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Pim de Voogt *Editor*

# Reviews of Environmental Contamination and Toxicology

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and Toxicology

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# Reviews of Environmental Contamination and Toxicology

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Vancouver, BC, Canada  
E-mail: peter.ross@vanaqua.org

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*In Memoriam: Dr. David Martin Whitacre  
1943–2015*

*David Whitacre leaves a continuing legacy of promoting science in agriculture to help increase farm production safely while maintaining stewardship of the land itself.*

*David was a native of Lebanon Ohio, born in 1943. He grew up on a farm where he developed a love of restoring old John Deere tractors, with a last count of 24, scattered over Illinois, Ohio, and North Carolina.*

*I first met David and his new wife, Trudy, at Wilmington College, Ohio, while seeking graduate entomology candidates for transfer to The Ohio State University. He completed his M.S. with me just before my move to the University of Arizona in Tucson in 1966. He was offered an assistantship in the Department of Entomology where I had just transferred as department head. He completed his requirements for the Ph.D. in record time. Then interested in pesticide toxicology, he rapidly moved into the industry circuit. He was board certified in General Toxicology in 1982, and he occupied various*

*industry positions including Sr. Vice President of Science for Novartis North America and Vice President of R&D for Sandoz in the United States. He also managed Research for Sandoz Crop Protection in Basle, Switzerland, for 2 years. He retired from Syngenta as VP of Development in 2001.*

*Wanting to split his time between restoring antique John Deere tractors and doing something intellectual, I persuaded him to coauthor The Pesticide Book which went thru three more editions. Still enjoying keeping his mind in his profession and being perfectly trained for the job, he became editor of Reviews and carried it thru 20 volumes including Volume 210.*

*While being prepared for open-heart surgery, David's heart stopped and couldn't be revived. He died on August 28, 2015, in Greensboro, NC, at the age of 71. He is survived by his wife Trudy of Summerfield, NC; son David Whitacre, research scientist with a PhD in molecular and cellular biology, San Diego, CA; daughter Dr. Elizabeth Whitacre, Emergency Medicine, Albuquerque, NM, and five grandchildren.*

*David was absolutely the best graduate student I ever guided and the most intelligent and fair-minded person I've had the pleasure of knowing.*

George Ware  
Professor Emeritus  
University of Arizona

# 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.

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 (eco)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 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 emerging contaminants and 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, nongovernmental organizations, or the private sector.

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

The ultimate role of publishing scientific environmental research is to enhance understanding of the environment in ways that allow the public to be better informed or, in other words, to enable the public to have access to sufficient information. Because the public gets most of its information on science and technology from internet, 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 an important 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, because 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. New legislation that will deal in an appropriate manner with this challenge is currently in the making or has been implemented recently, such as the REACH legislation in Europe. These regulations demand scientifically sound and documented dossiers on new chemicals.

*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, (eco)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 anthropogenic chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their scope.

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-in-Chief is recommended before volunteered review manuscripts are submitted. *Reviews* is registered in Web of Science™.

Inclusion in the Science Citation Index serves to encourage scientists in academia to contribute to the series. The impact factor in recent years has increased from 2.5 in 2009 to almost 4 in 2013. The Editor-in-Chief and the Editorial Board strive for a further increase of the journal impact factor by actively inviting authors to submit manuscripts.

Amsterdam, The Netherlands  
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Pim de Voogt



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# Contributors

**Hans Baveco** Environmental Risk Assessment Team, Alterra, Wageningen, The Netherlands

**Paul J. van den Brink** Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, Wageningen, The Netherlands

Environmental Risk Assessment Team, Alterra, Wageningen, The Netherlands

**Theo C. M. Brock** Environmental Risk Assessment Team, Alterra, Wageningen, The Netherlands

**Noël J. Diepens** Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, Wageningen, The Netherlands

**Sandra Ečimovic** Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

**Martine J. van den Heuvel-Greve** IMARES, Institute for Marine Resources and Ecosystem Studies, Wageningen UR, IJmuiden, The Netherlands

**Albert A. Koelmans** Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, Wageningen, The Netherlands

IMARES, Institute for Marine Resources and Ecosystem Studies, Wageningen UR, IJmuiden, The Netherlands

**Jacek Namieśnik** Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland

**Żaneta Polkowska** Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland

**Małgorzata Szopińska** Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland

**Mirna Velki** Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

# Prospective Environmental Risk Assessment for Sediment-Bound Organic Chemicals: A Proposal for Tiered Effect Assessment

Noël J. Diepens, Albert A. Koelmans, Hans Baveco, Paul J. van den Brink, Martine J. van den Heuvel-Greve, and Theo C.M. Brock

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N.J. Diepens (✉)  
Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands  
e-mail: [noel.diepens@wur.nl](mailto:noel.diepens@wur.nl)

A.A. Koelmans  
Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands  
IMARES, Institute for Marine Resources & Ecosystem Studies, Wageningen UR, P.O. Box 68, 1970 AB IJmuiden, The Netherlands

H. Baveco • T.C.M. Brock  
Environmental Risk Assessment Team, Alterra, P.O. Box 47, 6700 AA Wageningen, The Netherlands

P.J. van den Brink  
Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands  
Environmental Risk Assessment Team, Alterra, P.O. Box 47, 6700 AA Wageningen, The Netherlands

M.J. van den Heuvel-Greve  
IMARES, Institute for Marine Resources & Ecosystem Studies, Wageningen UR, P.O. Box 68, 1970 AB IJmuiden, The Netherlands

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## 1 Introduction

Aquatic sediments are an important part of the aquatic ecosystem, providing critical ecosystem services and functions (Wall 2004). The sediment compartment acts as a sink for hydrophobic organic chemicals, which can affect the services and functions provided. Therefore, sediment should be considered in prospective environmental risk assessment (ERA) whereas it is currently underrepresented.

Prospective ERA evaluates the future risks of a chemical stressor not yet released into the environment, by comparing outcomes of the exposure and effect assessment. The exposure assessment predicts exposure patterns and concentrations in environmental media such as sediment. The effect assessment describes the relationship between exposure concentration and effects for the assessed endpoints. The effect assessment is often performed in a tiered way, starting with a simple conservative assessment, and includes additional and more complex work only if necessary (Solomon et al. 2008).

Currently, in Europe, a conceptual prospective sediment ERA framework for hydrophobic organic chemicals is under development (ECHA 2014c; EFSA 2015). Such a framework requires clear protection goals, evidence-based concepts for linking exposure and effects, and a transparent tiered-effect assessment procedure for sediment organisms and processes. Furthermore, harmonization of data requirements, test protocols and sediment ERA frameworks between existing regulations/directives would be beneficial (Beketov et al. 2012; Diepens et al. 2014b; ECHA 2014c; EFSA 2015).

The aim of this paper is (1) to present an overview of current approaches for sediment ERA underlying European regulatory frameworks and (2) to provide guidance to establish a harmonised, prospective ERA framework for organic chemicals in sediments of freshwater, estuarine and marine ecosystems. In this paper we focus on prospective sediment ERA approaches in Europe, but we also address useful concepts developed in North America. Furthermore, we focus on the soft bottom sediment benthic community and briefly discuss the use of both artificial and field-collected sediments in conducting sediment toxicity tests for prospective ERA. This issue has been discussed before in Diepens et al. (2014b).

A synthesis of existing approaches and new scientific insights and data is provided, showing how a rational prospective assessment can be performed cost-effectively. After a short introduction in benthic ecology, our analysis starts by defining specific protection goals based on the ecosystem services concept, which in turn is based on the ecological role and functions provided by benthic organisms. We then present and discuss trigger values for sediment testing and data requirements within current European ERA frameworks. Current procedures for exposure and effect assessment, including the use of models, are presented and recommendations are given. Finally, several case studies are provided as ‘proof of concept’ and to illustrate the general features of the framework. The concepts underlying this paper were discussed with representatives of government, industry and academia during a workshop in Wageningen, the Netherlands, in February 2014 (for list of participants see Appendix 1). Discussions, remarks and recommendations from the workshop were used to improve this paper.

## 2 Benthic Ecology

Aquatic sediment is a complex heterogeneous matrix that covers a large part of earth’s surface (freshwater 0.5 %, marine 74 %) (Wall et al. 1997). In this paper, sediment is defined as all unconsolidated material of fine, medium and coarse grain

minerals and organic particles that make up the bottom of aquatic ecosystems (Adams et al. 1992; Palmer et al. 2000). The numerous benthic organisms that inhabit the sediment compartment fulfil a wide variety of crucial ecosystem functions. The benthic food chain and processes in the sediment compartment are not only connected with pelagic organisms and processes, but also with terrestrial soils. Soils, freshwater and marine sediments are closely interlinked as well, e.g. via groundwater systems, and have many functions in common (Wall 2004). Contamination and other anthropogenic pressures can negatively influence critical functions provided by benthic organisms. Protection of benthic organisms is essential for ecosystem functioning and the sustainable use of services provided by nature.

Landscape and local factors such as geology, hydrology, and water chemistry influence the sediment habitat and therewith the diversity and structure of benthic communities (Covich et al. 2004; Wall 2004). In general, sediment can be divided into two types: soft bottom sediments and hard substrates, each containing different benthic organism groups (Gérino et al. 2003). Low flow velocities and fine sediment particles characterize soft bottom sediments. Hard substrates are often found in high-energy areas, such as areas with high flow velocity and wave impact. In this paper we define benthic organisms as follows: organisms that spend their full life cycle, or an important part thereof, living on sediment (epibenthos) or in sediment (endobenthos). For these species, exposure via the sediment compartment may contribute to contaminant-mediated effects. This is not adequately covered by ERA's that are based on exposure in other environmental compartments.

Ecosystem processes performed by benthic organisms cover a wide range of temporal and spatial scales. On the micro scale, populations of microbenthos, which usually have a life cycle of hours to days (including bacteria, fungi, ciliate protozoans, flagellates, and diatoms), perform processes such as nitrogen and phosphate transformation, carbon mineralization and photosynthesis. Meiobenthos populations, which may have a life cycle of days to weeks (including nematodes, harpacticoid copepods, turbellarians, and Gastrotricha), regulate microbenthos populations and are characterized by a variety of feeding strategies (Gray 1981; Wall 2004). Macrobenthos populations, which have a life cycle of months to years (including rooted macrophytes and larger invertebrates such as crustaceans, larvae of dipterans, bivalves, and annelid worms), may act as ecosystem engineers by either mixing or stabilizing sediments. In addition, they produce organic matter (macrophytes in particular) and consume dead organic matter and associated microbenthos (detritivores) or serve as food for other benthic organisms (carnivores). For vertebrates such as fish, amphibians, birds and mammals, macrobenthos may be an important food source and consequently may be subject to exposure via food web transfer. Vertebrates may have a relatively large habitat range, and their life span may cover several years. Classification of benthic organisms based on size is not strictly coupled to taxonomic groups. This is because different species within a taxonomic group, and even different life stages of the same species, may belong to different size classes. For example, Gérino et al. (2003) classified macroinvertebrates in functional groups based on mechanical activities they perform, e.g. bioturbation or feeding strategies. More detailed information on the ecology

of benthic organisms is provided in review papers dealing with benthic bacteria (Nealson 1997), marine fungi (Hyde et al. 1998), marine meiobenthos (McIntyre 1969), micro- and meiobenthos (Fenchel 1978), and freshwater benthic invertebrate species (Covich et al. 1999).

### 3 Ecosystem Functions and Services Provided by Benthic Organisms

An overview of protection goals in EU directives is given by Hommen et al. (2010). Until now, in most documents underlying European regulations and directives, protection goals for benthic organisms have only been defined in general terms, except for pesticides (EFSA 2015). Defining specific protection goals is a crucial starting point in ERA. To operationalize the general protection goals mentioned in legislation, the ecosystem service concept has been proposed (Forbes and Calow 2013; Nienstedt et al. 2012). Ecosystem services are the stocks of natural capital from which humans benefit (Maltby 2013). The concept has been developed primarily as a communication tool to explain societal dependence on nature and as a framework to help decision makers implement policies and measures that support human wellbeing, including sustainable management of the environment. Specific protection goals for water organisms in edge-of-field surface waters subject to pesticide exposure were derived with this method by the European Food Safety Authority (EFSA) (2010b, 2013). In a recent European Chemicals Agency (ECHA) workshop (Helsinki, 2013), it was recognized that this concept could also be applied to derive specific protection goals for benthic ecosystems (ECHA 2014c). Wall (2004) provided an extensive overview of ecosystem functions and services in soils and sediments, whereas Levin et al. (2001) reviewed ecosystem functions provided by benthic communities in estuaries and coastal wetlands. Covich et al. (2004) reviewed the role of biodiversity in the functioning of freshwater and marine benthic ecosystems.

Based on these reviews, and following the approach originally developed by EFSA (2010b), we classified the ecosystem services provided by benthic organisms and ecosystems in freshwater and marine sediment into four groups according to the Millennium Ecosystem Assessment (MEA 2005) (Table 1):

- (1) provisioning ecosystem services i.e. products obtained by humans,
- (2) regulating ecosystem services i.e. regulating processes beneficial for humans,
- (3) cultural ecosystem services, i.e. important conditions for humans related to aesthetic, spiritual, educational and recreational values and benefits, and
- (4) supporting ecosystem functions, i.e. ecosystem functions that support ecosystem sustainability and therewith underlie the provisioning, regulating and cultural services.

**Table 1** Estimated importance of ecosystem services provided by freshwater and marine benthic organisms based on a subjective scale from low (1) to high (3), potentially influenced by organic contaminants

Millennium ecosystem category	Ecosystem services	Freshwater benthic ecosystems	Marine benthic ecosystems	Related soft bottom benthic organisms
Provisioning services (Products obtained by humans)	Food	2	3	Consumable benthic fish, shellfish, and macrophytes
	Fibre, construction materials	2	1	Emergent macrophytes (e.g. thatched roofs)
	Genetic resources	3	3	All species harvested by man
	Natural medicines and biochemical substances	1	2	Potentially all species
	Ornamental resources	2	2	Aquaria and garden pond macrophytes, invertebrates and vertebrates
	Fuels and energy	3	2	Peat, mangrove wood
	Biological products	2	2	Invertebrates for fish bait (e.g. <i>Nereis</i> sp., <i>Arenicola marina</i> , <i>Lumbriculus variegatus</i> )
Regulating processes beneficial for humans	Pest and disease regulation	1	1	Benthic fish and invertebrates (e.g. that control aquatic species that act as host for parasites and diseases)
	Sediment bioremediation	3	3	Bacteria, fungi, microfauna, macrophytes, bioturbating invertebrates and vertebrates
	Water purification	3	3	Bacteria, fungi, microfauna, macrophytes, bioturbating invertebrates and vertebrates
	Climate regulation	2	2	Bacteria (e.g. methane production)
	Shore, bank, and sediment stabilization	3	3	Macrophytes, biofilms (microbes and algae), sediment-stabilizing invertebrates
	Hydrological regulation	2	2	Macrophytes
	Pollination	2	1	Aquatic and semi-aquatic insects that pollinate vascular plants and that have benthic larval stages (e.g. Ephyridae)
	Air quality regulation	2	2	Rooted macrophytes and benthic algae
	Invasion resistance	2	2	All native benthic organisms having similar niche as invasive species

(continued)

**Table 1** (continued)

Millennium ecosystem category	Ecosystem services	Freshwater benthic ecosystems	Marine benthic ecosystems	Related soft bottom benthic organisms
Cultural services	Education and inspiration	3	3	All benthic organisms
	Aesthetic values	2	2	Benthic red list species
	Recreation and ecotourism	3	3	Rooted macrophytes, benthic fish,
	Spiritual and religious value	2	2	Potentially all species (including benthos)
	Cultural heritage	2	1	All characteristic benthic organisms of man-made aquatic ecosystems (e.g. channels, ditches, peat excavations)
Supporting functions (to facilitate other ES)	Sediment formation and structuring	3	3	Macrophytes, bioturbating invertebrates and vertebrates, bacteria and fungi
	Photosynthesis	3	3	Rooted macrophytes, benthic algae, photosynthesising bacteria in biofilms
	Primary and secondary food production	3	3	Benthic organisms
	Nutrient cycling	3	3	Benthic organisms
	Decomposition and mineralization	3	3	Benthic detritores and microbes
	Food web control mechanisms	3	3	Benthic vertebrates, invertebrates and microbes (including pathogens)
	Provision of habitat and shelter	3	3	Rooted macrophytes, biofilms

For each service, the most important benthic organism groups are identified

For each service provided by benthic organisms, we assessed the relative importance on this service on a subjective scale from low (1) to high (3). Moreover, we identified the ecosystem service providing units (SPUs), also referred to as key drivers by EFSA (2010b) and Nienstedt et al. (2012). SPUs are the main taxonomic groups of organisms providing each service (Table 1).

Freshwater and marine benthic ecosystems may provide similar ecosystem services (Table 1) and overall, similar taxonomic and/or functional groups of

benthic organisms provide these services. However, certain taxonomic groups are largely restricted to either freshwater sediments (e.g. insects) or marine sediments (e.g. Echinodermata). Important SPUs include microorganisms, benthic algae, benthic invertebrates, sediment rooted macrophytes, and benthic vertebrates.

### ***3.1 Dealing with Vulnerable Key Species***

Current approaches in prospective risk assessment aim to provide sufficient protection to a wide array of aquatic and benthic non-target species (see e.g. EFSA 2013, 2015). Vulnerable key species are of particular importance. When selecting indicator species for testing, it should be considered whether the lower-tier approaches (those based on standard test species and the application of an assessment factor) sufficiently protect these vulnerable benthic taxa. Vulnerable key species are species that fulfil a highly important role in the ecosystem, have a high risk of exposure (e.g. low avoidance potential), are very sensitive to chemical stress due to specific traits (e.g. poor detoxification mechanism, feeding habit, low elimination rate) and have a low recovery potential (e.g. low recolonisation potential, long generation times). These characteristics make it difficult to culture and test these species in the laboratory. Moreover, it is difficult to identify the most vulnerable key species of each SPU group and type of ecosystem, as many species have a high plasticity, fulfil a variety of functions and might change function depending on their life stage and/or type of ecosystem where they dwell. Furthermore, the vulnerability concept of benthic species and the impact of organic contaminants have not received much attention in the scientific literature. Two approaches to determine vulnerable key benthic species are possible. First, biological traits might be used to identify these species. A good example of such a trait-based approach to identify vulnerable aquatic invertebrates is provided by Rico and Van den Brink (2015). Trait-based approaches are less-well developed for microbes, but a noteworthy example may be nitrifying bacteria that oxidize nitrite to nitrate are slow growing specialists (Nealson 1997), which might therefore be good indicators of a vulnerable key group for benthic microbes. Second, the mode of action of the chemical might determine which main group of species is more sensitive. For instance, herbicides are designed to kill plants and would therefore be expected to mainly impact non-target benthic algae and macrophytes. However, even after identifying the most sensitive group to one herbicide, no single species is most sensitive to all herbicides (Giddings et al. 2013). An important research need is therefore to find a good method to identify vulnerable key benthic species.

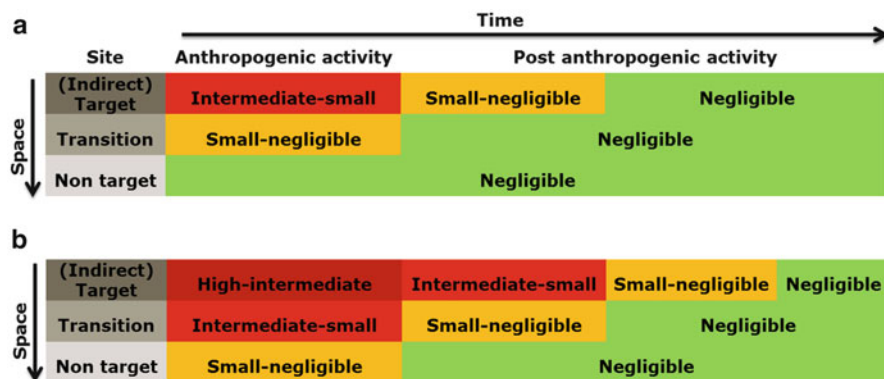
**Table 2** Proposed protection goals for benthic organisms with their ecological entity and attribute based on the ecosystem services concept

Organism group	Ecological entity	Attribute
Microorganisms	Functional group	Processes
Benthic algae	Population	Abundance, Biomass
Sediment rooted macrophytes	Population	Abundance, Biomass, Cover
Benthic invertebrates	Population	Abundance, Biomass
Benthic vertebrates	Individual to population	Survival, Growth, Abundance, Biomass

## 4 Specific Protection Goals for Sediment Risk Assessment

Specific protection goals for SPUs are presented in Table 2. These goals are defined in terms of the ecological entities and attributes to be protected. Ecological entities concern the relevant level of biological organization to consider and attributes determine which endpoint to assess (Nienstedt et al. 2012). Each specific protection goal must be addressed by a different ERA scheme. This is particularly the case when addressing spatial differentiation in specific protection goals with various options, such as a threshold option (accepting negligible impacts on sensitive endpoints only) or a recovery option (accepting temporal impacts followed by a return to the base line).

Microorganisms are of major importance for many functions such as nutrient cycling, decomposition and water purification (Palmer et al. 2000). The functional redundancy and recovery potential of microorganisms is high (Van Beelen and Doelman 1997). We therefore followed the proposal of Nienstedt et al. (2012) to protect microorganisms on the level of functional group and focused on functional measurement endpoints in ERA. However, generating quantitative data on microbial diversity in polluted sediments is still important, since this type of information likely provides insight into causal relationships between microbial composition and shifts in processes mediated by microbes (Diepens et al. 2014b). For benthic algae, macrophytes and invertebrates, we propose the population as the ecological entity to be protected, since the functional redundancy concept is more difficult to apply to several provisioning and cultural ecosystem services provided by these organisms. In particular, rooted aquatic macrophytes and benthic invertebrates might include vulnerable key species that require protection at the population level to guarantee the protection of structural and functional biodiversity of benthic communities. Again following the line of reasoning of Nienstedt et al. (2012) for benthic vertebrates, we selected the individual-to-population level as an ecological entity to avoid mortality due to acute toxicity and prevent suffering of individual animals due to sediment exposure. The SPUs that we have proposed for benthic organisms, as well as their ecological entities and attributes to be considered in the ERA of organic contaminants in sediments (Tables 1 and 2), are similar to those identified by EFSA (2013) in their derivation of specific protection goals for water organisms



**Fig. 1** Example for a strict (a) and a less strict (b) option to define the magnitude of acceptable effects on a temporal and spatial scale. Note that sediments not often are target sites for application of regulated organic chemicals, but often hot spot sites of sediment exposure can be identified

for prospective ERA of pesticides. Furthermore, for benthic organisms and prospective ERA of pesticides, EFSA (2015) adopted specific protection goals that are very similar to our proposal. Although the specific protection goals derived for pelagic and benthic organisms are very similar this does not mean that the environmental risks of benthic organisms are covered by the ERA schemes developed for pelagic aquatic organisms. Note that, dependent on the chemicals and use pattern, the temporal profile of exposure may be very different between the water column and the sediment. For instance, hydrophobic chemicals like many insecticides more often are subject to shorter term pulse exposures in the water column, whereas sediment-bound chemicals are subject to much slower temporal dynamics.

The acceptability of an effect can be specified for each SPU by quantifying the acceptable magnitude of an effect and the associated temporal and spatial scale. Figure 1 shows possible options for a spatial-temporal differentiation of acceptable effects. Defining the spatial scale for an appropriate sediment ERA, particularly the spatial scale of possible acceptable effects, can be challenging. In most cases, sediments and sediment organisms are not the target of chemical applications, but sediments can act as a sink for chemicals from elsewhere. For example, with the exception of rice paddy fields, agrochemicals such as pesticides are not directly applied in aquatic ecosystems, but edge-of-field surface waters (e.g. ditches) might be considered a transition zone between agricultural fields (target site) and larger surface waters such as lakes and rivers (non-target site). Moreover, exposure might be very heterogeneous, both horizontally (sediment surface) and vertically (depth of the sediment profile). For example, antibiotics and biocides are used in aquaculture cages, and these chemicals eventually reach the sediment (Rico et al. 2014; Telfer et al. 2006). In this case, it would be useful to consider the situation in a 3D profile and define the area under and around the cages as an indirect target area. A more complicated example concerns antifouling paints on ships, as they travel large distances. Consequently, contamination from antifouling substances has been found worldwide in sediments (Konstantinou and Albanis 2004). Harbours often

are sedimentation areas for contaminated particles (Koelmans et al. 2010; van Noort and Koelmans 2012) and might therefore be considered as a main accumulation site—or ‘hot spot’—for exposure to antifouling agents. Suspended solids also should be considered, which might carry contaminants away from the target or hot spot area. An important question is whether exposure via suspended solids should be addressed in aquatic or in sediment risk assessment schemes. A pragmatic approach could be to consider only settled particles in sediment ERA.

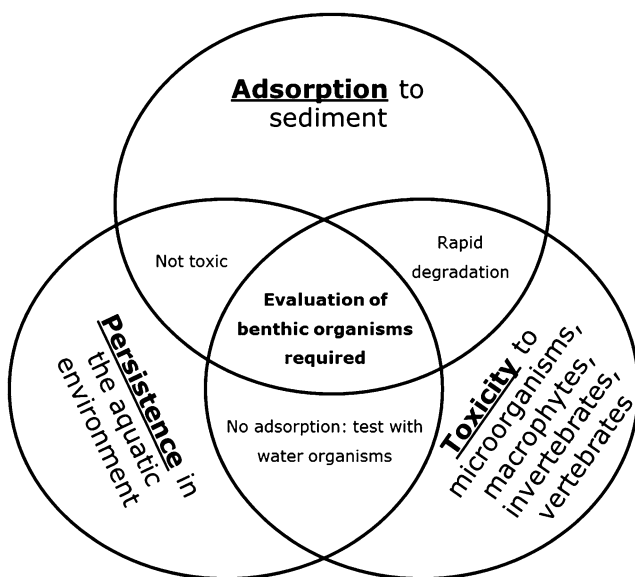
Thus, to sustain ecosystem structure and functioning, the effects of sediment-bound contaminants should be either preventable or reversible, even at target and/or hot spot sites. However, recovery of the selected attributes of the relevant ecological entities might be variable depending on the persistence of the chemical, its bioavailability and the ability of the affected benthic organisms to recover. Note that it is the responsibility of risk managers and policy makers to define the acceptable spatial and temporal effects.

## 5 Triggers for Prospective Sediment Risk Assessment in European Regulatory Frameworks

Ideally, triggers for conducting a sediment ERA should be based on the physico-chemical properties of the test compound that affect its adsorption and persistence in the sediment and on its toxicity potential for benthic organisms (Fig. 2). Maund et al. (1997) proposed the following triggers for sediment testing of pesticides: (1) an *adsorption trigger* consisting of an organic carbon-water partitioning coefficient ( $K_{oc}$ ) greater than or equal to 1000 (or  $\log K_{oc} > 3$ ), (2) a *persistence trigger* consisting of a laboratory aerobic soil half-life time greater than or equal to 30 days, and (3) a *toxicity trigger* consisting of a 48 h median effect concentration ( $EC_{50}$ ) to *Daphnia* of less than 1 mg/L or a 21 days no observed effect concentration (NOEC) of less than 0.1 mg/L in water-only toxicity tests.

Criteria that are currently required to trigger sediment toxicity testing differ between existing European regulations and directives dealing with prospective ERAs (Table 3). The persistence trigger (more than 10 % of the applied radioactivity of the parent in sediment after day 14) is used for pesticides and medicinal products for humans, while the adsorption trigger ( $\log K_{oc}$  or  $\log K_{ow} > 3$ ) is used for chemicals under REACH, biocides and veterinary medicinal products.

In most regulatory documents, except those for pesticides, the toxicity trigger for sediment ERA is initially based on equilibrium partitioning (EP) and toxicity data for pelagic organisms (Table 3). EP theory states that partitioning of a chemical between two phases is governed by the chemical affinity of each phase. For a more detailed description of the EP approach in sediment ERA, see Sect. 9.2 below. In a decision scheme for conducting sediment-spiked toxicity tests for pesticides recently proposed by EFSA (2015), the initial trigger to conduct sediment toxicity tests is based on measured/predicted exposure concentrations in the sediment



**Fig. 2** Theoretical basis for defining triggers for sediment toxicity studies based on Maund et al. (1997). The *circles* describe the three chemical characteristics that should be evaluated, and the overlap between the circles indicates the decision-making process for combinations of those characteristics

compartment and toxicity data of pelagic organisms. For pesticides, currently the toxicity trigger of a 21 days NOEC or  $EC_{10}$  for *Daphnia*  $<0.1$  mg/L is used, although another representative crustacean or insect may also be appropriate. Note, however, that EFSA (2015) proposed to use chronic toxicity data for algae and/or vascular plants as toxicity trigger in case of compounds with herbicidal properties that accumulate in sediments.

For veterinary medical products, a sediment ERA is not required if risks for pelagic aquatic invertebrates have not been demonstrated (Table 3). This disregards the fact that the environmental risks of hydrophobic veterinary chemicals for pelagic organisms may be predominantly acute, while those for benthic organisms will more often be chronic, at least if the chemical is persistent in the sediment compartment and remains bioavailable for a period that exceeds the generation time of benthic species.

Implementing a uniform set of triggers would improve harmonization between the guidance documents underlying the regulation/directives for various types of chemicals. A recent ECHA workshop recommended using a combination of triggers based on the physicochemical properties of the toxicant and the potential toxicity to benthic organisms (ECHA 2014c). In regulatory documents (see Table 3), hydrophobicity ( $\log K_{ow}$ ) and the organic carbon-water partitioning coefficient ( $\log K_{oc}$ ) are interchangeably used as triggers for the potential to adsorb to sediments from the water column. However, these are not equivalent; the values

**Table 3** Criteria that are currently required to trigger sediment toxicity testing as described in existing EU regulations and directives, and the guidelines accompanying these regulations

Regulation	Trigger	Reference
Regulation EC/1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	Sediment effect assessment is required if the chemical has a tonnage band $\geq 1000$ tonnes per manufacturer or importer per year and a $\log K_{oc}$ or $\log K_{ow}$ of $\geq 3$ .	ECHA (2008)
	<ul style="list-style-type: none"> <li><math>\log K_{ow} &gt; 3</math>: at least a screening assessment using the equilibrium partitioning (EP) method has to be performed.</li> </ul>	
	<ul style="list-style-type: none"> <li><math>\log K_{ow}</math> 3–5: the screening assessment using EP is considered appropriate, and no further testing is required if the risk quotient (<math>RQ = PEC_{sed}/PNEC_{sed,EP}</math>) <math>&lt; 1</math>.</li> </ul>	
	<ul style="list-style-type: none"> <li><math>\log K_{ow} &gt; 5</math> or a correspondingly high adsorption or binding behaviour: a more comprehensive sediment assessment is needed. If using the EP approach, the risk quotient (RQ) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment. If the RQ based on EP is <math>&gt; 1</math>, then a study, preferably long term, with benthic organisms using spiked sediment is recommended.</li> </ul>	
	For substances that are highly insoluble and for which no effects are observed in aquatic studies, the application of the equilibrium partitioning method is not possible. In this case, at least one sediment test has to be performed.	
Regulation EC/1107/2009 concerning the placing of Plant Protection Products on the market	Sediment toxicity tests with benthic organisms are required:	Sanco (2002), EFSA (2013)
	<ul style="list-style-type: none"> <li>if in the water-sediment fate study <math>&gt; 10</math> % of the applied radioactivity of the parent compound is present in the sediment at or after day 14 (OECD 308 (OECD 2002)), and the chronic toxicity value (<math>EC_{10}</math> or NOEC) derived from the 21 days <i>Daphnia</i> test (or another comparable chronic toxicity tests with a relevant crustacean or insect) is <math>&lt; 0.1</math> mg/L.</li> </ul>	
	<ul style="list-style-type: none"> <li>compounds applied more than once, with a potential for accumulation of residues in the sediment, should also be considered for sediment testing (Sanco 2002).</li> </ul>	
Directive 98/8/EC concerning the placing of biocidal products on the market <sup>a</sup>	A $\log K_{oc}$ or $\log K_{ow}$ of $\geq 3$ can be used as a trigger value for sediment effects assessment.	European Commission (2003b)
	If the RQ (based on EP) is $\geq 1$ , then testing of sediment organisms is recommended. For substances with a $\log K_{ow} > 5$ , the RQ (based on EP) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment.	
	If the RQ based on EP is $> 1$ , then a study, preferably long-term, with benthic organisms using spiked sediment, is recommended.	

(continued)

**Table 3** (continued)

Regulation	Trigger	Reference
Veterinary medicinal products	If the RQ for aquatic invertebrate is $\geq 1$ it is recommended to estimate the RQ for benthic organisms based on EP. If this RQ ( <i>based on EP</i> ) is $\geq 1$ , then testing of sediment organisms is recommended. For substances with a $\log K_{ow} > 5$ , the RQ ( <i>based on EP</i> ) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment.	VICH (2004)
	If the RQ based on EP is $> 1$ , then a study, preferably long term, with benthic organisms using spiked sediment is recommended.	
Guideline on the environmental risk assessment of medicinal products for human use	If a substance is not readily biodegradable and if the results from the water sediment study (OECD 308 (OECD 2002)) demonstrate significant shifting of the drug substance to the sediment, effects on sediment organisms should be investigated in Tier B. The criterion for sediment studies is met if more than 10 % of the substance at any time point after or at 14 days is present in sediment. A detailed strategy for further testing in order to refine the PNEC for the aquatic compartment can be found in the Technical Guidance document (European Commission 2003a).	EMA (2006)
	If the RQ ( <i>based on EP</i> ) is $\geq 1$ , then testing of sediment organisms is recommended. For substances with a $\log K_{ow} > 5$ , the RQ ( <i>based on EP</i> ) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment.	
	If the RQ based on EP is $> 1$ , then a study, preferably long term, with benthic organisms using spiked sediment is recommended.	

<sup>a</sup>Guidance documents underlying the new Biocidal Products Regulation (ECHA 2014b) are still in preparation

for  $\log K_{oc}$  can deviate substantially from  $\log K_{ow}$  (Koelmans et al. 2006, 2009; Mulligan et al. 2009; Poot et al. 2014; Seth et al. 1999). Because  $\log K_{oc}$  is a more direct measure for chemical binding to the sediment than  $\log K_{ow}$ , using  $\log K_{oc}$  is preferred. Considering the information presented in Table 3, a  $\log K_{oc}$  (preferred) or  $\log K_{ow}$  of  $\geq 3$  is generally used as a trigger value for sediment effect assessment. However, hydrophobic chemicals with a  $\log K_{oc}$  of  $\geq 3$  do not necessarily need to be persistent in the sediment compartment. Therefore, we also recommend using the results of Organisation for Economic Co-operation and Development (OECD) guideline 308 (OECD 2002) to assess the persistence of the chemical in the sediment. For this purpose, the persistence trigger, as used for pesticides and medical products ( $> 10\%$  of the substance is present in sediment at or after day 14), may be adopted for other chemicals as well.

A promising trigger to request sediment-spiked toxicity testing with benthic organisms is the EP approach that uses available chronic toxicity data for pelagic

organisms, at least if the taxonomic groups assessed for water ERA overlap with those required for sediment ERA. From Table 3 it appears that in regulatory documents for chemicals under REACH, biocides, and medical products (for veterinary and human use), the EP approach can be used as a screening method for chemicals with a  $\log K_{ow}$  3–5, and that when the EP approach is used for chemicals with a  $\log K_{ow} > 5$ , an extrapolation factor (EF) of 10 should be used to account for dietary uptake of the toxicant in the predicted no effect concentration for sediment based on the EP approach ( $PNEC_{sed;EP}$ ) derivation. If the risk quotient ( $RQ = PEC_{sed}/PNEC_{sed;EP} < 1$ ), then the environmental risks to benthic organisms are considered acceptable. The report of the ECHA workshop (ECHA 2014c), however, states that the EP approach is not valid for chemicals classified as ionizable, perfluorinated alkylated or insoluble. For these chemicals the  $PNEC_{sed}$  should be derived on the basis of spiked sediment toxicity tests with benthic organisms. In addition, this ECHA report recommended exploring the validity of the EP approach for other organic chemicals. This can be done by comparing the screening level  $PNEC_{sed;EP}$  with the  $PNEC_{sed}$  derived from spiked sediment toxicity tests for a number of representative chemicals. The ECHA workshop report (ECHA 2014c) also suggested additional sediment tests for chemicals with a  $\log K_{ow} > 5$ . As an initial screening approach in sediment ERA for pesticides, EFSA (2015) proposes the EP approach and an additional EF of 10 for benthic fauna to cover possible exposure due to ingestion of sediment particles. For benthic primary producers (e.g. rooted macrophytes) this additional EF of 10 is not considered necessary (EFSA 2015).

In order to explore the predictive value of the EP approach and the additional EF of 10, EFSA (2015) compared toxicity data of *Chironomus riparius* from water-spiked and sediment-spiked water-sediment toxicity tests for seven fungicides and insecticides. The calculated NOECs (in mg/kg sediment) based on the EP approach and the additional EF of 10 were for the tested insecticides a factor of 1.1–14 higher than the NOECs derived from sediment-spiked toxicity tests, while that was a factor of 1.8–135 for the tested fungicides. This suggests that, at least for pesticides, the EP approach and the additional EF of 10 results in a protective, but often conservative, estimate of sediment toxicity. EFSA (2015) also recommended evaluating the general applicability of the EP approach and the additional EF of 10 for a larger array of compounds and benthic species.

We recommend to further verify whether the EP approach and the EF of 10 can be considered a realistic worst case approach to derive a  $PNEC$  for benthic organisms and for different types of organic chemicals. Since the validation status of the EP approach has not yet been appropriately evaluated for a sufficient number of compounds, for the time being we propose using an EF of 10 to derive a  $PNEC_{sed;EP}$  for organisms that ingest sediment particles. The reasoning for this proposal is further elaborated in Sect. 9.2 below.

## 6 Data Requirements for Effect Assessment

### 6.1 Toxicity Data Requirements in European Regulatory Frameworks

If the triggers, described in Section 5, are met, toxicity data for benthic organisms are required (Table 4). Hommen et al. (2010) provided an overview of data requirements for aquatic ERA. Current regulations do not always specify the requirements for sediment toxicity testing. Data requirements for freshwater benthic organisms especially concern tests with *Chironomus* sp. and *Lumbriculus variegatus*. Macrophyte tests (e.g. using the rooted macrophyte *Myriophyllum spicatum* (OECD 2014)) are only required by the Plant Protection Products regulation when specific triggers are met for substances with an herbicidal mode-of-action. For marine systems, no specific test species are mentioned in regulatory documents as data requirements, although examples are given in some regulations.

From Table 4 it appears that the data requirements may concern a water-sediment test with *Chironomus* using either spiked water or spiked sediment. We suggest that the spiked sediment test should have priority in sediment ERA. Exposure via sediment in spiked water OECD toxicity tests, however, may also be considered appropriate if the concentration in the top sediment layer is measured (or adequately predicted) and the biotic activity of the test species is highest in this layer. If a chemical is not stable, then a mean or time-weighted average (TWA) concentration for the duration of the sediment toxicity test may be required. This is particularly the case if it cannot be demonstrated with high certainty that the decrease in the bioavailable fraction of the compound is faster in the sediment of the laboratory toxicity test than that predicted for sediments in the field. To obtain a more realistic worst case effect estimate, the chronic EC<sub>10</sub>/NOEC value can be calculated based upon the mean or TWA concentration of the chemical during the test. In case the bioavailable fraction of the compound in the sediment of the laboratory toxicity test decreases faster than that predicted (or measured) for field sediments, it may be appropriate to use the peak concentration in the sediment at the start of the sediment-spiked toxicity test as exposure metric in the effect estimate. The organic carbon (OC) content (%) of the sediment needs to be known to enable standardization of chemical concentration to OC or to express the toxicity value in terms of a fixed OC content per unit DW sediment.

Data requirements for prospective sediment risk assessment rely on official test protocols for standard test species. Diepens et al. (2014b) and Fojut et al. (2013) provided overviews of internationally accepted sediment tests for freshwater, estuarine, and marine invertebrates, as well as macrophytes. In the available protocol tests for marine/estuarine benthic organisms, amphipods seem to be overrepresented. For vertebrates, the whole-sediment toxicity test for larvae of the freshwater frog *R. pipiens* became available only in 2013 (ASTM 2013), so little experience has been acquired in conducting and interpreting this test. No official test guidelines exist for estuarine/marine rooted macrophytes and estuarine/marine vertebrates. Furthermore, no protocol tests for sediment-dwelling microbes are currently available. Most of the experience in tiered effect assessments therefore concerns benthic invertebrates.

