Volume 227

David M. Whitacre *Editor*

Reviews of Environmental Contamination and Toxicology

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Foreword

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

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

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

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

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

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

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

Coordinating Board of Editors

Preface

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

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

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

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

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

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

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

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

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

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

Summerfield, NC, USA David M. Whitacre

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Occurrence, Degradation, and Effect of Polymer-Based Materials in the Environment

Scott Lambert, Chris Sinclair, and Alistair Boxall

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1 Introduction

At a global scale, increasing human population and associated economic growth has lead to an increase in the demand for consumable goods such as those made from polymer-based materials (PBMs) (i.e., plastics and elastomers). During their

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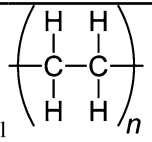
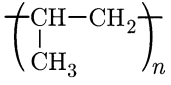
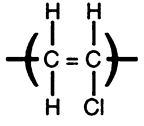
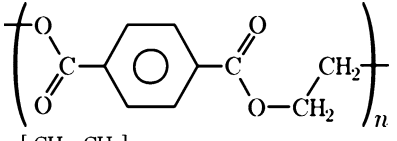
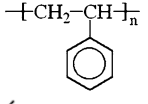
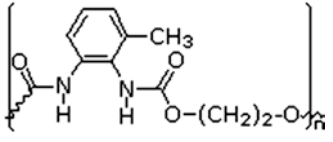
lifecycle PBMs can be released into the environment from a variety of sources. Once in the environment, PBMs are exposed to a variety of mechanical and chemical weathering processes. This causes a change to the PBM structure and facilitates the disintegration of the PBM into increasingly smaller fragments (Andrady 2011). Furthermore these materials are now thought to be contributing to the build-up of chemicals in the environment via the leaching of chemical additives that are used in the manufacturing process (Erren et al. 2009). The majority of physical effects data regarding bulk PBM items identifies them as presenting a hazard to mammals and birds as they can become entangled and/or mistake PBMs as a food source (Derraik 2002). The majority of ecotoxicity data regarding PBM additives has focused on the effects of compounds that are generally referred to as having endocrine disruptive potential, such as the phthalates (Oehlmann et al. 2009). However, receiving environments are potentially exposed to a combination of both these physical and chemical components, as well as substances produced during degradation processes. Therefore, PBMs and their associated degradation products may compromise the viability of organisms at all trophic levels. At the base of the food chain primary producers may be more sensitive to substances that have a biological action. Nonselective and filter-feeding consumers could be susceptible to ingesting both bulk PBMs and fragmented particles, leading to the potential passage up the food chain to secondary and tertiary consumers. Despite this concern, PBMs are regarded under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) as representing a low environmental concern because of their high molecular weight (ECHA 2012). However, the occurrence of PBMs and their associated chemical additives in the aquatic environment have been recognized as an emerging worldwide problem, and their impacts are now gaining a wider scientific and social audience (Hammer et al. 2012; Thompson et al. 2009).

The purpose of this article is to provide a broad bibliographical review of the research that addresses the use, release, occurrence, degradation and effects of PBMs and their associated chemical additives in aquatic and terrestrial environments. We address issues involving both the bulk polymer component of PBMs and the additive component.

2 Usage and Consumption

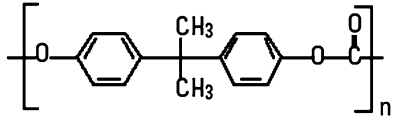
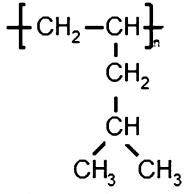
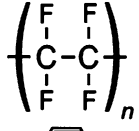
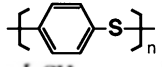
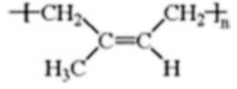
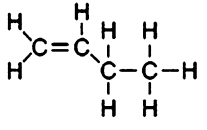
The PBMs used in society today are made from a broad class of materials that are both natural and synthetic in origin (Table 1). Natural polymers such as polyisoprene, derived from the tropical tree *Hevea brasiliensis*, are used to make natural rubber and latex products (Agostini et al. 2008). Petrochemical-based polymers are manufactured through a thermal splitting process termed “cracking” that separates oil and natural gas to produce different hydrocarbon monomers, such as ethylene and propylene (Chaudhuri 2010). World demand for petroleum derived polymers is estimated at 230 million t annually (Plastics-Europe 2010), with annual consumption estimated to be 26 kg per person (CIPET 2010). However, there are notable differences between geographic regions that result from differences in standards of

Table 1 Major polymer types and their uses

Polymer type	Uses	Structure
Polyethylene (PE)	Low density PE—Squeeze bottles, toys, carrier bags, high frequency insulation, chemical tank linings, heavy duty sacks, general packaging, gas and water pipes High density—chemical drums, toys, picnic ware, household and kitchenware, cable insulation, carrier bags, and food wrapping material	
Polypropylene (PP)	Food containers, microwavable meal trays, and in the auto industry	
Polyvinyl chloride (PVC)	Building, transport, packaging, electrical/electronic and healthcare applications	
Polyethylene terephthalate (PET)	Drinks bottles, oven-ready meal trays cable lining	
Polystyrene (PS)	Food containers, takeaway boxes, vending cups, plastic cutlery, protective packaging, and CD boxes	
Polyurethane (PUR)	Printing rollers, solid tires, wheels, shoe heels, car bumpers, as foams in mattress and car seats, and in biomedical applications	

(continued)

Table 1 (continued)

Polymer type	Uses	Structure
Polycarbonate (PC)	Bottles, utensils, containers, sheeting, electrical goods, and medical applications	
Polymethylpentene (PMP)	Medical ware, syringes, lamp covers, (good heat resistance), radar applications, encapsulation, and microwave food packaging	
Polytetrafluoroethylene (PTFE)	Nonstick coating, gaskets, bearings, high- and low-temperature electrical and medical applications, laboratory equipment, pump parts, and thread seal tape	
Polyphenylene Sulfide (PPS)	Electrical, automotive, cooking appliances, sterilizable medical, dental, and laboratory equipment, hair dryer grills and components	
Polyisoprene (NR)	Gloves, tires, rubber boots, rubber bands, pencil erases, hoses, belts floorings, and medical applications	
Polybutadiene	Tires, golf balls, and inner tubes	

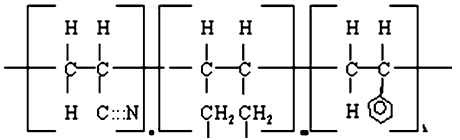
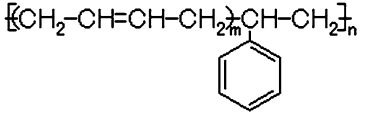
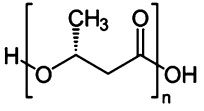
Acrylonitrile butadiene styrene (ABS)	Piping, musical instruments, golf club heads, automotive, medical devices for blood access, electrical devices, protective headgear, whitewater canoes, small kitchen appliances, and toys	
Styrene-butadiene (SBR)	Tires, shoes, building applications, and paper coating	
Polyhydroxyalkanoates (PHA)	Medical devices, such as cardiovascular patches, orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices bone implant material, drug release system, scaffold for tissue engineering, bulking and filling agents	

Table 2 Per capita consumption of polymers geographically (source: Central Institute for Plastic Engineering and Technology 2010)

Country	Polymer consumption per capita (kg)
India	5
China	12
South East Asia	10
Latin America	18
North America	90
West Europe	65
East Europe	10
Worldwide average	26

living, lifestyle, and income (Table 2). Polyolefins (i.e., polyethylene (PE; linear low density, low density, and high density), and polypropylene (PP)), account for ~60% of annual consumption followed by polyvinyl chloride (PVC) and polystyrene (PS) (Plastics-Europe 2010; Mutha et al. 2006). Packaging represents the most important application for PBMs and accounts for 40.1% of overall consumption, followed by building and construction (20.4%), automotive (7%), electrical and electronic equipment (5.6%), and other market sectors including leisure and agriculture (26.9%) (Plastics-Europe 2010; Mutha et al. 2006). Polyurethane (PUR) is a successful material for biomedical applications, where it is used to make artificial joints and flexible replacements for blood vessels and heart valves (Ghanbari et al. 2009). World demand for natural rubber (NR) is estimated at 10.97 million t annually; this demand is dominated by latex products (80.3%) such as medical and household products (NRS 2011). Other natural rubber uses include tires (9.2%), general rubber goods (7.2%), industrial rubber goods (3.2%), and footwear (0.2%) (NRS 2011).

Technological advances have seen the development of PBMs that have been altered to be more degradable. These PBMs can be broadly divided into three categories. First, are those that have a biodegradable ingredient, such as starch, which is added to the polymer matrix to link short strands of the polymer chain together (Drimal et al. 2007; Reddy et al. 2003). Second, nano clay composites are used to provide a favorable environment for growing microorganisms that can utilize the polymer matrix as a food source; montmorillonite clay has been reported to promote microbial growth by stabilizing pH in the polymer matrix (Reddy et al. 2009). Third are those produced from the bacterial fermentation of sugars and lipids that comprise a class of polymers that include polyhydroxyalkanoates (PHA), polylactides (PLA), aliphatic polyesters, polysaccharides, copolymers, and/or blends of the above. Reddy et al. (2003) have described these as being the most promising technological advances, because the polymer matrix is thought to be fully utilized by microbial communities.

High-performance composites are also an important market segment. These consist of a polymeric matrix and fillers that are designed to provide improved mechanical properties. Carbon fiber composites have been an important innovation for the aircraft industry, and have reduced aircraft weight and thereby reduced fuel use (Mulder 1998). Glass-fiber-reinforced polyester composites are used in shipping because of their impact resistance and light weight. PBMs are sometimes blended

to improve the deficient properties of traditional single-chemical polymers. When the properties of two or more incompatible polymers are desired in one blend, compatibilizers are employed. For example, blends of PP and acrylonitrile butadiene rubber (NBR) are desirable to combine the oil resistance and elastic properties of NBR and the low density and chemical resistance properties of PP, although their individual physical, mechanical, and chemical properties normally prevent this.

3 Bulk PBMs and the Environment

3.1 Environmental Release

PBMs may enter the environment from both ocean- and land-based sources. We address each of these in more detail below.

3.1.1 Ocean-Based Sources

Ocean-based sources include items lost or discarded from commercial fishing vessels, offshore oil or gas platforms and waste dumped by recreational boat users. Losses of cargo can also occur from shipping during bad weather events or accidents and items lost from improper loading, unloading, or onboard storage (Tharpes 1989). In the past, pre-production PE and PP pellets have reportedly been used on the decks of ships to reduce friction when moving large objects; as such, many of these pellets are washed from the deck and are dispersed by winds and ocean currents (Tharpes 1989). The dumping of wastes at sea has long been seen as a major issue and was prohibited under international legislation in 1973 (MARPOL 73/78 Annex V), which came into force in 1988 and regulates the operational discharges from shipping (do Sul and Costa 2007). One requirement of the MARPOL ruling is that under no circumstances are PBMs to be disposed of at sea, but the enforcement of this regulation is noted as being an issue (Ryan et al. 2009).

3.1.2 Land-Based Sources

General and Accidental Littering

On land, general and accidental littering are important routes of environmental entry of PBM debris (Gregory 2009). General littering is the direct dropping of litter, and dumping of items; for example, illegal dumping of waste that can then be transported by wind or from drainage and storm water runoff to ocean sinks (Tharpes 1989). Littering at festival sites is noted as an issue; especially from sites that have inadequate waste management systems (Cierjacks et al. 2012). Accidental littering, by contrast, results from windblown debris from bins, or from recycling and landfill

facilities (Tharpes 1989). Littering on land in the UK is covered by section 18 of the Clean Neighbourhoods and Environment Act of 2005, which came into force on 7th June 2005 and makes it an offense to litter on all public and private land and land covered by water (DEFRA 2012).

Landfills

Landfills are a major end-of-life disposal route for PBMs (Barnes et al. 2009). In most developed regions of the world, waste is collected, transferred to landfills and is typically covered with soil daily (Rayne 2008). However, in many developing regions waste materials are often disposed of in areas lacking adequate infrastructure, and are rarely or inadequately covered with soil (Rayne 2008). This increases the likelihood of windblown debris migrating from landfill sites. Rayne (2008) has also identified the increasing urbanization of Africa as a potential future problem, because it will increase the stress on limited waste management systems in this area of the world.

Sewage-Related Debris (SRD)

SRD also presents a source from which PBMs can enter the environment. In many countries, domestic inputs of household waste to the sewage system are largely uncontrolled. Therefore, PBMs associated with personal hygiene products, such as condoms, cotton buds (Ashley et al. 2005; Williams and Simmons 1999), and microscopic PE beads found in some hand cleaners and facial scrubs (Fendall and Sewell 2009), as well as microscopic fibers (acrylic) shed from cloths during washing (Zubris and Richards 2005) can constitute a portion of this waste stream. Larger items are generally removed by screening methods, but may enter the environment during sewage overflow events that occur during periods of heavy rainfall. The ability of sewage treatment works to process microscopic beads and fibers has now been questioned. Browne et al. (2011) recently sampled wastewater from domestic washing machines and demonstrated that a single garment can produce >1,900 fibers per wash. Microscopic beads and fibers can potentially pass through finer screening processes and enter the environment via sludge application and discharge of treated waters (Browne et al. 2011). Coarse screens, designed to remove large solids and debris items, typically have a mesh size of 6 mm, whereas fine screens typically have mesh sizes of 1.5–0.2 mm (EPA 2012).

Industrial Sources

Industrial sources of PBM waste include air-blasting technologies that use microscopic beads to strip paint from metallic surfaces and for cleaning engine parts; when discarded, they enter the environment through foul-water, or via transfer through sewage treatment processes (Derraik 2002). Low density polyethylene (LDPE) films

constitute a large-volume use of PBMs in agricultural crop production, and consequently they have become an important agricultural emission (Xu et al. 2006). Their application is thought to be one of the most important sources of PBM contamination of soils, because they become brittle and easily disintegrates, rendering their recovery difficult (Xu et al. 2006). Agriculture films can also contain light-sensitive additives, such as ferric and nickel dibutyldithio-carbamates, the ratio of which can be adjusted so that the film is usable during a specific growing season, after which the product begins to photo-degrade (Klemchuk 1990). This ultimately results in disintegration of the material, and when coupled with successive precipitation events the disintegrated particles can be washed into the soil where they accumulate (Klemchuk 1990).

3.1.3 Conclusion

The principal introduction routes of PBMs into the environment are most likely general littering, dumping of unwanted waste materials, migrations from landfill and during refuse collection (Gregory 2009; Teuten et al. 2009; Tharpes 1989). Routes of minor importance are potentially the weathering of PBM building materials. However, the importance of one particular source over another will depend on geographical location and infrastructure. For example, landfills are identified as a potential important source in areas of the world where infrastructure is lacking, but microscopic PE beads in facial scrubs are probably more important in more affluent regions. One must also be aware of the difficulties in determining the sources of PBM debris, because of the length of time it may have been in the environment and the distances it may have travelled. For a more in depth analysis of PBM origin see Hammer et al. (2012).

3.2 Environmental Occurrence

Upon their release to the environment PBMs are transported and distributed to various environmental compartments. The distances that an individual item will travel depends on its size and weight. Lightweight materials can be readily transported long distances via a windblown route or carried by freshwater to eventually accumulate in the oceans. During heavy rainfall events, roadside litter can be washed into drains and gullies, and, where the topography is favorable for it, can be carried to the sea. In this section, we review the literature in which the occurrence of polymers globally has been quantified.

3.2.1 Macro PBMs in the Oceans

Large items of PBM debris are termed “macroplastics” and have been generally categorized as items >5 mm in diameter, because this size provides an opportunity

to assess markings to trace an object to its origin. Marine habitats are highlighted as one of the most important sinks for macro PBMs (Browne et al. 2011; Derraik 2002; Thompson et al. 2009). PBMs are believed to contribute up to 80% of all anthropogenic debris in the oceans (Derraik 2002). A well documented example are preproduction PE and PP pellets that are transported from manufacturing plants to plastic injection factories, where they are melted and molded into consumer products. These pellets have been reported floating in coastal surface waters, and in the world's oceans, later to be washed ashore in nonindustrialized areas such as the South Pacific Islands (Derraik 2002; Gregory 1977; Moore 2008; Morris 1980). Lightweight items, such as PE bags, polystyrene foam items and polymer drinks bottles, inappropriately disposed of on land, can be readily transported long distances via a windblown route or carried by freshwater to eventually accumulate in the oceans (Ryan et al. 2009).

There are now a number of studies in which macro PBMs have been observed or collected floating on the ocean surface and laying on the seafloor (Table 3). These studies provide a snapshot, but do highlight PBMs as the dominate component of ocean debris. Geographical variability in ocean PBM debris has been highlighted by Barnes and Milner (2005), in their extensive study on the occurrence of drifting PBM debris in the Atlantic Ocean. These authors identified the English Channel as having the greatest number of debris items (10 to >100 items/km^2), 66% of which were a form of PBM. This study also established PBM debris to be an order of magnitude lower in both the Polar Regions, but the authors do highlight that the tropics and the West Atlantic were poorly sampled. One of the only documented cases of decreasing litter densities in the literature comes from Kuriyama et al. (2003), who reported a 45.3% decrease in the number of littered items on the seabed of Tokyo Bay between 1996 and 2000; the authors of this study hypothesized this to be a result of litter removal by bottom trawl fishing vessels.

3.2.2 Macro PBMs on Shorelines and on Land

Shorelines around the world have been found to accumulate debris, including island shorelines far from any centers of human activity (Table 4). Benton (1991) surveyed beach litter on Ducia Atoll in the south Pacific and found 953 items of debris over a 1.5 mile survey transect. This is one of the world's most remote islands, being 293 miles from the nearest inhabited location of Pitcarin Island, which in 1991 had a population of ~50 people. Another example comes from remote tropical beaches of Brazil, where PBMs have been found at densities of 9.1 items/m^2, accounting for 76% of the litter items found (Santos et al. 2009). Evidence of the increasing occurrence of PBMs is provided from in Scotland, where Caulton and Mocogni (1987) found 0.35 items of litter/m^2, with plastics accounting 29% of items found. Ten years later the same area of beach was surveyed and the density of litter was found to have increased to 0.8 items of litter/m^2, with PBMs accounting for 37% of items found (Velander and Mocogni 1998).

Table 3 Polymer-based materials as a component of marine debris

Location	Depth	Mean density of litter (items/km^2)	% Plastic items	Reference
North Atlantic and Europe				
Baltic sea	Sea floor	0.12	35.7	1
North Sea	Sea floor	0.15	48.3	1
Bay of Biscay	Sea floor	0.14	79.4	1
Celtic Sea	Sea floor	0.53	29.5	1
Adriatic Sea	Sea floor	0.38	69.5	1
English Channel	Surface	10–100	66	2
Sargasso Sea	Surface	3,500	100	3
Gulf of Mexico	Sea floor	Not stated	204 pieces	4
Mediterranean				
Malta				
Greece	15 m—seafloor	0–437 items	~65	5, 6, 7
France	40–1,448	0–78/ha	70.6	8
Pacific				
Central California (2007)	20–365 m	6,900	95	9
Southern California (2002)	20–365 m	320	41	9
Southern Chile	Surface	1–250	80	10
SE Pacific (Chile)	Surface	0–1.8	86.9	11
Brazil	Sea floor	2.9/100 m^2		12
North Pacific Gyre	Surface	334,271	100	15
Tokyo Bay (1996 and 2000)	Sea floor	338 and 185	90 and 90	13
Kodiak Island, Alaska (1994–1996)	Sea floor	Not stated	49 (1994), 59 (1995) and 47 (1996)	14
Middle East				
Jordan, Gulf of Aqaba	Coral Reef	2.8	42	16

References: 1 Galgani et al. (2000); 2 Barnes and Milner (2005); 3 Carpenter and Smith (1972); 4 Wei et al. (2012); 5 Katsanevakis and Katsarou (2004); 6 Koutsodendris et al. (2008); 7 Stefatos et al. (1999); 8 Galgani et al. (1996); 9 Watters et al. (2010); 10 Hinojosa and Thiel (2009); 11 Thiel et al. (2003); 12 Oigman-Pszczol and Creed (2007); 13 Kuriyama et al. (2003); 14 Hess et al. (1999); 15 Moore et al. (2001); 16 Abu-Hilal and Al-Najjar (2009)

The amount of PBM debris in the freshwater environment is less well documented, but one recent study reported on the distribution of PBM debris along the freshwater beaches of Lake Huron, Canada. In this study, 2,986 polymer pellets, 108 polymer fragments and 117 pieces of Styrofoam were found (Zbyszewski and Corcoran 2011). On land, urban littering is considered to be an important environmental and public issue (Seco Pon and Becherucci 2012), but it less well documented in the available literature. One researcher conducted a study in Nairobi, Kenya in 2001 and collected 4,834 plastic bags from 6 sites that measured 20 m × 50 m in size (Njeru 2006). A similar study was performed in Mar del Plata, Argentina, in which 20,336 items (14.27 items/m^2) of litter was recovered from study sites between April 2008 and March 2009; in this study, PBMs accounted for 22% of the recovered litter (Seco Pon and Becherucci 2012).

Table 4 Polymer-based materials as a component of shoreline debris

Location	Number of beaches	Mean density of litter (items/m^2)	% Plastic items	Reference
Europe				
Scotland (firth of forth) (1999 and 2007)	16 and 37	6.2 (max. Density)	46	1, 2
Scotland (Cramond) (1987 and 1998)	1	0.35 and 0.8, respectively	29.37 and 37.12, respectively	3, 4
Wales	1	Not stated	>50	5
Germany, Kachelotplate	1	Not stated	60.4	6
Mediterranean	32	36	Reported as most common item found	7
Russia	8	0.2	55.1	8
Inch Strand, Ireland	1	0.22	46	9
Mediterranean				
Malta	7	1.6–167 (max. 1,462)	Counts of pellets	10
Australasia				
Australia (Cable Beach)	1	0.5	14.65	11
Australia (Greater Sydney region)	6	0.2	89.8	12
Japan	18	3.4	72.9	8
Middle East				
Israel	6	0.03–0.88	70.6	13
Gulf of Oman	11	Ranged from 0.43 to 6.01, with a mean density of 1.79	61	14
North America				
West Indies	5	0.37	47	15
New Jersey, USA	1	728 items over 500 m transects (monthly mean)	~73	16
South America				
Chile	43	1.8	Reported as most common item found	17
Brazil	1–16	1–10 items	~57	18, 19, 20
Canada				
Nova Scotia	1 (70 m)	2,129 Items collected	86	21
Antarctic Peninsula				
Scotia Arc Islands	4	0–0.3	>70	22
Oeno Pitcarin	1	0.35	45	9
Ducia Atoll, South Pacific	1	0.12	38	23

References: 1 Storrier et al. (2007); 2 Velander and Mocogni (1999); 3 Caulton and Mocogni (1987); 4 Velander and Mocogni (1998); 5 Williams and Tudor (2001); 6 Liebezeit (2008); 7 Martinez-Ribes et al. (2007); 8 Kusui and Noda (2003); 9 Benton (1995); 10 Turner and Holmes (2011); 11 Foster-Smith et al. (2007); 12 Cunningham and Wilson (2003); 13 Bowman et al. (1998); 14 Claereboudt (2004); 15 Nagelkerken et al. (2001); 16 Ribic (1998); 17 Bravo et al. (2009); 18 Santos et al. (2009); 19 Silva-Cavalcanti et al. (2009); 20 Oigman-Pszczol and Creed (2007); 21 Walker et al. (2006); 22 Convey et al. (2002); 23 Benton (1991)

3.2.3 Micro PBMs in the Oceans

Particles <5 mm, formed as a result of the breakdown of larger materials, are now found floating on the ocean surface, mixed into the water column, and embedded in bottom sediments and beach sands (Colton et al. 1974; Thompson et al. 2004). These smaller particles are generally termed "microplastics" (Barnes et al. 2009; Moore 2008). However, it has recently been suggested that the term microplastics be redefined as items <1 mm to include particles only discernible by microscopy (Andrady 2011; Browne et al. 2011). The term "mesoplastic" should then be introduced to the scientific literature to account for items between 1 and 5 mm (Andrady 2011).

Colton et al. (1974) found PBM particles in 62% of surface plankton samples taken from the Atlantic Ocean (247 samples in total). Archived plankton samples, collected along routes between Aberdeen and the Shetlands and from Sule Skerry to Iceland as part of the continuous plankton recorder (CPR) survey, have also shown the presence of PBM particles and fibers in samples dating back to the 1960s (Thompson et al. 2004). This highlights the long-term trends first identified by Carpenter et al. (1972), who found fragmented polymer particles in surface nets while sampling the *Sargassum* (free-floating seaweed) community in the western Sargasso Sea. Furthermore, Carpenter et al. (1972) predicted that the increasing use and production of PBMs would lead to an increase in concentrations of these particles in the environment. In 2004, the CPR survey, the longest running plankton monitoring program in the North Sea and North Atlantic, added microplastic as their first nonbiological marine entity to their recordings (Richardson et al. 2006).

One area that has received particular attention is the subtropical accumulation zone in the North Pacific gyre. In this area, debris has accumulated at such high densities as a result of high atmospheric pressure and the clockwise rotation of ocean currents that forces debris into a central area where strong winds and currents diminish (Cooper and Corcoran 2010). Neuston sampling at 11 sites, using a mantra trawl, estimated a mean PBM abundance of 334,271 pieces km^2 (Moore et al. 2001). Items identified were fragments ranging in size from 0.44 to >4.76 mm, pellets, PP monofilament and Styrofoam pieces. In a study performed along Californian coastal waters, surface samples were collected with a manta trawl, mid-depth samples with a bongo net and bottom samples with an epibenthic sled, all having 333 μm nets; PBM debris density was found to be greatest near the bottom, and least in mid-depth zones (Lattin et al. 2004). This suggests that when measuring the occurrence of PBM debris it is important to establish whether the concentrations of a true sink or an intermediate pathway are being measured. A more recent study, focusing on the North Western Mediterranean Sea, found neuston PBM particles at an average abundance of 0.116 m^2 (Collignon et al. 2012). For an in-depth review on microplastics in the marine environment see Cole et al. (2011).

3.2.4 Micro PBMs on Shorelines and on Land

Infrared spectroscopy techniques have been utilized to identify fragment PBMs in the microscopic range by comparing spectra to those in a database of common polymers.

This technique was principally pioneered by Thompson et al. (2004), whose research identified synthetic fibers (PE, PP, polystyrene, nylon, and acrylonitrile butadiene styrene) in samples of beach sand and sub-tidal sediments from around the UK. Microscopic PBM granules and fibers have now been found in sediment at world heritage sites, such as the East Frisain Islands, where a maximum of 496 granules/10 g sediment has been observed (Liebezeit and Dubaish 2012). Further inland, sewage sludge application has been identified as a source of polymer fibers in agricultural soils. Zubris and Richards (2005) found polymer fibers were still present in field soils 15 years after application, with fibers also found in soil horizons below the depth of plowing, suggesting some potential for movement through the soil profile.

3.2.5 Conclusion

There have now been a number studies from around the world that have documented PBMs as the dominant component of shoreline, ocean, and terrestrial debris, although geographical differences in PBM occurrence have been noted. Research on microplastic as a component of beach sediments is also gaining increasing attention. However, microplastics as a component of freshwater sediments and soils are yet to be investigated. Lake and roadside habitats would seem a good place to start; items littered on lakes have less transportation potential, and the regular grass cutting roadsides receive in some countries would mean that littered items are quickly disintegrated by mowing equipment.

3.3 Environmental Degradation

Once in the environment PBMs are degraded through abiotic or biotic factors working together or in sequence; these processes cause the polymer matrix to disintegrate, resulting in the formation of fragmented particles of various sizes and leached additives (Fig. 1). There are now a number of studies whose authors have investigated the degradability of a range of PBMs under a range of exposure conditions (Table 5). In the following section we address the degradation of PBMs with a focus an studies that are environmental relevant.

3.3.1 Factors Affecting Degradation

Polymer Characteristics

Polymer characteristics play an important role in the degradation rate of PBMs. Those PBMs that contain ester linkages (e.g., polyester polyurethanes) are reported to be readily biodegraded by the action of esterases (Albertsson and Karlsson 1993). The molecular composition of a PBM also affects the hydrophobicity of the polymer surface, which in turn affects how easily microorganisms can attach themselves

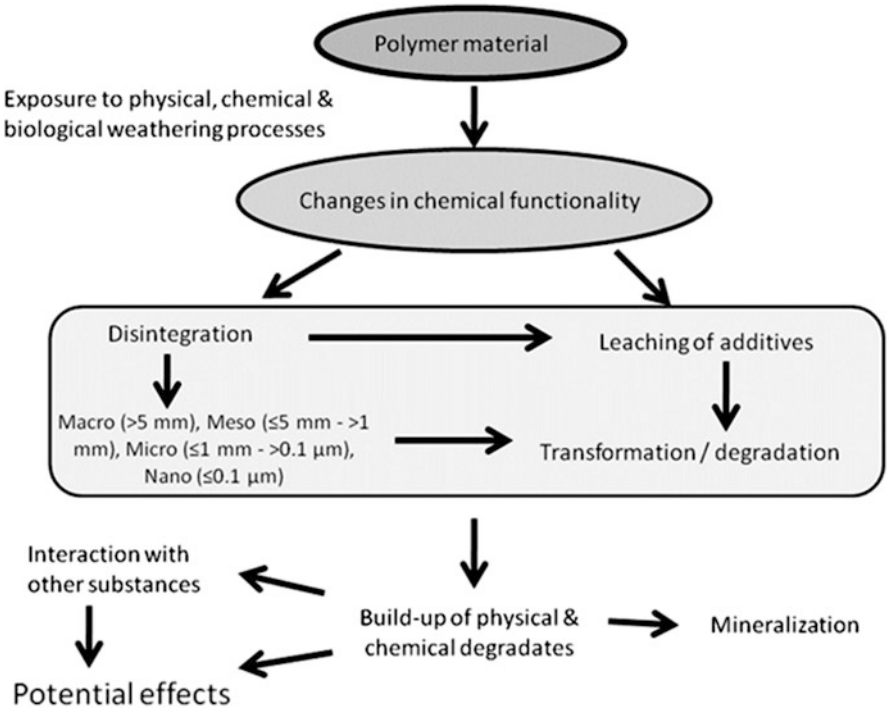


Fig. 1 Conceptual model illustrating degradation pathways for polymer materials

(Albertsson and Karlsson 1993). Complexity of a specific polymer structure (cross-linked polymers that form highly ordered networks) and composition (copolymers) can affect overall degradability by directly influencing the accessibility of enzymes (Artham and Doble 2008). PBMs with short and regular repeating units that have high symmetries and strong interchain hydrogen bonding (e.g., PE, PP, and polyethylene terephthalate (PET)), often limit accessibility and are less susceptible to enzyme attack (Artham and Doble 2008). Kumar et al. (2006) studied the degradability of ethylene–propylene copolymers, and found biotic degradability to decrease with increased ethylene content over a 6-month time period. Composition also affects how sensitive a polymer is to photo-degradation. Kaczmarek et al. (2007) used blends of poly (ethylene oxide) and pectin and found that after 20 h of exposure the blends most sensitive to UV irradiation were those with an equal weight-ratio of each polymer.

3.3.2 Abiotic Degradation

Photo-degradation

Under ambient conditions, photo-degradation is one of the primary means by which PBMs are damaged (Klemchuk 1990; Lucas et al. 2008). The main processes

Table 5 Selected polymer degradation studies in various environmental matrices

Type of material	Length of study	Main findings	Reference
Aquatic			
HDPE, LDPE and PP 1.5 mm think sheets	6 months	Samples were immersed in the Bay of Bengal at a depth of 3 m. Weight loss was greatest in LDPE sheets (2.5%), followed by HDPE (0.75%) and then PP (0.5%). Sites with higher dissolved oxygen had increased oxidation	16
PE glycols (PEGs) water soluble	135 days	Greater degradation was observed in freshwater media when compared to seawater media	3
PHA	42 days	Species of bacteria belonging to the phylogenetic groups of *Cytophaga-Flavobacterium-Bacteroides*, *g-Proteobacteria* and *b-Proteobacteria* were found to utilize PHA in a eutrophic reservoir	22
PUR	12 months	The degree of cross-linking effects PUR degradability in sea water. PUR with a heavily cross-linked network was very resistant to degradation	19
PHA	160 days	Film samples had 58% weight loss at a depth of 120 cm in the South China sea. Degrading microbes isolated from seawater were identified as *Enterobacter cloacae* sp. IBP_V001, *Bacillus* sp. IBP_V002, and *Gracilibacillus* sp. IBP_V003	44
Soil			
PE	10 years	Photooxidation processes produced carbonyl groups which were utilized by microorganisms to degrade the shorter segments of the PE chain	1
LDPE	12 months	After 12 months it was impossible to separate film residues from soil. No change in diversity of ammonia-oxidizing bacteria was detected	12
LDPE	220 days	Higher molecular weight components declined, but lower molecular weight components remained at the same level over the study period	13
LDPE starch blends	90 days	*P. chrysosporium* inoculated soils enhanced biodegradation. Uninoculated soil showed minimal biodegradation	14
PE compost bags	36 weeks	PE with 9% starch, LDPE and HDPE had 36, 2.1, and 1.3% weight loss, respectively	15
PP	12 months	Thermally pretreated PP showed greater weight loss, greater loss of tensile strength and greater changes in crystallinity than non pretreated PP	31

PHB and PHB/PP blends	90 days	PHB/PP blends had higher degradation than PHB and samples degraded quicker under alkaline conditions	33
PUR	28 days	Photolysis prior to biodegradation increased the rate of degradation	32
PUR	5 months	Sample had 95% loss in tensile strength. *Geomyces pannorum* and a *Phoma* sp. were the dominant species recovered from buried samples	40
Polyester films	40 weeks	Degradation was slower for soil buried samples than for solar exposed samples	39
PE and PCL	120 days	PE samples remained almost nonbiodegradable; PCL was shown to be biodegradable	34
Composting			
PE starch blends	125 days	Pure PE remained unchanged over the study period; PE with 40% starch had 25% surface erosion	23
2 Commercial biodegradable polymers	60 days	Starch-based polymer degraded quicker than synthetic polymer with a biodegradable additive	24
Ethylene-propylene copolymers	6 months	Photo-oxidative aging enhanced biodegradation. The composition of monomers in copolymers effects degradability and degradability decreased with increased ethylene content	25
PVC	6 months	*Phanerochaete chrysosporium* PV1 was able to utilize the PVC as a nutrient source	43
Landfill			
PVC	28 months	Found biodegradation rather than leaching accounted for the loss of plasticizers from PVC in landfill simulation	26
PHB, PCL, and PVC	120 days	Plastic volume reduction was more effective under aerobic conditions than anaerobic conditions	27
UV			
LLDPE	16 months outdoor study	UV stabilizer content increased resistance to weathering	8
LDPE	100 h	0.1% content of photo-degrading additives caused embrittlement after 100 h UV exposure	9
Blends of poly(ethylene oxide) and pection	20 h	Free radicals were formed under UV radiation (e.g., hydroxyl, alkoxyl, acyl radicals, or various macroradicals). Blends most sensitive to UV irradiation were those with an equal weight-ration of the blend	28
PUR	1 year	PURs with lactic acid and ethylene glycol degradable chain extenders showed greater mass loss over the study period, when compared to pure PUR	18

(continued)

Table 5 (continued)

Type of material	Length of study	Main findings	Reference
Polyester elastomer	100 h	Degradation occurred in the ether parts of the polymer chain	29
Degradation of nano and micro PLGA	100 days	Surface-associated poly vinyl alcohol (used as a stabilizer during formulation of particles) rather than particle size controlled degradation rate	30
PP		The distribution of photo-degradation products is not dependent on the conditions of irradiation	20
Thermal			
PE, PP, and PS		The presence of PE increased alkane content, PS led to higher aromatic content, PP favored alkene formation of end products	21
NR		Thermal oxidation of the polyisoprene is demonstrated by the formation of CO groups and cleavage of C–C bonds	36
Biodegradation			
PE	20 months	Samples with 5% and 8% starch were not susceptible to biodegradation in seawater	17
LDPE		Bacterial strains isolated from forest soils, most belonging to different genera of the proteobacteria group and three *Rhodococcus* strains, were able of adhering to the surface of oxidized LDPE film and were able to grow there	42
LDPE	15 months	Abiotic degradation produces an increase in carbonyl compounds overtime while the opposite was observed in biotically aged samples	2
LDPE	600 h UV and then 120 days bacterial culture	Oxygenated compounds and hydrocarbons are formed as a result of UV exposure making LDPE suitable for bacterial colonization. Bacterial strains were able to secrete extracellular surfactants which made the LDPE more bio-available	4
Linear LDPE and HDPE	60 days	Main degradation pathway was oxidative as shown by the addition of carbonyl group	5
Clay filled PE		Growth of microbes was significantly greater on PE with nano-clay composite than those without and oxidation was an important process in aiding the utilization of PE by microorganisms	6
PE starch blends	28 days	Demonstrated that fungal strains utilized starch in starch blends, but not the PE polymer	7

PE	4 months	Buried samples retained greater tensile strength than those exposed to sunlight	11
NR	8 weeks	Tread from a truck tire was degraded only slightly when it was used as the sole growth substrate for a strain *of Nocardia,* but its degradation was enhanced by addition of latex was readily utilized as a growth substrate	41
NR		The biodegradation mechanism of *Gordonia* strains was described as the scission of the *cis*-1,4 double bond by oxygen attack to produce carbonyl groups with an aldehyde on the one side and a ketone on the other side of each molecule	38
NR and SR		After incubation with *Nocardia* sp. DSMZ43191, *Streptomyces coelicolor*, *Streptomyces griseus*, bacterial isolate 18a, *Acinetobacter calcoaceticus,* and *Xanthomonas* sp. latex gloves had 11–18% weight loss	10
NR	10 weeks	*Bacillus* sp. SBS25 used NR as the sole source of carbon and was able to produce low molecular weight degradation products	35
NR		Of the microbial strains investigated Actino-bacteria were able to degrade NR	37

PE polyethylene, *PP* polypropylene, *PUR* polyurethane, *PCL* Polycaprolactone, *PHB* poly (Hydroxybutyrate), *PHA* polyhydroxyalkanoates, *NR* natural rubber, *SR* synthetic rubber

References: 1 Albertsson et al. (1987); 2 Albertsson et al. (1995); 3 Bernhard et al. (2008); 4 Roy et al. (2008); 5 Agamuthu and Faizura (2005); 6 Reddy et al. (2009); 7 Shang et al. (2009); 8 Al-Salem (2009); 9 Magagula et al. (2009); 10 Bode et al. (2001); 11 Williams and Simmons (1996); 12 Kapanen et al. (2008); 13 Xu et al. (2006); 14 Orhan and Buyukgungor (2000); 15 Orhan et al. (2004); 16 Sudhakar et al. (2007); 17 Rutkowska et al. (2002a); 18 Tatai et al. (2007); 19 Rutkowska et al. (2002b); 20 Philippart et al. (1997); 21 Pinto et al. (1999); 22 Volova et al. (2007); 23 Vieyra et al. (2013); 24 Mohee and Unmar (2007); 25 Kumar et al. (2006); 26 Mersiowsky et al. (2001); 27 Ishigaki et al. (2004); 28 Kaczmarek et al. (2007); 29 Nagai et al. (2005a); 30 Panyam et al. (2003); 31 Arkatkar et al. (2009); 32 Saad et al. (2010); 33 Pachekoski et al. (2009); 34 Cesar et al. (2009); 35 Cherian and Jayachandran (2009); 36 Agostini et al. (2008); 37 Rifaat and Yosery (2004); 38 Linos et al. (2000); 39 Kijchavengkul et al. (2010) 40 Cosgrove et al. (2007); 41 Tsuchii et al. (1997); 42 Koutny et al. (2009); 43 Ali et al. (2009); 44 Volova et al. (2011)

involved are chain scission and cross-linking reactions, when exposed to ultraviolet (UV) radiation (290–400 nm) or visible radiation (400–700 nm) (Al-Salem 2009). Most polymers tend to absorb high-energy radiation, which activates their electrons to higher reactivity and foments oxidation, cleavage, and other forms of degradation (Shah et al. 2008). The most damaging UV wavelength for a specific material depends on the bonds present; for polyethylene this is 300 nm and for polypropylene 370 nm (Singh and Sharma 2008). When exposed to UV radiation PE and PP films lose their mechanical integrity and tensile strength, which is accompanied by a decrease in their average molecular weight (Singh and Sharma 2008). UV absorption in PS has been found to occur at the benzene ring, causing loss of mechanical properties, chain scission, cross-linking reactions and is a precursor to oxidative degradation (Nagai et al. 1999). Nagai et al. (2005b) analyzed the photo-degradation of a polyether-polyester elastomer under laboratory conditions and found the degradation mechanism upon UV exposure was a selective degradation of the ether parts of soft segments in the polymer matrix, and resulted in the formation of ester, aldehyde, formate, and propyl end groups.

Thermal Degradation

Thermal degradation is the molecular deterioration of a polymer as a result of overheating, which causes bond scissions of the main polymer chain and results in a change in properties. This process affects the entire polymer and not just the polymer surface, and results in changes to molecular weight, loss of tensile strength, changes in crystallinity, reduced durability, embrittlement, changes in color, and cracking (Arkatkar et al. 2009). Thermally pretreated PP has shown enhanced biodegradation, when compared to non-pretreated samples after 12 months (Arkatkar et al. 2009). Thermal degradation of polyolefins (PP, LDPE & PET) at temperatures of 673, 773, 873, and 973 K were found to form tar-containing paraffinic structures in PP and LDPE, while aromatic structures were produced by pyrolysis of PET (Cit et al. 2010). The heat involved in the thermal degradation process also provides energy for the oxidation of carbon in the polymer backbone (Krzan et al. 2006).

Oxidative Degradation

Oxidation processes can be photo or thermally induced and are considered important, especially for non-hydrolyzable materials such as PE (Rutkowska et al. 2002a). The introduction of O_2 into the polymer matrix leads to the formation of OH and CO functional groups, which aid subsequent breakdown by biotic processes. The presence of O_3 in the atmosphere, even in small concentrations, accelerates the aging process of PBMs, because O_3 attacks covalent bonds to produce cross-linking reactions and/or chain scissions producing free radicals (Lucas et al. 2008).

Hydrolytic Degradation

The rate of hydrolysis is dependent on the presence of hydrolyzable covalent bonds such as ester, ether, anhydride, amide, carbamide (urea), or ester amide (urethane) groups in the polymer (Lucas et al. 2008). PBMs with these functionalities are able to absorb moisture (e.g., polyesters), which then promotes hydrolytic cleavage of the polymer chain (Krzan et al. 2006). Hydrolytic degradation of polyester occurs when positively charged hydrogen ions in acidic or negatively charged hydrogen ions in alkaline media attack the ester linkage, thus breaking the polyester chain (Iskander and Hassan 2001). This reduces the polymer chain length and alters its molecular weight distribution, which directly impacts the strength of the material. In addition to chain breakage, hydrolysis in alkaline media also causes surface erosion of polyesters, which is subsequently manifested by weight loss (Iskander and Hassan 2001).

Mechanical Disintegration

Mechanical disintegration is the breakdown of the material through the application of shear forces. This process is distinguished from degradation as the materials molecular bonds remain unchanged. Under field conditions, polymers are exposed to several forms of mechanical degradation that include aging and breakage from atmospheric weathering, water turbulences, freeze-thaw cycles, pressure due to burial under soil or snow, or damage inflicted by animals or birds.

3.3.3 Biotic Degradation (Biodegradation)

Abiotic processes act as an important first step in the degradation of PBMs as they result in a loss of mechanical properties and structural changes to the materials molecular bonds. These processes increase the surface area available for microbial colonization (Kijchavengkul et al. 2010; Lucas et al. 2008). The size of polymer molecules and their general lack of water solubility prevent microorganisms from transporting them into their cells, where most biochemical processes take place (Artham and Doble 2008). Biological processes involved in PBM disintegration start outside the microbial cell, with the secretion of extracellular enzymes (Artham and Doble 2008). These enzymes are too large to penetrate deep into the polymer, so act on the surface by cleaving the polymer chain via hydrolytic mechanisms (Palmisano and Pettigrew 1992). Biological processes are further enhanced by the formation of the aforementioned utilizable functional groups in the polymer chain (Albertsson et al. 1987; Nagai et al. 2005a). Over time, abiotic and biotic factors work together to further the degradation process. Chain scission reduces the molecular weight of the polymer, which in turn provides greater accessibility for moisture and oxygen to induce cross-linking reactions that cause the polymer structure to further weaken and become more susceptible to microbial activity (Kijchavengkul et al. 2010; Roy et al. 2008). When the molar mass of the polymer is sufficiently reduced to generate oligomers and then monomers that

are water soluble, the process of mineralization can begin. These substances are transported through the semipermeable outer membrane of the microorganisms, where they are assimilated as a carbon or nitrogen source through the appropriate metabolic pathway.

3.3.4 Degradation in the Natural Environment

Aquatic Environment

In the aquatic environment the mechanical disintegration of PBMs is facilitated by wave action and grinding with sediment particles, whereas changes in chemical functionality are driven by UV exposure. Floating debris has a greater exposure to sunlight and the oxidative properties of the atmosphere, which act alongside the hydrolytic properties of water to cause the material to become brittle and fragment. Sudhakar et al. (2007) immersed sheets of LDPE, HD (high density) PE, and PP of 1.5 mm thickness for 6 months in ocean waters of Bay of Bengal at a depth of 3 m, and found weight loss was greatest in LDPE sheets (2.5%), HDPE (0.75%), PP (0.5%). The authors of this study also found samples at sites with higher dissolved O_2 had increased oxidation. Rutkowska et al. (2002b) investigated the degradation of polyurethanes in the Baltic Sea over a period of 12 months and found the rate of degradation was dependent on the degree of cross-linking. In the deep ocean environment where sunlight and oxidative processes are missing, the rate of abiotic degradation is extremely low (Watters et al. 2010). Biodegradation in these environments is considered minimal, due to the reduced diversity and density of microbial communities (Browne et al. 2008; Watters et al. 2010). Therefore, PBMs do not readily biodegrade but rather disintegrate, breaking into smaller and smaller pieces (Barnes et al. 2009). In the absence of significant microbial degradation, the sediment compartment in both marine and freshwater environments could function as a continuing source of environmental exposure.

Soil Environment

Soil burial studies have been used as a method to evaluate the degradation of PBMs in the terrestrial environment. Soil type is an important factor affecting degradation; under laboratory conditions, polycaprolactone (PCL) degraded to a greater extent in clay soils than in sandy soils, owing to the great density of microbial communities associated with the clay soils (Cesar et al. 2009). However, when compared to solar exposed samples, buried samples degraded at a much slower rate. Kijchavengkul et al. (2010) buried polyester films in soil for 280 days and found minimal degradation when compared to solar exposed films. A similar result was also found by Williams and Simmons (1996) for PE strips that had been buried for 4 months; these strips retained greater tensile strength than samples that had been exposed to sunlight for 4 months.

The combined effect of multiple degradation processes has also been studied. For example, several authors have evaluated the effects of UV exposure prior to

conducting biodegradation studies under soil burial conditions. Saad et al. (2010) used PHB (polyhydroxybutyrate) films with a 0.1–0.12 mm thickness and found samples exposed to 9 h UV radiation showed ~52% weight loss after 28 days soil burial, compared to ~32% weight loss for samples without pre UV exposure. Sadi et al. (2010) also used PHB films (3 mm thickness) and found pre UV exposure increased the rate of degradation, but at a much slower rate due to the increased thickness of the film. These studies inform that abiotic pretreatment acts as a first step in weakening the polymer structure. This initiates the formation of oxygenated compounds and low molecular weight hydrocarbons, which are recognized by microbial communities and can be utilized as a food source (Roy et al. 2008). In sea water media, Sudhakar et al. (2008) also found thermal pretreatment enhanced the biodegradation of LDPE and HDPE by two marine microbes, namely, *Baccillus sphericus* and *Bacillus cereus*. Thermal processes are considered to contribute minimally to marine environmental disintegration of plastics because of the prevailing low water temperatures.

Biodegradation studies have tended to deal with the use of concentrated microbial cultures, with the aim of assessing a particular strain's ability to degrade a particular PBM. Actinomycetes are reported to be the main group of rubber degrading microbes, with *Bacillus* sp. SBS25 also reported as being capable of utilizing natural rubber as a sole carbon source (Cherian and Jayachandran 2009). Tsuchii et al. (1997) studied strains of *Nocardia* and observed that they only slightly degraded strips of tread cut from truck tires, when used as a sole carbon source. However, degradation of the tire was enhanced by the addition of more easily accessible carbon sources in the form of latex glove and unvulcanized rubber materials, which were readily utilized by the bacteria (Tsuchii et al. 1997).

Biological processes are affected by the amount and type of microorganisms present, their sensitivity to associated environmental parameters and the adaptability of the microbiota (Krzan et al. 2006; Palmisano and Pettigrew 1992). Koutny et al. (2009) isolated bacterial strains from forest soils, most belonging to different genera of the proteobacteria group and three *Rhodococcus* strains, and showed that commonly found bacteria were capable of adhering to and growing on the surface of oxidized LDPE film.

PBMs with a starch component are effectively hollowed out when exposed to microbial communities; this increases the surface to volume ratio allowing for higher oxygen and moisture permeability, enhancing both oxidative and hydrolytic processes (Rutkowska et al. 2002b). In theory, the released polymer fragments will have a greater surface area than the original polymer, allowing them to be further degraded by the microbiota. However, in the case of PE, microorganisms have been found to utilize the starch component, but are unable to utilize the remaining PE fragments, which remain nondegradable (Reddy et al. 2003). The starch is utilized by microorganisms, leaving behind a lace-like structure with reduced physical integrity. However, the molecular weight of the remaining material was not reduced sufficiently for permanent assimilation into the microbial biomass (Klemchuk 1990). Therefore, the remaining polymer matrix was no more biodegradable than the untreated polymer. This causes the disintegration of the polymer matrix, which generates many smaller particles and produces a wider distribution of polymer particles in the environment (Palmisano and Pettigrew 1992). PBMs, such as starch filled PE,

rather than being biodegradable are only biodisintegrated (Klemchuk 1990). However, there are no studies that quantify particle concentrations or particle sizes formed during polymer disintegration and degradation.

3.3.5 Conclusion

There is a broad literature dealing with the degradation of various polymer types under various conditions. Most of these studies were performed in the laboratory and had a major focus on samples exposed to high-energy UV irradiation. In the future, a needed focus is on test conditions that are environmental relevant, such as degradation in marine water and freshwater microcosms, so that samples are exposed to natural cycles of sunlight and temperature. This approach should also include the use of microbial communities' that represent natural conditions (e.g., agricultural soils of different types, freshwater and marine water), rather than concentrated cultures. Attention is also needed on testing materials of different thicknesses and determining if degradation half-lives can be calculated for PBM films, foams and bulkier items. The identification of microscopic PBM particles in environmental matrices (Sect. 3.2.4) highlights a need to establish whether nano-sized particles are also formed during the degradation of PBMs. This is a potentially important issue, given the current concerns regarding the environmental behavior and ecotoxicity of engineered nano-materials.

3.4 Environmental Effects

3.4.1 Entanglement and Ingestion

Once they enter the environment PBMs have the potential to mimic natural food sources (Fig. 2). Laist (1987) addressed this in one of the most frequently cited studies. This author identified 135 species of marine vertebrates and 8 species of invertebrates that are susceptible to entanglement, and 111 species of seabirds that are known to ingest plastic items. Hanni and Pyle (2000) and Page et al. (2004) also reported PBM packing loops as a threat to sea lions in California and fur seals in Australia, respectively; Bugoni et al. (2001) identified plastic bags as the main debris type ingested by sea turtles.

Seabirds are identified as particularly sensitive to PBM debris intake, and are known to accumulate high numbers of items in their stomachs. Robards et al. (1995) found species-specific differences for PBM ingestion in a colony-based survey of multiple species; however, the authors highlight that these differences may be because of geographical differences in PBM pollution. Surface-feeding and plankton-feeding divers are most at risk as they are more likely to confuse PBM items with their food source (Applegate et al. 2008). Petry et al. (2009) studied the stomach contents of 185 birds found dead during beach surveys from July 1997 and July 1998. They identified PBM items in 77% of the stomachs of Cory's Shearwater,

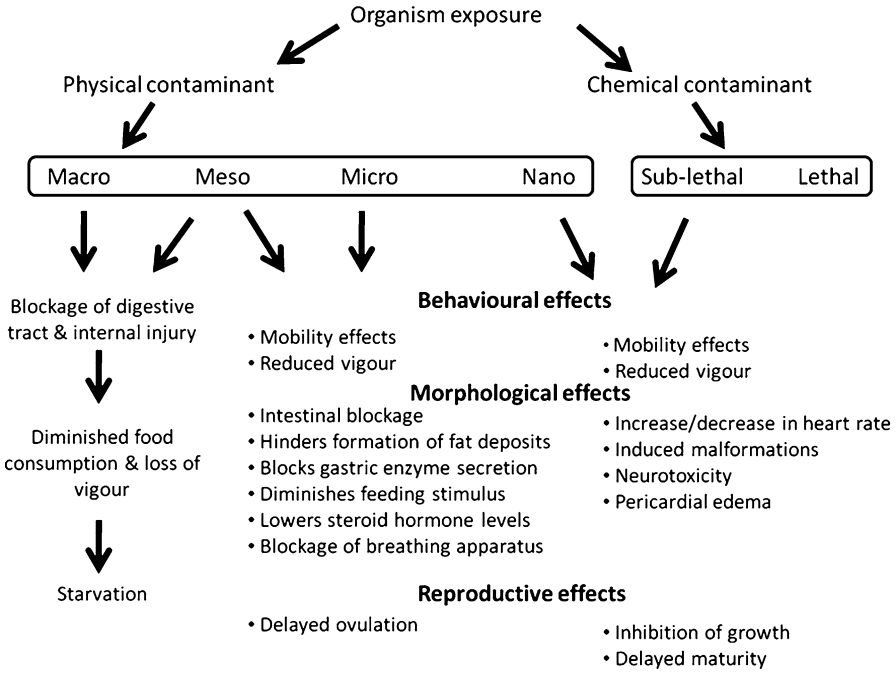


Fig. 2 Conceptual model illustrating the potential effects of degradates produced during the degradation of polymer-based materials

Calonectris diomedea, a pelagic seabird that winters in the waters off the state of Rio Grande do Sul in Southern Brazil. The most significant causes of mortality were from ingesting large PBM items, such as syringes, cigarette lighters and toothbrushes (Petry et al. 2009). The ingestion of such items causes obstruction of the digestive tract and internal injury, leading to diminished food consumption, loss of nutrition and eventually starvation and death (Bugoni et al. 2001; Derraik 2002).

