Most previous studies on biochar have focused on stabilizing soluble heavy metals, because such metals are important contaminants and can cause immediate environmental impact. Many other metal species also exist that need attention; these include those that are exchangeable, and those that are bound to carbonates, to Fe–Mn oxides, to organic matter, and to residual fractions (Mo et al. 2002). These metal species could be released into soil solution under certain conditions, or could be utilized by organisms through a diffusion effect, if sorbed onto soil particles. However, little research has been performed to investigate interactions between biochar and other chemical species of heavy metals, and such data are urgently needed.
2. Studies that address the complex interactions that occur among biochar, microorganisms and organic pollutants. Unlike heavy metals, most organic contaminants are biodegraded to a greater or lesser degree in soil. Such biodegradation can be decreased sharply by adding biochar as a soil amendment. Recently, it is reported that biochar can be utilized by microorganisms to increase microbial abundance and change microbial community composition. Doing so would enhance the biodegradation of organic pollutants. What is now needed is further work to explore the interactions that occur among biochar, microorganisms, and pollutants, and their combined effects on soil pollutants.
3. Research on biochar aging under different environmental conditions. Contaminated soil remediation is a long-lasting process. However, most research on biochar aging is limited to a 2-year period. More work is needed under natural environmental, as opposed to laboratory conditions, in particular, the influences of photodegradation, and the complicated interactions that may occur among the biochar, soil microorganisms, and plant components, as well as the effect of variable weather.
4. Research on the effect of biochar aging on pollutant sorption. Biochar “aging” via oxidation or mineralization in nature is inevitable. The effect of biochar aging on the stabilization of heavy metals and on organic contaminants is totally different. Studies on how to protect biochar from aging or how to utilize aged biochar to achieve a better effect on the contaminated soil remediation are essential.

6 Summary

Biochar is a carbon-rich material derived from incomplete combustion of biomass. Applying biochar as an amendment to treat contaminated soils is receiving increasing attention, and is a promising way to improve soil quality. Heavy metals are persistent and are not environmentally biodegradable. However, they can be stabilized in soil by adding biochar. Moreover, biochar is considered to be a predominant sorptive agent for organic pollutants, having a removal efficiency of about 1 order of magnitude higher than does soil/sediment organic matter or their precursor substances alone.

When trying to stabilize organic and inorganic pollutants in soil, several features of biochar's sorption capacity should be considered, viz., the nature of the pollutants to be remediated, how the biochar is prepared, and the complexity of the soil system in which biochar may be used. In addition, a significant portion of the biochar or some of its components that are used to remediate soils do change over time through abiotic oxidation and microbial decomposition. This change process is commonly referred to as "aging." Biochar "aging" in nature is inevitable, and aged biochar exhibits an effect that is totally different than non-aged biochar on stabilizing heavy metals and organic contaminants in soils.

Studies that have been performed to date on the use of biochar to remediate contaminated soil are insufficient to allow its use for wide-scale field application. Therefore, considerable new data are necessary to expand both our understanding of how biochar performs in the field, and where it can be best used in the future for soil remediation. For example, how biochar and soil biota (microbial and faunal communities) interact in soils is still poorly understood. Moreover, studies are needed on how to best remove new species of heavy metals, and on how biochar aging affects sorption capacity are also needed.

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Fusarial Toxins: Secondary Metabolites of *Fusarium* Fungi

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Contents

1 Introduction

Different types of fungi, belonging primarily to five genera (viz., *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Fusarium*), produce secondary metabolites that are called mycotoxins. There are also other genera, (viz., *Chaetomium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Phoma*, *Phomopsis*, *Pithomyces*, and *Stachybotrys*) that contain mycotoxin-producing fungi. Under favorable environmental conditions, when temperature and moisture are suitable, fungi proliferate and may produce secondary metabolites. These products have no biochemical significance for their own growth and development. The functions of mycotoxins

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have not been clearly established, but they are believed to play a role in eliminating other competing microorganisms in the same environment. They are also believed to help parasitic fungi invade host tissues. Toxigenic molds are known to produce one or more secondary metabolites, but not all molds are toxigenic and not all secondary metabolites from molds are toxic (Brase et al. 2009).

Fungi commonly enter the food chain through contaminated food and feed crops, mainly cereals, which become infested prior to and during harvest, or during (improper) storage. Although there are over 300 fungal toxins that have been isolated and chemically characterized, worldwide research has been focused on those forms that cause significant injuries to humans and animals. Therefore, there are only a few mycotoxins that are of practical relevance (Jakic-Dimic et al. 2010).

Even though there are geographic and climatic differences in the production and occurrence of mycotoxins, exposure to these substances occurs worldwide (Kuiper-Goodman 2004). The diseases that mycotoxins cause in animals and humans are called mycotoxicoses (Bryden 2012). Consumption of a mycotoxin-contaminated diet may induce acute and long-term chronic effects. Generally, the effects produced are teratogenic, carcinogenic, and/or estrogenic or immunosuppressive ones. However, the major problem associated with mycotoxin contamination of the animal feed supply chain is not acute disease episodes but reduced animal productivity. Direct consequences of consuming mycotoxin-contaminated animal feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (from immune-suppression), and reduced reproductive capacities, which all lead to economic losses. Although mycotoxins mainly affect grains, meat, milk, and eggs can be adversely affected as well. Consuming such products of animal origin has direct consequences for humans, because they are major human foods.

Our purpose in writing this review is to summarize and integrate the most significant data available about this important group of natural contaminants, because they have a major worldwide impact on feed and food safety and consequences for animal and human health. We also address measures pertinent to preventing and controlling fusarial toxins, and summarize the methods used to analyze for the presence of these toxins.

2 Fusarium Fungi

Fungi are a normal component of the microflora that exists in standing crops and stored feeds. Mycotoxin production varies with the fungal species present, agronomic practices used, the composition of the commodity and harvesting techniques used, and handling and storage conditions (Bryden 2009). Mycotoxins that adversely affect human or animal health are mainly found in postharvest crops such as cereal grains or forages. The preponderance of mycotoxins is produced by saprophytic fungi during cereal or other crop storage or by endophytic fungi during plant growth.

Table 1 Selected mycotoxigenic *Fusarium* species and the mycotoxins they produce

Species	Mycotoxin
F. culmorum	Aurofusarin, butenolide, chlamydosporol, culmorin, cyclonerodiol, cyclonerotriol, fusarins, moniliformin, trichothecenes, zearalenone
F. graminearum	Aurofusarin, butenolide, chlamydosporol, culmorin, cyclonerodiol, fusarins, trichothecenes, zearalenone
F. sporotrichioides	Aurofusarin, beauvericin, butenolide, culmorin, enniatins, fusarins, moniliformin, trichothecenes
F. crookwellense	Aurofusarin, butenolide, culmorin, cyclonerodiol, fusaric acid, fusarins, trichothecenes, zearalenone
F. acuminatum	Acuminatum, aurofusarin, beauvericin, chlamydosporol, enniatins, fusarins, moniliformin, trichothecenes
F. equiseti	Beauvericin, equisetin, fusarochromanone, moniliformin, trichothecenes, zearalenone
F. proliferatum	Beauvericin, enniatins, fumonisins, fusaric acid, fusaproliferin, moniliformin
F. verticillioides	Fumonisins, fusaric acid, fusarins, naphthoquinones
F. armeniacum	Beauvericin, fusarins, trichothecenes
F. pseudograminearum	Trichothecenes, zearalenone

The genus *Fusarium* was described by Link more than 200 years ago and currently contains over 20 species (De Hoog et al. 2000), some of which are among the most important toxigenic plant pathogenic fungi. *Fusarium* species infect important crops such as soft and durum wheat, barley, oats, rice, maize, potato, asparagus, mango, grasses, and other food and feed grains (Glenn 2007). *Fusarium* species produce long, multicellular, canoe-shaped or banana-shaped macroconidia. These large asexual conidia are the defining morphological characteristic of the genus. Many species also produce small, generally single-celled microconidia that range in shape from fusiform to oval to spherical. Additionally, some species produce thick-walled resistant chlamydospores that are important for long-term survival. Microconidia and macroconidia are important for wind and splash dispersal of the fungi. The conidia are also generally the propagules that result in infection of host plants.

Fusarium species are diverse in their host-associations and mycotoxin profiles, and individual *Fusarium* species are differentiated by using a range of morphological, molecular, and metabolic characteristics. *Fusarium* species cause root, stem, and ear rot, with severe economic crop yield reduction, often estimated at between 10 and 30% (Golinski et al. 2002; Logrieco et al. 2002; Uhlig et al. 2007). *Fusaria* are widespread in all cereal-growing areas of the world, but there are some geographical differences in their natural distribution, as well as of the corresponding mycotoxins they produce, which are influenced primarily by environmental conditions, and crop production and storage methods (Battilani et al. 2009). Selected mycotoxigenic *Fusarium* species and the mycotoxins they produce are presented in Table 1.

3 Fusarium Toxins

Fusarium toxins are produced in cereal grains during high moisture conditions at or near harvest time (Munkvold and Desjardins 1997; Sutton 1982). Wheat, triticale, and maize grains are especially vulnerable to *Fusarium* infection and are also frequently more highly contaminated with their secondary metabolites. In Table 2 we provide examples to illustrate the levels at which *Fusarium* mycotoxins normally appear in natural cereal samples (Döll and Dänicke 2011).