**Table 4** Data that are currently required for sediment toxicity testing as described in existing EU regulations and directives, and the guidelines accompanying these regulations

Regulation	What needs to be tested?	Reference
Regulation EC/1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	Long-term test with <i>Lumbriculus variegatus</i> using spiked sediment	ECHA (2014a)
	Long-term test with <i>Chironomus</i> sp. using spiked sediment	
	Long-term tests with a further benthic species using spiked sediment. Selection of third species should supplement the first two species in terms of habitat, feeding strategy, taxa or life-stage. For example, the amphipod <i>Hyalella azteca</i> or the nematode <i>Caenorhabditis elegans</i> could be used	
	For the marine compartment, the same testing strategy is followed. However, for this compartment more tests may be necessary to reduce the higher assessment factor applied if only limited data are available. For possible test species, refer to available protocol tests developed for estuarine/marine species.	
Regulation EC/1107/2009 concerning the placing of plant protection products on the market	OECD (2004a). OECD Guideline 218: Sediment—water chironomid toxicity test using spiked sediment; adopted 13 April 2004. OECD Publishing.	EFSA (2013)
	OECD (2007). OECD Guideline 225: Sediment—water <i>Lumbriculus</i> toxicity test using spiked sediment; adopted 16 October 2007. OECD Publishing.	
	ISO (2010) ISO/DIS 16191 Water quality - Determination of the toxic effect of sediment and soil on the growth behaviour of <i>Myriophyllum aquaticum</i> . International Organization for Standardization, Geneva.	
	ISO (2010). ISO/DIS 16191 Water quality—Determination of the toxic effect of sediment and soil on the growth of <i>Myriophyllum aquaticum</i> . International Organization for Standardization, Geneva.	
	OECD (2014). OECD guideline 239 spiked sediment test with <i>Myriophyllum spicatum</i> <i>Glyceria</i> —in preparation	

(continued)

**Table 4** (continued)

Regulation	What needs to be tested?	Reference
Directive 98/8/EC concerning the placing of biocidal products on the market <sup>a</sup>	For freshwater ERAs, long-term sediment tests with <i>Chironomus</i> sp., <i>Lumbriculus variegatus</i> and a third benthic test species differing in taxonomy and/or feeding habit are required.	European Commission (2003b)
	For estuarine/marine ERAs sub-chronic and chronic sediment toxicity tests for the following species are mentioned as example: <i>Corophium</i> sp., <i>Leptocheirus plumulosus</i> , <i>Neanthes</i> (=Nereis) sp., <i>Arenicola marina</i> , <i>Echinocardium cordatum</i> .	
Veterinary medicinal products	Freshwater sediment invertebrate species: OECD 219 (spiked water water-sediment <i>Chironomus</i> test) is normally used. If exposure is through sediment or adsorbed to soil in run-off, OECD 218 (spiked sediment test with <i>Chironomus riparius</i> ) should be used.	VICH (2004)
	Marine sediment invertebrate species: seek regulatory guidance (probably the standard protocol tests are referred to).	
Guideline on the environmental risk assessment of medicinal products for human use	Effects on a sediment dwelling organism ( <i>Hyalella</i> sp.; <i>Lumbriculus</i> sp. or <i>Chironomus</i> sp.) should be investigated.	EMEA (2006)

<sup>a</sup>Guidance documents underlying the new Biocidal Products Regulation (ECHA 2014b) are in preparation

## 6.2 Main Differences Between Existing OECD, ASTM and US-EPA Guidelines

As discussed by Faber and Bruns (2015) and EFSA (2015) one of the main differences between OECD technical guidelines for sediment-spiked toxicity tests and the corresponding guidelines from North America is the type of test sediment used. In the OECD test protocols pre-equilibrated artificial sediment is recommended whereas the US-EPA and ASTM technical guidelines recommend the use of field-collected sediment. In addition, the OECD and the US-EPA/ASTM

guidelines differ with respect to the recommended spiking procedure. The OECD recommends a pre-equilibrium period of 2–7 days (OECD 2004a, 2007) and US-EPA for freshwater sediment tests at least 1 month for persistent chemicals. However, sediment tests with industrial chemicals and tests with marine sediment should be started as soon as possible after spiking (EPA 1996a, b). During ageing, hydrophobic chemicals may become less bioavailable due to surface sorption to sediment particles and entrapment of the chemical within micropores and binding to organic complexes (e.g. Semple et al. 2003), which may affect exposure conditions in the tests. Differences in exposure conditions due to differences in sediment type (e.g. OC content) and aging may be partly accounted for by expressing the exposure concentrations in sediment as chemical mass per mass of OC, overlying water and preferable also in pore water (see above). Moreover, the use of (semi) static (OECD) versus semi static or flow through (US-EPA/ASTM) test systems is another main difference between the guidelines and can result in different exposure conditions. These differences hamper the comparability of results between sediment toxicity tests conducted according to different guidelines. Therefore, we recommend using pre-equilibrated artificial sediment for prospective toxicity testing, as described in Diepens et al. (2014b), or when field-collected sediment is used, to follow as much as possible the test design as currently proposed in OECD test guidelines with artificial sediment. However, we also support the recommendation of EFSA (2015) to initiate comparative studies to evaluate and understand the consequences of differences in OECD and US-EPA/ASTM guidelines (e.g. artificial vs. field-collected sediment; ageing period before starting sediment toxicity test; static vs flow through testing).

### 6.3 Recommendation for a Suite of Benthic Test Species

Sediment risk assessment should ideally include a set of sediment toxicity tests to cover a relevant number of representatives of benthic communities and focus on long-term exposure and chronic endpoints (Diepens et al. 2014b). Test exposure durations should depend on the generation time of the tested species (e.g. shorter for microorganisms than for invertebrates). Preferably, a chronic toxicity test should cover the full life-cycle of the test organism, or should at least cover its most sensitive life-stage.

An important question at stake is whether the current data requirements underlying European regulations are adequate and whether currently available standard test protocols are sufficient. Sediment toxicity tests should consider the SPUs and associated ecological entities as discussed in Sect. 4, depending on the mode-of-action of the organic chemical under evaluation. The current suite of standard test species used in the European prospective sediment ERA is limited and does not cover all SPUs; benthic microbes, rooted macrophytes and vertebrates receive hardly any attention (Table 4). For instance, for pesticides in Europe the prescribed Tier-1 benthic test species are *Chironomus riparius* (insect), *Lumbriculus*

*variegatus* (oligochaete worm), and for herbicidal compounds that accumulate in sediment *Myriophyllum* (macrophyte) species (EFSA 2013). It remains to be investigated whether the current Tier-1 approach based on chronic toxicity tests with these benthic standard test species, together with the proposed assessment factor, sufficiently covers the protection of all SPUs. Furthermore, a harmonized testing strategy between freshwater and estuarine/marine environment is not yet in place. For a suite of freshwater, estuarine and marine benthic test species and methods, including microorganisms, macrophytes and invertebrates, is referred to Diepens et al. (2014b). Since sediment toxicity testing with benthic vertebrates was not discussed in that review, this topic is addressed briefly in Sect. 9.8.

## 7 Factors Affecting the Exposure of Sediment-Dwelling Organisms

Exposure plays an important role in both sediment toxicity testing (the focus of this paper) and in predicting the field exposure concentrations in sediments. In this paper, exposure is defined as the external concentration of the chemical in environmental media potentially affecting sediment-dwelling organisms, together with the processes that affect its bioaccessibility and its bioavailability, including bioaccumulation.

For any organism, exposure is the net result of chemical uptake and depuration fluxes between the organism and its direct environment (see Diepens et al. 2014b and references therein). For benthic invertebrates, uptake may take place through fluxes from pore water, overlying water, and particle contact and ingestion (Diepens et al. 2014b; Selck et al. 2012). Transport to pore water takes place through desorption from the bulk sediment. If uptake through particle or food (prey) ingestion occurs, particle or diet composition is important. Depuration may include passive elimination, defecation, transformation and exudation. Chemical concentrations in organisms may also be reduced by growth dilution. For rooted macrophytes, partitioning to roots and shoots, translocation between roots and shoots and growth dilution are important (Diepens et al. 2014a). This means that uptake is a complex, time-dependent process, because the relative importance of the individual processes varies with environmental and life-stage changes over time. In addition, the relative importance of these uptake processes may differ between chemicals and benthic organisms.

In assessing exposure of benthic organisms, four types of influential factors are particularly important: chemical, biological, spatial and temporal (ECHA 2014c). These factors are addressed in the subsections below.

## 7.1 Chemical Factors

Traditional exposure assessment concepts use total sediment concentrations and the EP model for a first-tier screening approach to estimate exposure in field sediments (Diepens et al. 2014b; ECHA 2014c). Single sorption domain EP models, however, are known to work well only for partitioning of conventional organic substances to sediment amorphous organic matter phases. The EP model will not work for ionisable chemicals, perfluorinated alkylated substances, Non-Aqueous Phase Liquids (NAPLs), long aged sediments, or in the presence of sedimentary condensed organic matter pools like soot or black carbon (BC). Therefore, specific  $K_d$  models should be used to estimate exposure concentrations in field sediments (Diepens et al. 2014b; ECHA 2014c; Koelmans et al. 2006, 2009; Poot et al. 2014; Redman et al. 2014). If the traditional single domain EP approach is used, condensed organic matter phases may increase actual  $K_d$  values by two to three orders of magnitude, leading to a substantial overestimation of exposure (Koelmans et al. 2006). A realistic worst case approach would be to use a correction of one order of magnitude on the previous EP-based  $K_d$  values. For other chemicals, Quantitative Structure Property Relationship (QSPR) models can be used. These models are based on molecular descriptors, such as the Abraham parameters (Nguyen et al. 2005), and are available for many compound classes. For degradable compounds, however, exposure is dynamic in time, and it may be necessary to account for degradation products in the exposure assessment if they are also toxic. Sufficiently accurate predictive models to describe degradation in time or to translate laboratory degradation data into field-relevant rates have not been developed as yet.

## 7.2 Biological Factors

Species traits such as body size, lipid content, surface area-to-volume, respiratory strategies, diet, digestive processes and dietary assimilation affect bioaccumulation (Gaskell et al. 2007; Rubach et al. 2011) and thus internal exposure. Particle or food ingestion depends on diet and plays a dominant role for some benthic invertebrates such as *C. volutator* (Diepens et al. [Under revision](#)), *Lumbriculus variegatus* (Gaskell et al. 2007; Leppänen and Kukkonen 1998; [Sidney et al. in prep](#)), *Arenicola marina* (Diepens et al. [Under revision](#); Kaag et al. 1997) and *Macoma balthica* (Diepens et al. [Under revision](#); Kaag et al. 1997; McLeod et al. 2008; McLeod et al. 2007) whereas for other species such as *Ilyodrilus templetoni* (Lu et al. 2004) water uptake is dominant. For conventional organic substances, EP-based approaches predict biota sediment accumulation factors (BSAFs) values of approximately 1 or 2. For benthic invertebrates, however, much higher values are often observed (Besseling et al. 2013; Diepens et al. [Under revision](#); Hecht et al. 2004), which can be explained from food ingestion. A recent model analysis showed how actual parameter distributions contribute to this variation (Selck

et al. 2012). On the other hand, values much lower than 1 or 2 are sometimes observed (Koelmans et al. 2006; Moermond et al. 2005). This can be explained by binding to black carbon as mentioned above. In that case, the EP approach would be over-protective, unless a black carbon-inclusive EP approach is used. For organisms like benthic algae and sediment-rooted macrophytes, black carbon effects are similar, but food ingestion does not occur and thus will not add to variance in accumulation. Established models for invertebrates (Diepens et al. [Under revision](#); Hendriks 1995; Janssen et al. 2009; McLeod et al. 2008; Thomann et al. 1995) are available to quantify biological factors on BSAFs.

Experiments with the rooted macrophytes *Elodea canadensis* and *Myriophyllum spicatum* showed that an equilibrium state is not reached within 28 days, a timeframe that is even longer than the duration (7–14 days) of a standard macrophyte test (Diepens et al. 2014a). This means that maximum internal exposure might not be reached and that when conducting spiked sediment toxicity tests with rooted macrophytes, test durations should be increased. Alternatively, mechanistic models might be used as extrapolation tools to calculate maximum levels of internal exposure (Diepens et al. 2014a; Gobas et al. 1991; Heine et al. 2015; Vanier et al. 2001).

For any food web that includes the sediment compartment, exposure of sediment-associated chemicals along the food chain may occur. Whether or not a chemical will bioaccumulate and/or biomagnify depends on the hydrophobicity and persistence of the chemical, the feeding relationships and length of the chain, and the capacity to metabolise and eliminate the chemical by the respective species (Weisbrod et al. 2009). A novel approach to detect secondary poisoning is to directly assess the relative chemical fugacity in an organism at a certain trophic level by equilibrating its tissues with passive samplers in a closed system.

### 7.3 Spatial Factors

Both contaminant concentrations and presence of benthic organisms in field sediment are patchy (horizontally heterogeneous), and ‘exposure hot spots’ are present, which may be identified by appropriate spatial sampling strategies and geostatistics (ECHA 2014c). Similarly, colonization potentials of benthic organisms are influenced by spatial factors. This information is important for the development of realistic exposure assessment goals and exposure scenarios. An exposure scenario can be defined as the set of variables determining chemical exposure (De Laender et al. 2015). These exposure scenarios will yield spatially explicit exposure assessments on which spatially explicit ERA’s can be based. An alternative approach is to deal with spatial heterogeneity through probabilistic modelling (ECHA 2014c). This results in a point estimate of exposure for a heterogeneous region, where the heterogeneity is accounted for by the uncertainty interval in the point estimate.

Besides the abovementioned horizontal heterogeneity, vertical gradients may also affect the exposure of benthic organisms. Sediment exposure usually varies with sediment depth and, consequently, also relates to the biologically active layer, which may be different for various types of sediment-dwelling organisms. This means that vertical heterogeneity also has to be considered in ERA.

## **7.4 Temporal Factors**

Sediments can act as a buffer against fluctuations of chemical concentrations in the overlying water. Flushing or run-off events may cause sudden peaks in exposure in the water column and sequentially at the sediment-water interface and in the biologically relevant sediment top layer where exposure may last longer than in the water column (ECHA 2014c). This indicates that chronic exposure generally is more relevant for sediment assessment than acute exposures. The buffering is stronger for pore water concentrations than for near-sediment overlying water concentrations. Chemical exposure would thus be more variable in time for benthic species that are partly or fully exposed to overlying waters and suspended solids. Furthermore, the temporal dynamics of sediment re-suspension and deposition downstream may be relevant if re-deposited sediments are heavily contaminated.

As discussed before in Sect. 6.2, another important factor affecting exposure of benthic organisms to sediment-bound toxicants is the decrease in bioavailability due to ageing.

# **8 Exposure Concentration in Sediment ERA**

## **8.1 The Ecotoxicologically Relevant Concentration for Sediment-Dwelling Organisms**

In a prospective risk assessment, predicted no effect concentrations (PNEC) are evaluated against predicted environmental exposure concentrations (PECs), where the PEC/PNEC ratio often is used as an indicator of risk (Karman 2000). Lack of a clear conceptual basis for the interface between the exposure and effect assessment may lead to a low overall scientific quality of the risk assessment (Boesten et al. 2007). This interface is defined by EFSA (2005) and Boesten et al. (2007) as the concentration that correlates appropriately with ecotoxicological effects; it is called the ecotoxicologically relevant concentration (ERC). In prospective ERA, the ERC must be consistently applied so that sediment exposure estimates ( $PEC_{sed}$ ) and effect estimates for sediment-dwelling organisms (such as  $PNEC_{sed}$ ) can be compared. More specifically, the 'C' in the  $PEC_{sed}$  estimate should be consistent with the 'C' in the  $PNEC_{sed}$  estimate. From a theoretical point of view, the internal

concentration (body burden) at the target site in the benthic organism under evaluation would be the most appropriate ERC. Concentrations are hard to measure directly at the target site, especially for small animals. Therefore, whole body internal concentrations can be used (Di Guardo and Hermens 2013). In the vast majority of toxicity studies with benthic organisms, however, internal concentrations are not measured (Brock 2013) and in none of the regulatory guidelines is it given as a recommended measurement endpoint in ecotoxicological studies. Consequently, the 'C' in the  $PEC_{sed}$  and  $PNEC_{sed}$  estimates usually refers to external exposure concentrations.

An important question is whether the  $PEC_{sed}$  and  $PNEC_{sed}$  estimates should be expressed in freely dissolved chemical concentration in pore water, ingested particles or total sediment concentration. Since the bioavailability of organic toxicants may be affected by the OC content of the sediment, an additional question is whether the total sediment concentration should be normalized to standard sediment or expressed in terms of OC content of the dry sediment.

The current OECD sediment test protocols (OECD 218 (OECD 2004a), 219 (OECD 2004b), 225 (OECD 2007), 233 (OECD 2010)) advocate the use of artificial sediments containing 4–5 % peat, while EPA OPPTS 850.173.5 (EPA 1996b) advocates the use of clean, field-collected sediments. All protocols require the determination of OC content of the sediment, enabling the recalculation of effect concentrations based on OC content. In toxicity tests retrieved from the literature, different types of sediments varying in OC are used, hampering a direct comparison of test results. To allow comparison of sediment toxicity data from different sources, sediment toxicity data may be standardized to concentrations normalized on sediment OC content. An alternative approach might be to standardize all toxicity data to sediment with an organic matter content of 5 % (which equals approximately 2.5 % of OC), an approach often followed in Europe. The basic principle, however, is the same. To appropriately link exposure and effects, the  $PEC_{sed}$  and  $PNEC_{sed}$  estimates should be expressed either in terms of mg/kg DW standard sediment with a fixed OC content ( $=PEC_{sed-tot}$  or  $PNEC_{sed-tot}$ ) or in terms of mg/kg OC in dry sediment ( $=PEC_{sed-oc}$  or  $PNEC_{sed-oc}$ ). In our paper we have normalized the total concentration of the organic chemical in the sediment to organic carbon ( $PEC_{sed-oc}$  and  $PNEC_{sed-oc}$ ).

The sediment-water chironomid tests using spiked sediment (OECD Guidelines 218 (OECD 2004a) and 233 (OECD 2010)) specify that—as a minimum—the concentrations in overlying water, pore water, and sediment should be measured. According to OECD guideline 218, effect concentrations should be expressed as concentrations in sediment, based on dry weight, at the beginning of the test. OECD Guideline 233, however, does not explicitly specify on what basis the concentration in the  $L(E)C_X$  or NOEC values should be expressed, although in daily practice the concentration in the sediment at start of the test is generally used. The *L. variegatus* toxicity test using spiked sediment (OECD Guideline 225) specifies that the concentration in sediment and overlying water should be verified by measurement. The guideline also outlines a method for isolation and subsequent measurement of

chemicals in pore water. The effect concentration should be expressed in mg/kg sediment on dry weight basis (OECD 2007).

The United States Environmental Protection Agency (EPA) OPPTS 850.1735 Guideline (whole sediment acute toxicity invertebrates, freshwater) states that ‘Concentrations of spiked chemicals may be measured in sediment, interstitial water, and overlying water . . .’, but does not specify on what basis effect concentrations should be expressed, other than ‘In some cases it may be desirable to normalize sediment concentrations to factors other than dry weight, such as OC for non-ionic organic compounds or acid volatile sulfides for certain metals’ (EPA 1996b). The various guidelines lack clarity and are mutually inconsistent on these aspects.

The EFSA has recently published a Scientific Opinion on the assessment of exposure of organisms to pesticides in soils (EFSA 2010a). They recommend that the ERC should be reported both in concentration units of mass of pesticide per mass of dry soil and as a concentration in pore water (EFSA 2009, 2010a). If the rationale behind the recommended use of both measures of exposure would also apply to sediment, which seems likely, then this would suggest that toxicity data generated for sediment organisms should also be reported along with concentrations in pore water and in sediment mass or in sediment OC mass. This is not in line with OECD and EPA guidelines, where the most common recommendation is to report effect concentrations on the basis of sediment mass only. If the pore water concentration is not measured, or is difficult to measure, then an appropriate modelling approach to estimate pore water concentrations might be used. In a toxicity test the final response of the test organism in most cases will be influenced by the dynamics in exposure concentration during the test. We therefore propose as a minimum requirement to always measure exposure at the start and the end of the experiment. For organic chemicals that are expected to rapidly dissipate from sediment, we recommend measuring exposure concentrations, including ecotoxicologically relevant metabolites, at different time intervals during the test. Measurement of dynamics in exposure concentrations in pore water, total sediment, overlying water, and test organisms is advisable if chemical equilibrium is not reached between the different environmental compartments during the test period.

In conclusion, the  $PEC_{sed}$  and  $PNEC_{sed}$  used in the RQ should be expressed in the same type of concentration. Ideally, internal concentrations should be measured during the experiment. We recognise that in sediment-spiked toxicity tests, different exposure routes play a role, and that the relative importance of these routes depends on the properties of the chemical, the test organism and the test system. For the time being, and taking into account the current technical guidelines for sediment-spiked toxicity tests, we consider it a pragmatic and realistic worst-case approach to measure chemical concentrations in sediment (OC normalized), overlying water and pore water. This allows for flexibility when referencing toxicity estimates to OC mass concentration, pore water concentrations or a combination, dependent on the organism. Models may be used to calculate chemical concentrations in environmental compartments in which data is lacking.

## 8.2 Overview of Fate and Exposure Models

Fate models are essential for understanding and evaluating the required time for chemical equilibrium between sediment and pore water and to optimize other aspects of the tests, such as the water-sediment ratio, water renewal and pre-equilibration after spiking. There is a need for approaches to translate biodegradation process parameters obtained from lab tests to parameters that are relevant in the field. The development of passive samplers for more classes of chemical can provide more accurate input for such models.

Exposure models have been reviewed (Guillén et al. 2012; Koelmans et al. 2001; Pistocchi et al. 2010) and four basic approaches have been identified: multiple box models, single point multi-media models, numerical solutions to advection-dispersion transport models and meta-models. Geographic information system (GIS)-based modelling was proposed as a convenient fifth approach (Pistocchi et al. 2010). Single point multi-media models typically provide average concentrations in environmental compartments for a region or country using emission data and mass balance equations or material flow analysis (e.g. EUSES (Vermeire et al. 1997), SIMPLEBOX (Brandes et al. 1996)). However, spatially and temporally explicit models use more detailed and realistic process descriptions to simulate concentrations in aquatic systems as a function of place and time (e.g. DUFLOW (Clemmens et al. 1993), TOXSWA (Adriaanse 1996), GREAT-ER (Feijtel et al. 1997)). In exposure modelling of aquatic systems, single point multi-media models can be considered as a lower tier approach and spatially and temporally explicit models as a higher tier approach. For prospective ERAs, however, the development of exposure scenarios is a prerequisite to successfully apply exposure models. Consequently, more realistic exposure models are needed for emerging chemical classes like ionizable organics and polar substances; such models should also take degradation processes into account.

## 8.3 Linking Exposure to Effects in Sediment ERA

For exposure in chronic risk assessment, either the peak concentration (max) in total sediment normalized to organic carbon content ( $PEC_{sed-oc;max}$ ) or pore water ( $PEC_{sed-pw;max}$ ), or the TWA concentration in total sediment ( $PEC_{sed-oc;TWA}$ ) or pore water ( $PEC_{sed-pw;TWA}$ ) can be used to compare with the predicted no effect concentration for sediment based on chronic toxicity data (either  $PNEC_{sed-oc;ch}$  or  $PNEC_{sed-pw;ch}$ ). In the text below, when referring to  $PEC_{sed}$  and  $PNEC_{sed}$  estimates, this may be either the concentration in total sediment normalized to OC or in pore water.

In principle, the  $PEC_{sed;max}$  or  $PEC_{sed;TWA}$  should be lower than the  $PNEC_{sed;ch}$ . However, if using the  $PEC_{sed;TWA}$  in the risk assessment, the time window for the  $PEC_{sed;TWA}$  estimate should be equal to or shorter than the time window for the

chronic effect estimate that drives the risk (i.e. the duration of tests delivering the critical chronic  $EC_{10}$  values that drive the  $PNEC_{sed;ch}$ ). In addition, proof of reciprocity in toxicity tests should be provided in order to use the  $PEC_{sed;TWA}$  in the risk assessment. Reciprocity refers to Haber's law, which assumes that toxicity depends on the product of concentration and time (Giesy and Graney 1989; Karman 2000).

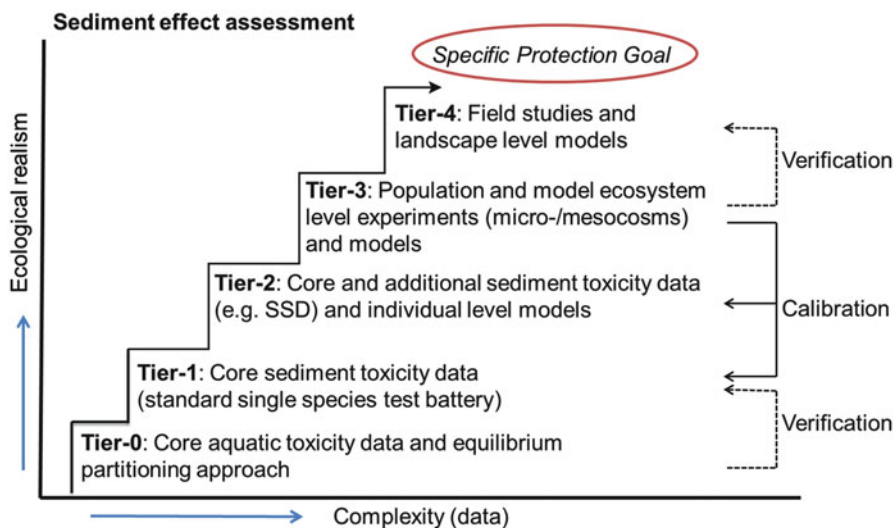
We recommend that the effect estimate derived from sediment toxicity tests be expressed in terms of TWA or mean exposure concentration during the test. However, in current sediment toxicity tests the effect estimate (such as  $EC_x$  and NOEC) is usually expressed in terms of initial exposure concentration. If the effect estimate is expressed in terms of initial exposure concentration, it should be shown that the exposure profile and bioavailable fraction in the toxicity test is worst-case relative to that in the field. Therefore, to assure a more realistic worst-case risk assessment when using the initial exposure concentration, the  $PEC_{sed;max}$  concentration should always be used.

## 9 Tiered Effect Assessment for Benthic Test Species and Spiked Sediments

### 9.1 Tiered Approach

In the tiered approach, test complexity and ecological realism increase when moving up tiers (Boesten et al. 2007; Posthuma et al. 2008; Solomon et al. 2008). This provides a cost-effective procedure, both for industry and regulatory agencies. The tiered system as a whole should be (i) appropriately protective, (ii) internally consistent, (iii) cost-effective and it should (iv) address the problem with a higher degree of realism and complexity when going from lower to higher tiers (see Fig. 3) (Boesten et al. 2007; Posthuma et al. 2008; Solomon et al. 2008). Furthermore, a tiered ERA scheme must be developed for each specific protection goal. An additional advantage of the tiered approach is that higher tiers can be used to calibrate the lower tiers (van Wijngaarden et al. 2015). Appropriate field observations may be used to verify the tiered effect assessment approach based on experimentation.

Below, a tiered ERA scheme for benthic invertebrates and rooted macrophytes is presented and discussed. Most data and experience with spiked sediment tests is available for these taxa. Despite the scarcity of spiked sediment toxicity tests with microorganisms and vertebrates, in this paper we also discuss sediment ERA approaches for these organisms. In principle, however, all tiers can be used for different groups of sediment organisms.



**Fig. 3** Schematic overview of a tiered approach in prospective risk assessment. In each tier an assessment factor (AF) may be necessary to derive a predicted no effect concentration (PNEC). The higher tiers can be used to calibrate the lower tiers (adapted from Diepens et al. (2014b))

## 9.2 Tier-0 Effect Assessment Based on Equilibrium Partitioning

Di Toro et al. (1991) showed that the bioavailability of non-ionic organic chemicals is a function of their distribution between environmental phases (e.g. organic matter and interstitial water). This understanding was the foundation for using EP to derive mechanistic sediment quality guidelines. Assuming that the toxicity of a non-ionic organic chemical is proportional to its concentration in water, then the sediment concentration of this chemical that will cause toxicity can be estimated if the relationship between the chemical concentration in the pore water and that in sediment is understood. The partitioning of a chemical between OC phase in the sediment and pore water can be represented by a simple equilibrium equation (European Commission 2011a):

$$C_{\text{sed-oc}} = C_{\text{pw}} * K_{\text{oc}} \quad (1)$$

In which  $C_{\text{sed-oc}}$  is the concentration of the chemical in the sediment per unit mass of OC ( $\mu\text{g/kg OC}$ ),  $C_{\text{pw}}$  is the concentration of the chemical in pore water ( $\mu\text{g/L}$ ) and  $K_{\text{oc}}$  is the partition coefficient of the chemical to sediment OC ( $\text{L/kg OC}$ ). When replacing  $C_{\text{pw}}$  by the predicted no effect concentration for surface water based on chronic toxicity data ( $\text{PNEC}_{\text{sw;ch}}$ ) derived for pelagic water organisms on basis of water toxicity tests, the  $C_{\text{sed-oc}}$  becomes the  $\text{PNEC}_{\text{sed;ch-EP}}$ .

An essential step in the application of the EP approach is the derivation of an appropriate  $K_{oc}$ , such as with OECD 106 (OECD 2000a). Because reported  $K_{oc}$  values may have a high variability, we recommend using the geometric mean value, since  $K_{oc}$  values usually show a log-normal distribution (EFSA 2014a). If no  $K_{oc}$  is available, then this value can be estimated from  $K_{ow}$  using quantitative structure-activity relationship (QSAR) models (European Commission 2003a).

Research in the past decade has shown that the EP theory does not accurately predict in situ partitioning (Morrison et al. 1996). This is because field  $K_{oc}$  values typically are two to three orders of magnitude higher than those in the laboratory due to the ubiquitous presence of condensed carbon phases, such as black carbon (Cornelissen et al. 2005; Koelmans et al. 2006; Moermond et al. 2005). Consequently, the chemical concentration in sediment that causes toxicity also will be two to three orders of magnitude higher. When the used  $K_{oc}$  value is based on sediment lacking a condensed carbon phase we recommend a worst case approach in Tier-0. This approach accounts for the effect of black carbon by using a  $K_{oc}$  value in Eq. (1), which is only ten times higher than the  $K_{oc}$  values traditionally used in the EP approach. This means that Eq. (1) will return toxic thresholds for sediments that are a factor of ten higher. Another shortcoming of the EP approach is that it neglects sediment ingestion as a relevant uptake pathway. EP also neglects specific species traits and is adequate only as long as the chemical transfer occurs through passive organic matter-water-lipid partitioning. EP-based approaches predict BSAF values of approximately 1 or 2. However, this has been shown to be inadequate for organisms such as the mayfly *Hexagenia* sp. with a BSAF up to 20 for PCB153 (Selck et al. 2012), the annelid *L. variegatus* with a BSAF up to 99 for chlorpyrifos (Jantunen et al. 2008), the marine amphipod *C. volutator* with a BSAF ranging from 16 to 218 for PCBs (Diepens et al. Under revision), the marine polychaete worm *A. marina* with a BSAF ranging from 10 to 40 for PCBs (Besseling et al. 2013; Diepens et al. Under revision) and the marine decapod *Chasmagnathus granulata* with a BSAF ranging from 0.1 to 44 for a range of organochlorine pesticides (Menone et al. 2004). These organisms thus accumulate up to two orders of magnitude higher concentrations than EP theory predicts. Therefore, to be protective, a Tier-0 approach should take this into account, and produce a toxic threshold in sediment that is a factor of 100 lower than calculated by the original Eq. (1). The two effects—the black carbon effect and the sediment ingestion effect—act in the opposite direction, and thus partly compensate for each other, but still yield a net effect of  $100/10 = 10$  as an extra safety factor to be applied to the effect threshold calculated for a Tier-0 for invertebrates and vertebrates that ingest sediment. For microorganisms, benthic algae and sediment-rooted macrophytes, ingestion does not play a role—but these organisms may have the capacity to extract a fast-desorbed fraction of the organic contaminant. For these organisms, we propose that the extra safety factor is not needed when using the EP approach. In Sect. 5 we already mentioned the research recommendation to verify whether the EP approach and the EF of 10 can be considered a realistic worst case approach to derive a PNEC for benthic fauna and for different types of organic chemicals.

### **9.3 Tier-1 Effect Assessment Based on Protocol Tests for Benthic Invertebrates and Macrophytes**

The following approach can be used to derive a chronic Tier-1 PNEC value based on sediment toxicity tests with the freshwater, estuarine and marine standard test species that were described in Sect. 6:

- 1: For the chemical of concern, collect the Tier-1 and additional toxicity data for (pelagic) water organisms in the compartment overlying water.
- 2: Identify the taxonomic group(s) of water organisms that is/are likely to be most sensitive.
- 3: Collect the available spiked sediment toxicity data for benthic freshwater and estuarine/marine standard test species (see sections above).
- 4: Determine whether the most sensitive taxonomic group for Tier-1 water column organisms is likely to be represented in the core data set of benthic test species according to standard protocols.
- 5: If so, use Table 5 to conduct the Tier-1 effect assessment for benthic organisms in freshwater and estuarine/marine ecosystems. If not, determine whether the most sensitive taxonomic group is also represented in the additional toxicity data, which can then be added to the core data set of benthic test species, or try other approaches (such as the EP approach).

### **9.4 Tier-2 Approach on Basis of Laboratory Toxicity Data for Standard and Additional Benthic Invertebrates and/or Rooted Macrophytes**

#### **9.4.1 Geometric Mean Approach**

If valid toxicity data from several species are available, but this number is too low to apply the species sensitivity distribution (SSD) approach, EFSA (2005, 2013) proposed the option of the geometric mean-AF approach. In this approach, the geometric mean toxicity value is calculated for species from the same taxonomic group (e.g. crustaceans, insects, annelids, nematodes, bivalves) and the same measurement endpoint (e.g. LC<sub>50</sub> values). The lowest geometric mean value for the various taxonomic groups is selected, and the same AF normally used in the Tier-1 effect assessment is applied. For the acute aquatic effect assessment of pelagic species exposed to insecticides, the geometric mean approach was recently calibrated by van Wijngaarden et al. (2015) with threshold concentrations for effects derived from aquatic micro/mesocosm tests. This study demonstrated that the geometric mean approach proposed by EFSA for acute effect assessment of insecticides provides sufficient protection to water organisms.

Given the requirements described above, the geometric mean approach could also be applied to sediment ERA that uses acute and/or semi-chronic LC<sub>x</sub> values for benthic species of the same taxonomic group and that have the same feeding

**Table 5** Proposal for assessment factors (AF) to be applied to the lowest sediment toxicity value for standard tests with spiked sediment and benthic organisms (adapted from EFSA 2013; ECHA 2008; European Commission 2011a)

Available data	AF
Three chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups, of which at least two test species, including the most sensitive, are representative for the ecosystem under evaluation (freshwater or marine/estuarine)	10 <sup>a</sup>
Three chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups, of which only the most sensitive is representative for the ecosystem under evaluation (e.g. freshwater test species for an marine/estuarine ERA)	30 <sup>a</sup>
Three chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups, of which one is representative for the ecosystem under evaluation (e.g. freshwater test species for an marine/estuarine ERA), but this species is not the most sensitive	50 <sup>a</sup>
Two chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups and representative for the ecosystem under evaluation (freshwater or marine/estuarine)	50
Two chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups of which one value each is representative for respectively freshwater and marine/estuarine ecosystems	100
Three chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups and not representative for the ecosystem under evaluation	100 <sup>a</sup>
Three semi-chronic (10 days) L(E)C <sub>10</sub> /NOEC values for different taxonomic/feeding groups and for standard benthic test species typical for the ecosystem (freshwater or marine/estuarine) under evaluation	30–100 <sup>a,b</sup>
Two chronic EC <sub>10</sub> /NOEC values for different taxonomic/feeding groups that are not representative for the ecosystem under evaluation (e.g. freshwater test species for an marine/estuarine ERA)	200
Three semi-chronic (10 days) L(E)C <sub>50</sub> values for different taxonomic/feeding groups and for standard benthic test species typical for the ecosystem (freshwater or marine/estuarine) under evaluation	30–100 <sup>a,b</sup>
Three semi-chronic (10 days) L(E)C <sub>50</sub> values for different taxonomic/feeding groups and not all test species are typical for the ecosystem (freshwater or marine/estuarine) under evaluation, but the most sensitive test species is typical.	30–100 <sup>a,b</sup>
Two semi-chronic (10 days) L(E)C <sub>50</sub> values for different taxonomic/feeding groups and for standard benthic test species typical for the ecosystem (freshwater or marine/estuarine) under evaluation	30–100 <sup>a,b</sup>

<sup>a</sup>For substances with a specific toxic mode of action (e.g. insecticides and herbicides) it may suffice to test two representative species of the potentially sensitive taxonomic group(s). This is demonstrated when the representative test species of the sensitive taxonomic group(s) that drive the risk are an order of magnitude more sensitive than the other test species in the chronic aquatic effect assessment for pelagic species

<sup>b</sup>For extrapolate semi-chronic toxicity data a range in AF is proposed to acknowledge differences in toxic mode-of-action and associated differences in time to onset-of-effects. An AF in the lower range may be selected for compounds with a short time to onset-of-effects and an AF in the higher range if latent effects likely will occur (informed by toxicity data of pelagic organisms and read across using data for compounds with a similar mode of action)

strategy. However, in the chronic effect assessment based on spiked sediment toxicity data, the geometric mean approach might be more difficult to use. This is because the chronic toxicity data for different species within the same taxonomic and/or feeding group in the majority of cases concern different measurement

endpoints—such as mortality, growth, biomass and emergence—in tests with different durations. Furthermore, the evaluation of the predictive value of the geometric mean approach by EFSA (2005) was predominantly based on acute toxicity data. Consequently, for the time being, we propose restricting the geometric mean approach for deriving a  $\text{PNEC}_{\text{sed;ch}}$  on the basis of (10 days) semi-chronic  $\text{L(E)C}_{50}$  values for benthic species of the same taxonomic group and with the same feeding strategy. For this purpose, an AF of 100–300 (if at least three taxa representative for the system under evaluation are available) or 200–500 (if less than three taxa representative for the system under evaluation are available) as proposed in Table 5 should be applied to the geometric mean  $\text{L(E)C}_{50}$  value for comparable semi-chronic toxicity of all species belonging to the most sensitive taxonomic group. An AF in the lower range may be selected for compounds with a short time to onset-of-effects and an AF in the higher range if latent effects likely will occur (informed by toxicity data of pelagic organisms and read across using data for compounds with a similar mode of action). In the future, when more chronic spiked sediment laboratory toxicity data become available for organic chemicals and benthic organisms of the same taxonomic group, as well as appropriate semi-field experiments to evaluate the ecological relevance of these laboratory data, the geometric mean approach to derive chronic sediment PNECs based on chronic toxicity data and sub-lethal endpoints can be reconsidered.

#### 9.4.2 Species Sensitivity Distribution (SSD) Approach

The SSD concept is an important probabilistic tool for ERA and accounts for differences in species sensitivity to different chemicals. SSDs are cumulative probability distributions of toxicity values for different species and assume a randomly distributed sensitivity for species. The model calculates hazardous concentration for  $x\%$  of the species ( $\text{HC}_x$ ). The use of the SSD approach in ERA is described in Posthuma et al. (2002). In current prospective ERA for pelagic water organisms, toxicity data for at least eight species (for pesticides EFSA 2013) and ten species (for other toxicants European Commission 2011b)—but preferably more—are needed to apply the SSD approach.

The predictive value of the SSD approach to avoid population and community-level effects predominantly has been evaluated for pelagic organisms and acute toxicity data whereas comparable toxicity data for benthic species are scarce. Whether the SSD approach based on chronic toxicity data and using measurement endpoints that often differ between species of different taxonomic groups, is over or under protective, remains to be investigated. Nevertheless, the SSD approach is widely accepted to set PNECs for pelagic organisms in technical guidance documents underlying European regulations and directives (e.g. EC 2011a; EFSA 2013). We, therefore, consider the SSD approach also feasible for sediment ERA.

Given the limited number of test protocols currently available for benthic species, as well as the limited published sediment toxicity data for organic

chemicals, it will be difficult to collect chronic toxicity data for more than ten benthic species. For sediment ERA, we propose—as a minimum—toxicity data for eight benthic species representing at least five different taxonomic/feeding groups, except when the ERA based on water organisms shows that a specific taxonomic group is at least an order magnitude more sensitive than other taxonomic groups. For example, this may be the case for toxicants with a specific toxic mode-of-action such as insecticides, for which arthropods (insects and crustaceans) are particularly sensitive, and herbicides, for which algae and macrophytes usually are the most sensitive groups. In case of organic toxicants with a specific toxic mode-of-action, the eight species with toxicity data to construct the SSD should preferably be selected from the sensitive taxonomic group(s) (EFSA 2013; Maltby et al. 2005; Maltby et al. 2009). We consider this minimum number of eight toxicity values as a reasonable and pragmatic solution to derive a chronic  $PNEC_{sed;ch}$  when using the SSD approach, but we also recommend applying an AF to the hazardous concentration to 5 % of the species tested as calculated from the SSD ( $HC_5$ ) to address the remaining uncertainty.

Since benthic species of freshwater and marine/estuarine ecosystems have many traits in common, we assume that sediment toxicity data for both freshwater and marine/estuarine benthic species can be combined to construct the SSD curve. Again, an AF may be applied to address the remaining uncertainty in deriving a  $PNEC_{sed;ch}$  for marine/estuarine benthic species based on an  $HC_5$  calculated from an SSD curve largely constructed with toxicity data from freshwater species and the other way around when deriving a  $PNEC_{sed;ch}$  for freshwater species mainly based on marine/estuarine data. Guidance for criteria that can be used to select the size of the AF is shown in Table 6. The use of the SSD approach is valid only if it has been verified that the selected toxicity data show an appropriate fit with the model used to calculate the SSD curve (e.g. the Anderson-Darling test for goodness-of-fit is accepted) (Aldenberg and Jaworska 2000; Aldenberg et al. 2002; Van Vlaardingen et al. 2004).

Preferably, to derive a  $PNEC_{sed;ch}$  based on the SSD approach, the SSD should be constructed with chronic  $EC_{10}/NOEC$  data addressing sub-lethal endpoints. However, if for an essential taxon, such as the species number eight in the SSD, a valid chronic toxicity value is missing but a valid semi-chronic toxicity value is available, then the approach described in Table 7 may be an option to derive the corresponding chronic  $EC_{10}/NOEC$ . The size of the EF to be applied should be based on read-across information on toxicity data for pelagic and benthic species and compounds with a similar toxic mode-of-action. EFs in the lower range may be appropriate for compounds with a short time to onset-of-effects (e.g. pyrethroid insecticides) while EFs in the higher range may be more appropriate for compounds with more latent effects e.g. if they have hormone disruptive properties (e.g. tributyltin). We recommend using this extrapolation approach for no more than two species in the chronic SSD curve, which means that minimal six species with chronic data is available. Another approach is to use semi-chronic data (e.g. 10 days  $L(E)C_{50}$  values) separately to construct an SSD and to calculate a corresponding semi-chronic  $HC_5$ . A  $PNEC_{sed;ch}$  can be estimated with the approach

**Table 6** Criteria, based on European guidance documents (EFSA 2013; European Commission 2011b), that can be used to select the size of the assessment factor (AF) to be multiplied with the median HC<sub>5</sub> (SSD approach) to derive a PNEC<sub>sed;ch</sub> for benthic organisms

AF	Criteria
1	• $\geq 10$ chronic toxicity data (spiked sediment)
	• $\geq 8$ different taxonomic/feeding groups <sup>a</sup>
	• $\geq 5$ taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine)
	• Lower limit HC <sub>5</sub> is less than a factor of 5 lower than the median HC <sub>5</sub>
2	• $\geq 10$ chronic toxicity data (spiked sediment)
	• $\geq 8$ different taxonomic/feeding groups <sup>a</sup>
	• $\geq 5$ taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine)
	• Lower limit HC <sub>5</sub> is more than a factor of 5 lower than the median HC <sub>5</sub> but less than a factor of 10
3	• $\geq 8$ chronic toxicity data (spiked sediment)
	• $\geq 5$ different taxonomic/feeding groups <sup>a</sup>
	• $\geq 4$ taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine)
	• Lower limit HC <sub>5</sub> is less than a factor of 10 lower than the median HC <sub>5</sub>
4	• $\geq 8$ chronic toxicity data (spiked sediment)
	• $\geq 5$ different taxonomic/feeding groups <sup>a</sup>
	• $\geq 4$ taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine)
	• Lower limit HC <sub>5</sub> is more than a factor of 10 lower than the median HC <sub>5</sub>
5	• $\geq 8$ chronic toxicity data (spiked sediment)
	• $\geq 5$ different taxonomic/feeding groups <sup>a</sup>
	• $< 4$ taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine)

<sup>a</sup>The default option is to select taxa belonging to different phylogenetic phyla or orders, unless (a) evidence is provided that a second benthic species selected for the same Phylum/Order has another feeding strategy, or (b) a specific taxonomic group is most sensitive (e.g. Arthropoda for insecticides). If (b), it suffices to select the required number of taxa from different Genera within the specific sensitive taxonomic group unless the second benthic species selected within a Genus has another feeding strategy (e.g. deposit feeder, suspension feeder, predator)

**Table 7** Proposed extrapolation factor to be applied to an individual semi-chronic or chronic toxicity value to estimate the corresponding chronic NOEC/EC<sub>10</sub> to be used in the SSD curve

Available toxicity value	Extrapolation factor
10 days LC <sub>50</sub>	10–30
10 days EC <sub>50</sub>	5–15
10 days NOEC	3–10
$\geq 21$ –28 days L(E)C <sub>50</sub>	2–5

described in Table 6 (but using semi-chronic instead of chronic toxicity data in the SSD) as well as an extra AF of 5–10. An AF in the lower range may be selected for compounds with a short time to onset-of-effects and an AF in the higher range for compounds with latent effects (read across).