Entanglement and ingestion of PBM debris in the terrestrial environment is not as well documented in the literature as it is in the marine environment; however, livestock are known to consume PBMs. In a recent study, inadequate pastures from drought was identified as a major cause of sheep and goats swallowing foreign objects in Birjand, Iran, with PBMs identified as the dominant foreign items consumed (Omidi et al. 2012). Foreign bodies such as plastic bags have also been highlighted as one of the many animal husbandry problems experienced by farmers in Southern Africa (Dreyer et al. 1999).

3.4.2 Ingestion of Fragmented Particles

The ingestion by a variety of organisms of micro size PBM particles has been reported (Fig. 2). Bern (1990) found that the crustacean zooplankton, *Bosmina coregoni*, did not differentiate between polystyrene beads (2 and 6 mm) and algae when

exposed to combinations of both objects. Thompson et al. (2004) exposed amphipods (detritivores), lugworms (deposit feeders), and barnacles (filter feeders) to microscopic plastic particles and found all three species ingested them within a few days. Browne et al. (2008) found microscopic polystyrene fragments (2 μm in diameter) were ingested by the mussel *Mytilus edulis* under laboratory conditions; these particles were then translocated from the gut to the circulatory system. Researchers have suggested that ingesting PBM particles could present a potential physical hazard leading to the following effects: intestinal blockage in fish, hindering formation of fat deposits, blocking gastric enzyme secretion, feeding stimulus diminution, lowering steroid hormone levels, and delaying ovulation that may cause reproductive failure (Ryan et al. 1988). The ingestion of microplastic particles by plankton-feeding species creates the potential for PBMs to pass up the food chain. Evidence that this occurs is seen from PBM particles having been recovered from fur seal scats on Macquaire Island (Eriksson and Burton 2003). It was hypothesized by the authors that these particles were consumed by a pelagic fish species, *Electrona subaspera*, which were then consumed by fur seals (Eriksson and Burton 2003).

3.4.3 Sorption of POPs to Particle Fragments

The ingestion of PBMs could provide a novel route of expose for chemicals that adsorb to the PBM surface. Persistent organic pollutants (POPs) (e.g., polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), some pesticides and polybrominated diphenyl ethers (PBDE)) have been shown to biomagnify in food webs, mimic natural hormones to cause reproductive disorders, and possibly increase the risk of disease (Ryan et al. 1988). Carpenter and Smith (1972) were the first to predict that PBM particles could be a factor to help explain the presence of PCBs in oceanic communities. They hypothesized that as polymers disintegrate into smaller particles, the surface area of the PBM would increase providing an increased surface for absorbing hydrophobic chemicals (Fig. 1). If the PBM particles are then taken up by organisms, the polymer-associated chemicals would also be transported into the organisms, possibly leaching into tissues and leading to long-term toxicity issues. Since then, polymer particles have increasingly been investigated as a vector for hydrophobic contaminants to enter the food web (Saal et al. 2008). Mato et al. (2001) found that PE and PP pellets (1–5 mm diameter) accumulated PCBs at concentrations up to 106 times that of the surrounding environment, while Ryan et al. (1988) found a positive correlation between ingested PBMs and PCB tissue concentration in seabirds, indicating transfer of these contaminants to organisms. Teuten et al. (2007) found that the pollutant phenanthrene (used to make dyes, plastics, pesticides, explosives, and drugs), was transmitted to the lugworm, *Arenicola marina*, by contaminated PE particles absorbed from seawater that were mixed into sediments inhabited by the worm. Other POPs such as the chlordanes, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) and heavy metals such as mercury, zinc, and lead have also been found to absorb to PBMs (Endo et al. 2005; Van et al. 2012). It has also recently been suggested that sorption

behavior of POPs to polymer surfaces is driven by polymer characteristics such as polymer type and density (Fries and Zarfl 2012). In their study, Fries and Zarfl (2012) found LDPE had higher diffusion coefficients than did high-density polyethylene (HDPE), meaning shorter equilibrium times for low density polymers. The knowledge that chemical contaminants adsorb to PBM particles creates the potential for novel uptake exposure routes, with the potential for indirect effects on PBM debris consumption.

3.4.4 Spread of Alien Species

It has been emphasized that PBM debris may provide a substrate for fouling organisms to be transported long distances, thereby contributing to species dispersal (Derraik 2002; Gregory 2009). Barnes and Milner (2005) reported sightings of an exotic species of barnacle, *Elminius modestus*, on debris in the northern Pacific, and Aliani and Molcard (2003) documented benthic invertebrates living on marine debris transported by wind and surface currents over the western Mediterranean Sea. PBM pellets (2–1.5 mm diam.) have also been identified as providing an oviposition site for the ocean-skater insect *Halobates*, and show that PBM debris may affect the dispersion of this species (Majer et al. 2012). However, Majer et al. (2012) do highlight temperature as a limiting factor with the geographical range of this species, as low water temperatures would prevent their full development. Barnes and Milner (2005) also tentatively suggested that the differences in water temperature could be a limiting factor in species dispersal.

3.4.5 Conclusion

Bulk PBMs are well documented as entanglement and ingestion hazards. The effects of microplastics are less well understood but research on uptake into aquatic organisms is starting to emerge. Microplastic uptake and effects on terrestrial organisms are yet to be investigated. Given that soils are highlighted as a potential sink (Sect. 3.2.4), it is likely that if soil dwelling organisms can ingest soil particles, they can also ingest microplastic particles. Research questions regarding the interaction of microplastics with POPs are also starting to emerge, but these issues are focused primarily on the aquatic environment. The terrestrial environment also needs to be considered, because the sorption of pesticides to microplastics in soil may also present an exposure route for pesticides to soil organisms.

4 Polymer Additives and the Environment

The types and functions of additives used in the production of PBMs are wide ranging. Some of the most important regarding their environmental impact are those that have an endocrine disruptive potential. These include chemicals or chemical

classes such as phthalates, brominated flame retardants (BFRs), and bisphenol A (BPA) (Moore 2008). Phthalate esters are primarily used as plasticizers to impart flexibility to the polymer matrix, and are also used in other products such as inks, lubricating oils, and as solvents in perfumes, paints and additives in hair-sprays, insect repellents, and home furnishings (Fatoki et al. 2010; Julinova and Slavik 2012; Teil et al. 2006; Yuan et al. 2002). In the past, the most important phthalate representative was di-(2-ethylhexyl) phthalate (DEHP), but due to restrictions on its use, others such as di-isodecyl phthalate (DIDP), di-isononyl phthalate (DINP), and di-*n*-butyl phthalate (DBP) are now commercially important (Clara et al. 2010; Tickner et al. 2001).

PVC resins are the most important polymer in terms of phthalate usage. PVC can be produced in two forms; the first is a plasticized from that makes the PVC flexible and the second is an unplasticized form (uPVC) used for the production of rigid materials. In the plasticized form, phthalates can account for 50% of total polymer weight (Mulder 1998; Oehlmann et al. 2009). Other PBMs that can incorporate phthalates include PET, polyvinyl acetates, cellulosic, and PUR (Teil et al. 2006).

BFRs are a diverse group of chemical mixtures that contain brominated organic compounds (Zhang et al. 2009). BFRs are commonly used in a variety of polymer products, such as computers, televisions, kitchen appliance casings, car trimmings, electrical insulation, polyurethane foams, as well as textiles to improve fireproof properties (de Wit 2002). There are approximately 80 different mixtures of BFRs used commercially, and until recently polybrominated diphenyl ethers (PBDEs) were the most widely used (Hu et al. 2009). In Europe and North America, restrictions on the use of PBDEs have lead to tetra-brominated bisphenol A (TBBPA) and hexabromocyclododecane (HBCD) becoming more commercially important (Hu et al. 2009).

BPA is widely used as a monomer in the production of commercial polycarbonate (Duong et al. 2010), and as an antioxidant and stabilizing material for polymer products (Yamamoto et al. 2001). Other additives that are used in PBMs include antimicrobial agents (used in food packaging to preserve shelf-life), and dyes and pigments (often used to improve aesthetic properties of the material) (Saron and Felisberti 2006). Recently, silver nanoparticles have been utilized as an antimicrobial agent in plastic food packaging materials. Nanosilver damages bacterial cells by weakening cell membranes and destroying enzymes that transport cell nutrients, therefore prolonging the shelf life of foodstuffs (Silvestre et al. 2011). Stabilizer technology has the aim to extend the service life of PBMs used in outdoor environments, especially in regions of the world that have high temperatures and long summer seasons (Al-Salem 2009). Solvents may also be applied to coat objects with plastic layers or to clean plastics before printing (Mulder 1998).

4.1 Fate of Additives

The phthalates are generally considered to be chemically stable over a wide temperature range and are easily dissolved in water (Clara et al. 2010), so tend to

adsorb to inorganic and organic particles such as plankton in the water column, before being deposited onto sediments (Larsson et al. 1986). The phthalates are not chemically bonded to the polymer matrix, and, hence, migrate from the products in which they are used by volatilization and enter the atmosphere. Once in the atmosphere they can undergo oxidative or photolytic reactions, followed by wet or dry deposition (Teil et al. 2006). The hydrolytic metabolites of DEHP have been identified as mono ethylhexyl phthalate (MEHP) and 2-ethanohexanol (2-EH) (Tickner et al. 2001).

BFRs are stable and resist degradation, but studies have shown that the higher brominated PBDEs will undergo degradation via de-bromination to more persistent lower-brominated compounds (Birnbaum and Staskal 2004). Such degradation occurs in sand, sediments, and soils under laboratory conditions (Birnbaum and Staskal 2004; Soderstrom et al. 2004). The half life of deca-BDE in sediments is estimated to be <30 min under UV light, 53 h under natural sunlight, and 150–200 h in soils (Soderstrom et al. 2004). The leachability of PBDEs from TV housings was found to be enhanced by the presence of dissolved organic matter in landfill leachate, but degradation rates of PBDEs varied from congener to congener (Kim et al. 2006).

TBBPA is reactively bonded to the polymer matrix and requires cleavage of covalent bonds before migration can take place. Photo-degradation and biodegradation occurs with TBBPA, and the breakdown products have been identified as tri-, di-, and mono-BBPA, as well as BPA (Debenest et al. 2010). In water, TBBPA derivatives are produced from the photochemical degradation and decomposition of the PBMs (Eriksson et al. 2004), whereas thermal degradation also leads to the formation of the above mentioned brominated species (Barontini et al. 2004). TBBPA is reported to have a half life of 7–81 days in water, depending on season, and 2 months under both aerobic and anaerobic conditions in soils and sediments (Birnbaum and Staskal 2004). HBCD has low water solubility and has been shown to persist in sediments (Remberger et al. 2004). Analysis of BFR residues in harbor seals sampled from the northwest Atlantic identified 16 congeners of PBDE at concentrations ranging from 35 to 19,500 ng/g lipid wt (Shaw et al. 2012). Shaw et al. (2012) also identified tissue-specific concentrations of an α-HBCD isomer that displayed significantly higher concentrations in the liver (2–279 ng/g lipid wt) than in the blubber (2–29 ng/g lipid wt).

The migration of BPA from commercially available polycarbonate baby bottles has been shown to range from 2.4 to 14.3 μg/kg, when bottles were filled with boiling water and left at ambient temperatures for 45 min, mainly during the first eight cycles of such use (Maragou et al. 2008). To put this into context the estimated dietary exposure for infants aged up to 1 year old ranges between 0.2 and 2.2 μg/kg-bwt/day, which is below the recently established tolerable daily intake (Maragou et al. 2008). Polycarbonate PBMs were also shown to exhibit accelerated leaching velocity of the BPA when exposed to salts in sea water (1.6 ng/mL/day at 20 °C and 11 ng/mL/day at 37 °C) compared to river water (0.2 ng/ml/day at 20 °C and 4.8 ng/ml/day at 37 °C) (Sajiki and Yonekubo 2003). The estimated half-life of BPA is up to 14 days in seawater (Robinson and Hellou 2009), with aerobic degradation of BPA constituting the most dominant degradation pathway, except when it is present in the atmosphere, and thereby susceptible to reaction with hydroxyl radicals (Staples et al. 1998).

4.2 Occurrence of Associated Additives

Chemical additives are used in the polymer manufacturing process to improve a materials performance, and such additives are dispersed within the three-dimensional porous structure of the polymer. These additives can be released to the environment during the manufacturing process, throughout a PBMs lifecycle and during subsequent PBM degradation processes. The rate at which additives are leached depends on the pore diameter of a particular polymer structure and the molecular size of the additives used; lower molecular weight additives move more easily through a polymer matrix that display larger pore size (Gopferich 1996). Various environmental samples have been analyzed for the presence of these additives, and they have been detected at various concentrations ranging from ng/L to mg/L (Table 6).

Table 6 Concentrations of compounds associated with the manufacturing of polymer products detected in various environmental matrices

Compound	Country	Concentration reported (min: max)	Reference
Plasticisers	*Surface water*		
DEHP	Chi, Ger, Ire, Jap, Neth, SA, Tai, US	n.d.–2.18 mg/L	1, 2, 3, 4, 5, 6, 7, 8
DBP	Chi, Neth, Tai	0.04–13.5 µg/L	2, 3, 5
DEP	SA	0.16–3.56 mg/L	7
DINP	Ire	0.14–1.89 µg/L	4
Bis(2-ethylhexyl) adipate	US	10 µg/L (max)	8
Triphenyl phosphate	US	0.22 µg/L (max)	8
Phthalic anhydride	US	1 µg/L (source: plastic manufacturing)	8
	River sediments		
DEHP	Can, Chi, Ger, Ire, Jap, SA, Tai	0.014–25.27 mg/kg	1, 2, 3, 4, 6, 7, 10
DBP	Can, Chi, Ire, SA, Tai	n.d.–0.89 mg/kg	2, 3, 4, 7, 9, 10
DINP	Can, Ire	n.d.–6.16 mg/kg	4, 10
DIDP	Ire	0.1–7.46 mg/kg	4
BBP	Can, Tai	< 0.3–220 µg/L	9, 10
	Sewage sludge		
DEHP	Aus	20–29 mg/kg	11
DMP	Aus	n.d.–89 µg/kg	11
DEP	Aus	< 40–130 µg/kg	11
BBP	Aus	120–380 µg/kg	11
DOP	Aus	58–180 µg/kg	11
	Wastewater effluent		
DEHP	Aus, Fra	ng–5.02 µg/L	11, 12
DEP	Aus	n.d.–1.1 ng/L	11
DMP	Aus	n.d.–0.19 ng/L	11
DBP	Aus	n.d.–2.4 ng/L	11

(continued)

Table 6 (continued)

Compound	Country	Concentration reported (min: max)	Reference
BBP	Aus	0.088–1.4 ng/L	11
DOP	Aus	n.d.–0.26 ng/L	11
	Untreated wastewater		
DEHP	Aus	3.4–34 ng/L	11
DEP	Aus	0.77–9.2 ng/L	11
DMP	Aus	n.d.–2.4 ng/L	11
DBP	Aus	n.d.–8.7 ng/L	11
BBP	Aus	0.31–3.2 ng/L	11
DOP	Aus	n.d.–1.1 ng/L	11
	Soil		
DEHP	Chi, Den	0.012–7.11 mg/kg	13, 14
DBP	Chi, Den	n.d.–1.56 mg/kg	13, 14
DEP	Chi	n.d.–2.61 mg/kg	13
	Stormwater		
DEHP	Aus, Swe	0.45–24 µg/L	11, 15
DEP	Aus	n.d.–0.27 µg/L	11
DMP	Aus, Swe	n.d.–0.3 µg/L	11, 15
DBP	Aus, Swe	<0.02–0.27 µg/L	11, 15
DIDP	Aus, Swe	n.d.–17 µg/L	11, 15
DINP	Aus, Swe	0.005–85 µg/L	11, 15
BBP	Aus, Swe	n.d.–0.33 µg/l	11, 15
DOP	Aus	n.d.–0.37 µg/L	11
	Rainwater		
DEHP	Fra	423 ng/L (mean)	16
DMP	Fra	116 ng/L (mean)	16
DEP	Fra	333 ng/L (mean)	16
DBP	Fra	592 ng/L (mean)	16
BBP	Fra	81 ng/L (mean)	16
DOP	Fra	10 ng/L (mean)	16
	Other		
Japan (aquatic vegetation)	DEHP	20–2,000 ug/kg	6
Taiwan (fish)	DEHP	2.4–253.9 mg/kg (dwt)	9
Bisphenol-A	*Surface water*		
BPA	Aust, Chi, Ita, Jap, Kor, Port, Swit, Tai, US	n.d.–39.4 µg/L	8, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27
	Sediments		
BPA	Ita	<2–118 µg/kg (dwt)	26
	Sewage sludge		
BPA	Can, Ger	0.001–1.36 mg/kg (dwt)	1, 28
	Wastewater effluent		
BPA	Aus, Aust, Gre, Port, Kor, Spa	0.0026–213.6 µg/L	20, 25, 26, 27, 28, 29, 30
	Landfill leachate		
	Jap	1.3–17,200 µg/L	31

(continued)

Table 6 (continued)

Compound	Country	Concentration reported (min: max)	Reference
Flame retardents	*Surface water*		
PBDE	Arg	n.d.	33
TBBPA	UK	140–3,200 pg/L	32
HBCD	UK	80–270 pg/L	32
Tri(dichlorisopropyl) phosphate	US	0.16 µg/L	8
Tri(2-chloroethyl) phosphate	US	0.54 µg/L	8
	River sediments		
PBDE	Bel, Swit	0.14–8,413 ng/g (dwt)	34, 35
TBBPA	UK, Jap, Swe	<0.2–270 µg/kg (dwt)	32, 36, 37
HBCD	UK, Jap, Swe	880–4,800 pg/g (dwt)	32, 34, 36
	Marine sediment		
TBBPA	Jap	5.5 ng/L	36
HBCD	Jap	<2–860 ng/L	36
	Sewage sludge		
TBBPA	Swe	31–56 µg/kg	37
	Landfill leachate		
TBBPA	Jap	0.3–540 ng/L	36
HBCD	Jap	<2–8 ng/L	36
	Soil		
PBDe	Arg	n.d.	33
	Other		
PBDE	Can (Crab, Sole, and Porpoise)	4–2,300 ng/g (lipid weight; lwt)	38, 39
TBBPA	UK (fish)	<0.29–270 pg/L (lwt)	32
HBCD	UK (fish)	14–290 ng/g (lwt)	32

n.d. not detected, *DEHP* Di(2-ethylhexyl)phthalate, *DEP* Di ethyl phthalate, *DMP* Di methyl phthalate, *DBP* Di-*n*-butyl phthalate, *DIDP* Di-isodecyl phthalate, *DINP* Di-isononyl phthalate, *BBP* Butyl Benzyl Phthalate, *DOP* Dioctyl phthalate, *BPA* Bisphenol-A, *PBDE* Polybrominated diphenyl ethers, *TBBPA* Tetrabromobisphenol A, *HBCD* Hexabromocyclododecane, *Arg* Argentina, *Aus* Austria, *Aust* Australia, *Bel* Belgium, *Can* Canada, *Chi* China, *Den* Denmark, *Fra* France, *Ger* Germany, *Gre* Greece, *Ire* Ireland, *Jap* Japn, *Kor* Korea, *Neth* Netherlands, *Port* Portugal, *SA* South Africa, *Spa* Spain, *Swe* Sweden, *Swit* Switzerland, *Tai* Taiwan, *UK* United Kingdom, *US* United States

References: 1 Fromme et al. (2002); 2 Yuan et al. (2002); 3 Zeng et al. (2008); 4 Kelly et al. (2010); 5 Peijnenburg and Struijs (2006); 6 Yuwatini et al. (2006); 7 Fatoki et al. (2010); 8 Kolpin et al. (2002); 9 Huang et al. (2008); 10 McDowell and Metcalfe (2001); 11 Clara et al. (2010); 12 Dargnat et al. (2009); 13 Hu et al. (2003); 14 –Vikelsoe et al. (2002); 15 Bjorklund et al. (2009); 16 Teil et al. (2006); 17 Wang et al. (2011); 18 Liu et al. (2011); 19 Ribeiro et al. (2009); 20 Ying et al. (2009); 21 Zhao et al. (2009); 22 Voutsa et al. (2006); 23 Duong et al. (2010); 24 Chen et al. (2010); 25 Pojana et al. (2007); 26 Arditsoglou and Voutsa (2010); 27 Fernandez et al. (2009); 28 Stasinakis et al. (2008); 29 Ko et al. (2007); 30 Furhacker et al. (2000); 31 Yamamoto et al. (2001); 32 Harrad et al. (2009); 33 Fontana et al. (2009); 34 Covaci et al. (2005); 35 Kohler et al. (2008); 36 Suzuki and Hasegawa (2006); 37 Sellstrom and Jansson (1995); 38 Ikonomou et al. (2002); 39 Luross et al. (2002)

Phthalates have been described as one of the most abundant and ubiquitous man-made chemicals in the environment (Liao et al. 2009; Martin and Voulvoulis 2009). Because they are not chemically bound to the polymer resin in which they are used, they tend to slowly migrate to the surface of the product and leach or evaporate from the end-product to the surrounding environment, both during and after the useful life of a specific product (Martin and Voulvoulis 2009). DEHP and DBP are the most commonly occurring phthalates. Residues of both have been detected in multiple environmental compartments: surface waters (Kelly et al. 2010), river sediments (Huang et al. 2008), sewage sludge, wastewater effluent, and untreated wastewater (Clara et al. 2010), rainwater (Teil et al. 2006), stormwater (Bjorklund et al. 2009), and agricultural soils (Hu et al. 2003). The other phthalates are generally considered to be of minor importance.

The concentration of phthalates reported to exist in surface waters have ranged from sub μg/L (e.g., Kelly et al. 2010) to high mg/L levels in contaminated hotspots (e.g., Fatoki et al. 2010), and to mg/kg in sediments (e.g., Kelly et al. 2010). Concentrations reported for agricultural soils in China (23 locations; 0.89–10.03 mg/kg) (Hu et al. 2003), were much higher than those found in agricultural soils in Denmark (2 locations; 0.3–1,900 μg/kg) (Vikelsoe et al. 2002). The concentrations observed for China were thought to be influenced by the use of agricultural films containing phthalates (Hu et al. 2003). The maximum concentration of DEHP in final effluent from a European Waste Water Treatment Plant (WWTP) was reported to be 182 μg/L, and was derived from a review of studies of estrogenic compounds that had a median concentration of 5.3 μg/L (Martin and Voulvoulis 2009). In the Venda region of South Africa, it has been reported by Fatoki et al. (2010) that PBMs are indiscriminately disposed of as a common practice. This has caused river water pollution by phthalates at levels ranging from 0.16 to 10.17 mg/L (Fatoki et al. 2010). This is noted by Fatoki et al. (2010) as an issue of concern, because water from these rivers and their associated dams are the primary sources of potable water. This poses a risk to human health, because people who drink water contaminated with such levels exceed the USEPA established safe limit for phthalates (<6 μg/L) over many years, and may develop liver and reproductive problems (USEPA 2012).

BPA can be released into the environment through sewage treatment effluent, landfill leachate (Wintgens et al. 2003), or degradation of polycarbonate polymers (Mohapatra et al. 2010). BPA residues are most commonly reported in surface waters and wastewater effluents, where they display concentrations up to 213.6 μg/L; sediments have been identified as being modest sinks (Wang et al. 2011) (Table 6). PBMs in landfills are thought to be a possible source of BPA in groundwater; in Japan, median concentration of 269 mg/L have been detected in sampled leachates (Yamamoto et al. 2001). The maximum concentration of BPA in final effluent from a European WWTP was reported to be 40.09 μg/L, with a median value of 0.36 μg/L (Martin and Voulvoulis 2009).

A review of the literature has indicted the PBDEs, TBBPA, and HBCD to be the most commonly occurring BFRs detected in environmental samples. PBDE and TBBPA differ in that PBDEs are generally used as an additive flame retardant, and thereby are not chemically bonded to the polymer matrix; in contrast, TBBPA is

primarily used as a reactive flame retardant and is covalently bonded to the polymer matrix (Alaee et al. 2003). Flame retardants, when used as additives (rather than a reactive compound), exhibit leaching and evaporation behavior similar to those displayed by the phthalates (Debenest et al. 2010). Levels in sediments are generally highest from urban and industrial areas, particularly downstream from WWTPs or from product manufacturing sites. Sellstrom and Jansson (1995) found high concentrations of TBBPA in sediments sampled downstream from plastic manufacturing factories (270 µg/kg dwt), in comparison to upstream sediments (34 µg/kg dwt), indicating the factory as the source. Harrad et al. (2009) reported similar concentrations of HBCD and TBBPA in water, sediments, and fish (see Table 6) from nine English lakes that had no major point-source inputs (i.e., from WWTP), with minimal seasonal variations and found aqueous concentrations were significantly correlated, but no common source was identified. Hites (2004) also provided a review of PBDE concentrations present in human samples (0.03–193 ng/g for milk; 0.44–6.03 ng/g for blood, lipid wt), outdoor and indoor air (82.6–1,780 pg/m^3), marine mammals (0.42–4,950 ng/g lipid wt), birds (124–7,510 ng/g lipid wt for gull eggs), and fish (6.31–7,200 ng/g lipid wt).

4.3 Toxicity of Chemical Additives

Once released from the degraded polymer matrix, chemical additives may become available for uptake by living organisms. The phthalates and BPA have been found to cause a range of effects on fish, crustacean, amphibian and bacteria species; effects include mortality, delayed maturity, reduced vigor, induced morphological deformations, and reduced reproduction (Table 7). DEHP represents the most widely studied phthalate and is regarded to be one of the most toxic of the class (Jonsson and Baun 2003). However, its metabolite MEHP, which is considered to be itself toxic, has not been widely studied. DEHP has displayed toxicity to rats through impaired testis development at high doses (Table 6). Other important phthalates, such as DMP, DEP, DBP, and BBP, also exhibit similar toxic effects to DEHP. The toxicity of some of the minor phthalates (i.e., DIDP, DNIP, and DOP) is less well researched, possibly because their concentrations in most aquatic environments are reported at low µg/L or less (Table 6). Oehlmann et al. (2009) published a comprehensive review of the effects of phthalates and BPA on wildlife, and highlighted the lack of long-term exposure or toxicity data at environmentally relevant concentrations, particularly in complex mixtures. Human exposure can occur through ambient environmental concentrations (Tickner et al. 2001). DEHP containing PVC, since the 1960s, has been used to produce a range for medical devices and in the construction industry (Rossi and Lent 2006; Tickner et al. 2001). Rossi and Lent (2006) have proposed the phasing out and replacement of PVC, and recommend a preference towards PBMs that do not contain hazardous additives such as PP and PE as means of reducing phthalates exposure.

Table 7 Selected studies in which the effects of commonly used polymer additives was reviewed

Compound	Organism	Findings	Reference
Phthalate	*Aquatic*		
DEHP	*Daphnia magna*	24 h EC_{50} 71.07 mg/L based on immobilization	1
	Chironomus tentans	24 h LC_{50} 438.96 mg/L based on death of individuals	1
	Danio rerio	5 g/kg after 10 day exposure via intraperitoneal injection caused increase in hepatosomatic index and levels of hepatic vitellogenin transcript, and a decrease in fertilization success of oocytes	2
	Salmo salar	1,500 mg/kg dosed via diet resulted in small incidence of juvenile intersex	3
	Oryzias latipes	No evidence of estrogenic effects at tested concentrations	4
	Cyprinus carpio	Disrupted synthesis of testosterone	5
	Mytilus galloprovincialis	21 day expose to 500 μg/L increased catalase and acyl-CoA oxidase activity and inhibition of superoxide and manganese superoxide dismutase	6
	Lumbriculus variegatus	Not acutely toxic	7
	Pseudokirchneriella subcapitata	72 h EC_{50} >0.003 mg/L based on inhibition of growth	8
	Vibrio fischeri	15 min EC_{50} >0.003 mg/L	8
	Hyalella azteca	No effect at concentrations tested	7
	Folsomia fimetaria	EC_{50} >5,000 mg/kg based on adult survival and reproduction, >1,000 mg/kg based on juvenile survival, growth and number of cuticles	16
	Escherichia coli and *Bacillus subtilis*	24 h low doses (<150 μg/ml) stimulated growth, doses >300 μg/mL showed morphological deformations	17
	Caenorhabditis elegans	24 h LC_{50} 22.55 mg/L based on mortality	18
	Adult Wistar rats	90 day dose of 500 mg/kg/d decreased weight and volume of testis	19
	Adult rats	Dose of 1 g/kg decreased testis weight and was linked with oxidative stress within testis	21
DMP	*Daphnia magna*	48 EC_{50} 284 mg/L based on immobility	8
	Chironomus tentans	10 day LC_{50} 68.2 mg/L	7
	Pseudokirchneriella subcapitata	72 h EC_{50} 228 based on inhibition of growth	8
	Vibrio fischeri	15 min EC_{50} 26.3 mg/L	8
	Hyalella azteca	10 day LC_{50} 28.1 mg/L	7
	Lumbriculus variegatus	10 day LC_{50} 246 mg/L	7

(continued)

Table 7 (continued)

Compound	Organism	Findings	Reference
DEP	*Daphnia magna*	48 EC_{50} 90 mg/L based on immobility	8
	Chironomus tentans	10 day LC_{50} 31 mg/L	7
	Pseudokirchneriella subcapitata	72 h EC_{50} 70.4 based on inhibition of growth	8
	Cyprinus carpio	96 h LC_{50} 48 mg/L based on induced testicular atrophy	10
	Vibrio fischeri	15 min EC_{50} 143 mg/L	8
	Hyalella azteca	10 day LC_{50} 4.21 mg/L	7
	Lumbriculus variegatus	10 day LC_{50} 102 mg/L	7
DBP	*Daphnia magna*	48 EC50 6.78 mg/L based on immobility	8
	Chironomus tentans	10 day LC_{50} 2.64 mg/L	7
	Cyprinus carpio	Disrupted synthesis of testosterone	10
	Oncorhynchus mykiss	No significant vitellogenin response	11
	Vibrio fischeri	15 min EC_{50} >7.4 mg/L	8
	Pseudokirchneriella subcapitata	72 h EC_{50} 2.52 based on inhibition of growth	8
	Xenopus laevis	96 h LC_{50} 14.5 mg/L based on mortality, 96 h EC_{50} 0.98 mg/L based on number of surviving tadpoles with a least 1 malformation	14
	Rana rugosa	Development of ovaries in 17% of males gonads exposed to 10 μM during days 19–23 after fertilization	15
	Folsomia fimetaria	EC_{50} 305 mg/kg based on adult survival, 19.4 mg/kg juvenile survival, 68 mg/kg reproduction, >10 mg/kg growth and number of cuticles	16
	Adult rats	2 week dose of 500 mg/kg/d by oral gavage decrease in body and testicular weight, 250 and 500 mg/kg/d decreased sperm count	20
	Hyalella azteca	10 day LC_{50} 0.63 mg/L, respectively	7
	Lumbriculus variegatus	10 day LC_{50} 2.48 mg/L, respectively	7

BBP	*Daphnia magna*	48 EC_{50} 2.43 mg/L based on immobility	8
	Danio rerio	6 μg/L found to induce changes in sperm motility. No effect on number of eggs spawned and viability of embryos at 8 h post fertilization	9
	Oncorhynchus mykiss	500 mg/kg dosed via intraperitoneal injection resulted in threefold increase in vitellogenin in males	11
	Pimephales promelas	No evidence of estrogenic effects at tested concentrations	12
	Vibrio fischeri	15 min EC_{50} >1.3 mg/L	8
	Hyalella azteca	10 day LC_{50} 0.46 mg/L	7
	Pseudokirchneriella subcapitata	72 h EC_{50} 0.96 mg/L based on inhibition of growth	8
	Lumbriculus variegatus	10 day LC_{50} 1.23 mg/L	7
	Gasterosteus aculeatus	31 day exposure to 100 μg/L caused behavioral alterations	36
DHP	*Lumbriculus variegatus*	Not acutely toxic	7
	Hyalella azteca	No effect at concentrations tested	7
DINP	*Oryzias latipes*	1 μg/g fish/day had no effect on reproduction or development at tested concentrations	13
DIDP	*Oryzias latipes*	1 μg/g fish/day had no effect on reproduction or development at tested concentrations	13
DOP	*Escherichia coli* and *Bacillus subtilis*	24 h low doses (<150 μg/ml) stimulated growth, doses >300 μg/ml showed morphological deformations	17
MEHP	*Daphnia magna*	48 EC_{50} 3.47 mg/L based on immobility	8
	Pseudokirchneriella subcapitata	51.9 mg/L based on inhibition of growth	8
	Vibrio fischeri	15 min EC_{50} 45.4 mg/L	8
	Mouse fetal oocytes	24 h 60% mortility in oocyte survival at 500 μM and 32% at 125 μM	22

(continued)

Table 7 (continued)

Compound	Organism	Findings	Reference
BPA	*Chironomus tentans*	24 h LC_{50} 3.264 mg/L and 96 h EC_{50} 2.7 mg/L based on mortality	1, 23
	Chironomus riparius	Chronic LOEC 1.0 mg/L based on reduction of larval wet weight and delay in molting	24
	Daphnia magna	24 h EC_{50} 0.237 mg/L based on immobilization, 21 day NOEC ≥3.16 mg/L based on reproduction rate, EC_{50} 16 mg/L based on reproductive tests, 48 h EC_{50} 10 mg/L based on immobilization	1, 25, 26, 27
	Hyalella azteca	42 day LOEC 1.1 mg/L based on number of offspring per female	23
	Marisa cornuarietis	96 h LC_{50} 2.24 mg/L (25°), >4.03 mg/L (22°) and no effect on fecundity, egg hatching, juvenile growth and reproduction at concentrations up to 0.64 mg/L. A significant decrease in female growth was observed at 0.64 mg/L	23, 29
	Oncorhynchus mykiss	50 mg/kg dosed by injection increased basal vitellogenin concentration by a factor of 700 after 6 days and 48 h EC_{50} 15 mg/L	11, 28
	Pimephales promelas	96 h LC_{50} 4.7 mg/L	27
	Acartia tonsa	LOEC 300 µg/L based on egg production	9
	Potamopyrgus antipodarum	4 Week EC_{50} 5.67 µg/kg embryo reproduction via sediment exposure	35
	Mytilus hemocytes	24 h EC_{50} 34.486 µM expressed as % lysosomal destabilization	34
	Menidia menidia	96 h LC_{50} 9.4 mg/L	27
	Mysidopsis bahia	96 h LC_{50} 1.1 mg/L	27
	Skeletonema costatum	96 h EC_{50} 1.0 mg/L based on cell count	27
	Oryzias latipes	LOEC 10 µg/L based on testis-ova	4
	Heteromyenia sp.	Abnormal growth observed at 16 mg/L, at 80 mg/L complete germination failure	33
	Eunapius fragilis	Abnormal growth observed at 16 mg/L	33
	Lemna minor	EC_{50} 20 mg/L based on frond density	23
	Vibrio fischeri	15 min EC_{50} 6.2 mg/L	28
	Pseudokirchneriella subcapitata	72 h EC_{50} 2.2 mg/L	28
	Thamnocephalus platyurus	24 h LC_{50} 19.9 mg/L	28
	Selenastrum capricornutum	96 h EC_{50} 2.7 mg/L based on cell count (3.1 mg/L for cell volume)	27
	Xenopus laevis (African clawed frog)	No effect found at concentrations tested	9

	Tigriopus japonicus	48 h EC_{50} 4.32 mg/L based on adult motility at 25°	32
	Brachionus calyciflorus	48 h LOEC 3.6 mg/L based on intrinsic rate of increase in offspring	23
	Brachydanio rerio	48 h EC_{50} 8.91 mg/L based on immobilization	32
	Hydra vulgaris	72 h LOEC 0.460 mg/L based on sublethal toxicity to polyps and 96 h EC_{50} 6.9 mg/L based on polyp survival	32
	Cnidarian test (Hydra attenuata assay)	96 h EC_{50} 19.9 mg/L	28
Flame retardants			
TBBPA	*Vibrio fischeri*	15 min EC_{50} 56.9 mg/L	28
TBBPA	*Pseudokirchneriella subcapitata*	72 h EC_{50} >250 mg/L	28
TBBPA	*Thamnocephalus platyurus*	24 h LC_{50} 8.3 mg/L	28
TBBPA	Cnidarian test (Hydra attenuata assay)	96 h EC_{50} 0.2 mg/L	28
TBBPA	*Rainbow trout*	48 h EC_{50} 13.9 mg/L	28
TBBPA	*Daphnia magna*	48 h EC_{50} 0.69 mg/L based on immobilization	30
TBBPA	*Oncorhynchus mykiss*	No significant vitellogenin response	11

DEHP Di(2-ethylhexyl)phthalate, *DEP* Di ethyl phthalate, *DMP* Di methyl phthalate, *DBP* Di-*n*-butyl phthalate, *DIDP* Di-isodecyl phthalate, *DINP* Di-isononyl phthalate, *BBP* Butyl benzyl phthalate, *DOP* Dioctyl phthalate, *DNP* Di-nonyl phthalate, *MEHP* Mono(2-ethylhexyl) phthalate, *BPA* Bisphenol-A, *TBBPA* Tetrabromobisphenol A

References: 1 Park and Choi (2007); 2 Uren-Webster et al. (2010); 3 Norman et al. (2007); 4 Metcalfe et al. (2001); 5 Thibaut and Porte (2004); 6 Orbea et al. (2002); 7 Call et al. (2001); 8 Jonsson and Baun (2003); 9 Oehlmann et al. (2009); 10 Barse et al. (2007); 11 Christiansen et al. (2000); 12 Harries et al. (2000); 13 Patyna et al. (2006); 14 Lee et al. (2005); 15 Ohtani et al. (2000); 16 Jensen et al. (2001); 17 Sandy et al. (2010); 18 Roh et al. (2007); 19 Dorostghoal et al. (2010); 20 Zhou et al. (2010); 21 Kasahara et al. (2002); 22 Bonilla and del Mazo (2010); 23 Mihaich et al. (2009); 24 Watts et al. (2003); 25 Caspers (1998); 26 Mu et al. (2005); 27 Alexander et al. (1988); 28 Debenest et al. (2010); 29 Forbes et al. (2008); 30 Liu et al. (2007); 31 Pascoe et al. (2002); 32 Marcial et al. (2003); 33 Hill et al. (2002); 34 Canesi et al. (2007); 35 Duft et al. (2003); 36 Wibe et al. (2004)

BFRs exposure has been found to inhibit growth of plankton and algae colonies and reduce zooplankton reproduction (Debenest et al. 2010). Mice and rat studies have shown liver disturbances, nervous system damage and decreased thyroxine levels; pentaBDE has been found to accumulate in certain predatory birds and mammals that are at the top of the food chain (Rhee et al. 2002). Another toxic compound that is associated with polymer manufacturing is zinc, which has been identified as the dominant toxicant in wastewater from rubber manufacturing factories (Park et al. 2008). Exposure of *Daphnia magna* to accelerators (e.g., zinc diethyl dithiocarbamate (ZDEC) and zinc mercaptobenzothiazole (ZMBT)) that are used to produce rubber and latex products gave 48 h EC_{50} values that were lower than those reported for DMP, DEP, DBP, BBP, and MEHP (Jonsson and Baun 2003) (Table 7). This higher toxicity level indicates that the risks associated with other additives compounds used in PBM manufacturing are also important.

4.4 Conclusion

The phthalates, BPA and BFRs are considered to be the most important PBM additives, because these are considered to be biologically active. To be effective these chemicals often have properties that make them resistant to photo-degradation and biodegradation. These properties imply a potential for accumulation and persistence in the environment, and as such there is a growing body of literature dealing with the environmental occurrence and effects of these compounds. However, there are many other PBMs that incorporate an even greater number of additive compounds, and the risks of these compounds also need to be evaluated. An example are the halogen-free flame retardants, which are of growing interest as replacements for the more traditional BFRs, and are the subject of an interesting and in-depth review by Waaijers et al. (2013). These authors highlight that the environmental behavior and ecotoxicological properties of many of these compounds are only known to a limited extent.

5 Recommendations for Future Research

Considerable information is now available on the environmental effects of PBMs. As described previously in this review, there are several emerging areas of interest that need future research attention. These include research to:

1. Better understand the sources and sinks for microscopic polymer particles (as highlighted by Browne et al. 2011); this research should address both terrestrial and freshwater sinks.
2. Establish appropriate degradation test strategies consistent with realistic environmental conditions, because the complexity of environmental systems is lost when only one process (e.g., hydrolysis) is assessed in isolation.

3. Establish appropriate analytical methods to characterize the formation and ecotoxicity of both the physical and chemical constituents formed during PBM degradation.
4. Evaluate the uptake and the long-term effects of very small polymer particles in both aquatic and terrestrial compartments.
5. Evaluate the extent to which different polymer characteristics (i.e., the molecular bonds present in different materials) influence sorption behavior of anthropogenic compounds, and how these characteristics influence ecotoxicity.

6 Summary

There is now a plethora of polymer-based materials (PBMs) on the market, because of the increasing demand for cheaper consumable goods, and light-weight industrial materials. Each PBM constitutes a mixture of their representative polymer/s and their various chemical additives. The major polymer types are polyethylene, polypropylene, and polyvinyl chloride, with natural rubber and biodegradable polymers becoming increasingly more important. The most important additives are those that are biologically active, because to be effective such chemicals often have properties that make them resistant to photo-degradation and biodegradation. During their lifecycle, PBMs can be released into the environment form a variety of sources. The principal introduction routes being general littering, dumping of unwanted waste materials, migration from landfills and emission during refuse collection. Once in the environment, PBMs are primarily broken down by photo-degradation processes, but due to the complex chemical makeup of PBMs, receiving environments are potentially exposed to a mixture of macro-, meso-, and micro-size polymer fragments, leached additives, and subsequent degradation products. In environments where sunlight is absent (i.e., soils and the deep sea) degradation for most PBMs is minimal.

The majority of literature to date that has addressed the environmental contamination or disposition of PBMs has focused on the marine environment. This is because the oceans are identified as the major sink for macro PBMs, where they are known to present a hazard to wildlife via entanglement and ingestion. The published literature has established the occurrence of microplastics in marine environment and beach sediments, but is inadequate as regards contamination of soils and freshwater sediments. The uptake of microplastics for a limited range of aquatic organisms has also been established, but there is a lack of information regarding soil organisms, and the long-term effects of microplastic uptake are also less well understood. There is currently a need to establish appropriate degradation test strategies consistent with realistic environmental conditions, because the complexity of environmental systems is lost when only one process (e.g., hydrolysis) is assessed in isolation. Enhanced methodologies are also needed to evaluate the impact of PBMs to soil and freshwater environments.

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References

Abu-Hilal A, Al-Najjar T (2009) Marine litter in coral reef areas along the Jordan Gulf of Aqaba, Red Sea. J Environ Manage 90(2):1043–1049

Agamuthu P, Faizura PN (2005) Biodegradability of degradable plastic waste. Waste Manage Res 23:95–100

Agostini DLS, Constantino CJL, Job AE (2008) Thermal degradation of both latex and latex cast films forming membranes—combined TG/FTIR investigation. J Thermal Analy Calorim 91(3):703–707

Al-Salem SM (2009) Influence of natural and accelerated weathering on various formulations of linear low density polyethylene (LLDPE) films. Mater Design 30(5):1729–1736

Alaee M, Arias P, Sjodin A, Bergman A (2003) An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ Int 29(6):683–689

Albertsson AC, Andersson SO, Karlsson S (1987) The mechanism of biodegradation of polyethylene. Polym Degrad Stabil 18(1):73–87

Albertsson AC, Karlsson S (1993) Aspects of biodeterioration of inert and degradable polymers. Int Biodeter Biodegrad 31(3):161–170

Albertsson AC, Barenstedt C, Karlsson S, Lindberg T (1995) Degradation product pattern and morphology changes as means to differentiate abiotically and biotically aged degradable polyethylene. Polymer 36(16):3075–3083

Alexander HC, Dill DC, Smith LW, Guiney PD, Dorn P (1988) Bisphenol-a—acute aquatic toxicity. Environ Toxicol Chem 7(1):19–26

Ali MI, Perveen Q, Ahmad B, Javed I, Razi-Ul-Hussnain R, Andleeb S, Atique N, Ghumro PB, Ahmed S, Hameed A (2009) Studies on biodegradation of cellulose blended polyvinyl chloride films. Int J Agric Biol 11(5):577–580

Aliani S, Molcard A (2003) Hitch-hiking on floating marine debris: macrobenthic species in the Western Mediterranean Sea. Hydrobiologia 503(1–3):59–67

Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62(8):1596–1605

Applegate B, Pallister C, Kennish JM (2008) Nature and sources of chemicals in marine plastic debris. Marine Debris Alaska: Coordinating Our Efforts 2009(1):53–56

Arditsoglou A, Voutsa D (2010) Partitioning of endocrine disrupting compounds in inland waters and wastewaters discharged into the coastal area of Thessaloniki, Northern Greece. Environ Sci Pollut Res 17(3):529–538

Arkatkar A, Arutchelvi J, Bhaduri S, Uppara PV, Doble M (2009) Degradation of unpretreated and thermally pretreated polypropylene by soil consortia. Int Biodeter Biodegrad 63(1):106–111

Artham T, Doble M (2008) Biodegradation of aliphatic and aromatic polycarbonates. Macromol Biosci 8(1):14–24

Ashley R, Blackwood D, Souter N, Hendry S, Moir J, Dunkerley J, Davies J, Butler D, Cook A, Conlin J, Squibbs M, Britton A, Goldie P (2005) Sustainable disposal of domestic sanitary waste. J Environ Eng-Asce 131(2):206–215

Barnes DKA, Milner P (2005) Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. Mar Biol 146(4):815–825

Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B: Biol Sci 364(1526):1985–1998

Barontini F, Marsanich K, Petarca L, Cozzani V (2004) The thermal degradation process of tetrabromobisphenol A. Indust Engineering Chem Res 43(9):1952–1961

Barse AV, Chakrabarti T, Ghosh TK, Pal AK, Jadhao SB (2007) Endocrine disruption and metabolic changes following exposure of Cyprinus carpio to diethyl phthalate. Pest Biochem Physiol 88(1):36–42

Benton T (1991) Oceans of garbage. Nature 352(6331):113

Benton TG (1995) From castaways to throwaways—marine litter in the Pitcairn Islands. Biol J Linnean Soc 56(1–2):415–422

Bern L (1990) Size-related discrimination of nutritive and inert particles by freshwater zooplankton. J Plankton Res 12(5):1059–1067
Bernhard M, Eubeler JP, Zok S, Knepper TP (2008) Aerobic biodegradation of polyethylene glycols of different molecular weights in wastewater and seawater. Water Res 42(19):4791–4801
Birnbaum LS, Staskal DF (2004) Brominated flame retardants: cause for concern? Environ Health Perspect 112(1):9–17
Bjorklund K, Cousins AP, Stromvall AM, Malmqvist PA (2009) Phthalates and nonylphenols in urban runoff: occurrence, distribution and area emission factors. Sci Total Environ 407(16):4665–4672
Bode HB, Kerkhoff K, Jendrossek D (2001) Bacterial degradation of natural and synthetic rubber. Biomacromolecules 2(1):295–303
Bonilla E, del Mazo J (2010) Deregulation of the Sod1 and Nd1 genes in mouse fetal oocytes exposed to mono-(2-ethylhexyl) phthalate (MEHP). Reprod Toxicol 30(3):387–392
Bowman D, Manor-Samsonov N, Golik A (1998) Dynamics of litter pollution on Israeli Mediterranean beaches: a budgetary, litter flux approach. J Coastal Res 14(2):418–432
Bravo M, Gallardo MD, Luna-Jorquera G, Nunez P, Vasquez N, Thiel M (2009) Anthropogenic debris on beaches in the SE Pacific (Chile): results from a national survey supported by volunteers. Mar Pollut Bull 58(11):1718–1726
Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC (2008) Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.). Environ Sci Technol 42(13):5026–5031
Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, Thompson R (2011) Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ Sci Technol 45(21):9175–9179
Bugoni L, Krause L, Petry MV (2001) Marine debris and human impacts on sea turtles in southern Brazil. Mar Pollut Bull 42(12):1330–1334
Call DJ, Markee TP, Geiger DL, Brooke LT, VandeVenter FA, Cox DA, Genisot KI, Robillard KA, Gorsuch JW, Parkerton TF, Reiley MC, Ankley GT, Mount DR (2001) An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures. Environ Toxicol Chem 20(8):1798–1804
Canesi L, Lorusso LC, Ciacci C, Betti M, Rocchi M, Pojana G, Marcomini A (2007) Immunomodulation of Mytilus hemocytes by individual estrogenic chemicals and environmentally relevant mixtures of estrogens: in vitro and in vivo studies. Aquat Toxicol 81(1):36–44
Carpenter EJ, Anderson SJ, Miklas HP, Peck BB, Harvey GR (1972) Polystyrene spherules in coastal waters. Science 178(4062):749–750
Carpenter EJ, Smith KL (1972) Plastics on the Sargasso Sea surface. Science 175:1240–1241
Caspers N (1998) No estrogenic effects of bisphenol A in Daphnia magna STRAUS. Bull Environ Contam Toxicol 61(2):143–148
Caulton E, Mocogni M (1987) Preliminary studies of man-made litter in the firth of forth, Scotland. Mar Pollut Bull 18(8):446–450
Cesar MEF, Mariani P, Innocentini-Mei LH, Cardoso E (2009) Particle size and concentration of poly(epsilon-caprolactone) and adipate modified starch blend on mineralization in soils with differing textures. Polym Test 28(7):680–687
Chaudhuri UR (2010) Petrochemicals. Fundamentals of petroleum and petrochemical engineering, Chemical Industries, CRC Press, pp 101–129
Chen T-C, Shue M-F, Yeh Y-L, Kao T-J (2010) Bisphenol A occurred in Kao-Pin River and its tributaries in Taiwan. Environ Monit Assess 161(1–4):135–145
Cherian E, Jayachandran K (2009) Microbial degradation of natural rubber latex by a novel species of Bacillus sp SBS25 isolated from soil. Int J Environ Res 3(4):599–604
Christiansen LB, Pedersen KL, Pedersen SN, Korsgaard B, Bjerregaard P (2000) In vivo comparison of xenoestrogens using rainbow trout vitellogenin induction as a screening system. Environ Toxicol Chem 19(7):1867–1874
Cierjacks A, Behr F, Kowarik I (2012) Operational performance indicators for litter management at festivals in semi-natural landscapes. Ecol Indic 13:328–337

CIPET (2010) Central Institute of Plastics Engineering and Technology—plastic industry statistics. http://cipet.gov.in/plastics_statics.html
Cit I, Sinag A, Yumak T, Ucar S, Misirlioglu Z, Canel M (2010) Comparative pyrolysis of polyolefins (PP and LDPE) and PET. Polym Bull 64(8):817–834
Claereboudt MR (2004) Shore litter along sandy beaches of the Gulf of Oman. Mar Pollut Bull 49(9–10):770–777
Clara M, Windhofer G, Hartl W, Braun K, Simon M, Gans O, Scheffknecht C, Chovanec A (2010) Occurrence of phthalates in surface runoff, untreated and treated wastewater and fate during wastewater treatment. Chemosphere 78(9):1078–1084
Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. Mar Poll Bull 62(12):2588–2597
Collignon A, Hecq JH, Glagani F, Voisin P, Collard F, Goffart A (2012) Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar Poll Bull 64:861–864
Colton JB, Knapp FD, Burns BR (1974) Plastic particles in surface waters of northwestern Atlantic. Science 185(4150):491–497
Convey P, Barnes DKA, Morton A (2002) Debris accumulation on oceanic island shores of the Scotia Arc. Antarctica Polar Biol 25(8):612–617
Cooper DA, Corcoran PL (2010) Effects of mechanical and chemical processes on the degradation of plastic beach debris on the island of Kauai, Hawaii. Mar Pollut Bull 60(5):650–654
Cosgrove L, McGeechan PL, Robson GD, Handley PS (2007) Fungal communities associated with degradation of polyester polyurethane in soil. Appl Environ Microbiol 73(18): 5817–5824
Covaci A, Gheorghe A, Voorspoels S, Maervoet J, Steen Redeker E, Blust R, Schepens P (2005) Polybrominated diphenyl ethers, polychlorinated biphenyls and organochlorine pesticides in sediment cores from the Western Scheldt river (Belgium): analytical aspects and depth profiles. Environ Int 31(3):367–375
Cunningham DJ, Wilson SP (2003) Marine debris on beaches of the Greater Sydney Region. J Coastal Res 19(2):421–430
Dargnat C, Teil MJ, Chevreuil M, Blanchard M (2009) Phthalate removal throughout wastewater treatment plant case study of Marne Aval station (France). Sci Total Environ 407(4): 1235–1244
de Wit CA (2002) An overview of brominated flame retardants in the environment. Chemosphere 46(5):583–624
Debenest T, Gagne F, Petit AN, Andre C, Kohli M, Blaise C (2010) Ecotoxicity of a brominated flame retardant (tetrabromobisphenol A) and its derivatives to aquatic organisms. Comp Biochem Physiol C: Toxicol Pharmacol 152(4):407–412
DEFRA (2012) Litter and refuse. Guidance on Part 4 of the Environmental Protection Act 1990 as amended by the Clean Neighbourhoods and Environment Act 2005. http://archive.defra.gov.uk/environment/quality/local/legislation/cnea/documents/litter-refuse.pdf
Derraik JGB (2002) The pollution of the marine environment by plastic debris: a review. Mar Pollut Bull 44(9):842–852
do Sul JAI, Costa MF (2007) Marine debris review for Latin America and the Wider Caribbean Region: from the 1970s until now, and where do we go from here? Mar Pollut Bull 54(8):1087–1104
Dorostghoal M, Zardkaf A, Dezfoolian A (2010) Chronic effects of di(2-ethylhexyl)phthalate on stereological parameters of testis in adult wistar rats. Iran J Basic Med Sci 13(4):170–176
Dreyer K, Fourie LJ, Kok DJ (1999) Assessment of cattle owners' perceptions and expectations, and identification of constraints on production in a peri-urban, resource-poor environment. Onderstepoort J Vet Res 66(2):95–102
Drimal P, Hoffmann J, Druzbik M (2007) Evaluating the aerobic biodegradability of plastics in soil environments through GC and IR analysis of gaseous phase. Polym Test 26(6):729–741
Duft M, Schulte-Oehlmann U, Weltje L, Tillmann M, Oehlmann J (2003) Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail Potamopyrgus antipodarum. Aquat Toxicol 64(4):437–449