The amount of toxin produced depends upon physical factors (viz., moisture, relative humidity, temperature, and mechanical damage), chemical factors (viz., carbon dioxide, oxygen, composition of substrate, and pesticides used), and biological factors (viz., plant variety, stress, insects, spore load, etc.). Moisture and temperature have a major influence on mold growth and mycotoxin production. Access to water is most critical to microbial growth; hence, the water content of a feed commodity, expressed as a moisture percentage, is an important measure of

Table 2 Levels at which *Fusarium* toxin contaminates European cereals (adapted from Döll and Dänicke 2011)

	No. of samples	Positive (%)	Maximum (mg/kg)
Deoxynivalenol			
Wheat	6,358	61	50.000
Maize	520	89	8.850
Barley	781	47	0.619
Oats	595	33	5.004
Rye	271	41	0.595
Zearalenone			
Wheat	847	30	0.152
Maize	824	79	6.492
Barley	226	5	0.053
Oats	377	20	1.310
Rye	84	5	0.024
T-2 toxin			
Wheat	1,417	21	0.160
Maize	293	28	0.255
Barley	502	3	0.280
Oats	464	16	0.550
Rye	62	21	0.193
Diacetoxyscirpenol			
Wheat	845	14	0.050
Maize	111	51	0.025
Fumonisin B_1			
Wheat	110	79	0.736
Maize	801	66	10.2
Fumonisin B_2			
Maize	544	51	1.268

microbial attraction to such feed. Pathogenic fungi that invade crops prior to harvest usually require higher moisture levels (200–250 g/kg) than other fungi to successfully infect and proliferate in feed during storage (130–180 g/kg). Therefore, most feedstuffs with moisture contents above 130 g/kg are susceptible to mold growth and mycotoxin formation (Bryden 2012; Jakic-Dimic and Nesic 2009, 2011b). In temperate climates, the *Fusarium* toxins are common contaminants of cereal crops. *Fusarium* toxins are stable at high temperatures and during storage, milling, processing, and cooking of food and feed; humans and animals are, therefore, always exposed to them to a certain degree (EFSA 2011a, b; Jakic-Dimic et al. 2009). Below we will describe several of the more important mycotoxins that are produced by *Fusarium*.

3.1 Zearalenone

Zearalenone (ZEA; ZON, F-2 toxin) is a phytoestrogenic compound known chemically as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid μ-lactone. It is a metabolite associated with several *Fusarium* species (*F. culmorum*, *F. equiseti*, and *F. verticillioides*), but *F. graminearum* is the species most responsible for producing the estrogenic effects commonly found in farm animals. Maize is the crop most often affected, although these fungi are also found in other crops such as wheat, barley, sorghum, and rye in various countries around the world. Whilst zearalenone is primarily a field contaminant, it may also occur under poor storage conditions. ZEA exhibits estrogenic activity and has been implicated in numerous mycotoxicoses in farm animals, especially pigs. Common clinical signs are vaginal and vulvar swelling, enlargement of mammary glands, and testicular atrophy, as well as other reproductive effects, such as decreased fertility, increased number of resorptions, and reduced litter size (EFSA 2011a; Nesic 2003; Nesic et al. 2008a).

The strong estrogenic effect of zearalenone results from its competition with 17 β-estradiol to bind cytosolic estrogen receptors present in the uterus, and in hypothalamus and mammary and pituitary glands (Abbes et al. 2006). It is acknowledged that ZEA is of a relatively low acute toxicity (Zinedine et al. 2007).

Although significant differences were found in the metabolic profile of ZEA among animal species, only limited data on this topic are available for man. In pigs and probably in humans, ZEA is rapidly adsorbed after oral administration and can be metabolized in intestinal cells. In these cells, ZEA is degraded into α-zearalenol (α-ZEA), β-zearalenol (β-ZEA), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL), which are subsequently conjugated with glucuronic acid (JECFA 2000). The ZEA derivatives (α-ZEA, β-ZEA, α-ZAL, and β-ZAL and zearalanone (ZAN)) can be detected in corn stems infected with *Fusarium* in the field and in rice culture (Zinedine et al. 2007). Recently, Schollenberger et al. (2006) reported the occurrence of α-ZEA and β-ZEA in corn by-products, corn silage, and soya meal at low levels.

ZEA is rapidly biotransformed and excreted in animals; therefore, its dietary intake from meat and meat products is probably of little significance (Creppy 2002). ZEA can be excreted into milk of lactating cows, when it is fed at high doses. Prelusky et al. (1990) reported that the maximum concentrations in the milk of one cow given an oral dose of 6,000 mg ZEA (equivalent to 12 mg/kg bwt) was 6.1 μg/L (ZEA), 4 μg/L (α-ZEA), and 6.6 μg/L (β-ZEA). However, neither ZEA nor its metabolites were found in the milk (<0.5 μg/L) of three lactating cows fed 50 or 165 mg ZEA (equivalent to 0.1 and 0.33 mg/kg bwt) for 21 days. Nor has ZEA been reported in eggs from commercial production. The main sources of dietary ZEA in humans and animals are wheat, rye, and oats in European countries, and corn, corn products, and wheat products in Canada and the USA. Considering the mean levels of ZEA in the principal foods and their consumption levels, the average daily intake in human adults of ZEA ranged from 0.8 to 29 ng/kg bwt, whereas small children have higher average daily intakes ranging from 6 to 55 ng/kg bwt/day (Minervini et al. 2005).

ZEA causes alterations in the reproductive tract of laboratory animals (mice, rat, guinea pigs, hamsters, and rabbits) and domestic animals. Various estrogenic effects like decreased fertility, increased embryolethal resorptions, reduced litter size, changed weights of adrenal, thyroid, and pituitary glands, and changes in serum levels of progesterone and estradiol have been observed; however, no teratogenic effects were found in mice, rats, guinea pigs, or rabbits (Bacha et al. 1993; JECFA 2000). Recent studies have demonstrated the potential for ZEA to stimulate growth of human breast cancer cells (Ahamed et al. 2001; Yu et al. 2005).

3.2 Fumonisins

Fumonisins are cancer-promoting metabolites of *F. proliferatum* and *F. verticillioides* that have a long-chain hydrocarbon unit (similar to that of sphingosine and sphinganine), which plays a role in their toxicity (Wang et al. 1992). Six fumonisins have been identified: fumonisins A1, A2, B1, B2, B3, and B4. The A series are amides, while B series have a free amine group. Fumonisin B1 (FB1) is the most significant of the fumonisins, in terms of toxicity and occurrence. FB1 is chemically described as 1,2,3-propanetricarboxylic acid, 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl]ester.

A range of toxic syndromes have been associated with exposure to fumonisins, including Equine Leukoencephalomalacia (ELEM), Porcine Pulmonary Edema (PPE), and hepatic and renal injury in most species tested. Fumonisin exposure also results in hemodynamic alterations that are considered to be involved in the pathogenesis of both ELEM and PPE (EFSA 2005). Poultry are relatively resistant to the toxic effects of FB1 (Bermudez et al. 1995). Fumonisins are both cytotoxic and carcinogenic to animals. The modes of such actions, however, are not completely understood. Wang et al. (1991) demonstrated that FB1 disrupts sphingolipid

metabolism by inhibiting sphingosine *N*-acyltransferase (ceramide synthase) in rat liver microsomes. It also has been shown that FB1 inhibits other intracellular enzymes, including protein phosphatases and arginosuccinate synthetase (Jenkins et al. 2000). Therefore, FB1 exerts its cytotoxicity by inhibiting sphingolipid metabolism, protein metabolism, and the urea cycle. The carcinogenic role of FB1 has been linked to the accumulation of sphingoid bases that cause unscheduled DNA synthesis (Schroeder et al. 1994), alteration of signaling by cAMP (Huang et al. 1995) and protein kinase C (Yeung et al. 1996), and disruption of normal cell cycling (Ramljak et al. 2000).

Esophageal cancer in humans has been linked to consumption of fumonisin-contaminated corn in South Africa (Gelderblom et al. 1992; Rheeder et al. 1992; Thiel et al. 1992) and China (Chu and Li 1994; Yoshizawa et al. 1994). Fumonisins have not been conclusively demonstrated to be carcinogens in humans, but there is epidemiological evidence of their involvement.

3.3 Moniliformin

Moniliformin (MON) is the potassium or sodium salt of 1-hydroxycyclobut-1-ene-3,4-dione, which is produced by at least 30 *Fusarium* species that have been isolated from different substrates and geographical areas (Abramson et al. 2001; De Nus et al. 1996; Fotso et al. 2002; Schütt et al. 1998), but mainly come from *F. proliferatum*. MON is usually found on corn kernels and can be transferred to next generation crops and can survive for years in the soil (Guzman and Casteel 1994). Although fumonisins and moniliformin are produced by the same fungal species, no structural resemblance is found between these toxins, although they frequently occur together in feeds.

Moniliformin is highly toxic and results in rapid death in chicks and rats (Battilani et al. 2009). Cardiac injury, with alterations in the cardiac electrical conductance, was shown to be a primary cause of mortality in birds after prolonged feeding with MON. Bird consumption of MON caused poor growth performance, increased serum pyruvate levels, and cardiopathy (Reams et al. 1997). Acute mortality and gross lesions, including ascites, hydropericardium, and myocardial pallor have been observed in broilers, turkeys, and ducklings (Engelhardt et al. 1989).

The cytotoxic action of moniliformin was attributed to the inhibition of pyruvate dehydrogenase (Gathercole et al. 1986). Moniliformin also has been shown to increase cardiac permeability in young rats and ducklings, suggesting a mechanism for inducing Keshan disease in humans (Zhang and Li 1989). Using rat cardiac tissues (Chen et al. 1990), moniliformin was shown to inhibit other enzymes, including glutathione peroxidase and glutathione reductase. It was suggested, therefore, that free radical metabolism in the heart was compromised from inhibition of these crucial enzymes.