### ***9.5 Tier-3 Approach Based on Semi-Field Experiments***

An important requirement for the use of micro/mesocosm test systems to derive a chronic PNEC value for sediment-dwelling organisms is that the concentration-response relationships for benthic organisms are expressed in terms of exposure concentrations measured in the sediment compartment. Lipophilic organic chemicals that enter aquatic ecosystems via the water compartment will easily sorb to sediment particles in the upper sediment layer. In addition, many benthic invertebrates can be found in this layer, because of more favourable food and oxygen conditions. Consequently, the measurement and/or calculation of exposure concentrations in micro/mesocosm test systems to derive concentration-response relationships for benthic organisms should focus not only on the overlying water column but also on the upper sediment layer of these test systems. However, it may be useful to measure the dynamics in exposure concentrations in different sediment layers because of variations in the habitat occupied by different benthic taxa. We propose measuring the dynamics in exposure concentration (freely dissolved pore water concentration; total concentration in sediment normalised on the basis of OC content) in different sediment layers, for example 0–1, 1–2.5, 2.5–5 and 5–10 cm. Depending on the habitat preference of the benthic organism at risk, the exposure concentration in the appropriate sediment layer can be selected (e.g. the 0–1 cm layer for epibenthos or 0–10 cm layer for rooted macrophytes).

We propose microcosm experiments with spiked sediment, in which the colonisation success by benthic organisms is studied, as an option for the third tier. A design with larger test systems (mesocosms) is possible but might be relatively labour-intensive due to the spiking procedure of sediment and the large volume of spiked sediment that is required. The advantage of using spiked sediments when constructing microcosm test systems is that the contaminant under investigation is homogeneously distributed in the sediment compartment, at least initially. A possible disadvantage of such a design is that the benthic community is not yet established when exposure starts. However, spiked sediment microcosm tests can be used to study the impact of different sediment concentrations on the colonization of the sediment compartment by benthic organisms (seeded or spontaneous) and on their dynamics in population densities. Since the exposure regime of organic chemicals that accumulate in sediments, and for which an ERA has to be performed, is long term, the duration of spiked sediment microcosm tests should be relatively long as well (at least several months), allowing a sufficiently long colonization period for most benthic invertebrates and rooted plants.

Alternatively micro/mesocosm test systems with a well-established aquatic community can be used by spiking the water compartment with the contaminant. The advantage of this approach is that benthic populations already present in the test systems become exposed. A disadvantage, however, is that initially the benthic organisms are primarily exposed via the overlying water, while in a later phase sediment exposure becomes more important. In addition, this experimental design requires a more detailed assessment of the dynamics in exposure concentrations in

different sediment layers and the overlying water. Expressing the treatment-related responses of benthic organisms in terms of sediment exposure concentrations most likely will result in a relatively worst case assessment for epi-benthic taxa in particular, since the initial high exposure via overlying water will also affect these organisms. Note that in spiked water micro/mesocosm tests, the peak concentration of the organic contaminant in the sediment compartment is usually measured days to weeks after the application (Crum and Brock 1994).

## ***9.6 Tier-4 Approach Based on Field Studies***

Currently, too little data and experience are available to give specific recommendations for a Tier-4 approach based on field studies. However, chemical and biological monitoring studies in the sediment compartment of aquatic ecosystems may be used as a quality check of prospective ERA procedures for sediment organisms. Due to the lack of data and experience we do not discuss Tier-4 any further.

## ***9.7 Effect Models to Supplement the Experimental Tiers***

Current ERA schemes focus largely on toxicity and bioaccumulation at the individual level, while specific protection goals, as proposed in Sect. 4, focus mainly on the population level. Effect models can be used to extrapolate results of experimental tiers, amongst others, in linking spatial-temporal variability from exposure to effect, in predicting concentration-response relationships at different levels of biological organisation and different spatial and temporal scales, and in addressing ecological recovery times, bioaccumulation in food-webs and food-web interactions in ecosystems (Forbes et al. 2011; Galic et al. 2010; Hommen et al. 2010; Koelmans et al. 2001; van Beusekom et al. 2006). Despite their ability to include and extrapolate effects that cannot be captured by the experimental tiers, effect models are rarely recommended in technical documents of ERA (Galic et al. 2010; Hommen et al. 2010).

Although a wide variety of effect models have been developed (Bartell et al. 2003; Galic et al. 2010; Koelmans et al. 2001; Pastorok et al. 2003; Schmolke et al. 2010), most of these models address specific scientific research questions and are not directly suitable in ERA. Limitations of the use of ecological models have been described by Rykiel (1996) and Scheffer and Beets (1994). The use of effect models in ERA and their potential to address the requirements of protection goals in EU directives have been assessed previously (Galic et al. 2010; Hommen et al. 2010). Recently, EFSA (2014b) published a scientific opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products, in which critical steps to implement the use of effect models in ERA were identified. *First*, a clear problem formulation is needed that

defines one or more specific questions according to the available data and specific protection goals and consider how the output matches with the specific protection goal. *Second*, the application domain of the model, and thus its predictive power, must to be considered to validate the broader conclusions based on model output. This means that either sufficient data should be available for model validation, or there is the potential to generate this data. *Third*, focal species must be selected, as not all species present in the ecosystem under evaluation can be modelled. Logically, these focal species should be vulnerable representatives of the main taxonomic groups of benthic organisms at risk. *Fourth*, realistic worst case environmental scenarios must be defined in relation to the specific protection goal and problem definition. An environmental scenario is a conceptual and quantitative description of the environmental system relevant to ERA, and has been defined by EFSA (2014b) as a combination of abiotic, biotic and agronomic parameters, thus including both exposure and effect. Scenarios from exposure models should be in line with those of the effect models, as they may share common variables (De Laender et al. 2015; Rico et al. 2015). EFSA (2014b) recommends that several scenarios should be considered, including a control/baseline and a toxic standard. A future research activity would be to develop and link scenarios in exposure and effect models that include the sediment compartment. For ERA, a set of freely available scientific sound robust models with a user friendly interface and a well-defined set of scenarios are needed (EFSA 2014b).

Currently, most effect models used in ERA focus on pelagic organisms and freshwater ecosystems, while marine systems (Galic et al. 2010), benthic organisms and the sediment compartment in general are usually disregarded. Below, we discuss effect models at the individual, population, ecosystem and spatial explicit level, which include benthic invertebrates and/or the sediment compartment or have the potential to do so.

### 9.7.1 Individual Level Models

Individual level models can be used as an addition to Tier 2. Given the characteristics of spatial and temporal variable exposure in the heterogeneous sediment compartment and the role of different exposure routes (e.g. exposure via pore water and food), the simplest models to use for linking exposure to effect at the individual level are TKTD models (e.g. GUTS) (Ashauer et al. 2006; Jager et al. 2011). TKTD models mechanistically account for time-varying exposure and effects of chemicals on individuals. More complex models that can be used are dynamic energy budget (DEB) models (Jager et al. 2013), which embed individual growth and development to account for growth dilution. For some freshwater benthic invertebrates (*Asellus aquaticus*, *Gammarus pulex*, and *C. riparius*), models that link exposure and effect have been developed and parametrized, while for other benthic species such as *M. balthica* (McLeod et al. 2008), uptake models exist but have not yet been linked to effect. Uptake, elimination and effects of contaminants are complicated for aquatic macrophytes

because roots in the sediment as well as leaf and stem surfaces in the water layer contribute to these processes (Diepens et al. 2014a; Heine et al. 2015). A model describing these processes has been developed for *E. canadensis* and *M. spicatum* (Diepens et al. 2014a) and for *M. spicatum* exposure has been coupled to effects (Heine et al. 2015).

### 9.7.2 Population-Level Models

Population models can be divided into three types: Lotka-Volterra type models, matrix models and individual-based models (IBM) (Galic et al. 2010), and can be used as an addition to the experimental Tier-3. IBMs are a convenient approach to deal with the complexity arising from complex life cycles of the organisms, seasonality and small- and large-scale spatial heterogeneity (Schmolke et al. 2010). Relevant population endpoints are recovery times after a peak exposure and population growth rate in case of chronic exposure and sub-lethal effects (Van der Ploeg et al. 2011). In the latter case, a more analytical approach to model structured populations is possible. Individual models can be connected to population models to link individual responses to chemical exposure (Baveco et al. 2014). For the freshwater (epi)benthic species (*Asellus aquaticus* and *Gammarus pulex*) and sediment dwelling species (*C. riparius*), models have been developed previously (Galic et al. 2013; Van den Brink et al. 2007). However, these models disregard sediment exposure via direct contact and ingestion of food and sediment particles. Because this may lead to an underestimation of actual exposure (Diepens et al. Under revision; Thomann et al. 1992), these models should be extended with exposure via this additional pathway. Recently, sediment uptake was explicitly added to a TKTD model integrated in an IBM to assess effect of sediment ingestion on the population level for *C. riparius* (Diepens et al. Submitted-a). This study showed that simultaneous exposure via water and ingestion of contaminated organic matter leads to a larger impact and a delayed recovery compared to exposure via water only. This highlights the importance of sediment and food ingestion as an exposure pathway for benthic invertebrates and underpins the need for sediment toxicity tests in ERA. For marine and estuarine organisms, *C. volutator* is the only benthic species for which a simple Leslie-matrix population model has been presented (Smit et al. 2006). This model has not yet been linked to exposure, which may constitute a direction for future research. Another possibility is to integrate the existing TKTD models for *M. spicatum* with an existing population model, such as that from Best and Boyd (1999).

### 9.7.3 Ecosystem Level Models

Ecosystem level models can be used as an addition to the experimental Tier 3. Only a few models have included higher levels of biological organisation, and mainly freshwater ecosystem models, such as AQUATOX (Park et al. 2008), have been applied in ERA (Galic et al. 2010). Food web accumulation modelling is a good

approach to assess secondary poisoning. Such models are flexible, usually well calibrated and have been evaluated. Several of these models, some including benthic organisms, have been confirmed and recommended for use in the regulatory context (Koelmans et al. 2001).

#### 9.7.4 Spatially Explicit Models

Spatially explicit models can be used as an addition to field studies in Tier 4. Depending on the combination of exposure pattern and species at hand, it may be important to explicitly consider spatiotemporal dynamics of both exposure and populations by modelling spatially-structured populations. This approach is relevant when there is a spatial differentiation in the exposure patterns, with some parts of the system being exposed to higher concentrations than others. Clearly, dealing with this heterogeneity becomes more urgent when larger systems, such as watersheds, are being considered. Also, the species at hand should have limited mobility relative to the scale of the system (Baveco et al. 2014). At the lowest level of spatial complexity, we may deal with relatively simple uniform systems representing streams, ditches and ponds, as in the FOCUS surface water scenarios (FOCUS 2007) used for edge-of-field evaluation of plant protection products, or patches of estuarine and marine ecosystems. Ultimately, the larger spatial scale can be considered, for instance addressing both exposure and population dynamics in a complex ditch system (Focks et al. 2014), a larger watershed or interconnected patches of an estuarine/marine ecosystem. For *Chironomus*, landscape-level approaches can be developed, possibly based on Galic et al. (2013) and Focks et al. (2014). In those studies, however, the focus was on the overlying water compartment. A future activity could be to integrate exposure via the sediment into the landscape/watershed level, for example by using the recently developed sediment-including IBM model for *C. riparius* (Diepens et al. Submitted-a).

### 9.8 Effect Assessment for Vertebrates

A comprehensive review on the use of fish for sediment toxicity assessment was given by Hallare et al. (2011). This review discusses the use of cell line assays, fish embryos, fish microarrays and whole fish tests. European Directive 2010/63/EU states that in the Member States of the European Union, testing with vertebrates should be minimized because of ethical considerations such as animal welfare. Therefore, as an animal friendly first-tier approach, cell line assays of vertebrate species can be used, such as the activated luciferase gene expression (CALUX) assay (Murk et al. 1996). These tests are designed to assess the sensitivity of a chemical for a specific mode of action such as dioxin-like activity or estrogenic activity (Houtman et al. 2006; Legler et al. 1999). However, we

consider the cell line assays not yet appropriate to be used in prospective ERA, since there is a lack of established cell lines. In addition, knowledge about the relationship between toxicant-induced cell line responses and effects on individuals and populations of vertebrates is insufficient (Bal-Price et al. 2014; Castano et al. 2003; Groothuis et al. 2015; Jha 2004; Lee and Steinert 2003). Therefore, an important topic for future research is the development of in vitro cell line assays and the evaluation of their ecotoxicological relevance. An alternative for cell line assays could be the sediment contact assay using zebrafish embryos (Hollert et al. 2003). Also newer approaches seem promising, including receptors and gene arrays in fish cells to identify the mode of action of sediment-bound chemicals (Hallare et al. 2011). As a more conventional Tier-1 assessment, the 10-day single species test (ASTM E2591 – 07 (ASTM 2013)) for amphibians may be used. Considering the very limited experience with benthic vertebrates, we will not provide a tiered ERA scheme for this group in this paper. However, the Tier-0 EP approach might provide a sufficiently conservative  $PNEC_{sed;ch}$  estimate for benthic vertebrates.

## 9.9 Effect Assessment for Microorganisms

Although advanced molecular techniques to determine functional and community responses exist, none of them are subjected to ring tests and described as standard tests (Diepens et al. 2014b). Moreover, experience with microorganisms in prospective sediment tests is limited. Several issues must be considered in a tiered ERA for microorganisms. Microorganisms might be negatively affected or stimulated by contaminants. Furthermore, functional redundancy is high among microorganisms. Consequently, even if there is a clear effect on the community composition, this may not result in an effect on their function (Van Beelen and Doelman 1997). This challenges the interpretation of the test outcomes, depending on which specific protection goal was adopted. Another challenge is to link exposure and effect, as microorganisms affect exposure by degradation and transformation of the contaminant. However, such feedback loops between toxicity and exposure play a role in all sediment tests, as it is very difficult to exclude microorganisms from a test system (Diepens et al. Submitted-b).

Although single species microbial tests do exist their ecological relevance requires support. A single species tests with *V. fischeri* test has been proposed within the first tier in retrospective risk assessment (Nendza 2002) and several International Organization for Standardization (ISO) tests with microorganisms are available. According to EFSA (2015), however, these tests are of limited use in prospective ERA and more research and method development is needed. The single-species test approach is hampered by the quite low representativeness of one species to the vast microbial geno- and phenotypic diversity of sediment systems. In addition, the overwhelming majority of microorganisms currently

cannot be cultured as pure culture isolates, but rather can be studied only in the context of more or less complex natural communities (EFSA 2015). Also ECHA (2014c) concluded that effects assessment for microbes should, when possible, evaluate the impact on the ecosystem or community level, rather than on single species level.

As a higher tier option, simple laboratory microcosm tests with spiked sediment in which functional endpoints of microbes, such as nitrification and denitrification, are determined can be used. These microcosm tests also allow the consideration of the community composition of microorganisms. For the terrestrial ERA, the nitrogen transformation test (OECD 216 (OECD 2000b)) is currently recommended. Ideally, a set of standard functional endpoints should be tested, guided by knowledge about the mode of action of the chemical. Another higher-tier option could be a microcosm or mesocosm study in which benthic invertebrates, macrophytes and microorganisms are tested simultaneously. For microorganisms, the same endpoints as in the laboratory microcosm can be used.

## 10 Sediment Effect Assessment: Case Studies

In this section we present three case studies with ivermectin, chlorpyrifos and tributyltin to investigate the tiered approach in sediment risk assessment as described above, with a focus on benthic invertebrates. These chemicals were selected based on data availability and type of chemical group. In the subsections below, a distinction is made between semi-chronic toxicity tests (test duration usually 10 days), and chronic toxicity tests (test duration usually  $\geq 21$ –28 days). However, not all tests reported in the literature as chronic considered sub-lethal endpoints and/or covered the whole life cycle (or the most sensitive life-stage) of the test organisms. All sediment toxicity data provided in the cases are expressed in  $\mu\text{g/g OC}$ , based on the OC of the sediment as reported in the original papers and/or assuming an OC content of 2.5 % in standard OECD sediment with a peat content of 4–5 %.

### 10.1 *The Pharmaceutical Ivermectin*

#### 10.1.1 Evaluation of Standard and Additional Toxicity Data for Pelagic Organisms and Ivermectin

The laboratory toxicity data for typical pelagic organisms and the pharmaceutical ivermectin are shown in Table 8.

It can be concluded from the information in Table 8 that invertebrate populations most likely are the most sensitive taxonomic group on which a chronic effects assessment for sediment-dwelling organisms should focus. Note that the reported

**Table 8** Toxicity data for typical water column organisms and the pharmaceutical ivermectin

Test species	Acute toxicity	Chronic toxicity	Reference
<i>Pseudokirchneriella subcapitata</i> (green alga)		72 h EC <sub>50</sub> ≥ 4 mg/L 72 h NOEC = 391 µg/L	Garric et al. (2007)
<i>Daphnia magna</i> (Crustacea)	48 h EC <sub>50</sub> = 5.7 ng/L	21 days NOEC = 0.0003 ng/L	Garric et al. (2007)
<i>Oncorhynchus mykiss</i> (fish)	96 h LC <sub>50</sub> = 3.0 µg/L		Halley et al. (1989)
<i>Salmo salar</i> (fish)	96 h LC <sub>50</sub> = 17 µg/L		Kilmartin et al. (1996)
Tier-1 PNEC <sub>sw;ch</sub>		0.0003/10 = 0.00003 ng/L	
Invertebrate community in mesocosms		10–97 days NOEC ≤ 30 ng/L	Sanderson et al. (2007)

toxicity values for the crustacean *Daphnia magna* are at least two orders of magnitude more sensitive than for the green alga and the fish. Another striking phenomenon is the high acute-to-chronic ratio that is reported for *Daphnia magna*. The Tier-1 PNEC<sub>sw;ch</sub> ( $3 \times 10^{-5}$  ng/L) is based on the application of an AF of 10 to the lowest chronic toxicity value (for *D. magna*).

### 10.1.2 Tier-0 Effect Assessment for Ivermectin on Basis of Equilibrium Partitioning

The following equation is used to calculate the PNEC<sub>sed;ch;EP</sub>:

$$\text{PNEC}_{\text{sed;ch;EP}} = \text{PNEC}_{\text{sw;ch}} * K_{\text{oc}} * 0.1 \quad (2)$$

In which PNEC<sub>sed;ch;EP</sub> is the concentration of the chemical in the sediment per unit mass of OC (µg/kg OC), PNEC<sub>sw;ch</sub> is the concentration of the chemical in pore water (µg/L) and  $K_{\text{oc}}$  is the partition coefficient of the chemical to sediment OC (L/kg OC). We selected the tier-1 PNEC<sub>sw;ch</sub> of  $3 \times 10^{-5}$  ng/L (Table 8) and a  $K_{\text{oc}}$  geometric mean of 12,497 L/kg ( $n = 5$ ) from a values range of 4000–25,800 L/kg (Krogh et al. 2008). The geometric mean  $K_{\text{oc}}$  value, resulting in PNEC<sub>sed;ch;EP</sub> value of  $3.75 \times 10^{-5}$  ng/g OC.

### 10.1.3 Tier-1 Effect Assessment for Benthic Organisms and Ivermectin

Chronic sediment toxicity data for three standard benthic freshwater organisms are available (insect, oligochaete, and nematode) (Table 9). In addition, the tests were conducted largely in accordance with internationally accepted guidelines: *C. riparius* (OECD 218), *L. variegatus* (OECD 225), and *C. elegans* (ISO

**Table 9** Sediment toxicity data for benthic organisms and the pharmaceutical ivermectin

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity value (µg/g OC)	Reference
<i>Chironomus riparius</i> Insecta (freshwater; OECD 218)	Mortality	10 days LC <sub>50</sub>	2.75	Egeler et al. (2010)
	Mortality	10 days LC <sub>10</sub>	1.46	
	Mortality	10 days NOEC	1.07	
	Individual dry weight	10 days NOEC	0.13	
	Female emergence	28 days EC <sub>50</sub>	0.39	
	<b>Female emergence</b>	<b>28 days EC<sub>10</sub></b>	<b>0.14</b>	
	Female emergence	28 days NOEC	0.27	
<i>Lumbriculus variegatus</i> Oligochaeta (freshwater: OECD 225)	Total dry weight	28 days EC <sub>50</sub>	131.86	Egeler et al. (2010)
	<b>Total dry weight</b>	<b>28 days EC<sub>10</sub></b>	<b>28.76</b>	
	Total dry weight	28 days NOEC	7.08	
<i>Caenorhabditis elegans</i> Nematoda (freshwater; ISO/CD 10872)	<b>Reproduction</b>	<b>4 days NOEC</b>	<b>4.31</b>	Liebig et al. (2010)
<i>Arenicola marina</i> Polychaeta (marine; non-standard test with field collected sediment)	Mortality	10 days LC <sub>50</sub>	16.48 <sup>a</sup>	Allen et al. (2007), Thain et al. (1997)
	Mortality	10 days NOEC	12.50	
	Mortality	100 days LC <sub>50</sub>	15.56	
	Casting	10 days EC <sub>50</sub>	5.19	
	Casting	10 days NOEC	2.16	
	Casting	100 days EC <sub>50</sub>	6.41	
	Casting	100 days NOEC	<0.43	
<i>Corophium volutator</i> Crustacea (marine; test with field collected sediment)	<b>Mortality</b>	<b>10 days LC<sub>50</sub></b>	<b>10.68<sup>a</sup></b>	Davies et al. (1998), Thain et al. (1997)
	Mortality	10 days NOEC	1.67	
	Mortality	28 days LC <sub>50</sub>	14.56	
<i>Asterias rubens</i> Echinodermata (marine; non-standard test with field collected sediment)	Mortality	10 days LC <sub>50</sub>	11,800	Davies et al. (1998)
	Mortality	10 days NOEC	2500	

The values in bold concern the standard toxicity data used in the Tier-1 effect assessment and were acquired in accordance with internationally accepted guidelines (see Table 2 in Diepens et al. 2014b)

<sup>a</sup>Geometric mean

10872). In the chronic effect assessment, 28 days  $EC_{10}$  values are preferred over 28 days NOEC values.

In Table 9, *C. riparius* shows lower toxicity values than *L. variegatus* and *C. elegans*. Selecting the 28 days  $EC_{10}$  of 0.14  $\mu\text{g/g}$  OC of *C. riparius* and the application of an assessment factor of 10 (Table 5) results in a Tier-1  $PNEC_{\text{sed;ch}}$  of 0.014  $\mu\text{g/g}$  OC for sediment-dwelling organisms in freshwater ecosystems. This Tier-1 PNEC value is lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 9, but is considerably higher than the Tier-0  $PNEC_{\text{sed;ch;EpP}}$  calculated above (Fig. 4).

For marine benthic organisms, toxicity data are available but the tests were not conducted according to standard test protocols, with the possible exception of the test with the crustacean *C. volutator*. The Tier-1  $PNEC_{\text{sed;ch}}$  for marine/estuarine benthic organisms can be derived on the basis of Table 5 in different ways. To demonstrate the concept of the table, we will show all possibilities. One option is to use the three chronic toxicity data for standard freshwater test species by applying an AF of 100 to the lowest chronic NOEC/ $EC_{10}$  (Table 5). Applying an AF of 100 to the 28 days  $EC_{10}$  of 0.14  $\mu\text{g/g}$  OC of *C. riparius* results in a Tier-1  $PNEC_{\text{sed;ch}}$  of 0.0014  $\mu\text{g/g}$  OC for sediment-dwelling organisms in marine/estuarine ecosystems. A second option is to use three semi-chronic toxicity data for marine organisms by applying an AF of 30–100 to the lowest semi-chronic 10 days  $L(E)C_{10}/\text{NOEC}$  (Table 5). In this case we selected an AF of 100 since the acute to chronic ratio for *Daphnia magna* was very large (Table 8). Applying an AF of 100 to the 10 days NOEC of 1.67  $\mu\text{g/g}$  OC of *C. volutator* results in a Tier-1  $PNEC_{\text{sed;ch}}$  of 0.0167  $\mu\text{g/g}$  OC for sediment-dwelling organisms in marine/estuarine ecosystems. A third option is to use three semi-chronic toxicity data for marine organisms by applying an AF of 100–300 to the lowest semi-chronic 10 days  $L(E)C_{50}$  (Table 5). Again we selected an AF in the higher range since the acute to chronic ratio for *Daphnia magna* was very large (Table 8). Applying an AF of 300 to the 10 days  $EC_{50}$  of 5.19  $\mu\text{g/g}$  OC of *C. volutator* results in a Tier-1  $PNEC_{\text{sed;ch}}$  of 0.0173  $\mu\text{g/g}$  OC for sediment-dwelling organisms in marine/estuarine ecosystems. Each of the Tier-1 PNEC values is lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 9 and again is considerably higher than the Tier-0  $PNEC_{\text{sed;ch;EpP}}$  calculated above. Options 2 and 3 based on marine species are very similar, but these options are an order of magnitude higher than the Tier-1  $PNEC_{\text{sed;ch}}$  derived for marine/estuarine ecosystems from the freshwater chronic toxicity data (due to the extra factor of 10 for the freshwater—marine extrapolation) (Fig. 4). To assess the  $PNEC_{\text{sed;ch}}$  for estuarine/marine benthic species, it is logical to prefer options 2 and 3, since these options use toxicity data for marine/estuarine benthic organisms.

### 10.1.4 Tier-2 Effect Assessment Based on Standard and Additional Test Species for Ivermectin

#### Geometric Mean Approach

When analysing the toxicity data presented in Table 9, the geometric mean approach cannot be used since all toxicity data concern test species from different taxonomic groups.

#### Species Sensitivity Distribution Approach

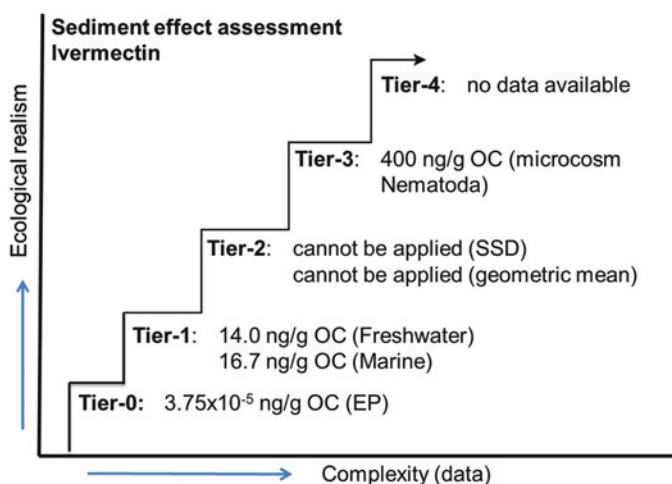
When analysing the toxicity data presented in Table 9, the SSD approach cannot be used since semi-chronic or chronic toxicity values for fewer than eight benthic species are available.

### 10.1.5 Tier-3 Effect Assessment Based on Micro/Mesocosm Experiments for Ivermectin

The effects of ivermectin exposure were investigated in indoor freshwater microcosms using ivermectin-spiked sediments, with a focus on the response of the nematode community (Brinke et al. 2010). An overall microcosm NOEC for Nematoda was observed at 0.4 µg/g OC. This value is approximately a factor of 10 lower than the 4 days NOEC observed for the nematode *C. elegans* in a laboratory test. To date, it remains a research question whether this NOEC for the populations of Nematoda is representative for populations of other potentially sensitive taxonomic groups (e.g. arthropods, Oligochaeta and Polychaeta)

### 10.1.6 Conclusions from the Ivermectin Toxicity Data for Benthic Organisms

- Applying the concept of EP to the  $PNEC_{sw;ch}$  (based on water toxicity data for pelagic organisms) results in a very conservative estimate of the  $PNEC_{sed;ch;EP}$  (Tier 0) (Fig. 4)
- The semi-chronic sediment toxicity data for freshwater and marine benthic organisms overlap
- The derived  $PNEC_{sed;ch}$  based on the Tier-1 approach (Table 5) was remarkably similar for freshwater and marine/estuarine species, at least when using the corresponding toxicity data
- In microcosms, the overall NOEC of the Nematode community was approximately a factor of 10 lower than the NOEC of the standard test nematode *C. elegans*



**Fig. 4** Predicted no effect concentration (ng/g OC) for ivermectin derived for different tiers

## 10.2 The Insecticide Chlorpyrifos

### 10.2.1 Evaluation of Standard and Additional Toxicity Data for Pelagic Organisms and Chlorpyrifos

The laboratory toxicity data for typical pelagic organisms and the insecticide chlorpyrifos are shown in Table 10.

It can be concluded from the information in Table 10 that invertebrate populations, and arthropods in particular, are probably the most sensitive taxonomic group on which a chronic effects assessment for sediment-dwelling organisms should focus. Note that the reported toxicity values for aquatic arthropods are at least one to two orders of magnitude lower than for algae and fish. The acute-to-chronic ratio for aquatic arthropods is approximately a factor of 10.

### 10.2.2 Tier-0 Effect Assessment for Chlorpyrifos Based on Equilibrium Partitioning

$K_{oc}$  values reported for chlorpyrifos have a geometric mean of 10,617 L/kg ( $n = 7$ ) in the range of 3000–25,565 L/kg (Gebremariam et al. 2012). Initially we selected the lower tier  $PNEC_{sw;ch}$  of 0.00046  $\mu\text{g/L}$  (see Table 10) and the abovementioned geometric mean  $K_{oc}$  value, resulting in a  $PNEC_{sed;ch;EP}$  value of 0.00049  $\mu\text{g/g OC}$  using Eq. (2). We then selected the higher-tier  $PNEC_{sw;ch}$  of 0.0033  $\mu\text{g/L}$  (see Table 10) and the abovementioned geometric mean  $K_{oc}$  value, resulting in a  $PNEC_{sed;ch;EP}$  value of 0.0035  $\mu\text{g/g OC}$  using Eq. (2). We consider this latter value to be more realistic, since it is based on higher-tier information.

**Table 10** Toxicity data for typical water column organisms and the insecticide chlorpyrifos

Test species	Acute toxicity	Chronic toxicity	Reference
<i>Skeletonema costatum</i> (marine diatom)		EC50 = 403 µg/L	Alterra database
<i>Daphnia magna</i> (Crustacea)	48 h EC <sub>50</sub> = 0.4 µg/L	21 days NOEC = 0.057 µg/L	Alterra database
<i>Chironomus riparius</i> (Insecta)	96 h EC <sub>50</sub> = 0.09 µg/L		Alterra database
<i>Americamysis bahia</i> (Crustacea)	96 h EC <sub>50</sub> = 0.04 µg/L	35 days NOEC = 0.0046 µg/L	Alterra database
<i>Oncorhynchus mykiss</i> (fish)	96 h LC <sub>50</sub> = 3.0 µg/L	21 days NOEC = 0.51 µg/L	Alterra database
Tier-1 PNEC <sub>sw:ch</sub>		0.00046 µg/L	Application of AF of 10 to the chronic NOEC of <i>A. bahia</i>
SSD aquatic arthropods	Acute HC <sub>5</sub> = 0.042 µg/L (n = 42)		Alterra database
Lowest NOEC micro/mesocosm	0.033–0.10 µg/L for arthropods (pulsed exposure)	0.01 µg/L for arthropods (more or less constant exposure)	Alterra database
Higher tier PNEC <sub>sw:ch</sub>		0.005 µg/L	Application of AF of 2 to threshold level of 0.01 µg/L in chronic micro/mesocosm study

### 10.2.3 Tier-1 Effect Assessment for Benthic Organisms and Chlorpyrifos

For one freshwater benthic insect species (*C. riparius*) a chronic sediment toxicity value is available (21 days NOEC of 0.32 µg/g OC), although this value was not derived according to standard guidelines (Table 11). Furthermore, 10 days LC<sub>50</sub> values are available for the freshwater insect *C. dilutus*, for the freshwater/marine amphipod *H. azteca*, for the estuarine amphipod *E. estuarius* and for the marine amphipod *A. abdita*. These tests were conducted essentially in accordance with USA guidelines.

The freshwater invertebrate species listed in Table 11 comprise only two taxonomic groups (insects and crustaceans) and the species *C. riparius*, *C. dilutus*, and *H. azteca*. The insect *C. riparius* showed the lowest toxicity values (21 days NOEC of 0.32 µg/g OC; 21 days LC<sub>50</sub> of 0.43 µg/g OC) but this test was not conducted according to standard test guidelines. However, the semi-chronic tests conducted with *C. dilutus* and *H. azteca* can be considered standard ASTM tests. Because of the specific mode of action of chlorpyrifos, the inhibition of acetylcholinesterase, two species are sufficient. Following the Tier-1 effect

**Table 11** Sediment toxicity data for benthic organisms and the insecticide chlorpyrifos

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity ( $\mu\text{g/g OC}$ )	Reference
<i>Chironomus riparius</i> Insecta (Freshwater; field collected sediment)	Mortality	4 days $\text{LC}_{50}$	1.58 <sup>a</sup>	Hoofman et al. (1993)
	Mortality	21 days $\text{LC}_{50}$	0.43 <sup>a</sup>	
	Mortality	21 days NOEC	0.32 <sup>a</sup>	
<i>Chironomus dilutus</i> Insecta (freshwater; ASTM E1706)	<b>Mortality</b>	<b>10 days <math>\text{LC}_{50}</math></b>	<b>7.19<sup>a</sup></b>	Ankley et al. (1994), Harwood et al. (2009)
<i>Hyalella azteca</i> Crustacea; Amphipoda (fresh/ estuarine: ASTM E1706)	<b>Mortality</b>	<b>10 days <math>\text{LC}_{50}</math></b>	<b>2.8<sup>a</sup></b>	Amweg and Weston (2007), Hintzen et al. (2009), Weston et al. (2009)
<i>Ampelisca abdita</i> Crustacea; Amphipoda (marine: ASTM E1367)	<b>Mortality</b>	<b>10 days <math>\text{LC}_{50}</math></b>	<b>15.9</b>	Anderson et al. (2008)
<i>Eohaustorius estuarius</i> Crustacea; Amphipoda (estua- rine: ASTM E1367)	<b>Mortality</b>	<b>10 days <math>\text{LC}_{50}</math></b>	<b>13.2</b>	Anderson et al. (2008)
<i>Amphiascus tenuiremus</i> Crustacea; Copepoda; field col- lected sediment	Mortality	4 days $\text{LC}_{50}$	1.74	Green et al. (1996)

The values in bold concern the toxicity data acquired essentially in accordance with internationally accepted guidelines (see Table 2 in Diepens et al. 2014b)

<sup>a</sup>Geometric mean

assessment according to Table 5, an AF of 100–300 has to be applied to the lowest 10 days  $\text{LC}_{50}$  value of *C. dilutus* and *H. azteca*. In this case we selected an AF of 100 since the toxicity data for pelagic organisms showed a relatively low acute to chronic ration, suggesting a fast time to onset-of-effects. The amphipod *H. azteca* (geomean 10 days  $\text{LC}_{50}$  of 2.8  $\mu\text{g/g OC}$ ) is the most sensitive, resulting in a Tier-1  $\text{PNEC}_{\text{sed;ch}}$  of 0.028  $\mu\text{g/g OC}$  for sediment-dwelling organisms in freshwater ecosystems. This Tier-1  $\text{PNEC}_{\text{sed;ch}}$  value is substantially lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 11. Furthermore, this Tier-1  $\text{PNEC}_{\text{sed;ch}}$  value is higher than the Tier-0  $\text{PNEC}_{\text{sed;ch;EP}}$  calculated from the lower tier  $\text{PNEC}_{\text{sw;ch}}$  and higher tier  $\text{PNEC}_{\text{sw;ch}}$  (Fig. 5).

In Table 11, semi-acute toxicity data for three marine/estuarine benthic organisms are shown. These data were acquired according to ASTM guidelines using the amphipods *H. azteca*, *A. abdita* and *E. estuarius*. These taxa comprise only one taxonomic/feeding group. However, when the 4 days LC<sub>50</sub> value for the marine copepod *A. tenuiremus* is included in the Tier-1 core data set, the marine toxicity data then comprise two feeding strategies and two taxonomic groups. The Tier-1 PNEC<sub>sed;ch</sub> for marine/estuarine benthic organisms can be derived by applying an AF of 100–300 to the lowest LC<sub>50</sub> for the combination *H. azteca*, *A. abdita*, *E. estuarius*, and *A. tenuiremus*. Again we selected an AF in the lower range because of the relatively low acute to chronic ratio for pelagic organisms. Although not a standard test species, the marine benthic copepod has the lowest LC<sub>50</sub> value (1.74 µg/g OC), resulting in a Tier-1 PNEC<sub>sed;ch</sub> of 0.0174 µg/g OC for sediment-dwelling organisms in marine/estuarine ecosystems. This Tier-1 PNEC<sub>sed</sub> value is substantially lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 11. Again, this Tier-1 PNEC<sub>sed;ch</sub> value is higher than the Tier-0 PNEC<sub>sed;ch;EP</sub> calculated from the lower tier PNEC<sub>sw;ch</sub>, but equals Tier-0 PNEC<sub>sed;ch;EP</sub> values calculated from the higher tier PNEC<sub>sw;ch</sub> (Fig. 5).

#### 10.2.4 Tier-2 Effect Assessment Based on Standard and Additional Test Species for Chlorpyrifos

##### Geometric Mean Approach

When analysing the data presented in Table 11, the geometric mean approach is only possible for the 10 days LC<sub>50</sub> values for the amphipods *H. azteca*, *A. abdita* and *E. estuarius*. The geometric mean LC<sub>50</sub> for these taxa is 8.4 µg/g OC. This value is higher than the 10 days LC<sub>50</sub> of 2.8 µg/g OC for *H. azteca* (the most sensitive species in the freshwater data set) and the 4 days LC<sub>50</sub> of 1.74 µg/g OC for *A. tenuiremus* (the most sensitive species in the marine/estuarine data set). Applying the geometric mean approach (AF of 100 as used in Tier-1 and the geometric mean LC<sub>50</sub> of 8.4 µg/g OC), results in a Tier-2 PNEC<sub>sed;ch</sub> values of 0.084 µg/g OC. This value can be used for both freshwater and marine taxa since for both types of organisms sufficient semi-chronic toxicity data are available.

##### Species Sensitivity Distribution Approach

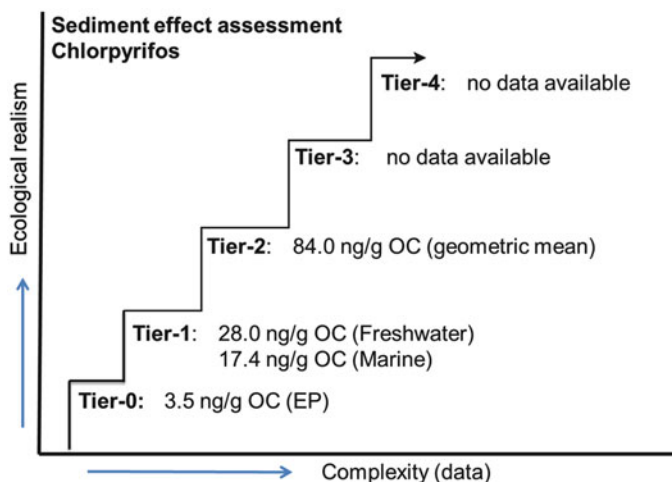
When analysing the toxicity data presented in Table 11, the SSD approach cannot be used since sediment toxicity data are available for fewer than eight benthic species.

### 10.2.5 Tier-3 Effect Assessment Based on Micro/Mesocosm Experiments

An appropriate micro/mesocosm test that allowed concentration-response relationships for benthic organisms and sediment exposure concentrations to be derived could not be found in the open literature.

### 10.2.6 Conclusions from the Chlorpyrifos Toxicity Data for Benthic Organisms for Chlorpyrifos

- Applying the concept of EP to the higher-tier  $PNEC_{sw;ch}$  (based on a microcosm test with a chronic exposure regime) results in a lower  $PNEC_{sed;ch;EP}$  (Tier-0) estimate when compared with the Tier-1  $PNEC_{sed;ch}$  estimates for both freshwater and marine/estuarine ecosystems (Fig. 5)
- The available sediment toxicity data are limited to arthropods and are predominantly semi-chronic in nature
- The sediment toxicity data for freshwater and marine benthic arthropods overlap



**Fig. 5** Predicted no effect concentration (ng/g OC) for chlorpyrifos derived for different tiers

## 10.3 The Biocide Tributyltin

### 10.3.1 Evaluation of Standard and Additional Toxicity Data for Pelagic Organisms and Tributyltin

The laboratory toxicity data for water organisms and long-term water exposure to the biocide tributyltin are shown in Table 12.

It can be concluded from the information in Table 12 that Mollusca are probably the most sensitive taxonomic group. However, the chronic toxicity values for aquatic arthropods are reported to be relatively low as well. The  $PNEC_{sw;ch}$  for pelagic organisms can be derived by applying an AF of 10 to the chronic NOEC of *Nucella lapillus*, resulting in a value of 0.0002 µg/L. This value is similar to the annual average quality standard (AA-QS) (0.0002 µg/L) derived for tributyltin compounds as part of the Water Framework Directive (European Commission 2005).

**Table 12** Chronic toxicity data for water organisms and the biocide tributyltin (data from IPCS 1999; EPA 1997; Hall et al. 2000)

Test species	Criterion	Chronic toxicity
Algae	IC <sub>50</sub> (primary production)	0.92–320 µg/L
<i>Daphnia magna</i> (Crustacea; Cladocera)	21 days NOEC (life cycle test)	0.14–0.25 µg/L
<i>Acartia tonsa</i> (Crustacea; Copepoda)	6 days geometric mean of NOEC/LOEC	0.014 µg/L
<i>Eurytemora affinis</i> (Crustacea; Copepoda)	13 days geometric mean of NOEC/LOEC (Life Cycle test)	<0.088 and 0.15 µg/L
<i>Acanthomysis scuppta</i> (Crustacea; Mysidae)	63 days geometric mean of NOEC/LOEC (Life Cycle test)	0.13 µg/L
<i>Mytilus edulus</i> (Mollusca; Bivalvia)	33 days geometric mean of NOEC/LOEC	0.017 µg/L
<i>Crassostrea gigas</i> (Mollusca; Bivalvia)	geometric mean of NOEC/LOEC Shell thickening	0.02 µg/L
<i>Nucella lapillus</i> (Mollusca; Gastropoda)	2 year geometric mean of NOEC/LOEC (imposex)	0.002 µg/L
<i>Oncorhynchus mykiss</i> (fish)	110 days; 20 % growth reduction	0.2 µg/L
<i>Pimephales promelas</i> (fish)	33 days geometric mean of NOEC/LOEC (Early life stage test)	0.26 µg/L
$PNEC_{sw;ch}$	Application of AF of 10 to the chronic NOEC of <i>Nucella lapillus</i>	0.0002 µg/L

### 10.3.2 Tier-0 Effect Assessment for Tributyltin Based on Equilibrium Partitioning

$K_{oc}$  values reported for tributyltin compounds have a geometric mean of 1317 L/kg ( $n = 16$ ) with a range of 188–2814 (Langston and Pope 1995). We selected the  $PNEC_{sw;ch}$  of 0.0002  $\mu\text{g/L}$  (see Table 12) and the geometric mean  $K_{oc}$  value of 1317 L/kg, resulting in a  $PNEC_{sed;ch;EP}$  value of  $2.63 \times 10^{-5}$   $\mu\text{g/g OC}$  using Eq. (2).