Duong CN, Ra JS, Cho J, Kim SD, Choi HK, Park JH, Kim KW, Inam E, Kim SD (2010) Estrogenic chemicals and estrogenicity in river waters of South Korea and seven Asian countries. Chemosphere 78(3):286–293

ECHA (2012) European Chemicals Agency. Guidance for monomer and polymers. Version 2.0. Guidance for the implementation of REACH. http://echa.europa.eu/documents/10162/13632/polymers_en.pdf

Endo S, Takizawa R, Okuda K, Takada H, Chiba K, Kanehiro H, Ogi H, Yamashita R, Date T (2005) Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: variability among individual particles and regional differences. Mar Pollut Bull 50(10):1103–1114

EPA (2012) Wastewater Technology Fact Sheet: screening and grit removal. http://water.epa.gov/aboutow/owm/upload/2004_07_07_septics_final_sgrit_removal.pdf

Eriksson C, Burton H (2003) Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. Ambio 32(6):380–384

Eriksson J, Rahm S, Green N, Bergman A, Jakobsson E (2004) Photochemical transformations of tetrabromobisphenol A and related phenols in water. Chemosphere 54(1):117–126

Erren T, Zeuss D, Steffany F, Meyer-Rochow B (2009) Increase of wildlife cancer: an echo of plastic pollution? Nat Rev Cancer 9(11):842

Fatoki OS, Bornman M, Ravandhalala L, Chimuka L, Genthe B, Adeniyi A (2010) Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects. Water Sa 36(1):117–125

Fendall LS, Sewell MA (2009) Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar Pollut Bull 58(8):1225–1228

Fernandez MP, Noguerol TN, Lacorte S, Buchanan I, Pina B (2009) Toxicity identification fractionation of environmental estrogens in waste water and sludge using gas and liquid chromatography coupled to mass spectrometry and recombinant yeast assay. Anal Bioanal Chem 393(3):957–968

Fontana AR, Silva MF, Martinez LD, Wuilloud RG, Altamirano JC (2009) Determination of polybrominated diphenyl ethers in water and soil samples by cloud point extraction-ultrasound-assisted back-extraction-gas chromatography—mass spectrometry. J Chromatogr A 1216(20):4339–4346

Forbes VE, Warbritton R, Aufderheide J, Van Der Hoeven N, Caspers N (2008) Effects of bisphenol a on fecundity, egg hatchability, and juvenile growth of Marisa cornuarietis. Environ Toxicol Chem 27(11):2332–2340

Foster-Smith J, Cefas ACB, Evans SM, Prince J (2007) Human impacts on Cable Beach, Broome (Western Australia). Coast Manage 35(2–3):181–194

Fries E, Zarfl C (2012) Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). Environ Sci Pollut Res 19(4):1296–1304

Fromme H, Kuchler T, Otto T, Pilz K, Muller J, Wenzel A (2002) Occurrence of phthalates and bisphenol A and F in the environment. Water Res 36(6):1429–1438

Furhacker M, Scharf S, Weber H (2000) Bisphenol A: emissions from point sources. Chemosphere 41(5):751–756

Galgani F, Souplet A, Cadiou Y (1996) Accumulation of debris on the deep sea floor off the French Mediterranean coast. Mar Ecol Prog Ser 142(1–3):225–234

Galgani F, Leaute JP, Moguedet P, Souplet A, Verin Y, Carpentier A, Goraguer H, Latrouite D, Andral B, Cadiou Y, Mahe JC, Poulard JC, Nerisson P (2000) Litter on the sea floor along European coasts. Mar Pollut Bull 40(6):516–527

Ghanbari H, Viatge H, Kidane AG, Burriesci G, Tavakoli M, Seifalian AM (2009) Polymeric heart values: new materials, emerging hopes. Trends Biotechnol 27:359–367

Gopferich A (1996) Mechanisms of polymer degradation and erosion. Biomaterials 17(2):103–114

Gregory MR (1977) Plastic pellets on New Zealand beaches. Mar Pollut Bull 8(4):82–84

Gregory MR (2009) Environmental implications of plastic debris in marine settings-entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos Trans R Soc B: Biol Sci 364(1526):2013–2025

Hammer J, Kraak MHS, Parsons JR (2012) Plastics in the environment: the dark side of a modern gift. Rev Environ Contam Toxicol 220:1–44

Hanni KD, Pyle P (2000) Entanglement of pinnipeds in synthetic materials at south-east Farallon Island, California, 1976–1998. Mar Pollut Bull 40(12):1076–1081

Harrad S, Abdallah MA-E, Rose NL, Turner SD, Davidson TA (2009) Current-use brominated flame betardants in water, sediment, and fish from English lakes. Environ Sci Technol 43(24): 9077–9083

Harries JE, Runnalls T, Hill E, Harris CA, Maddix S, Sumpter JP, Tyler R (2000) Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (pimephales promelas). Environ Sci Technol 34:3003–3011

Hess NA, Ribis CA, Vining I (1999) Benthic marine debris, with an emphasis on fishery-related items, surrounding Kodiak Island, Alaska, 1994–1996. Mar Poll Bull 38(10):885–890

Hill M, Stabile C, Steffen LK, Hill A (2002) Toxic effects of endocrine disrupters on freshwater sponges: common developmental abnormalities. Environ Pollut 117(2):295–300

Hinojosa IA, Thiel M (2009) Floating marine debris in fjords, gulfs and channels of southern Chile. Mar Pollut Bull 58(3):341–350

Hites RA (2004) Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. Environ Sci Technol 38(4):945–956

Hu J, Liang Y, Chen MJ, Wang XR (2009) Assessing the toxicity of TBBPA and HBCD by zebrafish embryo toxicity assay and biomarker analysis. Environ Toxicol 24(4):334–342

Hu XY, Wen B, Shan XQ (2003) Survey of phthalate pollution in arable soils in China. J Environ Monit 5(4):649–653

Huang PC, Tien CJ, Sun YM, Hsieh CY, Lee CC (2008) Occurrence of phthalates in sediment and biota: relationship to aquatic factors and the biota-sediment accumulation factor. Chemosphere 73(4):539–544

Ikonomou MG, Rayne S, Fischer M, Fernandez MP, Cretney W (2002) Occurrence and congener profiles of polybrominated diphenyl ethers (PBDEs) in environmental samples from coastal British Columbia, Canada. Chemosphere 46(5):649–663

Ishigaki T, Sugano W, Nakanishi A, Tateda M, Ike M, Fujita M (2004) The degradability of biodegradable plastics in aerobic and anaerobic waste landfill model reactors. Chemosphere 54(3):225–233

Iskander MG, Hassan M (2001) Accelerated degradation of recycled plastic piling in aggressive soils. J Compos Constr 5(3):179–187

Jensen J, van Langevelde J, Pritzl G, Krogh PH (2001) Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environ Toxicol Chem 20(5): 1085–1091

Jonsson S, Baun A (2003) Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environ Toxicol Chem 22(12):3037–3043

Julinova M, Slavik R (2012) Removal of phthalates from aqueous solution by different adsorbents: a short review. J Environ Manage 94(1):13–24

Kaczmarek H, Bajer K, Galka P, Kotnowska B (2007) Photodegradation studies of novel biodegradable blends based on poly(ethylene oxide) and pectin. Polym Degrad Stabil 92(11):2058–2069

Kapanen A, Schettini E, Vox G, Itavaara M (2008) Performance and environmental impact of biodegradable films in agriculture: a field study on protected cultivation. J Polym Environ 16(2):109–122

Kasahara E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, Tokuda M, Nakano Y, Inoue M (2002) Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl)phthalate. Biochem J 365:849–856

Katsanevakis S, Katsarou A (2004) Influences on the distribution of marine debris on the seafloor of shallow coastal areas in Greece (eastern Mediterranean). Water Air Soil Pollut 159(1–4): 325–337

Kelly MA, Reid AM, Quinn-Hosey KM, Fogarty AM, Roche JJ, Brougham CA (2010) Investigation of the estrogenic risk to feral male brown trout (Salmo trutta) in the Shannon International River Basin District of Ireland. Ecotoxicol Environ Saf 73(7):1658–1665

Kijchavengkul T, Auras R, Rubino M, Alvarado E, Montero JRC, Rosales JM (2010) Atmospheric and soil degradation of aliphatic-aromatic polyester films. Polym Degrad Stabil 95(2):99–107

Kim Y-J, Osako M, Sakai S-I (2006) Leachingcharacteristics of polybrominated diphenyl ethers (PBDEs) from flame-retardant plastics. Chemosphere 65:506–513

Klemchuk PP (1990) Degradable plastics—a critical-review. Polym Degrad Stabil 27(2):183–202

Ko EJ, Kim KW, Kang SY, Kim SD, Bang SB, Hamm SY, Kim DW (2007) Monitoring of environmental phenolic endocrine disrupting compounds in treatment effluents and river waters, Korea. Talanta 73:674–683

Kohler M, Zennegg M, Bogdal C, Gerecke AC, Schmid P, Heeb NV, Sturm M, Vonmont H, Kohler HPE, Giger W (2008) Temporal trends, congener patterns, and sources of Octa-, Nona-, and decabromodiphenyl ethers (PBDE) and hexabromocyclododecanes (HBCD) in Swiss lake sediments. Environ Sci Technol 42(17):6378–6384

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36(6):1202–1211

Koutny M, Amato P, Muchova M, Ruzicka J, Delort AM (2009) Soil bacterial strains able to grow on the surface of oxidized polyethylene film containing prooxidant additives. Int Biodeter Biodegrad 63(3):354–357

Koutsodendris A, Papatheodorou G, Kougiourouki O, Georgiadis M (2008) Benthic marine litter in four Gulfs in Greece, Eastern Mediterranean; abundance, composition and source identification. Estuar Coast Shelf Sci 77(3):501–512

Krzan A, Hemjinda S, Miertus S, Corti A, Chiellini E (2006) Standardization and certification in the area of environmentally degradable plastics. Polym Degrad Stabil 91(12):2819–2833

Kumar AP, Pandey JK, Kumar B, Singh RP (2006) Photo-bio-degradability of agro waste and ethylene-propylene copolymers composites under abiotic and biotic environments. J Polym Environ 14(2):203–212

Kuriyama Y, Tokai T, Tabata K, Kanehiro H (2003) Distribution and composition of litter on seabed of Tokyo Bay and its age analysis. Nippon Suisan Gakkaishi 69(5):770–781

Kusui T, Noda M (2003) International survey on the distribution of stranded and buried litter on beaches along the Sea of Japan. Mar Pollut Bull 47(1–6):175–179

Laist DW (1987) Impacts of marine debris: entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records. In: Coe JM, Rogers DB (eds) Marine debris—sources, impacts and solutions. Springer, New York, pp 99–139

Larsson P, Thuren A, Gahnstrom G (1986) Phthalate-esters inhibit microbial activity in aquatic sediments. Environ Poll Ser Ecol Biol 42(3):223–231

Lattin GL, Moore CJ, Zellers AF, Moore SL, Weisberg SB (2004) A comparison of neustonic plastic and zooplankton at different depths near the southern California shore. Mar Pollut Bull 49(4):291–294

Lee SK, Owens GA, Veeramachaneni DN (2005) Exposure to low concentrations of di-*n*-butyl phthalate during embryogenesis reduces survivability and impairs development of Xenopus laevis frogs. J Toxicol Env Health Part A 68(10):763–772

Liao CS, Chen LC, Chen BS, Lin SH (2009) Bioremediation of endocrine disruptor di-*n*-butyl phthalate ester by Deinococcus radiodurans and Pseudomonas stutzeri. Chemosphere 78(3):342–346

Liebezeit G (2008) Marine litter on the Kachelotplate, Lower Saxonian Wadden Sea. Senckenbergiana Maritima 38(2):147–151

Liebezeit G, Dubaish F (2012) Microplastics in beaches of the East Frisian islands Spiekeroog and Kachelotplate. Bull Environ Contam Toxicol 89(1):213–217

Linos A, Berekaa MM, Reichelt R, Keller U, Schmitt J, Flemming HC, Kroppenstedt RM, Steinbuchel A (2000) Biodegradation of cis-1,4-polyisoprene rubbers by distinct actinomycetes: microbial strategies and detailed surface analysis. Appl Environ Microbiol 66(4):1639–1645

Liu H, Liu X, Wang X, Wang X, Yu H (2007) Toxicity of BPA land TBBPA to Daphnia magna and zebrafish Brachydanio rerio. Huanjing Kexue 28(8):1784–1787

Liu Y, Guan Y-T, Tam NFY, Mizuno T, Tsuno H, Zhu W-P (2011) Influence of rainfall and basic water quality parameters on the distribution of endocrine-disrupting chemicals in coastal area. Water Air Soil Pollut 209(1–4):333–343

Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F, Nava-Saucedo JE (2008) Polymer biodegradation: mechanisms and estimation techniques. Chemosphere 73(4):429–442

Luross JM, Alaee M, Sergeant DB, Cannon CM, Whittle DM, Solomon KR, Muir DCG (2002) Spatial distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Laurentian Great Lakes. Chemosphere 46(5):665–672

Magagula B, Nhlapo N, Focke WW (2009) Mn2Al-LDH- and Co2Al-LDH-stearate as photodegradants for LDPE film. Polym Degrad Stabil 94(6):947–954

Majer AP, Vedolin MC, Turra A (2012) Plastic pellets as oviposition site and means of dispersal for the ocean-skater insect Halobates. Mar Pollut Bull 64(6):1143–1147

Maragou NC, Makri A, Lampi EN, Thomaidis NS, Koupparis MA (2008) Migration of bisphenol A from polycarbonate baby bottles under real use conditions. Food Addit Contam 25(3):373–383

Marcial HS, Hagiwara A, Snell TW (2003) Estrogenic compounds affect development of harpacticoid copepod Tigriopus japonicus. Environ Toxicol Chem 22(12):3025–3030

Martin OV, Voulvoulis N (2009) Sustainable risk management of emerging contaminants in municipal wastewaters. Philos Trans Royal Soc A: Math Phys Eng Sci 367(1904):3895–3922

Martinez-Ribes L, Basterretxea G, Palmer M, Tintore J (2007) Origin and abundance of beach debris in the Balearic Islands. Scientia Marina 71(2):305–314

Mato Y, Isobe T, Takada H, Kanehiro H, Ohtake C, Kaminuma T (2001) Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ Sci Technol 35(2):318–324

McDowell DC, Metcalfe CD (2001) Phthalate esters in sediments near a sewage treatment plant outflow in Hamilton Harbour, Ontario: SFE extraction and environmental distribution. J Gt Lakes Res 27(1):3–9

Mersiowsky I, Weller M, Ejlertsson J (2001) Fate of plasticised PVC products under landfill conditions: a laboratory-scale landfill simulation reactor study. Water Res 35(13):3063–3070

Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE, Potter T (2001) Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environ Toxicol Chem 20(2):297–308

Mihaich EM, Friederich U, Caspers N, Hall AT, Klecka GM, Dimond SS, Staples CA, Ortego LS, Hentges SG (2009) Acute and chronic toxicity testing of bisphenol A with aquatic invertebrates and plants. Ecotoxicol Environ Safe 72(5):1392–1399

Mohapatra DP, Brar SK, Tyagi RD, Surampalli RY (2010) Physico-chemical pre-treatment and biotransformation of wastewater and wastewater sludge—fate of bisphenol A. Chemosphere 78(8):923–941

Mohee R, Unmar G (2007) Determining biodegradability of plastic materials under controlled and natural composting environments. Waste Manage 27(11):1486–1493

Moore CJ (2008) Synthetic polymers in the marine environment: a rapidly increasing long-term threat. Environ Res 108:131–139

Moore CJ, Moore SL, Leecaster MK, Weisberg SB (2001) A comparison of plastic and plankton in the North Pacific central gyre. Mar Pollut Bull 42(12):1297–1300

Morris RJ (1980) Plastic debris in the surface waters of the south-Atlantic. Mar Pollut Bull 11(6):164–166

Mu XY, Rider CV, Hwang GS, Hoy H, Le Blanc GA (2005) Covert signal disruption: anti-ecdysteroidal activity of bisphenol a involves cross talk between signaling pathways. Environ Toxicol Chem 24(1):146–152

Mulder KF (1998) Sustainable consumption and production of plastics? Technol Forecast Soc Chang 58(1–2):105–124

Mutha NH, Patel M, Premnath V (2006) Plastics materials flow analysis for India. Resour Conserv Recy 47:222–244

Nagai N, Matsunobe T, Imai T (2005a) Infrared analysis of depth profiles in UV-photochemical degradation of polymers. Polym Degrad Stabil 88(2):224–233

Nagai Y, Ogawa T, Nishimoto Y, Ohishi F (1999) Analysis of weathering of a thermoplastic polyester elastomer II. Factors affecting weathering of a polyether-polyester elastomer. Polym Degrad Stabil 65(2):217–224

Nagai Y, Nakamura D, Miyake T, Ueno H, Matsumoto N, Kaji A, Ohishi F (2005b) Photodegradation mechanisms in poly(2,6-butylenenaphthalate-co-tetramethyleneglycol) (PBN-PTMG). I: influence of the PTMG content. Polym Degrad Stabil 88(2):251–255

Nagelkerken I, Wiltjer G, Debrot AO, Pors L (2001) Baseline study of submerged marine debris at beaches in Curacao, West Indies. Mar Pollut Bull 42(9):786–789

Njeru J (2006) The urban political ecology of plastic bag waste problem in Nairobi, Kenya. Geoforum 37(6):1046–1058

Norman A, Borjeson H, David F, Tienpont B, Norrgren L (2007) Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Arch Environ Contam Toxicol 52(2):235–242

NRS (2011) Natural rubber statistics. http://www.lgm.gov.my/nrstat/nrstats.pdf

Oehlmann J, Schulte-Oehlmann U, Kloas W, Jagnytsch O, Lutz I, Kusk KO, Wollenberger L, Santos EM, Paull GC, Van Look KJW, Tyler CR (2009) A critical analysis of the biological impacts of plasticizers on wildlife. Philos Trans R Soc B: Biol Sci 364(1526):2047–2062

Ohtani H, Miura I, Ichikawa Y (2000) Effects of dibutyl phthalate as an environmental endocrine disrupter on gonadal sex differentiation of genetic males of the frog Rana rugosa. Environ Health Perspect 108(12):1189–1193

Oigman-Pszczol SS, Creed JC (2007) Quantification and classification of marine litter on beaches along Armacao dos Buzios, Rio de Janeiro, Brazil. J Coastal Res 23(2):421–428

Omidi A, Naeemipoor H, Hosseini M (2012) Plastic debris in the digestive tract of sheep and goats: an increasing environmental contamination in Birjand. Iran Bull Environ Contam Toxicol 88(5):691–694

Orbea A, Ortiz-Zarragoitia M, Cajaraville MP (2002) Interactive effects of benzo(a) pyrene and cadmium and effects of di(2-ethylhexyl) phthalate on antioxidant and peroxisomal enzymes and peroxisomal volume density in the digestive gland of mussel Mytilus galloprovincialis Lmk. Biomarkers 7(1):33–48

Orhan Y, Buyukgungor H (2000) Enhancement of biodegradability of disposable polyethylene in controlled biological soil. Int Biodeter Biodegrad 45(1–2):49–55

Orhan Y, Hrenovic J, Buyukgungor H (2004) Biodegradation of plastic compost bags under controlled soil conditions. Acta Chim Slov 51(3):579–588

Pachekoski WM, Agnelli JAM, Belem LP (2009) Thermal, mechanical and morphological properties of poly (hydroxybutyrate) and polypropylene blends after processing. Mater Res-Ibero-Am J Mater 12(2):159–164

Page B, McKenzie J, McIntosh R, Baylis A, Morrissey A, Calvert N, Haase T, Berris M, Dowie D, Shaughnessy PD, Goldsworth SD (2004) Entanglement of Australian sea lions and New Zealand fur seals in lost fishing gear and other marine debris before and after government and industry attempts to reduce the problem. Mar Pollut Bull 49(1–2):33–42

Palmisano AC, Pettigrew CA (1992) Biodegradability of plastics. Bioscience 42(9):680–685

Panyam J, Dali MA, Sahoo SK, Ma WX, Chakravarthi SS, Amidon GL, Levy RJ, Labhasetwar V (2003) Polymer degradation and in vitro release of a model protein from poly(D, L-lactide-co-glycolide) nano- and microparticles. J Contr Release 92(1–2):173–187

Park EJ, Jo HJ, Kim HJ, Cho K, Jung J (2008) Effects of gamma-ray treatment on wastewater toxicity from a rubber products factory. J Radioanal Nucl Chem 277(3):619–624

Park SY, Choi J (2007) Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environ Int 33(6):817–822

Pascoe D, Carroll K, Karntanut W, Watts MM (2002) Toxicity of 17 alpha-ethinylestradiol and bisphenol A to the freshwater cnidarian Hydra vulgaris. Arch Environ Contam Toxicol 43(1): 56–63

Patyna PJ, Brown RP, Davi RA, Letinski DJ, Thomas PE, Cooper KR, Parkerton TF (2006) Hazard evaluation of diisononyl phthalate and diisodecyl phthalate in a Japanese medaka multigenerational assay. Ecotoxicol Environ Saf 65(1):36–47

Peijnenburg W, Struijs J (2006) Occurrence of phthalate esters in the environment of the Netherlands. Ecotoxicol Environ Saf 63(2):204–215

Petry MV, Kruger L, Fonseca VSD, Brummelhaus J, Piuco RD (2009) Diet and ingestion of synthetics by Cory's Shearwater Calonectris diomedea off southern Brazil. J Ornithol 150(3):601–606

Philippart JL, Sinturel C, Gardette JL (1997) Influence of light intensity on the photooxidation of polypropylene. Polymer Degrad Stabil 58(3):261–268

Pinto F, Costa P, Gulyurtlu I, Cabrita I (1999) Pyrolysis of plastic wastes. 1. Effect of plastic waste composition on product yield. J Anal Appl Pyrol 51(1–2):39–55

Plastics-Europe (2010) Plastics the facts 2010: an analysis of European plastics, production, demand and recovery for 2009. http://www.plasticseurope.org/documents/-document/20101006091310final_plasticsthefacts_28092010_lr.pdf

Pojana G, Gomiero A, Jonkers N, Marcomini A (2007) Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environ Int 33(7):929–936

Rayne S (2008) The need for reducing plastic shopping bay use and disposal in Africa. Afr J Environ Sci Technol 3:1–3

Reddy CSK, Ghai R, Rashmi T, Kalia VC (2003) Polyhydroxyalkanoates: an overview. Bioresour Technol 87(2):137–146

Reddy MM, Deighton M, Bhattacharya S, Parthasarathy R (2009) Biodegradation of montmorillonite filled oxo-biodegradable polyethylene. J Appl Polym Sci 113(5):2826–2832

Remberger M, Sternbeck J, Palm A, Kaj L, Stromberg K, Brorstrom-Lunden E (2004) The environmental occurrence of hexabromocyclododecane in Sweden. Chemosphere 54(1):9–21

Rhee GS, Kim SH, Kim SS, Sohn KH, Kwack SJ, Kim BH, Park KL (2002) Comparison of embryotoxicity of ESBO and phthalate esters using an in vitro battery system. Toxicol In Vitro 16(4):443–448

Ribeiro C, Tiritan ME, Rocha E, Rocha MJ (2009) Seasonal and spatial distribution of several endocrine-disrupting compounds in the Douro River Estuary, Portugal. Arch Environ Contam Toxicol 56(1):1–11

Ribic CA (1998) Use of indicator items to monitor marine debris on a New Jersey beach from 1991 to 1996. Mar Pollut Bull 36(11):887–891

Richardson AJ, Walne AW, John AWG, Jonas TD, Lindley JA, Sims DW, Stevens D, Witt M (2006) Using continuous plankton recorder data. Prog Oceanogr 68(1):27–74

Rifaat HM, Yosery MA (2004) Identification and characterisation of rubber degrading actinobacteria. Appl Ecol Environ Res 2(1):63–70

Robards MD, Piatt JF, Wohl KD (1995) Increasing frequency of plastic particles ingested by seabirds in the subarctic North Pacific. Mar Pollut Bull 30(2):151–157

Robinson BJ, Hellou J (2009) Biodegradation of endocrine disrupting compounds in harbour seawater and sediments. Sci Total Environ 407(21):5713–5718

Roh JY, Jung IH, Lee JY, Choi JH (2007) Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode Caenorhabditis elegcans. Toxicology 237(1–3):126–133

Rossi M, Lent T (2006) Creating safe and healthy spaces: selecting materials that support healing. http://www.healthybuilding.net/healthcare/CHD_RossiLent.pdf. Accessed 20 Aug 2012.

Roy PK, Titus S, Surekha P, Tulsi E, Deshmukh C, Rajagopal C (2008) Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. Polym Degrad Stabil 93(10):1917–1922

Rutkowska M, Heimowska A, Krasowska K, Janik H (2002a) Biodegradability of polyethylene starch blends in sea water. Polish J Environ Studies 11(3):267–271

Rutkowska M, Krasowska K, Heimowska A, Steinka I, Janik H (2002b) Degradation of polyurethanes in sea water. Polym Degrad Stabil 76(2):233–239

Ryan PG, Connell AD, Gardner BD (1988) Plastic ingestion and PCBs in seabirds—is there a relationship. Mar Pollut Bull 19(4):174–176

Ryan PG, Moore CJ, van Franeker JA, Moloney CL (2009) Monitoring the abundance of plastic debris in the marine environment. Philos Trans R Soc B: Biol Sci 364(1526):1999–2012
Saad GR, Khalil TM, Sabaa MW (2010) Photo- and bio-degradation of poly(ester-urethane)s films based on poly[(R)-3-Hydroxybutyrate] and poly(epsilon-Caprolactone) blocks. J Polym Res 17(1):33–42
Saal FS, Parmigiani S, Palanza PL, Everett LG, Ragaini R (2008) The plastic world: sources, amounts, ecological impacts and effects on development, reproduction, brain and behavior in aquatic and terrestrial animals and humans introduction. Environ Res 108(2):127–130
Sadi RK, Fechine GJM, Demarquette NR (2010) Photodegradation of poly(3-hydroxybutyrate). Polym Degrad Stabil 95(12):2318–2327
Sajiki J, Yonekubo J (2003) Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. Chemosphere 51(1):55–62
Sandy EH, Yao J, Zheng SX, Gogra AB, Chen HL, Zheng H, Yormah TBR, Zhang X, Zaray G, Ceccanti B, Choi MMF (2010) A comparative cytotoxicity study of isomeric alkylphthalates to metabolically variant bacteria. J Hazard Mater 182(1–3):631–639
Santos IR, Friedrich A, do Sul JAI (2009) Marine debris contamination along undeveloped tropical beaches from northeast Brazil. Environ Monit Assess 148(1–4):455–462
Saron C, Felisberti MI (2006) Influence of colorants on the degradation and stabilization of polymers. Quim Nova 29(1):124–128
Seco Pon JP, Becherucci ME (2012) Spatial and temporal variations of urban litter in Mar del Plata, the major coastal city of Argentina. Waste Manage (New York, NY) 32(2):343–348
Sellstrom U, Jansson B (1995) Analysis of tetrabromobisphenol a in a product and environmental-samples. Chemosphere 31(4):3085–3092
Shah AA, Hasan F, Hameed A, Ahmed S (2008) Biological degradation of plastics: a comprehensive review. Biotechnol Adv 26:246–265
Shang XY, Fu X, Chen XD, Yang LS (2009) Biodegradation of blends of polyethylene-octene elastomer with starches by fungi. J Appl Polym Sci 114(6):3574–3584
Shaw SD, Berger ML, Weijs L, Covaci A (2012) Tissue-specific accumulation of polybrominated diphenyl ethers (PBDEs) including deca-BDE and hexabromocyclododecanes (HBCDs) in harbor seals from the northwest Atlantic. Environ Int 44:1–6
Silva-Cavalcanti JS, de Araujo MCB, da Costa MF (2009) Plastic litter on an urban beach—a case study in Brazil. Waste Manage Res 27(1):93–97
Silvestre C, Duraccio D, Cimmino S (2011) Food packaging based on polymer nanomaterials. Prog Poly Sci 36:1766–1782
Singh B, Sharma N (2008) Mechanistic implications of plastic degradation. Polym Degrad Stabil 93(3):561–584
Soderstrom G, Sellstrom U, De Wit CA, Tysklind M (2004) Photolytic debromination of decabromodiphenyl ether (BDE 209). Environ Sci Technol 38(1):127–132
Staples CA, Dorn PB, Klecka GM, O'Block ST, Harris LR (1998) A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36(10):2149–2173
Stasinakis AS, Gatidou G, Mamais D, Thomaidis NS, Lekkas TD (2008) Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. Water Res 42(6–7):1796–17804
Stefatos A, Charalampakis M, Papatheodorou G, Ferentinos G (1999) Marine debris on the seafloor of the Mediterranean Sea: examples from two enclosed gulfs in western Greece. Mar Pollut Bull 38(5):389–393
Storrier KL, McGlashan DJ, Bonellie S, Velander K (2007) Beach litter deposition at a selection of beaches in the firth of forth, Scotland. J Coastal Res 23(4):813–822
Sudhakar M, Trishul A, Doble M, Kumar KS, Jahan SS, Inbakandan D, Viduthalai RR, Umadevi VR, Murthy PS, Venkatesan R (2007) Biofouling and biodegradation of polyolefins in ocean waters. Polym Degrad Stabil 92(9):1743–1752
Sudhakar M, Doble M, Murthy SP, Venkatesan R (2008) Marine microbe-mediated biodegradation of low- and high-density polyethylenes. Int Biodeter Biodegr 61:203–213
Suzuki S, Hasegawa A (2006) Determination of hexabromocyclododecane diastereoisomers and tetrabromobisphenol a in water and sediment by liquid chromatography/mass spectrometry. Anal Sci 22(3):469–474

Tatai L, Moore TG, Adhikari R, Malherbe F, Jayasekara R, Griffiths I, Gunatillake PA (2007) Thermoplastic biodegradable polyurethanes: the effect of chain extender structure on properties and in vitro degradation. Biomaterials 28(36):5407–5417

Teil MJ, Blanchard M, Chevreuil M (2006) Atmospheric fate of phthalate esters in an urban area (Paris-France). Sci Total Environ 354(2–3):212–223

Teuten EL, Rowland SJ, Galloway TS, Thompson RC (2007) Potential for plastics to transport hydrophobic contaminants. Environ Sci Technol 41(22):7759–7764

Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Bjorn A, Rowland SJ, Thompson RC, Galloway TS, Yamashita R, Ochi D, Watanuki Y, Moore C, Pham HV, Tana TS, Prudente M, Boonyatumanond R, Zakaria MP, Akkhavong K, Ogata Y, Hirai H, Iwasa S, Mizukawa K, Hagino Y, Imamura A, Saha M, Takada H (2009) Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc B: Biol Sci 364(1526):2027–2045

Tharpes YL (1989) International environmental law: turning the tide on marine pollution. U Miami Inter-Am L Rev 20(3):579–614

Thibaut R, Porte C (2004) Effects of endocrine disrupters on sex steroid synthesis and metabolism pathways in fish. J Steroid Biochem Mol Biol 92(5):485–494

Thiel M, Hinojosa I, Vasquez N, Macaya E (2003) Floating marine debris in coastal waters of the SE-Pacific (Chile). Mar Pollut Bull 46(2):224–231

Thompson RC, Moore CJ, vom Saal FS, Swan SH (2009) Plastic, the environment and human health: current consensus and future trends. Philos Trans Royal Soc 364(21):53–66

Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, McGonigle D, Russell AE (2004) Lost at sea: where is all the plastic? Science 304(5672):838

Tickner JA, Schettler T, Guidotti T, McCally M, Rossi M (2001) Health risks posed by use of di-2-ethylhexyl phthalate (DEHP) in PVC medical devices: a critical review. Am J Ind Med 39(1):100–111

Tsuchii A, Takeda K, Tokiwa Y (1997) Degradation of the rubber in truck tires by a strain of Nocardia. Biodegradation 7(5):405–413

Turner A, Holmes L (2011) Occurrence, distribution and characteristics of beached plastic production pellets on the island of Malta (central Mediterranean). Mar Pollut Bull 62(2):377–381

Uren-Webster TM, Lewis C, Filby AL, Paull GC, Santos EM (2010) Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. Aquat Toxicol 99(3):360–369

USEPA (2012) Basic information about Di(2-ethylhexyl) phthalate in drinking water. http://water.epa.gov/drink/contaminants/basicinformation/di_2-ethylhexyl_phthalate.cfm

Van A, Rochman CM, Flores EM, Hill KL, Vargas E, Vargas SA, Hoh E (2012) Persistent organic pollutants in plastic marine debris found on beaches in San Diego, California. Chemosphere 86(3):258–263

Velander K, Mocogni M (1999) Beach litter sampling strategies: is there a “best” method? Mar Pollut Bull 38(12):1134–1140

Velander KA, Mocogni M (1998) Maritime litter and sewage contamination at Cramond Beach Edinburgh—a comparative study. Mar Pollut Bull 36(5):385–389

Vieyra H, Aguilar-Mendez MA, San Martin-Martinez E (2013) Study of biodegradation evolution during composting of polyethylene-starch blends using scanning electron microscopy. J Appl Poly Sci 127(2):845–853

Vikelsoe J, Thomsen M, Carlsen L (2002) Phthalates and nonylphenols in profiles of differently dressed soils. Sci Total Environ 296(1–3):105–116

Volova TG, Gladyshev MI, Trusova MY, Zhila NO (2007) Degradation of polyhydroxyalkanoates in eutrophic reservoir. Polym Degrad Stabil 92(4):580–586

Volova TG, Boyandin AN, Vasil’ev AD, Karpov VA, Kozhevnikov IV, Prudnikova SV, Rudnev VP, Xuan BB, Dung VVT, Gitel’zon II (2011) Biodegradation of polyhydroxyalkanoates (PHAs) in the South China Sea and identification of PHA-degrading bacteria. Microbiology 80(2):252–260

Voutsa D, Hartmann P, Schaffner C, Giger W (2006) Benzotriazoles, alkylphenols and bisphenol a in municipal wastewaters and in the Glatt River, Switzerland. Environ Sci Pollut Res 13(5):333–341

Waaijers SL, Kong D, Hendriks HS, de Wit CA, Cousins IT, Westerink RHS, Leonards PEG, Kraak MHS, Admiraal W, Voogt WA, Parsons JR (2013) Persistence, bioaccumulation, and toxicity of halogen-free flame retardants. Rev Environ Contam Toxicol 222:1–71

Walker TR, Grant J, Archambault MC (2006) Accumulation of marine debris on an intertidal beach in an urban park (Halifax Harbour, Nova Scotia). Water Qual Res J Can 41(3):256–262

Wang L, Ying GG, Zhao JL, Liu S, Yang B, Zhou LJ, Tao R, Su HC (2011) Assessing estrogenic activity in surface water and sediment of the Liao River system in northeast China using combined chemical and biological tools. Environ Pollut 159(1):148–156

Watters DL, Yoklavich MM, Love MS, Schroeder DM (2010) Assessing marine debris in deep seafloor habitats off California. Mar Pollut Bull 60(1):131–138

Watts MM, Pascoe D, Carroll K (2003) Exposure to 17 alpha-ethinylestradiol and bisphenol A-effects on larval moulting and mouthpart structure of Chironomus riparius. Ecotoxicol Environl Safe 54(2):207–215

Wei CL, Rowe GT, Nunnally CC, Wicksten MK (2012) Anthropogenic "Litter" and macrophyte detritus in the deep Northern Gulf of Mexico. Mar Pollut Bull 64(5):966–973

Wibe AE, Fjeld E, Rosenqvist G, Jenssen BM (2004) Post exposure effects of DDE and BBP on feeding behaviour in threespine stickleback. Ecotox Environ Safe 57(2):213–219

Williams AT, Simmons SL (1996) The degradation of plastic litter in rivers: implication for beaches. J Coast Conserv 2:63–72

Williams AT, Simmons SL (1999) Sources of riverine litter: the River Taff, South Wales, UK. Water Air Soil Pollut 112(1–2):197–216

Williams AT, Tudor DT (2001) Litter burial and exhumation: spatial and temporal distribution on a cobble pocket beach. Mar Pollut Bull 42(11):1031–1039

Wintgens T, Gallenkemper M, Melin T (2003) Occurrence and removal of endocrine disrupters in landfill leachate treatment plants. Water Sci Technol 48(3):127–134

Xu G, Wang Q-H, Gu Q-B, Cao Y-Z, Du X-M, Li F-S (2006) Contamination characteristics and degradation behavior of low-density polyethylene film residues in typical farmland soils of China. J Environ Sci Health Part B-Pestic Contam Agric Wastes 41(2):189–199

Yamamoto T, Yasuhara A, Shiraishi H, Nakasugi O (2001) Bisphenol A in hazardous waste landfill leachates. Chemosphere 42(4):415–418

Ying GG, Kookana RS, Kumar A, Mortimer M (2009) Occurrence and implications of estrogens and xenoestrogens in sewage effluents and receiving waters from South East Queensland. Sci Total Environ 407(18):5147–5155

Yuan SY, Liu C, Liao CS, Chang BV (2002) Occurrence and microbial degradation of phthalate esters in Taiwan river sediments. Chemosphere 49(10):1295–1299

Yuwatini E, Hata N, Taguchi S (2006) Behavior of di(2-ethylhexyl) phthalate discharged from domestic waste water into aquatic environment. J Environ Monit 8(1):191–196

Zbyszewski M, Corcoran PL (2011) Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. Water Air Soil Pollut 220(1–4):365–372

Zeng F, Cui KY, Xie ZY, Liu M, Li YJ, Lin YJ, Zeng ZX, Li FB (2008) Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China. Environ Int 34(3):372–380

Zhang BZ, Guan YF, Li SM, Zeng EY (2009) Occurrence of polybrominated diphenyl ethers in air and precipitation of the Pearl River Delta, South China: annual washout ratios and depositional rates. Environ Sci Technol 43(24):9142–9147

Zhao JL, Ying GG, Wang L, Yang JF, Yang XB, Yang LH, Li X (2009) Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography-negative chemical ionization-mass spectrometry. Sci Total Environ 407(2):962–974

Zhou D, Wang H, Zhang J, Gao X, Zhao W, Zheng Y (2010) Di-*n*-butyl phthalate (DBP) exposure induces oxidative damage in testes of adult rats. Syst Biol Reprod Med 56(6):413–419

Zubris KAV, Richards BK (2005) Synthetic fibers as an indicator of land application of sludge. Environ Pollut 138(2):201–211

Effects of Addictive Substances During Pregnancy and Infancy and Their Analysis in Biological Materials

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Contents

1 Introduction

Addictive substance use is most prevalent in people who are of reproductive-age. In a national prevalence survey performed among pregnant women aged 15–44 years, 10.8% reported using alcohol, 17% reported smoking during pregnancy, and 4.4% reported abusing one or more illicit substances (Fig. 1) (SAMHSA 2011). Substance abuse for a pregnant woman is twice as dangerous as for others, because:

- She may harm her own health and impair her ability to support a successful pregnancy
- In utero exposure to substances of abuse either may affect fetal development or may induce physiological changes (e.g., organic and/or neurocognitive) to the child later in life (Narkowicz et al. 2012)

More than 75% of infants exposed to drugs later suffer from major medical problems. Similar problems result from excessive use of tobacco (cigarettes) during

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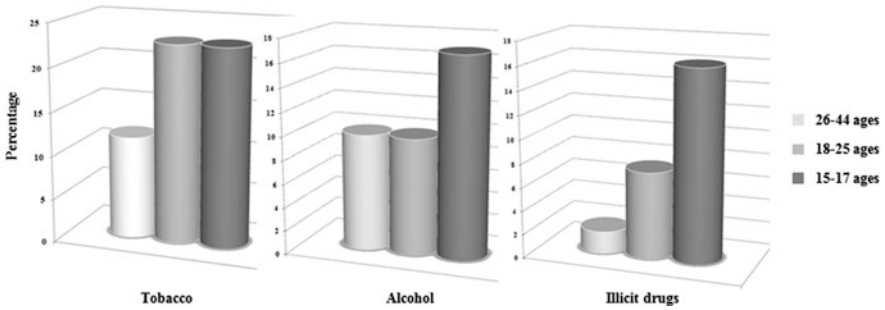

Fig. 1 Addictive substance abuse among pregnant women aged 15–44, by age, 2009–2010 combined (SAMHSA 2011)

pregnancy. The cost of treating drug-affected infants is twice the cost of medical care for non-affected infants (Huestis and Choo 2002). The incidence of obstetric complications are also higher among drug abusing mothers; therefore, assessing in utero drug exposure is quite relevant and important in providing for adequate care of the mother and the offspring of a fair segment of the population (Huestis and Cone 1998). The problem is quite serious, since 3% of women use illicit substances during pregnancy and about 54% of women use legal substances, including alcohol and tobacco, which could be harmful to a fetus (Scharnberg 2003).

When one wishes to assess the incidence of addition, several approaches are possible. For example, one can monitor maternal drug or cigarette consumption by performing periodic urinalysis, weekly sweat analysis or by analyzing patches or hair samples (Huestis and Cone 1998; Huestis and Choo 2002). Another approach is to monitor addictive substance exposure, or exposure to tobacco smoke by testing alternative (also defined as nonconventional) biological specimens from the fetus or the newborn, from the pregnant or nursing mother, or from both fetus and mother. The advantages of such specimen types are that they can be collected in a noninvasive way (except for amniotic fluid), and offer early exposure detection at different gestational periods. Obviously, several factors that concern both specimen and analyte need to be taken into account when selecting biological material for determination of addictive substances by a chosen analytical technique. Toxic substances that are absorbed circulate within the physiological fluids of the body, accumulate in tissues, or are excreted unchanged or as polar metabolites. Biological fluids are typically complex matrices, and require special procedures for sample preparation (Polkowska et al. 2004).

In this article, we present information on the effects of prenatal exposure to addictive substances, and on the prospects and difficulty of using different biological specimens for monitoring and assessing in utero exposure to illegal drugs, tobacco, and alcohol.

In Table 1 we describe the abbreviations used in this paper.

Table 1 Description of abbreviations used in this paper

Abbreviation	Description
6-AM	6-Acetylmorphine
AIDS	Acquired immunodeficiency syndrome
AMP	Amphetamine
BAR	Barbiturates
BE	Benzoylecgonine
BENZ	Benzodiazepines
BNE	Benzoylnorecgonine
C6G	Codeine-6-glucoranide
CNS	Central nervous system
COC	Cocaine
COCE	Cocaethylene
COMT	Catechol-O-methyltransferase
DI	Direct immersion
EC	Electrophoresis
ECG	Ecgonine
EDDP	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EEE	Ecgonine ethyl ester
EMDP	2-Ethyl-5-methyl-3,3-diphenylpyrroline
EME	Ecgonine methyl ester
EMIT	Enzyme multiplied immunoassay test
ETARA	Ethyl arachidonate
ETLAU	Ethyl laurate
ETLIN	Ethyl linoleate
ETMIR	Ethyl myristate
ETOLE	Ethyl oleate
ETPAL	Ethyl palmitate
ETSTE	Ethyl stearate
FAEE	Fatty acid ethyl esters
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorders
FPI	Fluorescence polarization immunoassay
GC	Gas chromatography
HER	Heroin
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HS	Headspace
IC	Ion chromatography
LC	Liquid chromatography
LLE	Liquid–liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
MAMP	Methamphetamine
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxy-N-ethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine

(continued)

Table 1 (continued)

Abbreviation	Description
METH	Methadone
MOR	Morphine
MS	Mass spectrometry
NAS	Neonatal abstinence syndrome
NCOC	Norcocaine
NMOR	Normorphine
RIA	Radio immunoassay
SIDS	Sudden infant death syndrome
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
THC	Δ-9-Tetrahydrocannabinol
THC-COOH	11-Nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid

2 Addictive Substance Use by Pregnant Women: A Social Problem

The term "addictive substances" normally refers to compounds that are illicit drugs, nicotine or alcohol. Addiction to these substances produces physical and psychological dependence in ways that cause health deterioration of the addict. In short, the drug user has a compulsive need to use the controlled substances for the purpose of functioning normally (SAMHSA 2011).

The percentage of women who use addictive substances is constantly growing. It has been shown that women aged 15–44 years (women of childbearing age) are the group that is the most frequent abusers of addictive substances. Such abuse is a major problem, not only because of the health impact to the pregnant woman and her offspring, but also because of the social costs it breeds, e.g., childcare neglect when the mother is dependent on addictive drugs (SAMHSA 2011).

As a result of drinking excessive alcohol or abusing illicit drugs, mothers are often deprived of their children, and consequentially the entire family suffers from the effects of the mother's addiction (Kissin et al. 2001). Infants born to mothers dependent on addictive substances often end up with Foster Families. Children of alcohol- or illicit drug-addicted women often do not receive adequate care during their first months of life, and are exposed to maltreatment. In addition, the costs of medical care for children born to addictive drug-abusing mothers, who used drugs during pregnancy, are higher than for children born to women not using drugs. Surveys conducted among the general public, show that up to 92% of respondents said that the mother has an ethical obligation during pregnancy to behave in a way that is not detrimental to the health and life of the fetus (Anderson et al. 1997).

A pregnant woman is a special organ of society, and therefore requires special care during pregnancy and during the course of her treatment for addiction. Indeed, specialized programs have been created to help pregnant women in their fight against addiction (Narkowicz et al. 2012). Such programs include the one being used at the Johns Hopkins Bayview Medical Center in Baltimore; this program

provides psychiatric and medical care to addicted pregnant women and these patients are under continuous ambulatory care.

Kissin et al. (2001) conceived and provided an index called the Addiction Severity Index, which characterizes seven basic factors that are involved with the life and social functioning of addicted individuals. These areas are (Kissin et al. 2001; SAMHSA 2011):

- Medical
- Employment/Support
- Drug
- Alcohol
- Legal
- Family/Social, and
- Psychiatric.

These factors often are extended to include child care, assistance and help with the *upbringing of children,* vocational training, education and counseling (Daley et al. 1998). Pregnancy can be a good moment in a woman's life, in which care for the offspring acts as a stimulus to facilitate the cessation of addiction (Huestis and Choo 2002).

3 The Effects of Prenatal Exposure to Addictive Substances

The effects of addictive substances on pregnant women may be classified into a chronology of categories: maternal effects; effects on the course of pregnancy and delivery, and effects on the fetus, newborn, and developing child (Marx et al. 2002). Substance abuse by pregnant women is one of the major problems of modern civilization and users of these substances can also be categorized according to what they abuse. These are women who:

- Use illicit drugs
- Smoke tobacco or utilize the so-called nicotine replacement therapy, and/or
- Drink alcoholic beverages

Each of these substance categories may adversely affect both the woman and her offspring. Illicit drugs are usually potent central nervous system stimulants, and their long-term consumption may destroy the whole organism. Excessive or chronic intake of illicit drugs and other addictive substances damage cells of the central nervous system. Neurons, in contrast to the other cells of the human body, do not easily regenerate, and therefore, one-time illicit drug consumption may produce lasting toxic damage to the body.

Tobacco smoking and its effects on mother and developing fetus is a common problem among pregnant women. A pregnant woman can either be exposed to tobacco smoke components by actively smoking or by being a passive smoker. An actively smoking woman is mainly exposed to mainstream smoke, which is absorbed via inhalation by mouth. In contrast, a passive smoker is exposed to the components of environmental tobacco smoke (ETS), which is a mixture of side stream and

exhaled mainstream smoke. ETS diffuses into the atmosphere and is diluted in ambient air, and undergoes various physical and chemical transformations that include reactions with the mouth during smoke inhalation. Another exposure source is side stream smoke that enters the environment from chemical substances not generated from burning tobacco (from the lit end of the cigarette between puffs) (Borgerdinga and Klusb 2005).

Excessive consumption of alcoholic beverages by pregnant women is another common societal problem. Ethyl alcohol acts primarily on the central nervous system, and at sufficient intake levels is poisonous. Alcohol poisoning is a life-threatening consequence if large amounts are consumed in a short period of time. This is because alcohol quickly moves from the bloodstream into every part of the body that contains water, including major organs like the brain, lungs, kidneys, and heart, and distributes itself equally both inside and outside of cells. Ethyl alcohol is rapidly metabolized to acetaldehyde, which is the most toxic compound arising from the decomposition of alcohol. Acetaldehyde, at sufficient levels in humans, may cause nausea, vomiting, and headache (Quertemont and Didone 2006). In Table 2, we summarize the health effects that may result from prenatal exposure to several addictive substances.

Table 2 Effects on pregnant mothers, fetuses, and newborns that result from in utero exposure to addictive substances

	Maternal effects	Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
Illicit drugs	Anemia-results from iron and folic acid deficiency	Obstetric complications	Over 75% of infants exposed to drugs have major medical problems versus only 27% of unexposed infants
	Central mechanism that controls appetite and hunger is inhibited	Increased morbidity and mortality	Almost 20% of drug-exposed babies are delivered prematurely
	Narcotics affect the absorption or utilization of ingested nutrients	Abortion and spontaneous abortion	Sudden infant death syndrome (SIDS)
	Illegal drug abuse during pregnancy increases a mother's risk of blood, heart, and skin infections, and other infectious diseases such as sexually transmitted diseases and human immunodeficiency virus (HIV)	Intrauterine death, placental insufficiency, placenta previa and abruptio placenta	Neonatal abstinence syndrome (NAS)
	Increased incidence of psychiatric disorders, for example chronic anxiety and depression, psychosis, personality changes, and delusions of paranoia	Premature rupture of membranes and premature delivery	Respiratory distress syndrome, congenital anomalies, and neurobehavioral changes

(continued)

Table 2 (continued)

	Maternal effects	Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
		Eclampsia	Fetal death
		Gestational diabetes	Premature birth
		Post partum hemorrhage and septic thrombophlebitis and intrauterine growth retardation	Birth defects
			Low birth weight, growth retardation
			Development disorders
			Long-term effects of illicit drugs abused during pregnancy, which are seen in older children, include: poor social adjustment, exhibit cognitive deficits, and learning disabilities
			Children and teenagers who were exposed to illicit drugs prenatally can be more irritable, have difficulty focusing attention, and have more behavioral problems
Environmental tobacco smoke	47–72% of women from various age groups suffer from dysmenorrhea	Smoking aggravates the symptoms of pregnancy	Neurodevelopmental and behavioral disturbances (from changes in the child's brain following fetal hypoxia)
	Both active and passive smoking adversely affects fertility	Smoking increases the risk of the child being lost	Low birth weight (the link between maternal smoking and birth weight is weaker during the early stages of pregnancy, becomes stronger as the pregnancy advances, and is strongest in the third trimester)
		Extrauterine pregnancies are more frequent	The thiocyanate ion, a metabolite of cyanide ions, inhibits iodine capture, which may inhibit the proper development of the brain and nervous system in infants
		Morphological damage to the placenta may become apparent as early as the first trimester of pregnancy, and irreversible changes, such as necrosis, are recognizable after the 9th week	Hyperactivity, reduced concentration

(continued)

Table 2 (continued)

	Maternal effects	Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
		Nicotine binds to acetylcholine, which controls the absorption of nutrients, volume of fluid, blood flow, and the vascularization of the placenta	Weak reaction to auditory stimuli in infants in the first week of life
		Chronic exposure to nicotine may cause the various known effects of tobacco smoking to manifest themselves in the fetus	Lower intelligence at preschool age
			Fetal exposure to nicotine can lead to addictive behaviors, and thus to smoking in adult life
			The action of irritants present in ETS may lead to chronic inflammation of the child's respiratory tract, which in turn may cause asthma
			Urinary tract disorders
			Sudden infant death syndrome (SIDS)
Alcohol	When women consume alcohol during pregnancy, the blood-alcohol content in the fetus reaches the same level as it does in the mother	Fetus is exposed not only to the teratogenic effects of alcohol, but also to the negative effects of the other factors that coexist in its mother's life	Sudden infant death syndrome
	A pregnant woman who consumes alcohol is also likely to follow a poor diet and exercise plan	Pregnant alcohol-exposed women are more likely to experience obstetric complications, and increased morbidity and mortality	FASDs (Fetal alcohol spectrum disorders) include physical, mental, behavioral, and/or learning disabilities with possible lifelong implications

(continued)

Table 2 (continued)

	Maternal effects	Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
	She may also have several other problems, including comorbid medical or psychiatric disorders such as depression, and social problems		Fetal alcohol syndrome (FAS) is the most clinically recognizable form of FASD characterized by: • Prenatal and postnatal growth retardation • Functional or structural central nervous system (CNS) abnormalities such as mental retardation and behavioral problems • A pattern of minor facial and skull anomalies including small eye openings, altered nose and forehead structure, an absent or elongated groove between the upper lip and nose, a thin upper lip, a flattened mid face, and under development of the upper or lower jaw FAS consequences are lifelong, and behavioral and learning difficulties are often greater than the degree of neurocognitive impairment

Sources: Chen et al. 2000; Dejmek et al. 2002; Eskenazi and Castorina 1999; Finnegan 1994; Gilmour et al. 2006; Huestis and Choo 2002; Jauniaux and Burton 2007; Jones 1974; Larkby and Day 1997; Marx et al. 2002; Miller and Hyatt 1992; Niemann and Anderson 2008; Otero et al. 2004; Phibbs et al. 1991; Rogers 2009; Vogel 1997; Zuckerman et al. 1989

4 Biomonitoring to Assess In Utero Exposures to Addictive Substances

Accurate identification of in utero exposure to addictive substances has important implications for the care of mothers and children. Maternal illicit drug use during pregnancy can be monitored by performing analyses on several key media; such media includes urine, sweat, oral fluid, and/or hair. The rate of drug absorption and disposition of addictive substances and metabolites into different matrices is dependent on the route of drug administration and on the physiochemical characteristics of the drug (Huestis and Choo 2002).