3.4 Trichothecenes

Trichothecenes are compounds containing sesquiterpene rings that are characterized by a 12,13-epoxy-trichothec-9-ene nucleus. They are produced by various *Fusarium* species, including *F. sporotrichioides*, *F. poae*, *F equiseti*, *F. acumninatum*, as well as species from the genera *Myrothecium*, *Cephalosporium*, *Verticimonosporum*, *Trichoderma*, *Trichothecium*, and *Stachybotrys* (EFSA 2011b). *Fusaria* produce type A and B trichothecenes. Types A and B are distinguished by the presence of an oxygen or carbonyl functional group at the C-8 position, respectively. The lack of the carbonyl group tends to make type A trichothecenes more toxic (Smith and Solomons 1994). The type A trichothecenes include T-2 toxin (T-2), HT-2 toxin (HT-2), neosolaniol (NEO), diacetoxyscirpenol (DAS), and monoacetoxyscirpenol (MAS), while type B trichothecenes include deoxynivalenol (DON, also known as vomitoxin) and its 3-acetyl and 15-acetyl derivatives (3AcDON and 15AcDON, respectively), nivalenol (NIV), and fusarenon X (FusX) (Krska et al. 2007). Although more than 150 trichothecenes have been identified, data about their natural occurrence in feed and food mainly concern T-2 toxin and HT-2 toxin, diacetoxyscirpenol (DAS), deoxynivalenol (DON or vomitoxin), and nivalenol (NIV). Both T-2 toxin and DAS are the most toxic and are soluble in nonpolar solvents (e.g., ethyl acetate and diethyl ether), whereas DON and its parent compound nivalenol are soluble in polar solvents such as alcohols (Trenholm et al. 1986). Trichothecenes are classified as gastrointestinal toxins, dermatotoxins, immunotoxins, hematotoxins, and gene toxins. Cytotoxicity of trichothecenes has been attributed to their potent inhibition of protein, RNA, and DNA synthesis. Other toxic effects of trichothecenes involve disruption of membrane transport and function, suppression of the immune response, and abnormal blood function effects (Hussein and Brasel 2001).

3.4.1 T-2 and HT-2 Toxins

T-2 and HT-2 toxins are mainly products of *Fusarium langsethiae*, but it may not be the only one producing these toxins, because other species, such as *Fusarium poae* or *Fusarium sporotrichioides* were also identified to possibly produce them. T-2 and HT-2 are toxic to all animal species, including humans. Symptoms of human intoxications are described as being Alimentary Toxic Aleukia (ATA), characterized by sepsis and hemorrhages and a general pancytopenia. One toxic effect exerted by the T-2 and HT-2 toxins is the inhibition of protein synthesis, which also affects immunoglobulin synthesis and, therefore, humoral immunity. Cell membrane functions and lipid peroxidation are also altered and account for many of the acute effects of T-2 and HT-2 toxins, including the necrotic lesions observed at contact sites. The systemic toxic effects that follow T-2 and HT-2 dietary exposure cause apoptosis of proliferating cells, such as bone marrow cells (inhibition of hematopoiesis) and cells of the immune system (lymphoid depletion) (EFSA 2011b). Comparable

symptoms have been described in farm animal species, often accompanied by local necroses in the upper gastrointestinal tract (Nesic et al. 2012). There are significant differences in the sensitivity of monogastric species and ruminants to the T-2 and HT-2 toxin, which is attributed to the effective presystemic elimination (de-epoxidation) of the toxins by the rumen of ruminant microflora (Jakic-Dimic and Nesic 2011a).

3.4.2 Deoxynivalenol

Deoxynivalenol (DON, vomitoxin) is produced by *F. graminearum*, *F. culmorum*, *F. crookwellense*, *F. sporotrichioides*, *F. poae*, *F. tricinctum*, and *F. acuminatum* (Pittet 1998). Intoxication with vomitoxin is manifested by a decrease in food intake or its refusal, vomiting, and digestive disorders with subsequent losses of weight gain. From a practical viewpoint DON is of outstanding importance among the B type trichothecenes because of its frequent occurrence at levels high enough to cause adverse effects, especially in pigs, which are the most susceptible. Ruminants and poultry are regarded to be less sensitive (Dänicke 2002; Dänicke et al. 2008; EFSA 2004a, b; Seeling and Dänicke 2005). Partial species differences were also found in DON metabolism. Susceptibility to DON is influenced by gender as well, i.e., males are more susceptible than females. A dose of 2 ppm DON in pig feed caused a reduction in feed conversion and body weight. Poultry tolerated 5 mg DON per ton of feed (5 ppb). A slight decrease in feed conversion was observed in dairy cows fed a diet containing 1% DON (Trenholm et al. 1984). The gastrointestinal system is the target organ of this toxin. In practice, the co-occurrence of DON and ZEA, or even additional mycotoxins in contaminated cereals exacerbates the management of affected animals (Döll and Dänicke 2011).

3.4.3 Diacetoxyscirpenol (DAS, Anguidine)

Diacetoxyscirpenol is one of the most toxic of the trichothecene mycotoxins. It is produced by certain species of *Fusarium* (e.g., *F. poae*, *F. semitectum*, *F. moniliforme*, *F. sporotrichioides*, *F. acuminatum*, *F. culmorum*, *F. crookwellense*, *F. venenatum*, *F. sambucinum*, *F. graminearum*, *F. equiseti*, *F. solani*, *F. roseum*, *F. tricinctum*, *F. avenaceum*, *F. langsethiae*, *F. compactum*, and *F. clamydosporum*; Omurtag et al. 2007; Schollenberger et al. 2007). A sublethal dose of the toxin resulted in cellular depletion and necrosis in the lymphopoietic organs, multifocal necrosis in the intestinal epithelium, and diffuse necrosis of germinal epithelium, with consequent progressive tubular degeneration of the testicles. After 3 exposure days, lymphopenia, neutropenia, and anemia were observed (Conner et al. 1986). The toxic effects of DAS in humans and animals are similar and include vomiting, diarrhea, hypotension, and myelosuppression (Battilani et al. 2009).

3.4.4 Nivalenol (NIV)

Nivalenol is primarily produced by *Fusarium cerealis* (*F. crookwellence*), *F. poae*, and *F. nivale* and to a lesser extent by *F. culmorum* and *F. graminearum* (Eriksen 2003). NIV belongs to type B trichothecenes, which are characterized by the presence of a carbonyl group at C-8 position. Nivalenol has often been reported in maize red ear rot throughout the European maize growing areas (Logrieco et al. 2002). It is a typical metabolite after dry and hot summers when harvest is performed earlier than usual (Pettersson 1996). Different NIV effects have been reported in acute toxicity studies. Such effects included bone marrow toxicity, erythropenia and slight leucopenia, hemorrhage and congestion in the intestine, and toxicity to lymphoid organs (Ryu et al. 1988), diarrhea, damage to epithelial mucous membranes of the intestine, the thymus, and testis (Ueno 1984). Major toxic effects produced in subacute, subchronic, and chronic toxicity experiments with NIV in mice were immunotoxicity, hematotoxicity, and reduced body weight gain, reduced feed intake, and organ weight changes (without histopathology findings). In subacute feeding studies with swine, NIV caused mild pathological changes in the gastrointestinal tract, spleen, and kidney, body weight gain, and food consumption (Pronk et al. 2002).

4 Prevention and Control of Fusarial Toxins

There are a number of approaches that can be taken to minimize mycotoxin contamination of the feed chain. They include prevention of fungal growth, and therefore mycotoxin formation, strategies to reduce or eliminate mycotoxins from contaminated feedstuffs or to divert contaminated products to low-risk uses.

Agricultural practices such as crop rotation and soil tillage are recommended to control plant contamination with *Fusarium* spp., even if these techniques are not always recognized as being efficient. In addition, removal, burning, or burial of crop residues is likely to reduce *Fusarium* inoculum for the following crop (Jouany 2007).

Because the contamination by *Fusaria* is most likely when the crop flowering stage occurs at the time of spore release, planting maize at earlier dates in temperate areas will often result in a lower contamination levels; this is true even if annual weather changes challenge the potential advantage of planting earlier (Blandino et al. 2009; Munkvold 2003). In wheat and barley, winter varieties develop and mature earlier than spring varieties and consequently have a reduced risk of *Fusarium* infection (Jouany 2007).

The harvest and postharvest control of pathogens is linked to the timing of harvest because, generally, earlier harvest results in lower concentrations of mycotoxins (Jones et al. 1981). Grain cutting height is another important factor in preventing postharvest contamination. Post harvest, damaged grains should be eliminated and the humidity level of the kernels lowered to reduce the possibilities of fungal infection and toxin production. Plant hydration and humidity are also important. A plant water activity <0.65 and a humidity level <14% in cereals usually limit

fungal growth; effectively, *Fusarium* spp. need 17–19% humidity to grow. The storage temperature after harvest has an effect on fungal growth too and, especially in silo storage temperature control is important. In particular, good ventilation that incorporates cooling and drying operations are necessary to avoid enhanced contamination during storage (Jouany 2007).

One strategy for controlling toxin production is to plant cereal varieties that are more resistant to injury by *Fusarium* spp. and insects. Fungal geneticists have unraveled the pathways and the genes responsible for synthesizing and regulating mycotoxin production, especially aflatoxin and the trichothecenes (Bhatnagar et al. 2008; Yu and Keller 2005). Information from this work may assist in developing plants that are resistant to toxin accumulation. What is sought is to achieve the success already achieved by incorporating Bt genes in maize hybrids, i.e., protection against insect attack (Wu et al. 2004). The transgenic Bt maize contains a gene from the soil bacterium *Bacillus thuringiensis*, which encodes for a protein delta-endotoxin that is toxic to common lepidopteran maize pests. These hybrids assist in managing mycotoxins because insect damage is often a major etiological factor in facilitating toxigenic fungal infection of crops (Dowd 1998). Bt maize is effective in reducing the incidence of fumonisin contamination, but is less effective in reducing deoxynivalenol contamination (Munkvold 2003). This response difference reflects different disease patterns and pathogens as deoxynivalenol is associated with Gibberella ear rot, whereas fumonisin production is associated with Fusarium ear rot, and the occurrence of Gibberella ear rot is not as strongly influenced by insect damage as is fumonisin accumulation (Munkvold and Desjardins 1997). Lower levels of *Fusarium* mycotoxins, fumonisin, and deoxynivalenol in Bt corn could have significant market and health impacts, both in the USA and around the world. It is estimated that at current planting levels, Bt corn saves farmers in the USA about $17 million annually through reduced fumonisin and deoxynivalenol damage alone (Wu et al. 2004).

Chemical control of the pathogen is difficult because, to be efficient, any fungicides applied must be totally lethal to *Fusarium* spp.; if not, they stimulate mycotoxin production in vitro (D'Mello et al. 1998).