### 10.3.3 Tier-1 Effect Assessment for Benthic Organisms and Tributyltin

An overview of the toxicity data for benthic invertebrates and spiked sediment tests with tributyltin is presented in Table 13. Note that in several of the studies reported in this table, toxicity values were expressed in terms of ng Sn/g DW sediment. These values were converted to  $\mu\text{g TBT/g OC}$  with a factor of 2.6 ( $=118.7/307.06$ ), derived by the division of the molecular mass of tin by the molecular mass of tributyltin.

**Table 13** Sediment toxicity data for benthic organisms and the biocide tributyltin

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity ( $\mu\text{g TBT/g OC}$ )	Reference
<i>Chironomus riparius</i> Insecta (Fresh; artificial sediment; semi-artificial sediment)	Mortality	28 days $LC_{50}$	227.9	Marinković et al. (2011)
	Mortality	28 days NOEC	76.0	
	<b>Male emergence time</b>	<b>28 days <math>EC_{10}^a</math></b>	<b>14.7</b>	
	Growth	10 days $EC_{50}$	750.3	Day et al. (1998)
	Growth	10 days NOEC	296.6	
<i>Hexagenia</i> Insecta (fresh; semi-artificial sediment)	Mortality	21 days $LC_{50}$	296.6	Day et al. (1998)
	Growth	21 days $EC_{50}$	104.7	
	<b>Growth</b>	<b>21 days NOEC</b>	<b>52.3</b>	
<i>Tubifex tubifex</i> Oligochaete (fresh; semi-artificial sediment)	Mortality	28 days $LC_{50}$	2320.8	Day et al. (1998)
	Growth	28 days $EC_{50}$	279.2	
	<b>Growth</b>	<b>28 days NOEC</b>	<b>122.1</b>	
<i>Hyaella azteca</i> Crustacea; Amphipoda (fresh/estuarine: field collected sediment; semi-artificial sediment)	Mortality	28 days $LC_{50}$	189.8	Bartlett et al. (2004)
	Mortality	70 days $LC_{50}$	121.3	
	<b>Mortality</b>	<b>70 days <math>LC_{10}^a</math></b>	<b>26.0</b>	
	Reproduction	70 days $EC_{50}$	30.9	Day et al. (1998)
	Growth	14 days $EC_{50}$	244.3	
	Growth	14 days NOEC	139.6	

(continued)

**Table 13** (continued)

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity ( $\mu\text{g}$ TBT/g OC)	Reference
<i>Potamopyrgus antipodarum</i> Mollusca; Gastropoda (freshwater; artificial sediment)	Mortality	28 days LC <sub>50</sub>	58.5	Duft et al. (2003)
	Mortality	56 days LC <sub>50</sub>	44.8	
	Total embryos development	28 days EC <sub>50</sub>	18.0	
	Total embryos development	56 days EC <sub>50</sub>	9.8	
	Total embryos development	28 days EC <sub>10</sub>	1.103	
	Total embryos development	56 days EC <sub>10</sub>	0.365	
<i>Corophium volutator</i> Crustacea; Amphipoda (marine; field collected sediment)	<b>Mortality</b>	<b>10 days LC<sub>50</sub></b>	<b>5.7</b>	Stronkhorst et al. (1999)
<i>Eohaustorius washingtonianus</i> Crustacea; Amphipoda (marine; field collected sediment)	Mortality	9 days LC <sub>50</sub>	170	Meador et al. (1997)
	Mortality	41 days LC <sub>50</sub>	78	
<i>Rhepoxynius abronius</i> Crustacea; Amphipoda (marine; field collected sediment)	Mortality	10 days LC <sub>50</sub>	3500	Meador et al. (1997)
<i>Armandia brevis</i> Polychaeta (marine; field collected sediment)	Mortality	10 days LC <sub>50</sub>	930	Meador et al. (1997)
	Mortality	42 days LC <sub>50</sub>	158.2	Meador and Rice (2001)
	Growth	42 days EC <sub>50</sub>	38.7	
	Growth	42 days EC <sub>10</sub>	5.9	
<i>Echinocardium cordatum</i> Echinodermata (marine; field collected sediment)	Mortality	14 days LC <sub>50</sub>	10.5	Stronkhorst et al. (1999)
	Mortality	28 days LC <sub>50</sub>	4.1	
	Mortality	28 days NOEC	2.94	
<i>Ruppia maritima</i> Aquatic macrophyte (marine; field collected sediment)	Relative growth rate	21 days EC <sub>10</sub> <sup>a</sup>	0.692	Jensen et al. (2004)

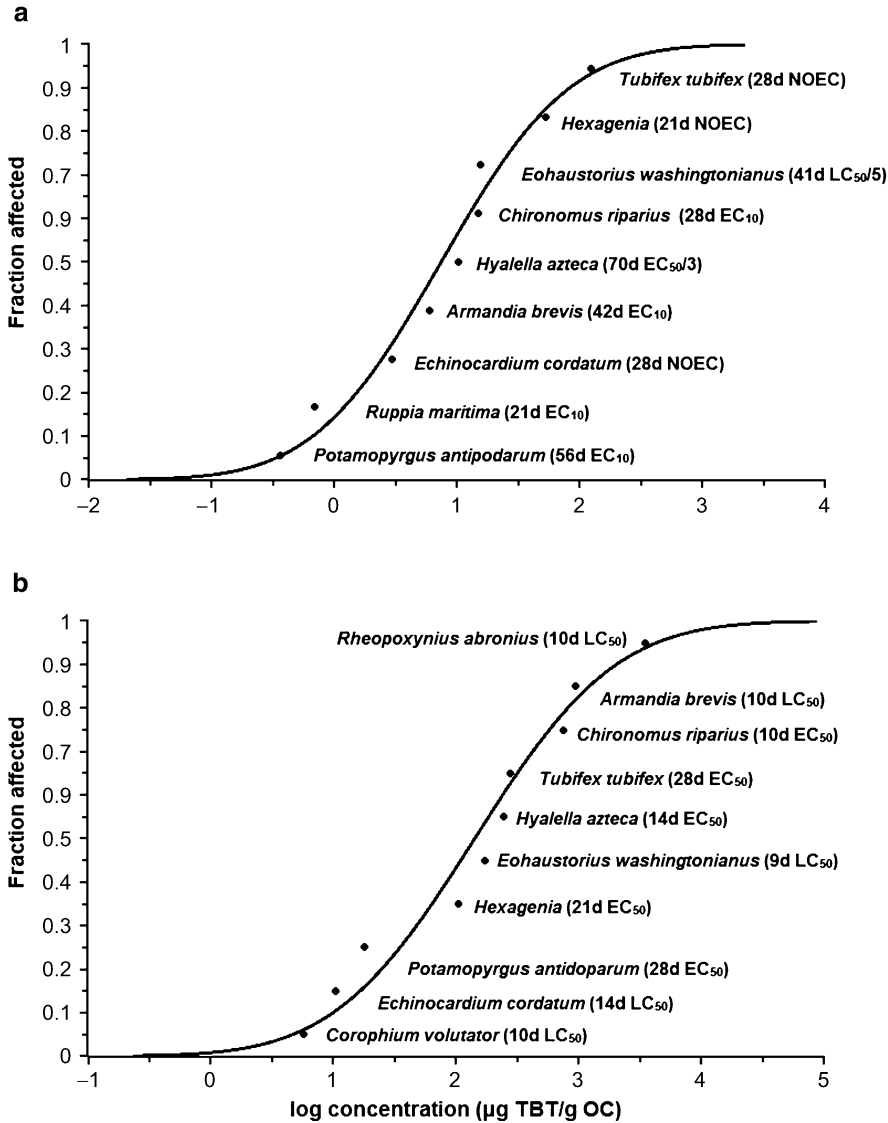
The values in bold concern toxicity data acquired essentially in accordance with internationally accepted guidelines (see Table 2 in Diepens et al. 2014b). Note that in several of the studies reported in this table, toxicity values were expressed in terms of ng Sn/g DW sediment

<sup>a</sup>Estimated value from graphs

The chronic NOEC/L(E)C<sub>10</sub> toxicity values for standard freshwater benthic invertebrates concern the insect *C. riparius* (28 days EC<sub>10</sub> of 14.7 µg TBT/g OC), the insect *Hexagenia* (21 days NOEC of 52.3 µg TBT/g OC), the crustacean *H. azteca* (28 days LC<sub>10</sub> of 26.0 µg TBT/g OC) and the oligochaete *T. tubifex* (28 days NOEC of 122.1 µg TBT/g OC) (Table 13). Another chronic toxicity value for a freshwater benthic organism concerns the freshwater snail *Potamopyrgus antipodarum* (56 days EC<sub>10</sub> of 0.365 µg TBT/g OC) (Table 13). Although the latter species is not a standard test species, it is considered a relevant Tier-1 test species, since the information presented in Table 12 shows that molluscs in particular are the most sensitive taxonomic group.

Following the Tier-1 effect assessment according to Table 5, an AF of 10 has to be applied to the lowest chronic NOEC/EC<sub>10</sub> value for the combination *C. riparius*, *Hexagenia*, *H. azteca*, *P. antipodarum* and *T. tubifex*. The snail *P. antipodarum* (56 days EC<sub>50</sub> of 0.365 µg TBT/g OC) is the most sensitive, resulting in a Tier-1 PNEC<sub>sed;ch</sub> of 0.0365 µg TBT/g OC for sediment-dwelling organisms in freshwater ecosystems. This value is considerably higher than the Tier-0 PNEC<sub>sed;ch;EP</sub> value mentioned above based on the EP concept (Fig. 6).

In Table 13, chronic NOEC/EC<sub>10</sub> values are available for four marine/estuarine benthic organisms: the amphipod *H. azteca*, the polychaete *Armandia brevis*, the echinoderm *E. cordatum* and the aquatic macrophyte *Ruppia maritima*. Only *H. azteca* is a standard test species. Furthermore, for one standard test species (the amphipod *C. volutator*) a 10 days LC<sub>50</sub> is available. However these taxa do not comprise Mollusca, the most sensitive taxonomic group mentioned in Table 13 (water exposure tests). Consequently, the freshwater snail *P. antipodarum* (56 days EC<sub>10</sub> of 0.365 µg TBT/g OC) was also considered when deriving a Tier-1 PNEC<sub>sed;ch</sub> for marine/estuarine ecosystems. Following the Tier-1 effect assessment according to Table 5, an AF of 10 has to be applied to the lowest chronic NOEC/EC<sub>10</sub> for the combination *H. azteca*, *A. brevis*, *E. cordatum*, *Ruppia maritima*, and *P. antipodarum*. The snail *P. antipodarum* (56 days EC<sub>10</sub> of 0.365 µg TBT/g OC) is the most sensitive, resulting in a Tier-1 PNEC<sub>sed;ch</sub> of 0.0365 µg TBT/g OC for sediment-dwelling organisms in estuarine/marine ecosystems. Again, this value is considerably higher than the Tier-0 PNEC<sub>sed;ch;EP</sub> value mentioned above based on the EP concept (Fig. 6). Alternatively, a Tier-1 PNEC<sub>sed;ch</sub> for marine/estuarine ecosystems can be derived by using the semi-chronic toxicity data for the amphipods *H. azteca* (14 days EC<sub>50</sub> of 244.3 µg TBT/g OC), *C. volutator* (10 days LC<sub>50</sub> of 5.7 µg TBT/g OC), *E. washingtonianus* (9 days LC<sub>50</sub> of 170 µg TBT/g OC), and *R. abronius* (10 days LC<sub>50</sub> is 3500 µg TBT/g OC), the polychaete *A. brevis* (10 days LC<sub>50</sub> is 930 µg TBT/g OC) and the echinoderm *E. cordatum* (14 days LC<sub>50</sub> is 10.5 µg TBT/g OC). These marine taxa comprise three taxonomic groups, so that an AF of 100–300 (see Table 5) can be applied to the lowest semi-chronic L(E)C<sub>50</sub> to derive a PNEC<sub>sed;ch</sub>. We selected an AF of 300 since the available toxicity data reveal latent effects and hormone-disrupting properties of TBT. Applying an AF of 300 to the lowest 10 days LC<sub>50</sub> (5.7 µg TBT/g OC for *C. volutator*) results in a PNEC<sub>sed;ch</sub> of 0.019 µg TBT/g OC for marine/estuarine benthic organisms. Note that this PNEC<sub>sed;ch</sub> value is lower than the Tier-1 PNEC<sub>sed;ch</sub> of 0.0365 µg TBT/g



**Fig. 6** Species Sensitivity Distribution (SSD) for tributyltin constructed with (a) (estimated) chronic EC<sub>10</sub>/NOEC values for freshwater and marine benthic invertebrates (n=9) and (b) semi-chronic L(E)C<sub>50</sub> values for freshwater and marine benthic invertebrates (n=10) (data from Table 14)

OC for sediment-dwelling organisms in estuarine/marine ecosystems derived on the basis of chronic toxicity data. However, the chronic Tier-1 PNEC<sub>sed;ch</sub> was selected in the effect assessment, since an assessment based on chronic toxicity data overrules that based on semi-chronic toxicity data.

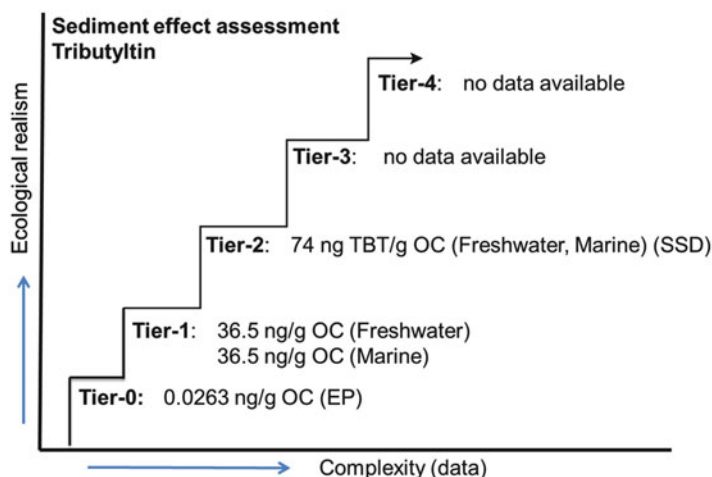
### 10.3.4 Tier-2 Effect Assessment Based on Standard and Additional Test Species for Tributyltin

#### Geometric Mean Approach

Considering the data presented in Table 13, and the criteria for the geometric mean approach mentioned in Sect. 9.4, this approach seems possible only for the 9–10 days  $LC_{50}$  values for the marine amphipods *C. volutator*, *E. washingtonianus*, and *R. abronius*, resulting in a geometric mean  $LC_{50}$  of 150.2  $\mu\text{g TBT/g OC}$  for these marine amphipod taxa. For two other marine taxonomic groups, a single semi-chronic  $LC_{50}$  value is available: for the polychaete *A. brevis* (10 days  $LC_{50}$  of 930  $\mu\text{g TBT/g OC}$ ) and the echinoderm *E. cordatum* (14 days  $LC_{50}$  of 10.5  $\mu\text{g TBT/g OC}$ ). The value for *E. cordatum* is lower than the geometric mean  $LC_{50}$  for marine amphipods, so this value has to be selected for the Tier-2  $PNEC_{\text{sed;ch}}$  derivation according to the geometric mean approach, although only a single value is available for Echinodermata. To derive a  $PNEC_{\text{sed;ch}}$ , an AF of 100–300 (see Table 5) can be applied to the geometric mean semi-chronic  $L(E)C_{50}$  value of the most sensitive taxonomic group. We selected an AF of 300 since the available toxicity data reveal latent effects and hormone-disrupting properties of TBT. Applying an AF of 300 (see Table 5) to the  $LC_{50}$  of 10.5  $\mu\text{g TBT/g OC}$  for *E. cordatum* results in a  $PNEC_{\text{sed;ch}}$  estimate of 0.035  $\mu\text{g TBT/g OC}$  for marine/estuarine benthic organisms. Note that for estuarine/marine benthic organisms this Tier-2  $PNEC_{\text{sed;ch}}$  (based on semi-chronic toxicity data) is somewhat higher than the Tier-1  $PNEC_{\text{sed;ch}}$  value of 0.019  $\mu\text{g TBT/g OC}$  based on semi-chronic toxicity data. Since the Tier-2  $PNEC_{\text{sed;ch}}$  value based on the geometric mean approach is somewhat lower than the Tier-1  $PNEC_{\text{sed;ch}}$  of 0.0365  $\mu\text{g TBT/g OC}$  for sediment-dwelling organisms in estuarine/marine ecosystems derived on basis of chronic toxicity data, the geometric mean approach in this case does not help to refine the effect assessment (Fig. 7).

#### Species Sensitivity Distribution Approach

Table 14 gives an overview of the  $PNEC_{\text{sed;ch}}$  derivation based on the SSD approach and by using the chronic or semi-chronic toxicity values presented in Table 14. Since chronic  $EC_{10}/NOEC$  are available for only seven species, the procedure described in Table 7 was used to estimate the chronic  $NOEC/EC_{10}$  based on chronic  $L(E)C_{50}$  values. To illustrate the SSD approach as recommended in Sect. 9.4, several SSDs were constructed. Two SSDs were constructed with chronic toxicity data, one with nine species (A in Table 14) and the other with eight species (B in Table 14). In addition, three SSDs were constructed with semi-chronic toxicity data for ten species (C in Table 14), nine species (D in Table 14) and eight species (E in Table 14). For all the SSDs constructed and summarized in Table 14, the Anderson-Darling test for normality was accepted at all levels, indicating that



**Fig. 7** Predicted no effect concentration (ng/g OC) for tributyltin for different tiers

the curves fitted the toxicity data well. Figure 6 presents the SSD curve constructed with chronic toxicity data for nine species of benthic freshwater and marine/estuarine organisms (A; upper panel) as well as the SSD curve constructed with semi-chronic toxicity data for ten species (B; lower panel).

The median  $HC_5$  values for tributyltin based on semi-chronic data are in most cases more than a factor 10 higher than the  $HC_5$  values based on chronic data. We proposed that a  $PNEC_{sed;ch}$  can be estimated using the semi-chronic  $HC_5$  by applying an AF according to the criteria mentioned in Table 6, as well as an extra AF of 5–10. Because of the hormone-disruptive properties of TBT we propose to select the extra AF in the high range (10).

The  $PNEC_{sed;ch}$  estimates based on the SSD approach as presented in Table 14 are remarkably similar between procedures that use the same number of species with chronic and semi-chronic toxicity data. For example the procedure using eight species with chronic toxicity data resulted in a  $PNEC_{sed;ch}$  of 0.055  $\mu\text{g TBT/g OC}$ , while the procedure using eight species with semi-chronic toxicity data resulted in a  $PNEC_{sed;ch}$  of 0.048  $\mu\text{g TBT/g OC}$  for freshwater taxa and 0.064  $\mu\text{g TBT/g OC}$  for marine taxa (Table 14). This suggests that the SSD approach as proposed in Sect. 9.4 works well. However, a  $PNEC_{sed;ch}$  preferably should be derived based on chronic toxicity data and a  $PNEC_{sed;ch}$  thus obtained overrules a  $PNEC_{sed;ch}$  derived based on semi-chronic toxicity data. The preferred chronic  $PNEC_{sed;ch}$  of 0.074  $\mu\text{g TBT/g OC}$  is higher the PNEC derived in Tier-0, Tier-1 and in the geometric mean approach in Tier-2 (Table 6).

The data presented in Table 14 also show that the median  $HC_5$  value increases and its confidence interval decreases if a larger number toxicity data is used to construct the SSD. This indicates that it may be rewarding in the Tier-2 effect assessment to generate spiked sediment toxicity data for a higher number of benthic taxa.

**Table 14** Overview of PNEC<sub>sed:ch</sub> values (µg TBT/g OC) for tributyltin derived by means of SSDs constructed with chronic or semi-chronic toxicity data for benthic organisms (see Table 13)

	Endpoints	No of species	No of taxonomic groups	Lower limit HC <sub>5</sub> (µg TBT/g OC)	Median HC <sub>5</sub> (µg TBT/g OC)	Upper limit HC <sub>5</sub> (µg TBT/g OC)	HC <sub>5</sub> /lower HC <sub>5</sub>	AF <sup>a</sup> fresh	AF <sup>a</sup> marine	SSD-PNEC <sub>sed:ch</sub> Fresh (µg TBT/g OC)	SSD-PNEC <sub>sed:ch</sub> Marine (µg TBT/g OC)
A	Chronic	9	7	0.024	0.296	1.145	12	4	4	0.074	0.074
B	Chronic	8	7	0.012	0.220	0.999	19	4	4	0.055	0.055
C	Semi-chronic	10	6	0.334	4.124	17.087	12	4*10 <sup>b</sup>	4*10 <sup>b</sup>	0.103	0.103
D	Semi-chronic	9	6	0.185	3.401	16.556	18	4*10 <sup>b</sup>	4*10 <sup>b</sup>	0.085	0.085
E	Semi-chronic	8	5	0.078	2.424	14.355	31	5*10 <sup>b</sup>	4*10 <sup>b</sup>	0.048	0.061

A: SSD constructed with chronic toxicity data for 9 species as presented in Fig. 6. B: SSD constructed with chronic toxicity data for 8 species similar to those presented in Fig. 6, except *Eohaustorius washingtonianus*. C: SSD constructed with semi-chronic toxicity data for 10 species similar to those presented in Fig. 7. D: SSD constructed with semi-chronic toxicity data for 9 species similar to those presented in Fig. 7 except *Hexagenia*. E: SSD constructed with semi-chronic toxicity data for 8 species similar to those presented in Fig. 7 except *Hexagenia* and *Tubifex tubifex*

<sup>a</sup>For criteria see Table 6

<sup>b</sup>An additional AF of 10 is applied to account for the extrapolation of semi-chronic toxicity data to chronic toxicity data

### 10.3.5 Tier-3 Effect Assessment Based on Micro/Mesocosm Experiments for Tributyltin

Appropriate spiked sediment micro/mesocosm tests could not be found.

### 10.3.6 Conclusions from the Tributyltin Toxicity Data for Benthic Organisms

- Applying the concept of EP to the  $PNEC_{sw;ch}$  (based on water toxicity data for pelagic organisms) results in a conservative estimate of the  $PNEC_{sed;ch;EP}$  (Tier-0) (Fig. 7)
- The chronic NOEC/EC<sub>10</sub> value (spiked sediment test) was lowest for a mollusc, which is in accordance with available toxicity data for water organisms and water exposure tests
- The sediment toxicity data for freshwater and marine arthropods overlap
- The toxicity data for both freshwater and marine benthic organisms can be used to construct an SSD with an appropriate fit
- The  $PNEC_{sed;ch}$  value for tributyltin derived on the basis of the SSD approach is approximately a factor of 2 higher than the Tier-1  $PNEC_{sed;ch}$

## 10.4 Main Outcomes from the Case Studies

In general, it can be concluded that the available sediment toxicity data are limited and the reported measurement endpoints are variable. Sediment toxicity data for freshwater and marine/estuarine benthic organisms often overlapped. Available data were mainly limited to arthropods and were predominantly sub-chronic in nature. For the insecticide chlorpyrifos, however, the focus on benthic arthropods is logical considering its specific toxic mode-of-action and the extensive dataset for water column organism, which indicates that aquatic arthropods are the sensitive taxonomic group

Applying the concept of EP to the  $PNEC_{sw;ch}$  (based on water toxicity data for pelagic organisms) results in a very conservative estimate of the  $PNEC_{sed;ch;EP}$  (Tier-0) for ivermectin and a conservative estimate for chlorpyrifos and tributyltin. For chlorpyrifos, however, by using the higher tier  $PNEC_{sw;ch}$  (on basis of a chronic micro/mesocosm study) in the equation then the Tier-0  $PNEC_{sed;ch;EP}$  resembles the Tier-1  $PNEC_{sed;ch}$  estimate for estuarine and marine species, but is a factor of 2–3 lower than the Tier-1  $PNEC_{sed;ch}$  for freshwater species. Aquatic data can provide good indicators for the most sensitive species group, as was shown for tributyltin, where the chronic NOEC/EC<sub>10</sub> value (spiked sediment test) was lowest for a mollusc, which is in accordance with available toxicity data for water organisms and water exposure tests.

The case studies illustrate that the geometric mean approach is of limited value in the chronic effect assessment for benthic organisms. However, these studies also show that toxicity data for both freshwater and marine benthic organisms can be used to construct an SSD with an appropriate fit. For tributyltin, the  $PNEC_{sed;ch}$  values derived on the basis of the SDD approach are approximately a factor of 2 higher than the Tier-1  $PNEC_{sed;ch}$ .

In microcosms in which the sediment was spiked with ivermectin, the overall NOEC of the nematode community was approximately a factor of 10 lower than the NOEC of the standard test nematode *C. elegans*.

## **11 Main Recommendations for Prospective ERA for Sediment-Bound Organic Chemicals and Outlook**

### ***11.1 Specific Protection Goals***

For benthic organisms we recommend adopting similar specific protection goals as developed for pelagic organisms. For benthic algae, macrophytes and invertebrates we propose to select the population as ecological entity to be protected and the functional group for microorganisms. For benthic vertebrates the ecological entity to be protected may be the individual—to population level.

### ***11.2 Triggers for Prospective Sediment ERA***

We recommend using a combination of triggers for sediment testing based on toxicity, persistence and adsorption. A trigger to request sediment-spiked toxicity testing with benthic organisms is based on the EP approach that uses available toxicity data for pelagic organisms and an extra extrapolation factor of 10 for benthic fauna that consume sediment particles. As a trigger for persistence,  $\log K_{oc}$  is preferred over  $\log K_{ow}$  since  $\log K_{oc}$  is a more direct measure for chemical binding to sediment. Because reported  $K_{oc}$  values may have a high variability, we recommend using the geometric mean value, as  $K_{oc}$  values usually show a log-normal distribution. For a persistence trigger we recommend using e.g. >10 % of the substance present in sediment at or after day 14 in an OECD guideline 308 test or >10 % of the annual dose applied occurring in sediment at the time of maximum  $PEC_{sed}$ .

### ***11.3 Linking Exposure to Effects***

For prospective toxicity testing, we recommend using pre-equilibrated artificial sediment, or when field-collected sediment is used to follow as much as possible the test design as currently proposed in OECD test guidelines with artificial sediment. During testing, we advise to measure chemical concentrations in total sediment (in units of mass of organic chemical per mass of dry sediment) and preferably also in pore water as well as in the overlying water and to measure the organic matter content (%) of the dry sediment. The  $PEC_{sed}$  and the  $PNEC_{sed}$  used in the risk quotient should be expressed in the same unit.

We recommend using a mean or TWA for the duration of the toxicity test if the chemical is not stable. However, if the bioavailable fraction of the compound in the sediment of the laboratory toxicity test decreases faster than that predicted (or measured) for field sediments, it may be appropriate to use the peak concentration in the sediment at the start of the sediment-spiked toxicity test as exposure metric in the effect estimate.

### ***11.4 The Tiered Approach in Effect Assessment***

Using the EP concept and chronic toxicity data for pelagic organisms is a worst case and cost-effective screening-level approach to evaluate the potential risks of sediment exposure to benthic organisms if the taxonomic groups assessed for water ERA overlap with those required for sediment ERA and the organic compounds are not ionizable, perfluorinated alkylated or insoluble. We propose using an extrapolation factor of 10 to derive a  $PNEC_{sed;EP}$  for organisms that ingest sediment particles to account for ingestion and binding to black carbon. It is recommended to verify whether the EP approach and the proposed extrapolation factor can be considered a realistic worst case approach to derive a PNEC for benthic fauna and for different types of organic chemicals.

Since benthic species of freshwater and marine/estuarine ecosystems have many traits in common, we assume that sediment toxicity data for both freshwater and marine/estuarine species can be used in the effect assessment, at least of the taxonomic group occurring in the ecosystem under evaluation.

Ideally, the Tier-1 effect assessment should be based on chronic  $EC_{10}/NOEC$  values for different taxonomic/feeding groups, of which at least two test species—including the most sensitive—are representative for the ecosystem under evaluation (freshwater or marine/estuarine). The Tier-1 PNEC is calculated by selecting the lowest  $EC_{10}/NOEC$  value and the application of an assessment factor of 10. For substances with a specific toxic mode-of-action (e.g. insecticides and herbicides) testing two representative species of the potentially sensitive taxonomic group (s) may suffice.

In the Tier-1 effect assessment based on internationally accepted protocol tests for benthic organisms it should be checked whether the most sensitive taxonomic group for Tier-1 water column organisms is likely represented in the core data set for benthic species. If not, it should be determined whether this taxonomic group is represented in the available additional toxicity data for benthic organisms and whether the quality of this information is high enough for use in the effect assessment.

If sediment-spiked toxicity data for both standard and additional benthic test species are available, a Tier-2 effect assessment might be based on the geometric mean and species sensitivity distribution (SSD) approaches. For the time being, we propose restricting the geometric mean approach for deriving  $PNEC_{sed}$  values on the basis of semi-chronic toxicity data (e.g. 10 days  $L(E)C_{50}$  values), while SSDs can be constructed either with semi-chronic or chronic toxicity data.

For the effect assessment based on the SSD approach we propose—as a minimum—to construct the SSD with toxicity data for eight benthic species representing at least five different taxonomic/feeding groups. However, when the ERA based on water organisms shows that a specific taxonomic group is at least an order of magnitude more sensitive than other taxonomic groups, the eight species should preferably be selected from the sensitive taxonomic group.

Preferably, the SSD should be constructed with chronic  $EC_{10}/NOEC$  data addressing sub-lethal endpoints. However, considering the scarcity of sediment-spiked toxicity data we propose the following. If for an essential taxon, such as the eighth species in the chronic SSD, a valid chronic toxicity value is missing but a valid semi-chronic (e.g. 10 days  $EC_{50}$  value) is available, an appropriate extrapolation factor might be used to derive a “surrogate”  $EC_{10}/NOEC$  value for this species that can be used in the chronic SSD. Furthermore, a  $PNEC_{sed;ch}$  on basis of an SSD fully constructed with (10 days) semi-chronic  $L(E)C_{50}$  values may be used by applying an appropriate extrapolation technique.

We propose microcosm experiments with spiked sediment, in which the colonisation success by benthic organisms is studied, as a possible Tier-3 option. Alternatively, micro-/mesocosm test systems with a well-established aquatic community can be used by spiking the water compartment with the contaminant. The latter experimental design requires a detailed assessment of the dynamics in exposure concentrations in different sediment layers and in the overlying water. Higher tiers should be used to calibrate the lower tiers. Moreover, effect model approaches should be integrated into the effect assessment. We recommend that these models link scenarios from exposure and effect models.

Sediments are often contaminated with a mixture of chemicals. Therefore, future efforts should be made to move from the current ERA, which is based on single substance exposure, to an approach that deals with multiple chemicals. The TKTD approach may be a good tool to deal with multiple exposures. Exposure to multiple stressors requires clear scenarios that combine exposure and ecology related elements (Beyer et al. 2014).

Moreover, the development of novel endpoints/techniques such as genomics, epigenetics, biomarkers, adverse outcome pathways, their ecological relevance and

impact on sediment risk assessment is an important future research activity (e.g. Ankley et al. 2010).

Overall, a holistic approach that combines experimental work and fate and effect modelling is needed to develop better and more cost-effective prognostic tools for sediment risk assessment.

## 12 Summary

Benthic organisms provide important ecosystem services and functions, and should therefore be protected. However, a broadly accepted framework for prospective ERA of sediment-bound organic chemicals is currently lacking. Such a framework requires clear protection goals, evidence-based concepts that link exposure to effects and a transparent tiered effect assessment. SPUs identified based on the ecosystem service concept are microorganisms, benthic algae, sediment-rooted macrophytes, benthic invertebrates and benthic vertebrates for both freshwater and marine sediments, which are similar to SPUs derived for the aquatic system. The proposed SPUs and their specific protection goals should be generally accepted and implemented to operationalize sediment risk assessment schemes.

There is an urgent need for harmonization of data requirements, test protocols and risk assessment frameworks between regulations/directives. The first step is to determine and agree on a set of harmonized triggers for sediment testing. These triggers should consist of a combination of chemical properties and toxicity triggers. When testing is required, sediment-spiked laboratory toxicity tests with pre-equilibrated artificial sediment as described in Diepens et al. (2014b) or field-collected sediment and standard and/or additional benthic test species should focus on long-term tests with chronic endpoints. The range of standard test species for sediment testing currently in use in Europe should be extended with species that differ in taxonomy, feeding traits and ecosystem, such as estuarine and marine species.

When defining guidance for both prospective exposure and effect assessment, chemical, biological, spatial and temporal factors should be taken into account in experimental and model approaches. For fate models there is a need for approaches to translate biodegradation process parameters obtained from lab tests to parameters that are relevant in the field. The development of passive samplers for more classes of chemical can provide more accurate input for such models. For prospective exposure modelling, more realistic exposure models are needed for emerging chemical classes like ionizable organics and polar substances; these models should also take degradation processes into account. Development of realistic exposure scenarios is a prerequisite to successfully apply exposure models.

To correctly link exposure and effect, the ERC for the  $PEC_{sed}$  and  $PNEC_{sed}$  used in the RQ should be expressed in the same type of concentration. Ideally, internal concentrations should be measured during the experiment. As advised in current technical guidelines, chemical concentrations in total sediment (in units of mass of

organic chemical per mass of dry sediment) and preferably also in pore water as well as the concentration in the overlying water and the organic matter content (%) of the dry sediment should be measured. Model approaches may be used to calculate chemical concentrations in environmental compartments in which data is lacking. For exposure in chronic risk assessment, either the  $PEC_{sed;max}$  or  $PEC_{sed;TWA}$  can be used to compare with the  $PNEC_{sed;ch}$ . Guidelines should give a clear and uniform description of the concentration that should be used both in exposure and effect assessment. They should also specify where (organism, water and sediment compartments, sediment layer) and when the exposure concentration should be measured.

For the first step in effect assessment, prior to actual testing, a cost-effective Tier-0 screening based on aquatic toxicity data and EP with an extrapolation factor of 10 that accounts for BC and ingestion is recommended (for benthic fauna only). This approach gives important information on the most sensitive groups and in some cases provides conservative protection levels. The case studies showed that this approach is moderately to very conservative for these chemicals. It is recommended to verify whether the EP approach and the extrapolation factor of 10 can be considered a realistic worst case approach to derive a PNEC for benthic fauna and for different types of organic chemicals.

In the Tier-1 approach to derive a  $PNEC_{sed}$ , spiked sediment laboratory toxicity testing with standard benthic test species and the application of an appropriate assessment factor (AF) is common practice. The size of the proposed AF to be applied depends on the number of available species with chronic and semi-chronic toxicity data and the taxonomy, feeding traits and ecosystem preference of the test species used.

Possible Tier-2 options are the geometric mean and SSD approach. Freshwater, estuarine and marine species can be combined in the Tier-2 approaches. For the time being, we recommend using the geometric mean approach only to conduct effect assessments based on acute/semi-chronic toxicity data (e.g. 10 days  $L(E)C_{50}$ 's) for test species in the same taxonomic group (e.g. benthic insects, crustaceans, oligochaetes or polychaetes). Whether the geometric mean approach can also be applied to chronic toxicity data of the same taxonomic group addressing different measurement endpoints still needs to be investigated. We propose that the SSD approach be used if toxicity data are available for eight or more benthic species. The SSD curve should be constructed with either chronic or acute/semi-chronic toxicity data. The derivation of a  $PNEC_{sed}$  based on the SSD approach is done by applying an appropriate AF to the  $HC_5$ . We propose basing the size of this AF on the number of species and quality of the available toxicity data used in the SSD. Ideally, the proposed assessment factors to derive PNECs for the Tier-1 and Tier-2 should be calibrated/validated with results of semi-field and field tests.

We propose microcosm experiments with spiked sediment, in which the colonisation success by benthic organisms is studied, as a Tier-3 option, although only limited experience is available with these types of tests. Alternatively, micro/mesocosm test systems with a well-established aquatic community can be used by spiking the water compartment with the contaminant. Effect models can be used

to complement experimental data to link exposure to effect at different levels of biological organization and at different spatial and temporal scales. In a regulatory context, scenarios relevant for aquatic ecosystems in different EU Member States using patterns of organic chemicals that integrate exposure and effects are a prerequisite. An important future research activity, therefore, would be to develop and link scenarios in exposure and effect models that include the sediment compartment and selected standard and appropriate vulnerable benthic species.

To evaluate the consistency of the tiered approach as described in this paper for the effect assessment of sediment exposure, the higher tiers (e.g. spiked sediment microcosm tests) should be used to calibrate the lower tiers. However, hardly any data for calibration of the tiered approach is currently available. Moreover, there is an urgent need to derive tiered ERA schemes for vertebrates and microorganisms, as insufficient data, methods and experience are currently available to do so. Also the development of novel endpoints/techniques such as genomics, epigenetics, biomarkers, adverse outcome pathways, their ecological relevance and impact on sediment risk assessment is an important future research activity.

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## Appendix 1: List of Workshop Participants

The workshop “Prospective Sediment Risk Assessment” was held on 24-02-14 in Wageningen, the Netherlands

Name	Surname	Affiliation	Country
Gertie	Arts	Alterra Wageningen UR	The Netherlands
Hans	Baveco	Alterra Wageningen UR	The Netherlands
Theo	Brock	Alterra Wageningen UR	The Netherlands
Eric	Bruns	Bayer	Germany
Noël	Diepens	Wageningen University	The Netherlands
Andreas	Focks	Alterra Wageningen UR	The Netherlands
Malyka	Galay-Burgos	Ecetoc	Belgium
Mick	Hamer	Syngenta	United Kingdom
Bruno	Hubesch	CEFIC	Belgium
Bart	Koelmans	Wageningen University	The Netherlands
Stuart	Marshall	Unilever	United Kingdom
Andreu	Rico	Wageningen University	The Netherlands
Mauricio	Rocha Dimitrov	Wageningen University	The Netherlands

(continued)

Name	Surname	Affiliation	Country
Cor	Schipper	Deltares	The Netherlands
Livia	Sidney	Wageningen University	The Netherlands
Hauke	Smidt	Wageningen University	The Netherlands
Ariadna	Szczybelski	Wageningen University	The Netherlands
Paul	Van den Brink	Alterra Wageningen UR/Wageningen University	The Netherlands
Martine	Van den Heuvel-Greve	IMARES Wageningen UR	The Netherlands
Bert	Van Hattum	VU University Amsterdam	The Netherlands
Erik	Verbruggen	RIVM	The Netherlands
Arjan	Wijdeveld	TU Delft	The Netherlands

## Appendix 2: List of Abbreviations

AF	Assessment factor
ASTM	American Society for Testing and Materials
BSAF	Biota sediment accumulation factor
C <sub>pw</sub>	Concentration of the chemical in pore water
C <sub>sed;oc</sub>	Concentration of the chemical in the sediment per unit mass of organic carbon
ECHA	European Chemicals Agency
EC <sub>x</sub>	Effect concentration x percent
EF	Extrapolation factor
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EP	Equilibrium partitioning
EPA	United States Environmental Protection Agency
ERA	Environmental risk assessment
ERC	Ecotoxicologically relevant concentration
GIS	Geographic information system
HC <sub>5</sub>	Hazardous concentrations to 5 % of the test species
IBM	Individual-based modelling
ISO	International Organization for Standardization
K <sub>d</sub>	Sediment-water partitioning coefficient
K <sub>oc</sub>	Organic carbon-water partitioning coefficient
K <sub>ow</sub>	Octanol-water partition coefficient
LC <sub>x</sub>	Lethal concentration x percent
NOEC	No effect concentration
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental exposure concentrations
PEC <sub>sed</sub>	Sediment exposure estimates

(continued)

PEC <sub>sed;max</sub>	Sediment exposure estimates based on peak concentration
PEC <sub>sed;TWA</sub>	Sediment exposure estimates based on time-weighted average concentration
PNEC	Predicted no effect concentration
PNEC <sub>sed</sub>	Effect estimates for sediment-dwelling organisms
PNEC <sub>sed;ch</sub>	Predicted no effect concentration for sediment based on chronic toxicity data
PNEC <sub>sed;ch;EP</sub>	Predicted no effect concentration for sediment based on chronic toxicity data calculated by equilibrium partitioning
PNEC <sub>sw;ch</sub>	Predicted no effect concentration for surface water based on chronic toxicity data
PPP	Plant Protection Products
QSAR	Quantitative Structure Activity Relationship
QSPR	Quantitative Structure Property Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	Risk Quotient ( $RQ = PEC/PNEC$ )
SPU	Service providing units
SSD	Species sensitivity distribution
TKTD	Toxicokinetic toxicodynamic
TWA	Time-weighted average
VICH	Veterinary International Conference on Harmonization

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# How Important Is Research on Pollution Levels in Antarctica? Historical Approach, Difficulties and Current Trends

Małgorzata Szopińska, Jacek Namieśnik, and Żaneta Polkowska

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## List of Acronyms

AFS	Atomic fluorescence spectrometry
CCAMLR	The Commission for the Conservation of Antarctic Marine Living Resources
CD	Conductometry detector
CFCs	Chlorofluorocarbons
CHLs	Chlordanes
COMNAP	Council of Managers of National Antarctic Program
CZE	Capillary zone electrophoresis

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M. Szopińska • J. Namieśnik • Ż. Polkowska (✉)  
Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology,  
11/12 Narutowicza St., Gdansk 80-233, Poland  
e-mail: [szopinska.malgorzata@gmail.com](mailto:szopinska.malgorzata@gmail.com); [chemanal@pg.gda.pl](mailto:chemanal@pg.gda.pl); [zanpolko@pg.gda.pl](mailto:zanpolko@pg.gda.pl)

DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethane
DDT	Dichlorodiphenyltrichloroethane
DLCs	Dioxin-like compounds
ECD	Electron capture detector
GC-MS	Gas chromatography–mass spectrometry
GPC	Gel permeation chromatography
HBB	Hexabromobenzene
HCB	Hexachlorobenzene
HCFCs	Hydrochlorofluorocarbons
HCHs	Hexachlorocyclohexanes
HPLC	High-performance liquid chromatography
IC	Ion chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IDMS	Isotope dilution mass spectrometry
LC-MS/MS	Liquid chromatography with tandem mass spectrometry detection
LOD	Limit of detection
LOQ	Limit of quantification
LRAT	Long-range atmospheric transport
NNA	Neuron activation analysis
OC	Organochlorine compound
OCP	Organochlorine pesticides
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzodioxins
PCDFs	Polychlorinated dibenzofurans
PCNs	Polychlorinated naphthalenes
PFBS	Perfluorobutane sulfonate
PFHxA	Perfluorohexanoic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
POPs	Persistent organic pollutants
QqQ	Triple quadrupole
SFC	Supercritical fluid chromatography
SML	Surface microlayer
TC	Thermal conductivity
TLC	Thin-layer chromatography
TOC	Total organic carbon
TOF	Time of flight analyzer
XRF	X-ray fluorescence

## Highlights

- Scientific interest in the issue of presence of pollutants in Antarctica steadily increasing since 1960.
- In various samples from Antarctica a variety of harmful pollutants were identified.
- The analytic methods, which are dedicated to determine POPs and metals in different matrices, need to be developed.
- Antarctica is prone to storage of POPs, which may also undergo remobilization processes.