Maternal blood was one of the first types of biological material that was analyzed to detect drugs. The analytical targets were either for illicit drug use during pregnancy, or for fetal exposure to drugs. However, the value of testing blood for drugs of abuse is limited because the window of detection is short and the fact that obtaining the sample is invasive (Lozano et al. 2007).

Other potential specimen types that can be monitored to evaluate the degree and type of drug exposure include the following: neonate cord blood, placenta, vernix, amniotic fluid, neonatal hair and urine, and meconium. As with maternal blood, measuring levels of drugs and their metabolites in cord blood reflects only fetal drug exposure during the previous hours or days before collection, and does not reflect chronic exposure during the entire gestation period. However, collecting other specimen types is preferred because obtaining neonatal plasma is invasive and difficult. The placenta and vernix have been rarely sampled for testing, but are currently under evaluation to determine their usefulness (i.e., noninvasiveness of collection and ready availability at delivery; Esteban and Castaño 2009; Lozano et al. 2007).

Because amniotic fluid is already formed in the first weeks of pregnancy, the presence of drugs in this fluid can reflect exposure during the early fetal life. Although it is dangerous for the fetus, amniotic fluid can be sampled at any time during pregnancy, if detecting either parent drugs or their metabolites are essential for protecting the fetus. Meconium testing has also been shown to be an effective and practical means of detecting in utero drug exposure. Analysis of meconium provides more complete information on drug exposure during pregnancy than does analysis of neonatal urine or cord blood. Recently, drug determination in meconium has been successfully applied to assess intrauterine exposure to addictive substances (Lozano et al. 2007).

5 Role of the Placenta in Biomonitoring of Addictive Substances

When performing biomonitoring studies on toxic substances, several liquid and solid tissue types are suitable for sampling. The particular tissues or fluids selected will depend on the goals of the experiment and what is available. Theoretically, the biological material that is selected for further research should fulfill the following criteria:

- Sample collection will not pose a risk to the health or life of the donor
- The amount of analyte to be sampled will be determined by currently available techniques
- The sample size is sufficient for analysis
- Sample collection is convenient
- Samples can be easily stored until analysis

Placental tissue has a key role, when used for biomonitoring and assessing the degree of in utero exposure to addictive substances. The placenta is an organ that connects the developing fetus to the mother, and provides the following main functions (Van der Aa et al. 1998):

- Nutrition
- Excretion
- Immunity
- Endocrine function, and
- Cloaking of the fetal immune system from that of the mother

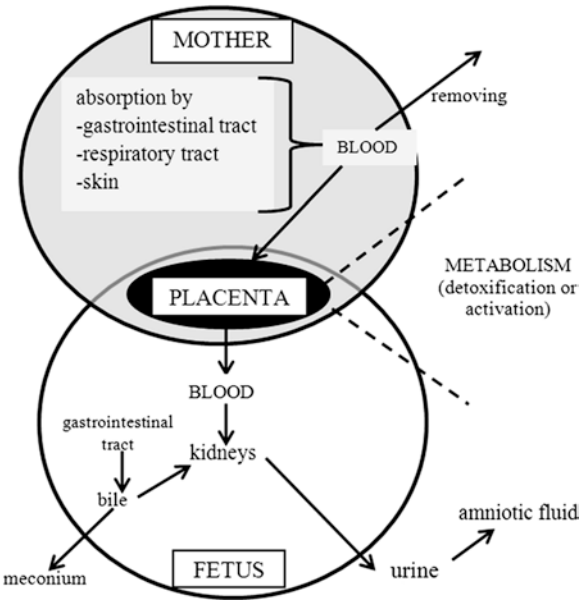

Fig. 2 Schematic of how xenobiotics are transferred from mother to child through the placenta

Unfortunately, the women who consume toxic illicit drugs, alcohol and their metabolites will transport these substances across their placentas, which can cause serious harm to the fetus (Leino et al. 2011). Figure 2 schematically depicts the manner in which addictive substances can be transferred from mother to child through the placenta.

Tobacco smoke contains toxic compounds that are readily soluble in water and may easily transgress the placental barrier. Although most of these substances can be removed by xenobiotic detoxification enzymes, the smoke components in tobacco may directly affect the villous cytotrophoblast.

The placenta, like the liver, may play an important role in metabolizing toxic substances. The cytochrome P450 system (CYP) is a family of enzymes that control the concentrations of many endogenous and exogenous substrates. CYP fulfill their role by actively metabolizing a wide variety of xenobiotics (e.g., drugs and other toxic chemicals). CYP also metabolize endogenous compounds, such as steroid hormones and arachidonic acid. This family of enzymes is composed by multiple subunits that differ in their amino acid sequences. In the human body, 19 enzymes from subfamily of CYP P450s have been discovered, most of which are located in the liver. However, several enzymes such as CYPlAl, CYPZFl, and CYP4Bl are associated largely with extrahepatic organs. The activity of CYP enzymes may lead to the formation of reactive metabolites with toxic consequences (sometimes carcinogenic). To date, the mechanism and function of particular forms of CYP enzymes in human placental tissue are not well known. However, the appearance of the CYPIAI enzyme has been observed in placental samples from women who smoked during pregnancy. In addition, the mRNA and protein of CYP3A7, the prominent

form in fetal liver, have been observed to also exist in the early-term placenta (Hakkola et al. 1996).

The use of addictive substances by woman during pregnancy results in changes to the placenta. Due to prenatal exposure to addictive substances, morphological damage of the placenta may be observed in the first trimester of pregnancy, and irreversible changes (e.g., necrosis) may be seen after the first 9 weeks of pregnancy (Jauniaux and Burton 2007). Hakkola et al. (1996) studied the expression of CYP P450 forms and they described the external appearance of the placenta. It has been proved that the placenta from women who smoked during pregnancy were calcified or thick. Similarly, the placentas from women who abused illicit drugs during pregnancy have been reported to be calcified.

The placenta is a good biological entity to use for biomonitoring and assessing the effects of prenatal exposure to toxic and addictive substances (Al-Saleh et al. 2011); the biomonitoring value of the placenta over blood or urine is that it can be used to assess long-term exposures (Myllynen et al. 2005). The placenta has other advantages for biomonitoring of toxic substances as well (Esteban and Castaño 2009), viz., samples can be taken noninvasively and it is a matrix that reflects the character of constant contact with both the mother and the fetus.

Protecting the fetus and serving as a barrier to entry of xenobiotics are not the only placental functions. Another function of the placenta is to transfer nutrients and oxygen from the mother to the fetus. The placenta also metabolizes chemical compounds, and thereafter assists in removing metabolites and waste products from the fetus.

There are several mechanisms responsible for the transport of addictive substances that are taken in by the mother. Among these mechanisms are passive diffusion, facilitated transport, active transport, pinocytosis, and phagocytosis. Most compounds enter the placenta by passive diffusion, which process is described by Fick's law (Myren et al. 2007). Myren et al. (2007) and Van der Aa et al. (1998) defined the factors that affect the rate of transport as including the:

- Xenobiotic concentration gradient between the circulatory system of the mother and that of the child
- Surface area of the exchange membrane
- Thickness of the endothelio-syncytial membrane
- Blood flow rate through the placenta
- pH of both the maternal and fetal blood
- Physicochemical properties of the individual chemical compounds
- Status of maternal and child health, and
- Rate of the metabolism of the xenobiotics present

Xenobiotics can also enter and pass through the placenta via facilitated transport. The diffusion processes is facilitated by carrier-mediated mechanisms that operate along a concentration gradient, without making use of an outside energy source. Only a few drugs are known to be transported by this mechanism (Myren et al. 2007).

Substances may cross the placenta by active transport as well. Active transport takes place against an electrochemical or concentration gradient, but extracts an energy cost during the process. Active transport is also carrier-mediated. A total of 20 different transport proteins have been detected in the human placenta (Myren et al. 2007).

Finally, transport may occur by pinocytosis or phagocytosis, in which the substance is invaginated into a cell membrane and is transferred to the other side of a

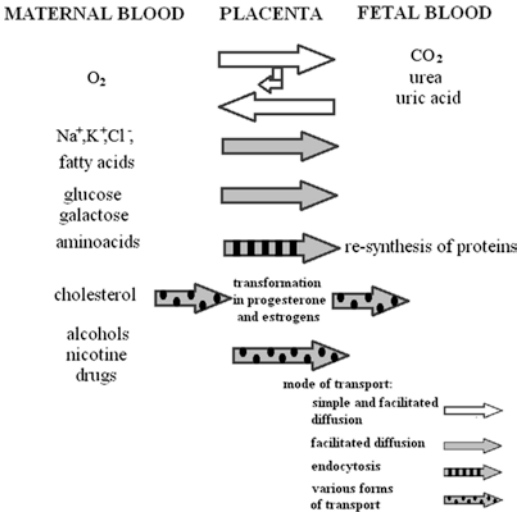

Fig. 3 Schematic of how various substances are transported to and through the placenta

membrane as an enclosed vesicle. This route of placental transfer of xenobiotics is the least important, mainly because this process is very slow (Myren et al. 2007).

In Fig. 3 we diagram the various mechanisms by which xenobiotics are transported between the circulatory systems of the mother and child.

6 Analysis of Addictive Substances in Biological Media

Above, we have described the important uses to which analytical drug residue data on pregnant mothers, their fetuses or the newborn can be put (Fig. 4). The methods used to perform these analyses (e.g., GC-MS and LC-MS) are quite sensitive and can be used to accurately measure addictive substances and their metabolites that are biomarkers of in utero drug exposure. Those abused substances and their metabolites that are most commonly used as biomarkers of in utero drug exposure are summarized in Table 3.

In Fig. 5, we show the stages that are involved when analyzing for xenobiotic residues of interest in biological media. Sample preparation is critical because the addictive substance analytes must be isolated from complex biological matrices such as tissues, or bodily fluids. The direction taken in sample preparation is determined by the physicochemical properties of the analytes and the complexity of the sample tissue or fluid from which they are to be extracted. The methods used to separate and purify the analytes in these specimens commonly utilize methods such as LLE, SPE, and SPME. If the parent compound or metabolites are polar, and must be detected by gas chromatography, appropriate derivatization to form volatile analytes must be added to the sample preparation steps.

Before identifying and quantifying analytes of interest, immunoassay methods may also be used as screening tests. The most popular immunoassay tests are: EMIT, RIA, and FPI. After a rapid screening is completed, specific and sensitive chromatographic methods are used to obtain more detailed information (Gray and

Fig. 4 Important factors gained from analyzing biological specimens from fetuses, newborns, and pregnant women (when these women have abused additive substances)

Table 3 Abused substances and their metabolites as biomarkers for biomonitoring of these substances

Abused substance	Toxic substance	Excretion substances—biomarkers
Tobacco smoke	Nicotine, hydrogen cyanide, formaldehyde, kadm, PHA, benzene	Nicotine, kotonina, *trans*-3′-hydroksykotynina, thiocyanate ion, formaldehyde, kadm, 1-hydroksy-benzo(a)piren, benzene, muconic acid, and S-phenyl mercapturic acid
Amphetamine group substances	Methamphetamine/ amphetamine	Methamphetamine, p-hydroxymethamphetamine, amphetamine, p-hydroxyamphetamine, glucuronide or glycine (hippuric acid) conjugate, benzoic acid, acid-labile precursor of benzyl methyl ketone, norephedrine, p-hydrohynorephedrine

(continued)

Table 3 (continued)

Abused substance	Toxic substance	Excretion substances—biomarkers
	MDMA/MDA/MDE	MDMA, MDA, MDE, 3,4-dihydroxymethamphetamine, 3,4-dihydroxyamphetamine, 4-hydroxy-3-methoxyamphetamine, 4-hydroxy-3-methoxymethamphetamine, 3,4-dihydroxyethylamphetamine
Opioids	Heroin	Heroin, morphine, 6-acetylmorphine
	Morphine	Morphine, morphine-3-glucuronide, morphine-6-glucuronide, morphine-3-sulfate, normorphine
	Methadone	Methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 2-ethyl-5-methyl-3,3-diphenylpyrroline
	Naloxone	Naloxone, naloxone-3-glucuronide
Cocaine	Cocaine	Cocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester, norcocaine
	Lidocaine	Monoethylglycinexylidide, glycine, xylidide
	Benzocaine	Benzocaine, acetylbenzocaine
Cannabis	Marijuana	THC, 11-OH-THC, 8-β-hydroxy THC, THC-COOH
	THC	
	Cannabidiol	Cannabidiol, THC, 11-OH-THC
	Cannabinol	Cannabinol, THC, 11-OH-THC
	THCV	THC, THCV
Alcohol	Ethanol	Acetaldehyde, ethyl glucuronide, ethyl sulfate, fatty acid ethyl esters

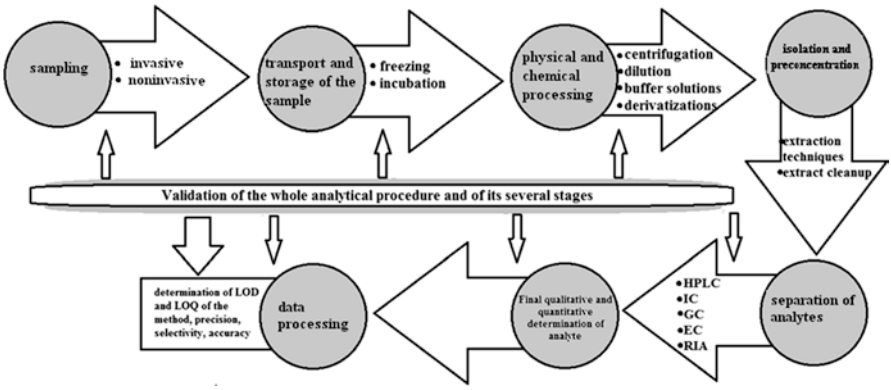

Fig. 5 General scheme for preparing and analyzing biological samples of complex matrices

Huestis 2007; Schütz et al. 2006). Although several chromatographic techniques are used to analyze for additive compounds in biological materials, the majority are based on GC-MS, LC-MS, or LC-MS-MS. In Table 4, we describe the characteristics of biological media commonly sampled from mothers and fetuses.

Table 4 Characteristics of biological materials normally selected for biomonitoring of addictive substances, and techniques used for their analysis

Biological materials	Characteristic	Advantages	Reference	Parent substnaces	Analytes	Detection levels	Concentration range	Sample preparation method(s)	Analysis method(s)	Reference
Mother's hair	Abused substance accumulates in hair during hair growth, and thereby is a unique measure of long-term, cumulative exposure to abused substances	Matrix is stable	Esteban and Castaño 2009	AMP, MAMP, BAR, BENZ, Cannabinoids, COC, Opiates, METH	Fatty acid ethyl esters (FAEE)	ng/mg	0.2–1.0	LLE	ELISA	Kulaga et al. 2010
	Possible pathways for drug incorporation into hair include: diffusion from capillaries to hair follicles; excretion onto the hair surface from sweat and sebum; and external contamination	Low cost						HS-SPME	GC-MS	
		Easy transport and storage		Nicotine	Nicotine, cotinine		Nicotine: <LOD-24.9	SPE	HPLC	Pichini et al. 1997
		Long prenatal exposure to certain drugs of abuse					Cotinine: <LOD-3.3			
		Can reflect exposures for the entire pregnancy		COC, BE	COC and metabolites, BE		40–2,000	SPE	LC-MS	López et al. 2007
		Large sample size								
		More sensitive and stable than other specimen types								
Oral fluid	Is a composite tissue consisting primarily of saliva, mixed with gingival fluid, buccal and mucosal transudates, cellular debris, bacteria, and residues of ingested products	Easy and noninvasive collection	López et al. 2007	Cannabis	THC	µg/L cut off	4.0	SPE	GC-MS ELISA	Gray et al. 2010
	Most prevalent substance form detected is parent compound, rather than its metabolites	Low cost								
	Several factors affect the abused-substance concentration in oral fluid			Nicotine	Cotinine		10.0		ELISA LC-MS	

Mother's blood	Bodily fluid delivers necessary substances such as nutrients and oxygen to cells and transports metabolic waste products away from those same cells	The ideal matrix for most chemicals from the constant contact with the entire organism	López et al. 2007	COC	6-AM, MOR, COD, COC, BE, COCE, AMP, MAMP, MDMA, MDA, THC, THC-COOH	ng/ml	MOR: 0.6–1.0	NA	GC-MS	Falcon et al. 2010
	Blood concentrations may closely reflect the amount of abused drug exposure to the fetus			Opiates			COC and metabolites: 9.2–23.3			
	Excessive blood collection may produce anemia	Restricted use in infants and children		METH			BE: 3.0–15.5			
				Cannabinoids			COCE: 0.5–0.8			
							THC: 0.2–2.7			
							THC-COOH: 0.1–0.8			
Breast milk	A complex physiological liquid that simultaneously provides nutrients and bioactive components	Noninvasive collection	López et al. 2007	METH	METH	µg/L	METH: 32.0–146.0	SPE	EI-GS-MS	Nikolaou et al. 2008
	Content: certain vitamins proteins, bioactive peptides, oligosaccharides, and organic (including fatty) acids	Used for monitoring because it provides information on exposure to both mother and fetus			EDDP		EDDP: 5.5–196.0			
	Extracting abused substance from breast milk is analytically challenging, because of its high protein and fat content, and its variable composition during the postpartum period				EMDP		EMDP: <LOD-3.17			
					NA					
				BE	BE		THC: 86 ng/mL THCOH: 5 ng/mL, METH: 97 ng/mL, EDDP: 8 ng/mL, MOR: 7 ng/mL	SPE	LC-MS-MS	Marchei et al. 2011
				COC	COC					
				MOR, 6-AM, COD, EDDP	MOR, 6-AM, COD, EDDP					
				MAMP, AMP, MDMA, MDA	MAMP, AMP, MDMA, MDA					
				Nicotine	Nicotine	µg/L	Nicotine, <LOD-513.5	LLE	LC-MS-MS	Pellegrini et al. 2007
					Cotinine		Cotinine:, <LOD-344.8			
					trans-3-hydroxycotinine,		*trans-3-hydroxycotinine*: <LOD-17.3			
					Cotinine-N-oxide		Cotinine-N-oxide: <LOD-18.4			

(continued)

Table 4 (continued)

Biological materials	Characteristic	Advantages	Reference	Parent substnaces	Analytes	Detection levels	Concentration range	Sample preparation method(s)	Analysis method(s)	Reference
Mother's urine	Sterile liquid by-product of the body secreted	The parent substance of abuse and its metabolites are present in urine	López et al. 2007	Opiates, COC	EME, BE, COCE, COC, mOHBE, pOHBE, NCOC, EEE	µg/L	COD: 25–136	NA	LC-MS	Shakleya et al. 2010
	Cellular metabolism generates numerous by-products, many rich in nitrogen, that require elimination from the bloodstream	Large sample size			MOR, M3G, M6G, 6-AM, NMOR, C6G, HER, COD		Free MOR: 53–3, 359, NMOR: 50–181, NCOD: 31–63			
		Easy and noninvasive collection		COC	EME, BE	mg/g creatinine	EME: 32.7–48; BE: 312–1,965, MOR: ND–2,909; COD: ND–8,666, METH: 98–2,925; EDDP: ND-13,575	LLE	FPIA	Vinner et al. 2003
				Opiates	6-AM, MOR, COD				EMIT	
				METH	METH, EDDP				GC-MS	
				Cannabinoids	THC-COOH					
Meconium	The first fecal matter passed by the neonate	Large sample size	López et al. 2007	Alcohol	ETMIR, ETPAL, ETOLE, ETSTE, ETLAU, ETLIN, ETARA	mg/g	ETMIR: 0–0.794; ETPAL: 0–1.746; ETOLE: 0–0.16.58; ETSTE: 0–0.934; ETLAU: 0–0.429; ETLIN: 0–5.715; ETARA: 0–1.168	SPE	GC-MS	Ostrea et al. 2006
	Composition: bile salt and acids, epithelial cells, lipids, mucopolysaccharides, and water	Easy and noninvasive collection		COC, heroin	COC, BE	ng/g	40–2,000	SPE	RIA	López et al. 2007
		Reflects exposures from the second and third trimesters of gestation			COD, MOR, 6-AM				GC-MS	
				Nicotine	Nicotine, cotinine, caffeine	ng/g	Cotinine: 20–86 Caffeine: 10–45	LLE, SPE	HPLC	Baranowski et al. 1998
				Nicotine	NIC, COT, *trans*-3-hydroxycotinine, NNIC, NCOT	ng/g	NIC: 101.4; COT : 94.7; *trans*-3-hydroxycotinine: 196.8; NNIC: 10.2; NCOT: 4.4	SPE	LC-MS-MS	Gray et al. 2008

Fetal hair	Hair residues could come from blood or from amniotic fluid	Samples can be stored at room temperature	López et al. 2007	Nicotine		ng/mg		SPE	HPLC	Pichini et al. 1997
	Hair starts growing at ~6 months of gestation and reaches the scalp surface approx. 3 weeks later	Reflects the third trimester of gestational exposure		Cotinine						
		Record of prenatal exposure available for as long as 4–5 months of postnatal life		COC	NA	mg/g	NA	LLA	ELISA	Vinner et al. 2003
				Opiates					GC-MS	
				Cannabinoids						
				METH						
				BENY						
				BAR						
Placenta	Source of fetoplacental circulation, between maternal and fetal blood, acts as a nutrient and waste exchanger	Large sample size	López et al. 2007	Nicotine	NIC, COT	NA	NA	SPE	GC-EI-MS	Joya et al. 2010
		Easy and noninvasive collection		Amphetamines, ecstasy	AMP, MAMP MDMA	NA	NA	SPE	GC-EI-MS	
				COC	COC, BE, COCE					
				Cannabinoids	THC-COOH					
				Opiates	METH, MOR					
				METH, EDDP, MOR, COD, 6-AM, COC, BE		ng/g		NA	LC-MS	De Castro et al. 2009
Amniotic fluid	Reflects a 20-week gestational content of maternal secretions, plus fetal secretions	Reflects long prenatal exposure to certain drugs of abuse	Lozano et al. 2007	COC	BE, EME, EEE, NCOC, COCE, mOHBE	µg/L	NA	NA	IC–MS/MS	Eyler et al. 2005
	Later, fetal urine and lung secretions are added to the amniotic fluid	Can be sampled at any time during pregnancy		BE, EME, EEE, NCOC, COCE, mOHBE		NA		SPE	HPLC-MS	Loughhead et al. 2006
	The amniotic fluid accumulates mainly water-soluble substances	The presence of drugs of abuse in this fluid may reflect exposure during the early fetal life							GS-MS	
	Traces of apolar parent compounds and their metabolites may also be present									

This description includes advantages and drawbacks, and provides literature references that address analytical procedures for determining markers of in utero drugs exposure in these specimens.

New techniques for analysis of addictive substances and their metabolites are routinely being developed; therefore, extraction efficiency, detection limits for addictive substances or their metabolites are improving, as are methods to analyze for substances in alternative matrices.

Although the wide spectrum of analytical methods available allows collecting important information on in utero exposure of addictive substances, there is still a dearth of knowledge about how addictive compounds are distributed in some human materials. Therefore, we propose that future research be performed to gather more robust data on addictive substances (tobacco smoke, illicit drugs and alcohol) in regard to their pharmacokinetics in humans, and how abused substances are mechanistically diffused among human tissues.

7 Summary

The use of addictive substances during pregnancy is a serious social problem, not only because of effects on the health of the woman and child, but also because drug or alcohol dependency detracts from childcare and enhances the prospect of child neglect and family breakdown. Developing additive substance abuse treatment programs for pregnant women is socially important and can help ensure the health of babies, prevent subsequent developmental and behavioral problems (i.e., from intake of alcohol or other additive substances such as methamphetamine, cocaine, or heroine) and can reduce addiction costs to society.

Because women of childbearing age often abuse controlled substances during their pregnancy, it is important to undertake biomonitoring of these substances in biological samples taken from the pregnant or nursing mother (e.g., blood, urine, hair, breast milk, sweat, oral fluids, etc.), from the fetus and newborn (e.g., meconium, cord blood, neonatal hair and urine) and from both the mother and fetus (i.e., amniotic fluids and placenta). The choice of specimens to be analyzed is determined by many factors; however, the most important is knowledge of the chemical and physical characteristics of a substance and the route of it administration. Maternal and neonatal biological materials reflect exposures that occur over a specific time period, and each of these biological specimens has different advantages and disadvantages, in terms of accuracy, time window of exposure and cost/benefit ratio.

Sampling the placenta may be the most important biomonitoring choice for assessing in utero exposure to addictive substances. The use of the placenta in scientific research causes a minimum of ethical problems, partly because its sampling is noninvasive, causes no harm to mother or child, and partly because, in any case, placentas are discarded and incinerated after birth. Such samples, when properly analyzed, may provide key essential information about fetal exposure to toxic

substances, and may provide the groundwork for protecting the fetus or newborn and the mother from further damage.

Several sensitive and specific bioanalytical methods are commonly utilized to accurately measure for drug biomarkers of in utero drug exposure. Moreover, several immunoassay methods are used to rapidly screen for drugs in many biological specimen types. However, results from immunoassays should be carefully interpreted, and should be confirmed by more specific and sensitive chromatographic methods, such as GC-MS or LC-MS. Although techniques for analysis of addictive substances are still being developed or are being refined, current methods are efficient and sensitive and provide valuable information on human exposures to addictive substances and their metabolites.

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References

Al-Saleh I, Shinwari N, Mashhour A, Mohamed Gel D, Rabah A (2011) Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women. Int J Hyg Environ Health 214:79–101

Anderson M, Elk R, Andres RL (1997) Social, ethical and practical aspects of perinatal substance use. J Subst Abuse Treat 14:481–486

Baranowski J, Pochopień G, Baranowska I (1998) Determination of nicotine, cotinine and caffeine in meconium using high-performance liquid chromatography. J Chromatogr B 707:317–321

Borgerdinga M, Klusb H (2005) Analysis of complex mixtures: cigarette smoke. Exp Toxicol Pathol 57:43–73

Chen C, Cho SI, Damokosh AI, Chen D, Li G, Wang X, Xu X (2000) Prospective study of exposure to environmental tobacco smoke and dysmenorrhea. Environ Health Perspect 108:1019–1022

Daley M, Argeriou M, McCarty D (1998) Substance abuse treatment for pregnant women: a window of opportunity? Addict Behav 23:239–249

De Castro A, Concheiro M, Shakleya DM, Huestis MA (2009) Simultaneous quantification of methadone, cocaine, opiates, and metabolites in human placenta by liquid chromatography-mass spectrometry. J Anal Toxicol 33:243–252

Dejmek J, Solansky I, Podrazilová K, Srám RJ (2002) The exposure of nonsmoking and smoking mothers to environmental tobacco smoke during different gestational phases and fetal growth. Environ Health Perspect 110:601–610

Eskenazi B, Castorina R (1999) Association of prenatal maternal or postnatal child environmental tobacco smoke exposure and neurodevelopmental and behavioral problems in children. Environ Health Perspect 107:991–1000

Esteban M, Castaño A (2009) Non-invasive matrices in human biomonitoring: a review. Environ Int 35:438–449

Eyler FD, Behnke M, Wobie K, Garvan CW, Tebbett I (2005) Relative ability of biologic specimens and interviews to detect prenatal cocaine use. Neurotoxicol Teratol 27:677–687

Falcon M, Valero F, Pellegrini M, Rotolo MC, Scaravelli G, Joya J, Vall O, García Algar O, Luna A, Pichini S (2010) Exposure to psychoactive substances in women who request voluntary termination of pregnancy assessed by serum and hair testing. Forensic Sci Int 196:22–26

Finnegan LP (1994) Perinatal morbidity and mortality in substance using families: effects and intervention strategies. Bull Narcotics 46:19–43

Gilmour MI, Jaakkola MS, London SL, Nel AE, Rogers CA (2006) How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. Environ Health Perspect 114:627–633

Gray T, Huestis M (2007) Bioanalytical procedures for monitoring in utero drug exposure. Anal Bioanal Chem 388:1455–1465

Gray TR, Eiden RD, Leonar KE, Connors GJ, Shisler S, Huestis MA (2010) Identifying prenatal cannabis exposure and effects of concurrent tobacco exposure on neonatal growth. Clin Chem 56:1442–1450

Gray TR, Shakleya DM, Huestis MA (2008) Quantification of nicotine, cotinine, trans-3′-hydroxycotinine, nornicotine and norcotinine in human meconium by liquid chromatography/tandem mass spectrometry. J Chromatogr B 863:107–114

Hakkola J, Pasanen M, Hukkanen J, Pelkonen O, Mäenpää J, Edwards RJ, Boobis AR, Raunio H (1996) Expression of xenobiotic-metabolizing cytochrome P450 forms in human full-term placenta. Biochem Pharmacol 51:403–411

Huestis MA, Choo RE (2002) Drug abuse's smallest victims: in utero drug exposure. Forensic Sci Int 128:20–30

Huestis MA, Cone EJ (1998) Alternative testing matrices. In: Karch SB (ed) Drug abuse handbook, 2nd edn. CRC Press, Boca Raton, FL, pp 799–857

Jauniaux E, Burton GJ (2007) Morphological and biological effects of maternal exposure to tobacco smoke on the feto-placental unit. Early Hum Dev 83:699–706

Jones J (1974) Nutrition, drugs and their interrelations. PA Health 35:3

Joya X, Pujadas M, Falcón M, Civit E, Garcia-Algar O, Vall O, Pichini S, Luna A, De la Torre R (2010) Gas chromatography–mass spectrometry assay for the simultaneous quantification of drugs of abuse in human placenta at 12th week of gestation. Forensic Sci Int 196:38–42

Kissin WB, Svikis DS, Morgan GD, Haug NA (2001) Characterizing pregnant drug-dependent women in treatment and their children. J Subst Abuse Treat 21:27–34

Kulaga V, Shor S, Koren G (2010) Correlation between drugs of abuse and alcohol by hair analysis: parents at risk for having children with fetal alcohol spectrum disorder. Alcohol 44:615–621

Larkby C, Day N (1997) The effects of prenatal alcohol exposure. Alcohol Health Res World 21:192–198

Leino O, Kiviranta H, Karjalainen AK, Kronberg-Kippilä C, Sinkko H, Larsen EH, Virtanen S, Tuomisto JT (2011) Pollutant concentrations in placenta. Food Chem Toxicol 54:59–69

Loughhead AM, Fisher AD, Newport DJ, Ritchie JC, Owens MJ, DeVane CL, Stowe ZN (2006) Antidepressants in amniotic fluid: another route of fetal exposure. Am J Psychiatry 163:145–147

Lozano J, García-Algar O, Vall O, De la Torre R, Scaravelli G, Pichini S (2007) Biological matrices for the evaluation of in utero exposure to drugs of abuse. Ther Drug Monit 29:711–734

López P, Bermejo AM, Tabernero MJ, Fernández P, Alvarez I (2007) Determination of cocaine and heroin with their respective metabolites in meconium by gas chromatography–mass spectrometry. J Appl Toxicol 27:464–471

Marchei E, Escuder D, Pallas CR, Garcia-Algar O, Gómez A, Friguls B, Pellegrini M, Pichini S (2011) Simultaneous analysis of frequently used licit and illicit psychoactive drugs in breast milk by liquid chromatography tandem mass spectrometry. J Pharm Biomed Anal 55:309–316

Marx JA, Hockberger RS, Wall RM, Adams J (2002) Rosen's emergency medicine: concepts and clinical practice, 5th edn. CV Mosby, St. Louis, pp 2396–2400

Miller WH, Hyatt MC (1992) Perinatal substance abuse. Am J Drug Alcohol Abuse 18:247–261

Myllynen P, Pasanen M, Pelkonen O (2005) Human placenta: a human organ for developmental toxicology research and biomonitoring. Placenta 26:361–371

Myren M, Mose T, Mathiesen L, Knudsen LE (2007) The human placenta—an alternative for studying fetal exposure. Toxicol Vitro 21:1332–1340

Narkowicz S, Polkowska Ż, Namieśnik J (2012) Analysis of markers of exposure to constituents of environmental tobacco smoke (ETS). Crit Rev Anal Chem 42:16–37

Niemann RA, Anderson DL (2008) Determination of iodide and thiocyanate in powdered milk and infant formulaby on-line enrichment ion chromatography with photodiode array detection. J Chrom A 1200:193–197

Nikolaou PD, Papoutsis II, Maravelias CP, Spiliopoulou CA, Pistos CM, Calokerinos AC, Atta-Politou J (2008) Development and validation of an EI-GC-MS method for the determination of methadone and its major metabolites (EDDP and EMDP) in human breast milk. J Anal Toxicol 32:478–484

Ostrea EM Jr, Hernandez JD, Bielawski DM, Kan JM, Leonardo GM, Abela MB, Church MW, Hannigan JH, Janisse JJ, Ager JW, Sokol RJ (2006) Fatty acid ethyl esters in meconium: are they biomarkers of fetal alcohol exposure and effect? Alcohol Clin Exp Res 30:1152–1159

Otero C, Boles S, Young NK, Dennis K (2004) Methamphetamine: addiction, treatment, outcomes and implications. U.S. Department of Health and Human Services, SAMHSA, Rockville, MD

Pellegrini M, Marchei E, Rossi S, Vagnarelli F, Durgbanshi A, Garcĺa-Algar Ó, Vall O, Pichini S (2007) Liquid chromatography/electrospray ionization tandem mass spectrometry assay for determination of nicotine and metabolites, caffeine and arecoline in breast milk. Rapid Commun Mass Spectrom 21:2693–2703

Phibbs CS, Bateman DA, Schwartz RM (1991) The neonatal costs of maternal cocaine use. J Am Med Assoc 266:1521–1526

Pichini S, Altieri I, Pellegrini M, Pacifici R, Zuccaro P (1997) The analysis of nicotine in infants' hair for measuring exposure to environmental tobacco smoke. Forensic Sci Int 84:253–258

Polkowska Ż, Kozłowska K, Witkowska E, Namieśnik J (2004) Materiały biologiczne pochodzenia ludzkiego jako źródło informacji o narażeniu człowieka na środowiskowe czynniki chemiczne. In: Collective work. Bioanalityka w ocenie zanieczyszczeń środowiska. CEEAM, Gdansk, pp 75–107

Quertemont E, Didone V (2006) Role of acetaldehyde in mediating the pharmacological and behavioral effects of alcohol. Alcohol Res Helth 29:258–266

Rogers JM (2009) Tobacco and pregnancy. Reprod Toxicol 28:152–160

SAMHSA (2011) Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings. NSDUH Series H-41, HHS Publication No. (SMA) 11–4658. Rockville, MD: Substance Abuse and Mental Health Services Administration. http://www.samhsa.gov/data/NSDUH/2k10NSDUH/2k10Results.htm. Accessed 7 June 2012

Scharnberg K (2003) Prosecutors targeting pregnant drug users; some fear women will shun treatment. Chi Trib Nov 23:C1

Schütz H, Paine A, Erdmann F, Weiler G, Verhoff MA (2006) Immunoassays for drug screening in urine. Forensic Sci Med Pathol 2:75–83

Shakleya DM, Dams R, Choo RE, Jones H, Huestis MA (2010) Simultaneous liquid chromatography-mass spectrometry quantification of urinary opiates, cocaine, and metabolites in opiate-dependent pregnant women in methadone-maintenance treatment. J Anal Toxicol 34:17–25

Van der Aa EM, Peereboom-Stegeman JH, Noordhoek J, Gribnau FW, Russel FG (1998) Mechanisms of drug transfer across the human placenta. Pharm World Sci 20:139–148

Vinner E, Vignau J, Thibault D, Codaccioni X, Brassart C, Humbert L, Lhermitte M (2003) Neonatal hair analysis contribution to establishing a gestational drug exposure profile and predicting a withdrawal syndrome. Ther Drug Monit 25:421–432

Vogel A (1997) Neurodevelopment: cocaine wreaks subtle damage on developing brains. Science 278:38–39

Zuckerman B, Frank DA, Hingson R, Amaro H, Levenson SM (1989) Effects of maternal marijuana and cocaine use on fetal growth. N Engl J Med 320:762–768

Genetic Structure and Diversity of Animal Populations Exposed to Metal Pollution

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Contents

1 Introduction

The introduction of toxic substances into the environment by anthropogenic or natural activities is widespread and causes significant perturbation. Therefore, increasing attention has been focused on better understanding the long-term ecological effects of chronically exposed populations, communities, and ecosystems. The increased understanding of such effects has resulted not only from enhanced biomonitoring activities but also from developing new toxicity and ecotoxicity data for various species.

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Genetic change in exposed populations is one of the more subtle effects of environmental exposures, and has potentially large, long-term effects (Van Straalen and Timmermans 2002). Thus, there are benefits for monitoring the genetic patterns of wild populations for assessing environmental impacts in natural systems (Belfiore and Anderson 1998; Medina et al. 2007).

Metals are among the most common substances released into the environment, and these compounds can have a profound effect on living organisms (Guttman 1994; Bickham et al. 2000; EPA 2000; Dimsoski and Toth 2001; Theodorakis 2001; Moore et al. 2004; WHO 2007; Tremblay et al. 2008). This is under-scored by the fact that metal-induced effects are among the top ten concerns of the US Environmental Protection Agency (EPA 2000). Metals affect natural populations in many ways. Genetic changes are one of the most important alterations that may occur, and when they occur, they are capable of disrupting the genetic equilibrium at all levels of biological organization.

Genetic structure represents the rearrangement of allelic and genotypic frequencies of populations and represents how genetic variation is distributed within and among populations. Evolution and the maintenance of genetic structure in space and time are dependent on natural selection forces, genetic drift, mating systems, recombination, mutations, and gene flow (Loveless and Hamrick 1984; Coutellec and Barata 2011). In addition to these evolutionary forces, how genetic variation is distributed within and among populations is determined by exterior factors, such as ecological phenomena, particularly habitat disturbance and fragmentation, and life history traits of the species (Barret and Khon 1991). Ecological disturbances may be of natural (e.g., geologic processes, volcanic eruptions, and fires) or anthropogenic (e.g., agricultural practices, mining, and other industrial activities) origin.

Genetic ecotoxicology is the study of xenobiotic-induced changes in the genetic material of natural biota. Direct alterations to genes and gene expression may occur from exposures, or the pollutants may induce selective effects on gene frequencies (Anderson et al. 1994). In this context, contaminant-induced selection and genetic bottlenecks are mechanisms by which the genetic structure of populations can become altered. Both factors may affect the adaptive ability of a contaminant-exposed population and may have consequences at the community and ecosystem levels (Gillespie and Guttman 1989; Theodorakis et al. 2000; Harper-Arabie et al. 2004; Athrey et al. 2007; Brown et al. 2009). Consequently, changes in diversity and genetic structure parameters may be used as bioindicators of ecosystem health, which is defined as a comprehensive, multiscale, dynamic, hierarchical measure of system resilience, organization, and vigor (Ehrenfeld 1992).

Although numerous studies address the ecotoxicity of metals, few have addressed the topic of genetic ecotoxicity. Among studies that have focused on environmentally stressed populations and their genetic population-level responses, two principal approaches have been utilized. In the first approach, genetic or molecular non-neutral markers are identified that are linked to resistance or sensitivity to environmental stressors, or a combination of both stressors, in select species. In the second approach, changes in genetic diversity parameters are addressed in the exposed populations, by using neutral molecular markers, such as allozymes, mitochondrial DNA analyses, RAPDs (random amplified polymorphic DNA), SSRs

(single sequence repeats) or microsatellites, and AFLPs (amplified fragment length polymorphic DNA) (D'Surney et al. 2001; Hoffman and Daborn 2007).

In this review, we endeavor to summarize the key work that has been performed to assess the effects of metals on the genetic pattern of several organisms; these effects are often a result of metal-induced environmental stress on natural animal populations. In genetic ecotoxicology, it is important to recognize the differences between genetic changes that are due to genotoxic or mutagenic mechanisms of action, genetic alterations due to ecological processes, such as genetic drift and bottlenecks, and environmental changes that alter genetic variability in natural populations, in terms of allele frequencies, heterozygosity levels and gene flow. Additionally, we address the potential relationship between exposure to chemical agents and changes in genetic structure, and the possible long-term consequences of chronically exposed populations. Another issue that we address is the use of sentinel species that are, or may be adequate to study genetic ecotoxicological questions.

Finally, we reach conclusions and make suggestions on what is required to strengthen this area of research, and we also propose a new class of biomarkers, termed "biomarkers of permanent effect." These biomarkers are useful tools to estimate ecosystem health through the evaluation of changes in structure and genetic diversity of the exposed populations.

2 Implications of Metal Toxicity on Population Genetics

Metals are among the most toxic elements to nearly all living organisms (EPA 2000; WHO 2007). The relationship between metal toxicity and a plethora of effects in living organisms is well established. Studies of populations exposed to metals in occupational or environmental settings were among the first to establish a quantitative relationship between effects and external exposure and/or internal dose received (Bernard 2008). The field of genetic toxicology is usually regarded as the study of the mechanisms of action of xenobiotics as regards their effects on DNA. The goal of research in this area is to assess the genetic-related risks posed to individuals by xenobiotics capable of inducing adverse health effects. For many years, studies in this field were focused on the effects of acute exposures to single toxicants at high doses. However, in genetic ecotoxicology, threats to populations and communities arising from chronic exposures to mixtures of chemical agents at lower doses (realistic exposures) are also of potential concern (Depledge 1994). Thus, establishing links between the molecular and cellular effects of metals and their possible consequences at higher levels of biological organization becomes truly necessary when attempting to understand population level responses to chemicals, such as metals. Bickham and Smolen (1994) defined the term "emergent effects." The research results they report explains that although the damage from xenobiotic exposure is at the cellular or subcellular levels, emergent effects are observed at higher levels of biological organization. However, the effects produced are not predictable by merely knowing the mechanism of action of the chemical agent in question. Therefore, these higher-level effects can be assessed in wild animal populations

only by using ecological indicators, such as shifts in the genetic pools of populations chronically exposed to environmental pollutants.

Because genetic variation is the basis for biodiversity and evolution (Duan et al. 2001; Medina et al. 2007), and because the loss of genetic variability may be permanent (depending on the population size and mutation rates), investigating how chemicals exert their effects on the genetic pool in exposed populations (changes in genetic structure) is a priority in environmental biomonitoring and conservation programs.

Various authors (Van Straalen 1999; Van Straalen and Timmermans 2002; Maes et al. 2005) have described ways in which chemical agents may alter the genetic variability of an exposed population, to wit: (1) toxicants can be genotoxic (i.e., they directly or indirectly alter the DNA molecule) or mutagenic (i.e., they increase mutation rates). Genotoxic substances can affect different DNA repair processes by interacting with the key enzymes responsible for DNA damage repair, and thereby increase mutation rates; (2) toxicants may favor more tolerant genotypes and/or eliminate intolerant genotypes, changing the genetic composition of the exposed population towards a higher mean tolerance; (3) toxicants may cause bottlenecks that reduce the size of a population; and (4) toxicants may alter exchange of migrant individuals among populations.

Therefore, studying genetic ecotoxicology endpoints are as important as many other endpoints when being employed to predict the risks of populations exposed to pollutants.

3 Genotoxic Effects Versus Genetic Changes Caused by Natural Processes

Understanding how contaminants affect population genetic parameters may provide key insights about the consequences of exposure at the population level of the analyzed species. Therefore, studying the genotoxic effects of metals should become a routine and vital component of ecotoxicology, along with performing biomonitoring and ecological risk assessment (Theodorakis 2001; Benton et al. 2002; Gardeström et al. 2008).

To achieve this goal, it is important to delineate between genetic changes that result from genotoxic exposure and genetic alterations that are a result of: evolutionary forces, different mating systems, ecological factors or species life history traits, environmental changes that alter the genetic variability in natural populations in terms of allele frequencies, or heterozygosity levels and gene flow.

DNA damage from, for example, the formation of DNA adducts, base pair modifications, DNA strand breaks, and chromosome rearrangements are among the most common biomarkers of early adverse effects from toxic exposure. These insults may have serious consequences on the health of the population. If these alterations occur in somatic cells, a number of immediate effects may occur, such as cell death, or the accumulation of mutations and/or transformation into a malignant phenotype (Weinstein 1988). DNA damage in somatic cells can reduce the longevity of the individual (Agarwal and Sohal 1994), alter the age structure of the population

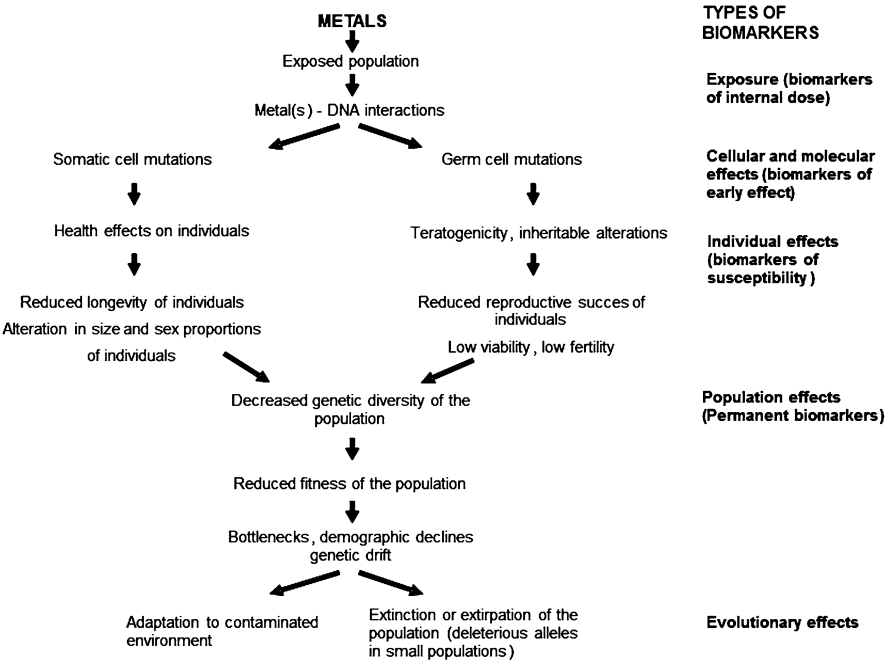

Fig. 1 Schematic representation of the relationships between the processes leading to decreased genetic diversity in animal populations exposed to metal pollution. Modified from Guttman (1994), Theodorakis (2001), and Staton et al. (2001)

(Theodorakis 2001), modify the size of the individual (individual size has implications for survival, fecundity, bioenergetics, and behavior; any perturbation in size structure has the potential to induce effects at the population level or above) or alter the sex proportion of the population (there may be gender-related responses to DNA damage) (Scheirs et al. 2006).

Because the DNA molecule is the unit of inheritance, DNA alterations (mutation and translocation) in germ cells can be passed to the next generation, causing teratogenicity, low viability, low fertility, and low reproductive success. Higher mutation rates produce damaging effects and may lead to a decrease in the average fitness of the population (Anderson et al. 1994; Guttman 1994; Belfiore and Anderson 1998; Bickham et al. 2000; Yauk et al. 2000; Theodorakis 2001; Maes et al. 2005; Medina et al. 2007; Gardeström et al. 2008). Therefore, because genotoxic alterations in somatic cells are numerous and affect adults, they affect the current population, whereas germ cell mutations from genotoxic stress, although less numerous, may affect future generations and have long-term effects. Hence, alterations in both cell types potentially affect the genetic composition of populations in multiple ways (Bickham and Smolen 1994; Belfiore and Anderson 1998; Yauk et al. 2000; Bickham et al. 2000) as depicted in Fig. 1.

The use of population genetic tools to analyze the effects of contaminants on a species is difficult and requires an extraordinarily detailed experimental design. To

discriminate between chemically-induced versus natural process-induced alterations in the genetic composition of natural populations, many authors (Endler 1995; Belfiore and Anderson 1998; Baker et al. 2001; Staton et al. 2001) have made recommendations to enhance the robustness of experimental design. First, using multiple reference sites along with multiple experimental sites is recommended (Ross et al. 2002). Second, correlating observed genetic effects with biomarkers for internal doses (i.e., contaminant levels in organs or tissues) and external exposures (i.e., levels of contaminants in air, soil, and water) is suggested. Third, it is recommended that site sampling be performed over time to establish the patterns resulting from non-contaminated factors, such as revolving ecological conditions and population cycles. To assess relationships between contaminant exposure and changes in genetic patterns, gradient effects should be evaluated (De wolf et al. 2004; Bourret et al. 2008; Durrant et al. 2011). Additionally, the use of biomarkers to detect early effects, such as different types of DNA damage, which are indicative of exposure to genotoxic contaminants in somatic cells and germ cells, should be used to determine the relationship between DNA damage and population genetic responses (Belfiore and Anderson 1998; Theodorakis 2001; Benton et al. 2002; Ross et al. 2002; Moore et al. 2004). Moreover, biomarkers should be chosen that reflect changes in the fitness of an organism (e.g., premature death, ability to mate, fecundity, viability of offspring) because these changes can have the greatest influence at the population level (Evenden and Depledge 1997).

Chen and Hebert (1999) suggested using molecular and phylogenetic tools to analyze the mutations via a technique termed "terminal branch haplotype analysis." A mutation originating from natural processes or one that is chemically induced should exhibit a low frequency, and its nucleotide sequence should differ by only one base pair from its more common ancestral haplotype. The analysis of nucleotide sequences should identify any new haplotypes originating at the branch tips of the phylogenetic tree. The variants that branch more deeply within the tree or differ by more than a single base substitution from the closest related haplotype are most likely the result of gene flow from close populations. This method could prove useful for studies that use population genetic analysis as a toxicological investigation tool (Bickham et al. 2000; Theodorakis 2001; Theodorakis et al. 2001). In addition to obtaining information about the effects of chemical agents on genetic diversity, the foregoing approaches may provide additional information on how dispersal and migration patterns influence the biological effects caused by contamination (Theodorakis 2001; Eeva et al. 2006; Gardeström et al. 2008).

4 Population Genetic Responses to Environmental Metal Stress

Population genetic responses to chemical exposures, especially metals, can have a profound effect on the genetic variability of chronically exposed populations. These responses are driven in two general directions: increased genetic variation resulting

from new mutations induced directly by the genotoxic agent(s), or decreased genetic variation resulting from population processes, such as bottlenecks or selection that will also alter allele and genotype frequencies in these populations. In both cases, the changes in genetic variation may result from adaptation to polluted environments (Bickham et al. 2000; Berckmoes et al. 2005; Maes et al. 2005; Gardeström et al. 2008; Durrant et al. 2011).

Since the year 2000, 33 studies were reported in the Hermes, PubMed and Biological abstracts databases, in which the genetic structure and diversity of animal populations exposed to metals were analyzed. Of these studies, nine were review articles (Bickham et al. 2000; Clements 2000; Belfiore and Anderson 2001; Staton et al. 2001; Theodorakis 2001; Van Straalen and Timmermans 2002; Medina et al. 2007; Morgan et al. 2007; Hoffmann and Willi 2008), and 24 were original research reports. Among these original reports, 15 aquatic and ten terrestrial ecosystems were examined.

In the aquatic ecosystem studies that were performed, 15 different species were analyzed, and a decrease in the genetic diversity of the exposed species was reported in ten of the publications. In contrast, no effects of metal pollution on the genetic diversity patterns of the exposed populations versus the reference population were reported in four of the studies.

A decreased genetic diversity in the exposed species was reported for the majority of the studies (73.3%). In contrast, metal pollution had no effect on the genetic diversity patterns of the exposed populations versus the reference population in four aquatic species (26.7%) (Table 1).

In the studies performed on terrestrial ecosystems, ten different species were analyzed, and decreased genetic diversity was reported for 40% of these exposed species. In addition, 40% of the exposed species exhibited increased genetic diversity, and genetic diversity was not affected by metal exposure in only two species (20%) (Table 1).

In the majority of the studies reviewed, mining activity, or processes related to mining constituted the source of the contamination. The most common metals found in the aquatic and terrestrial ecosystems were Cd, Zn, Cu, and Pb. However, the occurrence of these metals differed between the aquatic (Cd=Zn>Cu>Pb>Hg) and terrestrial (Cu>Cd>Pb>Zn>Ni) environments (Table 1).

Among the molecular markers used to assess the genetic diversity in the impacted populations, microsatellite markers were used (29.6%) most frequently, followed by allozyme electrophoretic techniques (25.9%), RAPD markers (18.5%), mitochondrial DNA analysis (14.8%) and other molecular markers (minisatellite mutations, electrophoretic analysis, and AFLP) (11.1%) (Table 1).

In general, most of the aquatic ecosystem studies performed during the last decade disclosed that reduced genetic diversity occurred in the animal populations exposed to a single metal or a mixture of metals; however, this pattern became less clear when terrestrial ecosystems were analyzed (Table 1).