Another control alternative is to utilize biological control with microbial antagonists or competitors to *Fusarium* spp. These can be integrated into contamination control strategies by spraying selected microbial competitors on plants at the flowering stage to eradicate or limit the growth of toxin producers (Jouany 2007). Some biological agents, such as some strains of *Bacillus subtilis*, *Bacillus thuringiensis*, *Candida*, *Pseudomonas,* or *Trichoderma* spp., are already approved in the European Union.

Biological methods or application of physical or chemical methods are different possibilities for the postharvest decontamination strategies. Many of these strategies are still at the study level. Farm feed storage and on-farm feeding systems can also contribute to mycotoxin exposure of the animals being fed. Simple measures can be used to significantly reduce the risk of mycotoxin exposure on the farm. Storage of grain at an appropriate moisture content (<130 g/kg), measuring grain temperature regularly, and inspecting for insects and wet spots regularly will limit the possibility

of fungal infection of feeds and feedstuffs. The risk of feed contamination will be reduced in animal units with rapid turnover of feed because there will be less time for fungal growth and toxin production. Recently, Australian researchers performed a survey (Moore et al. 2008), in which they investigated the on-farm occurrence of aflatoxin, deoxynivalenol, and zearalenone in cereal grains, forage, and straw. All three mycotoxins were found in all commodities, with zearalenone having the highest occurrence rate. Interestingly, grains had the lowest frequency of contamination but are often the only source of mycotoxins considered when examining a field toxicosis. These results highlight the potential risk of contamination of feedstuffs and forages, other than grain used in animal production. Moreover, the contamination of straw, which may be used as a roughage source in horse and ruminant diets, or as bedding for pigs, poultry, and horses, may also be a source of mycotoxin exposure on farms, as can grain dust (Degen 2011).

An important method for mycotoxin control is to alleviate and/or prevent harmful effects of mycotoxins already present in feed. To minimize the impact of mycotoxins one approach is to dilute feed with uncontaminated feedstuffs. Dilution of mycotoxin-contaminated grain with uncontaminated grain is one of the simplest and most widely utilized methods for improving feed intake and weight gain of animals. However the success of this approach depends on the degree of contamination, the dilution achieved, and the availability of a source of uncontaminated grain. In some countries, this practice is prohibited.

There is also the possibility of using various feed additives, which either adsorb mycotoxins on their surface or foment enzyme degradation of mycotoxins. The efficacy of alleviating harmful effects depends mostly on chemical structure of the adsorbent, as well as on the type of mycotoxin present. These are substances that are not resorbable from the gut. These substances act by physically binding some chemicals and blocking their resorption. Mineral adsorbents (e.g., hydrated sodium calcium aluminosilicate, sodium bentonit, dietary clay, and zeolites) and active charcol are among those commonly used for this purpose. The feasibility of utilizing organic adsorbents has also been examined, particularly esterified glucomanane which is isolated from the inner layer of yeast cell wall and which possesses significant adsorption capacity (Devegowda et al. 2004; Nesic 2003; Nesic et al. 2008a, b). Recently a new type of additive was developed which contains microorganisms that have the ability to enzymatically modify the mycotoxin structure (Fuchs et al. 2002; Nesic et al. 2011, 2012).

A program to control mycotoxin contamination from field to table is needed, and should involve applying the criteria of the HACCP (Hazard Analysis Critical Control Points) approach. This approach requires an understanding of the important aspects of interactions that occur at different levels of the food chain:

- Toxigenic fungi and crop plants interaction
- The on-farm plant production and crop harvest methods
- The production of livestock using grains and processed feeds
- Development of processed foods for human consumption
- Understanding the marketing and trade channels, including storage and delivery of foods to the consumer's table.

A good testing protocol for mycotoxins is necessary to manage all of the control points for finally being able to ensure a food supply free of toxic levels of mycotoxins for the consumer (Richard 2007).

5 Mycotoxin Detection

Mycotoxins present a major analytical challenge because of the range of chemical compounds they represent, and the array of feed matrices in which they are found. Analysis is essential for determining the extent of mycotoxin contamination, for risk analysis, for confirming the diagnosis of a mycotoxicosis and for monitoring mycotoxin mitigation strategies. Quantifying these compounds requires sophisticated laboratory equipment that includes high performance liquid chromatography, gas chromatography, gas chromatography/mass spectrometry, or liquid chromatography/mass spectrometry (Krska et al. 2008; Rahmani et al. 2009).

There are still several areas of mycotoxin analysis that require further study and refinement. These include improvements in commodity sampling techniques, performing analysis of conjugated toxins, and developing field or feed-mill screening techniques for feedstuffs.

Sampling is the single greatest source of error when quantifying mycotoxin contamination. The reason is that it is difficult to obtain feed samples representative of what may have caused a mycotoxicosis incident. Similarly, it is difficult to obtain representative samples to analyze for regulatory purposes from large grain consignments. These difficulties arise because of the uneven distribution of toxin within a commodity, in which mycotoxins occur (CAST 2003; Jakic-Dimic and Nesic 2011a; Whitaker 2003, 2006).

It has recently become apparent that there is a connection between "masked," "hidden," "bound," and/or conjugated mycotoxins in feedstuffs and the potential for animals to perform poorly. Mycotoxin conjugates may be formed as a result of plant metabolism (Berthiller et al. 2007; Gareis et al. 1990), but are not detected by using conventional analytical procedures. For example, zearalenone-4-glucoside, a conjugate of zearalenone and deoxynivalenol-3-glucoside, a conjugate of deoxynivalenol, can constitute up to 20% of the total content of the precursor mycotoxin in a feedstuff (Berthiller et al. 2005, 2006). It is likely that these conjugates will be hydrolyzed following ingestion, thereby increasing exposure to the precursor toxin. There is also evidence that ochratoxin A and fumonisins are conjugated by plants (Berthiller et al. 2007) and fumonisins may also be conjugated with sugars and proteins during food processing (Humpf and Voss 2004). Berthiller et al. (2009) reviewed the formation and determination of conjugated mycotoxins.

The development of immunological methods for mycotoxin detection (Pestka 1994), especially enzyme-linked immunosorbent assays (ELISA), although only semiquantitative, was a major step toward developing rapid, repeatable, and sensitive assays. These assays are suitable for field use and for screening feed commodities in feed mills. There are other approaches, most still experimental, that show

promise for rapid mycotoxin analysis without the need of sophisticated equipment. Such tests are flow-through ELISA, where the assay is conducted on a membrane or on gel-based columns, or lateral flow devices (LFD) and biosensors, and those based upon surface plasmon resonance (SPR) (Maragos and Busman 2010).

6 Summary

Exposure to mycotoxins occurs worldwide, even though there are geographic and climatic differences in the amounts produced and occurrence of these substances. Mycotoxins are secondary chemical metabolites of different fungi. They are natural contaminants of cereals, so their presence is often inevitable. Among many genera that produce mycotoxins, *Fusarium* fungi are the most widespread in cereal-growing areas of the planet. *Fusarium* fungi produce a diversity of mycotoxin types, whose distributions are also diverse. What is produced and where it is produced is influenced primarily by environmental conditions, and crop production and storage methods. The amount of toxin produced depends on physical (viz., moisture, relative humidity, temperature, and mechanical damage), chemical (viz., carbon dioxide, oxygen, composition of substrate, insecticides and fungicides), and biological factors (viz., plant variety, stress, insects, spore load, etc.). Moisture and temperature have a major influence on mold growth rate and mycotoxin production.

Among the most toxic and prevalent fusarial toxins are the following: zearalenone, fumonisins, moniliformin and trichothecenes (T-2/HT-2 toxin, deoxynivalenol, diacetoxyscirpenol, nivalenol). Zearalenone (ZEA; ZON, F-2 toxin) is a phytoestrogenic compound, primarily a field contaminant, which exhibits estrogenic activity and has been implicated in numerous mycotoxicoses of farm animals, especially pigs. Recently, evidence suggests that ZEA has potential to stimulate the growth of human breast cancer cells. Fumonisins are also cancer-promoting metabolites, of which Fumonisin B1 (FB1) is the most important. Moniliformin (MON) is also highly toxic to both animals and humans. Trichothecenes are classified as gastrointestinal toxins, dermatotoxins, immunotoxins, hematotoxins, and gene toxins. T-2 and HT-2 toxin, and diacetoxyscirpenol (DAS, anguidine) are the most toxic mycotoxins among the trichothecene group. Deoxynivalenol (DON, vomitoxin) and nivalenol although less toxic are important because they frequently occur at levels high enough to cause adverse effects.

The presence of mycotoxins in the animal diet can produce significant production losses. Any considerable presence of mycotoxins, in major dietary components, confirms the need to adopt a continuous prevention and control program. Such programs are usually based on several common approaches to minimize mycotoxin contamination in the food chain. Major strategies include preventing fungal growth and therefore mycotoxin formation, reducing or eliminating mycotoxins from contaminated feedstuffs, or diverting contaminated products to low risk uses. Because of the complexity of their chemical structures, mycotoxins also present a major analytical challenge. They are also found in a vast array of feed matrices.

Analysis is essential for determining the extent of mycotoxin contamination, for risk analysis, confirming the diagnosis of a mycotoxicosis and for monitoring mycotoxin mitigation strategies.

For the future, adequately controlling the mycotoxin problem in the livestock economy will depend on implementing appropriate agricultural management policies, as well as augmenting production and storage systems and analysis methods. Only such policies offer the opportunity to bring solid and long-lasting economical results to the livestock industry that is afflicted with the mycotoxin problem.

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Fungi Contamination of Drinking Water

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1 Introduction

Drinking water sources contain different kinds of biological pollutants, such as bacteria, viruses, protozoa, and fungi. Recently, researchers have reported the presence of fungi in drinking water (Goncalves et al. 2006a; Pereira et al. 2010), and have observed that these fungi affect the taste and odor of the water. A wide variety of fungi species have been isolated from drinking water. Some of these species are known to be strongly allergenic (e.g., causing skin irritation), or may cause infections in immune-suppressed individuals (e.g., those suffering from AIDS, cancer, asthma or other respiratory diseases, or recovering from organ transplants) (Hageskal et al. 2009; Green et al. 2003).