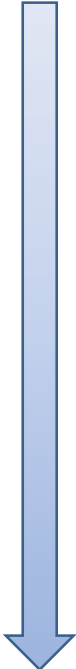
## 1 Introduction

The term “*Antarctica*” is used to define both the Antarctica continent itself as well as the Southern Ocean that surrounds the continent and the islands of this ocean. Antarctica is the most isolated continent; however, its specific location does not protect this area from negative impact of human activities (Aronson et al. 2011). A broad belt of the Southern Ocean’s waters constitutes a barrier, which makes it difficult to transport pollutants this way. Therefore, volatile and semi-volatile chemical compounds may reach Antarctica together with air masses moving in this direction (long-range atmospheric transport—LRAT) (Corsolini 2009). However, more and more attention has been recently paid to the determination of the size of the locally emitted contamination impact on Antarctic environment (Bengtson Nash et al. 2011).

The first information on the occurrence of anthropogenic pollutants comes from the 1960s and it pertains to the presence of dichlorodiphenyltrichloroethane (DDT) in sea organisms (Bargagli 2008). Further research pertained to chemical composition of samples of water, snow and ice and it included metal and ion determination. Since the 1960s, research on the presences of pollutants from the group of persistent organic pollutants (POPs), e.g. hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), aldrin, endrin, heptachlor and other pollutants in samples of living and non-living matter collected in Antarctica has been undertaken (Bargagli 2008; Corsolini 2009).

However, due to difficult climatic conditions, research pertaining to pollution analysis in this area was conducted on irregular basis. In recent decades, there has been a growing interest in the problems of pollutants present in samples from various elements of Antarctica’s ecosystem. Figure 1 presents milestones of events influencing the development of research on Antarctica (including ones influenced development of chemical research).

Urbanised areas, especially those with intensive agriculture, as well as tropical and subtropical regions, where spraying is used for disease vector control, are the main sources of POPs and heavy metals in the Southern Hemisphere. The increase in the usage of many POPs has been observed in the 1990s in Asian countries and Southern Pacific islands (Bargagli 2008). Some large amounts of polychlorinated biphenyls (PCBs) used in older electrical devices were also deposited as landfill in some developing countries. The heaviest user of DDT, toxaphene and lindane, has



1773	The first expedition of Captain James Cook to Antarctica
1897/98	A Belgian expedition was the first to spend winter in Antarctica
1882/83	First International Polar Year
1904	The first all-year Orcadas polar station was established by Argentinian Scientists
1932/33	Second International Polar Year
1957/58	International Geophysical Year
1958	Establishment of the Scientific Committee on Antarctic Research (SCAR)
1959	Signature of the Antarctic Treaty by 12 member states (at present ratified by 43 states)
1960	First information about the occurrence of pollutants in marine organisms (DDT)
1964	Agreed Measures for the Conservation of Antarctic Fauna and Flora, Antarctica Specially Protected Areas (ASPAs)
1972	The Convention for the Conservation of Antarctic Seals (CCAS)
1980	The Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR)
1988	Establishment of the Council of Managers of National Antarctic Programs (COMNAP)
1991	Protocol on environmental protection to the Antarctic Treaty (entered into force in 1998)
2007/08	Fourth International Polar Year

**Fig. 1** Milestones of events connected with the development of Antarctic research (Köler 2013; SCAR Information; Dastidar and Ramachandran 2008; Dodds 2010)

historically been in South America. A comprehensive report by UNEP in 2002 gives more precise data on air levels of POPs in the Southern Ocean and Antarctica (Bargagli 2008).

A critical comparison and discussion of results of the research conducted over decades is not easy, as over a period of more than 50 years, methods and techniques used for research have undergone continuous changes. Moreover, while conducting research on such a complex ecosystem, it is necessary to frequently verify any possible changes by comparing the data acquired during different research projects and at different times. However, this task often cannot be practiced as the results may be achieved with the use of analytical techniques which present extremely different degrees of accuracy and sensitivity (Magi and Tanwar 2014).

The study presents information on the dynamics of the development of polar research (covering main groups of pollutants) both in terms of its methodology and the scope of research on Antarctica (diversity of tested samples and analytes) conducted over the past decades by members of teams working at polar research stations.

## 2 The Presence of Pollutants in Antarctica's Environment

Polar ecosystems consist of several key species. Mutual relationships between individual elements of the environment are closely connected; therefore, the presence of pollutants in one of elements of the ecosystem may have a significant

influence on the functioning of the other ones. To become familiar with the influence of pollutants on the functioning of Antarctica's ecosystem, research is conducted on both abiotic and biological samples.

## **2.1 *Abiotic Environment***

Abiotic environmental media (fresh water and seawater, precipitation, glaciers, soils, etc.), as well as all processes and phenomena connected with changes occurring in individual elements of the environment (meteorological, geological, geochemical processes, etc.), play a significant role in transporting pollutants in Antarctica (Cipro et al. 2012). Elements of abiotic environmental media, such as snow, glaciers and polar catchment areas are sources of water for all organisms living in Antarctica. Antarctica's ecosystem has a very simple structure, therefore, even a small amount of pollution present in abiotic elements of nature may constitute a significant hazard for any individual plant and animal species because of absence of advanced detoxification mechanisms (Bengtson Nash et al. 2011).

### **2.1.1 Air**

The atmosphere plays an important role in transport of pollutants to polar areas. Over the past decade, a range of research has been conducted to determine mechanisms, which contribute to the presence of pollutants in Antarctica, as well as to distinguish between local sources of pollution and long-range atmospheric transport.

Information about Antarctica's air pollutants mostly comes from research conducted during cruises near Antarctica (Bengtson Nash et al. 2011) and is predominantly based on short-term (weeks–month) atmospheric monitoring (Kallenborn et al. 2013). Some of these data have been included in the assessment of global distribution of numerous POPs. However, due to the limited number of samples and non-continuous measurement periods, it is difficult to compare the results of air sample research conducted in Antarctica with the results of sample research from the Arctic region. A long-term atmospheric pollution monitoring in the polar regions is a significant scientific tool for assessing anthropogenic influences on the environment on a global scale. It enables the control or even changes of international legal regulations (Kallenborn et al. 2013).

The results of research on long-term monitoring of POPs were published in 2013 and focused on the concentrations of long-range transported contaminants (POPs) in the Antarctic environment. The research has revealed that the atmospheric long-range transport of polluted air masses is considered as the main source for the POPs monitored at Norwegian Troll station in Dronning Maud Land (Kallenborn et al. 2013). In the discussion about the presence of more volatile substances in Antarctica, as a source of it, long-range atmospheric transport is considered, while the presence of less volatile substances, which occur occasionally in Antarctic's air,

may rather indicate influence of local sources (Kallenborn et al. 2013). A particular impact of local sources is shown in the analysis of compounds from the polybrominated diphenyl ethers (PBDEs) group. Due to the fact that neither plastics nor PBDE manufacturing occur in Antarctica, the substantial indoor PBDE residues are likely to originate from losses of imported flame retarded plastic and electronic products. There are plenty of electronic devices in the research stations, but at the same time there is not much space for them. Moreover, the material transport to Antarctica is expensive (Hale et al. 2008). The first atmospheric measurement, which was constructed as a part of a new continuous monitoring effort, was presented in one of Australia's all-year research station—Casey Station (66°17' S 110°3' E). The results suggest a potential local source of the currently produced, involatile, decabrominated PBDE congener 209, which contributes to PBDE profiles in all the samples (Bengtson Nash et al. 2011).

These discussions prove that it is necessary to take additional precautions in order to stop further deterioration of the pristine air status in Antarctica caused by the human presence in this region.

### 2.1.2 Snow and Ice

In polar areas chemicals like POPs have been observed in seasonal snowpack and in older layers of firn and ice, providing accumulation time series (Herbert et al. 2006b).

During long-range atmospheric transport, pollutants may undergo decomposition and deposition processes, depending on the physicochemical properties of individual compounds.

The mechanisms of exchange of trace organic contaminants between the atmosphere and snow (both falling snow and standing snowpack) depend on the major processes like scavenging (vapour and particle) by falling snow, vapour sorption/desorption to the snow's surface, and diffusion of chemicals both into and out of the snowpack (Herbert et al. 2006b). These processes dictate the quantities of chemical compounds available to meltwater and in deeper areas (permanent snow and ice). Additionally, processes occurring after deposition, e.g. snow settling (fresh snow is gradually transformed into firn and then in a glacier layer, the volume of which becomes gradually reduced) are of importance. The snow-settling process is the first stage, during which compounds, e.g. from the polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) group, are adsorbed on snowflakes. These compounds due to their physicochemical properties are classified as semi-volatile compounds, may become released back to the gaseous phase during seasonal snowmelt or diffused into deeper snow layers (Wania 1997). This process *inter alia* depends on solubility (concentration of a given compound), the snow-air partitioning properties and the temperature gradient. The snow-air partitioning properties not only depend on the vapour pressure but also on the surface properties of the snow flakes/snow pack. These properties largely determine the sorption and diffusion processes (Herbert et al. 2006b).

Based on experimental diffusivities for a volatile tracer of sulfur hexafluoride in snowpack it was concluded that in the low-wind (up to 3 m/s) scenario the migration of sulfur hexafluoride in the snowpack can be largely attributed to diffusive transport, while at high wind speeds (up to 9 m/s) the chemical migration is largely due to advective transport (Albert and Shultz 2002). Snow and firn metamorphism processes depend on the temperature fluctuations. Grain growth may occur, which, in turn, increases the firn permeability. As a result of global migration of a broad range of compounds towards higher latitudes, they become accumulated in polar regions (Kozak et al. 2013). Systematic compound accumulation contributes to the formation of a pollutant reservoir. A large part of the pollutant load is stored in snow and ice. Chemical compounds, which may be trapped in polar areas, can constitute a long-term hazard due to the possibility of their subsequent release into the environment—the so-called reemission into the environment may occur (Herbert et al. 2006a). Quantities of pollutants released during the spring snowmelt could have significant influence on the quantities of pollutants present in both freshwater and marine system (Herbert et al. 2006b). This hypothesis is named “spring pulse” and currently researchers are working on the creation of snowmelt models concerning quantitative transport of pollutants from snow to other abiotic environmental media (Burniston et al. 2007; Herbert et al. 2006b; Wania et al. 1999).

### 2.1.3 Soil and Permafrost

For the study of air transported pollutants, soil samples are worthy of note materials because of their direct contact with the atmosphere. Antarctica’s soil may become polluted as a result of wet and dry deposition (LRAT) and accidental release of pollutants into the environment (oil spills) (Curtosi et al. 2007; Webster et al. 2003; Aisable et al. 2004).

The concentration limits of compounds in soil depend on the type of soil. Antarctica’s soil variability is mainly due to parent material, differences in land-surface age (range: from a few thousand to millions of years), topographic position and local climate (Aisable et al. 2004).

In general approach to the presence of pollutants in soil, permafrost and an active soil layer play an important role in migration of compounds in soil (Curtosi et al. 2007). An active soil layer and permafrost presence is a unique characteristic of polar areas. It is known that repeated freeze/thaw cycles occur in areas with an active layer of permafrost, as a result of which soil particles may undergo a slow process of screening. Small particles may migrate from the surface layer into deeper layers, while stones have a tendency to migrate from deeper layers to the surface. Pollutants are adsorbed mostly from the surface of particles with a smaller diameter. Research results show that the percentage (quantity) of small particles and their dynamics in the soil matrix are the key factors in determining the fate and degradation of pollutants, e.g. PAHs in Antarctic soil. In this way, thawing of the upper layer of the permafrost, which may be caused by global warming, will have widespread influence on the distribution of pollutants in this environment (Curtosi et al. 2007).

### 2.1.4 Catchment Areas

There are lakes and small streams, which thaw in the summer in small areas of Antarctica which are free from ice. Open water lakes in Antarctica are very rare due to low temperatures. However, the accumulation of pollutants also occurs in lakes and lake sediments. Much higher concentrations (as compared to concentrations of the same analytes in soil samples) of some compounds, e.g. HCH in lake sediments are probably determined by the nature of Antarctic lakes. Antarctic's lakes are formed from melting ice water, which is rich in atmospheric particles (trapped in it during formation) (Fuoco et al. 2009a; Vandal et al. 1998).

Another factor, which influences the level of pollutants in freshwater environment, is the transport of persistent chemicals by seabirds biovector. Higher concentration of POPs has been recorded in aquatic organisms from a seabird-affected lake. This is a proof that seabird-transported contaminants have been entering freshwater and thereby local food webs (Michelutti et al. 2010; Xie and Sun 2008). As long as detailed mechanism of pollution transfer by seabird's vectors are not widely described, further researches should be applied in this direction.

### 2.1.5 Ocean, Seas and Bottom Sediments

Oceans and seas plays a significant role in the circulation and removal of pollutants. Within Antarctica, the Antarctic Convergence Zone (also called the Antarctic Polar Front) is distinguished. It runs between 47°S and 62°S. It separates cold and less saline Antarctic waters from subantarctic waters. The zone may be the barrier for pollutants transported by sea (Bengtson Nash et al. 2011).

Relatively much attention was devoted to research targeted at estimating the degree of exchange of pollutants between the seawater surface (inter-phase) and the atmosphere and the role of seawater in the process of transporting chemical compounds to polar regions. The sea surface consists of layers, out of which the sea surface microlayer (SML) has been researched most broadly (0.1–0.001 mm). This is a place where pollutants, atmospheric particles and microorganisms accumulate. However, the majority of research projects focusing on measurements of pollutant content in SML samples were conducted using samples collected in coastal environments. There is very little data from open ocean samples (Fuoco et al. 2009a).

Another element of abiotic environmental media in the pollutant transportation process is bottom sediments. More hydrophobic organic compounds may undergo sorption on solid particles and microorganisms. Dead particles of organic matter and solid particles settle on the bottom and, thus, pollutants adsorbed on them accumulate in bottom sediments (Boutron et al. 1990). Pollutants present in bottom sediments may be re-emitted as a result of activity of bottom organisms and ocean currents. Thus, the bottom sediments can become secondary source of pollution.

## 2.2 *Biotic Environment*

Anthropogenic pollutants have an adverse effect on living organisms. Antarctic biota (e.g. seals and penguins) are particularly sensitive to contaminants. The natural stress on wildlife in extreme polar environments is often more severe than in temperate regions. Hence Antarctic species can be more vulnerable to the effects of pollutants in comparison with species which come from temperate regions (Schiavone et al. 2009a). Moreover, due to very simple structures of polar ecosystems, relationships between individual organisms are important in terms of pollution transfer. Mutual connections between individual species determine the way, in which pollutants are transported (Cipro et al. 2012).

### 2.2.1 *Plants*

Mosses and lichens are the main components of the terrestrial flora of Antarctica's ecosystem. Bryophytes are predominantly useful for monitoring the atmospheric pollution (metals and organochlorine compounds) because they have no protective waxy cuticles and no root system (Borghini et al. 2005). The content of pollutants present in samples of these plants largely depends on precipitation. Thus, they can play a very important role of biomonitors, i.e. indicators of long-term pollutant deposition (Fuoco et al. 2009a).

As mentioned above, pollutants present in the air may undergo dry or wet deposition, thus getting into Antarctica's environment. Plants absorb pollutants from the atmosphere (through their above-ground parts, especially leaves) or/and from the soil (through the roots). For compounds with strong hydrophobic properties, transport through solids seems to have little significance. Literature data may be the basis for concluding that the main mechanism of collecting pollutants from the environment is absorption from the surrounding air into the leaf surface of pollutants in the gaseous phase or the solid phase (through particles settled on plant surfaces) (e.g. Borghini et al. 2005; Mão de Ferro et al. 2014; Poblet et al. 1997; Wu et al. 2014; Yogui and Sericano 2008; Yogui et al. 2011). Pollutants get into plants through stomata or leaf epidermis. Furthermore, the process of "assimilating pollutants" into plants is influenced by a range of physicochemical factors (e.g. partial pressure of water vapour, the numerical value of the octanol/water partition coefficient and the water/octanol partition coefficient), environmental factors (e.g. the temperature, precipitation, wind speed) and plant properties (e.g. the species, fat content, leaf morphology) (Yogui and Sericano 2008; Yogui et al. 2011).

### 2.2.2 *Crustaceans, Benthic Organisms and Fishes*

Antarctica's ecosystem has a very simple structure. Organisms at higher levels of the trophic chain depend on several key species, such as the Antarctic silverfish (*Pleuragramma antarcticum*) and the Antarctic krill (*Euphausia superba*). The

Antarctic silverfish and the Antarctic krill are the main sources of food for many maritime species of birds and mammals. As a result of the mutual relationship between the size of the krill and silverfish populations and the size of the populations of other species, a decrease in the krill and silverfish population size may have a negative impact on the entire environment of Antarctica's marine ecosystem (Corsolini et al. 2002b). As a result of close relationships between individual species, POPs are present in every level of the trophic chain (Corsolini et al. 2002b). The phenomenon of biomagnification plays a more important role than bioaccumulation itself in the case of Antarctic fish. Lower pollutant concentrations are observed in samples of fish, for which krill is the staple food. Values of harmful compound concentrations increase if invertebrates or other fish are the main source of food (Weber and Goerke 2003).

In pelagic fish a downward trend in concentrations of some persistent organic pollutants (e.g. HCB, dieldrin) is visible (Van den Brink et al. 2011). It contrasts distinctly with steady or increasing concentrations levels in benthic organisms. Transfer of contaminants between Antarctic pelagic and benthic food webs is associated with seasonal sea-ice dynamics and thus with different climatic conditions. This fact may hinder the predictability of future trends of emerging compounds in the Antarctic ecosystem (e.g. the brominated compounds). The discrepancy in trends between pelagic and benthic organisms still remains the question whether the total environmental burden of contaminants in the Antarctic ecosystem is declining or increasing (Van den Brink et al. 2011).

### 2.2.3 Seabirds

Marine birds are another link in the food chain, where penguins constitute the most numerous group. They belong to key-species in Antarctica's ecosystem. Penguins feed mainly on krill and also on fish (depending on krill's accessibility). Researchers have reported that predators may be a sink for chemicals (special for volatile and toxic ones) and this may pose an important environmental problem (Corsolini et al. 2007).

Penguins (Adèlie and Emperor) spend their whole life in the Southern Ocean, while marine bird species, such as migrating snow petrel, south polar skua, brown skua are species migrating all over Antarctica. In both cases, results of samples researched from these species could reflect the condition of their ecosystems (Corsolini et al. 2011). The aforementioned bird species rely on all krill species and the Adèlie penguin eats the most krill (Corsolini et al. 2011). The Emperor penguin also eats a lot of fish as well as crustaceans and cephalopods. The south polar skua feeds on penguins' eggs and chicks and it also eats Antarctic silverfish krill (over 80 %). In the nesting season, on the other hand, skuas depend on food found on land. The brown skua also relies on sea food (Corsolini et al. 2011). Moreover, the research results concerning detection of POPs in seabirds' eggs (including penguin and south polar skua eggs) proved the transfer of POPs from mothers to eggs (Corsolini et al. 2002a).

The most important link between Antarctic marine, freshwater and terrestrial ecosystems constitutes seabirds. In fact, they maintain the development of terrestrial flora due to the high amount of nutrients deposited by seabirds on the land (e.g. by guano). Seabirds usually transport loads of pollution. Unfortunately, endocrine mechanisms are still poorly investigated in free-living organisms, despite the fact, that contaminants have endocrine disrupting properties. In the scientific literature there is surprisingly only few data on the effect of age on contaminant levels, despite the fact that long-lived organisms are thought to be highly sensitive to pollution. Therefore, it is not clear if seabirds accumulate POPs with increasing age (Tartu et al. 2015).

Comparing research results concerning pollution in birds' tissues from other areas of the world, shows that POPs concentrations in penguins are relatively low (Corsolini et al. 2007). In relation to species and sex, different chemical accumulation patterns are observed. Penguins are showing low detoxifying capacities and therefore studies on their xenobiotic metabolism should be carried out (Corsolini et al. 2007).

#### 2.2.4 Marine Mammals

During the evaluation of contamination presence in the marine mammals' tissues scientists should bear in mind the migratory habits of these organisms. Some species of marine mammals (including cetaceans) exist in Antarctica' seawaters in summer time and then go northward during winter, while other species, e.g. some seals, spend their entire life cycles in the Southern Ocean and on the Antarctic coasts. In migrating organisms what may affect the amount of pollution in Antarctic organisms is the forage or breed during summer, as well as exposition to pollutants in more contaminated areas during winter. Species and individuals staying in anthropized areas during migration contribute to greater exposure to contamination compared with those that stay in Antarctica all year round. Furthermore, pollution (like POPs) accumulation in marine mammals depends on some other factors including metabolism (Corsolini 2009).

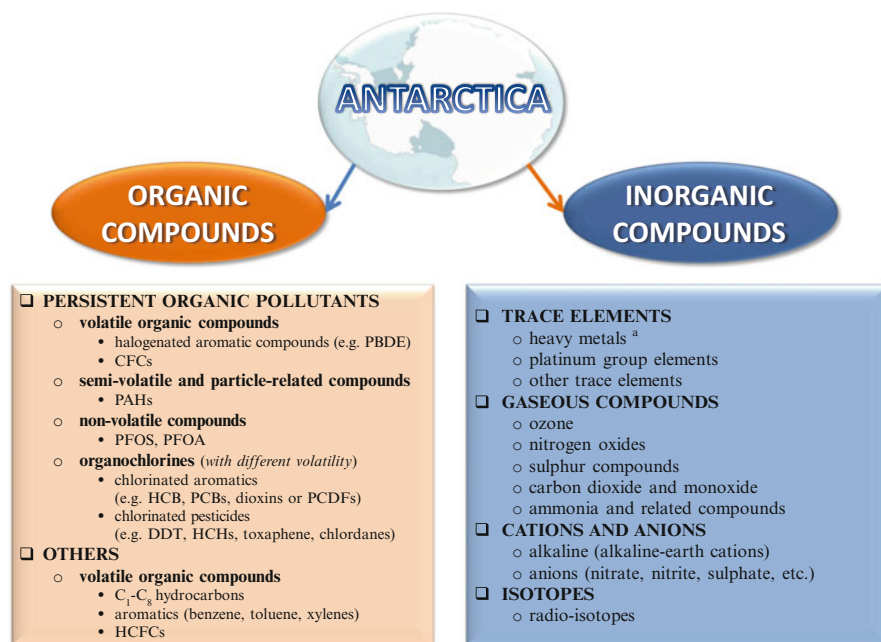
Marine mammals differ from the land ones with a high lactation transfer of all lipophilic substances (including pollutants) to young animals (Schiafone et al. 2009a; Trumble et al. 2012). This mostly results from an increased fat content in the mother's milk (Schiafone et al. 2009a). For cetacea and pinnipeds a vast majority (approx. 90 %), of the total amount of chloroorganic pollutants occurring in newborns are transferred in the mother's milk (Cipro et al. 2012). Due to the position of mammals in the trophic chain of the marine environment, a relatively long life and an increased demand for energy, the pinniped species can be treated as an indicator (reference) species for the examination of harmful effects of pollutant bioaccumulation in organisms (Cipro et al. 2012).

Marine mammals have been exposed also *inter alia* to heavy metals. Scientists are devoting particular attention to mercury because of its toxicity as well as the fact that it is widespread within the environment, and can be biomagnified in marine food chains. Very important is also the fact that Hg is available mainly because of human activities (e.g. Jerez et al. 2011). However, data of concentrations of Hg in seals and other vertebrates of Antarctica's are sparse (Szefer et al. 1993). Moreover, most of the attention in marine mammals' research is devoted to the identification of organic contaminants. Some reports lead even to observation of an increasing trend of PCBs and chlorinated pesticides: HCB, HCHs, chlordanes (CHLs), DDTs in minke whales (*Balaenoptera bonaerensis*) feeding on Antarctic krill between 1984/1985 and 1992/1993 (Aono et al. 1997). Concentration of DDTs, PCBs and HCB have been reported in various species of marine mammals during last decades. However, data on the presence of other POPs (including new emerging ones, like poly- and per fluorinated organic compounds (PFCs)), even if it was reported in oceanic and lake water samples (Cai et al. 2012), in marine mammals tissues are still scarce (Corsolini 2009).

Only a few of the hundreds of thousands of different industrial chemicals produced on a world scale have been studied and reported in the Antarctic environment. Antarctica's trophic chains are relatively simple and short and therefore understanding the detailed information on the levels of pollutants in different parts of the environment (including abiotic part) is very important. Animals at the top of the food webs depend on a few key species. Therefore affecting one of these key species could have a devastating impact on the whole ecosystem.

### 3 Types of Pollutants Present in Antarctica's Environment

Anthropogenic pollutants in Antarctica may come from global (LRAT) and local sources. Global sources include industrialised sites situated all over the Southern Hemisphere, from which pollutants are transported to Antarctica by various routes (Bargagli 2008). Local sources, on the other hand, include, amongst other things, scientific activities which are connected with the use of waste incineration plant, fuel consumption, sewage production, developing tourism and related intensification of ship transport (Cincinelli et al. 2009). The most polluted areas include areas around historic bases and polar stations where soil is often polluted by fuel remains, solid waste and household sewage (Negri et al. 2006; Webster et al. 2003). Anthropogenic pollutants are present in various elements of the environment in Antarctica. Because of their specific (also hazardous) properties POPs and heavy metals are described in this article in detail. However, authors do not include any chapter about general sources, properties and toxicity of pollutant groups determined in various types of samples collected from the Antarctic environment. This information has been given in other literature sources (e.g. Aisable et al. 2004; Borghesi et al. 2008; Cincinelli and Dickhut 2011; Corsolini 2009; Fuoco et al. 2012; Houde et al. 2011; Ma et al. 2014; Planchon et al. 2002; Vecchiato et al. 2015).



<sup>a</sup>including mercury itself and its transformation products (e.g. methylmercury-organic compound)

**Fig. 2** The group of chemical compounds identified in Antarctica

Despite the fact that environmental studies represent only a small part of scientific research in Antarctica (Magi and Tanwar 2014), polar explorers are increasingly also interested in chemical research. Figure 2 shows a group of chemical compounds that are of interest to researchers in Antarctica (after Walton et al. 2001).

In the discussion on the presence of organic compounds in the Antarctic environment, scope of interest is mainly focused on POPs like HCB, PCBs, DDTs, PBDE and PAHs. Over the past decades, sporadic research also pertained to identification and determination of compounds such as: CHL, dioxins, dioxin-like compounds (DLCs), PFCs, pesticides (dieldrin, mirex, heptachlor, endosulfan), aliphatic hydrocarbons, n-alkanes and cumulative parameters such as total organic carbon (TOC) in various environmental samples.

The presence of metals in remote Antarctica is not, as it was thought previously, limited only to lead and copper, but also other includes metallic elements, metalloids and radioactive elements, such as: V, Cr, Mn, Zn, Co, Ag, Cd, Ba, Bi, U, Pt, Ir, Rh, Mo, Tl, As, Sb (Hong et al. 2012; Soyol-Erdene et al. 2011).

## 4 Detailed Information Pertaining to Analytical Research in Antarctica

For a long time Antarctica was not available to scientists mainly because of the specificity of its location. Initial research was aimed at getting to know geological properties of the area. With time, also meteorological, magnetic and botanic research was undertaken and in recent years, chemical research was also conducted. The implementation of this research requires enormous involvement and determination on the part of scientists, mostly due to very difficult weather conditions (Köler 2013).

### 4.1 *History of Research on the Chemical Composition of Samples from Antarctica*

Research conducted in Antarctica has always been interdisciplinary. One area of research includes actions connected with determining the chemical composition of biotic and abiotic samples. Initially, it was research using classical analytical techniques; however, the scope of determined compounds has been expanded over time. Table 1 presents the historical calendar pertaining to the development of the scope of analytical research of the Antarctic environment conducted up to the end of 1989.<sup>1</sup>

### 4.2 *Pollution Concentration Levels Over Decades*

The scope of analytical researches conducted over individual decades is differentiated both in respect of the place of research and types of samples and analytes which are determined in them. Monitoring of the environment allows for reliable observation changes and information contained in publications pertain to individual parts of the Antarctic ecosystem and various groups of pollutants. At present, scientists devote a lot of attention to research on pollutant levels in Antarctica's environment; however, there are still areas which have not been researched in this respect. Figure 3 shows the percentage of most commonly studied regarding the presence of contaminants in the environment of Antarctica up to end of 2014.

In this article, the authors pay particular attention to the research on determination of persistent organic compounds and heavy metals in different samples from

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<sup>1</sup> Analytical research is applied in Antarctic since the early 1960s. (that gives 55 years period of research). Hence authors decided to designate first three decades as historic ones (up to the end of 1989). During this period only few data has been published, hence this period is three decades long.

**Table 1** Historical calendar pertaining to the development of the scope of analytical research of the Antarctic environment conducted up to the end of 1989

Year	Sampling place	Analytes or subject of research	Type of sample	The analytical techniques	Literature
1960	n/a <sup>a</sup>	DDTs	Adelie penguins, crabeater seal	n/a	Bargagli (2008)
1963	Princess Elizabeth Land	Trace metals: Sr, Br,	Lake water	n/a	Burton (1981)
1964	South Victoria Land	Element: I	Lake water	n/a	Burton (1981)
1966	Mc Murdo Dry Valley	Chemical composition ( $\text{Cl}^-$ , $\text{Mg}^{2+}$ , $\text{Ca}^{2+}$ , C –biocarbonate ion concentration) temperature, density, solar radiation penetrating the ice, conductivity	Lake water	Classical analytical techniques (titration), selenium photo-electric cell, a bolometer, remote control conductivity probe	House et al. (1966)
1967	McMurdo Station, Hut Point Peninsula Saddle, Taylor Valley, Ross Ice Shelf, Mt. Discovery	$\text{NO}_2$ , $\text{SO}_2$ aldehydes	Air	Portable air sampling apparatus	Fischer et al. (1967)
	South Victoria Land	Trace metals: Mn, Fe, Mo, Pb, Zn, Bi, Rb, Cs	Lake water	n/a	Burton (1981)
1969	Pacific Ocean	PCBs	Sea birds	n/a	Risebrough et al. (1969)
	Interior of The Antarctic Continent	Pollutant lead aerosols, terrestrial dusts and sea salts	Snow strata	n/a	Murozumi et al. (1969)
	Plateau Station	DDT	Surface snow	n/a	Peterle (1969)
1972	Mimv, Vostok	Cl, Na, Mg, K, Ca, Mg, Fe	Surface firn	Atomic absorption, neutron activation	Boutron et al. (1972)
	Doumer Island, Antarctic Peninsula	PCBs	Penguin eggs	n/a	Risebrough and Carmignani (1972)
1975	Halley Bay	DDT	Snow	n/a	Peel (1975)
1976	n/a	DDTs, PCBs	Snow penguin eggs	n/a	Aono et al. (1997), Risebrough et al. (1976)

(continued)

**Table 1** (continued)

Year	Sampling place	Analytes or subject of research	Type of sample	The analytic techniques	Literature
1978	East Antarctic, South Polar Station And Dome C	Sulfate $\text{SO}_4^{2-}$	Snow	Classical analytical techniques (titration)	Delmas and Boutron (1978)
1978/1979	Ross Island and the Wrightand Taylor Valleys	Fe, Hg	Sediments, clays and rock fines	Atomic absorption spectrophotometry	Siegel et al. (1981)
1980	Mc Mundo	Environmental assessment of Antarctic research	Rocks, ice cores, soil samales, meteorites, certain biota, fossils	n/a	Myers et al. (1980)
	King Edward Cove	Aliphatic hydrocarbons, PAHs	Plants, soil, fresh-water sediment, zooplankton	Gas chromatography-mass spectrometry (GC-MS)	Platt and Mackie (1980)
	James Ross Island	Acidity	Precipitation	pH determination or titration	Delmas and Gravenhorst (1983)
1981	Signy Island, King Edward Cove	Aliphatic and aromatic hydrocarbons	Marine benthic invertebrates	n/a	Clarke and Law (1981)
	Syowa Station	DDTs, PCBs	Fish (whole body)	Gas chromatography—electron capture detector (GC-ECD)	Subramanian et al. (1983)
1982	The Geographic South Pole	Na, Mg, K, Ca, Fe, Al, Mn, Pb, Cd, Cu, Zn and Ag	Snow layers	Atomic absorption techniques	Boutron (1982)
1983	n/a	DDTs, PCBs	Fish tissues	n/a	Aono et al. (1997)
	Ross Sea	DDTs, PCBs	Weddell seal blubber	n/a	Aono et al. (1997)
	East Antarctica	Pb	Snow cores	Isotope dilution mass spectrometry (IDMS)	Boutron and Patterson (1983)
1984	The Coast On Riiser-Larsenisen Ice Shelf	Ions (for example: $\text{SO}_4^{2-}$ , $\text{Na}^+$ )	Snow profiles	n/a	Gjessing (1984)
	n/a	DDTs, PCBs	Mink whale liver, Ross seal blubber	n/a	Aono et al. (1997)

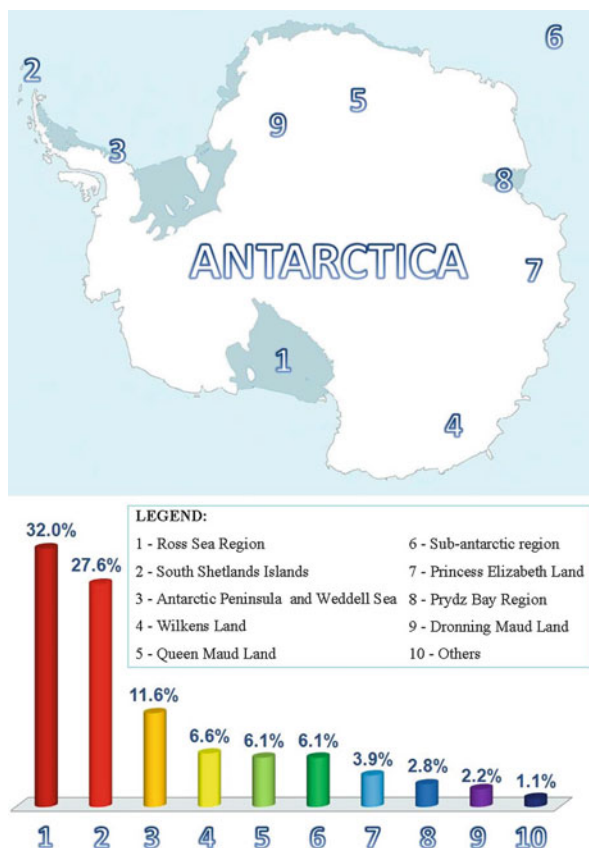
Year	Areas of the Antarctic ice cap	Heavy metals (Pb, Cd, Cu, Zn, Ag)	Snow	Atomic absorption techniques	Author
1979	n/a	Trace metals and chlorinated hydrocarbons	Ross seal tissues	Atomic absorption techniques, gas-liquid chromatograph fitted with ECD	Boutron (1979)
1984	East Antarctica	Cd, Cu, Zn, Au, Se, $\text{SO}_4^{2-}$	Prehistoric ice	n/a	Boutron et al. (1984)
	n/a	$\text{Na}^+$ , $\text{NH}_4^+$ , $\text{K}^+$ , $\text{Cl}^-$ , $\text{NO}_3^-$ , $\text{SO}_4^{2-}$	Snow and ice	Ion chromatography (IC)	Legrand et al. (1984)
1986	Antarctic Peninsula	Chlorinated hydrocarbon residues (HCB, HCH isomers, p,p' DDT, DDE, PCB congeners)	Lichen and moss samples	n/a	Bacci et al. (1986)
	Adelie Land	$\text{Na}^+$ , $\text{NH}_4^+$ , $\text{K}^+$ , $\text{Cl}^-$ , $\text{NO}_3^-$ , $\text{SO}_4^{2-}$ , $\text{Mg}^{2+}$	Precipitation	IC	Legrand and Delmas (1986)
	n/a	DDTs, PCBs	Penguin tissues	n/a	Aono et al. (1997)
1987	Ross Sea, Wilkes Land	Normal alkanes (n-C-C <sub>36</sub> ), isoprenoid hydrocarbons (i-C <sub>15</sub> , i-C <sub>16</sub> , i-C <sub>18</sub> , i-C <sub>19</sub> , and i-C <sub>20</sub> ) triterpanes (C <sub>27</sub> -C <sub>32</sub> ), and (C <sub>27</sub> -C <sub>29</sub> )	Quaternary sediment	n/a	Kvenvolden et al. (1987)
	Syowa Station, Antarctica	Heavy metals	Tissue of the Weddell seal	n/a	Yamamoto et al. (1987)
	n/a	$\text{NH}_4^+$ , $\text{F}^-$ , $\text{COOH}^-$ , $\text{CHOO}^-$ , $\text{CH}_3\text{SO}_3^-$ , $\text{F}^-$ , $\text{NH}_4^+$ ions	Ice	IC	Saigne et al. (1987)
1988	The South Shetland Islands	Pb	Aerosols	IDMS	Völkering et al. (1988)
	The Ekström ice shelf, "Georg-von-Neumayer" station	Heavy metals	Surface snow	IDMS, differential pulse anodic stripping voltammetry (DPASV)	Völkering and Heumann (1988)
					(continued)

Table 1 (continued)

Year	Sampling place	Analytes or subject of research	Type of sample	The analytic techniques	Literature
1989	Weddell Sea, Antarctic Peninsula	HNO <sub>3</sub>	Surface snow	IDMS	Neubauer and Heumann (1988)
	Sections Of the Byrd Station	Liquid conductivity, acidity, sulfate, nitrate, aluminum, and sodium concentrations	Ice containing tephra (volcanic ash) layers	n/a	Palais (1988)
	Vostok Station	Na <sup>+</sup> , NH <sub>4</sub> <sup>+</sup> , K <sup>+</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup> , H <sup>+</sup> , Cl <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup>	Ice core	n/a	Legrand et al. (1988)
	Mc Muurdo Sound	Fatty acids, -alcohols, n-alkanes, PAH	Marine sediments	n/a	Venkatesan (1988)
	n/a	Organochlorine pesticides, PCBs and mercury	Seabird eggs and tissues	n/a	Luke et al. (1989)
	The coastal area of Antarctica	SO <sub>4</sub> <sup>2-</sup> /Na <sup>+</sup> , SO <sub>4</sub> <sup>2-</sup> /Cl <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> /Mg <sup>2+</sup>	Snow	n/a	Gjessing (1989)
	Wright Valley, Antarctica	Mn, Fe, Co, Ni, Cu, Cd	Fresh water	n/a	Green et al. (1989)

<sup>a</sup>no data

**Fig. 3** The percentage of areas most commonly studied regarding the presence of contaminants up to end of 2014 in the Antarctic environment



Antarctica because of the toxic properties and the threat which is associated with their presence in the polar environment. Table 2 presents general information on xenobiotics determined in samples collected from various parts of the Antarctic ecosystem.

In the discussion pertaining to the presence of pollutants in Antarctica, it is very important to become familiar with accurate levels of concentration present in individual elements of both, biotic and abiotic, environments. Table 3 (A, B, C) data referring to levels of detected contamination present in the whole Antarctic environment and Fig. 4 presents a summary of POPs and heavy metals concentration levels determined in various elements of Antarctica's environments during three time periods (up to end of 2014).