Among the reported studies, very different animal populations (from copepods to wild birds), environments (aquatic vs. terrestrial), exposure conditions (single or metal mixtures), types of metals, and degrees of disturbance (intensity, duration of exposure, affected area, and magnitude) were analyzed. Because the large number

Table 1 Summary of studies published since the year 2000, in which the genetic diversity of animal populations exposed to metal pollution are addressed

Species (common and scientific name)	Classification (phylum: order)	Genetic diversity and structure parameters	Changes in genetic diversity patterns	Technique	Study conditions	Type of metal and concentration	Comments	Reference
	Aquatic ecosystem							
Acorn bernacle (*Balanus glandula*)	Arthropoda: Sessilia	Diversity indices, diversity by rarefaction analysis, proportion of shared haplotypes	Genetic diversity decreased significantly in exposed populations	RAPD	Field: levels of pollutants in the sediment (N.R.)	Heavy metal mixture (N.R.)	Pollution at the impacted sites may reduce genetic diversity among aquatic invertebrate populations. Individuals at impacted sites were more likely to share haplotypes than were those from reference sites	Ma et al. (2000)
Amphipod (*Hyalella azteca*)	Arthropoda: Amphipoda	Genetic distance, genetic variation at different loci	No relationship was found between levels of heterozygosity and metal exposure (was not formally evaluated using statistical methods). Increased genetic divergence	Allozyme	Laboratory: 1-month-old H. azteca were used to evaluate each metal: Cd (μg/L) and Zn (mg/L)	Water: (mean ± S.D.) Cd (37.10 ± 2.52), Zn (5.77 ± 0.30)	Suggests the potential use of genetic distance as a water bioindicator	Duan et al. (2001)
Prawn (*Leander intermedius*)	Arthropoda: Decapoda	BSI	Decreased genetic diversity was of prawn population at the polluted site versus one reference population, and diversity significantly lower than that of all reference populations	RAPD	Laboratory: levels of trace metals in food pellets at one contaminated site (μg/g). Prawns were fed metal mixtures in varing concentrations ranging from 0.14 to 26 times that found in contaminated site	Food pellets: (range) Cd (7.8–4,580), Zn (0–136,197), Cu (18.9–1,975), Pb (0–34,552), Mn (022359)	These observations highlight the need to include various reference populations in genetic population studies and the need to assess the influence of pollution on the genetic diversity of more than one species	Ross et al. (2002)
Isopod (*Platynympha longicaudata*)	Arthropoda: Isopoda	BSI	Decreased genetic diversity of isopod populations at polluted sites versus all reference populations	RAPD	Laboratory: levels of trace metals in food pellets at one contaminated site(μg/g). Isopods were fed metal mixtures in varing concentrations ranging from 0.14 to 26 times that found in the contaminated site	Food pellets: (range) Cd (7.8–4,580), Zn (0–136,197), Cu (18.9–1,975), Pb (0–34,552), Mn (022359)	These observations highlight the need to include various reference populations in genetic population studies and the need to assess the influence of pollution on the genetic diversity of more than one species	Ross et al. (2002)

Harpacticoid copepod (*Attheyella crassa*)	Arthropoda: Harpacticoida	Fixation index, heterozygosity	Decreased genetic diversity in experimental groups	AFLP	Field: analyzed metals in surface sediments (top 0.5–1 cm) at two polluted sites (μg/g dry wt)	Sediment: (range) Zn (1,110–2,966), Cu (614–1,039), Pb (55–1,080), Hg (n.a.–28.6)	Toxicant exposure can reduce genetic diversity and cause population differentiation	Gardeström et al. (2008)
Periwinkle (*Littorina littorea*)	Mollusca: Mesogastropoda	Heterozygosity, Fst	No relationship was found between levels of locus-specific heterozygosity and metal exposure	RAPD	Field: levels of pollutants in soft tissue, in seven sites (μg/g^{-1} dry wt)	Soft tissue: (range) Ag (0.81–4.85), As (7.96–22.92), Cd (0.92–5.23), Co (0.0–2.15, Cr (0.11–1.48), Cu (68.2–176.0), Fe (337.0–1,214.0), Mn (19.64–113.7), Ni (3.43–7.43), Pb (0.861.67), Zn (59.6–106.0)	Authors suggest that selection, rather than bottleneck effects, induced by less favorable leaving conditions at polluted sites are responsible for the genetic patterning	De Wolf et al. (2004)
Gasteropod (*Littorina brevicula*)	Mollusca: Mesogastropoda	Haplotype distribution and diversity	Decreased genetic diversity in experimental group. Haplotype diversity was significantly lower in polluted environments	Sequencing analysis (mtDNA)	Field: analyzed metals in seawater (μl/L), sediment (μg/g) and organism (μg/g) in three polluted sites	Seawater: (range) Cd (0.010–1.705), Zn (1.70–35.12), Cu (0.61–24.6), Pb (0.042–0.120). Sediment: (range) Cd (2.71–11.2), Zn (144–230), Cu (37.6–47.8), Pb (41.0–56.8). Organism: Cd (11.81), Zn (88), Cu (126), Pb (1.76)	Observed emergent effects from pollution at population level, taking into account rare haplotypes	Kim et al. (2003)
Snail (*Pleurocera canaliculatum*)	Mollusca: Caenogastropoda	Allele and genotype frequencies, heterozygosity	Decreased heterozygosity in experimental group versus reference group	Allozyme	Field: whole-body of P. canaliculatum from five sites (μg/g dry wt)	Whole-body: (range) Hg (0.678–4.257)	Reinforces the use of allozyme analysis as a marker of contamination and possible selection for pollution resistance	Benton et al. (2002)
Bay mussel (*Mytilus galloprovinciali*)	Mollusca: Mytiloida	Diversity indexes, diversity by rarefaction analysis, proportion of shared haplotypes	Genetic diversity decreased significantly in exposed populations	RAPD	Field: levels of pollutants in the sediment (N.R.)	Heavy metal mixture (N.R.)	Pollution at the impacted sites may reduce genetic diversity among aquatic invertebrate populations. Individuals at impacted sites were more likely to share haplotypes than were those from reference sites	Ma et al. (2000)

(continued)

Table 1 (continued)

Species (common and scientific name)	Classification (phylum: order)	Genetic diversity and structure parameters	Changes in genetic diversity patterns	Technique	Study conditions	Type of metal and concentration	Comments	Reference
Marsh frogs (*Rana ridiunda*)	Chordata: Anura	Haplotype and nucleotide diversity, Fst, Nm	Genetic diversity decreased significantly in exposed populations	Sequencing analysis (mtDNA)	Field: March frogs were collected from of eight exposed and three reference sites. Hg concentrations in fresh water sediments (ppm)	Fresh water sediments Hg (1.49) and a complex mixture of chemical pollutants	The authors conclude that the observed loss of diversity is likely the results of population declines, and the environmental degradation is most likely cause of the regional reductions of genetic diversity	Matson et al. (2006)
Yellow perch (*Perca flavescens*)	Chordata: Perciformes	Heterozygosity, allelic diversity and richness, Fst, Rst and IR	Genetic diversity decreases along a gradient of increasing Cd and Cu contamination IR: presented the opposite tendency as the more contaminated individuals were more diverse than the less contaminated ones in contaminated and reference populations	SSR's	Field: liver of P. flavescens from 20 sites (µg/g dry wt)	Liver: (range)[a] Cd (2–37), Cu (20–185)	Chronic exposure to metal contamination have impacted genetic diversity among populations of wild yellow perch, which may affect the capacity of populations to respond to environmental changes	Bourret et al. (2008)
Brown trout (*Salmo trutta*)	Chordata: Salmoniformes	Allelic richness , Fst, Ho and He, Nm	Genetic diversity estimates did not support a negative correlation between population genetic diversity and increasing metal pollution	SSR's	Field and Laboratory: water metal concentrations ($\mu g/L^{-1}$).	Water: (two conditions) (1) Cu (94), Zn (760) (2) Cu (4), Zn (28)	Population genetic analysis indicated that metals were not a barrier to gene flow within the river. The metal tolerance trait exhibited by this Brown trout population may represent an important component of the species genetic diversity in the region	Durrant et al. (2011)

Gudgeon (*Gobio gobio*)	Chordata: Cypriniformes	Na, Ho, He, genetic and genotypic differentiation	Ho, He, and Na did not differ between contaminated and reference sites	Allozyme and SSR's	Field: metal concentrations of the surface water in four sites were analyzed (µg/L)	Surface water: (range) Cd (20–30), Zn (1,500–2,100)	Long-term exposure to metals can induce changes at the population genetic level in natural fish populations, which can be detected both at microsatellite as well as at allozyme loci	Bervoest and Blust (2003)
Least killifish (*Heterandria formosa*)	Chordata: Cyprinodontiformes	Heterozigosity	Decreased genetic diversity in three experimental populations versus reference populations, only two decreased significantly	SSR's	Laboratory: Immature fish were exposed to Cd (µg/L) until at least 50% of individuals had died	Water: Cd (6)	Loss of genetic variation in Lab populations is taken into account when extrapolating from lab to natural populations	Athrey et al. (2007)
European eel (*Anguilla anguilla*)	Chordata: Anguilliformes	Allele and genotype frequencies, levels of polymorphisms and heterozygosity	Decreased genetic variability in strongly polluted eels	Allozyme and SSR's	Field: heavy metals in muscle tissue of *A. anguilla* from 16 sites in three rivers. Concentration were expressed in µg kg^{-1} (Hg, Cd, Pb, Ni, Cr, As and Se) or mg kg^{-1} (Cu and Zn) wet weight	Muscle tissue: (range) Cd (1.5–23.1), Zn (17.0–32.5), Cu (0.3–1.2), Pb (5.0–95.3), Hg (59.6–245.2), Ni (5.0–94.0), Cr (135.3–823.7), As (135.0–704.0), Se (329.0–1,556.0)	Significant and negative correlation between metals and fitness, suggesting an impact of pollution on the health of subadult eels	Maes et al. (2005)
	Terrestrial ecosystem							
Earthworms (*Lumb ricus rub ellus*)	Annelida: Haplotaxida	Allele and genotype heterozygosity	Increased in frequencies, heterozygosity exposed compared to reference individuals. Allele and genotypic frequencies did not differ between groups	Electrophoretic analysis	Field: Concentration of heavy metals in soils (ppm) were monitored yearly in each plot from 1978 to 1993	Soil: (range)Cd (1.3–2.7), Zn (81.0–140.5), Cu (16.9–36.0), Pb (23.1–48.0)	Certain alleles and genotypes may be more sensitive to the effects of heavy metals	Peles et al. (2003)
Enchytraeid worm (*Cognettia sphagnetorum*)	Annelida: Haplotaxida	Allele and genotype frequencies, H, D	Decreased genetic diversity in experimental group (H, unique genotypes, except D)	Allozyme	Laboratory: soil used in the laboratory experiments (after 15 weeks incubation) with Cu (mg kg^{-1} dry matter)	Soil: (mean ± S.E.) Total Cu (2,000 + 30.1) Extractable Cu (183 + 3.3)	Greater diversity and more unique genotypes in the population living in the uncontaminated site	Haimi et al. (2006)

(continued)

Table 1 (continued)

Species (common and scientific name)	Classification (phylum: order)	Genetic diversity and structure parameters	Changes in genetic diversity patterns	Technique	Study conditions	Type of metal and concentration	Comments	Reference
Land snail (*Cepaea nemoralis*)	Mollusca: Stylommatophora	Polymorphic loci, allelic richness, Ho, He, ϕ	Increased observed heterozygosity (Ho) in exposed compared to reference plots	Allozyme	Field: Concentration of heavy soil: (mg kg^{-1} dry soil)	Soil: (mean ± D.E.) metals in sediment soils Cd (9.7 ± 6.4), Cr (269 ± 209), Cu soil) (190 ± 89), Ni (95 ± 18), Pb (397 ± 249), Zn (1,520 ± 846)	Observed patterns of genetic variation may be explained by the action of genetic drift, pollution-mediated selection, restricted gene flow, or a combination of these processes	Jordaens et al. (2006)
Land snail (*Succinea putris*)	Mollusca: Stylommatophora	Polymorphic loci, allelic richness, Ho, He, ϕ	Genetic diversity is not affected by metal pollution	Allozyme	Field: concentration of heavy metals in sediment soils (mg kg^{-1} dry soil)	Soil: (mean ± D.E.) Cd (9.7 ± 6.4), Cr (269 ± 209), Cu(190 ± 89), Ni (95 ± 18), Pb (397 ± 249), Zn (1,520 ± 846)	Observed patterns of genetic variation may be explained by the action of genetic drift, pollution-mediated selection, restricted gene flow, or a combination of these processes	Jordaens et al. (2006)
Amphipod sandhopper (*Talitrus saltador*)	Arthropoda: Amphipoda	Average gene diversity over loci mean number of pair-wise differences	Decreased in Hg exposed populations. Population from sites with High Hg availability had the lowest values of genetic diversity	flSSR's	Field: metal concentrations in tissue of A. sandhopper (ppm) and sand (ppm) from eight sites were analyzed	Sandhopper: (range) Cd (0.40–1.74), Cu (40.6–73.6), Hg (0.07–0.21) Sand: (range)[a] Cd (0.01–0.24), Cu (1.0–4.0), Hg (0.02–0.11)	Validate the use of fl-SSR markers in genetic studies in sandhoppers and support the "genetic erosion hypothesis" by showing the negative influences of Hg contamination on genetic diversity	Ungherese et al. (2010)
Crab (*Pachygrapsus marmoratus*)	Arthropoda: Decapoda	Ho, He, allelic richness, number of private alleles, standardized mean d^2 (parental similarity)	Decreased genetic variability in P. marmoratus from polluted sites. A significantly lower percentage of unrelated individuals, than populations from unpolluted sites	SSR's	Field: metal concentration in gills and hepatopancreas in P. marmoratus adult males (μg/g wet wt)	Gills: (approx) As (5), Pb (2.2), Cd (0.2), Cu (75) Hepatopancreas: (approx) As (20), Pb (1.4), Cd (0.48), Cu (340)	This study supports the "genetic erosion" hypothesis for metal heavy exposure in natural environments	Fratini et al. (2008)

Insectivourus paserines (*Ficedula hypoleuca*)	Chordata: Passeriformes	Nucleotide diversity	Decreased nucleotide diversity in F. hypoleuca in polluted sites	Sequencing analysis (mtDNA)	Field: metal concentration in feathers of F. hypoleuca from three sites were analyzed (µg/g dry wt)	Feather: (range) Cd (0.04–0.17), Zn (134.4–185.4), Cu (11.6–15.3), Pb (1.02–23.6), Ni (3.82–12.9), Al (39.1–53.74), As (0.24–8.41), Cr (0.90–2.96), Sn (0.72–1.28)	Genetic diversity depends on species and their metabolism	Eeva et al. (2006)
Pied flycatcher (*Parus major*)	Chordata: Passeriformes	Nucleotide diversity	Increased nucleotide diversity in P. major in polluted sites suggesting high mutation rates	Sequencing analysis (mtDNA)	Field: heavy metal concentration in feathers of P. major from one site were anualized	Feather: (mean ± S.E.) Cd (0.03±0.00), Zn (132.4±2.56), Cu (15.9±1.15), Pb (2.00±0.64), Ni (9.18±1.06), Al (45.5±3.92), As (1.10±1.10), Cr (0.82±0.06), Sn (3.44±0.20)	Genetic diversity depends on species and their metabolism	Eeva et al. (2006)
Herring gulls (*Larus argentatus*)	Chordata: Charadriiformes	Mutation rates, number of bands scored	Mutation rates increase significantly in steel sites versus urban and rural sites	Multilocus DNA fingerprinting	N.R.	Steel mills	Demonstrate significant risk for induced germ line mutations in zones with steel operations	Yauk et al. (2000)
Wood mouse (*Apodemos silvaticus*)	Chordata: Rodentia	Heterozygosity, allele richness, Fst, gene flow	Genetic diversity is not affected by metal pollution. Gene flow among populations restricted	SSR's	Field: heavy metals in soil from seven sites. Concentration were expressed in µg/g (Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn) or mg/g (Al and Fe) dry wt	Soil: (range) Cd (0.07–26.25), Zn (4.19–224.66), Cu (2.64–133.89), Pb (18.18–693.27), Ni (0.93–15.87), Al (0.69–13.70), Ag (0.06–4.02), As (3.25–26.25), Co (0.32–6.71), Cr (2.36–113.78), Mn (4.10–107.87), Fe (1.17–59.39)	Genetic diversity in the wood mouse populations is not affected by the heavy metal pollution. Pollution induced stress is not intense enough, or insufficient time has passed since the onset of pollution stress to induce a population genetic response	Berckmoes et al. (2005)

RAPD random amplified polymorphic DNA, *AFLP* amplified fragment length polymorphism, *SSR's* sequence simple repeats or microsatellite markers, *BSI* band-sharing index, *IR* internal relatedness, *fISSR's* fluorescence inter-simple sequence repeat, *N.R.* not reported, *Fst, Rst and ϕ* genetic differentiation, *Ho* observed heterozygosity, *He* expected heterozygosity, *Na* number of alleles per locus, *H* Shannon–Wiener index, *D* Simpson index, *Nm* number of migrants

[a]Approximate data (data represented by bar column plots in the original reference)

of variables makes comparisons difficult, each study compared results with its own geographical reference site(s). In this review, we did not find means to make valid comparisons among studies on genetic diversity parameters.

There are many explanations for what causes reduced genetic diversity in exposed populations. Genotypic selection may affect genetic change at the population level. In addition, changes in population size may produce genetic bottlenecks and possibly genetic drift of the population. Finally, changes in the demographic patterns and reduced migration rates may reduce the genetic diversity (Van Straalen and Timmermans 2002). Therefore, ecological processes, such as bottlenecks resulting from the genotoxic effects of metals, the selection of tolerant and the elimination of intolerant genotypes, or the reduction in offspring that contribute to the next generation may lead to a decrease in the genetic variation within populations that are chronically exposed to polluted environments. Because genetic variability is the basis for adaptation by natural selection, it is generally accepted that the loss of genetic variability makes it more difficult for a population to adapt to future environmental changes. Any reduced variation can lead to an increased extinction rate (Anderson et al. 1994; Bickham et al. 2000; Tremblay et al. 2008) (Fig. 1).

The loss of genetic diversity in populations subjected to anthropogenic stress is referred to as "genetic erosion" and this may be a factor of concern in assessing the risk of toxic chemicals (Van Straalen and Timmermans 2002). Fratini et al. (2008) and Ungherese et al. (2010) validated the use of molecular markers in genetic studies to support the "genetic erosion hypothesis," by showing that metal contamination has negative influences on genetic diversity. In contrast, Eeva et al. (2006) reported increased genetic variation in populations impacted by metal pollution. The free-living insectivorous passerine (*P. major*) populations living near a smelter exhibited statistically higher nucleotide diversity than did a reference population in an unpolluted site, suggesting that high mutation rates occur in contaminated environments. Additionally, Peles et al. (2003) reported higher levels of heterozygosity in an exposed population compared with the reference population. The report showed that the percentage of earthworms (*Lumbricus rubellus*) in the highest heterozygosity class was four times higher in the exposed than in the reference population. Bourret et al. (2008) assessed the level of heterozygosity, allelic richness, diversity, and internal relatedness (IR), a measure of individual genetic diversity in yellow perch (*Perca flavescens*) populations. A negative correlation was observed between each of the genetic diversity parameters and the metal concentrations. In contrast, the levels of IR indicated that the more contaminated individuals were genetically more diverse than the less contaminated individuals in both the contaminated and reference populations. These results suggest that the less inbred perch were more tolerant to metal contamination under certain circumstances. The authors explained that, under these circumstances, one would predict that individual fitness will increase with individual genetic diversity, and consequently, the selective pressures exerted by Cd contamination should favor the maintenance of higher genetic diversity within the contaminated populations.

Reports of increased genetic diversity in exposed populations support the hypothesis that the vast majority of mutations that negatively affect fitness are expected to

be deleterious; an increased mutation rate of a population will also increase its mutational load. Additionally, several field studies have demonstrated that mutations accumulate more rapidly in environments that are more polluted (Yauk and Quinn 1996; Clements 2000; Rogstad et al. 2003; Gardeström et al. 2008; Peles et al. 2003). Therefore, it is expected that populations that are chronically exposed to pollutants will likely experience a steady decrease in fitness from an increasing mutational load, which ultimately has the potential to drive a population to extinction (Lynch et al. 1995).

Because individual fitness should increase with an individual's genetic diversity, another possible scenario is based on the assumption that selective pressures favor more genetically diverse populations. Thus, contaminated populations may contain higher levels of genetic diversity (Bourret et al. 2008).

When using different genetic diversity endpoints, variable results (e.g., increased or decreased genetic variability) can be explained in a variety of ways:

1. Differences in response to environmental stress have been attributed to species susceptibility; as stated earlier, different responses to stress among populations of a single species have been documented to occur (Diamond et al. 1991; Eeva et al. 2006). Moreover, different species in the same polluted environment produce diverse results, which have been attributed to differences in species metabolism (Eeva et al. 2006).
2. Populations that belong to different ecosystems (terrestrial vs. aquatic) will exhibit different responses, mainly because the routes of exposure and the bioavailability of metals are different between the systems.
3. The use of different techniques to analyze the genetic parameters can produce different results. The majority of researchers have examined genetic variation using microsatellite markers and at allozyme loci. As stated previously, many microsatellite loci are considered to be one of the best molecular markers (Yauk and Quinn 1996; Athrey et al. 2007; Tremblay et al. 2008); their high mutation rates and high variability make them one of the most sensitive markers for analyzing genetic variability within and between populations that were exposed to different concentrations of genotoxins. Additionally, the quantification of the genetic variation at allozyme loci using electrophoretic techniques is the second most frequently used method. Most allozyme studies emphasize the impacts of heavy metals on allozyme diversity in aquatic organisms, because of the extensive pollution of aquatic ecosystems with metals, and because there is evidence of many metals inhibiting or altering enzymatic activities (Nevo et al. 1983; Benton et al. 2002; Keane et al. 2005; Maes et al. 2005). The results from studies of mosquito fish (Chagnon and Guttman 1989; Diamond et al. 1989; Newman et al. 1989; Roark et al. 2001) and aquatic invertebrates (Nevo et al. 1978; Battaglia et al. 1980; Gillespie and Guttman 1989; Patarnello et al. 1991; Ma et al. 2000; Benton et al. 2002; Kim et al. 2003; Keane et al. 2005; Maes et al. 2005; Gardeström et al. 2008) suggest that genotypic frequencies at allozyme loci are affected by contaminant exposure, although there is not a unique response pattern. Single metals and mixtures of metals may elicit different responses

among the array of genotypes at a locus and among populations of a single species (Diamond et al. 1991; Lee et al. 1992). However, because the genetic pool of a population is constantly modified by natural processes, such as mutations, gene flow, genetic drift, and natural selection, the cause–effect relationships between genetic alterations measured using molecular markers and environmental stress are difficult to establish using organisms collected in the field (Medina et al. 2007). Thus, different techniques may yield different results.

4. The chemical agents under investigation can also affect the results. Heavy metals have numerous mechanisms of action. Their toxicological properties vary depending on the compound, concentration, route of exposure, type of exposure (mixtures or single agents) and metabolism. Thus, results may vary because of the metal or metal-mixture analyzed. Moreover, it is important to consider that responses to metal stress may be influenced by other classes of chemical agents (e.g., polycyclic aromatic hydrocarbons) that may also occur in polluted environments. In such cases, reciprocal interactions, cascades, and indirect mechanisms can enhance or suppress the expected responses (Benedetti et al. 2007).

A number of researchers, who published the papers outlined in this review, measured metal concentrations in soil or water but did not measure the internal dose of the metals in tissues or organs of the exposed individuals (Duan et al. 2001; Peles et al. 2003; Berckmoes et al. 2005; Haimi et al. 2006; Jordaens et al. 2006; Matson et al. 2006; Athrey et al. 2007; Gardeström et al. 2008; Durrant et al. 2011). Internal metal concentrations may not have been measured because small body size (of the organisms involved) may have made such measurements difficult. In other studies, the internal dose, but not the external dose, was measured (Benton et al. 2002; Maes et al. 2005; Eeva et al. 2006; Bourret et al. 2008). However, both the internal dose and external doses were measured in several studies (Kim et al. 2003; Ungherese et al. 2010), although two of these studies do not specify the type of metals examined or the internal concentrations found (Ma et al. 2000; Yauk et al. 2000). Moreover, in the majority of the studies, the exposure conditions, such as the type and duration of the exposure, are not well characterized.

Despite these shortcomings, most authors report that metal-polluted environments affect the genetic structure of impacted animal populations. Bickham et al. (2000) suggested that the observed genetic effects are independent of the mechanism of action of the chemical agents involved. We think this assertion should be taken with caution, both because it is controversial and needs further analysis. Certainly, genetic structure effects may result from toxic exposures. However, it is yet to be established whether the accepted mechanism of toxic action of chemical agents are independent of observed genetic pattern effects for any given metal-exposed population. From the available studies reviewed, it is clear that the authors of future genetic ecotoxicological studies should better describe the chemical agents under study, and better detail the exposure conditions (external and internal metal concentrations).

The majority of studies performed during the last decade that have assessed population genetic responses have demonstrated adverse effects. In particular, populations inhabiting environments with higher levels of contamination have exhibited lower genetic diversity and population differentiation, lower reproductive success,

reduced adaptive potential and lower fitness. Therefore, it appears that there is a potential association between metal contamination and changes in the genetic structure of exposed populations (Table 1). Unfortunately, there are only a limited numbers of such studies, in which the genetic diversity of terrestrial ecosystems impacted by metal pollution, have been analyzed.

5 Genetic Markers for Assessing Genetic Variability in Environmentally Impacted Populations

The application of DNA sequencing and polymerase chain reaction-based technologies over the last 20 years has revolutionized the science of generating high throughput genetic markers (D'Surney et al. 2001).

Molecular markers are observable traits (their expression indicates the presence or absence of certain genes) that play an important role in estimating the genetic diversity among individuals by comparing the genotypes at a number of polymorphic loci (Arif and Khan 2009). A number of molecular markers have been applied to genetic ecotoxicological research, including nuclear and mitochondrial DNA analyses, such as allozymes, restriction fragment length polymorphisms (RFLPs), SSRs, RAPDs, the DNA sequencing of mtDNA, and AFLPs (Table 1).

One of the oldest techniques used to assess genetic variability in natural populations is to analyze the electrophoretic shifts in the charge characteristics of enzymes produced by amino acid substitutions, namely allozyme analysis. The majority of allozymes exhibit codominant inheritance and the variants are attributed to nucleotide substitutions that induce replacement of charged amino acids. This technique can detect one-third of amino acid substitutions. However, the generally low level of polymorphisms at allozyme loci often limits their resolving power for detecting population differences (Keane et al. 2005). Despite its limited resolution, allozyme analysis remains the simplest and most rapid technique for surveying genetic diversity in single-copy nuclear genes (Bickham et al. 2000).

The RFLP method uses restriction enzymes to detect variations in the primary structure of DNA. The number of bases in the restriction site and the genome-based composition determine the number of restriction sites. RFLP probes are usually loci and alleles defined by a specific probe–enzyme combination (Lowe et al. 2004). These markers are codominant, and a major advantage of RFLP probes is that they make it possible to detect DNA and organelle DNA polymorphisms in total DNA extracts. In addition, RFLP results are highly repeatable, and large amounts of variation can be detected. However, the RFLP method requires large quantities of DNA and only a limited number of suitable nDNA markers are available. Moreover, the detection of RFLPs is expensive and time-consuming (Lowe et al. 2004).

SSRs are widely used to analyze for genetic structure and variability. SSRs are short tandem repeats of mononucleotide to tetranucleotide repeats, which are assumed to be randomly distributed throughout the nuclear and mitochondrial genomes. The SSR method detects length variations that result from changes in the

number of repeated units, and their mode of inheritance is codominant. Mutations in SSRs are high compared with other DNA markers. SSRs are regarded to be one of the best molecular markers (Yauk and Quinn 1996; Athrey et al. 2007; Tremblay et al. 2008), due to their high mutation rates and high variability, which make them sensitive markers for analyzing genetic variability within and between populations. Unfortunately, identifying SSRs is expensive and requires cloning and sequencing. Although SSR primer pairs appear to be species-specific, cross-species amplification has been demonstrated, albeit with reduced variability being observed.

RAPDs utilize single decamer oligonucleotide primers to amplify regions of the genome by polymerase chain reaction (PCR). RAPD primers contain a random sequence and are relatively short, and many of them are used to sample the whole genome. Sites in the genome that are flanked by perfect or imperfect inverted repeats permit multiple annealing of the primers. The primer annealing sites occur throughout the genome, from single-copy DNA sequences to multiple-copy DNA sequences, and in coding and noncoding regions. RAPDs are cheap, simple to use, require no sequence information, and a large number of putative loci can be obtained when using them. However, there are numerous disadvantages associated with these molecular markers; RAPDs are dominant markers, meaning that they cannot distinguish heterozygotes from homozygotes at the phenotypic level, and their degree of reproducibility is low. Additionally, the primer structure, product competition, product homology, allelic variation, genome sampling, and non-independence of the loci are examples of other weaknesses associated with this methodology (Lowe et al. 2004; Arif and Khan 2009). To overcome these disadvantages, modifications to the technique have been proposed, such as sequence characterized amplified regions (SCARs) and randomly amplified microsatellite polymorphisms (RAMPO) (Lowe et al. 2004).

One of the most powerful tools in modern molecular population genetics is the nucleotide sequence analysis of mitochondrial DNA (mtDNA) (Bickham et al. 2000). The mitochondrial protein-coding regions are regarded to be powerful markers for genetic diversity analysis. The most studied of the mitochondrial genes for genetic diversity analyses include cytochrome b (cyt b), NADH dehydrogenase subunit 5, and mitochondrial cytochrome oxidase I (COI). Additionally, the highly polymorphic noncoding region of mtDNA, termed the control region (CR or D-loop), has been used in genetic diversity analyses because of its role in the replication and transcription of mtDNA. The D-loop region exhibits higher variation levels than the protein-coding regions because of the reduced functional constraints and the relaxed selection pressure. The advantages of the sequence approach include the ability to target different mitochondrial genes, thereby selecting for targets that have an appropriate evolutionary rate and higher resolution by revealing the nucleotide sequence. Moreover, an advantage of the PCR-RFLP analysis of mtDNA is that homozygosity and heterozygosity values and allele/genotype frequencies can be determined for the genetic loci analyzed (Bickham et al. 2000; D'Surney et al. 2001; Arif and Khan 2009).

AFLPs are multilocus markers that involve the selective amplification of a subset of restriction fragments generated by the digestion of DNA with restriction enzymes, followed by ligation to specific adapters. Similar to RAPDs, these markers are

dominant, although codominant AFLP markers may be detected because of small insertions or deletions in the restriction fragments (Lowe et al. 2004; Arif and Khan 2009). Compared with RAPDs and SSRs, AFLP markers can generate ten times the number of potential markers per genome (D'Surney et al. 2001). Comparing the results obtained from using SSRs, mtDNA, or AFLPs (Lucchini 2003) suggests that AFLPs could be very useful for evaluating genetic diversity. Because they are easily amplified in any species, AFLP markers may prove to be a valuable tool for estimating genetic diversity in animal populations.

All of the aforementioned molecular markers have applications in genetic ecotoxicology studies. Because none of the markers is ideal, marker choice should be based on the hypothesis that is being tested, the properties of the marker system, the organism under investigation, and the resources that are available for the research project.

6 Use of Sentinel Organisms for Genetic Ecotoxicological Studies

An important step in establishing links between pollution effects and population level responses is the utilization of sentinel organisms or bioindicator species. Sentinel organisms are a set of taxa that can be utilized to survey locales for increased mutation stressors (Yauk and Quinn 1996).

A variety of organisms have been studied for their potential to be biological indicators of different forms of chemical pollution. Certain species are known to be highly sensitive, either in their physiological response to contaminants, or by their ability to accumulate metals in a dose-dependent manner. These organisms respond to the environmental stress caused by one or more pollutants by changing their morphology and/or metabolism, and the nature of such changes are observable and measurable. For bioindicators to be sensitive, it is often necessary that the xenobiotic of interest be accumulated (Markert et al. 1999).

In many cases, sentinel species are used to assess risk to species that may be closely related evolutionarily or may occupy a similar niche within an ecosystem. In general, many species of wild animals (especially aquatic organisms) have been used as sentinel organisms in ecotoxicological studies with metals. Examples of sentinel species include mosquitofish (*Gambusia affinis*) (Roark et al. 2001), many isopod species, copepods, and gastropods (Ross et al. 2002; Storelli and Marcotrigiano 2005; Gardeström et al. 2008), earthworms (*Lumbricus rubellus*) (Peles et al. 2003), many nematode species (Ekschmitt and Korthals 2006), zebra mussels (*Dreissena polymorpha*) (Sues et al. 1997), garden snails (*Helix aspersa*) (Nedjoud et al. 2009), various species of sea birds (Burger and Gochfeld 2004), two crayfish species (*Austropotamobius pallipes* and *Pacifastacus leniusculus*) (Antón et al. 2000), and many species of prawns, mussels, and oysters (Ma et al. 2000; Ross et al. 2002; Storelli and Marcotrigiano 2005).

Although they are key components of ecosystems and occupy a variety of niches, few small mammalian species have been used as sentinel organisms. Small mammals are however attractive sentinel organismal candidates, because they are important nutrient recyclers, influence plant and insect communities, and serve as prey for numerous predators (Levengood and Heske 2008). Several adverse effects have been documented to occur in small mammals after chronic exposure to metals. Among these effects are teratogenesis, genotoxic-related diseases, and reproductive alterations (Baranski 1987; Talmage and Walton 1991; Sunderberg and Okarsson 1992; Eisler 1997; Husby et al. 1999; Bisser et al. 2004).

Other factors that make small mammals ideal for studying pollution effects are their wide geographical distribution and abundance, the fact that adults remain established in the same localized area, they exhibit generalized food habits, short life spans, and high reproductive rates, and they are easily captured (Talmage and Walton 1991; Pascoe et al. 1994; Laurinolli and Bendell-Young 2006; Levengood and Heske 2008). Moreover, small mammals play an important role in food chains and are considered to be intermediates for metal transfer to higher trophic levels (Talmage and Walton 1991; Levengood and Heske 2008). In addition, mammals accumulate metals in their tissues when they live in or near smelters (Anthony and Koslowski 1982; Smith and Rongstad 1982; Beyer et al. 1985; Ma et al. 1991; Beyer and Storm 1995; Levengood and Heske 2008), mine tailings (Cooke et al. 1990; Laurinolli and Bendell-Young 2006), and metal-processing industries (Johnson et al. 1978; Kisseberth et al. 1984).

Another advantage of using small mammals as sentinel species is our knowledge of their genome, which permits developing more than 100 polymorphic microsatellite markers (Mullen et al. 2006) to evaluate genetic structure parameters.

Many organisms are exposed to complex mixtures of contaminants that represent a broad spectrum of different compounds. Consequently, it is likely that, when compared with humans, many animal species have far higher exposure to these substances (Hebert and Murdoch-Luiker 1996), and therefore may be ideal models for surveys that attempt to quantify genotoxic, mutagenic, or ecotoxicological effects.

If we are to successfully predict ecosystem health effects, a multispecies approach for selecting sentinel organisms (different types of sentinels) is needed, and is more suitable for studying pollutant effects above the population level.

7 Conclusions and Future Perspectives

The greatest challenge in genetic ecotoxicology is to demonstrate a convincing link between contaminant effects and responses at higher levels of biological organization. The studies that have assessed biomarkers of genetic diversity in animal populations, as they relate to ecosystem health, are limited in number, and most of the information derived from such studies has focused on aquatic ecosystems. Moreover, a clear relationship between contaminant effects and population-level responses are often lacking, as are mechanistic explanations. Thus, the results of many studies demonstrate

correlation but not causation, which suggests that despite the fact that metal contamination is present, other factors are causing the differences in the mutation rates.

The goal is to ensure reproducible and reliable results, and to produce more accurate data for providing a deeper understanding of the relationship between metal exposure and alterations in the genetic diversity of impacted populations. To achieve this, we suggest that researchers include the following parameters in each future study they perform whenever possible:

1. Describe the chemical nature of each pollutant in detail. In addition, the metal concentrations that appear in soil, air or water must be assessed. It is also essential to identify each of the metals involved in an exposure, rather than only referring to "metal mixtures" or "sites with heavy metal pollution."
2. Supplement any ecotoxicology data on populations with data at the community and ecosystem level. Such data are important because indicators at different levels of biological organization provide different types of information essential to achieving a more robust ecological risk assessment (Clements 2000). The use of biomarkers for ecotoxicological studies has become a matter of priority and should be strengthened. It is particularly important in future studies to employ biomarkers for better assessing internal doses (metal concentration in tissues, organs, or biological fluids), early effects (genotoxicity assays), and susceptibility (genetic polymorphisms) in both somatic and germ cells. Integrating biomarkers into genetic ecotoxicology surveys will provide solid evidence of the ecological effects of pollution, because they may reflect metal bioaccumulation levels that exist in the population. Because of their prognostic properties, biomarkers are also useful for linking alterations at molecular and cellular levels with ecologically relevant responses.
3. Expand the use of sentinel organisms by utilizing different species in future studies. Many of the major principles underlying molecular or population genetic processes are conserved across all five kingdoms of living organisms. Therefore, it is feasible to extrapolate ecological effects that occur in a selected few species of model organisms (Theodorakis 2001), especially sentinel organisms, to all organisms. Moreover, using several sentinel organisms, a "multispecies approach," would enhance the ability to extrapolate results to higher levels of biological organization.
4. Under field conditions, the experimental design should include gradients of environmental metal contamination with several reference sites, in order to enhance the ability to identify cause–effect relationships.
5. Increase sampling of reference populations that are in close proximity to exposed populations. Such research will increase the possibility that the observed changes in the genetic structure and diversity of the exposed population are the result of exposure to a polluted environment. Sampling in close geographical proximity reduces the possibility that the observed changes between or among populations will result from phylogeographic processes.
6. Expand the use of genetic structure parameters to infer the fate of exposed populations. Bickham et al. (2000) suggested that, "because population genetic

changes are expected to be independent of the mechanisms of toxicity, and sensitive indicators of transgenerational effects, they represent the ultimate biomarker of effect." Because genetic changes, especially the loss of genetic variability, might be permanent (depending on the population size and mutation rates) once variability is lost, the population cannot recover to what it was before the environmental impact. Furthermore, strong evidence suggests that genetic population diversity may be a useful biomarker of ecosystem health. For these reasons, those engaged in this emerging field of study should concentrate on finding new biomarkers, namely "biomarkers of permanent effect." Genetic variability may be used as a "biomarker of permanent effect," which we define as "measures of changes or alterations in biological or/and chemical processes that once altered will not recover or will not be the same as they were originally" (as in the case of loss of genetic variability), and will result in permanent effects on populations.

7. Seek opportunities to move ecotoxicology and biomarker research toward a more holistic approach (Chapman 2002). One such opportunity is to utilize the power of genomics as a tool to improve the understanding of toxicant impact on natural populations. In this context, "ecotoxicogenomics" will benefit from the application of high-throughput technology, in which changes in the expression of hundreds to thousands of genes (genomics), proteins (proteomics), and metabolites (metabolomics) are assessed simultaneously. Such methodologies add value to classical whole-organism testing methods, because they provide information on the molecular basis of exposure, and act as "early warning" signs that permit both more accurate classification of chemical exposures, and better prediction of the mode of action and the development of novel biomarkers. These approaches are addressed in a number of recent publications (Poynton et al. 2007; Watanabe et al. 2008; Roh et al. 2009; Villenueve et al. 2012). Moreover, these methods provide a better understanding of how to extrapolate data from the laboratory to the field and from a few sentinel species to the whole-ecosystem (Lee et al. 2008).

Finally, because genetic variability is the basis for adaptation by natural selection and is one of the pillars of biodiversity and evolution (Anderson et al. 1994; Van Straalen and Timmermans 2002), attention must be paid to understanding the effects of xenobiotic exposure. Moreover, just demonstrating genetic, biochemical or physiological responses to toxicants may not be sufficient to protect wildlife from diversity loss or extinction; rather, a real effort must be undertaken to discover their effects on populations, communities, and ecosystems. Expanded interdisciplinary research, along with more detailed study designs, will be required to resolve the complex genetic ecotoxicology issues posed by environmental pollution.

8 Summary

Studying the genetic diversity of wild populations that are affected by pollution provides a basis for estimating the risks of environmental contamination to both wildlife, and indirectly to humans. Such research strives to produce both a better

understanding of the underlying mechanisms by which genetic diversity is affected, and the long-term effects of the pollutants involved.

In this review, we summarize key aspects of the field of genetic ecotoxicology that encompasses using genetic patterns to examine metal pollutants as environmental stressors of natural animal populations. We address genetic changes that result from xenobiotic exposure versus genetic alterations that result from natural ecological processes. We also describe the relationship between metal exposure and changes in the genetic diversity of chronically exposed populations, and how the affected populations respond to environmental stress. Further, we assess the genetic diversity of animal populations that were exposed to metals, focusing on the literature that has been published since the year 2000.

Our review disclosed that the most common metals found in aquatic and terrestrial ecosystems were Cd, Zn, Cu and Pb; however, differences in the occurrence between aquatic (Cd=Zn>Cu>Pb>Hg) and terrestrial (Cu>Cd>Pb>Zn>Ni) environments were observed. Several molecular markers were used to assess genetic diversity in impacted populations, the order of the most common ones of which were SSR's>allozyme>RAPD's>mtDNA sequencing>other molecular markers.

Genetic diversity was reduced for nearly all animal populations that were exposed to a single metal, or a mixture of metals in aquatic ecosystems (except in *Hyalella azteca*, *Littorina littorea*, *Salmo trutta*, and *Gobio gobio*); however, the pattern was less clear when terrestrial ecosystems were analyzed.

We propose that future research in the topic area of this paper emphasizes seven key areas of activity that pertain to the methodological design of genetic ecotoxicological studies. Collectively, these points are designed to provide more accurate data and a deeper understanding of the relationship between alterations in genetic diversity of impacted populations and metal exposures. In particular, we believe that the exact nature of all tested chemical pollutants be clearly described, biomarkers be included, sentinel organisms be used, testing be performed at multiple experimental sites, reference populations be sampled in close geographical proximity to where pollution occurs, and genetic structure parameters and high-throughput technology be more actively employed. Furthermore, we propose a new class of biomarkers, termed "biomarkers of permanent effect," which may include measures of genetic variability in impacted populations.

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References

Agarwal S, Sohal R (1994) DNA oxidative damage and life expectancy in houseflies. Proc Nat Acad Sci U S A 91:1232–1235

Anderson S, Sadinski W, Shugart L, Brussard P, Depledge M, Ford T, Hose J, Stegeman J, Suk W, Wirgin I, Wogan G (1994) Genetic and molecular ecotoxicology: a research framework. Environ Health Perspect 102:3–8

Anthony R, Koslowski R (1982) Heavy metals in tissues of small mammals inhabiting waste-water-irrigated habitats. J Environ Qual 11:20–22

Antón A, Serran T, Angulo E, Ferrero G, Rallo A (2000) The use of two species of crayfish as environmental quality sentinels: the relationship between heavy metal content, cell and tissue biomarkers and physico-chemical characteristics of the environment. Sci Total Environ 249:239–251

Arif I, Khan H (2009) Molecular markers for biodiversity analysis of wildlife animals: a brief review. Animal Biodiv Conser 32:9–17

Athrey N, Leberg P, Klerks P (2007) Laboratory culturing and selection for increased resistance to cadmium reduce genetic variation in the least killfish, *Heterandria Formosa*. Environ Toxicol Chem 26:1916–1921

Baker RJ, Bickham AM, Bondarkov M, Gaschak SP, Matson CW, Rodgers BE, Wickliffe JK, Chesser RK (2001) Consequences of polluted environments on population structure: the bank vole (*Clethrionomys glareolus*) at Chornobyl. Ecotoxicology 10:211–216

Baranski B (1987) Effect of cadmium on prenatal development and on tissue cadmium, copper, and zinc concentrations in rats. Environ Res 42:54–62

Barret S, Khon J (1991) Genetic and evolutionary consequences of small populations in plant: implications for conservation. In: Folk D, Holsinger K (eds) Genetics and conservation of rare plants. Oxford University Press Inc, New York, pp 3–30

Battaglia B, Bisol P, Rodino E (1980) Experimental studies on the genetic effects of the marine pollution. Helgol Meer 33:587–595

Belfiore N, Anderson S (1998) Genetic patterns as a tool for monitoring and assesment of environmental impacts: the example of genetic ecotoxicology. Environ Monit Assess 51:465–479

Belfiore N, Anderson S (2001) Effects of contaminants on genetic patterns in aquatic organisms: a review. Mutat Res 489:97–122

Benedetti M, Martuccio G, Fattorini D, Canapa A, Barucca M, Nigro M, Regoli F (2007) Oxidative and modulatory effects of trace metals on metabolism of polycyclic aromatic hydrocarbons in the Antarctic fish *Trematomus bernacchii*. Aquatic Toxicol 85:167–175

Benton M, Malott M, Trybula J, Dean D, Guttman S (2002) Genetic effects of mercury contamination on aquatic snail populations: allozyme genotypes and DNA strand breakage. Environ Toxicol Chem 21:584–589

Berckmoes V, Scheirs J, Jordaens K, Blust R, Backeljau T, Verhagen R (2005) Effects of environmental pollution on microsatellite DNA diversity in wood mouse (*Apodemus sylvaticus*) populations. Environ Toxicol Chem 24:2898–2907

Bernard A (2008) Biomarkers of metal toxicity in population studies: research potential and interpretation issues. J Toxicol Environ Health Part A 71:1259–1265

Bervoest L, Blust R (2003) Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. Env Pollut 126:9–19

Beyer W, Pattee O, Sileo L, Hoffman D, Mulhern B (1985) Metal contamination in wildlife living near two zinc smelters. Environ Pollut Series A 38:63–86

Beyer W, Storm G (1995) Ecotoxicological damage from zinc smelting at Palmerton, Pennsylvania. In: Hoffman DJ, Rattner BA, Burton GA, Cairns JC (eds) Handbook of ecotoxicology. CRC Press, Inc, Boca Raton, pp 596–608

Bickham J, Smolen M (1994) Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. Environ Health Perspect 102:25–28

Bickham J, Sandhu S, Hebert P, Chikhi L, Athwal R (2000) Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutat Res 463:33–51

Bisser J, Vogel L, Berger J, Hjelle B, Loew S (2004) Effects of heavy metals on immunocompetence of white-footed mice (*Peromyscus leucopus*). J Wildlife Dis 40:173–184

Bourret V, Couture P, Campbell P, Bernatchez L (2008) Evolutionary ecotoxicology of wild yellow perch (*Percha flavescens*) populations chronically exposed to a polymetallic gradient. Aquat Toxicol 86:76–90

Brown A, Hosken D, Balloux F, Bickley L, LePage G, Owen S, Hetheridge M, Tyler C (2009) Genetic variation, inbreeding and chemical exposure-combined effects in wildlife and critical considerations for ecotoxicology. Philos Trans R Soc Lond B Biol Sci 364:3377–3390

Burger J, Gochfeld M (2004) Marine birds as sentinels of environmental pollution. Ecohealth 1:263–274

Chagnon N, Guttman S (1989) Differential survivorship of allozyme genotypes in mosquitofish populations exposed to copper or cadmium. Environ Toxicol Chem 8:319–326

Chapman PM (2002) Integrating toxicology and ecology: putting the eco into cotoxicology. Mar Poll Bull 44:7–15

Chen JZ, Hebert PDN (1999) Terminal branch haplotype analysis: a novel approach to investigate newly arisen variants of mitochondrial DNA in natural populations. Mutat Res-DNA Repair 434:219–231

Clements W (2000) Integrating effects of contaminants across levels of biological organization: an overview. J Aquat Ecosyst Stress Recovery 7:113–116

Cooke J, Andrews S, Johnson M (1990) Lead, zinc, cadmium, and fluoride in small mammals from contaminated grassland established on fluorspar tailings. Water Air Soil Pollut 51:43–54

Coutellec M, Barata C (2011) An introduction to evolutionary processes in ecotoxicology. Ecotoxicology 20:513–523

Depledge M (1994) Genotypic toxicity: implications for individuals and populations. Environ Health Perspect 102:101–104

De Wolf H, Blust R, Backeljau T (2004) The population genetic structure of *Littorina littorea* (Mollusca: Gastropoda) along a pollution gradient in the Scheldt estuary (The Netherlands) using RAPD analysis. Sci Total Environ 325:59–69

Diamond S, Newman M, Mulvey M, Dixon P, Martinson D (1989) Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Baird and Girard), during acute exposure to inorganic mercury. Environ Toxicol Chem 8:613–622

Diamond S, Newman M, Mulvey M, Guttman S (1991) Allozyme genotype and time-to-death of mosquitofish, *Gambusia holbrooki*, during acute inorganic mercury exposure: a comparison of populations. Aquat Toxicol 21:119–134

Dimsoski P, Toth G (2001) Development of DNA-based microsatellite marker technology for studies of genetic diversity in stressor impacted populations. Ecotoxicology 10:229–232

D´Surney S, Shugart L, Theodorakis C (2001) Genetic markers and genotyping methodologies: an overview. Ecotoxicology 10:201–204

Duan Y, Guttman S, Oris J, Bailer A (2001) Differential survivorship among allozyme genotypes of *Hyalella azteca* exposed to cadmium, zinc or low pH. Aquat Toxicol 54:15–28

Durrant CJ, Stevens JR, Hogstrand C, Bury NR (2011) The effect of metal pollution on the population genetic structure of brown trout (*Salmo trutta* L.) residing in the River Hayle, Cornwall, UK. Environ Pollut 159:3595–3603

Eeva T, Belskii E, Kuranov B (2006) Environmental pollution affects genetic diversity in wild bird populations. Mutat Res 608:8–15

Ehrenfeld D (1992) Ecosystem health and ecological theories. In: Norton BG, Heskell B (eds) Ecosystem health. New goals for environmental management. Island Press, Washington, DC, pp 135–143

Eisler R (1997) Zinc hazards to plants and animals with emphasis on fishery and wildlife resources. In: Chemisinoff PN (ed) Ecological issues and environmental impact assessment. Gulf Publ Co, Houston, pp 443–537

Ekschmitt K, Korthals G (2006) Nematodes as sentinels of heavy metals and organic toxicants in the soil. J Nematol 38:13–19

Endler J (1995) Multiple-trait coevolution and environmental gradients in guppies. Trends Ecol Evol 10:22–29

EPA Environmental Protection Agency (2000) Innovative remediation technologies: field scale demonstration projects in North America.