Mycotoxins cause an array of health problems in both human and animals, and some mycotoxins are known to be carcinogenic and capable of impairing the immune system. Characteristically, Stakheev et al. (2011) reported that the trichothecene toxins inhibit protein biosynthesis and induce chromosomal changes. Humans that consume grains, food or water that contains certain mycotoxins are subject to developing serious maladies, such as alimentary toxic aleukia. The degree to which a fungus will be mycotoxigenic depends on several factors that include species and strains of fungus involved, composition of the environmental matrix in which they appear (Fernandez-Cruz et al. 2010).

Paterson et al. (1997) detected aflatoxins in water from a cold water storage tank and found that the aflatoxins B_2,G_2 were present. Gromadzka et al. (2009) detected the zearalenone mycotoxin in surface waters, groundwater and wastewater in Poland, and found that naturally occurring low molecular weight organic matter in the water affected analytical recovery rates of zearalenone.

Moreover, humans may acquire fungal several diseases or develop allergies directly by inhaling mold spores and hyphal fragments, which may become aerosolized in indoor air when contaminated water passes through showerheads, taps, or toilet cisterns (Green et al. 2003). Such respiratory exposure to potentially harmful species (Hageskal et al. 2009; Pereira et al. 2010), or dietary exposure to mycotoxin-contaminated food and feed products potentially pose an acute risk to both human and animal health (Mishra et al. 2003; Green et al. 2003; Kanzler et al. 2007).

It is our purpose in this review to address the risks that arise from the occurrence of fungi in drinking water. We address both the fungi themselves and the major secondary mycotoxin metabolites produced by the filamentous fungi. In addition, we describe the fungi that are known to be hazardous, and the nature of the risks they pose to human health, when consumed in contaminated water, food, or are inhaled. Finally, we review selected studies, in which efforts have been made to control fungi or their toxic metabolisms in aquatic environments.

2 Sources and Types of Fungi Found in Water

In numerous studies, water samples from a variety of sources have been collected and examined for the presence of fungi. Water sources sampled have included surface water (Anaissie et al. 2003; Pereira et al. 2010; Hageskal et al. 2006), ground water (Pereira et al. 2010; Hageskal et al. 2006; Sammon et al. 2010; Gottlich et al. 2002; Kanzler et al. 2007), spring water (Pereira et al. 2010; Grabinska-Loniewska et al. 2007), water from treatment plants (Sammon et al. 2010), tap water (Goncalves et al. 2006a; Hageskal et al. 2007; Kanzler et al. 2007), water from hospital installations (Arvanitidou et al. 1999; Hageskal et al. 2007), municipal water supply systems (Grabinska-Loniewska et al. 2007; Anaissie et al. 2003; Doggett 2000), storage tank and shower water, mineral water (Cabral and Pinto 2002), and water consumed by school children (Nasser 2004). In addition, Anaissie et al. (2003) examined water samples collected from a bone marrow transplant unit, and from cold- and hot-water storage tanks, and showers and sinks in patient's hospital rooms. Among these various sources, fungi were frequently detected in the water samples, although the frequency of such detections was much higher in surface and spring water samples than in groundwater samples (Pereira et al. 2010). The most common genera of fungi occurring in the various water sources tested (e.g., reservoirs, mains, and treated water at water treatment facilities) belonged to the following genera: *Cladosporium*, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Pithomyces*, *Alternaria*, *Peacilomyces*, *Acremonium*, *Epicoccum*, and *Curvularia* (Sammon et al. 2010). It was also noted that *Aspergillus* was the most common genus recovered from all parts of water supply systems (Arvanitidou et al. 2000). Hageskal et al. (2006) and Grabinska-Loniewska et al. (2007) found that fungal flora was dominated by species representing the genera *Penicillium*, *Trichoderma*, and *Aspergillus*, but Gottlich et al. (2002) and, in a separate study, Goncalves et al. (2006a) noted the dominance of *Phialophora*, *Exophiala*, *Acremonium*, *Penicillium*. Cabral and Pinto (2002) studied fungi in bottled mineral water and isolated the fungal species *Penicillium* sp., *Cladosporium cladosporioides*, and *Alternaria alternata*. Nasser (2004), studying water samples from schools, discovered *Aspergillus*, *Fusarium*, and certain other fungal species, but in very low numbers. Kanzler *et al*. (2007) noted that in drinking water the fungal taxa that occurred most frequently were *Cladosporium* spp., *Basidiomycetes* spp., and *Penicillium* spp. These authors commented that drinking water serves as a reservoir for fungi, and opportunist species thereof can cause infections in immune-suppressed individuals. Abdel-Hameed et al. (2008) reported fungi representing *Aspergillus flavus*, *A. parasiticus*, *Penicillium*, *Fusarium*, and *Trichoderma* appeared in samples collected from air and water environments. Anaissie et al. (2003) noted that species of *Aspergillus* and *Penicillium* were the fungi most often found. Paterson et al. (2006) also reported that these same two genera, along with *Verticillium*, were frequently found fungi. In addition, Anaissie et al. (2003) noted that these fungi were recovered in high numbers from the aqueous environment of hemodialysis apparatus, implying a potential risk for hemodialysis patients. Doggett (2000) and Grabinska-Loniewska et al. (2007) investigated the biofilms present in municipal water distribution systems and

found filamentous fungi in numbers ranging from 4.0 to 25.2 CFU cm^2. Saad et al. (2004) extracted DNA from biofilms on painted surfaces, and Goncalves et al. (2006b) investigated fungi on biofilms resident in water distribution systems by using fish and calcofluor staining technique to detect the fungi. Paterson et al. (2006) reported that *Penicillium* spp., a filamentous fungus, displayed their highest numbers in winter, and the authors believed that mycotoxin produced by this fungus may affect the prevalence of biofilms. Problems associated with the growth of filamentous fungi in water systems include unsightly appearance, blocked distribution pipes, odors, pigment buildup, increased presence of potentially pathogenic and allergy-causing fungi, and mycotoxin production. Gottlich et al. (2002) studied the presence of fungi using standard hygiene indicators versus other microorganisms, and reported no correlation between the occurrence of fungi with *Escherechia coli* or other coliform bacteria. However, Sammon et al. (2010) found a significant positive correlation between the frequency at which filamentous micro-fungi, yeast, and bacteria existed, when the combined data from all sample sites were analyzed. In general, most researchers performing the studies described above revealed that fungi occurred in the water matrices they had studied, and indicated that many of these isolated fungi have highly negative effects on human health.

Fungi exist commonly in various aqueous environments and play potentially crucial roles in nutrient and carbon cycling. Fungi may be leached or released from agricultural land, soil, or air, to contaminate surface water. Water contaminated with fungi may reach tap water (i.e., homes, hospitals, etc.) after being processed for disinfection through Water Treatment Plants (WTP). One wonders why such treatment does not sufficiently remove all fungi from water. The partial answer is that many fungal species that appear in surface and top water often reside in the water system pipes as biofilms. The fungi in such biofilms are resistant to standard water treatment processes. So, fungi in biofilms are available to periodically recontaminate water sources that end up as tap water.

3 Methods Used to Isolate Fungi

At present, there is no standard method for isolating fungi (using media) from water for enumeration, although several researchers have recommended culturing, and isolation approaches to enumerate fungi. Among these latter methods, the most common used is membrane filtration by using Millipore TM (0.45 μm pore size and 47 mm diam) (Arvanitidou et al. 1999; Cabral and Pinto 2002; Anaissie et al. 2003; Hageskal et al. 2006; Grabinska-Loniewska et al. 2007; Kanzler et al. 2007; Sammon et al. 2010; Pereira et al. 2010). Goncalves et al. (2006a), however, used swabbing and baiting, in addition to filtration to isolate fungi. Moreover, several authors used the direct plate-spread method which requires water volumes of 0.1–1 ml (Pereira et al. 2010; Abdel-Hameed et al. 2008; Gottlich et al. 2002; Kanzler et al. 2007). Different types of culture media have also been used to enumerate fungi; examples are Czapek-Dox Agar and Dichloran Rose Bengal Chloramphenicol (DRBC) (Pereira et al. 2010), and Sabouraud Dextrose Agar

(Arvanitidou et al. 1999; Anaissie et al. 2003), although they required adding antibiotics to prevent bacterial growth (Sammon et al. 2010; Kanzler et al. 2007). Malt Extract Agar (MEA) was used by Cabral and Pinto (2002), Kanzler et al. (2007) and Sammon et al. (2010). Hageskal et al. (2006) used Dichloran—18% Glycerol Agar for isolating fungi, while Gottlich et al. (2002) used culture plates with Blood Agar Base for isolating bacteria and fungi. Goncalves et al. (2006a) used half-strength corn meal, Neopeptone–Glucose Rose Bengal Aureomycin (NGRBA), and Oomycete Selective Agars for enumerating colony-forming units, and Nasser (2004) used Cellulose Czapek's and Glucose Czapek's media to study fungi collected from school children. Finally, Abdel-Hameed et al. (2008) used Rose Bengal Streptomycin Agar and Starch Casein Agar for counting waterborne fungi and actinomycetes.

Depending on what culture media or process is used, some fungi species may not be successfully isolated from water, even if present. Moreover, the method used for isolation of fungi may affect the frequency results recorded for the fungi. The filter membrane method is better than the direct plate-spread method for studying fungal occurrence in water than the other methods. The reason for this is that the volume of water in the filter membrane method is higher than that used for the plate-spread method. The swabbing method is best for studying the presence of biofilms in surface pipes.

4 Identification of Fungi

Fungi are a diverse group of organisms belonging to the kingdom Eumycota. This kingdom comprises five phyla: Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota. Some fungi are primarily adapted to aquatic environments, and are naturally found in water. Such aquatic fungi are called zoosporic, and many belong to the phylum Chytridiomycota (Hageskal et al. 2009). There are two methods for identifying fungi: conventional and molecular methods. The conventional method is to differentiate fungi from microscopic examination of typical morphological features (e.g., characteristics of spores and hyphae and colony color as they appear on media). Even today, fungi are commonly recognized, or are initially identified on the basis of their microscopic characteristics. Because microscopic identification of fungi requires considerable experience, Hageskal et al. (2006) suggested a second identification method, which entails subculturing the fungi on a suitable agar medium, whenever possible, after which the molds can be phenotypically identified to the species level by microscopic examination (Pitt and Hocking 2009; Lesli and Summerell 2006; Webster and Weber 2007). In addition, such examination may rely on staining techniques (e.g., lacto-fuchsin or fluorescent Mag fura, if needed) that better reveal the microscopic nature of the fungi under light microscopic examination (Cox and Thomas 1999; Santos et al. 2010).