As is showed in Fig. 4 studies on the determination of the pollutants concentrations in biotic and abiotic samples over the decades are irregular. It makes presentation of concentrations trends very difficult. However, as a main source of air contamination the LRAT from Africa, South America or Australia (Negoița et al. 2003) is administered. Nevertheless, the year-round operation of stations

**Table 2** Summary of literature data on results of analytical research on various types of (a) abiotic and (b) biotic samples collected in Antarctica in three time periods

Abiotic samples													
Type of sample	Time range within which the results were published	Analytes										Literature	
		OCP				PCBs	PBDEs	PAHs	PFCs	Metals	Other <sup>b</sup>		
		DDTs	HCHs	HCB	Other <sup>a</sup>								
Air	Up to 1989	x											Sen Gupta et al. (1996), Fischer et al. (1967)
	1990–1999	x	x	x	x	x		x			x		Larsson et al. (1992), Kallenborn et al. (1998), Caricchia et al. (1995), Bidleman et al. (1993), Riddlein and Heumann (1995)
	2000–2014	x	x	x	x	x		x			x		Cincinelli et al. (2009), Cabrerizo et al. (2013), Galbán-Malagón et al. (2013a, c), Gambaro et al. (2005), Li et al. (2012), Choi et al. (2008), Montone et al. (2003), Baek et al. (2011), Montone et al. (2005), Kallenborn et al. (2013), Dickhut et al. (2005), Ma et al. (2014), Fischer et al. (2002), Sprovieri et al. (2002)

Snow	Up to 1989	x	x							x							Kang et al. (2012), Sen Gupta et al. (1996), Delmas and Boutron (1978), Boutron and Patterson (1983), Wania et al. (1998), Boutron et al. (1972), Aono et al. (1997), Risebrough et al. (1976), Völkner and Heumann (1988)
	1990–1999									x							Suttie and Wolff (1992), Görlach and Boutron (1992), Wolff et al. (1999), Vandal et al. (1995), Capelli et al. (1998), Vandal et al. (1998)
	2000–2014		x							x		x					Kang et al. (2012), Vecchiato et al. (2015), Fuoco et al. (2012), Nemirovskaya (2006), Antony et al. (2011), Cai et al. (2012), Zoccolillo et al. (2007), Edwards et al. (2001), Planchon et al. (2002), Planchon et al. (2001), Thamban and Thakur (2013), Fortner et al. (2011), Witherow and Lyons (2008), Han et al. (2013), Velde et al. (2005), Burn-Nunes et al. (2011), Vallelonga et al. (2010)
Ice	Up to 1989																Boutron et al. (1984)
	1990–1999																Green et al. (1992), Hong et al. (1998), Vandal et al. (1995)
	2000–2014												x				Nemirovskaya (2006), Vallelonga et al. (2010), Jiratu et al. (2009)

(continued)

Table 2 (continued)

Fresh waters	Up to 1989	x	x							x		x	Sen Gupta et al. (1996), Burton (1981), Platt and Mackie (1980), Green et al. (1989)
	1990–1999										x		Vandal et al. (1998)
	2000–2014										x	x	Cai et al. (2012), Mão de Ferro et al. (2013)
Seawater	Up to 1989											x	Platt and Mackie (1980)
	1990–1999											x	Guerra et al. (2013), Stortini et al. (2009), Cripps (1992), Green et al. (1992), Niemistö and Perttälä (1995)
	2000–2014	x	x	x				x		x		x	Cincinelli et al. (2009), Cincinelli et al. (2008), Stortini et al. (2009), Bicego et al. (1996), Galbán-Malagón et al. (2013b), Fuoco et al. (2009b), Zhang et al. (2013), Ahrens et al. (2010), Cai et al. (2012)
Polonya water	Up to 1989												–
	1990–1999	x	x					x					Sen Gupta et al. (1996)
	2000–2014												–
Sediments	Up to 1989								x			x	Platt and Mackie (1980), Venkatesan (1988), Merlin et al. (1989), Siegel et al. (1981)
	1990–1999	x	x					x				x	Fuoco et al. (1996), Sen Gupta et al. (1996), Risebrough et al. (1990), Green et al. (1992), Vandal et al. (1998),

																				Yuguang and Junlin (1991), Giordano et al. (1999), Ciaralli et al. (1998), Crespi et al. (1993), Niemi and Perttinen (1995), Ahn et al. (1996), Alam and Sadiq (1993), Santos et al. (2005), Lenihan (1992)
	2000–2014	x	x	x	x				x											Zhang et al. (2013), Klánová et al. (2008), Montone et al. (2001), Curtosi et al. (2007), Dauner et al. (2014), Kim et al. (2006), Negri et al. (2006), Crockett and White (2003), Martins et al. (2010), Siegel et al. (1981), Santos et al. (2007), Ribeiro et al. (2011), Ribeiro et al. (2010), Waheed et al. (2001), Negri et al. (2006), Ianni et al. (2010), Huang et al. (2014), Townsend and Snape (2008), Sun et al. (2013), Vodopivec et al. (2015), Curtosi et al. (2010), Andrade et al. (2001)
Soil	Up to 1989								x											Platt and Mackie (1980)
	1990–1999		x	x	x				x											Aislabie et al. (1999), Mazzera et al. (1999), Kennicutt et al. (1995),

(continued)

Table 2 (continued)

																		Fuoco et al. (1996), Carrasco and Predez (1991), Bargagli et al. (1995), Bargagli et al. (1999)
	2000–2014	x	x	x														Borghini et al. (2005), Klánová et al. (2008), Cabrerizo et al. (2012), Negoita et al. (2003), Curtosi et al. (2007), Kang et al. (2012), Webster et al. (2003), Lu et al. (2012)
Biotic samples																		
Type of sample	Time range within which the results were published	Analytes						PCBs	PBDEs	PAHs	PFCs	Metals	Other <sup>d</sup>	Literature				
		OCP		HCHs	HCB	Other <sup>c</sup>												
Krill	Up to 1989																	
	1990–1999	x	x	x				x										Sen Gupta et al. (1996), Aono et al. (1997), Bargagli (2008), Petri and Zauke (1993)
	2000–2014	x	x	x	x			x										



Table 2 (continued)

Seabirds	Up to 1989	x					x				x		Sen Gupta et al. (1996), Risebrough et al. (1969), Aono et al. (1997), Luke et al. (1989), Bargagli (2008)
	1990–1999	x	x					x					Sen Gupta et al. (1996), Court et al. (1997), Aono et al. (1997), Inomata et al. (1996)
	2000–2014	x	x	x			x	x	x	x	x		Corsolini et al. (2006), Corsolini et al. (2011), Schiavone et al. (2009a), Geisz et al. (2008), Cipro et al. (2010), Yogui and Sericano (2009), Taniguchi et al. (2009), Van den Brink et al. (2011), Corsolini et al. (2007), Busnes et al. (2006), Llorca et al. (2012), Van den Brink et al. (2011), Senthil et al. (2002), Tao et al. (2006), Taniguchi et al. (2009), Brasso and Polito (2013), Santos et al. (2006), Smichowski et al. (2006), Jerez et al. (2013a), Jerez et al. (2011), Bargagli (2001)

Marine mammals	Up to 1989	x					x				x		Schiavone et al. (2009a), Aono et al. (1997), Bargagli (2008), Risebrough and Carmignani (1972), McClurg (1984), Yamamoto et al. (1987)
	1990–1999					x					x		Aono et al. (1997), Schiavone et al. (2009a), Malcolm et al. (1994), Moreno et al. (1997), Aono et al. (1997), Szefer et al. (1993)
	2000–2014	x				x	x			x	x		Cipro et al. (2012), Schiavone et al. (2009c), Schiavone et al. (2009a), Corsolini et al. (2002a), Trumble et al. (2012), Krahn et al. (2007), Bengtson Nash et al. (2010), Senthil et al. (2002), Tao et al. (2006), Santos et al. (2006)
Algae	Up to 1989												–
	1990–1999										x		Moreno et al. (1997)
	2000–2014	x					x		x		x		Caberizo et al. (2012), Runcie and Riddle (2004), Santos et al. (2006)
Antarctic lichens	Up to 1989												–
	1990–1999										x		Poblet et al. (1997), Olech et al. (1998), Upreti and Pandey (1994), Bargagli et al. (1999)
	2000–2014	x					x			x	x		Cipro et al. (2011), Caberizo et al. (2012), Yogui and Sericano (2008),

(continued)

Table 2 (continued)

Antarctic mosses	Up to 1989																Yogui et al. (2011), Mão de Ferro et al. (2013), Osyczka et al. (2007), Santos et al. (2006)
	1990–1999	x	x	x													–
	2000–2014	x	x	x													Focardi et al. (1991), Bargagli et al. (1995)
																	Yogui and Sericano (2008), Yogui et al. (2011), Borghini et al. (2005), Cipro et al. (2011), Cabrerizo et al. (2012), Mão de Ferro et al. (2013), Osyczka et al. (2007), Santos et al. (2006)

<sup>a</sup>Heptachlor epoxide and nanochlor<sup>b</sup>Sulphate, methyl Hg, n- alkanes, aliphatic hydrocarbons, chlorinated terphenyls, and TOC<sup>c</sup>Mirex, dieldrin, endosulfan (I/II)<sup>d</sup>PCNs, PCDDs, PCDFs; sulphate, methyl Hg, n- alkanes, aliphatic hydrocarbons, chlorinated terphenyls, TOC

Authors decided to summarise the first three decades of analytical research as historic ones (up to the end of 1989). During this period only few data has been published, hence this period is three decades long. The period between 1990 and 1999 was relatively abundant in terms of applied analytical research, so it has been limited to only one decade. The last period contains years from 2000 up to present times. Authors do not see the need to separate the last decade (incomplete), so this period is 15 years long

**Table 3** Detailed literature data on results of analytical research on (a) main POPs; (b) remaining organic compounds; (c) heavy metals in various types of biotic and abiotic samples collected in Antarctica in three time periods

Main POPs detected in biotic samples									
Type of sample	Sample	Localization	Range or average concentrations (±standard deviation, if available)					PAHs	Literature
			DDTs <sup>a</sup>	PCBs <sup>b</sup>	HCHs <sup>c</sup>	HCB	PBDEs <sup>d</sup>		Unit
Fish	Antarctic fishes; whole body ( <i>Pagothenia borchgrevinkii</i> , <i>Trematomus bernacchii</i> , <i>T. hanonii</i> , <i>T. Newnesi</i> , <i>T. Borchgrevinkii</i> )	near Syowa Station	0.03–1.9	0.08–0.77	–	–	–	–	µg/g wet wt
		King Edward Cove	–	–	–	–	–	0.01–0.5	ng/g wet wt
	Antarctic cod; flesh ( <i>Notathenia rossii</i> )		–	–	–	–	–	0.01–0.11	ng/g wet wt
	Antarctic cod; liver ( <i>Notathenia rossii</i> )		–	–	–	–	–	–	ng/g wet wt
Seabirds	Chinstrap penguin ( <i>Pygoscelis antarcticus</i> )	–	0.63–4.27	–	–	–	–	–	pg/g
	Macaroni penguin ( <i>Eudyptes chryscolopus</i> )	–	500	–	–	–	–	–	pg/g
Marine mammals	Migrating snow petrel ( <i>Pagodroma nivea</i> )	–	600	–	–	–	–	–	pg/g
	Crabeater seals ( <i>Lobodon carcinophagus</i> )	–	7–17	–	–	–	–	–	ng/g
Data reported from 1990 up to 1999									
Crustaceans	Krill ( <i>Euphausia superba</i> )	Dakshin Gangotri, Queen Maud Land	31.1–44.4	146.9–166.2	141.3–164.3	–	–	–	pg/g dry wt
		–	0.56	<1.0	0.028	0.30	–	–	ng/g wet wt
Seabirds	Penguin; feathers	Dakshin Gangotri, Queen Maud Land	30.8–35.7	105.8–113.6	103.6–112.8	–	–	–	pg/g dry wt
		Cape Bird, Ross Island	12.1–97.4	18.7–110.6	–	12.5–57.2	–	–	ng/g dry wt
	Adelie penguin; eggs ( <i>Pygoscelis adeliae</i> )		24.3 ± 12.8	618.0 ± 506.0	–	15.9 ± 3.9	–	–	ng/g dry wt
	Adelie penguin; liver ( <i>Pygoscelis adeliae</i> )		263.4 ± 209.2	2546.0 ± 1675.0	–	49.6 ± 26.0	–	–	ng/g dry wt
	South polar skuas; liver ( <i>Catharacta macconnicki</i> )		30.8–972.3	43.2–1583.6	<LOD <sup>e</sup> –39.3	42.3–1159.7	–	–	ng/g
Data reported from 1990 up to 1999									
Seabirds	Gentoo penguin; fat tissue ( <i>Pygoscelis papua</i> )	Admiralty Bay, King George Island	30.8–972.3	43.2–1583.6	<LOD <sup>e</sup> –39.3	42.3–1159.7	–	–	ng/g
	Adelie penguin; fat tissue ( <i>Pygoscelis adeliae</i> )								

(continued)

Table 3 (continued)

Marine mammals	Mink whale, blubber ( <i>Balaenoptera acutorostrata</i> )	–	27–380	13–210	0.67–5.7	39–290	–	ng/g wet wt	Aono et al. (1997)
Flora	Antarctic mosses	Key Island, Ross Sea and Antarctic Peninsula	0.2–0.5	<5–16	0.2–1.7	0.3–0.8	–	ng/g dry wt	Focardi et al. (1991)
Data reported from 2000 up to 2014									
Crustaceans	Krill ( <i>Euphausia superba</i> )	Ross Sea	0.22–2.30	85.27–282.29	–	0.2–0.6	–	ng/g wet wt	Consolini et al. (2002b)
			0.07–0.10	–	0.28 ± 0.04	0.23 ± 0.01	0.17–0.23	ng/g wet wt	Consolini et al. (2006)
			–	–	0.001–0.32	0.006–0.06	–	ng/g wet wt	Cincinelli et al. (2009)
			–	1.9	–	–	–	ng/g wet wt	Consolini et al. (2002a)
			0.05–0.79	4.66–13.6	–	<LOD–0.06	–	ng/g wet wt	Cipro et al. (2010)
Phytoplankton	–	Southern Ocean areas (Weddell, South Scotia, and Bellingshausen Seas)	–	2.67 ± 0.86–14.07 ± 12.72	0.16 ± 0.17–1.81 ± 2.11	1.64 ± 2.4–4.34 ± 4.34	–	ng/g dry wt	Galbán-Malagón et al. (2013b)
			–	–	0.45 ± 0.38–1.24 ± 1.65	2.9 ± 1.76	–	ng/g lipid wt	Chiuchio et al. (2004)
Benthic organisms	<b>Antarctic invertebrates</b> ( <i>Protelipidia murrayi</i> , <i>Penagone vignoni</i> , <i>Bathyploes natans</i> , <i>Molpadia muscidus</i> )	Western Antarctic Peninsula	0.26 ± 0.15	0.84–10.3	0.14–0.35	0.87 ± 0.34	–	ng/g lipid wt	Zhang et al. (2013)
			–	–	–	–	–	ng/g lipid wt	Hale et al. (2008)
			–	–	–	–	–	ng/g lipid wt	
	<b>Antarctic invertebrates</b> <i>clams</i> ( <i>Laternula elliptica</i> ), <i>sea stars</i> ( <i>Odonaster validus</i> ), <i>sea urchins</i> ( <i>Sterechinus neumayeri</i> ), <i>sponges</i> ( <i>Halictona</i> sp. and <i>Homaxinella balfourensis</i> ), <i>proboscis worms</i> ( <i>Parborlasia corrugatus</i> )	Mc Murdo Sound	–	–	–	–	356 ± 196	ng/g lipid wt	

Fish	Silverfish; larva ( <i>Pleurogramma antarcticum</i> )	Ross Sea	1.51–2.03	497.81–509.88	–	0.88–4.04	–	ng/g wet wt	Corsolemi et al. (2002b)
	Silverfish; adults ( <i>Pleurogramma antarcticum</i> )		0.04–5.70	16.2–1050.58	–	0.07–14.93	–		
	Silverfish ( <i>Pleurogramma antarcticum</i> )		–	138	–	–	–	ng/g wet wt	Corsolemi et al. (2002a)
	Rockcod; whole body ( <i>Trematomus bernacchii</i> )		0.02–2.53	–	0.03–0.17	1.35 ± 1.24	0.15–0.16	ng/g wet wt	Corsolemi et al. (2006)
	Rockcod; muscle ( <i>Trematomus bernacchii</i> )		0.11–1.1	–	0.03–1.23	1.44 ± 0.45	0.02–0.06	–	
	Rockcod; ( <i>Trematomus bernacchii</i> )		–	–	–	–	1520–1840	ng/g lipid wt	Hale et al. (2008)
	Antarctic fish ( <i>Chionodraco hamatus</i> , <i>Chaemopsoccephalus gunnari</i> , <i>Gymnoscapellus nicholsi</i> , <i>Trematomus eulipoides</i> )		–	–	–	–	0.001–0.13	ng/g wet wt	Borghesi et al. (2009)
	Crocodile icefish; muscle ( <i>Chionodraco hamatus</i> )		–	0.07–0.95	–	–	(0.085–0.300)	ng/g wet wt	Borghesi et al. (2008)
	Crocodile icefish; liver ( <i>Chionodraco hamatus</i> )		–	0.75–3.30	–	–	(0.001–0.320)	–	
	Emerald rockcod; muscle ( <i>Trematomus bernacchii</i> )		–	(0.35–4.20)	–	–	(0.220–0.530)	–	
	Emerald rockcod; liver ( <i>Trematomus bernacchii</i> )		–	(5.20–28)	–	–	(0.500–1.100)	–	
	Antarctic fish; liver ( <i>Gobionotothen gibberifrons</i> , <i>Chaemopsoccephalus gunnari</i> , <i>Chaenoccephalus acerans</i> )	Elephant Island, South Shetland Islands	5–13	0.4–2	–	15–20	–	ng/g lipid wt	Weber and Goerke (2003)
	Sharp-spined notothen ( <i>Trematomus pennellii</i> )	Ross Sea	–	111–175	–	–	–	ng/g wet wt	Corsolemi et al. (2002a)
	Notothenioids fish; muscle ( <i>Trematomus newnesi</i> , <i>Notothenia coriiceps</i> , <i>Notothenia rossii</i> )	Potter Cove, King George Island	<LOD–7.31	<LOQ–8.33	<LOQ–3.44	–	<LOQ–8.53	ng/g lipid wt	Lana et al. (2014)
	Notothenioids fish; liver ( <i>Trematomus newnesi</i> , <i>Notothenia coriiceps</i> , <i>Notothenia rossii</i> )		<LOD–10.5	<LOQ–7.00	<LOQ–0.99	–	<LOQ–73.6	ng/g lipid wt	Lana et al. (2014)
	Notothenioids fish; gonads ( <i>Trematomus newnesi</i> , <i>Notothenia coriiceps</i> , <i>Notothenia rossii</i> )		<LOQ–98.8	<LOQ–46.9	2.41–24.2	–	<LOQ–4.86	ng/g lipid wt	Lana et al. (2014)
	Notothenioids fish; gills ( <i>Trematomus newnesi</i> , <i>Notothenia coriiceps</i> , <i>Notothenia rossii</i> )		<LOQ–43.0	<LOQ–14.8	1.57–9.95	–	<LOQ–39.8	ng/g lipid wt	Lana et al. (2014)

(continued)

Table 3 (continued)

<b>Penguin adèle, preen oil (<i>Pygoscelis adeliae</i>)</b>	Hop Island	–	1–37	–	–	2–567	–	–	ng/g lipid wt	Van den Brink et al. (2011)
<b>Southern fulmar, preen oil (<i>Fulmarus glacialis</i>)</b>	Hop Island	–	1–40	–	–	1–314	–	–	ng/g lipid wt	Van den Brink et al. (2011)
<b>Penguin blood (pooled together)</b> <b>Penguins adèle (<i>Pygoscelis adeliae</i>)</b> <b>Gentoo penguin (<i>Pygoscelis papua</i>)</b> <b>Chinstrap penguin (<i>Pygoscelis antarcticus</i>)</b>	Admiralty Bay, King George Island	2.7–16	1.5–17	–	–	0.4–20	0.0017–1726	–	ng/g wet wt	Corsolini et al. (2007)
<b>Migrating snow petrel, eggs (<i>Pagodroma nivea</i>)</b>	Ross Sea	3.64–10.83	15.23–22.66	0.03–0.37	–	10.43–15.40	–	–	ng/g wet wt	Corsolini et al. (2011)
<b>South polar skua, eggs (<i>Catharacta maccormicki</i>)</b>	Ross Sea	<LOD–64.75	1.69–64.23	<LOD–0.080	–	9.23–43.39	–	–	ng/g wet wt	Corsolini et al. (2011)
	Antarctic Peninsula	–	–	–	–	–	19.0–558	–	ng/g lipid wt	Yogui and Sericano (2009)
<b>South polar skua, blood (<i>Catharacta maccormicki</i>)</b>	Dronning Maud Land	0.4–40.9	1.0–50.5	<0.1–6.5	–	0.6–21.2	–	–	ng/g lipid wt	Bustnes et al. (2006)
<b>Migrating brown skua, eggs (<i>Catharacta antarctica</i>)</b>	Bransfield Strait	0.09–27.87	31.28–68.62	<LOD–0.04	–	1.80–27.49	–	–	ng/g wet wt	Corsolini et al. (2011)
<b>Migrating brown skua, fat tissue (<i>Catharacta antarctica</i>)</b>	King George Island, South Shetland	6118 ± 3813	19,720 ± 9620	1.22–3.11	–	573 ± 278	–	3375 ± 1588	ng/g lipid wt	Taniguchi et al. (2009)
<b>Antarctic tern, fat tissue (<i>Sterna vittata</i>)</b>	King George Island, South Shetland	524 ± 205	613 ± 187	<0.12–2.60	–	601 ± 256	–	5744 ± 2546	ng/g lipid wt	Taniguchi et al. (2009)
<b>Blue-eyed shag, fat tissue (<i>Phalacrocorax atriceps</i>)</b>	King George Island, South Shetland	374	282	1.33	–	161	–	3961	ng/g lipid wt	Taniguchi et al. (2009)
<b>Snowy sheathbill, fat tissue (<i>Chionis alba</i>)</b>	King George Island, South Shetland	468	297	<0.12	–	282	–	4090	ng/g lipid wt	Taniguchi et al. (2009)

(continued)

**Table 3** (continued)

Flora	Antarctic lichen ( <i>Usnea</i> spp.)	King George Island	0.353 ± 0.04	7.76 ± 2.3	0.205 ± 0.08	0.141 ± 0.10	0.236 ± 0.05	—	ng/g dry wt	Cipro et al. (2011)
	Antarctic lichens ( <i>Usnea Antarctica</i> )	Southern Shetlands	0.003–0.01	0.043–0.61		0.002–0.31	—	15–40	ng/g dry wt	Cabrerizo et al. (2012)
	Antarctic lichens ( <i>Usnea aurantiaco-atra</i> )	Admiralty Bay, King George Island,	—	—	—	—	139 ± 33.6	—	pg/g dry weight	Yogui and Sericano (2008)
	Antarctic lichens ( <i>Usnea aurantiaco-atra</i> )		—	—	—	—	262 ± 48.7	—		Yogui et al. (2011)
	Antarctic lichens ( <i>Usnea Antarctica</i> )		—	—	—	—	192 ± 93.9	—		Yogui and Sericano (2008)
	Antarctic lichens ( <i>Usnea aurantiaco-atra</i> )		—	—	—	—	262 ± 48.7	—		Yogui et al. (2011)
	Antarctic mosses ( <i>Santonia uncinata</i> )		—	—	—	—	818 ± 270	—		Yogui and Sericano (2008)
	Antarctic mosses ( <i>Santonia uncinata</i> )		—	—	—	—	1022 ± 348	—		Yogui et al. (2011)
	Antarctic mosses ( <i>Santonia uncinata</i> )		—	—	—	—	718	—		
	Antarctic mosses ( <i>Syntrichia princeps</i> )		—	—	—	—	276	—		
	Antarctic mosses ( <i>Brachythecium</i> sp.)		—	—	—	—	328	—		
	phanerogam ( <i>Colobanthus quitensis</i> )		—	—	—	—	—	—		
	Antarctic mosses ( <i>Bryum argenteum</i> , <i>Pottia heinii</i> , <i>Ceratodon purpureus</i> )	Victoria Land	0.54–7.9	23–34	0.18–4.0	0.82–1.95	—	—	ng/g dry wt	Borghini et al. (2005)
	Antarctic mosses ( <i>Brachitecium</i> sp. <i>Syntrichia princeps</i> <i>Santonia uncinata</i> )	King George Island	<LOQ–1.73	7.76–18.6	<LOQ–1.20	0.141–1.06	0.276–0.893	—	ng/g dry wt	Cipro et al. (2011)
	Antarctic mosses ( <i>Santonia uncinata</i> )	Southern Shetlands	0.005–0.04	0.04–0.76	—	0.21–0.12	—	4.4–34	ng/g dry wt	Cabrerizo et al. (2012)
	Hair grass ( <i>Oeschampsia antarctica</i> )	Southern Shetlands	0.061–0.09	0.39–2.40	—	0.080–0.20	—	6–10	ng/g dry wt	Cabrerizo et al. (2012)
	Pearl-wort ( <i>Colobanthus quitensis</i> )	Shetlands	0.04	0.31	—	0.04	—	9.5	ng/g dry wt	
	Green algae ( <i>Prasiola crispa</i> )		0.08	0.86	—	0.033	—	7		
	Red snow algae		0.28	3.07	—	0.67	—	141		

Main POPs detected in abiotic samples

Type of sample	Localization	Range or average concentrations (±standard deviation, if available)							UNIT	LIT
		DDTs	PCBs	HCHs	HCB	PBDEs	PAHs			
Data reported in 80th years and earlier	Air	–	150	–	–	–	–	–	pg/m <sup>3</sup>	Sen Gupta et al. (1996)
	Snow	–	–	2300–4900	–	–	–	–	pg/L	Kang et al. (2012)
	–	0.63–4.27	–	–	–	–	–	–	–	Sen Gupta et al. (1996)

(continued)

Table 3 (continued)

Fresh water	–	1.3–30.7	–	1665	–	–	–	pg/L	Sen Gupta et al. (1996)
Sediments	King Edward Cove	–	–	–	–	–	2–220	ng/g dry wt	Platt and Mackie (1980)
Data reported from 1990 up to 1999									
Air	Ross Island	1.0–2.0	12.2	25.8	–	–	–	pg/m <sup>3</sup>	Larsson et al. (1992)
Air (ambient air)	Signy Island	0.07–0.40	0.02–17	22	–	–	–	pg/m <sup>3</sup>	Kallenborn et al. (1998)
Air (atmospheric particulates)	Terra Nova Bay	–	–	–	–	–	<1.5–689	pg/m <sup>3</sup>	Caricchia et al. (1995)
Soil	McMurdo Sound—Dry Valley Region (fuel-oil contaminated area)	–	–	–	–	–	46–3398	ng/g dry weight	Aislabie et al. (1999)
Sediments	McMurdo Station	–	–	–	–	–	28–27,000	ppb	Mazzera et al. (1999)
	Victoria Land	–	–	–	–	–	6267–6339	ppm	Kennicutt et al. (1995)
		–	60	–	–	–	–	pg/g dry wt	Fuoco et al. (1996)
	Ross Sea	–	80	–	–	–	–	pg/g dry wt	Fuoco et al. (1996)
	Winter Quarters Bay	–	100–1400	–	–	–	–	ng/g	Risebrough et al. (1990)
Seawater	Victoria Land	–	120	–	–	–	–	pg/g dry wt	Fuoco et al. (1996)
	–	605.03–844.9	–	92.9–123.2	–	–	–	pg/g	Sen Gupta et al. (1996)
	Admiralty Bay, King George Island	–	–	–	–	–	0.003–0.011	µg/L	Bicego et al. (1996)
Polynya water	Dakshin Gangotri	24.8–26.5	96.8–103.8	85.6–90.7	–	–	–	pg/L	Sen Gupta et al. (1996)

Data reported from 2000 up to 2014

Air (gase phase)	Western Ross Sea	–	–	0.1–1.05	7.23–20.39	–	–	pg/m <sup>3</sup>	Cincinelli et al. (2009)
	Western Antarctic Peninsula	–	–	0.06–2.98	11.9–32.1	–	–		Dickhut et al. (2005)
	Livingston Island (Antarctica)	–	4–29	–	–	–	–		Cabrerizo et al. (2013)
	South Scotia Sea	–	–	1.63 ± 1.52–1.70 ± 2.16	49.71 ± 8.19	–	–		Galbán-Malagón et al. (2013a)
	Weddell	–	–	0.16 ± 0.14–0.87 ± 0.88	11.93 ± 15.77	–	–		
	Livingston Island	–	–	0.79 ± 0.77–2.27 ± 0.68	10.30 ± 4.81–11.97 ± 2.67	–	–		
	Southern Ocean, Antarctic Peninsula	–	1–70	–	–	–	–		Galbán-Malagón et al. (2013c)
	Terra Nova Bay	–	<LOD–0.25	–	–	–	–		Gambaro et al. (2005)
	Southern Ocean	–	–	–	–	–	0.03–4.58	ng/m <sup>3</sup>	Cabrerizo et al. (2014)
	Southern Ocean, Antarctic Peninsula	–	0.04–0.4	–	–	–	–	pg/m <sup>3</sup>	Galbán-Malagón et al. (2013c)
Air	King George Island	–	1.66–6.5	–	–	0.67–2.98	–	pg/m <sup>3</sup>	Li et al. (2012)
		–	<LOD–117.8	–	–	–	–		Choi et al. (2008)
		–	<LOD–33.2	–	–	–	–		Montone et al. (2003)
		–	–	2.5–3.65	–	–	–		Back et al. (2011)
	Antarctic Ocean	<2.7–5.2	<2.3–22.8	<2.7–4.6	3.3–25.3	–	–		Montone et al. (2005)
	Dronning Maud Land	0.02–0.20	–	0.02–0.46	22	–	–		Kallenborn et al. (2013)
Snow	Dome Fuji, East Antarctica	–	–	17.5–137.0	<LOD–182	–	–	pg/L	Kang et al. (2012)
	Victoria Land	–	110–580	–	–	130–340	0.65–140	pg/L	Vecchiato et al. (2015)
Snow/finn core	Talos Dome	–	0.03–0.24	–	–	–	0.35–4.6	ng/L	Fuoco et al. (2012)

(continued)

**Table 3** (continued)

Snow-ice cover	Russian Antarctic stations ( <i>Molodezhnaya, Cosmonaut Sea, Progress, Prydz Bay, Commonwealth Sea, and Mirnyi, coastal part of the Davis Sea</i> )	–	–	–	–	<10	ng/L	Nemirovskaya (2006)
Seawater	Western Ross Sea	–	–	0.61–8.79	1.72–16.24	–	pg/L	Cincinelli et al. (2009)
	Ross Sea	–	–	–	–	–	ng/L	Cincinelli et al. (2008)
	Ross Sea	–	23–45	–	–	–	pg/L	Fuoco et al. (2009b)
	Gerlache Inlet sea	–	–	–	–	–	ng/L	Stortini et al. (2009)
	<b>Southern Ocean areas (Weddell, South Scotia, and Bellingshausen Seas)</b>	–	1.34 ± 0.398–3.727 ± 1.466	0.189 ± 0.09–3.132 ± 4.031	0.281 ± 0.078–0.976 ± 0.828	–	pg/L	Galbán-Malagón et al. (2013b)
Porewater	Western Antarctic Peninsula	0.11–1.00	0.06–3.4	–	0.63–6.7	–	pg/L	Zhang et al. (2013)
Sediments	Western Antarctic Peninsula	–	0.003–0.35	–	–	–	ng/g dry wt	Zhang et al. (2013)
	James Ross Island	0.19–1.15	0.32–0.83	0.14–0.76	0.95–4	–	ng/g	Kláňová et al. (2008)
	Admiralty Bay	–	<0.05–57	–	–	–	ng/g dry wt	Montone et al. (2001)
	King George Island, Potter Cove	–	–	–	–	–	ng/g dry wt	Curtosi et al. (2007)
	McMurdo Sound	–	–	–	–	–	ng/g	Dauner et al. (2014)
Sediment cores	Winter Quarters Bay, Mc Murdo Station	–	11–21	–	–	–	ng/g	Kim et al. (2006)
	Admiralty Bay, King George Island	–	<LOD–4299	–	–	–	ng/g	Negri et al. (2006)
		–	–	–	–	–	ng/g	Crockett and White (2003)
		–	–	–	–	–	ng/g	Martins et al. (2010)
		–	–	–	–	–	ng/g	

Soil	Victoria Land	0.053–0.086	0.36–0.59	–	0.034–0.17	–	–	ng/g dry wt	Borghini et al. (2005)
	James Ross Island	0.51–3.68	0.51–1.82	0.49–1.34	2.41–7.75	–	34.9–171	ng/g	Klánová et al. (2008)
	Southern Shetlands	LOQ–0.20	0.005–0.15	–	<LOQ–0.07	–	0.16–3718	ng/g dry wt	Cabrerizo et al. (2012)
	East Antarctic coast	0.11–1.22	0.20–0.41	0.86–4.69	–	–	12 ± 1– 1182 ± 113	ng/g dry wt	Negoita et al. (2003)
	Potter Cove, Jubany Station	–	–	0.09–40.1	0.02–25.28	–	–	ng/g	Kang et al. (2012)
		–	–	–	–	–	19–42	ng/g dry wt	Curtosi et al. (2007)

<sup>a</sup>DDTs: p,p'-DDE; o,p'-DDT; p,p'-DDD; p,p'-DDT

<sup>b</sup>pCBs: congeners (penta-CB: 99, 101, 105, 118; hexa-CB: 128, 138, 146, 149, 151, 153, 156; hepta-CB: 170, 171, 174, 177, 180, 183, 187; octa-CB: 194, 195, 199; nona-CB: 206; deca-CB: 209)

<sup>c</sup>HCHs: α-HCH; β-HCH; γ-HCH

<sup>d</sup>pBDEs: BDE-47; BDE-99; BDE-100 and others congeners (nos: 28, 153, 154, 183)

<sup>e</sup>LOD—limit of detection

<sup>f</sup>LOQ—limit of quantification

**Table 3b** Detailed literature data on results of analytical research on (a) main POPs; (b) remaining organic compounds; (c) heavy metals in various types of biotic and abiotic samples collected in Antarctica in three time periods

Various chemical compounds identified in biotic samples					
Type of sample	Compound groups/ determined compounds	Range or average concentrations ( $\pm$ standard deviation, if available)	Unit	Literature	
Data reported in 80th years and earlier					
Crustaceans	<b>Krill</b> ( <i>Euphausia superba</i> )	n-alkanes	0.5	$\mu\text{g/g}$ wet wt	Platt and Mackie (1980)
Benthic organisms	<b>Bivalve</b> ( <i>Yoldia eighisti</i> )	n-alkanes	0.4		
Fish	<b>Antarctic cod; flesh</b> ( <i>Notothenia rossii</i> )	n-alkanes	0.05		
	<b>Antarctic cod; liver</b> ( <i>Notothenia rossii</i> )	n-alkanes	1.9		
Marine mammals	<b>Crabeater seal, tissues</b> ( <i>Lobodon carcinophagus</i> )	PCDF's	3.7–6.1	pg/g lw	Schiavone et al. (2009c)
	<b>Minke whale; blubber</b> ( <i>Balaenoptera Acutorostrata</i> )	CHLs <sup>g</sup>	9.6–59	ng/g wet wt	Aono et al. (1997)
Flora	<b>terrestrial plants</b> ( <i>Tortula robusta</i> , <i>Rostkovia magellanica</i> , <i>Festuca contracta</i> <i>Poa flabellata</i> , <i>Acaena magellanica</i> )	n-alkanes	15.7–420.5	$\mu\text{g/g}$ wet wt	Platt and Mackie (1980)
Data reported from 1990 up to 1999					
Crustaceans	<b>Krill</b> ( <i>Euphausia superba</i> )	CHLs	68	pg/g wt wt	Aono et al. (1997)
Marine mammals	<b>Minke whale; blubber</b> ( <i>Balaenoptera Acutorostrata</i> )	CHLs	18–75	ng/g wet wt	Aono et al. (1997)
	<b>Antarctic fur seal; tissues</b> ( <i>Arctocephalus gazella</i> )	Dioxins	15.7	pg/g wet wt	Schiavone et al. (2009c)
Data reported from 2000 up to 2014					
Crustaceans	<b>Krill</b> ( <i>Euphausia superba</i> )	OCP	Chlordanes <sup>h</sup>	ng/g wet wt	(Cipro et al. (2010)
			Drins <sup>i</sup>		
		Dioxin	total PCDD/DFs	27	Senthil et al. (2002)

Fish	<b>Antarctic fishes; muscle</b> ( <i>Chionodraco Hamatus</i> , <i>Trematomus Bernacchii</i> )	Dioxins	PCDDs	2.69–5.8	pg/g wet wt	Borghesi et al. (2008)
		DLCs	PCDFs	1.53–1.68		
		Dioxins	PCDDs	4.6–4.94		
		DLCs	PCDFs	1.25–2.3		
	<b>Antarctic fishes; liver</b> ( <i>Chionodraco Hamatus</i> , <i>Trematomus Bernacchii</i> )					
	<b>Antarctic fishes; (Trematomus pennelli, Chionodraco hamatus)</b>	Dioxin	Total PCDD/DFs	11–17	pg/g	Senthil et al. (2002)
	<b>Emerald rockcod: (Trematomus bernacchii)</b>	OCP	CHLs	2.61 ± 2.07	ng/g wet wt	Corsolini (2009)
	<b>Antarctic fishes; liver</b> ( <i>Champsocephalus gunnari</i> , <i>Gobionotothen gibberifrons</i> , <i>Chanocephelus aceratus</i> )	OCP	Mirex	1–7	ng/g lipid wt	Weber and Goerke (2003)
	<b>Penguin; dung (Pygoscelis papua)</b>	Surfactants	PFCs <sup>j</sup>	0.63–603	ng/g	Llorca et al. (2012)
	<b>Penguin; muscle tissues (Pygoscelis papua)</b>	Surfactants	PFCs	<LOQ <sup>i</sup> –2.28	ng/g	Llorca et al. (2012)
Seabirds	<b>Penguin; preen oil (Pygoscelis adeliae)</b>	OCP	Dieldrin	2–24	ng/g lipid wt	Van den Brink et al. (2011)
	<b>Penguins; fat tissue (Pygoscelis adeliae, Pygoscelis papua, Pygoscelis antarctica)</b>	OCP	Dieldrin	47.1 ± 12.4	ng/g lipid wt	Cipro et al. (2012);
			Mirex	26.4 ± 20.2		Cipro et al. (2010);
			Dieldrin	26.4 ± 19.1		Corsolini
			Mirex	90.6 ± 70.6		et al. (2007); Senthil et al. (2002),
	<b>Penguin; blood (Pygoscelis adeliae)</b>	Surfactants	PFOS	<0.1	ng/ml	Tao et al. (2006); Taniguchi
	<b>Penguin; blood (Pygoscelis adeliae, Pygoscelis papua Pygoscelis antarctica)</b>	Dioxins	PCDDs	0.7–103	pg/g wet wt	et al. (2009)
		DLCs	PCDFs	0.8–194		
						(continued)

Table 3b (continued)

<b>Penguin: eggs</b> ( <i>Pygoscelis adeliae</i> , <i>Pygoscelis papua</i> <i>Pygoscelis antarctica</i> )	Surfactants	PFOS	<0.1–8.8	ng/g
	OCP	Chlordanes	0.32–7.57	ng/g wet wt
		Mirex	0.67–6.37	
		Drins	0.06–35.8	
	Dioxins	eoni PCDD/DFs	23	pg/g
	Surfactants	PFOS	<0.24–1.36	ng/ml
	Surfactants	PFOS	2.08–3.12	ng/g
	OCP	Chlordanes	977 ± 445	ng/g lipid wt
		Oxychlordane	408 ± 169	
		Dieldrin	254 ± 158	
		Mirex	2210 ± 1590	
<b>Brown skua: fat tissue</b> ( <i>Catharacta Antarctica</i> )		Chlordanes	80.6 ± 47.1	
		Oxychlordane	44.2 ± 21.0	
		Dieldrin	<0.48–23.0	
		Mirex	260 ± 58	
<b>Antarctic tern: fat tissue</b> ( <i>Sterna vittata</i> )	OC	Chlordanes	3.05	
		Oxychlordane	<0.24	
		Dieldrin	<0.48	
		Mirex	89.2	
<b>Blue-eyed shag: fat tissue</b> ( <i>Phalacrocorax Ariceps</i> )	OCP	Chlordanes	468	
		Oxychlordane	63.3	
		Dieldrin	22.4	
		Mirex	149	
<b>Snowy sheathbill: fat tissue</b> ( <i>Chionis alba</i> )	OCP	Chlordanes	468	
		Oxychlordane	63.3	
		Dieldrin	22.4	
		Mirex	149	

Marine mammals	<b>Whitechinned petrel; pectoral muscle</b> ( <i>Procellaria aequiactialis</i> )	surfactants	PFOS	1.2–2.0	ng/g	Llorca et al. (2012)
	<b>Southern fulmar; preen oil</b> ( <i>Fulmarus glacialisoides</i> )	OCP	Dieldrin	1–38	ng/g lipid wt	Van den Brink et al. (2011)
	<b>Seals; tissues (muscle, blubber, fur)</b>	OCP	Drins	18.4–82.4	ng/g lipid wt	Cipro et al. (2012); Bengtson Nash et al. (2010); Schiavone et al. (2009a, c); Corsolini et al. (2002b)
	<b>Antarctic fur seal</b> ( <i>Arctocephalus gazella</i> )		Endosulfan (I/II)	2.09–21.15		
	<b>Other seal species</b> ( <i>Leptonychotes weddelli</i> , <i>Lobodon carcinophagus</i> )		Mirex	5.53–17.0		
			Chlordanes	9.5–78.2		
			PCNs	0.01–3.08		
		–			ng/g wet wt	
		Dioxins	PCDDs	3.5–53.6		
		DLCs	PCDFs	8.5–96.4		
		Surfactants	PFCs	<0.4–2.0	ng/g	
	<b>Seals; liver</b>	Pesticides	Drins	6.88	ng/g lipid wt	
	<b>Southern elephant seal</b> ( <i>Mirounga leonine</i> )	OCP	Endosulfan (I/II)	2.72		
	<b>Antarctic fur seal</b> ( <i>Arctocephalus gazella</i> )		Mirex	16.2		
			Chlordanes	37.7		
		Dioxins	PCDDs	10.6	ng/g wet wt	
		DLCs	PCDFs	153.7		
	<b>Weddell seal; liver</b> ( <i>Leptonichotes weddelli</i> )	Surfactants	PFCs	<0.4–12.6	ng/g	Senthil et al. (2002)
		Dioxin	total PCDD/DFs	8.9	pg/g	
	<b>Southern elephant seal; blood</b> ( <i>Mirounga leonine</i> )	PFCs	PFOS	<0.08–3.52	ng/ml	Tao et al. (2006)

(continued)

**Table 3b** (continued)

Various chemical compounds identified in abiotic samples					
Type of sample	Compound groups/determined compounds	Range or average concentrations (± standard deviation, if available)	Unit	Literature	
Data reported in 80th years and earlier					
Snow	–	Sulphate	50–100	ng/g	Delmas and Boutron (1978)
Soil	Hydrocarbons	n-alkanes	0.6	µg/g	Platt and Mackie (1980)
Freshwater sediments	Hydrocarbons	n-alkanes	0.9–1.7		
Seawater	Hydrocarbons	n-alkanes	5.8		
Data reported from 1990 up to 1999					
Air	OCP	Heptachlor epoxide	0.52	pg/m <sup>3</sup>	Bidleman et al. (1993); Kallenborn et al. (1998)
		Chlordanes + nanochlors	1.8		
		Chlordanes	0.04–0.9		
Seawater	Hydrocarbons	n-alkanes	2.6–7.6	µg/L	Guerra et al. (2013)
		n-alkanes	353–968	ng/L	Stortini et al. (2009)
		n-alkanes	2.6–7.6	µg/L	Cripps (1992)
Seawater (particulate matter)	Hydrocarbons	Aliphatic hydrocarbons	0.07–0.17	µg/L	Green et al. (1992)
Marine sediment	OC	Chlorinated terphenyls	30–1200	ng/g	Risebrough et al. (1990)
	Hydrocarbons	Aliphatic hydrocarbons	45–48	µg/g	Green et al. (1992)
	Organic carbon	TOC	0.34	g %	Vandal et al. (1998)
Sea ice	Hydrocarbons	Aliphatic hydrocarbons	1.9–12.5	mg/m <sup>2</sup>	Green et al. (1992)