Evenden AJ, Depledge MH (1997) Genetic susceptibility in ecosystems: the challenge for ecotoxicology. Environ Health Perspect 105:849–854

Fratini S, Zane L, Ragionieri L, Vannini M, Cannicc S (2008) Relationship between heavy metal accumulation and genetic variability decrease in the intertidial crab *Pachygrapsus marmoratus* (Decapoda; Grapsidae). Est Coast Shelf Sci 79:679–686

Gardeström J, Dahl U, Kotsalainen O, Maxson A, Elfwing T, Grahn M, Bengtsson B, Breitholtz M (2008) Evidence of population genetic effects of long-term exposure to contaminated sediments: a multi-endpoint study with copepods. Aquat Toxicol 86:426–436

Gillespie R, Guttman SI (1989) Effects of contaminants on the frequencies of allozymes in populations of the central stoneroller. Environ Toxicol Chem 8:309–317

Guttman S (1994) Population genetic structure and ecotoxicology. Environ Health Perspect 102:97–100

Haimi J, Knott K, Selonen S, Laurikainen M (2006) Has long-term metal exposure induced changes in life history traits and genetic diversity of the enchtraeid worm *Cognettia sphagnetorum*. Environ Poll 140:463–470

Harper-Arabie R, Wirth E, Fulton M, Scott G, Ross P (2004) Protective effects of allozyme genotype during chemical exposure in the grass shrimp, *Palaemonetes pugio*. Aquat Toxicol 70:41–54

Hebert PM, Murdoch-Luiker M (1996) Genetic effects of contaminant exposure-towards an assessment of impacts on animal populations. Sci Total Environ 191:23–58

Hoffman A, Daborn P (2007) Towards genetic markers in animal populations as biomonitors for human-induced environmental change. Ecol Lett 10:63–76

Hoffmann A, Willi Y (2008) Detecting genetic responses to environmental change. Nat Rev Genet 9:421–429

Husby M, Hausbeck J, McBee K (1999) Chromosomal aberrancy in white-footed mice (*Peromyscus leucopus*) collectd on abandoned coal strip mines, Oklahoma, USA. Environ Toxicol Chem 18:919–925

Johnson M, Roberts R, Hutton M (1978) Distribution of lead, zinc, and cadmium in small mammals from polluted environments. Oikos 30:153–159

Jordaens K, De Wolf H, Van Houtte N, Vandecasteele B, Backeljau T (2006) Genetic variation in two land snails Cepaea nemoralis and Succinea putris (Gastropoda, Pulmonata), from sites differing in heavy metal content. Genetica 128:227–239

Keane B, Collier M, Rogstad S (2005) Pollution and genetic structure of North American populations of the common dandelion (*Taraxacum officinale*). Environ Monit Assess 105:341–357

Kim S, Rodriguez M, Suh J, Song J (2003) Emergent effects of heavy metal pollution at a population level: *Littorina brevicula* a study case. Mar Pollut Bull 46:74–80

Kisseberth W, Sunderberg J, Nyboer R, Reynolds J, Kasten S, Beasley V (1984) Industrial lead contamination of an Illinois wildlife refuge and indigenous small mammals. J Am Vet Med Assoc 185:1309–1313

Laurinolli M, Bendell-Young L (2006) Copper, zinc, and cadmium concentrations in *Peromyscus maniculatus* sampled near an abandoned copper mine. Environ Contam Toxicol 30:481–486

Lee C, Newman M, Mulvey M (1992) Time to death of mosquitofish (*Gambusia holbrooki*) during acute inorganic mercury exposure: population structure effects. Arc Environ Contam Toxicol 22:284–287

Lee JS, Raisuddin S, Schlenk D (2008) Kryptolebias marmoratus (Poey 1880): a potential model species for molecular carcinogenesis and ecotoxicogenomics. J Fish Biol 72:1871–1889

Levengood J, Heske E (2008) Heavy metal exposure, reproductive activity, and demographic patterns in white-footed mice (*Peromyscus leucopus*) inhabiting a contaminated wetland. Sci Total Environ 389:320–328

Loveless M, Hamrick J (1984) Ecological determinants of genetic structure in plant population. Annu Rev Ecol Syst 15:65–95

Lowe A, Harris S, Ashton P (2004) Ecological genetics: design, analysis and application. Blackwell Publishing, Oxford, 313 p

Lucchini V (2003) AFLP: a useful tool for biodiversity conservation and management. Comptes Rendus Biol 326:43–48

Lynch M, Conery U, Bürger R (1995) Mutation accumulation and the extinction of small population. Am Nat 146:489–518

Ma W, Denneman W, Faber J (1991) Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. Arch Environ Contam Toxicol 20:266–270

Ma X, Cowles D, Carter R (2000) Effect of pollution on genetic diversity in the Bay Mussel *Mytilus galloprovincialis* and the acorn bernacle *Balanus gladula*. Mar Environ Res 50:559–563

Maes G, Raeymaekers J, Pampoulie C, Seynaeve A, Goemans G, Belpaire C, Volckaert F (2005) The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: relationship between heavy metal bioaccumulation, condition and genetic variability. Aquat Toxicol 73:99–114

Markert B, Wappelhorst O, Weckert V, Herpin U, Siewers U, Friese K, Breulmann G (1999) The use of bioindicators for monitoring the heavy-metal status of the environment. J Radioanal Nucl Chem 240:425–429

Matson C, Lambert M, McDonald T, Autenrieth R, Donnelly K, Islamzadeh A, Politov D, Bickham J (2006) Evolutionary toxicology: population-level effects of chronic contaminant exposure on the marsh frogs (*Rana ridibunda*) of Azerbaijan. Environ Health Perspect 114:547–552

Medina M, Correa J, Barata C (2007) Micro-evolution due to pollution: possible consequences for ecosystem responses to toxic stress. Chemosphere 67:2105–2114

Moore M, Depledge M, Readman J, Leonard D (2004) An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. Mutat Res 552:247–268

Morgan AJ, Killie P, Sturzenbaum SR (2007) Microevolution and ecotoxicology of metals in invertebrates. Environ Sci Technol 41:1085–1096

Mullen L, Hirschmann R, Prince K, Glenn T, Dewey M, Hoekstra H (2006) Sixty polymorphic microsatellite markers for the oldfield mouse developed in *Peromyscus polionotus* and *Peromyscus maniculatus*. Mol Ecol Notes 6:36–40

Nedjoud G, Houria B, Rachid R, Amira D, Reda D (2009) Impact of pollution by industrial metallic dust on bio-accumulator organism *Helix aspersa*. Global Vet 3:276–280

Nevo E, Shimony T, Libni M (1978) Pollution selection of allozyme polymorphisms in Barnacles. Experientia 34:1562–1564

Nevo E, Lavie B, Ben-Shlomo R (1983) Selection of allelic isozyme polymorphisms in marine organisms: patern, theory and application. In: Ratazzi MC, Scandalios JC, Whitt GS (eds) Isozymes: current topics in biological and medical research. Genetics and Evolution AR Liss, New York, pp 69–92

Newman M, Diamond S, Mulvey M, Dixon P (1989) Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Baird and Girard) during acute toxicant exposure: a comparison of arsenate and inorganic mercury. Aquat Toxicol 15:141–156

Pascoe G, Blanchet R, Liner G (1994) Bioavailability of metals and arsenic to small mammals at a mining waste-contaminated wetland. Arch Environ Contam Toxicol 27:44–50

Patarnello T, Guinez R, Battaglia B (1991) Effects of pollution on heterozygosity in the bernacle *Balanus amphitrite* (Cirripedia: Thoracica). Mar Ecol Prog Ser 70:237–243

Peles JD, Towler WI, Guttman SI (2003) Population genetic structure of earthworms (*Lumbricus rubelluz*) in soils contaminated by heavy metals. Ecotoxicology 12:379–386

Poynton H, Varshavky J, Chang B, Cavigiolio G, Chan S, Holman P, Loguinov A, Bauer D, Komachi K, Theil E, Perkins E, Hughes O, Vulpe C (2007) Daphnia magna ecotoxicogenomics provides mechanistic insights into metal toxicity. Environ Sci Technol 47:1044–1050

Roark S, Andrews J, Guttman S (2001) Population genetic structure of the western mosquitofish *Gambusia Affinis*, in a highly channelized portion of the San Antonio River in San Antonio, TX. Ecotoxicology 10:223–227

Rogstad S, Keane B, Collier M (2003) Minisatellite DNA mutation rate in dandelions increases with leaf-tissue concentrations of Cr, Fe, Mn, and Ni. Environ Toxicol Chem 22:2093–2099

Roh J, Sim SJ, Yi J, Park K, Chun K, Ryu D, Choi J (2009) Ecotoxicity of silver nanoparticles on the soil nematode Caenorhabditis elegans using functional ecotoxicogenomics. Environ Sci Technol 43:3933–3940

Ross K, Cooper N, Bidwell J, Elder J (2002) Genetic diversity and metal tolerance of two marine species: a comparison between populations from contaminated and reference sites. Mar Poll Bull 44:671–679

Scheirs J, De Coen A, Covaci A, Beernaert J, Kayawe V, Caturla M, De Wolf H, Baert P, Van Oostveldt P, Verhagen R, Blust R, De Coen W (2006) Genotoxicity in wood mice (*Apodemus sylvaticus*) along a pollution gradient: exposure age and gender related effects. Environ Toxicol Chem 25:2154–2162

Smith G, Rongstad O (1982) Small mammal heavy metal concentrations from mined and control sites. Environ Poll 28:121–134

Staton J, Schizas N, Chandler G, Coull B, Quattro J (2001) Ecotoxicology and population genetics: the emergence of "phylogeographic and evolutionary ecotoxicology". Ecotoxicology 10:217–222

Storelli M, Marcotrigiano G (2005) Bioindicator organisms: heavy metal pollution evaluation in the ionian sea (Mediterranean Sea-Italy). Environ Monit Assess 102:159–166

Sues B, Tarashewski H, Rydlo M (1997) Intestinal fish parasites as heavy metal bioindicators: a comparison between *Acanthocephalus lucci* and zebra mussel, *Dressena polymorpha*. Bull Environ Contam Toxicol 59:14–21

Sunderberg J, Okarsson A (1992) Placental and lactational transfer of mercury from rats exposed to methylmercury in their diet: speciation of mercury in the offspring. J Trace Elem Exp Med 5:47–56

Talmage S, Walton B (1991) Small mammals as monitors of environmental contaminants. Rev Environ Contam Toxicol 119:47–145

Theodorakis C, Swartz C, Rogers W, Bickham J, Donnely K, Adams S (2000) Relationship between genotoxicity, mutagenicity, and fish community structure in a contaminated stream. J Aquat Ecosyst Stress Recovery 7:131–143

Theodorakis C (2001) Integration of genotoxic and population genetic endpoints in biomonitoring and risk assessment. Ecotoxicol 10:245–256

Theodorakis CW, Bickham JW, Lamb T, Medica PA, Barret LT (2001) Integration of genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the nevada test site, USA. Environ Toxicol Chem 20:317–326

Tremblay A, Lesbarreres D, Merritt T, Wilson C (2008) Genetic structure and phenotypic plasticity of yellow perch (*Perca flavescens*) populations influences by habitat, predation, and contamination gradients. Integr Environ Assess Manag 4:264–266

Ungherese G, Mengoni A, Somigli S, Baroni D, Focardi S, Ugolini A (2010) Relationship between heavy metals pollution and genetic diversity in Mediterranean population of the sandhopper *Talitrus saltator* (Montagu) (Crustaceae, Amphipoda). Environ Pollut 158:1638–1643

Van Straalen N (1999) Genetic biodiversity in toxicant-stressed populations. Prog Environ Sci 1:195–201

Van Straalen N, Timmermans M (2002) Genetic variation in toxicant-stressed populations: an evaluation of the "genetic erosion" hypothesis. Hum Ecol Risk Assess 8:983–1002

Villenueve D, Garcia-Reyero N, Escalon B, Jensen K, Cavallin J, Makynen E, Durhan E, Kahl M, Thomas L, Perkins E, Ankley G (2012) Ecotoxicogenomics to support ecological risk assessment: a case study with bisphenol A in fish. Environ Sci Technol 46:51–59

Watanabe H, Kobayashi K, Kato Y, Oda S, Abe R, Tatarazako N, Iguchi T (2008) Transcriptome profiling in crustaceans as a tool for ecotoxicogenomics. Cell Biol Toxicol 24:641–647

Weinstein I (1988) The origins of human cancer: molecular mechanism of carcinogenesis and their implications for cancer prevention and treatment. Cancer Res 48:4135–4143

WHO. World Health Organization (2007) Health risks of heavy metals from long range transboundary air pollution, European Committee

Yauk C, Quinn J (1996) Multilocus DNA fingerprinting reveals high rate of heritable genetic mutation in herring gulls nesting in an industrialized urban site. Proc Natl Acad Sci U S A 93: 12137–12141

Yauk C, Fox G, McCarry B, Quinn J (2000) Induced minisatellite germline mutations in herring gulls (*Larus argentatus*) living near steel mills. Mutat Res 452:211–218

Bioamplification as a Bioaccumulation Mechanism for Persistent Organic Pollutants (POPs) in Wildlife

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Contents

1 Introduction

Bioaccumulation describes the process by which anthropogenic chemicals are taken up by organisms from their environment and diet and are subsequently assimilated and distributed into tissues (Arnot and Gobas 2003; Borgå et al. 2004; Mackay and Fraser 2000). Thus, bioaccumulation is a central framework within ecotoxicology, because it

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helps define the maximum concentration that can be achieved by an organism in its tissues, relative to the exposure media, and helps determine the potential chemical dose/toxicity experienced by an individual. Therefore, understanding the dynamic processes that regulate chemical bioaccumulation in animals is essential for protecting species, ecosystems, and ultimately human health (Arnot and Gobas 2004; Kelly et al. 2004).

The rationale for studying the bioaccumulation of persistent organic pollutants (POPs) derives mainly from the high potential some organisms (and associated food webs) have to bioaccumulate them. Several reviews have been published on the bioaccumulation of POPs in biota, and the approaches used to model their bioaccumulation (Barber 2003, 2008; Connell 1988; Gobas 1993a; Gobas and Morrison 2000; Mackay and Fraser 2000; Nichols et al. 2009; Selck et al. 2012; Thomann 1981; Thomann et al. 1992; Walker 1990). State of the art POP bioaccumulation models continue to evolve and now incorporate many diverse concepts. These concepts include: hydrophobicity-driven equilibrium partitioning (DeBruyn and Gobas 2007; Gobas et al. 1986; Hamelink et al. 1971; Mackay 1982, Neely et al. 1974; Veith et al. 1979), bioavailability constraints related to chemical sequestration in abiotic organic and inorganic carbon matrices (Black and McCarthy 1988; Cornelissen et al. 2005; Lohmann et al. 2005), biomagnification related to chemical exposure from food, and complex food web feeding relationships (Alonso et al. 2008; Arnot and Gobas 2004; Borgå et al. 2012; Campfens and Mackay 1997; Gobas et al. 1993b; Morrison et al. 1997; Thomann et al. 1992), and biological vectors as sources of POPs introduction to and from ecosystems (Ewald et al. 1998; Gregory-Eaves et al. 2007; Krummel et al. 2003). More recently, biological mechanisms across a given species' life cycle have also been described (Hickie et al. 1999, 2005, 2007; Ng and Gray 2009; Sijm et al. 1992; Yordy et al. 2010; Zhao et al. 2007).

The focus of this review is to draw attention to a non-steady state, non-equilibrium mechanism of bioaccumulation, herein described as bioamplification. In this paper, we apply bioamplification to POPs. The term bioamplification has recently been coined to define the condition in which an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate those chemicals (Daley et al. 2009, 2011). This causes an increase in the chemical concentration in the organism and in its tissues, when concentrations are expressed on a wet weight basis. A much greater increase in the concentration of chemical occurs, when tissue residues are expressed on a lipid-normalized basis. Although the term bioamplification has been used previously in ecotoxicology to describe mercury biomagnification (Potter et al. 1975), and is sometimes used as the French translation of biomagnification, the past use of this term has been largely superseded by "biomagnification" (Connolly and Pedersen 1988). Notably, bioamplification is mechanistically distinguished from other bioaccumulation mechanisms (e.g., bioconcentration and biomagnification, see Sect. 2 below) in that concentrations of chemical become enriched in the organism without a change in the chemical mass balance in the animal, or when the concentration enrichment exceeds the mass increase of a chemical. MacDonald et al. (2002) explained this process as being analogous to solvent depletion and one of several mechanisms (e.g., biomagnification) that contributes to "amplification" of chemical residues in organism tissues. Although bioamplification

is an independent bioaccumulation process that interacts with both bioconcentration and biomagnification processes, it is rarely acknowledged or specifically addressed in risk assessments or in the bioaccumulation modelling literature.

2 Bioamplification as an Independent Bioaccumulation Mechanism

The POPs literature has classically defined bioaccumulation as being contributed to by two main processes: bioconcentration and biomagnification. Both of these processes are individually defined by how chemical exposures to the animal occur (see below). The two processes are commonly distinguished from one another on the basis that bioconcentration is often modeled as an equilibrium partitioning process (Barber 2003; Mackay 1982), whereas biomagnification is modeled as a non-equilibrium kinetic process (Gobas et al. 1988). For clarity, the differences between bioconcentration, biomagnification, and bioamplification are defined and described below.

Bioconcentration describes the diffusive transport of chemicals across respiratory surfaces (Barber et al. 1988; Barber 2003; Leblanc 1995; Neely et al. 1974). Through bioconcentration, the organism accumulates and eventually equilibrates with its respired media via respiratory exchange and can approach or achieve a chemical fugacity similar to its respired media (Di Toro et al. 1991; Landrum et al. 2001). Chemical fugacity is defined by the criteria for establishing chemical equilibrium in reference to both the chemical concentration and partitioning capacity of the sample (Mackay 1979; Schwarzenbach et al. 1993). For biological samples, chemical fugacity (Pa) is calculated as the chemical concentration (C_{org}, mol/m^3) divided by the equilibrium distribution coefficient for the chemical of interest between the sample and air (Z_{org}, mol/m^3/Pa) (Mackay 1979; Mackay and Paterson 1981). When the chemical fugacity is equal between two interacting environmental media, the samples are considered to be in equilibrium with one another. This consideration of chemical equilibrium cannot be deduced by direct comparison of wet weight chemical concentrations. However, kinetic-based bioconcentration models can be used to describe the approach of chemical fugacity toward equilibrium in an organism relative to its respired media.

Equilibrium partitioning-based bioconcentration models have shown a high degree of success for predicting POP bioaccumulation in laboratory bioconcentration tests, when water is the predominant exposure route (Di Toro et al. 1991; Landrum et al. 2001; Mackay 1982; Meylen et al. 1999). Under field conditions, the equilibrium partitioning model appears to be best suited to negligibly biotransformed hydrophobic organic chemicals over a log K_{OW} range of approximately 3–5 (Barber 2008; Gobas and Morrison 2000; Meylen et al. 1999). Several comprehensive reviews of, and modelling approaches to bioconcentration have been completed (Arnot and Gobas 2006; Barber 2003; Barron 1990; Connell 1988; Devillers et al. 1998; Gobas and Morrison 2000; Mackay and Fraser 2000). Bioconcentration can be complicated when organisms respire water from both overlying and pore waters that are associated

with sediments (DiToro et al. 1991), and when these different water sources exhibit differences in chemical fugacity (DeBruyn and Gobas 2004). Furthermore, the bioconcentration process is complicated by the presence of constituents in water and sediments (viz., suspended solids, dissolved organic matter, and/or black carbon) that alter chemical bioavailability and the freely dissolved concentrations of chemical in the respired media (Black and McCarthy 1988; Cornelissen et al. 2005).

Biomagnification is a non-equilibrium bioaccumulation process, and is commonly modeled under steady state conditions (Drouillard 2008). Biomagnification occurs when chemical exposures occur via ingestion of contaminated food (Connolly and Pedersen 1988). For negligibly biotransformed, highly hydrophobic chemicals, biomagnification can result in the chemical fugacity of an animal exceeding that of its food (DeBruyn and Gobas 2006; Gobas et al. 1993b; Kelly et al. 2007a). This can translate into elevated chemical fugacities in the organism, compared to the respired environmental media (Connolly and Pedersen 1988; Morrison et al. 1997). The relative importance of bioconcentration and biomagnification to the uptake and overall chemical bioaccumulation potential varies and depends on several factors that include the following: chemical hydrophobicity, chemical elimination rates, differences in chemical fugacity between ingested food and respired media, and whether the chemical fugacity in the animal is well below, approaching, or exceeds the chemical fugacity in its respired media. The increased chemical fugacity in organisms resulting from contaminated food exposures can further propagate through successive trophic levels in a food web, and produce non-equilibrium food web biomagnification (Connolly and Pedersen 1988). Food web biomagnification of POP compounds has been widely demonstrated to occur for multiple animal species from both aquatic (Oliver and Niimi 1988; Russell et al. 1999a) and terrestrial (Czub and McLachlan 2004; Kelly and Gobas 2001, 2003; McLachlan 1996) food webs. A number of models have described the biomagnification of POPs in organisms and food webs (Arnot and Gobas 2004; Drouillard et al. 2012; Gobas et al. 1988, 1999; Kelly et al. 2004; Mackay and Fraser 2000; McLachlan 1996; Schlummer et al. 1998).

Both bioamplification and biomagnification can be empirically distinguished from bioconcentration as mechanisms that raise the chemical fugacity of the organism above that of its respired media and that of its food. Bioamplification is distinguished from biomagnification in that the chemical fugacity in an animal is increased without a change in the chemical uptake rate into the organism. This specifically occurs when the animal experiences a rapid decrease in the partitioning capacity of its tissues at a rate that exceeds the chemical elimination rate. The result is that the chemical fugacity of the organism experiences an increase, even though the total mass of chemical in the organism does not change, or, if it does change, it does so to a lesser extent than the fugacity change that was measured. Therefore, bioamplification occurs when there is a shift from steady state to non-steady state conditions as precipitated by a rapid weight loss event. Bioamplification may also occur during the uptake portion of the non-steady state bioaccumulation curve. In this case, bioamplification increases the chemical fugacity of the animal over what would be normal for a non-steady state uptake trajectory, if no change in partitioning capacity was experienced by the animal. Bioamplification is more difficult to distinguish during

the uptake phase of the non-steady state bioaccumulation process. Properly defining bioamplification under this scenario requires simultaneous determination of partitioning capacity changes, as well as characterizing changes in the mass balance of chemical in the animal. As in the steady state case above, bioamplification is verified when the change in chemical fugacity of the animal over a period of time exceeds the change in chemical mass balance in the animal over the same time interval.

A chief characteristic of bioamplification is that it results from weight loss. Negative growth in an organism and reduced partitioning capacity for the chemical under study are specific characteristics of bioamplification. Although growth dilution has long been adopted within bioaccumulation models, growth is commonly assumed to be constant and positive (Clark et al. 1990; Gobas 1993a). Notwithstanding, growth is known to be highly dynamic over an animal's life cycle, and is highly influenced by ecological and physiological factors (Blais et al. 2003; Chiuchiolo et al. 2004; Czub and McLachlan 2004; DeBruyn and Gobas 2006; Hickie et al. 1999; MacDonald et al. 2002; Ng and Gray 2009; Norstrom et al. 2007). Growth dilution (or biodilution) becomes a non-steady state process when the growth rate of an organism changes over a period of time that is shorter than the time required for the animal to re-achieve steady state with a chemical in its environment (McLachlan 1996). Although it has not been well studied, many animal species are known to lose weight during certain periods of their life histories. These weight loss events drive bioamplification, which represents the opposite of growth dilution (Clark et al. 1990; Kelly et al. 2004). Unless the animal dies, weight loss is predominantly a temporary condition, and is therefore often ignored or negated in bioaccumulation models. Therefore, bioamplification is not typically considered in steady state bioaccumulation models, because weight loss is not a component of the model algorithms. Arguably, excluding weight loss from bioaccumulation models has reduced interest in evaluating bioamplification as a separate phenomenon in natural systems (Gabrielsen et al. 1995; MacDonald et al. 2002).

Bioamplification, bioconcentration, and biomagnification are attenuated by chemical elimination. Chemical elimination restricts the types of chemicals and organisms for which bioamplification is likely to occur. Although bioamplification will always occur during weight loss events, the extent depends on the rate of chemical elimination and the loss of partitioning capacity. Bioamplification will be maximized for POPs in aquatic food webs that (1) exhibit high hydrophobicity, because hydrophobicity inversely correlates with chemical elimination (Kelly et al. 2007a; Paterson et al. 2007ab), and (2) for chemicals that undergo little or no metabolic biotransformation (Boon et al. 1989, 1994; Rasmussen et al. 1990; Safe 1994). Consequently, bioamplification will be most prominent for chemicals that have high log K_{OW} (>6.5) values, and exhibit food web biomagnification (Clark et al. 1990). For terrestrial food webs, chemicals that possess a log K_{OW} >2 and a log K_{OA} (octanol–air partition coefficient) >6 should be regarded as having a high bioamplification potential, because they display slow respiratory elimination (Drouillard et al. 2012; Kelly et al. 2007a). From the organismal perspective, bioamplification is expected to occur in those species that exhibit: (1) pronounced weight or lipid loss at specific times during their life cycles and (2) slow elimination kinetics of chemicals relative

to the time frame required to produce weight loss. In the latter case, the extent of bioamplification achieved by an individual is expected to be correlated with animal body size, inversely related to the metabolic biotransformation capacity of the animal and, in most cases, be greater for terrestrial than aquatic organisms (Drouillard and Norstrom 2000; Fisk et al. 1998; Kelly and Gobas 2001, 2003; Kelly et al. 2007a; Paterson et al. 2007b).

3 Measurement of Bioamplification

The manner in which bioamplification is characterized is similar to how chemical bioconcentration factors (BCF), bioaccumulation factors (BAF), and biomagnification factors (BMF) are quantified. In particular, the degree of bioamplification is characterized by expressing the ratio of chemical fugacity in an animal ($f_{\mathrm{org}(t)}$) relative to a reference state. Specifically, the reference state refers to the chemical fugacity of the animal prior to the weight loss event ($f_{\mathrm{org}(t-1)}$). Hence, the bioamplification factor (BAmF) in fugacity notation is defined as:

$$BAmF = \frac{f_{org(t)}}{f_{org(t-1)}} \tag{1}$$

Alternatively, bioamplification can be expressed as the ratio of lipid-normalized or lipid-equivalent chemical concentrations for the animal post versus pre-weight loss events (see below).

If the animal has achieved steady state with its environment prior to the initial sampling event, a BAmF >1 provides evidence that bioamplification has occurred. Bioamplification can be further confirmed if both the BAmF >1 and the mass balance of chemical in the animal has not changed. Under non-steady state uptake conditions, the BAmF will always exceed 1, even when bioamplification does not occur. Under these circumstances, bioamplification can only be distinguished from bioconcentration and biomagnification by factoring in both the chemical mass balance in the animal and the BAmF ratio. When the magnitude of the BAmF is >1 and also exceeds the change in chemical mass balance in the organism, both following and prior to weight loss, bioamplification can be regarded to have occurred. Finally, under conditions of net chemical elimination, which may occur after an animal switches to a less contaminated diet, BAmFs >1 are always indicative of bioamplification. Since the status of an organism (i.e., steady state, non-steady state net uptake, or non-steady state net depuration) is rarely known when samples are collected in the field, BAmFs should generally be interpreted in conjunction with the chemical mass balance determined in the animal over the same time interval.

BAmFs are best described as changes in chemical fugacity, but lipid-normalized and lipid-equivalent ratios may be used as surrogate measures for chemical fugacity. Differences in the partitioning capacity of animals and their respective tissues are commonly calculated on the basis of the lipid content of the sample (Mackay and

Paterson 1981). Thus, correcting or normalizing for lipid content standardizes the fugacity capacity of different biological sample types. This normalization permits the comparison of relative chemical fugacities between differing sample types (Clark et al. 1988, 1990; Mackay 1991). BAmFs, expressed as the ratio of lipid-normalized concentrations in an animal at two time intervals, are expressed as:

$$BAmF = \frac{C_{org(t)}}{C_{org(t-1)}} \times \frac{X_{lipid(t-1)}}{X_{lipid(t)}} \tag{2}$$

Where $C_{org(t)}$, $C_{org(t-1)}$ are the chemical concentrations (ng/g wet weight) in the animal at the two time intervals, and $X_{lipid(t)}$, and $X_{lipid(t-1)}$ refer to the mass fraction of lipid (g/g body weight) in the animal at each time interval, respectively.

Bioamplification is most often a direct consequence of lipid loss. Consequently, when studying this phenomenon it is important to accurately quantify lipids. However, it is important to note that the lipid content of tissue samples is usually operationally defined by the analytical method used to determine the lipids. Lipids are usually extracted from tissue samples with solvents that are then evaporated, and then subjected to gravimetric analysis (Drouillard et al. 2004; Randall et al. 1998). Since solvent combinations used to extract lipids vary among studies, it is important to maintain consistency of method for lipid analysis in any comparative study. However, some lipid determination methods provide better surrogate measures of partitioning capacity than others. For example, Randall et al. (1998) recommended that the Bligh and Dyer (1959) technique, which uses a chloroform–methanol solvent mixture for total lipid extraction, be used for lipid normalization, because this method co-extracts both polar and neutral lipids (McElroy et al. 2011). Drouillard et al. (2004) compared lipid extractions from chloroform–methanol and dichloromethane–hexane in fish tissues and found the two methods to produce different lipid results. However, when the authors compared lipid-normalized POP concentrations between tissues of individual fish, it was found that the dichloromethane–hexane procedure best compensated for differences in tissue partitioning capacities. Specifically, lipid-normalized POP concentrations between tissues were lower, when lipids from the dichloromethane–hexane extraction procedure were utilized. The authors concluded that solvent mixtures that extract neutral rather than total lipids are most appropriate when the lipid result is used to measure POP partitioning capacity of tissues. Unfortunately, lipid determination methods are rarely standardized across studies, and most typically utilize the same solvent combinations as for chemical extraction.

More recently, researchers have recognized that neutral lipids are not the only contributors to partitioning capacity of hydrophobic POPs in animal tissues. Biological sample partitioning capacities can be underestimated when the lipid content of the sample drops below 1% (DeBruyn and Gobas 2007; Gobas et al. 1999). Gobas et al. (1999) defined non-lipid organic matter (NLOM), essentially referring to lean dry protein (LDP) content, as having a partitioning capacity of approximately 3% of that provided by neutral lipids. DeBruyn and Gobas (2007) later revised this estimate to define the partitioning capacity of lean dry matter as having 5% of the partitioning capacity of neutral lipid. For organisms or tissue samples having low lipid content, the lipid-equivalent concentration is suggested to better

represent chemical fugacity than lipid normalized concentrations. However, obtaining estimates of NLOM or LDP requires analyzing both the neutral lipid content and moisture content of the sample. Expressing the BAmF on a lipid-equivalents basis is calculated as follows:

$$BAmF = \frac{C_{org(t)}}{C_{org(t-1)}} \times \frac{\left(X_{lipid(t-1)} + 0.05X_{LDP(t-1)}\right)}{\left(X_{lipid(t)} + 0.05X_{LDP(t)}\right)} \tag{3}$$

Where $X_{\mathrm{LDP}(t-1)}$ and $X_{\mathrm{LDP}(t)}$ refer to the mass fraction of lean dry protein in the sample at each time interval. Lean dry protein content (g) is typically calculated by subtracting the water content (g) and lipid content (g) from the wet weight (g) of the sample.

4 Bioamplification Case Studies

Animal energetics encompasses the processes through which individuals acquire, assimilate, and allocate their food resources (Humphries et al. 2004; McNab 2001). To compensate for metabolic demands that are imposed by periods of energy imbalances in an animal's life cycle, animals are frequently required to rely on accumulated somatic energy reserves. These periods, although perhaps brief, are expected to coincide with weight loss and bioamplification. In addition to generating water from catabolism, lipids are the most calorically rich tissue reserves, providing up to eight times the total energy available from protein and carbohydrate stores (McWilliams et al. 2004). Consequently, animals that maximize their lipid reserves prior to periods of nutritional stress are those most likely to successfully endure the event. The rates at which lipids are mobilized during a bioenergetic bottleneck depends on several key factors that include an animal's field metabolic rate as influenced by basal metabolism, thermoregulation costs and activity, and the duration of the bottleneck itself (Humphries et al. 2004). Although lipids are a critical component for surviving energetically demanding events, protein turnover is also required for muscle repair. Moreover, species that undergo periods of intense exercise (i.e., migrations) require large protein stores (Golet and Irons 1999). Body size also matters, because large-bodied individuals often better withstand prolonged starvation events from their higher ratio of somatic energy reserves to metabolic rate (Bystrom et al. 2006). Bioamplification of hydrophobic organic chemicals is predicted to occur frequently in nature, because mobilization of somatic lipid stores, and to a lesser extent, lean dry protein, represents the primary response of animals under conditions of an energy imbalance.

Fasting occurs in nearly all major taxonomic groups and often extends over prolonged periods (Wang et al. 2006). Many animal species inhabit highly variable environments where nutrient abundance and quality are inconsistent and periods of starvation are common (Kirk 1997). Winter is often the most significant and recurring period of energy imbalance experienced by animals during their life history. Nutrient abundance is a particular constraint for species inhabiting north temperate

or Arctic climatic zones, where cold temperature periods last several months and dominate the annual temperature cycle (Fort et al. 2009; Webster and Hartman 2007). Low temperatures, decreased food and water availability, and potential ice cover all exacerbate the energetic imbalances that animals experience during overwintering events. Animals often respond to suboptimal cold temperatures by employing specific strategies such as migration or hibernation, both of which entail bioenergetic consequences and can be associated with weight loss (Yahner 2012). Bioenergetic bottlenecks also result from natural physiological events that occur during the animals' life history. This includes events such as metamorphosis, molting, and reproduction (Golet et al. 2000; Lambert and Dutil 2000; Orlofske and Hopkins 2009). Although less common, weight loss may also result from disease.

In the following sections, we review bioamplification across taxonomic groups by examining laboratory and field studies that have been performed during different life stages or under different developmental conditions. These stages or conditions include embryo and larval development, metamorphosis, reproduction, overwintering, hibernation, migration and at other times of nutritional stress (e.g., disease). In Table 1, we present a list of selected cases studies from the literature that demonstrate bioamplification.

4.1 Bioamplification During Embryo and Juvenile Development

In addition to representing a highly dynamic period of tissue differentiation and animal development, embryo development for oviparous species is characterized by a dependence on maternally provided food resources. During this period, the mobilization of endogenous lipid and protein reserves by the developing embryo has the potential to generate conditions suitable for POPs bioamplification (Kleinow et al. 1999). Over the past 10 years, evidence for embryonically derived bioamplification has been observed among avian and fish species. For example, Drouillard et al. (2003) developed a bioenergetic model for herring gull (*Larus argentatus*) embryos during the egg incubation period that predicted a steady decline in egg lipid mass during incubation. During this life stage, lipid-normalized polychlorinated biphenyl (PCB) concentrations that were quantified in developing chicks increased as a function of incubation date (Drouillard et al. 2003). Significantly, for pipping chicks, lipid-normalized PCB concentrations were higher than those quantified in the egg laying females (Drouillard et al. 2003). However, following hatching and the initiation of exogenous feeding, growth dilution quickly attenuated the extent of bioamplification occurring in growing chicks (Drouillard et al. 2003).

For fish species such as yellow perch (*Perca flavescens*) and Chinook salmon (*Oncorhynchus tshawytscha*), life history characteristics can differ substantially during the embryo and larval development periods (Scott and Crossman 1973). Yellow perch spawn in the spring and are iteroparous (characterized by multiple reproductive events), wherein the larval stage emerges as free swimming individuals that are capable of external feeding (Post and McQueen 1988). In contrast,

Table 1 A list of case studies demonstrating bioamplification across taxonomic groups

Species	Life history	Sex	Field/experimental	Chemicals[a]	Tissues	$BAmF_{max}$	Reference
Invertebrates							
Mayflies (*Hexagenia* spp.)	Reproductive flight	M	Field	PCBs	Whole	3.4	(Daley et al. 2011)[b]
Amphibians							
Green frogs (*Rana clamitans*)	Metamorphosis	M/F	Experimental	PCBs	Whole	4.3	(Leney et al. 2006a)[c]
Green frogs (*Rana clamitans*)	Hibernation	M/F	Experimental	PCBs	Whole	1.4	(Angell and Haffner 2010)[c]
Fish							
Sockeye salmon (*Oncorhynchus nerka*)	Migration	M/F	Field	PCBs, PCDD/Fs	Muscle, gonad, liver	9.7	(DeBruyn et al. 2004)[b]
Europeans eel (*Anguilla anguilla* L)	Migration	F	Experimental	PCBs	Muscle	14.0	(Van Ginneken et al. 2009)[b]
Chinook salmon (*Oncorhynchus tshawytscha*)	Larval development	M/F	Experimental	PCBs, OCs	Whole	4.9	(Daley et al. 2012)[d]
Arctic Char (*Salvelinus alpinus*)	Overwintering	M/F	Experimental	PCBs	Liver, kidney	6.8	(Jorgensen et al. 1999)[d]
Yellow perch (*Perca flavescens*)	Incubating eggs	M/F	Experimental	PCBs	Whole	5.4	(Daley et al. 2009)[c]
Common sole (*Solea solea*)	Larval development	M/F	Experimental	PCBs	Whole	10.1	(Foekema et al. 2012)[d]
Yellow perch (*Perca flavescens*)	Overwintering	M/F	Experimental	PCBs	Whole	2.3	(Paterson et al. 2007a, b)[d]
Pacific salmon (*Oncorhynchus* spp)	Migration	M/F	Field	PCBs, OCs, PCDD/Fs	Muscle, gonad, liver	10	(Kelly et al. 2011)[d]
Birds							
Kittiwakes (*Rissa tridactyla*)	Reproduction	F	Field	PCBs	Liver, brain, fat	4.8	(Henriksen et al. 1996)[d]
Herring Gull (*Larus argetatus*)	Incubating eggs	M/F	Experimental	PCBs	Embryo	3.3	(Drouillard et al. 2003)[d]
Greater scaup (*Aythya marila*)	Overwintering	M	Field	PCBs, OCs	Muscle, fat pad	5.5	(Perkins and Barclay 1997)[d]
Common eiders (*Somateria mollisima*)	Incubation fast	F	Field	PCBs, OCs	Blood	6.1	(Bustnes et al. 2010)[d]
White Carneau pigeons (*Columba livia*)	Starvation	M/F	Experimental	PCBs	Blood, liver, kidney, brain, muscle, whole	5.7	(DeFreitas and Norstrom 1974)[d]

Mammals							
Harp seals (*Phoca groenlandica*)	Reproductive fast	F	Field/experimental	PCBs, OCs	Blood, blubber	7.2	(Lydersen et al. 2002)[d]
(*Zalophus californianus*)	Domoic acid fast	M/F	Field/experimental	PCBs, OCs, PBDEs	Blubber	2.5	(Hall et al. 2008)[d]
Polar bears (*Ursus maritimus*)	Summer fast	M/F	Field	PCBs, OCs	Fat	1.5	(Polischuk et al. 2002)[d]
Grizzly bears (*Ursus arctos horribilis*)	Hibernation	M/F	Field	PCBs, OCs, PBDEs	Fat	3.8	(Christensen et al. 2007)[d]
Elephant seals (*Mirounga angustirostris*)	Juvenile development	M/F	Field	PCBs	Blubber, blood	1.9	(Debier et al. 2006)[d]
Sea otters (*Enhydra lutris nereis*)	Infectious disease	M/F	Field	PCBs, OCs	Liver, kidney, brain	6.3	(Nakata et al. 1998)[d]
Humans (*Homo sapiens*)	Restricted diet	M/F	Field/experimental	PCBs, OCs	Blood	1.3	(Pelletier et al. 2002)[d]
Humans (*Homo sapiens*)	Restricted diet	M/F	Field/experimental	PCBs, OCs	Blood, fat	1.4	(Chevrier et al. 2000)[d]
Grey seals (*Halichoerus grypus*)	Lactation	F	Field	PCBs	Blood, blubber	2.5	(Debier et al. 2003)[d]
Grey seals (*Halichoerus grypus*)	Lactation	F	Field	PCBs, OCs	Blood, blubber	2.7	(Sormo et al. 2003)[d]

[a]Chemical acronyms: *PCBs* polychlorinated biphenyls, *OCs* organochlorines, *PCDD/Fs* polychlorinated dibenzodioxins/furans, *PBDEs* polybrominated diphenyl ethers
[b]BAmFs (bioamplification factors) were calculated or reported using a lipid equivalents ratio from the available data found in the corresponding citation
[c]BAmFs were calculated or reported using a fugacity ratio from the available data found in the corresponding citation
[d]BAmFs were calculated or reported using a lipid normalized ratio from the available data found in the corresponding citation

Chinook salmon are semelparous (characterized by a single reproductive episode before death), spawn in the fall, and allocate substantial maternal resources into eggs to enhance their survival during periods of prolonged ice cover (Bull et al. 1996; Kaitaranta and Ackman 1981; Russell et al. 1999b; Wiegand 1996). Following hatching, larval Chinook salmon tend to remain on gravel substrates and are nourished primarily by endogenous yolk reserves, because availability of external food remains low. These differences in the provisioning and timing of the use of maternal lipids produce important consequences for bioamplification during the early life stages of different fish species.

Daley et al. (2009) quantified the changes that occurred in proximate composition and PCB concentrations in yellow perch eggs during embryo development. These authors demonstrated a loss of egg dry mass over time and a decline in lipid content. Significantly, PCB fugacities quantified in yellow perch eggs at later stages of incubation were an average 2.7-fold (range: 1.8–5.4) higher than measured in newly fertilized eggs (Daley et al. 2009). For Chinook salmon eggs, no declines in lipid content were observed during the incubation period, and no concomitant bioamplification was observed (Daley et al. 2012). However, during the free swimming larval stage, juvenile Chinook salmon fry exhibited steady decreases in lipid content over time, which resulted in significant PCB bioamplification increases. For these fry, a maximum lipid-equivalent BAmF of 4.9 was determined by the end of yolk resorption (Daley et al. 2012). A similar magnitude of bioamplification has been observed in the early life stages of sole (*Solea solea*), whereby the lipid-normalized PCB concentrations peaked just prior to first feeding. The lipid-normalized concentrations in the late yolk sac larvae were approximately eightfold to tenfold higher than in the newly hatched larvae (Foekema et al. 2012).

Bioamplification-related increases in POP concentrations have also been observed during the development of juvenile grey (*Halichoerus grypus*) and northern elephant (*Mirounga angustirostris*) seals (Addison and Stobo 1993; Debier et al. 2006). Northern elephant seal pups are generally abruptly weaned from the mother, but can fast for up to 2.5 months and potentially lose up to 30% of their post-weaning body mass (Debier et al. 2006). During this period, lipid- normalized PCB concentrations quantified in blubber samples from elephant seal pups exhibit bioamplification, in addition to elevated blood serum PCB levels (Debier et al. 2006). Bioamplification-related increases in PCB concentrations have also been demonstrated during the post-weaning fast period in the blubber tissues of juvenile grey seals ranging from 0 to 13 months of age (Addison and Stobo 1993). Decreased POP concentrations were observed when grey seal pups resumed feeding (Addison and Stobo 1993). This was similar to the growth dilution observed for herring gull chicks and yellow perch larvae on initiation of feeding (Drouillard et al. 2003; Daley et al. 2009).

These studies demonstrate that bioamplification typically generates chemical concentrations that increase during embryo and juvenile development until independent external feeding is initiated by the animal. Such bioamplification trends can also be log K_{OW} dependent, whereby the more hydrophobic chemicals achieve the greatest BAmFs during larval development (Daley et al. 2012). Embryonic and larval/juvenile development also represents one of the periods in an animal's life

history, when they are most sensitive to the toxic effects from chemical exposure. As such, bioamplification could represent an important mechanism that mediates chemical toxicity during this critical developmental period (Daley et al. 2012; Foekema et al. 2012).

4.2 Bioamplification During Metamorphosis

During metamorphosis, several changes to an animal's biochemical, metabolic, and physiological functions may occur. In an experimental study using green frog (*Rana clamitans*) tadpoles, Leney et al. (2006a) dosed individuals with a PCB-Aroclor® mixture approximately three weeks prior to the onset of metamorphosis. Because tadpoles do not feed during metamorphosis, endogenous lipid mobilization was observed during this period, along with bioamplification of the PCB dose (Leney et al. 2006a). Bioamplification factors were maximized during tadpole metamorphosis by approximately 4.5-fold for the most hydrophobic PCB congeners (Leney et al. 2006a).

Aquatic insects have also demonstrated bioamplification during metamorphosis from pupae to larval life stages. Bartrons et al. (2007) reported an approximate threefold increase, when comparing mean dry weight POP concentrations between pupae and larval stages across four species of aquatic invertebrates. It was concluded that the increases in POP concentrations were due to the lack of feeding and subsequent loss of body weight during metamorphosis (Bartrons et al. 2007). During metamorphosis from juvenile instar to adult life stages, non-feeding emergent chironomids have been observed to increase PCB concentrations by approximately 4.6-fold, although this ratio was expressed on a fresh weight basis (Larsson 1984). Similarly, Harkey and Klaine (1992) documented increased concentrations of the pesticide chlordane in adult *Chironomus decorus* following metamorphosis. Given the wide range of invertebrate species that undergo multistage metamorphosis, it is likely that this phenomenon occurs commonly across this taxonomic group.

4.3 Bioamplification During Reproduction

Reproduction represents a substantial energy investment for most taxonomic groups and often encompasses substantial periods of feeding, fasting, and, for mammalian species, a lactational period that entails significant energetic costs. For example, female elephant seals follow a prolonged feeding period with a 3-month period that includes fasting, competition for mates and breeding (Schneider 2004). In some phocid species, up to 40% loss of body mass occurs during reproductive activities, in comparison to the non-breeding season (Lydersen et al. 2002). These species have a unique life history in that females undergo an extensive lactation period, which is consequently followed by molting, and both of these events incur

substantial energetic costs (Nilssen et al. 1995). For these individuals, the highest body condition indices occur prior to lactation and breeding (Lydersen et al. 2002). Individual females in poor physical condition during molting were determined to have blood PCB concentrations that were 7.2-fold higher than those measured from individuals having higher body lipid content (Lydersen et al. 2002). Increases in serum- and milk-PCB concentrations have also been documented in blood and milk samples collected from lactating female grey seals (Debier et al. 2003; Sørmo et al. 2003). Additionally, adult harp seals (*Phoca groenlandica*) that fasted over a 28-day period were observed to lose up to 24 kg of body mass and also exhibited significant increases in blood POP concentrations by the termination of the fasting period (Lydersen et al. 2002). In nature, weight losses that occur during reproduction are often compounded when the animal molts, which could lead to BAmFs higher than those reported above (Lydersen et al. 2002).

Several avian species, including common eiders (*Somateria mollissima*), kittiwakes (*Rissa tridactyla*), and Adelie penguins (*Pygoscelis adeliae*), experience lengthy fasting periods during reproduction (Bustnes et al. 2010; Henriksen et al. 1996; Subramanian et al. 1986). Female common eiders in Arctic regions have been demonstrated to lose approximately 25% of their body mass and 35% of lipid reserves during the egg incubation period (Bustnes et al. 2010). Similarly, female kittiwakes can lose up to 20% of their body mass from the pre-breeding to late chick rearing stages of reproduction (Henriksen et al. 1996). For these species, the bioamplification-related changes in lipid-normalized POP concentrations ranged from 1.7- to 8.2-fold across tissues, including blood, liver, brain, and adipose lipid reserves (Henriksen et al. 1996; Bustnes et al. 2010). Subramanian et al. (1986) indicated that maximum BAmFs of 3.5 and 2.8 can be achieved by male and female Adelie penguins, respectively, during this species reproductive fasting period. Subramanian et al. (1986) also observed similar bioamplification-related changes in POP concentrations among liver, muscle, and brain tissues in Adelie penguins during reproduction.

Reproductive activities also often include courtship rituals and rigorous competition between male individuals, in efforts to ensure mating success. In the mayfly genera *Hexagenia* spp., male individuals engage in intensive mating swarms that lead to significant reductions in lipid reserves between the sub-imago and imago life stages (Daley et al. 2011). Mayflies have a unique life history in that the majority of their lifespan is spent burrowed in lake and river sediments. Mayflies emerge from sediments as sub-imagos and rapidly molt into their final reproductive adult imago stage (Edmunds et al. 1976). Adult male Ephemeropteran mayfly species, including *H. Limbata* and *H. rigidia*, also do not feed following their emergence and rely exclusively on accumulated lipid reserves during their search for mates. Daley et al. (2011) demonstrated that during this reproductive flight, male individuals lose up to 50% of their lipid reserves, whereas females lose <10%. Further, lipid-normalized PCB-congener concentrations quantified in male mayflies were observed to bioamplify by roughly a factor of 2 during reproductive swarm events. This contrasts BAmFs of approximately 1 for PCB concentrations quantified in female mayflies which do not expend as much energy in reproductive swarms (Daley et al. 2011).

4.4 Bioamplification During Overwintering

In addition to the metabolic challenges associated with thermoregulation for both poikilotherms and homeotherms, reduced food availability often accompanies the extended cool–cold temperatures associated with winter months. For extreme latitude fish species such as Arctic charr (*Salvelinus alpinus*), lipid reserves can be reduced by up to 80% during the overwinter period (Jobling et al. 1998). Jorgensen et al. (1999) experimentally confirmed that PCB concentrations in the tissues of starved Arctic charr demonstrate bioamplification. Further, redistribution of the chemical from fat/muscle into liver and brain tissues occurred during overwintering (Jorgensen et al. 2002). Similar observations have been made for sole (*Solea solea*), whereby up to fourfold bioamplification of PCB concentrations in liver and muscle tissues occurred during experimental starvation trials and in wild collected overwintering fish (Boon and Duinker 1985). Overwintering mesocosm studies have also confirmed the bioamplification of PCBs by overwintering yellow perch (Paterson et al. 2007a, b). Specifically, PCB lipid-normalized bioamplification factors ranging from 1.7 to 2.3 were generated during the overwintering months. This bioamplification was consistent with a lack of chemical elimination and depletion of lipid reserves during the cold water period (Paterson et al. 2007a, b).

Avian and mammalian species, including the greater scaup (*Aythya mariya*), bald eagles (*Haliaeetus leucocephalus*), and Arctic fox (*Vulpes lagopus*), have also demonstrated POP bioamplification during the overwinter season. Seasonal lipid mobilization by greater scaup and bald eagles resulted in POP bioamplification factors in muscle and adipose tissues that were up to 5.5-fold during overwinter periods (Elliott et al. 1996; Perkins and Barclay 1997). Arctic foxes rely on sea bird forage during the spring and summer; however, as these prey items migrate from Arctic regions during the winter, individual foxes often face starvation (Fuglei and Oritsland 1999; Sonne et al. 2009). During winter starvation, a significant negative relationship between animal lipid content and POP concentrations was observed, which is consistent with POP bioamplification (Fuglei et al. 2007). Similar observations have also been made when comparing POP patterns quantified in winter-collected older lean foxes versus younger fatter individuals (Wang-Andersen et al. 1993).

4.5 Bioamplification During Hibernation

Hibernation is generally characterized as a behavioral and/or physiological mechanism invoked by species for enduring prolonged overwinter events and periods of low food availability. Current examples of hibernation-induced bioamplification include bat (Clark and Prouty 1977; Clark and Krynitsky 1983), amphibian (Angell and Haffner 2010), and bear species (Christensen et al. 2007), but is also likely to occur for the majority of animal taxa that undergo a state of hibernation, torpor, or estivation.

For bear species such as the grizzly (*Ursus arctos horribilis*), hibernation is referred to as pseudo-hibernation because the animal maintains a body temperature within a few degrees of normal; however, the potential for chemical elimination via defecation

and urination is minimized, because they are unlikely to feed (Hock 1960; Lyman et al 1982; Nelson 1978; Svihla and Bowman 1954). In a study of hibernating grizzly bears, there was an average 2.21 lipid-normalized concentration effect for ΣPCBs in post-hibernation compared to pre-hibernation bears (Christensen et al. 2007). For POPs such as dichloro-diphenyl-trichloroethane (DDT), which can be metabolized, no significant increases in chemical residue were demonstrated (Christensen et al. 2007).

Unlike other bear species, polar bears (*Ursus maritimus*) remain active over the winter months, while searching for prey during the ice cover season. However, polar bears are regarded to endure a "walking hibernation" or pseudo-hibernation (Nelson et al. 1983; Ramsay et al. 1991). This occurs during the summer months when animals experience lengthy periods of low food availability from the lack of ice cover (Chow et al. 2011). Polischuk et al. (2002) sampled polar bears in July and August and demonstrated a BAmF of approximately 1.3-fold for lipid-normalized ΣPCBs, compared to animals sampled in the winter from September to November. The highest BAmFs of 1.5 was determined for female bears with nursing cubs (Polischuk et al. 2002). Elevated levels of POPs have also been quantified in blood samples collected from polar bears during the summer months, indicating the potential mobilization of these chemicals from inert fat stores into the circulatory system (Knott et al. 2011; Polischuk et al. 1995, 2002).

Some of the earliest observations of POP bioamplification during hibernation were reported for a range of bat species (Clark and Prouty 1977; Clark and Krynitsky 1983). Hibernation in many bat species commonly occurs from early autumn to spring, and the animals typically rely on fat reserves to survive and recover during this period (Fenton and Barclay 1980). The depletion of lipid reserves during hibernation has been observed for big brown (*Eptesicus fuscus*), little brown (*Myotis lucifugus*), and eastern pipistrelle (*Pipistrellus flavus*) bats (Clark and Prouty 1977; Clark and Krynitsky 1983). Critically, concentrations of POPs, including the DDT metabolite dichloro-diphenyl-dichloroethylene (DDE), have been demonstrated to bioamplify significantly during this hibernation (Clark and Prouty 1977; Clark and Krynitsky 1983). Of particular significance, mortalities in bat populations have been observed coincident with animal arousal from hibernation, when lipid reserves are at their lowest, and thus, the potential for bioamplification is maximized (Clark 1981).

Northern latitude amphibian species also respond to harsh winter conditions by entering a hibernation state. Recently, Angell and Haffner (2010) sampled hibernating green frogs from October–January to quantify changes in PCB dose kinetics and animal lipid contents. The lipid content of frogs declined through the sampling period, with maximum BAmFs of 1.4 occurring for more recalcitrant hydrophobic ($\log K_{OW}$ >6.5) PCB congeners (Angell and Haffner 2010).

4.6 Bioamplification During Migration

Migration is a ubiquitous life strategy that is demonstrated in nearly all major taxonomic groups, including birds, mammals, fishes, reptiles, amphibians, and insects. Among these taxa, fishes and birds provide primary examples of extensive long

distance migrations that require energy demands from animals that are sufficient to bioamplify POPs.

Anadromous fish such as salmonid species often migrate long distances to return to natal streams and rivers to spawn. For example, the Yukon River Chinook salmon population is known to swim distances over 3,000 km during their migration (Beacham et al. 1989). Such extensive migrations have been demonstrated to deplete 90% of somatic lipid reserves in some individuals that return to spawning sites (Hendry and Berg 1999). Such extensive loss of somatic energy reserves has been demonstrated to bioamplify POPs, including PCBs, up to tenfold in migrating sockeye salmon (*Oncorhynchus nerka*) over the combined duration of the migration and spawning events (DeBruyn et al. 2004b; Ewald et al. 1998; Kelly et al. 2007b, 2011). Similar examples of POP bioamplification have been demonstrated for migrating Atlantic (*Salmo salar*) (Hansson et al. 2009) and Chinook salmon populations (Kelly et al. 2011). Of particular significance, the magnitude of PCB bioamplification by salmonid species is positively correlated with both migration distance and chemical log K_{OW} value (DeBruyn et al. 2004; Kelly et al. 2011). Similar to Pacific salmonids, the catadromous European eel (*Anguilla anguilla*) often returns to spawning grounds that are thousands of kilometers from feeding areas, and these animals tend to fast during migration (Olivereau and Olivereau 1997). This species can spend between 5 and 18 years in river- and coastal-feeding grounds prior to migrating to oceanic spawning grounds (Belpaire and Goemans 2007). Lipid-normalized concentrations of the organochlorine insecticide lindane have been quantified at a level of 9,255 ng/g, in pre-spawning European eel populations (Belpaire and Goemans 2007). Van Ginneken et al. (2009) demonstrated that the depletion of lipid reserves in more actively swimming European eels resulted in PCB congener bioamplification up to 14-fold higher than levels found in resting individuals. Eels also demonstrated little capacity for eliminating chemicals during such non-feeding events (Duursma et al 1991). Many Anguillid eels, such as the European and American eel (*Anguilla rostrata*) are at-risk species for conservation, and the very high POP concentrations and low lipid reserves have been identified as factors contributing to eel population declines (Belpaire and Goemans 2007).

Numerous bird populations undertake lengthy migrations. However, less is known about how bird migration influences bioamplification, mainly because of difficulties associated with measuring POP concentrations and body condition during migration events. Migration patterns among bird species also differ substantially, resulting in widely different energy demands. For many bird species, migration involves flight over relatively large food-limited areas, thus providing minimal opportunities for refueling (Klaassen 1996). Additionally, in larger non-gliding migratory species, active flight, rather than wind-assisted gliding, serves to more rapidly deplete energy reserves (Klaassen 1996). During such long distance migrations, wind direction and climate also affect metabolic costs. For example, headwinds increased the consumption of lipid stores (Colabuono et al. 2012; Piersma 2002). Such rapid depletion of lipid stores during migration has been observed to result in the redistribution of POPs among lipid, liver and muscle tissues

in Antarctic migratory species (Colabuono et al. 2012). Critically, overall animal body condition, as indicated by lipid content and starvation status, have been determined to be important factors in the redistribution of POPs in animal tissues during bird migration (Colabuono et al. 2012; Sodergren and Ulfstrand 1972).