The primary technique for identifying fungi is still based on morphology, and physiological tests (e.g., characteristics of fungi in culture media or their metabolism characteristics). Sometimes conventional methods for identifying fungi are difficult to use. Therefore, in such cases, the more recently developed molecular

Table 1 Some universal PCR primers that have been used to study the diversity of fungi

rRNA	Primer sequence	Target	References
NS1	GTAGTCATATGCTTGTCTC	18S	White et al. (1990)
NS2	GGCTGCTGGCACCAGACTTGC		
NS3	GCAAGTCTGGTGCCAGCAGCC		
NS4	CTTCCGTCAATTCCTTTAAG		
NS5	AACTTAAAGGAATTGACGGAAG		
NS6	GCATCACAGACCTGTTATTGCCTC		
NS7	GAGGCAATAACAGGTCTGTGATGC		
NS8	TCCGCAGGTTCACCTACGGA		
nu-SSU-0817	TTAGCATGGAATAATRRAATAGGA		Borneman and Hartin (2000)
nu-SSU-1196	TCTGGACCTGGTGAGTTTCC		
nu-SSU-1536	ATTGCAATGCYCTATCCCCA		
ITS1	TCCGTAGGTGAACCTGCGG	ITS	White et al. (1990)
ITS2	GCTGCGTTCTTCATCGATGC		
ITS5	GGAAGTAAAAGTCGTAACAAGG		
ITS3	GCATCGATGAAGAACGCAGC		
ITS4	TCCTCCGCTTATTGATATGC		
NL209	AAGCGCAGGAAAAGAAACCAACAG	28S	Zuccaro et al. (2004)
NL912	TCAAATCCATCCGAGAACATCAG		
NL359	GGACGCCATAGAGGGTGAGAGC		
ITS1-F	CTTGGTCATTTAGAGGAAGTAA		Gardes and Bruns (1993)
TW14	GCTATCCTGAGGGAAACTTC		Taylor et al. (1999)
Ctb6	GCATATCAATAAGCGGAGG d		
TW13	GGTCCGTGTTTCAAGACG		

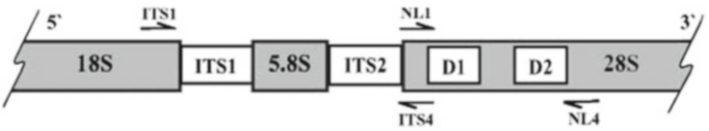


Fig. 1 The mapping of a typical fungal rRNA gene showing the positions of variable regions and universal primers designed to amplify these regions of DNA. Adapted from White et al. (1990)

methods can be employed to identify fungi to species level, or to study fungal diversity. Molecular methods are relatively easy to use and only require having suitable primers for the fungi under study. In molecular methods, many researchers rely only on universal primers. White et al. (1990) designed such primers for identifying fungi (see Table 1, and Fig. 1). The other primers that have been used to identify fungi include the following: ITS primer (Viaud et al. 2000; Pereira et al. 2010; Hageskal et al. 2006; Pedersen et al. 1997; Wilson et al. 2004; Manter and Vivanco 2007; Borman et al. 2008), the NS primer (Zhou et al. 2000), 18S primer (Kowalchuk et al. 1997; Borneman and Hartin 2000), and the 28S primer (Zuccaro et al. 2004; Gardes and Bruns 1993; Taylor et al. 1999), among others.

Many researchers have designed primers by using software designed for the purpose, to wit, e.g., primer 5, DNAMAN. Such software programs need the sequence of the gene under study. However, these primers must be highly sensitive and specific if they are to be successfully used to identify fungi. Llorens et al. (2006) developed molecular approaches for systematically studying fungi, and several authors (Zhang et al. 2006; Lefevre et al. 2010; Fredlund et al. 2008; Lievens et al. 2006; Savazzini et al. 2008; Selma et al. 2008) used such approaches to study the fungal diversity in the environment. Ludwig and Rudi (2010), Hageskal et al. (2006), Nicolaisen et al. (2009), and Stakheev et al. (2011) used a real-time PCR-based system for detecting and quantifying the most common *Fusarium* species.

To use molecular methods, the DNA source must originate from spores (Saad et al. 2004), or from mycelia (Płaza et al. 2004; Vesper et al. 2008; Ludwig and Rudi 2010).

Some past studies specifically targeted mycotoxigenic fungi from different environment media (e.g., *Aspergillus* spp., which produces aflatoxins) and *Fusarium* spp., which produces several mycotoxins (i.e., fumonision, trichothecence, T2-toxin, etc.). Yu et al. (2004) reported the naming scheme for the aflatoxins pathway genes of aflatoxigenic fungi (*Aspergillus* spp.) according to the substrates converted by these gene products (Fig. 2). Similarly, Shapira et al. (1996) designed three genes, *ver-1*, *omt-1*, and *apa-2*, coding for key enzymes and a regulatory factor in aflatoxin biosynthesis. Manonmani et al. (2005) designed a primer to the study *aflR* gene, and Somashekar et al. (2004) examined primers specifically targeting *aflR* and *omt* genes of the aflatoxin biosynthesis pathway. Paterson (2006a) suggested that *aflP*, or *aflQ* are more logical choices for identifying aflatoxins, while Rodrigues et al. (2009) suggested *aflD*(nor1) and *aflQ*(ord1) is more suitable for identification of aflatoxin

Many researchers have investigated *Fusarium* strains (e.g., *F. verticilliioides* and *F. proliferatum*) of fungi that produce fumonisin. Mishra et al. (2003) developed a fluorescent-based polymerase chain reaction assay that allowed for rapid and reliable identification of five toxigenic and pathogenic *Fusarium* species. Wilson et al. (2004) investigated trichothecene produced by *F. sporotrichioides* and *F. langsethiae* in wheat grain. Stakheev et al. (2011) used a real-time PCR method to study the most common mycotoxin—deoxynivalenol (DON)—content of infected grain samples. In other studies, Suanthie et al. (2009) used multiplex quantities real-time PCR to study mycotoxigenic fungi in the environment. This new method allows simultaneous detection and quantification of multiple fungal species in a single experiment; among genera of mycotoxigenic fungi detected by this method are species of *Aspergillus*, *Penicillium*, and *Fusarium*. Proper application of this new method may help reduce the probability of mycotoxin-contaminated food from entering the food chain. Eriksson et al. (2009) developed another method for multiplex detection and quantification of microbes, called the Padlock Probe Methodology, and it works in conjunction with QPCR (quantitative-PCR) and Luminex.

It is difficult for fungal taxonomists to differentiate fungi by using conventional methods, and doing so successfully requires long experience. The difficulty arises from the similarity of colony morphological characteristics, and sometimes the inadequacy of media culture dye-staining techniques to differentially stain fungi

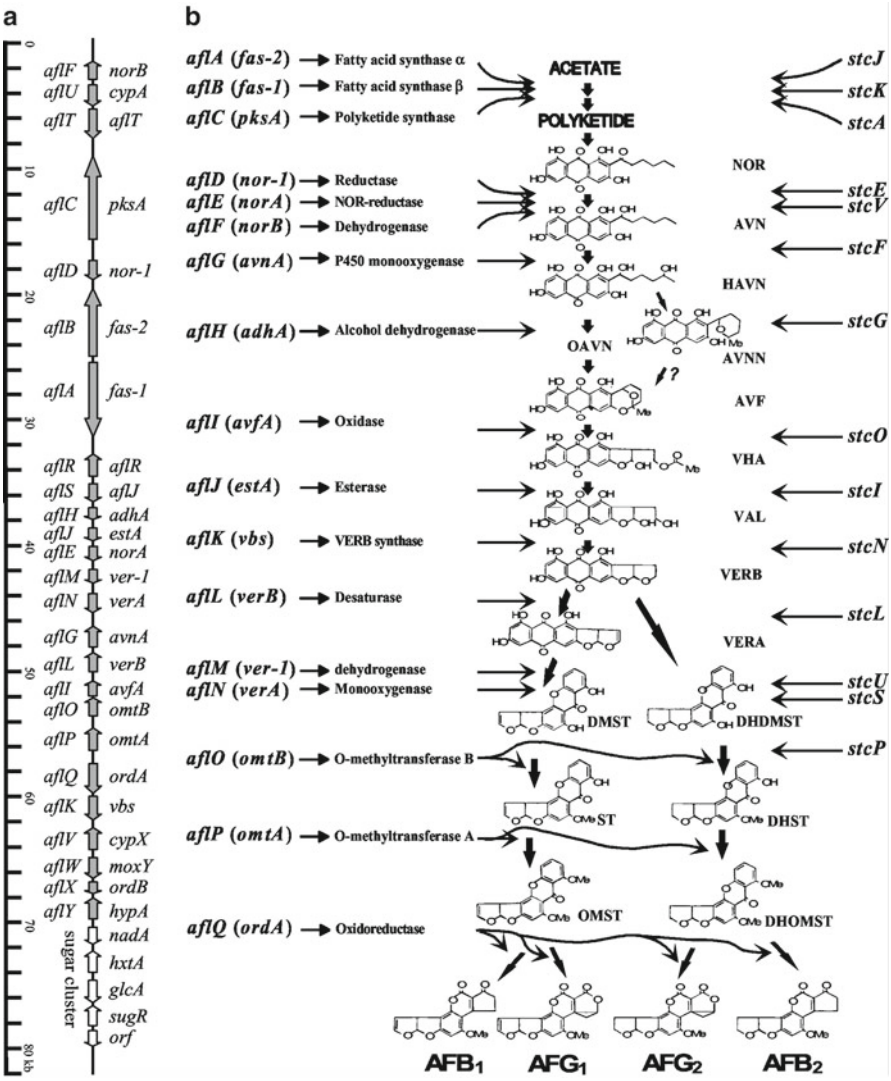


Fig. 2 Clustered genes (**a**) and the aflatoxin biosynthetic pathway (**b**) (Yu et al. 2004)

during growth. Moreover, taxonomists have difficulty in microscopic differentiation of spore size, color etc. Making it even more difficult is the fact that some species of fungi cannot be isolated in the lab, and some of these species do not produce spores. Hence, the development of the PCR method opened the door to gain more knowledge for understanding and identifying many new fungi species. However, skills for using this new technique must be refined and suitable primers must be developed for it to advance. Notwithstanding the foregoing, we still believe it is better to employ both conventional and molecular methods when studying fungi or fungal metabolites in water.