Data reported from 2000 up to 2014					
Air (gas phase)	Pesticides	Heptachlor	<1–19.1	pg/m <sup>3</sup>	Dickhut et al. (2005)
		Heptachlor epoxide	<0.3–20.7		
Air	–	Chlorinated paraffin	3.7–20.8	pg/m <sup>3</sup>	Ma et al. (2014)
	Pesticides	Aldrin and dieldrin	12.2–88.5	pg/m <sup>3</sup>	Baek et al. (2011)
	Hydrocarbons	Akryl nitrates	<LOD–1.11	ppt(v)	Fischer et al. (2002)
	Organic carbon	TOC	88–928	µg/L	Antony et al. (2011)
Snow	Surfactants	PFCs	1129.2–2491.3	pg/L	Cai et al. (2012)
	Hydrocarbons	Chlorinated hydrocarbons	<LOD–380	µg/g	Zoccolillo et al. (2007)
	Surfactants	PFCs	2121.8–5767.9	pg/L	Cai et al. (2012)
	Surfactants	PFCs	<3.0–51	pg/L	Ahrens et al. (2010)
Fresh water	Surfactants	PFCs	531.9–15,284	pg/L	Cai et al. (2012)
Surface water	Hydrocarbons	n-alkanes	0.03–0.41	µg/g	Dauner et al. (2014)
Seawater	Hydrocarbons	n-alkanes	1.1–2.1	µg/g	Negri et al. (2006)
Sediments	Hydrocarbons	Total hydrocarbons	81–144		

<sup>a</sup>CHLs: oxychlordane + cis-chlordane + trans-nonachlor + cis – nonachlor  
<sup>b</sup>Chlordanes: heptachlor + epoxides + oxychlordane + α- and β-chlordane  
<sup>c</sup>LOD—limit of detection  
<sup>d</sup>Drins: aldrin + endrin + dieldrin + isodrin  
<sup>e</sup>PFCs: PFHxA, PFOA, PFNA, PFBS, PFOS  
<sup>f</sup>LOQ—limit of quantification



Fish	<b>Notothenia; muscle</b> ( <i>Notothenia coriiceps</i> )	Antarctic Peninsula	–	<0.05	–	–	–	0.04–0.5	0.01–0.10	–	–	1.00–6.70	µg/g wet wt	Moreno et al. (1997)
Marine mammals	<b>Antarctic fur seal; liver</b> ( <i>Arctocephalus gazella</i> )	Bird Island, South Georgia; Sub-Antarctic	0.1	350	–	1	–	263	215	–	–	384	ng/g dry wt	Malcolm et al. (1994)
		Antarctic Peninsula	–	1.90–3.50	–	–	–	11.90–16.20	4.10–7.60	–	–	25.30–38.40	µg/g wet wt	Moreno et al. (1997)
	<b>Antarctic fur seal; muscle</b> ( <i>Arctocephalus gazella</i> )	Antarctic Peninsula	–	<0.05	–	1.10–1.50	–	–	<0.05	–	–	12.70–25.70	µg/g wet wt	Moreno et al. (1997)
	<b>Antarctic fur seal; kidney</b> ( <i>Arctocephalus gazella</i> )	Antarctic Peninsula	–	3.70–5.90	8.40–22.30	–	–	–	0.20–0.30	–	–	23.80–38.90	µg/g wet wt	Moreno et al. (1997)
	<b>Antarctic fur seal; fat</b> ( <i>Arctocephalus gazella</i> )	Antarctic Peninsula	–	<0.05	0.20–0.60	–	–	–	<0.05	–	–	3.20–8.40	–	
	<b>Crabeater seal; muscle</b> ( <i>Lobodon carcinophagus</i> )	Antarctic Peninsula	–	–	–	–	–	–	0.27–6.2	–	–	–	µg/g dry weight	Szefer et al. (1993)
	<b>Crabeater seal; liver</b> ( <i>Lobodon carcinophagus</i> )	Antarctic Peninsula	–	–	–	–	–	–	1.7–16.3	–	–	–	–	
	<b>Crabeater seal; kidney</b> ( <i>Lobodon carcinophagus</i> )	Antarctic Peninsula	–	–	–	–	–	–	1.7–2.5	–	–	–	–	
	<b>Crabeater seal; muscle</b> ( <i>Hydrurga leptonyx</i> )	Antarctic Peninsula	–	–	–	–	–	–	1.06–3.22	–	–	–	–	
	<b>Leopard seal; liver</b> ( <i>Hydrurga leptonyx</i> )	Antarctic Peninsula	–	–	–	–	–	–	8.78–18.1	–	–	–	–	
	<b>Leopard seal; kidney</b> ( <i>Hydrurga leptonyx</i> )	Antarctic Peninsula	–	–	–	–	–	–	4.64–6.05	–	–	–	–	
	<b>Weddell seal; muscle</b> ( <i>Leptonychotes weddellii</i> )	Antarctic Peninsula	–	–	–	–	–	–	1.18–3.61	–	–	–	–	
	<b>Weddell seal; liver</b> ( <i>Leptonychotes weddellii</i> )	Antarctic Peninsula	–	–	–	–	–	–	21.1–48.8	–	–	–	–	
	<b>Weddell seal; kidney</b> ( <i>Leptonychotes weddellii</i> )	Antarctic Peninsula	–	–	–	–	–	–	9.67–15.9	–	–	–	–	
	<b>Southern elephant seal; muscle</b> ( <i>Mirounga leonine</i> )	Antarctic Peninsula	–	0.05–0.12	–	–	–	0.57–0.68	0.17–0.19	–	–	24.37–28.58	µg/g wet wt	Moreno et al. (1997)
	<b>Southern elephant seal; skin</b> ( <i>Mirounga leonine</i> )	Antarctic Peninsula	–	<0.05	–	–	–	0.49–0.52	0.09–0.14	–	–	25.07–30.72	–	
	<b>Southern elephant seal; fat</b> ( <i>Mirounga leonine</i> )	Antarctic Peninsula	–	<0.05	–	–	–	0.54–1.46	<0.05	–	–	0.30–0.84	–	
	<b>Minke whale; blubber</b> ( <i>Balaenoptera acutorostrata</i> )	–	262.88–2050	0.03–0.28	–	1.4–8.9	–	1.63–93	0.08–0.43	6.77–443	0.7–9.5	3.64–104	ng/g wet wt	Aono et al. (1997)

(continued)

**Table 3c** (continued)

Flora	<b>Algae</b> ( <i>Desmarestia</i> sp., <i>Durvillea</i> Antarctica, <i>Adenocystis</i> sp., <i>Ascoseyria</i> sp., <i>Cytosphaera</i> sp., <i>Iridaea</i> sp., <i>Leptosomia simplex</i> st.)	Antarctic Peninsula	–	0.05–2.02	–	–	–	0.10–4.32	–	–	–	–	–	2.12–27.31	µg/g dry wt	Moreno et al. (1997)
	<b>Antarctic mosses</b> ( <i>B. argentatum</i> , <i>B. pseudotriquetrum</i> , <i>Ceratodon purpureus</i> <i>Pottia heinlii</i> )	Edmonson Point	–	0.10–0.92	–	–	–	–	0.05–0.15	–	–	–	0.3–1.4	–	µg/g dry wt	Bargagli et al. (1995)
	<b>Antarctic lichens</b> ( <i>Usnea aurantiacoatra</i> )	King George Island	262.88–1364.8	<LOD–0.015	–	–	–	1.63–5.79	–	6.77–39.16	–	–	<LOD–2.76	3.64–17.92	µg/g dry wt	Poblet et al. (1997)
	<b>Antarctic lichens</b> ( <i>Usnea antarctica</i> )	King George Island	283.37–1115.1	<LOD–0.03	–	–	–	2.17–9.49	–	15.65–56.03	–	–	<LOD–2.85	5.52–21.43	µg/g dry wt	Poblet et al. (1997)
	<b>Antarctic lichens</b> ( <i>Usnea decussata</i> )	Trishvil Hill base, East Antarctica	4966–12,760	–	–	–	4.2–3.36	45–93	–	–	–	–	4–160	–	ppm	Olech et al. (1998)
		Victoria Land	802 ± 402	0.21 ± 0.11	–	–	1.3 ± 0.6	5.3 ± 5.1	–	11.8 ± 3.9	–	–	0.54 ± 0.34	18.6 ± 4.1	µg/g dry wt	Bargagli et al. (1999)
Data reported from 2000 up to 2014																
Zooplankton	<b>Whole body amphipoda</b> ( <i>Parameira orrug</i> )	Windmill Island	–	7.2 ± 2.7	–	–	–	–	0.07 ± 0.03	–	–	–	–	–	µg/g dry wt	Bargagli (2008)
	<b>Whole pooled across species</b>	Windmill Island	–	3.4 ± 2.3	–	–	–	–	0.07 ± 0.03	–	–	–	–	–	–	–
Crustaceans	<b>Krill</b> ( <i>Euphausia superba</i> )	Admiralty Bay	72	–	–	–	–	–	34.6*	–	–	–	–	50.2	µg/g; *ng/g	Santos et al. (2006)
	<b>Pooled across species</b> ( <i>Bonellia gigantea</i> , <i>Chirimedeon femoratus</i> , <i>Gondogeneia antarctica</i> )		388–1108	–	–	–	–	–	35.0–37.0	–	–	–	–	62.1–84.1	–	–



**Table 3c** (continued)

<b>Penguin Adèle; liver</b> ( <i>Pygoscelis adeliae</i> )	Potter Cove, King George Island	–	–	18 ± 1	269 ± 10*	10.0 ± 0.2	–	202 ± 9*	–	µg/g; *ng/g dry wt	Smichowski et al. (2006)
	South Shetland Islands	1364.01 ± 351.09	0.12 ± 0.06	92.06 ± 74.53	–	12.01 ± 5.80	0.01 ± 0.01	0.04 ± 0.07	133.88 ± 71.42	µg/g dry wt	Jerez et al. (2013a)
	Potter Cove, King George Island	–	<0.07*	6.4 ± 0.4	–	1.5 ± 0.1	–	121 ± 7*	–	µg/g; *ng/g dry wt	Smichowski et al. (2006)
	South Shetland Islands	154.97 ± 66.71	0.01 ± 0.02	5.52 ± 1.97	–	1.13 ± 0.40	0.04 ± 0.03	0.04 ± 0.10	104.34 ± 49.70	µg/g dry wt	Jerez et al. (2013a)
<b>Penguin Adèle; bone</b> ( <i>Pygoscelis adeliae</i> )	South Shetland Islands	277.18 ± 135.74	0.01 ± 0.004	57.81 ± 35.82	–	10.57 ± 8.76	0.41 ± 0.41	0.04 ± 0.10	227.01 ± 121.11	µg/g dry wt	Jerez et al. (2013a)
	Antarctic Peninsula	59.74 ± 45.26	0.04 ± 0.02	13.41 ± 2.6	–	1.3 ± 1.16	0.55 ± 0.55	0.64 ± 1.09	82.45 ± 13.10	µg/g dry wt	Jerez et al. (2011)
	Terra Nova Bay	–	–	–	–	0.82 ± 0.13	–	–	–	–	Bargagli (2001)
	Admiralty Bay	87	–	–	–	1401.4*	–	–	61.5	µg/g; *ng/g	Santos et al. (2006)
<b>Chinstrap penguin; kidney</b> ( <i>Pygoscelis antarctica</i> )	South Shetland Islands	79.80 ± 62.22	0.13 ± 0.08	13.32 ± 8.22	–	2.01 ± 0.52	0.05 ± 0.03	0.24 ± 0.38	61.11 ± 20.30	µg/g dry wt	Jerez et al. (2013a)
	South Shetland Islands	397.49 ± 82.35	0.54 ± 0.29	13.64 ± 2.28	–	10.19 ± 2.63	0.08 ± 0.06	0.14 ± 0.02	92.83 ± 32.19	µg/g dry wt	Jerez et al. (2013a)
	South Shetland Islands	2075.44 ± 1745.28	0.11 ± 0.08	95.10 ± 48.67	–	11.42 ± 3.24	0.07 ± 0.07	0.18 ± 0.02	132.20 ± 64.40	µg/g dry wt	Jerez et al. (2013a)
	South Shetland Islands	328.59 ± 102.73	0.01 ± 0.01	6.82 ± 1.20	–	2.55 ± 1.53	1.83 ± 2.67	0.20 ± 0.06	105.08 ± 55.41	µg/g dry wt	Jerez et al. (2013a)
<b>Chinstrap penguin; bone</b> ( <i>Pygoscelis antarctica</i> )	South Shetland Islands	117.49 ± 40.10	0.004 ± 0.001	0.71 ± 0.36	–	12.50 ± 2.13	3.82 ± 2.52	0.14 ± 0.02	235.01 ± 40.62	µg/g dry wt	Jerez et al. (2013a)
	South Shetland Islands	173.86 ± 173.09	0.02 ± 0.03	18.57 ± 2.78	–	2.25 ± 3.17	0.13 ± 0.10	0.06 ± 0.04	94.99 ± 5.29	µg/g dry wt	Jerez et al. (2013a)
	Antarctic Peninsula	164.26 ± 149.75	0.1 ± 0.05	19.23 ± 3.65	–	3.26 ± 2.68	1.18 ± 1.1	1.76 ± 1.74	97.27 ± 21.35	µg/g dry wt	Jerez et al. (2011)
	South Shetland Islands	302.35 ± 103.68	0.20 ± 0.05	14.26 ± 4.33	–	7.54 ± 3.47	0.06 ± 0.05	0.0008	125.43 ± 12.60	µg/g dry wt	Jerez et al. (2013a)
<b>Gentoo penguin; kidney</b> ( <i>Pygoscelis papua</i> )	South Shetland Islands	854.55 ± 136.61	0.08 ± 0.04	142.40 ± 63.85	–	10.51 ± 3.74	0.01 ± 0.01	0.0008	152.91 ± 45.53	µg/g dry wt	Jerez et al. (2013a)

(continued)

**Table 3c** (continued)



**Table 3c** (continued)

Data reported from 1990 up to 1999												
Air (aerosol particles)												
Snow and firm	Antarctic Ocean	502–9550	1.3–41.6	–	16–218	–	–	–	78–224	14.2–75.8	–	Ridlein and Heumann (1995)
	Dolleman Island	–	0.08	–	–	4	–	–	–	–	0.4	Suttie and Wolff (1992)
	Adelie Land	–	0.3	–	–	5	–	–	–	–	4	Görlach and Boutron (1992)
Surface snow	Coats Land; Queen Maud Land	–	0.1	–	–	3.5	–	–	–	–	1.5	Wolff et al. (1999)
	Dome C	–	–	–	–	–	0.13–0.50	–	–	–	–	Vandal et al. (1995)
	Victoria Land	–	–	–	–	–	0.07–0.71	–	–	–	–	Capelli et al. (1998)
Precipitation (snow)	Lake Hoare	–	–	–	–	–	0.5–5	–	–	–	–	Vandal et al. (1998)
	Fresh water	–	–	–	–	–	3.3–6.8	–	–	–	–	
Seawater	Weddel Sea	–	45–102	–	–	162–358	–	–	–	87–461	–	Niemistö and Pertteli (1995)
Glacial streams	Lake Hoare	–	–	–	–	–	2.7–4.8	–	–	–	–	Vandal et al. (1998)
	Ice cores	–	0.11–0.63	–	–	0.06–0.45	–	–	–	0.58–4.5	0.42–<100	Hong et al. (1998)
Soil	Dome C	–	–	–	–	–	0.19–2.21	–	–	–	–	Vandal et al. (1995)
	Antarctic Peninsula	–	1.0–8.0	1.2–28	17–86	63–570	–	329–1138	2.9–47	<14–82	40–1301	Carrasco and Prenz (1991)
	northern Victoria Land	12,760–48,540	0.05–0.37	–	8–68	7–37	0.01–0.09	77–1356	3–29	4.5–36	29–121	Bargagli et al. (1995)
	Victoria Land	3.16 ± 0.67	0.21 ± 0.19	–	56.8 ± 27.0	38.0 ± 42.0	–	546 ± 156	–	11.3 ± 7.05	1.9 ± 21.8	Bargagli et al. (1999)

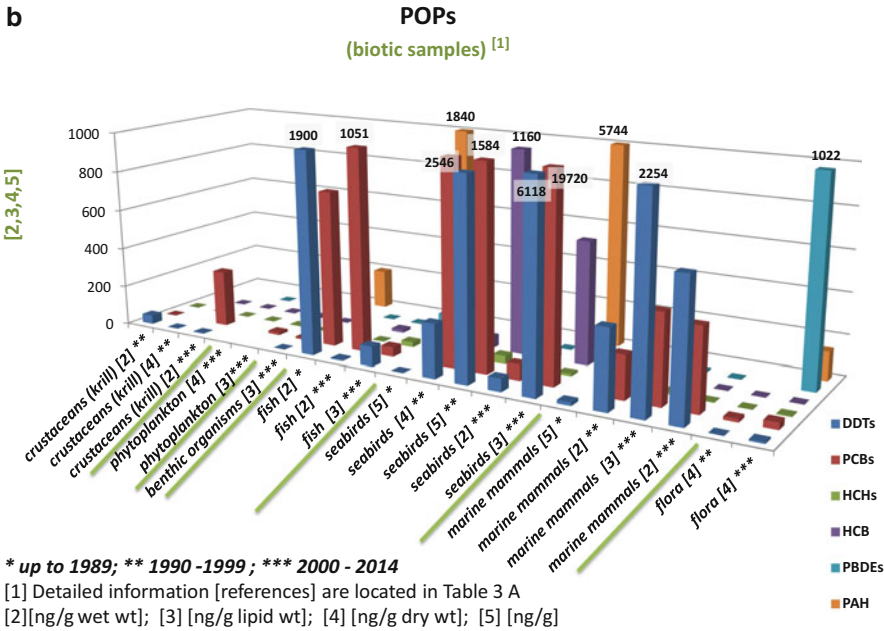
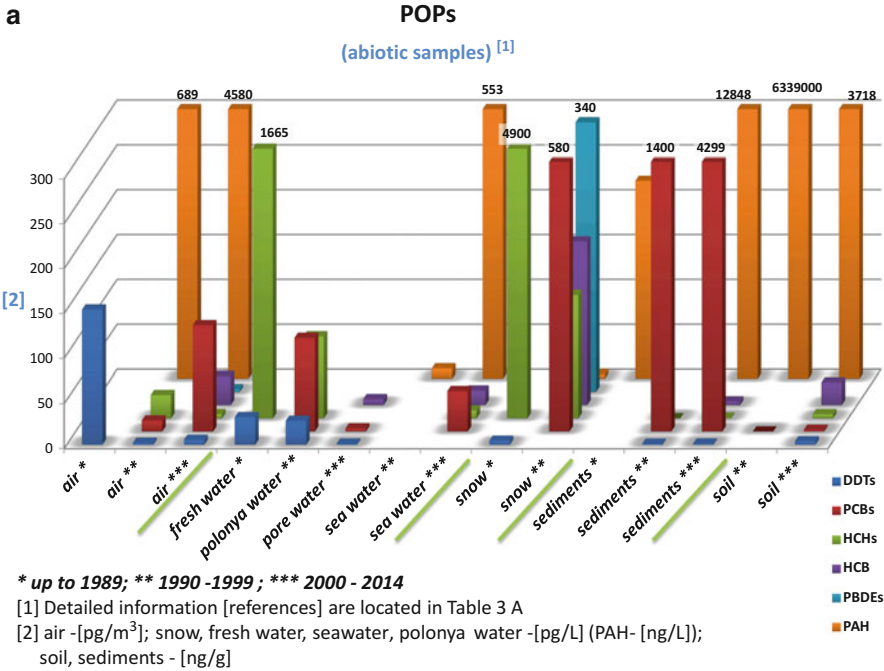


**Table 3c** (continued)

Surface snow	Ingrid Christensen Coast, East Antarctica	0.23–2.88	–	0.01–0.18	0.04–0.55	0.14–4.6	–	0.04–1.66	–	–	1.31–14.45	µg/L	Thamban and Thakur (2013)
Snow pit	Antarctic Taylor Valley glaciers (Commonwealth, Canada, Howard)	–	<0.057–0.53	–	–	<0.56–190	–	–	–	0.029–13	–	nM	Fortner et al. (2011)
	Glacier, Taylor Valley, Victoria Land	–	–	–	–	–	0.3–40	–	–	–	–	pg/g	Witherow and Lyons (2008)
	Dome Fuji	–	–	–	–	–	<0.32–2.93	–	–	–	–	pg/g	Han et al. (2013)
Firn core	Victoria Land	–	–	–	–	–	–	–	–	1.5–21.2	–	pg/g	Velde et al. (2005)
	Victoria Land	–	–	–	–	–	–	–	–	1.1–10.3	–	pg/g	Velde et al. (2005)
	Law Dome; Wilkes Land	–	–	–	–	–	–	–	–	0.08–5.2	–	pg/g	Burn-Nunes et al. (2011)
Ice core	–	–	–	–	–	–	–	–	–	0.5–124	–	nM	Edwards et al. (2006)
	–	–	–	–	–	–	–	–	–	0.21–7.00	–	pg/g	Vallelonga et al. (2002)
	Dome C	–	–	–	–	–	<1–65	–	–	–	–	pg/g	Jiratu et al. (2009)
Sediments	–	–	–	–	–	–	–	–	–	0.36–13.4	–	pg/g	Vallelonga et al. (2010)
	Ferraz station, The King George Island	6.15	–	–	40	44	–	442	5.1	11.5	52	µg/g	Santos et al. (2005)
	–	–	–	–	25–52	–	–	–	–	–	87–134	µg/g	Santos et al. (2007)
	Admiralty Bay	–	0.4–0.9	–	7–12	47–84	–	–	3–10	3–11	44–89	µg/g	Ribeiro et al. (2011)
	–	–	–	–	–	80–91	–	–	–	–	50–57	µg/g	Ribeiro et al. (2010)

	Princess Regnhold Coast	–	–	–	40–342	–	–	–	–	–	–	–	–	–	26–134	µg/g	Waheed et al. (2001)
	McMurdo Sound	–	0.03–0.46	–	–	0.9–100	–	<0.001–0.087	–	–	–	–	–	0.34–12.5	17–156	ng/g	Negri et al. (2006)
	Ross Sea	–	0.1–1.6	–	12–97	10–38	–	–	–	–	10–46	–	–	4–20	52–144	µg/g	Iamni et al. (2010)
	Amanda Bay, East Antarctic	–	–	–	–	22.3–35.3	–	55.5–281*	–	–	–	–	–	20.5–23.8	138–652	µg/g, *ng/g	Huang et al. (2014)
	Antarctic Station, Casey	–	–	–	–	–	–	–	–	–	–	–	–	5–26	–	µg/g	Townsend and Snape (2008)
	Prydz Bay	–	0.254–0.421	–	–	–	–	–	–	–	–	–	–	–	34.6–96.6	µg/g	Sun et al. (2013)
	Potter Cove	32,800–34,100	0.56–0.69	–	4.2–6.5	54–82	–	–	690–700	–	–	–	–	4.9–5.8	52–63	µg/g	Vodopivec et al. (2015)
		19,665	0.25	–	7.0	103	–	–	798	–	–	–	–	7.6	56		Curtosi et al. (2010)
		5.15–21.39	–	–	4.11–8.11	73.37–156.3	–	–	0.79–1.13*	–	–	–	–	2.29–5.52	44–96–63.02	µg/g; *ng/g	Andrade et al. (2001)
	near Lake Vanda	1.00	–	–	–	28	–	–	104	11.2	3.9	24	–	–	–	µg/g	Webster et al. (2003)
	Fildes Peninsula, King George Island,	43,255–70,534	0.04–0.34	–	17.10–64.90	51.10–176.50	0.0081–0.0601*	449–1401	7.18–25.03	2.76–60.52	–	–	–	–	41.57–80.65	µg/g, *ng/g	Lu et al. (2012)
	The King George Island	2.92	–	–	–	–	–	1100	60.7	–	–	–	–	–	–	µg/g	Machado et al. (2001)

<sup>e</sup>LOD—limit of detection



**Fig. 4** Contamination concentration levels during three time periods: (a) POPs in abiotic samples, (b) POPs in biotic samples, (c) heavy metals in abiotic samples, (d) heavy metals in biotic samples

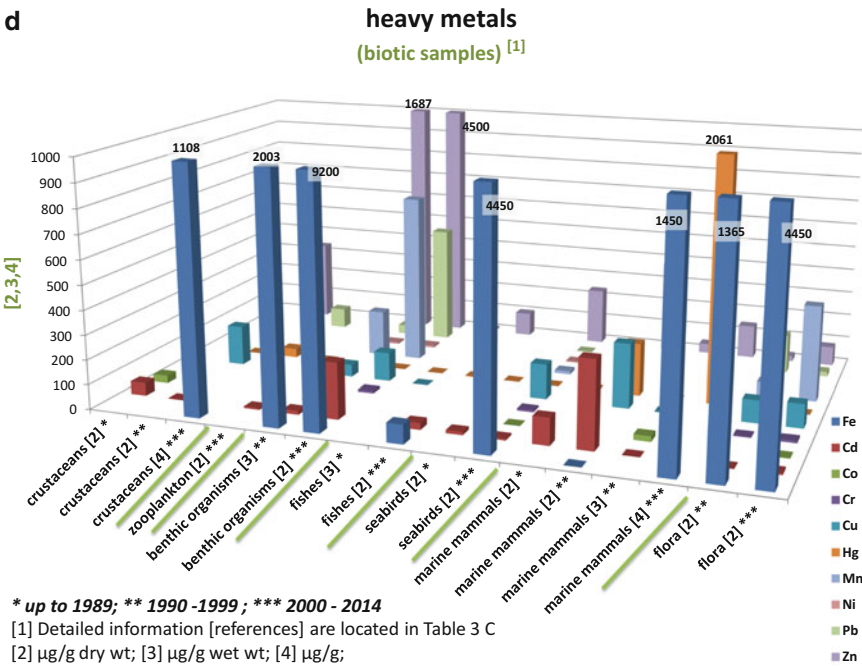
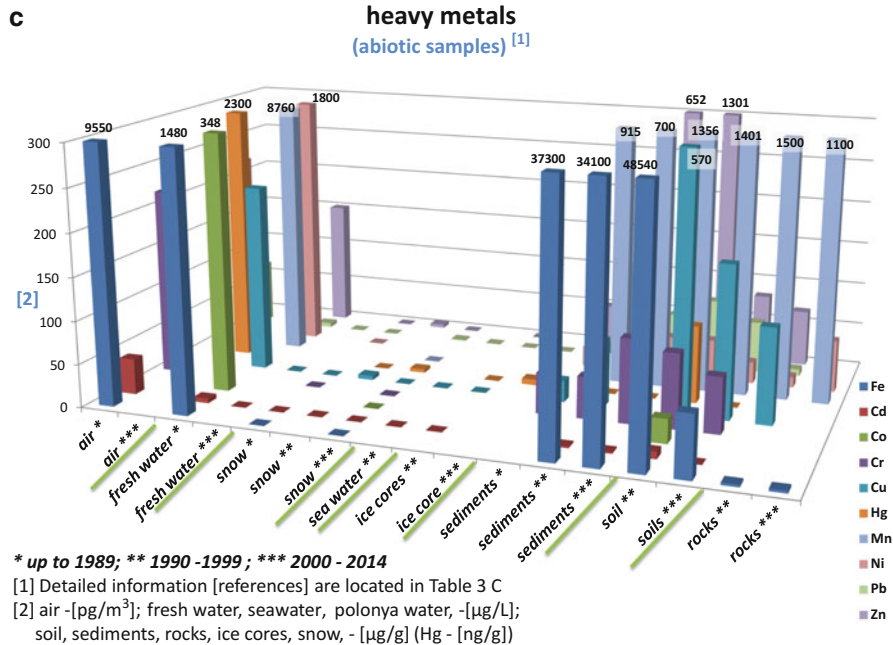


Fig. 4 (continued)

and activities of tourists and scientists can result in the detectable contaminants (PBDE, PFAS) in most stations' areas in Antarctica (Cai et al. 2012). Every part of the abiotic environment (as well as Antarctica's atmosphere and reservoirs: soil and snow) are currently closely coupled. These parts, affecting each other, have a tendency for re-volatilization of POPs to the atmosphere. These are so called secondary sources of pollution. However it is not known to what extent this remobilization is a part of a seasonal cycle with volatilization during summer and deposition during winter (Cabrerizo et al. 2013). Glacial melt may carry pollutants to nearby lakes and the adjacent coastal marine areas, thereby spreading the contamination and increasing its impacts (Majer et al. 2014). Glacier meltwater can be a current source of pollution to Antarctica's marine food web as a result of an unexpected consequence of climate change (Geisz et al. 2008). Therefore the monitoring and remediation of this scenario is essential. The active layer/permafrost transition zone was revealed to be a low-permeability barrier to downward migration of chemical compounds (Curtosi et al. 2007). Near Antarctica's stations exhibiting PAHs contamination in soils, this behaviour highlights the risk for coastal marine environments (Curtosi et al. 2007). An analysis of stations' emissions and transect sampling of abiotic matrices are carried out. The research provides indication as to the significance of research stations as local sources of POPs contamination (Bengtson Nash et al. 2010). Only few studies have determined PCB and organochlorine pesticides (OCP) concentrations in sediments in Antarctica (Zhang et al. 2013). Pollution in marine sediments are the end result of a long term accumulation and this is not directly correlating with activities on land. Unfortunately, pollutants in sediments will persist for many years to come (Kim et al. 2006), hence it is necessary to control the levels of pollution in every part of abiotic environment including sediments.

Referring to abiotic research the monitoring programs need to be extended to facility points far from major bases, assessing the extent of contamination in order to prevent local pollution episodes. This kind of studies should verify the hypothesis of a decline of PCBs in the last decade in Antarctica (Vecchiato et al. 2015).

In the discussion of biological research, what is important is using organisms for monitoring. Atmospheric monitoring of POPs using conventional instrumental methods is expensive and difficult. Scientists can overcome this limitation using biomonitoring methods and thereby provide reliable information assessing the impact of pollutants on the biota and various ecosystems. Most popular in Antarctica is using mosses to define the relationship between the concentrations of POPs in Antarctic environment and in the atmosphere (Wu et al. 2014). It should be noted, based on PBDEs studies, that mosses can accumulate more POPs than lichens (Yogui et al. 2011).

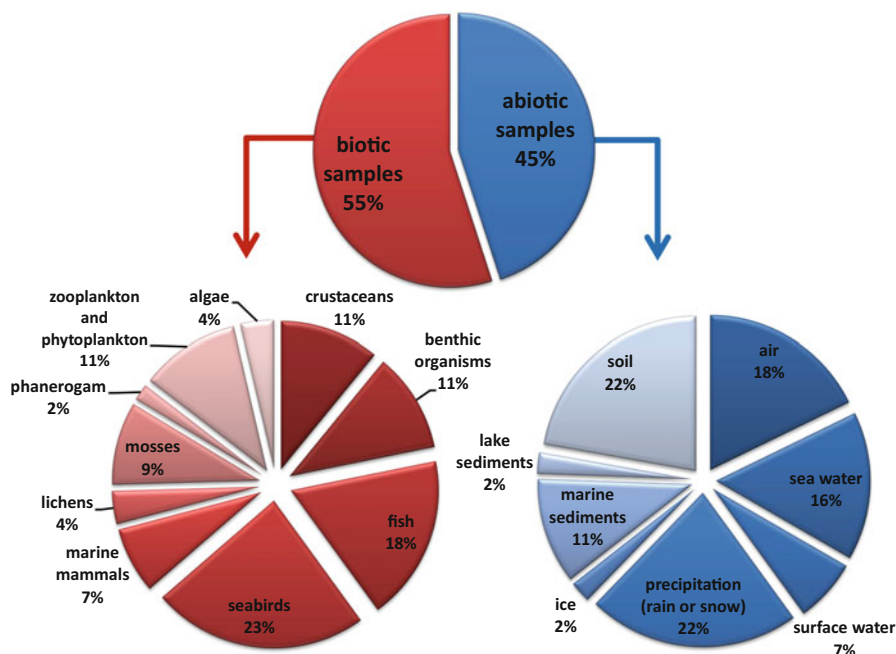
Equally important is the transport of pollution between organisms. Collected data can be useful to notice that the high concentrations of POPs encountered in the brown skua is certainly correlated to its migratory habits as well as its high trophic level position (Taniguchi et al. 2009). A useful tool to trace migration behaviour of seabirds and marine mammals can be the research of POPs levels in tissues (Kallenborn et al. 2013). Moreover, the transfer of contaminants between

Antarctica's pelagic and benthic organisms is associated with seasonal sea-ice dynamics (Van den Brink et al. 2011). The concentrations of organochlorines in penguin eggs may be toxicologically insignificant, but more studies are needed to assess the real health risks associated with these levels of pollutants because Antarctica's seals and penguins are more sensitive to contaminants than those living in temperate regions (Schiavone et al. 2009b).

In a comprehensive approach to the issue of the presence of pollutants in Antarctica, it is also very important to become familiar with accurate levels of heavy metals in this environment. In a discussion of heavy metals in abiotic environment, the geochemical characteristics of the area should be further investigated, in particular, the transport of metals as particulate or soluble fraction from the terrestrial to the marine environment (Vodopivec et al. 2015). Based on lead isotopic data, Southern South America is an important source of dust deposited in Antarctica's ice (Vallelonga et al. 2010). Moreover, based on results of research on ice cores, anthropogenic activities have become the most important source of heavy metals in Antarctica (Yin et al. 2006). Antarctica is a kind of a sink for heavy metals (e.g. Hg). Considering long atmospheric lifetime and the ability to deposit and be re-emit from soil and oceans, the ability of heavy metals to bioaccumulate suggests that their deposition would indeed have a serious effect for environment (Sprovieri et al. 2002).

Referring to heavy metals present in biological samples, particular attention should be paid to the biomagnification process which depends upon the food web (high trophic level animals have a higher content than lower trophic level ones) (Moreno et al. 1997). The presence of potentially toxic elements (such as Cd and Hg) in penguins suggest, that the accumulation of elements depends on the geochemical characteristics of the area, age of individuals and also on their diet (mainly krill) (Smichowski et al. 2006). Moreover, the results of research indicated, that a slight increase in Mn and Cr levels in Antarctica could be related mainly to human presence (usage of combustibles and oil contamination). Other studies indicate common sources of pollution (such as Cr, Ni, Pb, Mn, Cd or As), which are correlated with anthropogenic activities (plane and ship trips related to the tourism industry) (Jerez et al. 2013a). Feathers can be an important identifiers of the absorbed heavy metals (e.g. Pb) in penguins (Jerez et al. 2013b). For a better understanding of spatio-temporal trends feathers of Antarctic penguins, put together with other penguin tissues, are useful tools for long-term monitoring of trace elements in Antarctic marine environment (Jerez et al. 2011).

Furthermore mercury and its transformation products (e.g. methylmercury), because of their high bioaccumulation properties, should be investigated more precisely. A quantitative understanding of pathways and mechanisms that affect the transport of mercury from sources to ecosystems as well as the conversion of mercury to methylmercury, and their bioaccumulation in food webs are fundamental to evaluating and managing human and wildlife health risks in a local and global scale (Driscoll et al. 2013). The observations that have been made in polar marine ecosystems showed progressive increase in mercury concentrations in the food web (Bargagli et al. 1998). The role of Antarctic coastal ecosystems as sink in the global



**Fig. 5** Classification of analytical research according to types of environmental samples collected in the years 2000–2014

mercury cycle can be enhanced by the global warming and the possible change in the ice coverage together with increasing anthropogenic emissions of gaseous mercury in countries of the Southern Hemisphere (Bargagli et al. 2007). It clearly demonstrates the need for understanding how climatic variability and anthropogenic disturbances (e.g., increases of population, perturbations to food chains, changes in other air pollutants) affect mercury and methylmercury concentrations in Antarctic ecosystems (Driscoll et al. 2013; Bargagli 2008). Research data on pollutant levels has been enhanced during last two decades. Figure 5 presents information on the proportion of various types of analytical research in a general number of studies aimed at getting to know the degree of pollution of the Antarctica's environment during the last two decades.

The most popular research locations were the areas of the Antarctic Peninsula (including South Shetland Islands) and Ross Sea. A little more attention (55 % of contemporary research) is paid to tests of biological samples, mostly due to the interest in the actual influence of pollutants on Antarctica's ecosystem and becoming familiar with new directions of pollutant movement in the food web. Research on the chemical composition of inorganic samples (45 % of contemporary research), is equally important, as elements of abiotic environmental media are the first link in the pollutant movement process in Antarctica.

### ***4.3 Analytical Techniques in the Study of the Antarctic Environment***

Together with the development of science and instruments, various analytical procedures and techniques were used in analytical practice to test environmental samples (abiotic and biotic) collected in Antarctica.

Nowadays, Antarctica's researchers have gained access to many different analytical techniques of scope detection, power and robustness, which they couldn't even dream of some decades ago (Caroli 2001). For the chemical elements they can use: atomic absorption spectrometry (AAS) with flame or electrothermal (another name—graphite furnace (GF)) atomisation, inductively coupled plasma—atomic emission spectrometry (ICP-AES), inductively coupled plasma—optical emission spectrometry (ICP-OES), atomic fluorescence spectrometry (AFS), mass spectrometry (MS) with different ionization sources (e. g. ICP), X-ray fluorescence spectrometry (XRF), neutron activation analysis (NAA), ion-selective electrodes and isotope dilution mass spectrometry (IDMS). For organic substances, depending on properties of organic substances, analysts can choose one of the following techniques: gas chromatography (GC), high performance liquid chromatography (HPLC), thin layer chromatography (TLC), supercritical fluid chromatography (SFC) and gel permeation chromatography (GPC) with several detection systems (electron capture (EC), flame ionization (FI)), thermal conductivity (TC), flame photometry (FP), infrared spectroscopy (IR), UV absorption spectrophotometry, fluorescence (F), capillary zone electrophoresis (CZE) and MS (Caroli 2001). To determine ionic compound concentration the analysts use ion chromatography (IC) with various types of detection (e.g. conductometry detector (CD), ICP).

Applications with impressively high-resolution and full scan performances were made possible by modern instrumental configuration, that is hybrid mass spectrometers. Quantification of highly polar organic pollutants without derivatization, lower than the ppt level (nanogram per liter or per kilogram of matrix) in environmental samples, is possible by the use of tandem mass spectrometry combined with liquid chromatography. The measurement of emerging contaminants in environmental analysis are performed using the achievements of liquid chromatography—mass spectrometry (LC-MS) like the more recent advancements in triple quadrupole (QqQ), linear ion trap, time of flight analyzer (TOF) and Orbitrap mass spectrometers (Magi and Tanwar 2014).

Generally, the analysts are warned of pushing the instrumental method beyond its intrinsic limits, in terms of limits of detection, optimal working range and applicability to specific groups of substances. Otherwise, the rapid increase in the overall uncertainty associated with the experimental data will be observed soon (Caroli 2001).

Polar regions are an excellent place to study some natural phenomena as well as historical trends mostly due to the large distance between them and anthropogenic emissions sources. The concentration of micro-constituents or micro-pollutants in

polar regions is rather low and therefore it is necessary to develop some analytical methods of high sensitivity.

The chemical specification of such a variety of samples requires scientific experience and skills from different areas of science. The wide choice of analytical techniques, from the classical to the most innovative ones, which are available nowadays, offers the scientists an opportunity to face challenging qualitative and quantitative determinations. What is more, some more precise chemical information can be achieved by developing hyphenated methodologies, which means the combination of different instrumental techniques (Magi and Tanwar 2014).

Nowadays the most useful analytical tool seems to be the mass spectrometry, which was designed for determining a wide range of compounds present in environmental samples. In combination with such techniques as gas or liquid chromatography, it creates the possibility of specifying the organic (GC-MS, LC-MS) as well as inorganic compounds (ICP-MS) with a large degree of sensitivity and selectivity. Another advantage of such an analytical solution is the fact that MS provides more chemical information using a minimum amount of sample than any other analytical method (Gasparics and Maria 2000; Magi and Tanwar 2014; Planchon et al. 2001).

Determination of organic contaminants in various matrices is usually performed using chromatographic techniques (Plotka et al. 2013). Actual trend in chromatography is development of multidimensional approaches (e.g. Ouyang et al. 2015; Seeley and Seeley 2013). Multidimensional chromatography is a technique for isolating and identifying volatile (GC) and semi-volatile (GC and LC) organic compounds present in complex mixtures during one analytical cycle. Hence this techniques coupled with mass spectrometry can provide an important tool in a future monitoring of organic chemicals in Antarctica. Therefore, because of a low concentration of chemical compounds in complex matrices (feathers, leathers and internal organs of organisms) (Magi and Tanwar 2014), Antarctica poses a real challenge of developing innovative analytical approaches as well as improving MS instrument performances.

#### ***4.4 Impact of Research Station Activities on Pollution Levels***

Research stations are and will be an inseparable element of the Antarctic environment. Individual polar stations have a different nature. A detailed description of the operations of polar stations is presented in Table 4. The influence that each station can have on Antarctic environment is related with length of time it has been operated or/and number of people present at station etc. This information is given regularly each year by Council of Managers of National Antarctic Program (COMNAP) on its webpage (e.g. [COMNAP Information](#)). It is also important that the development of research (the use of the station and the construction of new facilities) should not additionally contribute to environmental pollution. There are numerous ways of operating stations without polluting the environment. The

**Table 4** Characteristics of polar stations operating in Antarctica (SCAR Information)

Division according to the infrastructure	
Type of infrastructure	Description
Station	– consists of durable buildings and mechanical services, – buildings are equipped with power supply and water and sewage systems
Camp	– more basic and less durable sleeping facilities are situated at the camp (tents, shelters), – these places are often used only for a few seasons,
Refuge	– has a permanent nature, – usually small and easy to install single huts
Airfield	– infrastructure (camp or shelter) is situated near the airport, it is usually connected with it, – not distinguished according to the size
Depot	– for storing food, fuel and other things
Division according to the specificity of operations	
Specificity of operations	Description
Year-round	– operate both in summer and in winter
Seasonal	– operate in summer
Closed	– the facility does not exist any more
Temporarily closed	– the facility has been closed on a temporary basis, ready to be re-opened, if necessary
Closed stations	– stations closed for an indefinite period of time – the facility can be renovated and/or re-used at any time

Princess Elisabeth Antarctica Station is an example of a station that virtually has no impact on the environment. At this station, electricity is produced using photovoltaic panels, solar collectors and wind turbines. The use of renewable sources of energy in Antarctica in the twenty-first century should not be a sign of modernity in this area, but a necessity. Reduction of potential anthropogenic pollution sources to a minimum allows to obtain reliable research results, in particular in research on long-range atmospheric transport of pollutants ([Polar Foundation Information](#)). The results of work on the design process of a photovoltaic (PV)-wind power system were recently published. This system could be installed in very challenging ambient conditions. This work has been done in the French-Italian Antarctic Base (Concordia Base). Work in this scope should be continued in other polar bases. Pollution can affect important research activities in this area (e.g. astronomical observations, studies of physics of atmosphere and Earth science). The ambient conditions significantly affect the quality of the research results. Usage of renewable energy leads to reduce usage of diesel generator and thereby leads to preserve an ecosystem, which is mandatory for heritage of the humanity (Boccaletti et al. [2014](#)).

## 5 Summary and Conclusions

The environment of polar regions is characterised by the lowest pollution levels in the world. However, the growing number of studies on the presence of a broad range of chemical compounds in various elements of the Antarctic environment may indirectly indicate the scale of the problem of growing symptoms of global human influence in this area.