4.7 Other Events Leading to Bioamplification

For some animal species, aphagia often occurs with the onset of disease, and this condition can generate the negative energy imbalance that is consistent with energetic bottlenecks and weight loss. For example, California sea lions (*Zalophus californianus*) exposed to the domoic acid neurotoxin cease feeding and can lose substantial body mass and blubber content (Hall et al. 2008). During a 12-day aphagia period, individuals exposed to this neurotoxin lost approximately 16% of their body mass and exhibited bioamplification (of PCBs, DDT, brominated flame retardants, and other POP compounds) in blubber tissues (Hall et al. 2008). Stranded sea otters (*Enhydra lutris nereis*), which died from disease and emaciation, exhibited higher lipid-normalized DDT concentrations than did animals that died from acute trauma (Nakata et al. 1998). Similar patterns of POP bioamplification have been observed in stranded dolphins, relative to those caught as by-catch (Chou et al. 2004). The patterns of PCB bioamplification were consistent with higher levels of lipid mobilization in the diseased and starving animals that were found stranded (Chou et al. 2004). Evidence for POP bioamplification has also been observed in diseased black-backed gulls (*Larus fuscus fuscus*) and little brown bats (Hario et al. 2004; Kannan et al. 2010). In addition, weight loss and starvation experiments have demonstrated POP bioamplification in humans (Chevrier et al. 2000; Pelletier et al. 2002; Tremblay et al. 2004; Walford et al. 1999), birds (Defreitas and Norstrom 1974; Ecobichon and Saschenbrecker 1969; Stickel et al. 1984), fish (Antunes et al. 2007), and rodents (Dale et al. 1962; Jandacek et al. 2005; Ohmiya and Nakai 1977).

5 Modelling POP Bioamplification

Non-steady state bioaccumulation models, and in some cases multi-life stage bioaccumulation models, have been described for POP compounds in marine mammals (Czub and McLachlan 2007; Hickie et al. 1999, 2000, 2005, 2007; Yordy et al. 2010), birds (Clark et al. 1987, 1988; Drouillard et al. 2003; Norstrom et al. 1986a, 2007), and fish (Drouillard et al. 2009; Foekema et al. 2012; Ng and Gray 2009; Sijm et al. 1992). However, relatively few of the studies just cited were focused explicitly on interpreting bioamplification peaks resulting from model simulations. Drouillard et al. (2003) used a non-steady state embryo bioaccumulation model to contrast differences in lipid-normalized PCB concentrations in herring gull embryos through

incubation. The above simulation demonstrated that pipping-aged chicks can achieve bioamplification factors of 3.1 and 1.5, compared to fresh eggs and laying females, respectively. Foekema et al. (2012) applied an analogous approach to model hydrophobic POP bioaccumulation in the embryo stage of a fish, the common sole (*Solea solea*). Based on their simulations, the authors concluded that at the end of the yolk-sac stage, sole larvae can achieve bioamplification factors of between 2 and 4, compared to spawning parent fish for compounds having log K_{OW} values exceeding 5.

There are few examples, in which non-steady state bioaccumulation models have been applied to adult life stages that incorporate realistic seasonal changes in whole body lipids, and assess the impact of this on POPs bioamplification. Norstrom et al. (2007) highlighted the importance of including seasonal changes in lipid content as a model variable, because of the close interaction that exists between whole body lipid content and POP toxicokinetics. Incorporating the effects of seasonal changes on lipid content is particularly important as it relates to chemical elimination by animals. For example, Clark et al. (1988) used the herring gull bioenergetic and toxicokinetic model (described in Clark et al. 1987; Norstrom et al. 1986a, b) to predict pronounced seasonal changes in dieldrin and mirex chemical fugacities as they related to decreases in adult bird fat stores that occur each spring. Similarly, Czub and McLachlan (2007) applied a non-steady state bioaccumulation model to POPs in ringed seals. This model predicted seasonal bioamplification factors of 2 or more in seals that resulted from the blubber loss that occurs during molting in the spring and early summer. Drouillard et al. (2009) established a non-steady state model for yellow perch that accounted for seasonal lipid changes and its effect on PCB depuration kinetics. Importantly, this last study showed that winter weight loss, as experienced by aquaculture-reared yellow perch, exceeded the rate of PCB elimination, enabling bioamplification to occur.

Model Simulations. To further demonstrate the application of multi-life stage, non-steady state bioaccumulation models to predict bioamplification, two case studies are presented in the appendices for a fish and bird using previously reported models. For fish, a modified version of the yellow perch bioenergetic and toxicokinetics model reported by Drouillard et al. (2009) was adopted. The model was extended to include growth over most of the lifespan (age 1–9) of this species. Yellow perch ages as high as 7–9 years have been reported for North American populations, including the populations inhabiting the Great Lakes (Scott and Crossman 1998). Growth rates, temperature-dependent specific growth and temperature-dependent changes in whole body lipid content and lean dry protein were incorporated as described in Appendix 1. The fish bioenergetics sub-model used proximate composition, growth, and body size data in conjunction with water temperatures to predict daily food consumption and gill ventilation rates as described by Drouillard et al. (2009). Since the Drouillard et al. (2009) model was limited to describing chemical elimination, the model was modified to incorporate chemical uptake from water and food, by using equations described by Arnot and Gobas (2004). Simulations were initiated using a 1-year-old male fish that was assumed to be in equilibrium with water for a chemical having a log K_{OW} of 6.5. The chemical was assumed to not be

subject to biotransformation by fish. Fish growth was simulated over an 8-year period, with fish fed a diet of constant proximate composition, energy density, and chemical concentration throughout the simulation. The chemical concentration in the diet was set such that the diet was in equilibrium with an assumed constant water concentration. Two simulation scenarios were modeled. In the baseline scenario, a constant temperature of 21 °C was maintained throughout the simulation, and hence, did not allow for seasonal weight loss to occur. The second scenario, referred to as the dynamic temperature scenario, allowed temperature to vary by season according to an annually repeating temperature profile recorded for Southern Ontario, Canada. Appendix 1 provides a full description of the yellow perch bioaccumulation model, parameters and algorithms used for model calculations.

For birds, a modified version of the herring gull bioenergetic and toxicokinetic model as described by Norstrom et al. (2007) was utilized (Appendix 2). The model was formulated to simulate what would occur in a male bird to avoid having to incorporate depuration of maternal residues to eggs, and to predict maximum bioamplification factors for this species. The model was adapted to predict chemical residues across multiple life stages, commencing with a pipping chick, followed by chick growth through fledging, to a subadult male life stage and reproductively active adult life stage over an 8-year simulation period. The bioenergetic portion of the model predicted daily growth, proximate composition, and feeding rate of a male bird as described in Norstrom et al. (1986b). The toxicokinetic sub-model predicted chemical uptake from food and chemical elimination at daily intervals as described in Clark et al. (1987). A slight modification to the model was that the plasma/fat partition coefficient (K_{PF}) was redefined as a plasma/lipid equivalent partition coefficient to be consistent with the yellow perch model. This consideration reflects the current understanding of chemical partitioning within organisms (Debruyn and Gobas 2007). The values for K_{PF} and the plasma clearance constant (k'_{pc}) were chosen from those reported for Mirex to provide a non-biotransformed, highly hydrophobic chemical comparable to the fish simulations. As in the case of the yellow perch model, two simulations were performed. In the baseline simulation, temperature and photoperiod were kept constant at 21 °C and 12 h/d across seasons and years. The seasonal scenario relied on an annually repeating temperature and photoperiod profile from Lake Ontario during 1997. The model was initialized using a fresh egg concentration predicted from a 10-year old female adult model simulation (Clark et al. 1988). This female bird was fed a constant diet of the same concentration and energy density as had been used for male simulations. The female was also subjected to equivalent temperature and photoperiod profiles. Bioamplification of fresh egg residues in the pipping embryo was estimated by multiplying the fresh-egg lipid-equivalent concentration by a factor of 3.1 as suggested by Drouillard et al. (2003). The bird was subsequently modeled to feed on a constant food source of similar proximate composition, energy density and chemical concentration over the duration of the study. A full description of model parameters and algorithms for the herring gull simulations are presented in Appendix 2.

In Fig. 1, we summarize the yellow perch model output for the baseline and seasonal temperature scenarios. Body weight of the 1–8-year-old fish varied between

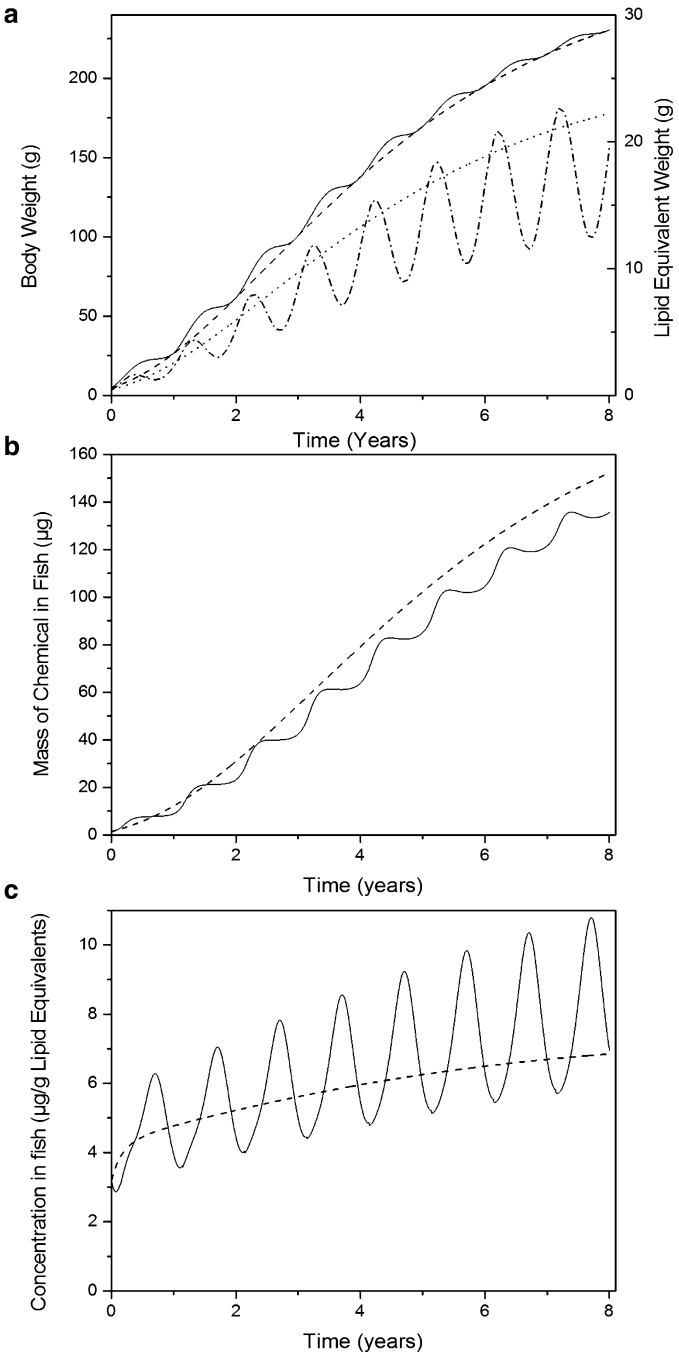

Fig. 1 Yellow perch model simulations for baseline and seasonal temperature scenarios. (**a**) Body weight (g) is simulated from a baseline constant temperature (*dashed line*) and a dynamic temperature scenario (*solid line*). Lipid-equivalents (g) are simulated for a baseline constant temperature (*dotted line*) and dynamic temperature scenario (*long dash-dot*). (**b**) Mass of chemical in the fish (µg) is presented for the baseline constant temperature (*dotted line*) and dynamic temperature scenario (*solid line*). (**c**) Chemical concentration (ng/g lipid-equivalents) is simulated for a baseline constant temperature (*dotted line*) and dynamic temperature scenario (*solid line*)

4.8 and 230 g for the two scenarios (Fig. 1a). The upper limit of this range is similar to the 257 g body weight value reported for 8+-year-old yellow perch collected from the Bay of Quinte, Ontario, Canada (Scott and Crossman 1998). For the dynamic temperature scenario, body weights were marginally higher in the summer months, as a result of the temperature dependence of fish feeding rates and growth during warm water seasons (Kitchell et al. 1977). In contrast, winter growth approached zero. Differences in growth between simulations were more pronounced when comparing model predictions of fish whole-body lipid-equivalent weights over time. The baseline simulation produced lipid-equivalent weight estimates at the upper range of those predicted during the summer of the dynamic temperature scenario. These upper range estimates resulted from the high constant temperature used in the simulation. Lipid-equivalent weights in the baseline scenario mirrored body weight trends, whereas % lipid was constant at 8.6%. In the seasonal scenario, lipid-equivalent weights showed strong seasonal cycles. In each year of the simulation, the % lipid decreased to a minimum value of 4.3% in the winter and to a maximum value of 9.3% in the summer. These values corresponded to a 2.1-fold difference in lipid content on a per annum basis.

Changes in the total fish body burden of chemical from the two scenarios are provided in Fig. 1b. Fish consumed less food during the winter and had lower lipid-equivalent weights in the dynamic temperature simulation. Consequently, the total chemical mass accumulated by fish under the seasonal simulation was lower than that observed for the baseline scenario. The magnitude of these differences also increased with fish age. Figure 1c contrasts lipid-equivalent concentrations of the chemical in fish under the two scenarios. Lipid-equivalent concentrations were predicted to increase with age over the duration of the baseline scenario simulation. This observation is consistent with non-steady state bioaccumulation. This occurred since fish did not reach their maximum size as predicted by the von Bertalanffy growth model. The dynamic temperature scenario predicted pronounced seasonal oscillations for chemical concentrations in fish that were opposite those observed for whole body lipid-equivalents. Specifically, lipid-equivalent concentrations peaked on Feb. 1 of each simulation year, coincident with animal minima for lipid-equivalent weights (Fig. 1a). In contrast, the lowest annual lipid-equivalent concentrations occurred on July 17th of each year, when lipid-equivalent weights were maximized.

In Table 2, we summarize the metrics for comparing model outputs from the yellow perch constant and dynamic temperature scenarios. Both of these scenarios predicted a non-steady state for fish over the simulation duration. Thus, temporal changes in chemical mass balance and lipid-equivalent concentrations are required to demonstrate the occurrence of bioamplification. The first column of Table 2 describes the ratio of maximum to minimum chemical mass (ng) in the fish for each year of the simulations. This mass balance ratio exceeded 1 in all simulation years and tended to be higher for the dynamic temperature scenario. BAmF values exceeded 1 in all simulations and approached a near constant value (1.89–1.90) for the later years of the dynamic temperature scenario. Moreover, BAmF values exceeded the chemical mass balance ratios for years 3–8 during this scenario, validating the occurrence of bioamplification. Bioamplification occurred despite the fact that the organism was

Table 2 Model predicted changes in age specific mass balance, lipid equivalents, and BAmFs in yellow perch over the simulation duration

Year interval	ΔX_{fish} max/min chemical mass (ng) in fish over a given year	BAmF max/min lipid equivalents concentration in fish over a given year	Across simulation ratio of the peak lipid equivalent concentration between scenarios[a]
0–1	6.77 (8.27)[b]	2.19 (1.49)[b]	1.36
1–2	2.61 (2.54)	1.98 (1.09)	1.38
2–3	1.82 (1.76)	1.96 (1.08)	1.42
3–4	1.50 (1.45)	1.95 (1.06)	1.46
4–5	1.33 (1.29)	1.93 (1.05)	1.49
5–6	1.23 (1.20)	1.91 (1.04)	1.53
6–7	1.16 (1.14)	1.90 (1.03)	1.56
7–8	1.12 (1.09)	1.89 (1.02)	1.59

[a]Expresses the ratio of the maximum lipid equivalent concentration determined in a given year for the dynamic temperature simulation scenario to the lipid equivalent concentration determined on the same day for the constant temperature scenario

[b]First number corresponds to the dynamic temperature scenario. Value in parentheses corresponds to the constant temperature scenario

still in the uptake stage of the bioaccumulation curve (Fig. 1b). In contrast, BAmF values for the constant temperature scenario were close to 1 (1.02) by the last simulation year and did not exceed the chemical mass balance ratio. This demonstrates that bioamplification did not occur during the constant temperature scenario.

Herring gull model outputs are provided in Fig. 2. Temporal changes in whole body and lipid-equivalent weights are depicted in Fig. 2a. Juveniles grew rapidly during the first 90 days of the simulations. Under the dynamic temperature scenario, body weight and lipid-equivalent weights fluctuated according to an annual cycle each year following fledging. After the first simulation year, herring gull whole body and lipid-equivalent weights changed annually by 1.07- and 1.67-fold, respectively. Seasonal changes in herring gull lipid-equivalent weights were opposite those observed for fish. Specifically, herring gull lipid-equivalent maxima occurred in the winter while minima occurred during the summer. Lipid-equivalent weights were always higher in the dynamic temperature scenario, relative to the baseline simulation, due to the high temperature (21 °C) selected for constant temperature simulation.

Figure 2b contrasts the magnitude of chemical mass accumulated by birds under the two scenarios. For the dynamic temperature scenario, male birds accumulated higher chemical mass, relative to that predicted under the baseline scenario. This occurred because of the higher lipid content predicted for birds under the dynamic temperature scenario. Bird chemical mass approached an asymptote between years 2 and 4 of the simulation, with seasonal oscillations in chemical mass occurring after year 4. These oscillations were associated with courtship feeding and chick rearing costs that caused seasonal changes in animal food consumption rates. These behaviors contribute to increased chemical uptake followed by lags in elimination, with a gradual return to steady state by the following year. Unlike fish, rapid early growth by fledging chicks facilitated the steady state condition for the chemical

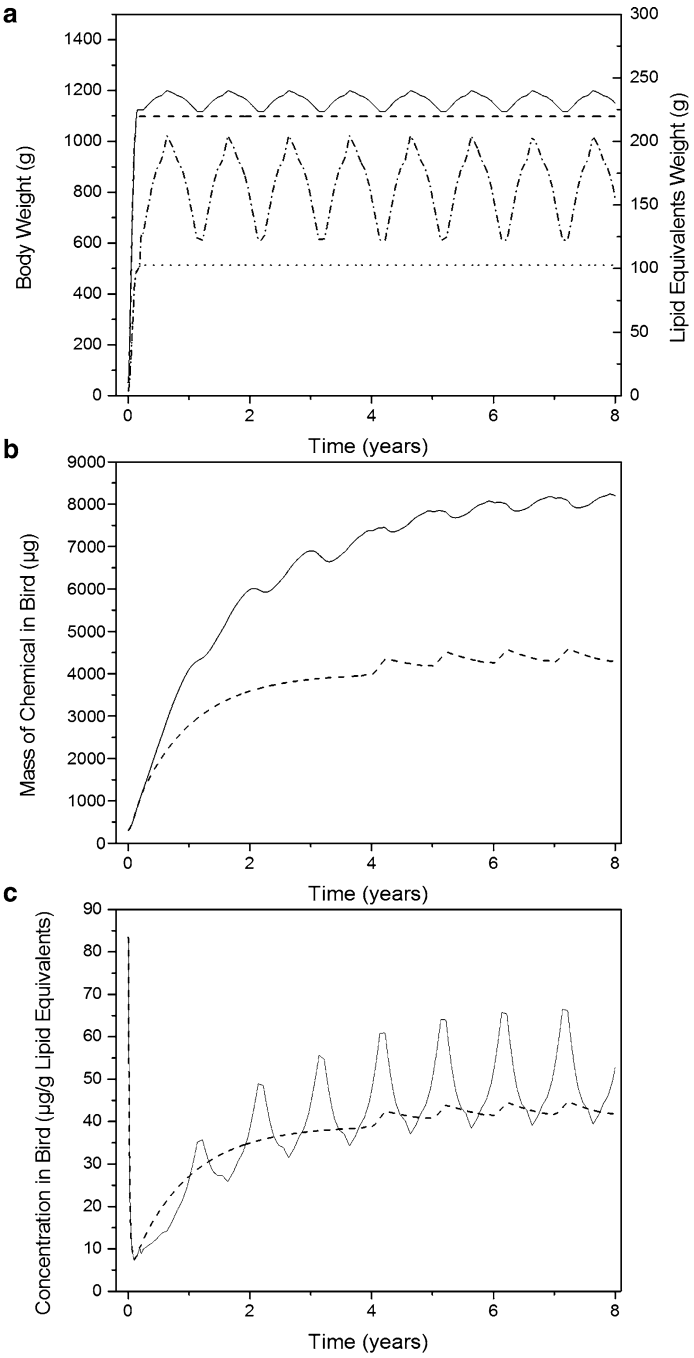

Fig. 2 Herring-gull model-simulations for baseline and seasonal temperature scenarios. (**a**) Body weight (g) is simulated from a baseline constant temperature (*dashed line*) and a dynamic temperature scenario (*solid line*). Lipid equivalents (g) are simulated for a baseline constant temperature (*dotted line*) and dynamic temperature scenario (*long dash-dot*). (**b**) Mass of chemical in the fish (µg) is presented for the baseline constant temperature (*dotted line*) and dynamic temperature scenario (*solid line*) (**c**) Chemical concentration (ng/g lipid-equivalents) is simulated for a baseline constant temperature (*dotted line*) and dynamic temperature scenario (*solid line*)

Table 3 Model predicted changes in age specific mass balance, lipid equivalents, and BAmFs in male herring gulls over the simulation duration

Year interval	ΔX_{fish} max/min chemical mass (ng) in fish over a given year	BAmF max/min lipid equivalents concentration in fish over a given year	Across simulation ratio of the peak lipid equivalent concentration between scenarios[a]
0–1	11.17 (13.92)[b]	12.76 (8.65)[b]	1.88
1–2	1.45 (1.30)	1.48 (1.30)	1.22
2–3	1.16 (1.08)	1.55 (1.08)	1.38
3–4	1.11 (1.03)	1.62 (1.03)	1.47
4–5	1.07 (1.09)	1.64(1.09)	1.45
5–6	1.05 (1.08)	1.67 (1.08)	1.51
6–7	1.04 (1.07)	1.68 (1.07)	1.52
7–8	1.04 (1.07)	1.69 (1.07)	1.53

[a]Expresses the ratio of the maximum lipid equivalent concentration determined in a given year for the dynamic temperature simulation scenario to the lipid equivalent concentration determined on the same day for the constant temperature scenario

[b]First number corresponds to the dynamic temperature scenario. Value in parentheses corresponds to the constant temperature scenario

under the baseline scenario. In contrast, steady state was not achieved until 5 years of age for birds under the dynamic temperature scenario. Greater seasonal oscillations in the magnitude and duration of chemical mass balance under the dynamic temperature scenario were attributed to temperature-dependent changes in feeding rates and reproduction-associated foraging costs occurring after year 4.

Trends in lipid-equivalent chemical concentrations throughout the herring gull life history are provided in Fig. 2c. Distinct bioamplification occurs for pipping chicks during the first 90 days of growth under both temperature scenarios. Under the baseline scenario, pipping chicks commenced with a lipid-equivalent concentration of 53.85 μg/g, which exceeds the maximum achieved by adult males (44.68 μg/g) by 1.2-fold. For the dynamic temperature scenario, pipping chicks had an initial lipid-equivalent concentration of 101.1 μg/g, which was 1.5-fold higher than the maximum predicted for adult males (66.9 μg/g). Although chemical mass in birds continued to increase with time (Fig. 2b), rapid growth dilution and a diet switch from highly contaminated yolk lipids to less contaminated external prey resulted in a precipitous drop in chick lipid-equivalent concentrations by day 37 (7.92 μg/g). These results demonstrate the importance of temporal lipid dynamics on chemical bioamplification in adult birds, chicks and at the time of maternal transfer.

In Table 3, we summarize the simulation metrics for the herring gull chemical concentrations, under the constant and dynamic temperature scenarios. BAmF values did not exceed the chemical mass balance ratio during any simulation year of the baseline scenario. Moreover, excluding year 1, BAmF values and chemical mass balance ratios were equal for each simulation year, suggesting an absence of bioamplification for older birds under this simulation. For the dynamic temperature simulation, BAmF values exceeded chemical mass balance ratios and progressively increased from 1.48 to 1.69 from years 1 to 8. These results confirm the occurrence of bioamplification in herring gulls under the dynamic temperature scenario.

Both models demonstrated the capacity of fish and birds to bioamplify hydrophobic, negligibly transformed chemicals throughout their life histories. This occurs primarily due to age-related changes in growth and seasonal weight loss. The temporal bioamplification trends for fish and birds were different, because fish feed less in winter and have reduced body fat content, whereas birds increase food intake, thereby enhancing fat deposits in winter. These differences are expected to occur, because ectothermic and endothermic species respond differently to low temperature exposures. Under the dynamic temperature scenario, BAmFs for both fish and birds exceeded the maximum ratio of chemical mass balance by simulation years 7–8. These results confirm that bioamplification occurred as it is defined in this review. Specifically, the rate of weight loss exceeded that for chemical depuration. For birds, bioamplification patterns were complex across the life stages included in the simulations. Pipping chicks exhibited a pronounced bioamplification peak during early growth, followed by reduced bioamplification events that were associated with temporal changes in subadult foraging costs and reproductive activities for adult birds. Similar bioamplification peaks are likely to be present in pre-hatched yellow perch embryos as described by Daley et al. (2009) and modeled in larval sole by Foekema et al. (2012). However, such simulations were not included in the current study owing to the absence of empirical information that described larval fish growth and proximate composition for the first simulation year. Additional factors (e.g., spawning costs related to mate competition and/or changes in food availability) probably further contributed complexity to the fish bioaccumulation curve. However, these factors were not included in the model structure.

Additional research during the full life cycle of different species would prove beneficial for identifying critical exposure periods to hydrophobic chemicals that are associated with bioamplification events. Development of such models requires a comprehensive knowledge of changes in animal bioenergetics, proximate composition, and food types and availability that occur at both seasonal and annual temporal scales. Insights into other toxicokinetic parameters (e.g., chemical exchange efficiency terms related to dietary assimilation, uptake and depuration across gills, and organism/fecal exchange) are also critical to establishing such a model structure. Although the theory behind extrapolating toxicokinetic parameters to multiple organisms, based on chemical attributes, is relatively well established, gaps do remain in our understanding of age and diet-related effects on such toxicokinetic parameters (Arnot and Gobas 2004). In contrast, limited empirical data are available for describing the physiological and proximate composition characteristics of species across a range of natural habitats and over multiple life stages. Such paucity of information hinders the development of full life cycle chemical bioaccumulation as exemplified in this review. Finally, it should be noted that field sampling rarely considers seasonal dynamics in animal lipid content, when timing of animal collection is arranged. Seasonally staggered sampling programs are required to track bioamplification over seasonal temperature cycles. However, most temporal biomonitoring programs sample animals during restricted time periods (e.g., open water, summer months), because of sampling logistics and the need to characterize interannual temporal chemical bioaccumulation trends (Bhavsar et al. 2010; Gewurtz et al. 2011; Richman et al. 2011; Roose et al. 1998).

6 Implications of Bioamplification

Several of the case studies outlined in Sect. 4 demonstrate a central consequence of bioamplification: POP residues in the animal are redistributed from metabolically inert lipid reserves to more toxicologically sensitive tissues. Loss of animal lipid reserves has been associated with proportional increases in xenobiotic chemical concentrations in blood, liver, muscle, and brain tissues (Bustnes et al. 2010, 2012; Debier et al. 2003, 2006; Henriksen et al. 1996; Jørgensen et al. 1999, 2002; Lydersen et al. 2002; Perkins and Barclay 1997; Subramanian et al. 1986). For example, DeFreitas and Norstrom (1974) starved pigeons for 7 days and observed a net mass transfer of PCBs from adipose into brain, liver, and muscle tissues. Henriksen et al. (1996) showed a steady decrease in body mass and lipids for breeding kittiwakes, which resulted in a quadrupling of PCB concentrations in brain tissue. Such changes occur because much of an animal's capacity for chemical bioaccumulation is defined by the mass of adipose stores and it is these tissues that experience the greatest decreases during weight loss. Consequently, a twofold decrease in animal partitioning capacity causes an equivalent increase in chemical fugacity. This leads to commensurate increases in chemical wet weight concentrations in blood and other lean tissues. Furthermore, lean tissue POP concentrations change rapidly following weight loss events, because the time required to achieve inter-tissue equilibrium is short (~1 week; Braune and Norstrom 1989; Clark et al. 1987; Norstrom et al. 1986a), despite the time being long for the organism to achieve steady state with its environment (Drouillard et al. 2001). For a more detailed explanation of how toxicokinetic processes regulate target site concentrations and the mechanisms of toxic action of POPs, readers are referred to the review articles of Escher et al. (2011), McCarty et al. (2011) and McElroy et al. (2011).

POP residues in blood are generally more available to target organs than those present in adipose stores, and thus blood residues pose greater toxicological risk (Knott et al. 2011). Studies involving insects, fish, and mammals have shown significant positive relationships between animal lipid content and the LD_{50} value for chemicals, indicating that toxicity increases with a decreasing animal lipid content (Geyer et al. 1990, 1993). Birds have been shown to experience enhanced POP toxicity following periods of dramatic weight loss (Ecobichon and Saschenbrecker 1969; Gabrielsen et al. 1995; Stickel and Stickel 1969). Van Velzen et al. (1972) observed that birds exposed to DDT doses from 100 to 300 μg/g, or subjected to weight loss from reduced food availability, exhibited no mortality. However, the combined effects of weight loss and DDT exposure caused significant mortality in brown-headed cowbirds (*Molothrus ater*). Fish having a low body lipid content (and poor condition) have been demonstrated to experience greater negative effects from PCB exposure (25–2,500 μg/kg food/day) than those having a higher lipid content and better condition (Quabius et al. 2000). These studies emphasize that the toxicity experienced by animals from POP exposure is best correlated to chemical fugacity rather than the whole body or tissue specific wet weight concentration.

POP bioamplification has the potential to modify chemical biotransformation rates, thereby enhancing toxic metabolite formation via first and second order kinetic

processes. Bioamplification also has the potential to alter POP bioaccumulation signatures in the animal's tissues (Boon et al. 1989; Walker 1990). For example, the redistribution of PCBs from deep blubber layers into the bloodstream of bottlenose dolphins was suggested to induce cytochrome P450 mono-oxygenases, leading to enhanced production of PCB metabolites (Montie et al. 2008). As discussed in Sect. 4.4, Jorgensen et al. (1999, 2002) studied overwinter emaciation in Arctic charr and saw an increase in PCB concentrations in more sensitive tissues (e.g., kidney and brain). This long-term food deprivation in overwintering charr also led to marked increases in biomarker responses during this period of PCB redistribution. Leney et al. (2006b) demonstrated the onset of PCB biotransformation in green frogs immediately following metamorphosis, a period when bioamplification is maximized for this species. However, it was not determined whether biotransformation was a consequence of bioamplification, or was from the ontogenetic changes associated with metamorphosis (Leney et al. 2006b). Christensen et al. (2007) reported that fasting animals, such as hibernating bears, experience prolonged exposure to toxic biotransformation products, from reduced capacity to eliminate them via urine and fecal egestion during this dormant state. Unfortunately, the information available is too limited to draw general conclusions on the specific interactions that take place between POP bioamplification, chemical biotransformation rates, and metabolite-mediated toxicity responses.

Because there are many bioenergetic bottlenecks at critical and highly sensitive periods of an animal's life cycle, bioamplification of POPs may increase chemical toxicity during these susceptible periods. Bioenergetic imbalances and associated weight loss events are predicted to increase stress and enhance possibilities of chemical/stress toxicity interactions (Fuglei et al. 2007; Knott et al. 2011). For example, several POPs are known to interfere with behavioral responses such as predator avoidance (Schulz and Dabrowski 2001; Weis and Weis 1987). Therefore, bioamplification of POPs during larval and/or fry development may reduce the capacity for predator avoidance, when these animals become free-swimming individuals and are more susceptible to predation. Further, ontogenetic shifts from endogenous to exogenous food resources are often associated with elevated mortality. Thus, bioamplification could potentially augment mortality at this critical period for larval fish. While stranded cetaceans often have higher total POP burdens than healthy animals do, it is unknown what role bioamplification plays in such mortalities (Chou et al. 2004). Amphibian metamorphosis represents a period of substantive changes in gene transcription and translational activities, in addition to significant physiological and metabolic reorganization (Kawahara et al. 1991). Subsequently, the potential for chemical interference during gene expression is higher during metamorphosis than during other life stages.

7 Conclusions

In this review, we have demonstrated that bioenergetic bottlenecks and environmental stressors influence POP exposure dynamics for many taxonomic groups that utilize different life history strategies. The main conclusions of this review are:

1. Bioamplification occurs when an animal loses body mass and chemical partitioning capacity at a faster rate than it can eliminate contaminants.

   Bioamplification, explicitly, is a non-equilibrium, non-steady state process, in which chemical residues in animal tissues become concentrated from reductions in chemical partitioning capacity that occur due to rapid loss of body lipids, without a commensurate loss of chemical mass.
2. Bioamplification leads to the mobilization of POPs from inert storage sites (e.g., adipose tissue) to other more sensitive tissues.

   A major implication of bioamplification is that it increases chemical fugacity in the animal's tissues. Several case studies demonstrate that this increase in fugacity results in the redistribution of contaminants from inert lipid stores (adipose) to more toxicologically sensitive tissues.
3. Bioamplification often occurs during sensitive life stages in the animal's life history.

   We have demonstrated that bioamplification often produces enhanced POP fugacities and tissue-specific chemical concentrations at critical periods in an animal's life history. Common critical behavioral, ontogenetic and physiological events, when bioamplification has been demonstrated, include embryo development, juvenile life stages, metamorphosis, reproduction, migration, overwintering, hibernation, and disease.
4. As a bioaccumulation process, bioamplification is additive to bioconcentration and biomagnification mechanisms of chemical exposure.

   As outlined in model simulations (Sect. 5), bioamplification can result in animals achieving the same magnitude of chemical concentrations predicted by steady state bioaccumulation models, prior to the animal actually achieving steady state with its exposure media (Figs. 1c and 2c). Bioamplification can increase the chemical concentration in an animal to levels that exceed the maximum concentration predicted for a given species by steady-state biomagnification models.
5. Examples of bioamplification of POPs can be found across the animal kingdom.

   Case studies of bioamplification are presented and include examples across a range of animal taxa including invertebrates, amphibians, fishes, birds, and mammals. Occasionally, BAmFs were observed to approach or exceed BMFs reported for POPs compounds. For example, observed BMFs for POPs in fish species often range from 5 to 10, whereas migrating salmon and eels have been shown to achieve BAmF's that exceed 10 (Table 1).

8 Future Directions

Bioamplification results in elevated POP fugacities and chemical concentrations during critical periods of the life history of many animal species. Consequently, understanding the dynamics of bioamplification and how behavioral, ontogenetic, and physiological changes during an animal's life history alter critical tissue

residues is important to bioaccumulation and risk assessment studies. Unfortunately, bioamplification is rarely considered in such studies. Most POP bioaccumulation models fail to fully consider the full scope of ecological, climatic, and physiological variables that regulate POP kinetics and bioaccumulation. In recent years, several authors have called for acquiring more robust datasets and for integrating more thorough life history information, as means to better calibrate and validate models (DeBruyn and Gobas 2006; Muir and de Wit 2010; Ng and Gray 2009; Norstrom et al. 2007). In our view, the major data gaps that require attention in future bioamplification research include the following:

1. Parameterization of life-stage specific toxicokinetic parameters and mapping their interactions with changing environmental conditions.
2. Understanding seasonal and life-stage specific growth, weight loss, and proximate composition changes under realistic environmental conditions.
3. Investigating the influence of multiple stressors (e.g., habitat alterations, climate change, species invasions, etc.) on growth, weight loss, and proximate composition in a given species.
4. Development of full lifecycle (embryo to adult) non-steady state bioaccumulation models.

Addressing these data gaps is not only essential for understanding and predicting POP bioaccumulation and biomagnification in food webs, but also for protecting wildlife, ecosystem, and human health.

9 Summary

Persistent organic pollutant bioaccumulation models have generally been formulated to predict bioconcentration and biomagnification. A third bioaccumulation process that can mediate chemical fugacity in an organism is bioamplification. Bioamplification occurs when an organism loses body weight and the chemical partitioning capacity occurs at a rate that is faster than the chemical can be eliminated. Although bioamplification has not been widely recognized as a bioaccumulation process, the potential consequences of this process are significant. Bioamplification causes an increase in chemical fugacity in the animal's tissues and results in the redistribution of contaminants from inert storage sites to more toxicologically sensitive tissues. By reviewing laboratory and field studies, we have shown in this paper that bioamplification occurs across taxonomic groups that include, invertebrates, amphibians, fishes, birds, and mammals. Two case studies are presented, and constitute multi-life stage non-steady state bioaccumulation models calibrated for yellow perch and herring gulls. These case studies were used to demonstrate that bioamplification is predicted to occur under realistic scenarios of animal growth and seasonal weight loss. Bioamplification greatly enhances POP concentrations and chemical fugacities during critical physiological and behavioral events in an animal's life history, e.g., embryo development, juvenile stages, metamorphosis,

reproduction, migration, overwintering, hibernation, and disease. Consequently, understanding the dynamics of bioamplification, and how different life history scenarios can alter tissue residues, may be helpful and important in assessing wildlife hazards and risks.

10 Appendix 1: Description of the Yellow Perch Model and Associated Simulations

Simulations were performed by finite difference, using a daily time step. The model was set up using a Microsoft Excel spreadsheet, and the calculation algorithms and/or constants for each variable of the model are summarized in Table 4. The model was initialized by assuming that day 1 corresponded to a 1-year-old fish (365 days old) hatched on May 20 of the previous year. The model was initialized at this life stage, because of a lack of data on growth, proximate composition changes and chemical toxicokinetics in larvae fish during the first year. The model was run for 2,920 days (i.e., 8 years from the simulation initialization). In the simulations, fish growth was allowed to change as a function of age, following the precedent of a von Bertalanffy model fitted to 22 North American populations of yellow perch (Jackson et al. 2008). The von Bertalanffy model was used to predict the length (cm) of fish at a given age (in days) for the constant temperature scenario. Body length (cm) was converted to a body weight (g) value, by using a linear regression equation fitted to the $\log_{10}$ of body length versus $\log_{10}$ of body weight (this approach relied on unpublished data that was generated for aquaculture-reared yellow perch; see Table 4).

For the dynamic temperature scenario, growth rates from the baseline simulations were further modified to make growth rates temperature dependent, whereas overall growth rates generally matched those of the constant temperature simulation. The modifications to the basic growth model are described as follows. On May 20 of each year (i.e., reflecting the assumed birthday of the fish), the body weight of the fish was set to be equal to the body weight estimated for the constant temperature simulation. Body weights for other days in the year were calculated by a finite difference method as: $BW_{(t)} = BW_{(t-1)} + GC_{YI} \times T$; where GC_{YI} is the temperature-dependent growth constant for a given year interval and T is the water temperature (°C). The GC_{YI} was iteratively fit for each year class so that the predicted body weight of fish at the next May 20th date corresponded to the body weight predicted under the constant temperature scenario. The fitted coefficients for each year class are described in the footnotes of Table 4.

Whole body lipid contents (g lipid per g body weight) were predicted from the temperature-dependent algorithm used for medium and large yellow perch described in Drouillard et al. (2009). For the constant temperature simulations, all fish had a constant fractional lipid content of 0.086 (by weight). For the dynamic temperature scenario, lipid content ratios varied between 0.043 and 0.093, depending on the temperature. Water content of the fish was predicted from the negative correlation between %moisture and %lipid as described in Drouillard et al. (2009). The lean dry

Table 4 Model parameters and variables used in yellow perch non-steady state bioaccumulation simulations

Model parameter/variable	Constant temperature simulation	Variable temperature simulation
Chemical log K_{OW}	6.5	6.5
C_w—Concentration in water (ng/mL)	0.001	0.001
C_{food}—Concentration in food (ng/g ww)[a]	79.85	79.85
$f_{LEQ(food)}$—Lipid equivalent content of food	0.025	0.025
$f_{L(food)}$—Lipid content of food	0.015	0.015
$f_{LDP(food)}$—Lead dry protein content of food	0.205	0.205
E_D—Energy density of food (kJ/g)	4.28	4.28
E_{diet}—Energy assimilation from diet	0.644	0.644
E_{food}—Digestion efficiency of food[b]	0.863	0.863
$C_{fish(0)}$—Initial concentration in fish (ng/g ww)[c]	305.07	269.02
$f_{LEQ(EX)}$—Lipid equivalent content of feces	0.0053	0.0053
T—Temperature (°C)	21	Variable[d]
L—Body length of fish (cm)[e]	Variable with age	NA
BW—Body weight of fish (g)[f,g]	$BW = 10^{(\log (L) \times 3.267 - 2.30)}$	Temperature dependent growth[g]
$f_{L(fish)}$—Lipid content of fish[h]	0.0863	Temperature dependent
$f_{w(fish)}$—Water content of fish[i]	$f_{w(fish)} = -0.68 \times f_{L(fish)} + 76.9$	$-0.68 \times f_{L(fish)} + 76.9$
$f_{LDP(fish)}$—Lean dry protein content of fish	$f_{LDP(fish)} = 1 - f_{w(fish)} - f_{L(fish)}$	$f_{LDP(fish)} = 1 - f_{w(fish)} - f_{L(fish)}$
$f_{LEQ(fish)}$—Lipid equivalents content of fish	$f_{LEQ(fish)} = f_{L(fish)} + 0.05 f_{LDP(fish)}$	$f_{LEQ(fish)} = f_{L(fish)} + 0.05 f_{LDP(fish)}$
ΔX_L—Daily lipid growth increment (g)	$\Delta X_L = X_{L(t)} - X_{L(t-1)}$	$\Delta X_L = X_{L(t)} - X_{L(t-1)}$
ΔX_{LDP}—Daily protein growth increment (g)	$\Delta X_{LDP} = X_{LDP(t)} - X_{LDP(t-1)}$	$\Delta X_{LDP} = X_{LDP(t)} - X_{LDP(t-1)}$
ΔG_L—Energy cost of lipid growth (kJ/d)	$\Delta G_L = \Delta X_L$ 39.3 always positive or 0	$\Delta G_L = \Delta X_L \times 39.3$ term may be positive or negative for weight gain or weight loss
ΔG_P—Energy cost of protein growth (kJ/d)	$\Delta G_P = \Delta X_L \times 18$ always positive or 0	$\Delta G_P = \Delta X_L \times 18$ term may be positive or negative due to weight gain or weight loss
D_{O2}—Oxycalorific coefficient (kJ/g O_2)	14.3	14.3
SMR × A—Standard metabolic rate and activity cost multiplier (kJ/g BW/d)[j]	$SMR \times A = 0.01 \times BW^{0.72} \times e^{(0.19 \times T)} \times 0.024 \times D_{O2} \times 1.5/BW$	$SMR \times A = 0.01 \times BW^{0.72} \times e^{(0.19 \times T)} \times 0.024 \times D_{O2} \times 1.5/BW$
SDA—Specific dynamic action (kJ/g BW/d)[j]	$SDA = 0.172 \times (SMR \times A + \lvert \Delta G_L \rvert + \lvert \Delta GP \rvert)$	$SDA = 0.172 \times (SMR \times A + \lvert \Delta G_L \rvert + \lvert \Delta GP \rvert)$
U—Excretion (kJ/g BW/d)[j]	$U = 0.0253 \times T^{0.58} \times e^{-0.299} \times (SMR \times A + \lvert \Delta G_L \rvert + \lvert \Delta GP \rvert)$	$U = 0.0253 \times T^{0.58} \times e^{-0.299} \times (SMR \times A + \lvert \Delta G_L \rvert + \lvert \Delta GP \rvert)$

Q_{feed}—Food consumption (kJ/g BW/d)	$Q_{feed} = \pm\Delta G_L \pm \Delta G_P + SMR \times A + SDA + U$	$Q_{feed} = \pm\Delta G_L \pm \Delta G_P + SMR \times A + SDA + U$
R—Respiration (kJ/g/BW/d)[j]	$R = SMR \times A + SDA \times U$	$R = SMR \times A + SDA \times U$
C_{O2}–Dissolved oxygen concentration in water (g/mL)[j]	$14.45–0.413 \times T + 5.56 \times 10^{-3} \times T^2/1 \times 10^{-6}$	$14.45–0.413 \times T + 5.56 \times 10^{-3} \times T^2/1 \times 10^{-6}$
E_{O2}—Oxygen efficiency across gills	0.6	0.6
G_v—Gill ventilation (mL/g/d)	$G_v = R/(D_{O2} \times C_{O2} \times E_{O2})$	$G_v = R/(D_{O2} \times C_{O2} \times E_{O2})$
G_{feed}—Food ingestion rate (g/g/d)	$G_{feed} = Q_{feed}/E_D$	$G_{feed} = Q_{feed}/E_D$
G_{ex}—feces excretion rate (g/g/d)	$G_{ex} = G_{feed} \times E_{food}$	$G_{ex} = G_{feed} \times E_{food}$
K_{BW}—Biota/water partition coefficient	$K_{BW} = K_{OW} \times f_{LEQ(fish)}$	$K_{BW} = K_{OW} \times f_{LEQ(fish)}$
K_{BEX}—Biota/feces partition coefficient	$K_{BEX} = f_{LEQ(EX)}/f_{LEQ(fish)}$	$K_{BEX} = f_{LEQ(EX)}/f_{LEQ(fish)}$
AE_w—Chemical extraction efficiency across gills	0.54	0.54
AE_{food}—Dietary chemical assimilation efficiency	0.6	0.6
IN_{Wat}—Daily chemical intake from water (ng/d)	$IN_{Wat} = G_v \times C_w \times AE_w \times BW$	$IN_{Wat} = G_v \times C_w \times AE_w \times BW$
IN_{food}—Daily chemical intake from food (ng/d)	$IN_{food} = G_{feed} \times C_{food} \times AE_f \times BW$	$IN_{food} = G_{feed} \times C_{food} \times AE_f \times BW$
OUT_{wat}—Daily loss chemical to water (ng/d)	$OUT_{Wat} = G_v \times AE_w \times BW \times C_{fish(t-1)}/K_{BW}$	$OUT_{Wat} = G_v \times AE_w \times BW \times C_{fish(t-1)}/K_{BW}$
OUT_{EX}—Daily loss of chemical to feces (ng/d)	$OUT_{EX} = G_{ex} \times AE_{food} \times BW \times C_{fish(t-1)}/K_{BW}$	$OUT_{EX} = G_{ex} \times AE_{food} \times BW \times C_{fish(t-1)}/K_{BW}$
$X_{fish(t)}$—Mass of chemical in fish at time t (ng)	$X_{fish(t)} = X_{fish(t-1)} + IN_{Wat} + IN_{food} - OUT_{Wat} - OUT_{EX}$	$X_{fish(t)} = X_{fish(t-1)} + IN_{Wat} + IN_{food} - OUT_{Wat} - OUT_{EX}$
$C_{(t)}$—Concentration in fish at time t (ng/g lipid equivalents)	$C_{(t)} = X_{fish(t)}/(BW \times f_{LEQ(fish)})$	$C_{(t)} = X_{fish(t)}/(BW \times f_{LEQ(fish)})$

[a]Food was assumed to be in equilibrium with water. $C_{food} = K_{OW} \times C_w \times f_{LEQ}$, where C_W is the concentration of chemical in water and f_{LEQ} is the lipid equivalent fraction in food

[b]Digestion efficiency of food refers to the reduction in volume of feces per unit of food consumed. Estimation based on a lipid assimilation efficiency of 0.92, lean dry protein assimilation efficiency of 0.6, and water assimilation of 0

[c]Year 1 fish on day 364 were assumed to be in equilibrium with water. $C_{fish(o)} = K_{OW} \times C_w \times f_{LEQ}$, where C_W is the concentration of chemical in water and f_{LEQ} is the lipid equivalent fraction in food

[d]Variable temperature involved a repeating temperature cycle measured in mesocosm tanks in Windsor, Ontario as described in (Drouillard et al. 2009). Day 1 of the simulation corresponded to May, 20 with a temperature of 16.0 °C

[e]Von Bertalanffy Growth curve for yellow perch reported by (Jackson et al. 2008). Total Length (cm) = $[280.5 \times (1 - e(-0.332 \times (Age + 0.031))]/10$, where Age is in days

[f]Body weight versus length relationship was based on seasonally collected yellow perch from ponds in southwestern Ontario (Unpublished data)

[g]Growth rates were fitted to be temperature dependent. For each birthday of the fish (May 20, years 1–8), the body weight was set to the body weight used for the constant temperature simulation. A temperature and year dependent growth constant (GC_{YI}) was fitted for each year interval to achieve the target body weight by the next anniversary. The equation was: $BW = GC_{YI} \times T + BW_{(t-1)}$, where GC is specific to each year class (0.0046; 0.0074; 0.0083; 0.0079; 0.0067; 0.0054; 0.0043; and 0.003 for years 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, and 8+) and T is the water temperature (°C)

[h]Calculated using a temperature-dependent algorithm reported for medium and large yellow perch by (Drouillard et al. 2009). % Lipid = $0.23 \times T + 3.8$. f_{lipid} = % Lipid/100

[i]Relationship between moisture content of yellow perch and lipid content of yellow perch reported by (Drouillard et al. 2009)

[j]Based on yellow perch algorithms for SMR, SDA and U reported by (Drouillard et al. 2009)

weight of fish was subsequently estimated by subtracting the water and lipid weights from the fish body weight. Daily changes in whole body lipid and lean dry weights were used to estimate growth increments, growth energy costs and lipid equivalent contents for each time step (see Table 4 for details). Daily estimates of food consumption rates (g food consumed per g body weight per day) and gill ventilation rates (mL water ventilated per g body weight per day) for yellow perch were calculated from temperature and size dependent algorithms as described in Drouillard et al. (2009) and detailed in Table 4.

The uptake portion of the toxicokinetics sub-model used the calculation methods of Arnot and Gobas (2004). Uptake flux of chemical into fish from water (ng/d) was estimated as the product of gill ventilation rate (mL per g body weight per day), chemical extraction efficiency across gills (unitless), chemical concentration in water (ng per mL), and body weight (g) of fish. In both scenarios, the recommended value of 0.54 was used for the chemical extraction efficiency across gills. The uptake flux of chemical into fish from food (ng/d) was estimated as the product of the feeding rate, dietary assimilation efficiency of the chemical, chemical concentration in food and body weight of fish. A constant value of 0.6 was used as the dietary assimilation efficiency value of the chemical. Chemical loss from fish (ng/d) via gills and feces were estimated as detailed in Drouillard et al. (2009), and described in Table 4. For simplicity of the simulations, the same chemical extraction efficiency across gills was used as the uptake algorithm. Similarly, the organism/feces chemical exchange efficiency was set to be equal to the dietary chemical assimilation efficiency described above.

Yellow perch model simulations were performed to predict bioaccumulation and bioamplification of a negligibly metabolized POP compound having a log K_{OW} of 6.5. Two different simulation scenarios were established to compare bioamplification under an artificial baseline condition of constant temperatures and a seasonally dynamic scenario that is consistent with temperate aquatic ecosystems. The baseline scenario involved a simulation, in which temperature was kept constant (21 °C; at the species optimum) across seasons and years. The seasonal scenario involved a simulation, in which an annual temperature profile that is consistent with measurements made in aquaculture ponds of Southern Ontario, was applied to predict the seasonal variation in lipid content and its impact on chemical bioamplification. The same annual temperature profile was cycled across simulation years. In both simulations, the model was initialized by assuming Age 1 fish were in chemical equilibrium with water. Fish were assumed to feed on a stable food source (constant in its proximate composition, energy density and chemical concentrations) over the duration of the simulation. The concentration of chemical in food was set so that the food was in equilibrium with the concentration of chemical in water.

The yellow perch non-steady state bioaccumulation model was developed using a Microsoft Excel spreadsheet. The model is a finite difference model run at a daily time steps for 2,920 days. The model was initialized with a temperature of 15.9 °C and fish aged 365 d (May 20, year 1), body weight of 4.84 g, lipid content of 7.5%, moisture content of 71.8%. The initial fish concentration was set to be in equilibrium with water. Food concentration was set to be in equilibrium with water. The food concentration we held constant throughout the experimental duration.

11 Appendix 2: Description of the Herring Gull Model and Associated Simulations

The herring gull bioenergetic and toxicokinetics model is described in several papers (Norstrom et al. 1986a, b, 2007; Clark et al. 1987, 1988; Drouillard et al. 2003). Most commonly, the model has been used to describe non-steady bioaccumulation of POPs in adult female life stages over multi-year periods. However, the basic algorithms for chick growth and bioenergetics of early and late life stages for both sexes are detailed in Norstrom et al. (1986b). For simplicity, the herring gull model simulations were formulated for male birds to circumvent the need to consider chemical depuration by egg laying, and to maximize predictions of bioamplification in the species. The model was expanded to include three linked life-stages: a chick stage (post-pipping to fledging), immature adult (post fledging to 3.8 years), and a reproductive adult (3.8 years to 8 years) stage. The chick stage immediately experiences bioamplification from maternally deposited residues (Drouillard et al. 2003), followed by growth dilution until fledging. The subadult male experiences seasonal temperature variation and proximate composition, but does not participate in reproductive activities (such as courtship, or the feeding and foraging costs associated with rearing a clutch of chicks). Adult males experience additional foraging costs associated with the later activities. Although herring gulls have much longer life spans than the 8-year period of the model simulation, an 8-year duration was selected for consistency with yellow perch simulations.