5 Fungal-Produced Toxins

Some toxins that are produced by fungi pose risks to humans and animals. Therefore, it is important to study the occurrence of fungi that produce and exude them into drinking water. One important class of agents produced by such fungi is the mycotoxins (Fig. 3a–c).

Water from different sources may contain a variety of microorganisms that include bacteria, viruses, protozoa, and fungi. Many researchers have investigated how bacteria and viruses, and even protozoa are metabolically activated to produce entities that are toxic to humans and animals. For example, *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* all produce toxic secondary metabolites (Manonmani et al. 2005), and *A. parasiticus* (Rodrigues et al. 2009) is a strong producer of AFB (aflatoxins B) and AFG (aflatoxins G). Similarly, *A. fumigatus* is an opportunistic nosocomial pathogen that often causes fatal pneumonia, and invasive aspergillosis (IA) in immune-suppressed patients (Spreadbury et al. 1993). Fernandez-Cruz et al. (2010) and Stakheev et al. (2011) studied the effects of mycotoxins (e.g., specifically the trichothecenes) and reported that these toxins inhibited protein biosynthesis and induced chromosomal changes. Consuming any products containing such toxin-contaminated grain entailed great risk of developing serious maladies, including Alimentary Toxic Aleukia (ATA). ATA is a hemorrhagic syndrome that resulted in the death of thousands of people during the second world war in the former Soviet Union. ATA is caused by the T2 toxin, which is produced by *Fusarium sporotrichioides* (Wang and Groopman 1999; Hageskal et al. 2006). Results from these studies produced several important observations: fungal contaminants cause off tastes and odors in water, and affect food and beverages, as well. In addition, people exposed to fungal contaminants are afflicted with skin irritation and allergic reactions, and the incidence of opportunistic systematic mycosis is enhanced in immune-compromised patients. Finally, any mycotoxins present among the fungal contaminants are known to be genotoxic, which is a risk factor for any human that consumes mycotoxins in water or food.

A wide variety of fungal genera (and species) have been isolated from water. Some of these (*Penicillium*, *Trichoderma*, and *Aspergillus*) are known to be strongly allergenic and can induce skin irritation, or may cause infections in immune-suppressed individuals (e.g., AIDS, cancer, and organ-transplant patients, or those with asthma or other respiratory problems) (Arruda et al. 1990; Maurya et al. 2005). Similarly, when fungal contaminated water passes through showerheads, taps, or toilet cisterns, mold spores and hyphal fragments may be aerosolized into indoor air (Green et al. 2003). This could result in respiratory exposure of human inhabitants to potentially harmful species (Hageskal et al. 2009; Pereira et al. 2010). Such respiratory exposure, when combined with the probability of dietary exposure to mycotoxin-contaminated water, food, and feed products pose an acute risk to human and animal health. The risks of most concern from exposure to mycotoxins are carcinogenicity, and potentially impairment of the immune system (Ostry 2008; IARC 2002; Fink-Gremmels 1999).

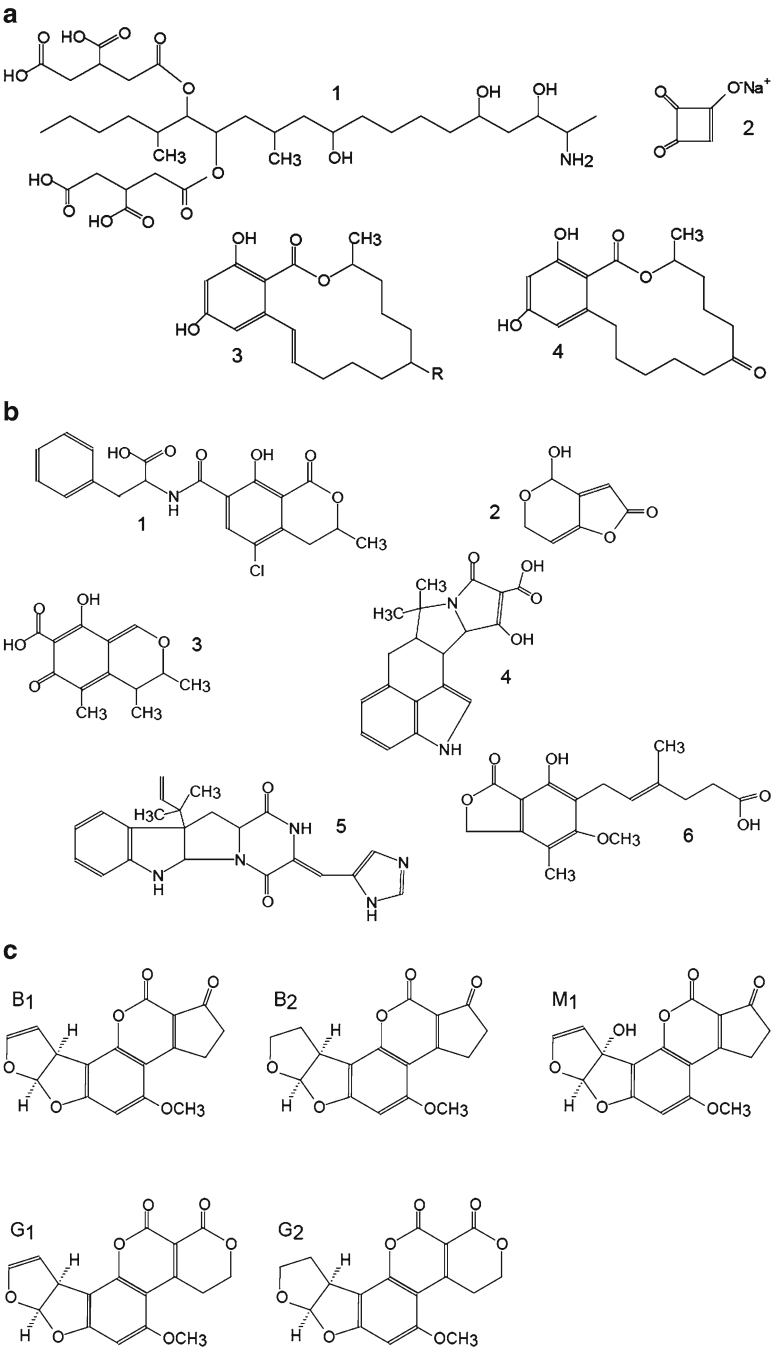
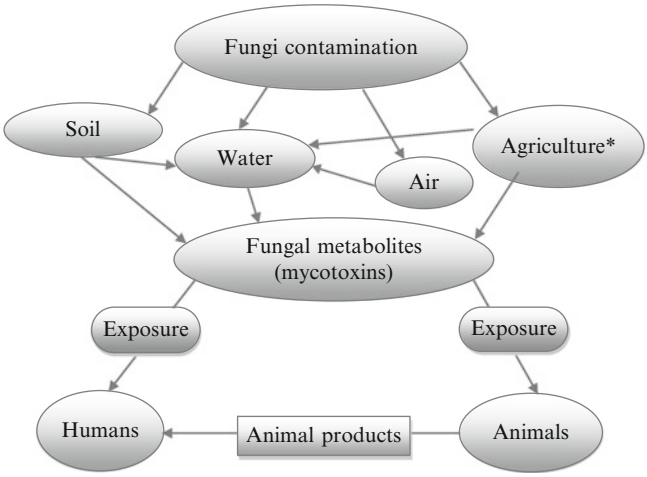


Fig. 3 (**a**) Structures of some *Fusarium* mycotoxins: FUM B1 (1) Moniliformin (usually as sodium or potassium salt) (2) Zearalenone {ZON} (R=O), and Zearalenol {ZOL} (R–OH), and (3) a possible Zearalanone metabolite {ZAN} (4). (**b**) Structures of some important mycotoxins produced by *Penicillium* and *Aspergillus* species: Ochratoxin A {OTA}(1), Patulin {PAT}(2), Citrinin {CIT} (3), Cyclopiazonic Acid {CPA} (4), Roquefortin C {RQ} (5), Mycophenolic acid {MPA} (6). (**c**) Structures of aflatoxins B1, B2, M1, G1, and G2



*Agriculture commodities such as cereals, oil, forage, feed, etc.

Fig. 4 A flow diagram showing the source, movement in the environment of fungi, and their metabolites that eventually reach humans and animals

Recent outbreaks of diseases caused by fungi (Mishra et al. 2003) are known to be a great problem for the agricultural industry, and potentially threaten the global food supply. Paterson et al. (1997) quantified mycotoxins present in water from a cold water storage tank and detected aflatoxins B_2 and G_2. Gromadzka et al. (2009), in Poland, noted the presence of the toxin zearalenone in surface waters, groundwater and wastewater; the levels this author found in water samples ranged from 0 to 43.7 ng L^{-1}.

Other mycotoxins (e.g., deoxynivalenol, zearalenone, and some zearalenone (ZEN) metabolites) are known to possess strong estrogenic activity that is associated with causing hyperestrogenism and physiological alterations of the reproductive tract (Abid-Essefi et al. 2004). Moreover, ZEN affected cell proliferation and inhibited protein and DNA syntheses in a concentration-dependent manner. It appeared that ZEN inhibited DNA synthesis before protein synthesis was affected (Abid-Essefi et al. 2004).

Paterson (2006b) reported that fungal toxins could possibly be used as biochemical weapons if placed by protagonists in drinking water, or non-potable water, because such media would rapidly disperse such "weaponized" mycotoxins. The threat that contaminated drinking water would pose is obvious. But toxin dispersal in non-potable water may pose its own degree of threat from inhaling shower spray or aerosols or from workplace exposures, where abundant water may be employed in farm irrigation, car washing facilities, etc. Although water quality for livestock is generally considerably lower than for human consumption, the intentional contamination of it by mycotoxins may nevertheless constitute a potential route of for a terrorist attack (Fig. 4).

6 Controlling Fungi in Water Sources

Aflatoxins or other fungal toxins may be degraded by physical, chemical, or biological methods (Dimitrokallis et al. 2008). Also addressed in some studies is how drinking water can be processed in water treatment plants to reduce the danger of contaminating fungi.

Sammon et al. (2010) showed that coagulation/flocculation, sand filtration, and chlorination was highly effective in removing microfungal contaminants from raw water, although recontamination will occur if supplementary chlorination of all water service reservoirs is not routinely carried out to prevent growth of fungi or reduce fungal metabolites (e.g., geosmin, 2-methylisoborneol (2-MIB), and methyl tert-butyl ether (MTBE)) in drinking water. Lin et al. (2003) noted that the presence of chlorine substantially reduced concentrations of geosmin, 2-MIB, and MTBE. Similarly, Wilson et al. (2005) tested the effects of chlorine dioxide gas on growth of some fungi, and showed that it inactivated all organisms except for *C. globosum* colonies, although some Ascospores may also have been destroyed. Pereira et al. (2013) studied the effectiveness of free chlorine for deactivating some species of fungi, and established rate constants for such deactivation.

Nourmoradi et al. (2012) studied the effect of UV irradiation on selected *Aspergillus* spp. Chun et al. (2010) studied the effect UV-C irradiation on inactivation of the food-borne pathogens population, and suggested that it can be useful for improving microbial safety of stored food, without impairing quality. Begum et al. (2009) suggested that UV-C irradiation can effectively inactivate spores of *A. flavus*, *P. corylophilum*, *E. rubrum*, and *A. niger*, but the efficacy of UV-C radiation against fungal spores varied significantly among genera and method of irradiation exposure. *A. niger* is more resistant to UV irradiation than other genera such as *A. flavus*, *Mucor* spp., and *Penicilluim* spp. Moreover, Hijnen et al. (2006) showed that *Aspergillus* spp. are less sensitive to UV irradiation than are cells of vegetative bacteria species, such as *Campylobacter jejuni* and *Legionella penumophia*; however, *Aspergillus* spp. are more sensitive than bacterial spore-forming species such as *Bacillus subtilis* and *Clostridium perfringens*.

Xiong et al. (2010) investigated neutralized and acidic electrolyzed oxidizing water, and noted significantly different fungicidal treatment effectiveness against *Aspergillus flavus*, when targeting conidia; the normal cellular functioning of K^+ and Mg^{2+} in *A. flavus* conidia was damaged. Similarly, Young et al. (2006) investigated the degradation of ten trichothecene mycotoxins from exposure to aqueous ozone. They discovered that all studied mycotoxins degraded readily; at pH 7–8 the degree of reactivity was dependent upon the carbon 8 oxidation state, whereas at pH 9, there was little or no reaction.

An attempt at biocontrol was made by using either bacterial or other forms of fungi to prevent mycotoxin-activation or fungal growth. Dimitrokallis et al. (2008) reported that many microorganisms including bacteria, yeasts, and molds are able to remove or degrade small amounts of aflatoxin in food and feed. These authors also attempted to use nontoxogenic fungal strains to degrade some mycotoxins.

Abrunhosa et al. (2002) observed that the fungi, *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, and *Penicillium*, most frequently isolated from grapes, have significant Ochratoxin A degradation capabilities. Moreover, Hend et al. (2006) noted that *Aspergillus carbonarius*, *A. niger aggregate*, and *A. japonicus*, were assessed for Ochratoxin A (OTA) degradation capacities in Czapek, and yeast extract broth (CYB).

Varga et al. (2005) noted that Ochratoxin A was successfully degraded by some *Rhizopus* isolates, and *Rhizopus* isolates were able to degrade more than 95% of Ochratoxin A within 16 days. A *R. stolonifer* isolate could also effectively decompose Ochratoxin A on moistened wheat. Further studies are in progress to identify the enzymes and genes responsible for Ochratoxin detoxification and to transfer these genes to other *Rhizopus* isolates or microbes, which could be used safely for decontaminating cereal products. El-Nezami et al. (1998) investigated the ability of selected dairy strains of lactic acid bacteria to remove aflatoxin B1(AFB1), and documented significant removal of AFB1. Krishnamurthy and Shashikala (2006) studied the control of aflatoxin B1 production by using a spore suspension of *T. harzianum*, *A. niger*, and a combination of both, which greatly reduced the level of aflatoxin B1. Alberts et al. (2006) and Teniola et al. (2005) examined the biodegradation of AFB1 by using *Rhodococcus erythropolis* in liquid cultures, and their results indicated that the 90% of AFB1 was degraded within 4 h at 30 °C, whilst after 8 h AFB1 was practically not detectable.

Studies were conducted on the use of some fungi to control mycotoxins producing fungi. For example, *Trichoderma* species were used as biocontrol agents against seed-associated pathogenic *Aspergilli* and *Fusaria* (Calistru et al. 1997; Yates et al. 1999). Another example was the use of white rot fungi, to degrade Aflatoxin B1 by laccase enzyme from *T. versicolor* and *A. niger* (Alberts et al. 2006). Velazhahan et al. (2010) studied the effects of aromatic seed extracts (e.g., Ajowan seed), and showed that AFG1 was degraded to a maximum of 65%. The dialyzed *T. ammi* extract was more effective than the crude extract, which was capable of degrading >90% of the toxin. Campbell et al. (2006) used gallic acid, from *hydrolysable tannins* in the pellicle of walnut kernels, dramatically inhibited biosynthesis of aflatoxin by *Aspergillus flavus*. Zorlugenc et al. (2008) investigated the control of fungi and aflatoxins and showed that gaseous ozone was more effective than ozonated water for reduction of aflatoxin B1and microbial counts.

Few authors have investigated the effect of treating drinking water in WTP, or which treatment processes are effective for removing/decreasing fungi in water. It is known, however, that some fungi are difficult to control, because they regrow in water systems as biofilms. Fungi from such biofilms are subject to release and consumption by humans who drink tap water, or indirectly through inhaling aerosols produced from showerheads, etc. Mycotoxins exposure may cause carcinogenicity or may affect immune-suppressed patients, like AIDS or organ transplant patients. Using biocontrol species to control fungi or the mycotoxins they produce has a negative side, i.e., the biocontrol organism or its mycelium may induce allergenicity or respiratory problems. Therefore, new solutions are still needed to adequately control fungi and the mycotoxins that they can release into drinking water.

7 Conclusions

Some of the most important points we have gleaned from conducting this literature review on water contamination by fungi are presented below:

1. Fungi are major contaminants of surface waters
2. The types and amounts of fungi found in surface waters are variable
3. Fungi are often associated with soil and air, which themselves either enter or release their contents to water. Therefore, both soil and air are sources of fungi that contaminate water
4. The occurrence of fungi in water causes problems, such as odors, taste, and release of chemicals into water by some fungal species (e.g., pigments such as γ-carotene, lycopene, and xanthophylls).
5. Researchers have used different methods or media to isolate fungi from water. The different isolation procedures have limitations in accurately detecting fungi in the water, and may selective in detecting some fungal species or missing others.
6. PCR methods to identify fungi have made fungal identification a little easier. However, this procedure is fraught with some difficulties, because the sequences for some fungi in the gene-bank database are not yet available.
7. When fungal contaminated water passes through showerheads, taps, or toilet cisterns, mold spores and hyphal fragments may be aerosolized into indoor air. Such aerosols could be inhaled by human inhabitants and cause harmful effects.
8. It may be possible to use certain fungal toxins as biochemical weapons.

The types of research needing more attention in the future include the following:

- The occurrence of fungi in water systems as biofilms, and how such biofilms form in water systems.
- What different treatment processes can be used to remove/decrease the amounts of mycotoxins in water.
- How to prevent fungal infection of hospital water particularly the water used in organ transplant units and hemodialysis patient rooms.

8 Summary

Aquatic fungi commonly infest various aqueous environments and play potentially crucial roles in nutrient and carbon cycling. Aquatic fungi also interact with other organisms to influence food web dynamics. In recent decades, numerous studies have been conducted to address the problem of microorganism contamination of water. The major concern has been potential effects on human health from exposure to certain bacteria, viruses, and protozoa that inhabit water and the microbial metabolites, pigments, and odors which are produced in the water, and their effects on

human health and animals. Fungi are potentially important contaminants because they produce certain toxic metabolites that can cause severe health hazards to humans and animals. Despite the potential hazard posed by fungi, relatively few studies on them as contaminants have been reported for some countries.

A wide variety of fungi species have been isolated from drinking water, and some of them are known to be strongly allergenic and to cause skin irritation, or immunosuppression in immunocompromised individuals (e.g., AIDS, cancer, or organ transplant patients). Mycotoxins are naturally produced as secondary metabolites by some fungi species, and exposure of humans or animals to them can cause health problems. Such exposure is likely to occur from dietary intake of either food, water or beverages made with water. However, mycotoxins, as residues in water, may be aerosolized when showering or when being sprayed for various purposes and then be subject to inhalation. Mycotoxins, or at least some of them, are regarded to be carcinogenic. There is also some concern that toxic mycotoxins or other secondary metabolites of fungi could be used by terrorists as a biochemical weapon by adding amounts of them to drinking water or nondrinking water. Therefore, actions to prevent mycotoxin contaminated water from affecting either humans or animals are important and are needed. Water treatment plants may serve to partially accomplish this, by first filtering the water and finally by adding disinfection treatments adequate to remove or mitigate fungi or their toxic metabolites.

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