For each life stage, the bioenergetic sub-model predicts growth, proximate composition, and food consumption as outlined in Norstrom et al. (1986a). The toxicokinetics model only considers chemical uptake from food, since air uptake by birds is negligible (Drouillard et al. 2012). Similar to the yellow perch model, the uptake flux of chemical into the bird from food (ng/d) was estimated as the product of the feeding rate, dietary assimilation efficiency of chemical, chemical concentration in food, and body weight of fish. A constant value of 0.9 was used as the dietary assimilation efficiency value for the chemical and was derived from data collected for ring doves (Drouillard and Norstrom 2000). The toxicokinetic parameters necessary to describe chemical elimination included the plasma/fat partition coefficient (K_{PF}) and plasma clearance coefficient (k'_{pc}). For model simulations, the values of K_{PF} and k'_{pc} for mirex, measured in juvenile herring gulls (Clark et al. 1987), were used and were assumed to be constant across the different life stages. Mirex was chosen to represent a highly hydrophobic POP that is negligibly biotransformed in birds. A modification to the herring gull model not applied in previous publications of the model was that the chemical outflux was measured by multiplying the k'_{pc} by the body weight and lipid equivalent concentration of chemical in the animal tissues. Past iterations of the module used the lipid normalized concentration. This change was made to make the model more consistent with the yellow perch model. However, it should be noted that the fish and bird models differ fundamentally in how elimination flux is treated. In the herring gull model, k'_{PC} is a constant, and elimination flux varies over the year only as a result of changes in the proximate

composition (lipid equivalent content) of the animal. In the fish model, elimination flux of the chemical depends on variation in proximate composition, as well as variation in gill ventilation and feeding rates. These differences result in a decoupling of feeding rates and chemical elimination in birds that causes lags in return to steady state, following sudden shifts in feeding rates. This is exemplified in Fig. 2c for the constant temperature, post-adult male simulation. No attempts were made to harmonize the two model organisms into a single toxicokinetic model, because we preferred to preserve the characteristics and attributes of the models that had been addressed in their original publications.

As for the yellow perch models, two simulation scenarios were established for herring gulls. The baseline simulation kept temperature and photoperiod constant at 21 °C and 12 h/d across seasons and years. The seasonal scenario used temperature and photoperiod data from Lake Ontario that had been collected during 1997. The model used monthly mean temperature and photoperiod data and interpolated temperature and photoperiods for each day of the simulation. The model cycled the same annual temperature profile across all years in each simulation. The model was initialized using a fresh egg concentration predicted from a 10-year adult female model simulation (Clark et al. 1988), wherein the female bird was fed a constant diet of the same concentration and energy density as that used for male simulations. The female simulation used a constant temperature and photoperiod to initialize the constant temperature simulation, and a variable temperature and photoperiod equivalent to the Lake Ontario profile to initialize the dynamic temperature simulation. The simulated fresh egg concentration (μg/kg wet weight) from adult female simulations was multiplied by the egg weight (85 g), and was divided by the fresh egg lipid content (7.2 g) as reported by Drouillard et al. (2003) to estimate the lipid normalized egg concentration. Bioamplification of fresh egg residues in the pipping embryo was accounted for by multiplying the fresh egg lipid normalized concentration by a factor of 3.1 (Drouillard et al. 2003), and multiplied by the lipid equivalent content of the newly pipped chick to determine the total mass of chemical in the chick. The bird was grown out and was assumed to feed on a constant food source of proximate composition, energy density, and chemical concentration that was similar for the duration of the study. A full description of model parameters and algorithms employed is presented in Table 5.

The herring gull toxicokinetic model output was copied onto a Microsoft Excel spreadsheet. The model is a finite difference model and was run at a daily time steps for 3,137 days. The model was initialized with a 1-day-old pipping male chick hatched on May 29, 1997. The chick sub-model was used to calculate growth, proximate composition, bioenergetics, and chemical toxicokinetics between days 1 and 88. The output from the chick sub-model was linked to a subadult male model between days 89 and 1,398, i.e., up to 4 years. In the subadult model, immature birds were assumed to not participate in reproductive activities and therefore had no courtship feeding or chick provisioning costs. The output from the subadult male model was linked to a reproductive adult male model which covered simulation days 1,399–3,136.

Table 5 Model parameters and variables used in herring gull non-steady state bioaccumulation simulations

Model parameter/variable	Constant temperature simulation	Variable temperature simulation
Parameters common to chick and adult sub-models		
K_{pf}—Plasma/fat distribution coefficient[a]	0.0067	0.0067
k'_{pc}—Plasma clearance coefficient (L/kg/d)[a]	0.041	0.041
C_{food}—Concentration in food (ng/g ww)	79.85	79.85
E_D—Energy density of food (kcal/g)	1.24	1.24
E_{diet}—Energy assimilation from diet[b]	0.85	0.85
AE_{food}—Dietary chemical assimilation efficiency[c]	0.9	0.9
T—Temperature (°C)[d]	21	Variable by season
PP—Photoperiod (h daylight/d)[d]	12	Variable by season
Chick sub-model (days 1–88)		
$C_{chick(0)}$—Initial concentration in pipping chick (ng/g lipid weight)[e]	54,012	101,243
BW—Time dependent body weight of chick[f]	$BW = 1{,}193 \times \exp^{[-\exp(-0.075\times(Age-16.3))]}$	$BW = 1{,}193 \times \exp^{[-\exp(-0.075\times(Age-16.3))]}$
I_w—Water content of chick per g of lean dry weight (g/g)[g]	$I_W = 4.255–0.061$ age (days 3–36). See footnote	$I_W = 4.255–0.061$ age (days 3–36). See footnote
I_L—Lipid content of chick per g of lean dry weight (g/g)[b]	0.24	0.24
LDW—Lean dry weight of chick (g)	$LDW = BW/(1 + I_W + I_L)$	$LDW = BW/(1 + I_W + I_L)$
X_{lipid}—Mass of lipid in chick (g)	$X_{lipid} = LDW \times I_L$	$X_{lipid} = LDW \times I_L$
X_{LEQ}—Mass of lipid equivalents in chick (g)	$X_{LEQ} = X_{lipid} + 0.05 \times LDW$	$X_{LEQ} = X_{lipid} + 0.05 \times LDW$
$Q_{feed(chick)}$—Food consumption rate of chick (kcal/d)[h]	Estimated on a daily basis Days 1–88. See footnote for details	Estimated on a daily basis Days 1–88. See footnote for details
G_{feed}—Food ingestion rate (g/g/d)	$G_{food} = Q_{feed(chick)}/E_D$	$Q_{food} = Q_{feed(chick)}/E_D$
IN_{food}—Daily chemical intake from food (ng/d)	$IN_{food} = G_{feed} \times C_{food} \times AE_{food}$	$IN_{food} = G_{feed} \times C_{food} \times AE_{food}$
OUT_{EX}—Daily loss of chemical (ng/d)	$OUT_{EX} = k'_{pc} \times BW \times C_{(t-1)} \times K_{pf}$	$OUT_{EX} = k'_{pc} \times BW \times C_{(t-1)} \times K_{pf}$
$X_{chick(t)}$—Mass of chemical in chick at time t (ng)	$X_{chick(t)} = X_{chick(t-1)} + IN_{food} - OUT_{EX}$	$X_{chick(t)} = X_{chick(t-1)} + IN_{food} - OUT_{EX}$
$C_{(t)}$—Concentration of chemical in chick at time t (ng/g lipid weight)	$C_{(t)} = X_{chick(t)}/X_{LEQ}$	$C_{(t)} = X_{chick(t)}/X_{LEQ}$
Immature male sub-model (days 89–1,397)		
f_{lipid}—Fraction of lipid in body[b]	0.079	$f_{lipid} = (-0.27 \times T + 3.4)/100$

(continued)

Table 5 (continued)

Model parameter/variable	Constant temperature simulation	Variable temperature simulation
LDW—Lean dry weight of male (g)[b]	314	314
BW—Body weight of male (g)	1,098	$BW = LDW \times 3.22/(1-f_{lipid})$
X_{lipid}—Lipid weight of male (g)	86.97	$X_{lipid} = BW \times f_{lipid}$
X_{LEQ}—Lipid equivalent weight of male (g)	102.67	$X_{LEQ} = X_{lipid} + 0.05 \times LDW$
ΔX_L—daily growth increment of lipid (g)	0	$\Delta X_{lipid} = X_{lipid(t)} - X_{lipid(t-1)}$
MR—Metabolic rate due to basal metabolism and thermoregulation (kcal/d)	141.6	$SMR = (3.35 + 0.05PP) \times BW^{0.544} - ((0.141 + 0.006PP) \times BW^{0.3})T$
ΔG_L Energy cost of lipid growth (kJ/d)	0	$\Delta G_L = \Delta X_L \times 9.4$ Term may be positive or negative due to weight gain or weight loss
F—Foraging costs (kcal/d)[i]	55.1	$F = (0.28/(1-0.28)) \times (MR \pm \Delta G_L)$
Q_{feed}—Food consumption rate of subadult male (kcal/d)	$Q_{feed} = MR + F$	$Q_{feed} = \pm \Delta G_L + MR + F$
G_{feed}—Food ingestion rate (g/g/d)	$G_{feed} = Q_{feed}/E_D$	$G_{feed} = Q_{feed}/E_D$
IN_{food}—Daily chemical intake from food (ng/d)	$IN_{food} = G_{feed} \times C_{food} \times AE_{food}$	$IN_{food} = G_{feed} \times C_{food} \times AE_{food}$
OUT_{EX}—Daily loss of chemical (ng/d)	$OUT_{EX} = k'_{pc} \times BW \times C_{(t-1)} \times K_{pf}$	$OUT_{EX} = k'_{pc} \times BW \times C_{(t-1)} \times K_{pf}$
$X_{subadult(t)}$—Mass of chemical in subadult male at time t (ng)	$X_{subadult(t)} = X_{subadult(t-1)} + IN_{food} - OUT_{EX}$	$X_{subadult(t)} = X_{subadult(t-1)} + IN_{food} - OUT_{EX}$
$C_{(t)}$—Concentration at time t (ng/g lipid equivalents weight)	$C_{(t)} = X_{suzbadult(t)}/X_{LEQ}$	$C_{(t)} = X_{subadult(t)}/X_{LEQ}$
Mature male sub-model (days 1,398–3,137)		
f_{lipid}—Fraction of lipid in adult tissues[b]	0.079	$f_{lipid} = (-0.27 \times T + 13.4)/100$
LDW—Lean dry weight of adult male (g)[b]	314	314
BW—Body weight of adult male (g)	1,098	$BW = LDW \times 3.22/(1-f_{lipid})$
X_{lipid}—Lipid weight of adult male (g)	86.97	$X_{lipid} = BW \times f_{lipid}$
X_{LEQ}—Lipid equivalent weight of male (g)	102.67	$X_{LEQ} = X_{lipid} + 0.05 \times LDW$
ΔX_L—Daily growth increment of lipid (g)	0	$\Delta X_L = X_{lipid(t)} - X_{lipid(t-1)}$
MR—Metabolic rate due to basal metabolism and thermoregulation costs (kcal/d)	141.6	$SMR = (3.35 + 0.05PP) \times BW^{0.544} - ((0.141 + 0.006PP) \times BW^{0.3})T$
ΔG_L Energy cost of lipid growth (kJ/d)	0	$\Delta G_L = \Delta X_L \times 9.4$ Term may be positive or negative due to weight gain or weight loss
F—Foraging costs (kcal/d)[i]	55.1	$F = (0.28/(1-0.28)) \times (MR \pm \Delta G_L)$

CF—Courtship feeding costs (kcal/d)[j]	Estimated on a daily basis for days March 27–April 25 during years 4–8 of the simulation. See footnote	Estimated on a daily basis for days March 27–April 25 during years 4–8 of the simulation. See footnote
CR—Chick rearing costs (kcal/d)[k]	Estimated on daily basis for days May 28–Aug 24 during years 4–8 of the simulation. See footnote	Estimated on daily basis for days May 28–Aug 24 during years 4–8 of the simulation. See footnote
	$CR = [0.28/(1-0.28) \times (Q_{feed(chick)})] \times N_{chicks}/2$	$CR = [0.28/(1-0.28) \times (Q_{feed(chick)})] \times N_{chicks}/2$
Q_{feed}—Food consumption rate of subadult male (kcal/d)	$Q_{feed} = MR + F + CF + CR$	$Q_{feed} = \pm \Delta G_L + MR + F + CF + CR$
G_{feed}—Food ingestion rate (g/g/d)	$G_{feed} = Q_{feed}/E_D$	$G_{feed} = Q_{feed}/E_D$
IN_{food}—Daily chemical intake from food (ng/d)	$IN_{food} = G_{feed} \times C_{food} \times AE_{food}$	$IN_{food} = G_{feed} \times C_{food} \times AE_{food}$
OUT_{EX}—Daily loss of chemical (ng/d)	$OUT_{EX} = k'_{pc} \times BW \times C_{(t-1)} \times K_{pf}$	$OUT_{EX} = k'_{pc} \times BW \times C_{(t-1)} \times K_{pf}$
$X_{adult(t)}$—Mass of chemical in subadult male at time t (ng)	$X_{adult(t)} = X_{adult(\tau-1)} + IN_{food} - OUT_{EX}$	$X_{adult(t)} = X_{adult(t-1)} + IN_{food} - OUT_{EX}$
$C_{(t)}$—Concentration at time t (ng/g lipid equivalents weight)	$C_{(t)} = X_{adult(t)}/X_{LEQ}$	$C_{(t)} = X_{adult(t)}/X_{LEQ}$

[a]Equivalent value for herring gulls as reported for mirex in (Clark et al. 1987)

[b]Estimate for herring gulls as reported in (Norstrom et al. 1986a, b)

[c]Reported dietary assimilation efficiency of PCBs in ring doves from Drouillard and Norstrom (2000)

[d]Average monthly values of temperature and photoperiod for Lake Ontario during 1997 were used as recommended for the default model inputs in the visual basic version of the Herring gull model. The same monthly means were repeated across the different years of the simulation

[e]The herring gull visual basic model was run for an adult female with an initial bird concentration of zero; birds were fed a constant diet containing 79.85 ng/g of chemical for 8 years until steady state was achieved. The model predicted egg concentration after 8 years was 2,763 ng/g wet weight. The fresh egg concentration was converted to a lipid normalized egg concentration of 32,619 ng/g lipid weight using a fresh egg weight of 85 g and egg lipid content of 7.2 g (Drouillard et al. 2003). The fresh egg concentration was multiplied by 3.1 to account for bioamplification in the embryo as reported for herring gull chicks at the time of pipping (Drouillard et al. 2003)

[f]Male chick Gompertz growth equation reported by (Norstrom et al. 1986a, b). Chick age is in days. Growth curve used until day 58 when predicted chick weight became equivalent to adult male weight

[g]The water content per lean dry weight content was set to 2.81 and 3.84 for day 1 and 2. Between days 3 and 36, it was estimated according to $I_W = 4.255–0.061$ age (days 3–36) as reported in (Norstrom et al. 1986a, b). The parameter was then held constant at a value of 2 for days 37–89. Beyond day 89, the subadult model was used and does not require this parameter

[h]Based on total energy intake per chick per day for herring gull chicks extrapolated from Fig. 2 of Norstrom et al. (1986a, b) between days 1 and 70. Between days 71 and 89, value held constant at the adult sub-model start value of 232.6 kcal/d

[i]Foraging coefficient established at 0.28 as recommended for Great Lakes herring gulls by Norstrom et al. (1986a, b). Constant of 9.4 represents the energy per gram of fat (kcal/g)

[j]Courtship feeding costs were interpolated on a daily basis from data presented in Table 5 of Norstrom et al. (1986a, b)

[k]Chick rearing costs consider the additional foraging cost associated with feeding a clutch of chicks (kcal/d). Daily energy requirements of chicks are established in footnote h. The foraging cost increment for the adult male is given by: $CR = [(0.28/(1-0.28)) \times (C_{chick})] \times N_{chicks}/2$, where C_{chick} is the daily energy intake of each chick (kcal/d), 0.28 is the foraging coefficient (see footnote i) and N_{chick} is the number of chicks (3) reared each year. The function is divided by 2 because it is assumed that adult male birds contribute to 50% of the food provisioning for a clutch of chicks

References

Addison RF, Stobo WT (1993) Organochlorine concentrations and burdens in grey seal (*Halichoerus grypus*) blubber during the first year of life. J Zool Lond 230:443–450

Alonso E, Tapie N, Budzinski H, Leménach K, Peluhet L, Tarazona JV (2008) A model for estimating the potential biomagnification of chemicals in a generic food web: preliminary development. Env Sci Pollut Res 15:31–40

Angell RA, Haffner GD (2010) Polychlorinated biphenyl elimination rates and changes in chemical activity in hibernating amphibians. Environ Toxicol Chem 29:700–707

Arnot JA, Gobas FAPC (2003) A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs. QSAR Comb Sci 22:337–345

Arnot JA, Gobas FAPC (2004) A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environ Toxicol Chem 23:2343–2355

Arnot JA, Gobas FA (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev 14:257–297

Antunes P, Gil O, Ferreira M, Vale C, Reis-Henriques MA (2007) Depuration of PCBs and DDTs in mullet under captivity clean conditions. Chemosphere 57:58–64

Barber MC, Suarez LA, Lassiter RR (1988) Modeling bioconcentration of nonpolar organic pollutants by fish. Environ Toxicol Chem 7:545–558

Barber MC (2003) A review and comparison of models for predicting dynamic chemical bioconcentration in fish. Environ Toxicol Chem 22:1963–1992

Barber MC (2008) Dietary uptake models used for modeling the bioaccumulation of organic contaminants in fish. Environ Toxicol Chem 27:755–777

Barron MG (1990) Bioconcentration. Will water-borne organic chemicals accumulate in aquatic animals? Environ Sci Technol 24:1612–1618

Bartrons M, Grimalt JO, Catalan J (2007) Concentration changes of organochlorine compounds and polybromodiphenyl ethers during metamorphosis of aquatic insects. Environ Sci Technol 41:6137–6141

Beacham TD, Murray CB, Withler RE (1989) Age, morphology, and biochemical genetic variation of Yukon river chinook salmon. Trans Am Fish Soc 118:46–63

Belpaire C, Goemans G (2007) Eels: contaminant cocktails pinpointing environmental contamination. ICES J Mar Sci 64:1423–1436

Bhavsar SP, Gewurtz SB, McGoldrick DJ, Keir MJ, Backus SM (2010) Changes in mercury levels in Great Lakes fish between 1970s and 2007. Environ Sci Technol 44:3273–3279

Black MC, McCarthy JF (1988) Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants by the gills of rainbow trout (*Salmo gairdneri*). Environ Toxicol Chem 7:593–600

Blais JM, Wilhelm F, Kidd KA, Muir DCG, Donald DB, Schindler DW (2003) Concentrations of organochlorine pesticides and polychlorinated biphenyls in amphipods (*Gammarus lacustris*) along an elevation gradient in mountain lakes of western Canada. Environ Toxicol Chem 22:2605–2613

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Boon JP, Duinker JC (1985) Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (*Solea solea*) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquat Toxicol 7:119–134

Boon JP, Eijgenraam F, Everaarts JM, Duinker JC (1989) A structure-activity relationship (SAR) approach towards metabolism of PCBs in marine animals from different trophic levels. Mar Environ Res 27:159–176

Boon JP, Oostingh I, van der Meer J, Hillebrand TJ (1994) A model for the bioaccumulation of chlorobiphenyl congeners in marine mammals. Eur J Pharmacol 270:237–251

Borgå K, Fisk AT, Hoekstra PF, Muir DCG (2004) Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. Environ Toxicol Chem 23:2367–2385

Borgå K, Kidd KA, Muir DCG, Berglund O, Conder JM, Gobas FAPC, Kucklick J, Malm O, Powell DE (2012) Trophic magnification factors: considerations of ecology, ecosystems and study design. Int Environ Assess Manage 8:64–84

Braune BM, Norstrom RJ (1989) Dynamics of organochlorine compounds in herring gulls: 3 tissue distribution and bioaccumulation in Lake Ontario gulls. Environ Toxicol Chem 8:957–968

Bull CD, Metcalfe NB, Mangel M (1996) Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. Proc R Soc London Ser B 263:13–18

Bustnes JO, Moe B, Herzke D, Hanssen SA, Nordstad T, Sagerup K, Gabrielsen GW, Borgå K (2010) Strongly increasing blood concentrations of lipid-soluble organochlorines in high arctic common eiders during incubation fast. Chemosphere 79:320–325

Bustnes JO, Moe B, Hanssen AA, Herzke D, Fenstad AA, Nordstad T, Borga K, Gabrielsen GW (2012) Temporal dynamics of circulating persistent organic pollutants in a fasting seasbird under different environmental conditions. Environ Sci Technol 46:10287–10294

Bystrom P, Andersson J, Kiessling A, Eriksson LO (2006) Size and temperature dependent foraging capacities and metabolism: consequences for winter starvation mortality in fish. Oikos 115:43–52

Campfens J, Mackay D (1997) Fugacity-based bodel of PCB bioaccumulation in complex aquatic food webs. Environ Sci Technol 31:577–583

Chevrier J, Dewailly E, Ayotte P, Despres JP, Tremblay A (2000) Body weight loss increase plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. Int J Obese Related Metab Disord 24:1272–1278

Chiuchiolo AL, Dickhut RM, Cochran MA, Ducklow HW (2004) Persistent organic pollutants at the base of the Antarctic marine food web. Environ Sci Technol 38:3551–3557

Chou CC, Chen YN, Li CS (2004) Congener-specific polychlorinated biphenyls in cetaceans from Taiwan waters. Arch Environ Contam Toxicol 47:551–560

Chow BA, Hamilton J, Cattet MR, Stenhouse G, Obbard ME, Vijayan MM (2011) Serum corticosteroid binding globulin expression is modulated by fasting in polar bears (*Ursus maritimus*). Comp Biochem Physiol Part A Mol Integr Physiol 158:111–115

Christensen JR, MacDuffee M, Yunker MB, Ross P (2007) Hibernation-associated changes in persistent organic pollutant (POP) levels and patterns in British Columbia grizzly bears (*Ursus arctos horribilis*). Environ Sci Technol 41:1834–1840

Clark DR, Prouty RM (1977) Experimental feeding of DDE and PCB to female big brown bats (*Eptesicus fuscus*). J Toxicol Environ Health 2:917–928

Clark DR (1981) Bats and environmental contaminants: a review. U.S. Department of the Interior (Special Scientific Report # 235). Fish and Wildlife Service, Washington, DC, pp 1–29.

Clark DR Jr, Krynitsky AJ (1983) DDT: recent contamination in New Mexico and Arizona? Environment 25:27–31

Clark TP, Norstrom RJ, Fox GA, Won HT (1987) Dynamics of organochlorine compounds in herring gulls (*Larus argentatus*): II. A two-compartment model and data for ten compounds. Environ Toxicol Chem 6:547–559

Clark T, Clark K, Paterson S, Norstrom R, Mackay D (1988) Wildlife monitoring, modeling and fugacity. Environ Sci Technol 22:120–127

Clark KE, Gobas FAPC, Mackay D (1990) Model of organic chemical uptake and clearance by fish from food and water. Environ Sci Technol 24:1203–1213

Colabuono FI, Taniguchi S, Montone RC (2012) Organochlorine contaminants in albatrosses and petrels during migration in South Atlantic Ocean. Chemosphere 86:701–708

Connell DW (1988) Bioaccumulation behavior of persistent organic chemicals with aquatic organisms. Rev Environ Contam Toxicol 102:117–154

Connolly JP, Pedersen CJ (1988) A thermodynamic based evaluation of organic chemical accumulation in aquatic organisms. Environ Sci Technol 22:99–103

Cornelissen G, Gustafsson Ö, Bucheli TD, Jonker MTO, Koelmans AA, van Noort PCM (2005) Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ Sci Technol 39:6881–6895

Czub G, McLachlan MS (2004) A food chain model to predict the levels of lipophilic organic contaminants in humans. Environ Toxicol Chem 23:2356–2366

Czub G, McLachlan MS (2007) Influence of the temperature gradient in blubber of bioaccumulation of persistent lipophilic organic chemicals in seals. Environ Toxicol Chem 26:1600–1605

Dale WE, Gaines TB, Hayes WJ Jr (1962) Storage and excretion of DDT in starved rats. Toxicol App Pharmacol 4:89–106

Daley JM, Leadley TA, Drouillard KG (2009) Evidence for bioamplification of nine polychlorinated biphenyl (PCB) congeners in yellow perch (Perca flavescens) eggs during incubation. Chemosphere 75:1500–1505

Daley JM, Corkum LD, Drouillard KG (2011) Aquatic to terrestrial transfer of sediment associated persistent organic pollutants is enhanced by bioamplification processes. Environ Toxicol Chem 30:2167–2174

Daley JM, Leadley TA, Pitcher TE, Drouillard KG (2012) Bioamplification and the selective depletion of persistent organic pollutants in Chinook salmon larvae. Environ Sci Technol 46:2420–2426

DeBruyn AM, Gobas FAPC (2004) Modelling the diagenetic fate of persistent organic pollutants in organically enriched sediments. Ecol Model 179:405–416

DeBruyn AMH, Ikonomou MG, Gobas FAPC (2004) Magnification and toxicity of PCBs, PCDDs, and PCDFs in upriver migrating Pacific Salmon. Environ Sci Technol 38:6217–6224

DeBruyn AMH, Gobas FAPC (2006) A bioenergetic biomagnification model for the animal kingdom. Environ Sci Technol 40:1581–1587

DeBruyn AMH, Gobas FAPC (2007) The sorptive capacity of animal protein. Environ Toxicol Chem 26:1803–1808

Debier C, Pomeroy PP, Dupont C, Joiris C, Comblin V, Le Boulenge E, Larondelle Y, Thome JP (2003) Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halicheorus grypus*. Mar Ecol Prog Ser 247:237–248

Debier C, Chalon C, Le Boeuf BJ, De Tillesse T, Larondelle Y, Thomé JP (2006) Mobilization of PCBs from blubber to blood in northern elephant seals (*Mirounga angustirostris*) during the post-weaning fast. Aquat Toxicol 80:149–157

DeFreitas AS, Norstrom RJ (1974) Turnover and metabolism of polychlorinated biphenyls in relation to their chemical structure and the movement of lipids in the pigeon. Can Physiol Pharm 52:1081–1094

Devillers J, Domine D, Bintein S, Karcher W (1998) Fish bioconcentration modeling with log P. Toxicol Mech Meth 8:1–10

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR (1991) Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ Toxicol Chem 10:1541–1583

Duursma EK, Nieuwenhuize J, Van Liere JM, de Rooy CM, Witte JIJ, Van der Meer J (1991) Possible loss of polychlorinated biphenyls from migrating European silver eels: a 3 month simulation experiment. Mar Chem 36:1–4

Drouillard KG, Norstrom RJ (2000) Dietary absorption efficiencies and toxicokinetics of polychlorinated biphenyls in ring doves following exposure to Aroclor mixtures. Environ Toxicol Chem 19:2707–2714

Drouillard KG, Fernie KJ, Smits JE, Bortolotti GR, Bird DM, Norstrom RJ (2001) Bioaccumulation and toxicokinetics of 42 polychlorinated biphenyl congeners in American kestrels (*Falco sparverius*). Environ Toxicol Chem 20:2514–2522

Drouillard KG, Norstrom RJ, Fox GA, Gilam A, Peakall DB (2003) Development and validation of a herring gull embryo toxicokinetic model for PCBs. Ecotoxicology 12:55–68

Drouillard KG, Hagen H, Haffner D (2004) Evaluation of chloroform/methanol and dichloromethane/hexane extractable lipids as surrogate measures of sample partition capacity for organochlorines in fish tissues. Chemosphere 55:395–400

Drouillard KG (2008) Biomagnification. In: Jorgensen SE, Fath BD (eds) Ecotoxicology vol 1 of encyclopedia of ecology, vol 5. Elsevier, Oxford, pp 441–448

Drouillard KG, Paterson G, Haffner D (2009) A combined food web toxicokinetic and species bioenergetic model for predicting seasonal PCB elimination in yellow perch (*Perca flavescens*). Environ Sci Technol 43:2858–2864

Drouillard KG, Paterson G, Liu J, Haffner GD (2012) Calibration of the gastrointestinal model to predict maximum biomagnification potentials of polychlorinated biphenyls in a bird and fish. Environ Sci Technol 46:10279–10286

Ecobichon DJ, Saschenbrecker PW (1969) The redistribution of stored DDT in cockerels under the influence of food deprivation. Toxicol Appl Pharmacol 15:420–432

Edmunds GF Jr, Jensen SL, Berner L (1976) The mayflies of North and Central America. University of Minnesota Press, Minneapolis, MN, USA

Elliott JE, Wilson LK, Langelier KW, Norstrom RJ (1996) Bald eagle mortality and chlorinated hydrocarbon contaminants in livers from British Columbia, Canada, 1989–1994. Environ Pollut 94:9–18

Escher BI, Ashauer R, Dyer S, Hermens JL, Lee JH, Leslie HA, Mayer P, Meador JP, Warne MS (2011) Crucial role of mechanisms and modes of toxic action for understanding tissue residue toxicity and internal effect concentrations of organic chemicals. Integr Environ Assess Manag 7:28–49

Ewald G, Larsson P, Linge H, Okla L, Szarzi N (1998) Biotransport of organic pollutants to an inland Alaska lake by migrating sockeye salmon (*Oncorhynchus nerka*). Arctic 51:40–47

Fenton MB, Barclay MR (1980) Myotis lucifugus. Mamm Sp 142:1–95

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DC (1998) Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environ Toxicol Chem 17:951–961

Foekema EM, Fischer A, Parron ML, Kwadijk C, de Vries P, Murk AJ (2012) Toxic concentrations in fish early life stages peak at a critical moment. Environ Toxicol Chem 31:1381–1390

Fort J, Porter WP, Grémillet D (2009) Thermodynamic modelling predicts energetic bottleneck for seabirds wintering in the northwest Atlantic. J Exp Biol 212:2483–2490

Fuglei E, Øritsland NA (1999) Seasonal trends in body mass, food intake and resting metabolic rate, and induction of metabolic depression in arctic foxes (*Alopex lagopus*) at Svalbard. J Comp Physiol B 169:361–369

Fuglei E, Bustnes JO, Hop H, Mørk T, Björnfoth H, van Bavel B (2007) Environmental contaminants in arctic foxes (*Alopex lagopus*) in Svalbard: relationships with feeding ecology and body condition. Environ Pollut 146:128–138

Gabrielsen GW, Skaare JU, Polder A, Bakken V (1995) Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. Sci Total Environ 160(161):337–346

Gewurtz SB, Backus SM, Bhavsar SP, McGoldrick DJ, de Solla SR, Murphy EW (2011) Contaminant biomonitoring programs in the Great Lakes region: review of approaches and critical factors. Environ Rev 19:162–184

Geyer H, Scheuntert I, Rapp K, Kettrup A, Korte F, Greim H, Rozman K (1990) Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and total body fat content in mammals. Toxicology 95:97–107

Geyer HJ, Steinberg CE, Scheunert I, Brueggemann R, Schuetz W, Kettrup A, Rozman K (1993) A review of the relationship between acute toxicity (LC50) of gamma-hexachlorocyclohexane (gamma-HCH, lindane) and total lipid content of different fish species. Toxicology 83:169–179

Gobas FAPC, Opperhuisen A, Hutzinger O (1986) Bioconcetration of hydrophobic chemicals in fish: relationship with membrane permeation. Environ Toxicol Chem 5:637–646

Gobas FAPC, Muir DCG, Mackay D (1988) Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere 17:943–962

Gobas FAPC (1993) A model for predicting the bioaccumulation of hydrophobic organic chemicals in aquatic food-webs: application to Lake Ontario. Ecol Model 69:1–17

Gobas FAPC, Zhang X, Wells R (1993) Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environ Sci Technol 27:2855–2863

Gobas FAPC, Wilcockson JB, Russell R, Haffner GD (1999) Mechanism of biomagnification in fish under laboratory and field conditions. Environ Sci Technol 33:133–141

Gobas FA, Morrison HA (2000) Bioconcentration and biomagnification in the aquatic environment. In: Boethling RS, Mackay D (eds) Handbook of property estimation methods for chemicals, environmental and health sciences. CRC Press, Boca Raton, FL, pp 189–231

Golet GH, Irons DB (1999) Raising young reduces body condition and fat stores in black-legged kittiwakes. Oecologia 120:530–538

Golet GH, Irons DB, Costa DP (2000) Energy costs of chick rearing in Black-legged kittiwakes (Rissa tridactyla). Can J Zool 78:982–991

Gregory-Eaves I, Demers MJ, Kimpe L, Krummel EM, Macdonald RW, Finney BP, Blais JM (2007) Tracing salmon-derived nutrients and contaminants in freshwater food webs across a pronounced spawner density gradient. Environ Toxicol Chem 26:1100–1108

Hall AJ, Gulland FMD, Ylitalo GM, Greig DJ, Lowenstine L (2008) Changes in blubber contaminant concentrations in California sea lions (*Zalophus californianus*) associated with weight loss and gain during rehabilitation. Environ Sci Technol 42:4181–4187

Hamelink JL, Waybrant RC, Ball RC (1971) A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. Trans Am Fish Soc 100:207–214

Hansson MC, Persson ME, Larsson P, von Schantz T (2009) Polychlorinated biphenyl (PCB) load, lipid reserves and biotransformation activity in migrating Atlantic salmon from River Mörrum, Sweden. Environ Pollut 157:3396–3403

Harkey GA, Klaine SJ (1992) Bioconcentration of trans-chlordane by the midge, *Chironomus decorus*. Chemosphere 24:1911–1919

Hario M, Hirvi J, Hollmén T, Rudbäck E (2004) Organochlorine concentrations in diseased versus healthy gull chicks from the northern Baltic. Environ Pollut 127:411–423

Henriksen EO, Gabrielsen GW, Skaare JU (1996) Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of body-lipids associated with reproduction. Environ Pollut 92:27–37

Hendry AP, Berg OK (1999) Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Can J Zool 77:1663–1675

Hickie BE, Mackay D, de Koning J (1999) A lifetime pharmacokinetic model for hydrophobic contaminants in marine mammals. Environ Toxicol Chem 18:2622–2633

Hickie BE, Kingsley MCS, Hodson PV, Muir DCG, Beland P, Mackay D (2000) A modelling-based perspective on the past, present, and future polychlorinated biphenyl contamination of the St. Lawrence beluga whale (*Delphinapterus leucas*) population. Can J Fish Aquat Sci 57(suppl 1):101–112

Hickie BE, Addison R, Muir DCG (2005) Development and application of bioaccumulation models to assess persistent organic pollutant temporal trends in arctic ringed seal (*Phoca hispida*) populations. Sci Total Environ 351–352:417–430

Hickie BE, Ross PS, Macdonald RW, Ford JK (2007) Killer whales (*Orcinus orca*) face protracted health risks associated with lifetime exposure to PCBs. Environ Sci Technol 41:6613–6619

Hock RJ (1960) Seasonal variation and physiologic functions of Arctic ground squirrels and black bears. Comp Zool Bull 124:155–171

Humphries MM, Umbanhowar J, McCann KS (2004) Bioenergetic prediction of climate change impacts on northern mammals. Integr Comp Biol 44:152–162

Jackson ZJ, Quist MC, Larsheid LG (2008) Growth standards for nine North American fish species. Fisheries Manag Ecol 15:107–118

Jandacek RJ, Anderson N, Liu M, Zheng S, Yang Q, Tso P (2005) Effects of yo-yo diet, caloric restriction, and olestra on tissue distribution of hexachlorobenzene. Am J Physiol Gastrointest Liver Physiol 288:292–299

Jobling M, Johansen SJS, Foshaug H, Burkow IC, Jorgensen EH (1998) Lipid dynamics in anadromous Arctic charr, *Salvelinus alpinus* (L.): seasonal variations in lipid storage depots and lipid class composition. Fish Physiol Biochem 18:225–240

Jørgensen EH, Bye BE, Jobling M (1999) Influence of nutritional status on biomarker responses to PCB in the arctic charr (Salvelinus alpinus). Aquat Toxicol 44:233–244

Jørgensen EH, Foshaug H, Andersson P, Burkow IC, Jobling M (2002) Polychlorinated biphenyl toxicokinetics and P4501A responses in anadromous Arctic charr during winter emaciation. Environ Toxicol Chem 21:1745–1752

Kaitaranta JK, Ackman RG (1981) Total lipids and lipid classes of fish roe. Comp Biochem Physiol Part B Biochem Mol Biol 69:725–729

Kannan K, Hun Yun S, Rudd RJ, Behr M (2010) High concentrations of persistent organic pollutants including PCBs, DDT, PBDEs and PFOS in little brown bats with white-nose syndrome in New York, USA. Chemosphere 80:613–618

Kawahara A, Baker BS, Tata JR (1991) Developmental and regional expression of thyroid-hormone receptor genes during *Xenopus* development. Development 112:933–943

Kelly BC, Gobas FAPC (2001) Bioaccumulation of persistent organic pollutants in lichen–caribou–wolf food chains of Canada's central and western Arctic. Environ Sci Technol 35:325–334

Kelly BC, Gobas FAPC (2003) An Arctic terrestrial food chain bioaccumulation model for persistent organic pollutants. Environ Sci Technol 37:2966–2974

Kelly BC, Gobas FAPC, McLachlan MS (2004) Intestinal absorption and biomagnification of organic contaminants in fish, wildlife and humans. Environ Toxicol Chem 23:2324–2336

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC (2007a) Food web-specific biomagnification of persistent organic pollutants. Science 317:236–239

Kelly BC, Gray SL, Ikonomou MG, Macdonald JS, Bandiera SM, Hrycay EG (2007b) Lipid reserve dynamics and magnification of persistent organic pollutants in spawning sockeye salmon (Oncorhynchus nerka) from the Fraser River, British Columbia. Environ Sci Technol 41:3083–3089

Kelly BC, Ikonomou MG, MacPherson N, Sampson T, Patterson DA, Dubetz C (2011) Tissue residue concentrations of organohalogens and trace elements in adult Pacific salmon returning to the Fraser River, British Columbia, Canada. Environ Toxicol Chem 30:367–376

Kirk KL (1997) Life-history responses to variable environments: starvation and reproduction in planktonic rotifers. Ecology 78:434–441

Kitchell JF, Stewart DJ, Weininger D (1977) Application of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J Fish Res Bd Can 34:1922–1935

Klaassen M (1996) Metabolic constraints on long-distance migration in birds. J Exp Biol 199:57–64

Kleinow K, Baker J, Nichlos J, Gobas F, Parkerton T, Muir DCG, Monteverdi G, Mastrodone P (1999) Exposure, uptake and disposition of chemicals in reproductive and developmental stages of selected oviparous vertebrates. In: Di Giulo RT, Tillittt DE (eds) Reproductive and developmental effects of contaminants in oviparous vertebrates. Society of Environmental Toxicology and Chemistry, Pensacola, FL, USA, pp 9–111

Knott KK, Schenk P, Beyerlein S, Boyd D, Ylitalo G, O'Hara TO (2011) Blood based biomarkers of selenium and thyroid status indicates possible adverse biological effects of mercury and polychlorinated biphenyls in Southern Beaufort Sea polar bears. Environ Res 111:1124–1136

Krummel EM, Macdonald RW, Kimpe LE, Gregory-Eaves I, Demers MJ, Smol JP, Finney B, Blais JM (2003) Aquatic ecology: delivery of pollutants by spawning salmon. Nature 425:255–256

Lambert Y, Dutil JD (2000) Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Can J Fish Aquat Sci 57:815–825

Landrum PF, Tigue EA, Kane Driscoll SB, Gossiaux DC, Van Hoof PL, Gedeon ML, Adler M (2001) Bioaccumulation of PCB congeners by *Diporeia spp*. kinetics and factors affecting bioavailability. J Great Lakes Res 27:117–133

Larsson P (1984) Transport of PCBs from aquatic to terrestrial environments by emerging chironomids. Environ Poll 34:283–289

Leblanc GA (1995) Trophic-level differences in bioconcentration of chemicals: implications in assessing environmental biomagnification. Environ Sci Technol 29:154–160
Leney J, Drouillard KG, Haffner DG (2006a) Does metamorphosis increase the susceptibility of frogs to highly hydrophobic contaminants? Environ Sci Technol 40:1491–1496
Leney JL, Balkwill KC, Drouillard KG, Haffner GD (2006b) Determination of polychlorinated biphenyl and polycyclic aromatic hydrocarbon elimination rates in adult green and leopard frogs. Environ Toxicol Chem 25:1627–1634
Lohmann R, MacFarlane JK, Gschwend PM (2005) Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York Harbor sediments. Environ Sci Technol 39:141–148
Lydersen C, Wolkers H, Severinsen T, Kleivane L, Nordøy ES, Skaare JU (2002) Blood is a poor substrate for monitoring pollution burdens in phocid seals. Sci Total Environ 292:193–203
Lyman CP, Willis SJ, Malan A, Wang LCH (1982) Hibernation and torpor in mammals and birds. Academic, New York, NY
MacDonald R, Mackay D, Hickie B (2002) Contaminant amplification in the environment. Environ Sci Technol 36:456–462
Mackay D (1979) Finding fugacity feasible. Environ Sci Technol 13:1218–1223
Mackay D, Paterson S (1981) Calculating fugacity. Environ Sci Technol 15:1006–1014
Mackay D (1982) Correlation of bioconcentration factors. Environ Sci Technol 16:274–278
Mackay D (1991) Multimedia environmental models: the fugacity approach. Lewis Publishers, Chelsea, MI, pp 1–257
Mackay D, Fraser A (2000) Bioaccumulation of persistent organic chemicals: mechanisms and models. Environ Pollut 110:375–391
McCarty LS, Landrum PF, Luoma SN, Merten AA, Shephard BK, van Wezel AP (2011) A review of tissue residues in environmental toxicology. Integr Environ Assess Manag 7:7–27
McElroy AE, Barron MG, Beckvar N, Driscoll SBK, Meador JP, Parkerton TF, Preuss TG, Steevens JA (2011) A review of the tissue residue approach for organic and organometallic compounds in aquatic organisms. Integr Environ Assess Manag 7:50–74
McLachlan MS (1996) Bioaccumulation of hydrophobic chemicals in agricultural food chains. Environ Sci Technol 30:252–259
McNab BK (2001) The physiological ecology of vertebrates: a view from energetics. Cornell University Press, Cornell
McWilliams SR, Guglielmo C, Pierce B, Klaassen M (2004) Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J Avian Biol 35:377–393
Meylen WM, Howard PH, Boethling DA, Printup H, Gouchie S (1999) Improved method for estimating bioconcetration/bioaccumulation factor from octanol/water partition coefficient. Environ Toxicol Chem 18:664–672
Montie EW, Fair PA, Bossart GD, Mitchum GB, Houde M, Muir DCG, Letcher RJ, McFee WE, Starczak VR, Stegeman JJ, Hahn ME (2008) Cytochrome P4501A1 expression, polychlorinated biphenyls and hydroxylated metabolites, and adipocyte size of bottlenose dolphins from the Southeast United States. Aquat Toxicol 86:397–412
Morrison HA, Gobas FAPC, Lazar R, Whittle DM, Haffner GD (1997) Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in western Lake Erie. Environ Sci Technol 31:3267–3273
Muir DCG, De Wit CA (2010) Trends of legacy and new persistent organic pollutants in the circumpolar Arctic: overview, conclusions, and recommendations. Sci Total Environ 40:3044–3051
Nakata H, Kanaan K, Jing L, Thomas NJ, Tanabe S, Giesy JP (1998) Accumulation pattern of organochlorine pesticides and polychlorinated biphenyls in southern sea otters (*Enhydra lutris nereis*) found stranded along coastal California, USA. Environ Pollut 103:45–53
Neely W, Branson D, Blau G (1974) Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ Sci Technol 8:1113–1115

Nelson RA (1978) Urea metabolism in the hibernating black bear. Kidney Int 13:S177–S179
Nelson RA, Folk GE Jr, Pfeiffer EW, Craighead JJ, Jonkel CJ, Steiger DL (1983) Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. Intl Conf Bear Res Manage 5:284–290
Ng CA, Gray KA (2009) Tracking bioaccumulation in aquatic organisms: a dynamic model integrating life history characteristics and environmental change. Ecol Model 220:1266–1273
Nichols JW, Bonnell M, Dimitrov SD, Escher BI, Han X, Kramer NI (2009) Bioaccumulation assessment using predictive approaches. Int Environ Assess Manage 5:577–597
Nilssen KT, Haug T, Potelov V, Stasenkov VA, Timoshenko YK (1995) Food habits of harp seals (*Phoca groenlandica*) during lactation and moult in March–May in the southern Barents Sea and White Sea. ICES J Mar Sci 52:33–41
Norstrom RJ, Clark TP, Jeffrey DA, Won HT, Gilman AP (1986a) Dynamics of organochlorine compounds in herring gulls (*Larus argentatus*): I. Distribution and clearance of [14C]DDE in free-living herring gulls. Enviro Toxicol Chem 5:41–48
Norstrom RJ, Clark TP, Kearney JP, Gilman AP (1986b) Herring gull energy requirements and body constituents in the Great Lakes. Ardea 74:1–23
Norstrom RJ, Clark TP, Enright M, Leung B, Drouillard KG, MacDonald CR (2007) ABAM, a model for bioaccumulation of POPs in birds: validation for adult herring gulls and their eggs in lake Ontario. Environ Sci Technol 41:4339–4347
Olivereau M, Olivereau JM (1997) Long-term starvation in the European eel: effects and responses of pituitary growth hormone-GH and somatolactin-SL secreting cells. Fish Physiol Biochem 17:261–269
Oliver BG, Niimi AJ (1988) Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ Sci Technol 22:388–397
Orlofske SA, Hopkins WA (2009) Energetics of metamorphic climax in the pickerel frog (*Lithobates palustris*). Comp Biochem Physiol A Mol Integr Physiol 154:191–196
Ohmiya Y, Nakai K (1977) Effect of starvation on excretion distribution and metabolism of DDT in mice. Tobuku J Exp Med 122:143–153
Paterson G, Drouillard KG, Haffner GD (2007a) PCB elimination by yellow perch (*Perca flavescens*) during an annual temperature cycle. Environ Sci Technol 41:824–829
Paterson G, Drouillard KG, Leadley TA, Haffner GD (2007b) Long-term PCB elimination by three sizes classes of yellow perch (*Perca flavescens*). Can J Fish Aquat Sci 64:1222–1233
Pelletier C, Doucet E, Imbeault P, Tremblay A (2002) Associations between weight loss-induced changes in plasma organochlorine concentrations, serum T3 concentration, and resting metabolic rate. Toxicol Sci 67:46–51
Perkins CR, Barclay JS (1997) Accumulation and mobilization of organochlorine contaminants in wintering greater scaup. J Wildl Man 61:444–449
Piersma T (2002) Energetic bottlenecks and other design constraints in avian annual cycles. Integr Comp Biol 42:51–67
Polischuk SC, Letcher RJ, Norstrom RJ, Ramsay MA (1995) Preliminary results on the kinetics of organochlorines in western Hudson Bay polar bear (Ursus maritimus). Sci Total Environ 160(161):465–472
Polischuk SC, Norstrom RJ, Ramsay MA (2002) Body burdens and tissue concentrations of organochlorines in polar bears (*Ursus maritimus*) vary during seasonal fasts. Environ Pollut 118:29–39
Post JR, McQueen DJ (1988) Ontogenetic changes in the distribution of larval and juvenile yellow perch (*Perca flavescens*): a response to prey or predators? Can J Fish Aquat Sci 45:1820–1826
Potter L, Kidd D, Standiford D (1975) Mercury levels in Lake Powell. Bioamplification of mercury in man-made desert reservoir. Environ Sci Technol 9:41–46
Quabius ES, Nolan DT, Allin CJ, Wendelaar Bonga SE (2000) Influence of dietary exposure to polychlorinated biphenyl 126 and nutritional state on stress response in tilapia (Oreochromis mossambicus) and rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem 19: 2892–2899

Ramsay MA, Nelson RA, Stirling I (1991) Seasonal changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. Can J Zool 69:298–302

Randall RC, Young DR, Lee H II, Echols SF (1998) Lipid methodology and pollutant normalization relationships for neutral nonpolar organic pollutants. Environ Toxicol Chem 17:788–791

Rasmussen JB, Rowan DJ, Lean DRS, Carey JH (1990) Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can J Fish Aquat Sci 47:2030–2038

Richman LA, Hobson G, Williams DJ, Reiner E (2011) The Niagara River mussel biomonitoring program (*Elliptio complanata*): 1983–2009. J Great Lakes Res 37:213–225

Roose P, Cooreman K, Vyncke W (1998) PCBS in cod (*Gadus morhua*), flounder (*Platichthys flesus*), blue mussel (*Mytilus edulis*) and brown shrimp (*Crangon crangon*) from the belgian continental shelf: relation to biological parameters and trend analysis. Chemosphere 37:2199–2210

Russell R, Gobas FAPC, Haffner GD (1999a) Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environ Toxicol Chem 18:1250–1257

Russell R, Gobas FAPC, Haffner GD (1999b) Maternal transfer and in-ovo exposure of organochlorines in oviparous organisms: a model and field verification. Environ Sci Technol 33:416–420

Safe SH (1994) Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149

Schlummer M, Moser GA, McLachlan MS (1998) Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. Toxicol Appl Pharm 152:128–137

Schneider JE (2004) Energy balance and reproduction. Physiol Behav 81:289–317

Schulz R, Dabrowski JM (2001) Combined effects of predatory fish and sublethal pesticide contamination on the behavior and mortality of mayfly nymphs. Environ Toxicol Chem 20:2537–2543

Schwarzenbach RP, Gschwend PM, Imboden DM (1993) Environmental organic chemistry. Wiley, Toronto, ON, Canada, 681 pp

Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Fish Res Board Can, Ottawa, pp 1–966

Scott WB, Crossman EJ (1998) Freshwater fishes of Canada. Galt House Pub, Oakville, Canada

Selck H, Drouillard K, Eisenreich K, Koelmans AA, Palmqvist A, Ruus A, Salvito D, Schultz I, Stewart R, Weisbrod A, van den Brink NW, van den Heuvel-Greve M (2012) Explaining differences between bioaccumulation measurements in laboratory and field data through use of a probabilistic modeling approach. Integr Environ Assess Manag 8:42–63

Sijm DTHM, Seinen W, Opperhulzen A (1992) Life-cycle biomagnification study in fish. Environ Sci Technol 26:2162–2174

Sodergren A, Ulfstrand S (1972) DDT and PCB relocate when caged robins use fat reserves. Ambio 1:36–40

Sonne C, Wolkers H, Rigét FF, Jensen JB, Teilmann J, Jenssen BM, Fuglei E, Ahlstrøm Ø, Dietz R, Muir DC, Jørgensen EH (2009) Mineral density and biomechanical properties of bone tissue from male Arctic foxes (*Vulpes lagopus*) exposed to organochlorine contaminants and emaciation. Comp Biochem Phys C 149:97–103

Sørmo EG, Skaare JU, Lydersen C, Kovacs KM, Hammill MO, Jenssen BM (2003) Partitioning of persistent organic pollutants in grey seal (*Halichoerus grypus*) mother-pup pairs. Sci Total Environ 302:145–155

Stickel L, Stickel W (1969) Distribution of DDT residues in tissues of birds in relation to mortality, body condition, and time. Ind Med 38:44–53

Stickel WH, Stickel LF, Dyrland RA, Hughes DL (1984) Aroclor 1254 residues in birds: lethal levels and loss rates. Arch Environ Contam Toxicol 13:7–13

Subramanian AN, Tanabe S, Hidaka H, Tatsukawa R (1986) Bioaccumulation of organochlorines (PCBs and p, p,-DDE) in Antarctic Adelie penguins Pygoscelis adeliae collected during a breeding season. Environ Pollut 40:173–189

Svihla S, Bowman HC (1954) Hibernation in the American black bear. Am Midland Nat 52:248–252

Thomann RV (1981) Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can J Fish Aquat Sci 38:280–296

Thomann RV, Connolly JP, Parkerton TF (1992) An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. Environ Toxicol Chem 11:615–629

Tremblay A, Pelletier C, Doucet E, Imbeault P (2004) Thermogenesis and weight loss in obese individuals: a primary association with organochlorine pollution. Int J Obes 28:936–939

Van Ginneken V, Palstra A, Leonards P, Nieveen M, Berg H, Flik G, Spannings T, Niemantsverdriet P, Thillart G, Murk T (2009) PCBs and the energy costs of migration in European silver eel (*Anguilla anguilla*). Aquat Toxicol 92:213–220

Van Velzen AC, Stiles WB, Stickel LF (1972) Lethal mobilization of DDT by cowbirds. J Wildl Manage 36:733–739

Veith GD, DeFoe DL, Bergstedt BC (1979) Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Res Board Can 36:1040–1048

Walford RL, Mock D, MacCallum T, Laseter JL (1999) Physiologic changes in humans subjected to severe, selective calorie restriction for two years in biosphere 2: health, aging, and toxicological perspectives. Toxicol Sci 52:61–65

Walker C (1990) Persistent pollutants in fish-eating sea birds—bioaccumulation, metabolism and effects. Aquatic Toxicol 17:293–324

Wang-Andersen G, Skaare JU, Prestrud P, Steinnes E (1993) Levels and congener pattern of PCBs in Arctic fox, *Alopex lagopus*, in Svalbard. Environ Pollut 82:269–275

Wang T, Hung CCY, Randall DJ (2006) The comparative physiology of food deprivation: from feast to famine. Annu Rev Physiol 68:223–251

Webster JS, Hartman KJ (2007) Possible seasonal population bottlenecks in brook trout (*Salvelinus fontinalis*) in central Appalachian headwater streams. J Freshwat Biol 22:177–187

Weis JS, Weis P (1987) Pollutants as development toxicants in aquatic organisms. Environ Health Perspct 71:77–85

Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Rev Fish Biol Fish 6:259–286

Yahner RH (2012) Winter strategies. In: Wildlife behaviour and conservation. Springer, New York, pp 139–143

Yordy JE, Wells RS, Balmer BC, Schwacke LH, Rowles TK, Kucklick JR (2010) Life history as a source of variation for persistent organic pollutants (POP) patterns in a community of common bottlenose dolphins (*Tursiops truncates*) resident to Sarasota Bay, FL. Sci Total Environ 408:2163–2172

Zhao RB, Sun DY, Fu S, Wang XF, Zhao RS (2007) Bioconcentration kinetics of PCBs in various parts of the lifecycle of the tadpole *Xenopus laevis*. J Environ Sci 19:374–384

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