Over the past decade, the scope of tested samples has been extended; however, the type of pollutants identified in individual samples (in the years 2000–2014) differs from the previous decades, *inter alia* is enhanced to new emerging pollutants. Most of the information about the presence of pollutants in biotic samples pertains to samples of Antarctic mosses krill, molluscs and invertebrates, various fish species and maritime birds—mostly penguins. Research of biotic samples have a special value as more and more attention is devoted to the phenomenon of bioaccumulation and its consequences within one plant or animal species as well as to biomagnification in the food chain. Research data about pollutants detected in abiotic samples are also important mainly due to direct and continuous contact with Antarctic biota.

A significant part of research is targeted at the occurrence of POPs compounds in the environment (Fuoco et al. 2009a, b). A possibly exhaustive list of information pertaining to POPs present in Antarctica's environment is possible only for several groups of compounds (HCB, PCB, DDTs, PBDE and PAHs). Their presence may largely result from the activity of research stations and the development of tourism. Over the past decades, sporadic research also pertained to identification and determination of compounds, such as: CHL, dioxins, DLC, PFCs, pesticides (dieldrin, mirex, heptachlor, endosulfan), aliphatic hydrocarbons, n-alkanes and cumulative parameters such as TOC in various environmental samples. In the future emerging pollutants exhibiting characteristics of persistence comparable to POPs should also be considered in long term monitoring.

Heavy metals are global pollutants and can reach almost any location on Earth. They come from natural, volcanic or geological sources, or as a result of anthropogenic activities. Accordingly with increasing human presence in Antarctic region the presence of metals in this area is becoming an issue that needs to be more investigated. Especially issues like: understanding of pathways and mechanisms that affect the transport of mercury from sources to ecosystems, the conversion of mercury to methylmercury, and its bioaccumulation state in food webs should be continuously studied.

Regrettably, data on pollutants in Antarctica's environment are dispersed in many magazines. It is worth mentioning that over the years different methods of POPs quantification have been used. Often information is scarce or lacking on the biology of the sampled species (age, sex, nutritional status, reproductive status, etc.). This makes data difficult to compare (Trumble et al. 2012). The fact that research results are presented in various units (g/g wet wt, g/g dry wt., etc.) is a further inconvenience, as it also makes it difficult to compare results of studies

conducted in various areas of Antarctica. To overcome this problem, some of scientists have presented their results expressed in multiple units (Court et al. 1997; Yogui et al. 2011); unfortunately very few researchers have done so.

Research on the influence of research stations on the pollution levels in the surrounding environment is also important. Detailed research in this areas leads to differentiate sources of pollutants between influence of local sources and global sources (LRAT). Additionally polar stations should implement usage of renewable energy in whole possible areas. This kind of solution of energy production leads to reduced usage of diesel generators and thereby lead preservation of the polar ecosystem.

The analysis of available information allows for concluding that human activity on a local and global scale leads to affecting and/or degradation of Antarctic ecosystems. The basic direction for contemporary Antarctic research pertaining to pollutants should be:

- carrying out the long term atmospheric monitoring for main POPs and new emerging pollutants coupled with meteorological data,
- carrying out the long-term monitoring of man-made chemicals (as well as new emerging pollutants monitoring) in Antarctic abiotic environment and endemic species in order to follow the future trends of global contamination,
- the detailed description of remobilization processes and “second sources” (e.g. melting glaciers) of pollutant in polar areas,
- the enlargement of research using non-invasive samples (like feathers and preen oil) as a useful tool to POPs and heavy metals monitoring,
- the determination of reaction and tolerance individual pollution levels for Antarctica’s fauna and flora towards individual anthropogenic chemicals (examination of the toxicological sensitivity of Antarctic key species),
- the detailed description of environmental fate (including biotic and abiotic environment) and negative effects on Antarctic ecosystem of anthropogenic compounds,
- the development of innovative analytical approaches improving the limits of detection of chemical compounds in various abiotic and biological matrices.

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# Important Issues in Ecotoxicological Investigations Using Earthworms

Mirna Velki and Sandra Ečimović

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M. Velki, Ph.D. (✉) • S. Ečimović, Ph.D.  
Department of Biology, Josip Juraj Strossmayer University of Osijek,  
Cara Hadrijana 8/A, Osijek 31000, Croatia  
e-mail: [mirna.velki@gmail.com](mailto:mirna.velki@gmail.com); [sandra@biologija.unios.hr](mailto:sandra@biologija.unios.hr)

**Abstract** The importance and beneficial effects of earthworms on soil structure and quality is well-established. In addition, earthworms have proved to be important model organisms for investigation of pollutant effects on soil ecosystems. In ecotoxicological investigations effects of various pollutants on earthworms were assessed. But some important issues regarding the effects of pollutants on earthworms still need to be comprehensively addressed. In this review several issues relevant to soil ecotoxicological investigations using earthworms are emphasized and guidelines that should be adopted in ecotoxicological investigations using earthworms are given. The inclusion of these guidelines in ecotoxicological studies will contribute to the better quantification of impacts of pollutants and will allow more accurate prediction of the real field effects of pollutants to earthworms.

**Keywords** Ecotoxicology • Earthworms • Soil ecosystems • Risk assessment • Biomarker responses • Toxic effects • Pollutants • Pollutant mixtures • Hormesis • Microcosmic systems • Experimental conditions • Temperature change

## 1 Introduction

Earthworms beneficially affect the soil structure and quality and consequently play a significant role in the functioning of the soil ecosystems. Also, earthworms are important model organisms in soil ecotoxicological investigations. They have been used as model organisms for assessment of adverse effects of various pollutants. However, some important issues regarding the effects of pollutants on earthworms require attention and further in-depth investigation. This review gives emphasize on several issues in earthworm ecotoxicology that are of essential importance for soil ecotoxicological investigations and risk assessment but are poorly explored and understood to date. Precisely, the importance of assessment of following issues in earthworm ecotoxicology is discussed—the linkage of responses at different levels of biological complexity, the assessment of the effects of pollutant mixtures, the occurrence and detection of the hormetic effect, the necessity of inclusion of microcosmic systems in ecotoxicological investigations and the possible effects of exposure temperature on the strength of toxic effects of pollutants. By addressing these issues it will be possible to gain a more in-depth understanding of the effects of pollutants on earthworms and soil ecosystem, as well as to improve the assessment of environmental risks of soil pollutants.

## 2 Role of Earthworms in Soil Ecosystems

The role and the importance of earthworms in the functioning of soil ecosystems is well-established. Earthworms can represent a major fraction of the soil invertebrate biomass (>80 %) and are considered as ecosystem engineers in many terrestrial ecosystems (Lee 1985; Edwards and Bohlen 1996). Earthworms play an important role in numerous soil processes and are regarded as useful indicators of soil health and quality (Edwards 2004). Many studies investigated the interactions of earthworms with soil physical conditions, with plants, with soil microorganisms and with other soil invertebrates. These studies demonstrated that earthworms impact the soil physical properties and structure, cause changes in nutrient availability and soil respiration, affect the biomass and composition of soil microorganisms, density of other soil invertebrates, composition of plant communities and aboveground food webs mainly due to their activities (Abbott and Parker 1981; Martin 1982; Doube et al. 1994; Bohlen and Edwards 1995; Fraser et al. 2003; Wurst et al. 2003; Eisenhauer et al. 2007, 2009, 2010; Boyer et al. 2013; Doan et al. 2013; etc.). The burrowing activities of earthworms cause changes in the soil structure and earthworms have important function in soil formation—they consume organic matter, fragment it, mix it with soil mineral particles and form water-stable aggregates (Edwards 2004). In addition, they play a role in decomposition, mineralization processes and in carbon storage or protection from decomposition in stable aggregates (Brown et al. 2000). The stability of aggregates is used as an indicator of soil structure (Six et al. 2000) and is a key factor for physical soil fertility (Abiven et al. 2009).

Besides their immense role in soil functioning, earthworms are important for the investigation of pollutant effects on soil ecosystems. Earthworms are continuously exposed to pollutants present in the soil (via ingestion and passive absorption through their skin) and are suitable species for ecotoxicological assessment of soil pollution (e.g. Reinecke and Reinecke 2004; Sanchez-Hernandez 2006; Zhou et al. 2007; Schreck et al. 2008; Hirano and Tamae 2011; Lionetto et al. 2012). In addition to the beneficial effects on soil functioning and common usage as model organisms in ecotoxicological investigations, it has been shown that earthworms act as promoters of soil enzyme activities (Tao et al. 2009; Jusselme et al. 2013; Sanchez-Hernandez et al. 2014). Since it was demonstrated that earthworms also increase activities of pesticide-detoxifying esterases in soil, the presence of earthworms could have direct benefit for pesticide bioremediation (Sanchez-Hernandez et al. 2014). This indicates that the presence of earthworms can affect the concentration of pollutants, i.e. earthworms can contribute to the reduction of pollutant concentrations in soil and thus may play a role in decreasing the negative effects of pollutants on soil ecosystems. Although more research is needed on this subject, this study shows that earthworms have potential to act as pesticide scavengers and reduce the concentration of pesticides in soil so their presence in agro-ecosystems is highly desirable.

Earthworms also have important roles in maintaining soil quality and in promoting the ecological functioning of the agro-ecosystems, so the reduction in earthworm populations might in different ways negatively affect the soil functioning. Taking this into account, and considering that soil and crop management practices can influence many soil properties that affect earthworms, such as effects of tillage practices to earthworms (e.g. Chan 2001; Birkás et al. 2004; Riley et al. 2008; Ernst and Emmerling 2009; Crittenden et al. 2014; Pelosi et al. 2014), wider adoption of methods that have less negative influence on earthworm biomass and biodiversity should be taken into consideration. Generally, it is advisable to strive towards preservation of earthworm populations in agricultural areas and to implement appropriate soil ecosystem management practices favoring earthworms.

### 3 Advances in Earthworm Ecotoxicology

The most important topics of ecotoxicological studies include investigation of the exposure routes, distribution, transport and accumulation of pollutants in the ecosystem and its compartments; uptake, transformation and elimination of pollutants in the environment and the evaluation of the qualitative and quantitative effects of pollutants on living organisms at all levels of ecosystem organization. Conduction of ecotoxicological studies is of great importance since it provides data useful for the risk assessment. Assessment of environmental risks of soil pollutants is particularly important as an integral part of the overall protection of the soil ecosystems, and is a necessary part of the legislation and all forms of regulations whose ultimate purpose is environmental safety.

Developments in soil ecotoxicology started with observations of pesticide effects on soil invertebrates in the 1960s (van Gestel 2012). Due to their characteristics and lifestyle, earthworms are being commonly used in standardized toxicity tests, as well as model organisms in ecotoxicological studies. The field of ecotoxicological investigations with usage of earthworms as model organisms has undergone a significant progress. From the first standardized test with earthworms (OECD 1984) to the present, there has been a significant shift—from measurement of mortality as only end-point, to measurement of a battery of biomarkers, i.e. monitoring changes at molecular and biochemical level, detection of histological changes, observation of behavioral changes and monitoring changes at the levels of populations and communities. Also, along with the usage of standardized toxicity tests, the application of model microcosmic systems, which enable more realistic conditions of earthworm exposure to pollutants, is gradually increasing (Reinecke and Reinecke 2007; Santos et al. 2011a, b; Wu et al. 2012; Velki et al. 2014; etc.). In addition, besides the usage of *Eisenia fetida* and *Eisenia andrei*, commonly used species in laboratory experiments that are usually ecologically not relevant in the environment, the importance and necessity of usage of other earthworm species has been recognized and effects of pollutants to earthworm

species from all ecological categories (epigeic, endogeic and anecic) are being investigated (LaCourse et al. 2009; van Gestel et al. 2009; Ellis et al. 2010; Tripathi et al. 2010a, b; Vejares et al. 2010; Calisi et al. 2011; Dittbrenner et al. 2011; Kılıç 2011; Klobučar et al. 2011; Velki and Hackenberger 2012, 2013a; Calisi et al. 2013; Leveque et al. 2013; Giska et al. 2014; Velki et al. 2014; etc.).

The effects of various chemical pollutants on earthworms were often the subject of research. Effects of pesticides (e.g. Venkateswara Rao and Kavitha 2004; Capowiez et al. 2010), polyaromatic hydrocarbons (e.g. Brown et al. 2004), metals (e.g. Morgan et al. 2004; Calisi et al. 2013), etc. were assessed, but there is still a large number of uninvestigated substances. Also, some very important aspects of the effects of pollutants on earthworms require further investigation. For example, in case of investigation of the effects of pesticides on earthworms, there are knowledge gaps linked to the lack of representativeness in terms of investigated pesticides and earthworm species (studies on effects of pesticides that are currently being used on earthworm species actually present in the environment are needed), and lack of studies conducted under realistic conditions in terms of soil, pesticide dose and experimental duration (Pelosi et al. 2013). Velki et al. (2014) addressed all of the current knowledge gaps. In addition, most studies are conducted only under laboratory conditions so it is essential to develop and apply tests which will more resemble the conditions in the environment (addressed in detail in Chap. 6). More recently, the usage of omics methods (e.g. Jones et al. 2008) for the toxicity assessment and measuring the effects of emerging pollutants in earthworms, especially nanoparticles and nanomaterials (Hu et al. 2010, 2014; Heckmann et al. 2011; Hooper et al. 2011; Shoults-Wilson et al. 2011a, b; El-Temsah and Joner 2012; Tsyusko et al. 2012; Hayashi et al. 2013), provided new aspects of toxicant effects on earthworms. The assessment of the effects of these pollutants on soil ecosystems is mainly in the initial stage. Ultimately it will be necessary to assess their long-term effects on earthworms at all levels of biological organization. In this context, the further application of omics approach and development of adequate protocols will certainly be a compulsory part of the risk assessment of these pollutants.

## **4 Biomarker Responses and Issue of Linkage to Higher Level Responses**

### **4.1 Biomarkers**

The risk assessment of soil pollution cannot be based solely on the chemical analysis of pollutants present in the soil (Sanchez-Hernandez 2006). The monitoring of the type and quantity of pollutants that enter into the soil is extremely consuming due to the complexity and costs resulting from the identification of such chemical substances. Since pollutants can be present in soil at concentrations below the detection limit of the analytical techniques, it is sometimes not possible

to determine their exact concentration. In addition, various factors (such as bioavailability and biomagnification) affect the overall toxicity of pollutant. Consequently, only from the data about the pollutant concentration it is not possible to accurately assess whether the pollutant will have measurable effects on soil ecosystems. So in order to assess the impact of pollutants, it is necessary to determine the direct effects of pollutants (i.e. its bioavailable fraction) on organisms and this is possible by measuring biomarkers. The term biomarker represents a measurable biological response of an organism to pollutant exposure and/or the effects of pollutants on the organism (Kurelec 1998). Biomarkers can be classified as markers of exposure, effects, and susceptibility (WHO 2001) and can be measured at molecular, biochemical, cellular, or physiological levels of biological organization (Ricketts et al. 2003). The utilization of biomarkers is of great importance for the risk assessment because changes detected at lower levels of biological organization can serve as a sensitive and early indicators of possible effects at higher levels of biological organization (Spurgeon et al. 2005), and can provide information about the mode of action of pollutants (Kammenga et al. 2000). The application of biomarkers as tools for evaluation of the effects of pollution is becoming more common and some biomarkers are already included in environmental monitoring programs.

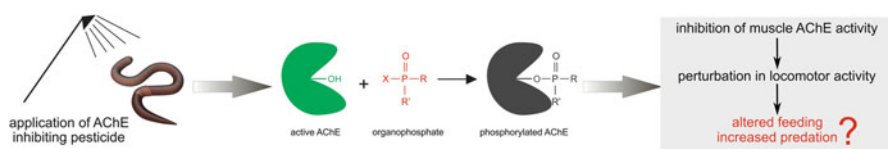
## ***4.2 Changes at Different Levels of Biological Organization***

For effective protection of ecological systems it is necessary to promptly detect the occurrence of changes. Since the probability of the repair of the ecosystem decreases with time after the entrance of a pollutant into the system, early detection of biological effects at lower levels of biological organization (molecules, cells, tissues) allows identification of changes and effective action in terms of reparation of higher biological levels (population, ecosystem). Changes at molecular level represent the earliest response of an organism to pollutant exposure and measurement of molecular biomarkers enables the assessment of impact of bioavailable fractions of pollutants, determination of the direct effects of pollutants and identification of interactions between pollutants and organisms (Sarkar et al. 2006). On the other hand, changes at the population level have much higher ecological relevance compared to changes at lower levels, but may be detected only a long period after the exposure when the probability of system repair is very small. So it is clearly evident that there is a need for establishment of links between responses at lower and higher levels in order to enable early detection of changes and at the same time to predict changes that will occur later at higher levels of biological organization. Currently, there is a lack of knowledge on the interconnection of the early biological responses with chemical exposure to ecological responses at the population and community level (Ankley et al. 2010).

### ***4.3 Establishing the Link Between Responses at Different Levels of Biological Complexity***

Studies conducted on effects of different pollutants showed that earthworms are impacted by pollutants at all organizational levels. For earthworms, a wide range of biomarkers have been developed such as monitoring of reproductive output, histopathological alterations, behavioral changes, changes in enzyme activities, gene expression, etc. And although there are numerous studies assessing effects of various pollutants on earthworms by measuring end-points at different levels, only few attempted to establish a link between these responses. Rodríguez-Castellanos and Sanchez-Hernandez (2007) proposed the inhibition of acetylcholinesterase (AChE) activity and carboxylesterase (CES) activity as potential biomarkers of pesticide toxicity at behavioral and reproductive levels. The inhibition of AChE activity was proposed as a biomarker directly implicated in behavior perturbation and the relationship between AChE inhibition and behavior perturbation has been investigated in invertebrates and vertebrates. Earthworm body wall muscles represent vertebrate-like cholinergic neuromuscular junctions (Rosenbluth 1972) which contain the enzyme AChE for regulating the synaptic transmission, and the correlation between inhibition of muscle AChE activity by anti-ChE pesticides and perturbation in locomotor activity has been demonstrated in the study of Gupta and Sundararaman (1991). The activity of CES was proposed as a biomarker that could be related to reproductive fitness. CES over-expression is a common feature in the male reproductive system of organisms as dissimilar as rodents, bivalve molluscs and insects, so Mikhailov and Torrado (1999) suggested that CES activity levels in the male reproductive system could be a determinant in the local protective mechanism for sperm differentiation and maturation against pesticides. Many studies showed that pesticides currently used in agriculture are able to cause reproductive toxicity in earthworms, and the toxic effects on the reproductive system could be correlated to CES activity levels in this tissue. More investigations are needed to enable establishment of link between changes at molecular or biochemical level and changes at higher levels.

In most studies the issue of establishing a direct link between sub-individual biomarkers and adverse effects at individual or population level was a secondary objective of research. Future studies should focus particularly on this issue and endeavor to design the experiment with the main aim of establishing these links. For instance, one of the aims could include the determination of the linkage of AChE inhibition not only to changes in behavior, but also to changes in population parameters through changes in feeding activity or predation susceptibility (e.g. inhibition of AChE activity in earthworms can cause perturbations in locomotor activity which could lead to altered feeding and increased predation) (Fig. 1). Establishing the link between low and high levels responses in earthworms is of great importance for soil risk assessment since it will enable to predict effects of pollutants on earthworm populations based on measurements of early earthworm responses, i.e. molecular or biochemical biomarkers.



**Fig. 1** Inhibition of acetylcholinesterase (AChE) activity leads to perturbation in locomotor activity and possibly to changes in population parameters through changes in feeding activity or predation susceptibility

## 5 Effects of Mixtures

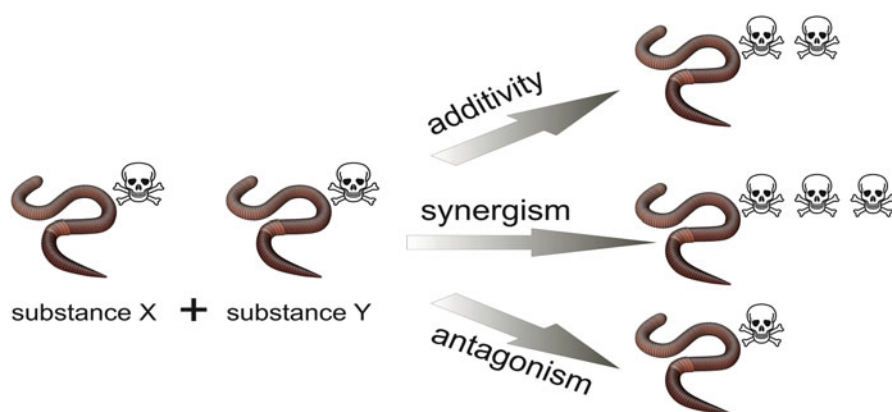
### 5.1 Environmental Pollutants as Mixtures

It is generally acknowledged that environmental pollutants exist as mixtures. Of course, the investigation of effects of mixtures is more demanding and challenging compared to effects of single compounds, especially in soil systems that are very complex. Due to occurrence of pollutant mixtures in the soil, the interactions between compounds of the mixtures and the effects of mixtures on soil components, it is of immense importance to assess the pollutant mixture effects. Spurgeon et al. (2010) provided a framework for investigation of mixture effects which highlights the importance of processes involved in determining external exposure, toxicokinetics and toxicodynamics.

The effects of mixtures and the responses of the organisms are consequences of biological activity, bioavailability, characteristics of biochemical processes in organisms and possible interactions of the components in the mixture. Interactions between pollutants in a mixture can occur at different levels and finally lead to higher or lower toxicity compared to the individual compounds. In the soil, a pollutant may affect the level of binding of another pollutant and change its availability to organisms. In organisms, a pollutant may change the detoxification process of another pollutant and therefore affect its toxicity. Consequently, the results obtained from assessment of single compound effects may lead to inaccurate estimation of the effects of pollutant mixtures present in the environment.

### 5.2 Effects of Mixtures on Earthworms

Effects of pollutant mixtures on earthworms have been investigated (Lydy and Linck 2003; Schreck et al. 2008; Gomez-Eyles et al. 2009; Zhou et al. 2011; Belmeskine et al. 2012; Stepić et al. 2013a, b; Wang et al. 2015; etc.) and different interactions between mixture compounds—e.g. additivity, synergism, antagonism—have been identified (Fig. 2). However, these studies were performed only under laboratory conditions and there is a lack of studies investigating the effects of mixtures of earthworms in terms of realistic environment.



**Fig. 2** Interactions between mixture compounds and changes in toxicity

Recently Schnug et al. (2014, 2015) investigated effects of three biocides using a soil multi-species test system and applying biocides individually and as a mixture on an earthworm community in the field. The results of these studies emphasize the importance of combining structural and functional responses, as well as different life-stages of multiple species, and imply that simple laboratory studies and more complex field and/or semi-field studies may complement one another in the risk assessment process.

### 5.3 Assessment of Effects of Mixtures

Current practices consider only the effects of single compounds (and mostly under controlled conditions), but for the soil risk assessment it is required to also address the mixture effects. Since it has been already demonstrated that due to synergistic interactions or potentiation effects mixtures can have increased toxicity to earthworms, there is an urgent need for assessment of mixture effects to earthworms. The number of studies investigating effects of mixtures on earthworms is low and a first prerequisite is to increase the number of studies dealing with issue. Secondly, it is necessary to increase the investigations of effects of pollutant mixtures to earthworms under environmentally relevant conditions (in terms of exposure methods, selection of mixture compounds, concentration, duration of exposure) and integrate the obtained data with data obtained using laboratory toxicity tests.

Besides the application of environmentally relevant tests, the aforementioned framework should be also implemented since insight on toxicokinetics and toxicodynamics will provide a better understanding of the mechanisms of interactions. Gaining knowledge on the mechanisms of interactions between compounds in the mixture would enable better prediction of its effects. This is particularly

important in the case of mixtures with different types of pollutants (e.g. mixture of pesticide and metal), as well as in the case of more complex mixtures where several compounds are present, e.g. ternary and quaternary mixtures. For example, Schnug et al. (2013) investigated the toxicity of a ternary biocide mixture to the reproduction and adult survival of two consecutive generations of earthworms and emphasized the need for more advanced mixture toxicity prediction models that consider degradation kinetics and changes in toxic effects over time.

When risk assessment of mixtures is required, testing every possible mixture combination to determine interaction and derive actual joint effects is impossible, and the prediction of the effects of mixtures is a challenging task for researchers. From the data obtained in the studies conducted so far it is not possible to make a general prediction on the effects of new untested mixtures. One of the possibilities to reduce the number of combinations to be tested is to determine the “realistic mixtures” i.e. to focus on the pollutants that actually pose a risk and determine the combinations and concentrations of pollutants that can realistically be found in the environment. After selecting and testing the mixtures of interest, different strategies can be used for analyzing of obtained data, as well as for prediction of cases with interactions between compounds. One possibility is to use mechanistic approaches, such as physiological-based pharmacokinetic modeling, to predict cases where interaction may be expected (Cahill et al. 2003). Also, Jonker et al. (2005) described a framework for analyzing patterns in the data and significance testing of statistical interactions (i.e. deviations from some standard model). This approach may be useful as a first step, but the descriptive nature precludes a mechanistic interpretation of the results, and therefore does not provide a better understanding of mixture toxicity (Jager et al. 2010). We share the opinion that understanding the effects of mixtures cannot be achieved by descriptive methods, but requires a biology-based approach for sublethal endpoints. Biology-based models are effective tools in estimating and managing ecological risks (e.g. Pastorok et al. 2003) and Jager et al. (2010) proposed biology-based mixture analysis for sublethal effects which considers toxicokinetics (going from external concentration to target site) and toxicodynamics (going from target site to effects on specific endpoints). Although more data has to be analyzed, this approach seems to be promising in mixture ecotoxicity.

Assessment of mixture effects is extremely complex and demands additional experimental investigations and development of tools that will be able, based on the mechanism of action and identification and structure elucidation, to give insight into the possible interactions between mixture compounds. By knowing the interactions between mixture compounds, as well as toxicokinetics and toxicodynamics of particular compounds, the use of compounds that are known to cause (or are anticipated to cause) a substantial increase in overall toxicity could be avoided. Also, improvement of existing and development of new assessment tools (e.g. prediction models) will contribute to quantifying pollutant mixture effects and reducing the uncertainties in current soil risk assessment arising from considering only the single compound effects.

## 6 Occurrence of Hormetic Effect

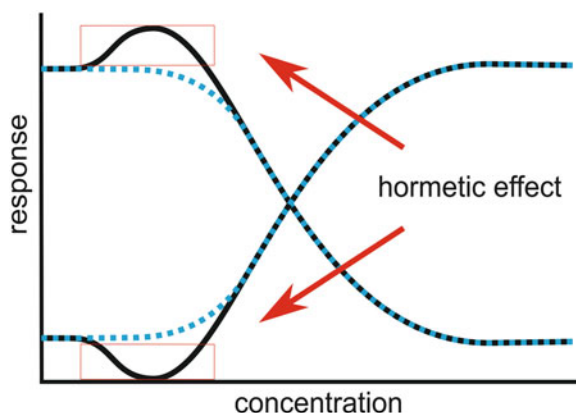
### 6.1 Hormesis

Pollutants in the environment are most commonly present at very low concentrations. However, effects of pollutants are usually investigated only at effect levels, whereas effects of concentrations below the predicted no-effect-concentrations often remain unknown. Although for long time it was considered that these sub-effective concentrations of toxic substances have no effect on organisms, today it is well-known that such concentrations of toxic substances can lead to the opposite effect than effect caused by the higher concentrations, i.e. to the appearance of hormetic effect (Calabrese and Baldwin 2002; Calabrese 2008) (Fig. 3). The hormetic dose response is often described as either an inverted U- or J-shaped dose response, depending on the end-point measured, but it is best described as a dose-time-response, in which there is an initial disruption of homeostasis (i.e. toxicity) followed by a modest overcompensation response which eventually leads to a re-establishment of homeostasis (Calabrese 2003). Previous studies have shown that this effect, i.e. hormetic dose-response model, in toxicology is much more common than the threshold model (Calabrese and Baldwin 2003). Therefore, when investigating effects of pollutants, it is particularly important to also test the sub-effective concentrations (i.e. hormetic concentrations) and to determine the possibility of occurrence of hormesis.

### 6.2 Hormetic Effect in Earthworms

Regarding the investigations on earthworms, a hormetic effect was recorded in several studies after exposure to different pollutants. Using standardized toxicity tests organophosphate pesticides caused a hormetic effect on AChE and CES

**Fig. 3** Hormetic effect—opposite effect of low and high concentrations



activity (Hackenberger et al. 2008; Velki and Hackenberger 2012, 2013b); formalin caused a hormetic effect on AChE activity (Hackenberger et al. 2012); cadmium caused a hormetic effect on superoxide dismutase (SOD) and catalase (CAT) activity (Zhang et al. 2009) and on CYP3A4 activity (Cao et al. 2012); phenylpyrazole and neonicotinoid insecticide caused a hormetic effect on reduction of biomass (Alves et al. 2013). Regarding the hormetic response detection under environmentally relevant conditions, in the study of Łaszczyca et al. (2004) earthworms were sampled in the environment and biphasic (but statistically insignificant) responses of AChE, CES and CAT activities in earthworms from zinc, lead and cadmium polluted areas were recorded, whereas Velki et al. (2014) recorded a hormetic effect of organophosphate pesticides on AChE (and CES) activity using soil microcosmic system. The occurrence of hormesis demonstrates an overcompensation response of the organism elicited by exposure to low concentrations of pollutants (Calabrese and Baldwin 2003). Detection of a hormetic effect in microcosmic systems is of great importance for future ecotoxicological research and biomonitoring studies since in realistic soil environments pollutants are found in low concentrations that could potentially cause occurrence of hormetic effect. Also, in environmental biomonitoring, the hormetic effect can theoretically be anticipated as a marker of exposure to sub-effective concentrations (for example, in case of detection of effect opposite to the expected an exposure to sub-effective concentrations of pollutants and occurrence of hormetic effect may be suspected).

### 6.3 *Hormetic Effect in Ecotoxicological Investigations*

Considering that the possibility of occurrence of hormesis in earthworms has been established, when earthworms are used for ecotoxicological parameters research, models that include hormetic effect should be taken into account. Although hormetic effects in earthworms after exposure to different pollutants has been recorded, the occurrence of hormesis in earthworms is generally poorly studied. Due to the potential significance of the hormetic effect in ecological risk assessment, the occurrence and the role of this phenomenon in earthworms should be thoroughly investigated. As pointed out by Calabrese (2003) the concept of hormesis changes the way of thinking about risk assessment. Namely, hormesis argues that there are meaningful biological effects below the toxicological no observed adverse effect level (NOAEL). So there is a challenge to use appropriate experimental designs and endpoints measured that will be able to detect and appropriately interpret the responses below NOAEL.

Although sometimes hormesis is characterized as positive or beneficial effect of low concentrations of some substances, however, this is not correct—hormetic effect is a response characterized by opposite effects of low and high concentrations. As reviewed by Calabrese and Baldwin (2003), in the context of dose-response studies it is difficult to determine the concept of a beneficial effect. Biological systems have a high complexity and beneficial effects are often seen with reference

to a specific and relative setting. So what may be beneficial at the individual level due to low-dose exposures may be harmful at population level, e.g. longevity may be enhanced at low doses but at the expense of fecundity. In case of earthworms, it was determined that different organophosphate pesticides cause a hormetic effect on AChE activity, i.e. exposure to low concentrations cause an increase in AChE activity, whereas exposure to high concentrations cause inhibition of AChE activity (Hackenberger et al. 2008; Velki and Hackenberger 2012, 2013b; Velki et al. 2014). Although it is clear that inhibition of AChE activity negatively affects earthworms due to consequent impairment in synaptic transmission, it is not known whether the increase in AChE activity caused by low doses in the long-term will be positive for earthworms. For example, it is possible that the increase in AChE activity will lead to consumption of more energy and in long-term sense it could adversely affect earthworm growth. So in order to determine the overall effect of pollutants it is essential to assess effects of sub-effective concentrations of pollutants on earthworms. This includes the determination of the mechanism of action and changes at molecular, biochemical and physiological level, as well as determination of changes occurring at higher levels up to the level of earthworm populations.

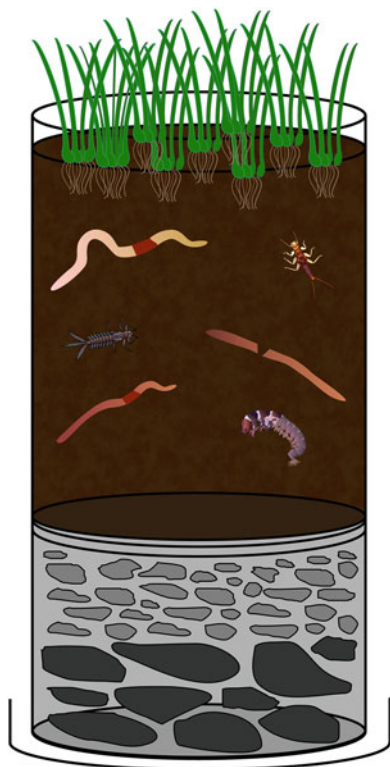
Therefore, for the proper risk assessment it is necessary to include the investigation of the effects of sub-effective concentrations of pollutants at different levels of biological organization, as well as the chronic exposure to these concentrations in order to determine the long-term effects of such concentrations on earthworms. This means that in ecotoxicological testing it is not sufficient to assess only the effects of predicted or measured effective concentrations (which has so far been the most common case), yet it is required to include the assessment of sub-effective concentrations. Taking hormesis into account will certainly lead to reconsideration of testing results of some pollutants that were regarded as non-harmful due to presence in low concentrations in the environment. Also, as previously mentioned, pollutants in the environment exist as mixtures and it is crucial to investigate the possibility of hormetic effects of mixtures on responses of earthworms in the environment in order to adequately assess the risk posed to earthworms, as well as to other soil organisms.

## **7 Inclusion of Microcosmic Systems in Ecotoxicological Investigations**

### **7.1 *Characteristics of Soil Microcosm***

A soil microcosm (Fig. 4) is a system that consists of a certain amount of soil with several species of organisms which make up a significant component of soil biocenosis and are characteristic for the area from which the soil originates. A soil microcosm serves for the assessment of pollutant effects at different levels of the biological organization (Burrows and Edwards 2002). The soil for the microcosm can be prepared by various methods (sieving, sterilization, mixing,

**Fig. 4** Schematic representation of soil microcosmic system



etc.) or can be transferred intact from the environment into the laboratory. The main advantage of applying microcosmic systems in ecotoxicological studies, compared to the classic toxicological tests, is the assessment of pollutant effects under conditions close to environmental conditions and consequently the reduction of the possibility of an inaccurate estimation of the pollutant's adverse effects.

In ecotoxicological studies using earthworms as model organisms, the advantage of application of microcosms is the possibility of usage of earthworm species belonging to all ecological categories (epigeic, endogeic and anecic). Because soil microcosms provide conditions necessary for normal activities and behavior of all earthworm species (e.g. vertical depth of soil column necessary for anecic species), it is possible to simultaneously expose earthworm species from different ecological categories and assess their susceptibility to pollutant exposure. This is of immense importance since it was determined that species belonging to different ecological category may have significantly different sensitivities to particular pollutants (e.g. Ma and Bodt 1993; Langdon et al. 2005; Robidoux et al. 2004; Lukkari et al. 2005; Velki and Hackenberger 2012; Velki et al. 2014). Also, it is known that pollutants in the environment are not homogeneously distributed in the soil, so the exposure of an organism depends on its position in the soil. Unlike in standardized toxicity tests where test substances are homogeneously distributed

at/in the exposure medium, in the microcosm the test substance is applied at a surface. The concentration of test substance in the microcosm differs in different layers of soil and depends on its physical and chemical properties. Consequently, in the microcosmic system, just as in the environment, exposure of earthworms will depend on their location in the soil, i.e. the ecological category they belong to. But although soil microcosms provide advantages compared to standardized laboratory tests, it is important to emphasize that soil microcosmic systems are still artificial systems and not real ecosystems. Although they give a more realistic data about pollutant effects on earthworms in the environment compared to standardized toxicity tests, it must be taken into account that the generalization of the results obtained in soil microcosmic systems to real soil ecosystems is limited.

## ***7.2 Soil Microcosm in Ecotoxicological Studies***

Soil microcosms have been applied for the assessment of adverse effects of different pollutants on earthworms. Santos et al. (2011a, b) investigated the effects of insecticides, herbicides and acaricides on the growth reduction and biomass of earthworms, whereas Burrows and Edwards (2002) investigated effects of fungicides on earthworm biomass. Adverse effects of organophosphate insecticides on biomarker responses of earthworms were determined in studies of Reinecke and Reinecke (2007) and Velki et al. (2014). Microcosmic systems were also used for investigation of the effects of metals (Wu et al. 2012), volatile organic compounds (An 2005) and polycyclic aromatic hydrocarbons (Bogomolov et al. 1996) on earthworms. Looking at the total number of studies focusing on the effects pollutants on earthworms, the application of the soil microcosm is just in its initial stage. Due to the above mentioned characteristics of soil microcosms and their advantages compared to standardized laboratory tests, it is necessary to include the usage of soil microcosms in the assessment of the adverse effects of known pollutants, and especially in the assessment of adverse effects of emerging pollutants.

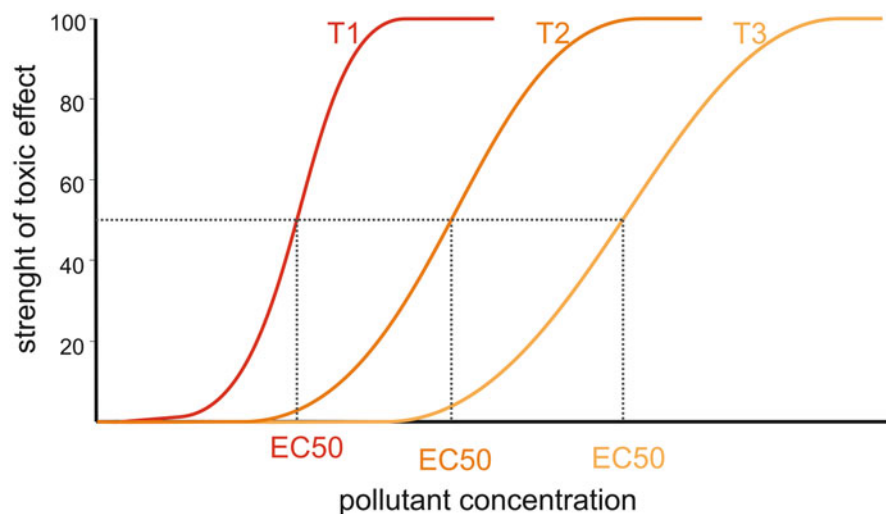
Application of microcosmic systems in soil ecotoxicological research provides significant advantages compared to standardized toxicity tests, such as realistic distribution of pollutants in the soil, simultaneous monitoring of changes at the different levels of biological organization (e.g. measurement of biomarker responses and monitoring of behavior), etc., thus enabling the implementation of experiments under environmentally more relevant conditions. It is certainly important to stress out that soil microcosmic systems are laboratory model systems, rather than ecosystems, and therefore the conditions in the microcosm are by no means identical to environmental conditions. The biological complexity of soil microcosms is lower compared to that in the real environment, but the usage of soil microcosm is still recommended since it shares some common features with real ecosystems and provides rapid testing under conditions close to the conditions of

the realistic environment. Future ecotoxicological studies should aim to improve and optimize the usage of soil microcosms in terms of increasing its biological complexity and aiming at developing systems with greater capabilities of generalization and extrapolation of the results to the situation in the environment. This could be done, for example, by including several trophic levels in a soil microcosm and assessing the effects of pollutants not only to earthworms, but also to other components of the soil ecosystem (e.g. microorganisms, other invertebrates, plants) in order to obtain broader insight into the overall impact of pollutants. Therefore, the application of such microcosmic system should be an integral part of the soil ecotoxicological studies since the obtained results can contribute to the quantification of the impact of pollutants on the environment and increase the predictive power of such studies for the soil risk assessment.

## **8 Effects of Temperature Change on Toxic Effects of Pollutants and Biomarker Responses in Earthworms**

### ***8.1 Effects of Temperature Change***

Organisms in the environment have to cope with environmental fluctuations of different abiotic factors. Many of them have developed a number of adaptations (biochemical and molecular mechanisms, behavioral changes, etc.) that enable continuous adjustment to fluctuating environmental conditions. Regarding the abiotic factors, temperature is one of the most important regulatory factors in ecotoxicology. In ectotherms, the environmental temperature affects the physiological and biochemical processes, and changes in temperature can act as a stressor and lead to changes in the physiological status of the organism. Previous studies have shown that changes in temperature can affect the behavior of earthworms, lead to changes in survival, growth, development and cause changes in metabolic enzymes (e.g. Presley et al. 1996; Fayolle et al. 1997; Wever et al. 2001; Perreault and Whalen 2006; Tripathi et al. 2011). So it is very likely that changes in temperatures will affect the enzymatic processes that determine the metabolism and detoxification of pollutants. In addition to the biotic component, temperature changes can affect the fate and transport of pollutants in the environment. For example, degradation of pesticides is slow at lower temperatures and faster at higher temperatures (e.g. Pal et al. 2006), leading to different stability and retention in the environment which can affect the pesticide toxicity. It is obvious that changes in temperatures at which earthworms are exposed to pollutants in the environment can ultimately lead to changes in the strength of the toxic effect (Fig. 5).



**Fig. 5** Changes in temperature may lead to changes in the strength of the toxic effects of pollutants

## 8.2 Interactions Between Temperature Change and Pollutant Toxicity to Earthworms

Holmstrup et al. (2010) provided a comprehensive review on the interactions between effects of environmental chemicals and natural stressors, including the interactions between effects of temperature stress and chemicals in different organisms. Regarding the earthworms, in many studies effects of various pollutants on earthworms have been assessed (see Sect. 3); however, there is a smaller number of studies investigating the effects of pollutants under different (stressful) temperatures and the data on the impact of exposure temperature on the final effects of pollutants and biomarker responses of earthworms are scarce. In case of the effects of temperature on metal toxicity to earthworms, Khan et al. (2007) found that an increase in temperature caused an increase in the toxicity of metals Pb, Cu and Zn to *Lumbricus terrestris*. They hypothesized that the increase in toxicity at higher temperatures may be due to limiting the scope of aerobic metabolism (oxygen extraction, transport, and utilization) via quantitative and qualitative effects on hemoglobin. Svendsen et al. (2007) investigated the short-term survival, reproduction and physiological responses of *Lumbricus rubellus* exposed to metal contaminated field soils under different laboratory temperatures and physiological responses of earthworms collected from the field in three different seasons, however no effect of temperature on metal toxicity was determined. Synergistic interaction between freezing temperatures and Cu has been observed in *Dendrobaena octaedra* and *Aporrectodea caliginosa*, and between freezing temperatures and Hg in *D. octaedra* (Holmstrup et al. 1998; Bindesbøl et al. 2005, 2009a, b). Bindesbøl et al. (2009a) hypothesized

that reduced tolerance to freezing temperatures in Cu-exposed earthworms may be due to changes in membrane phospholipids and consequently membrane damage. Wieczorek-Olchawa et al. (2002) investigated effects of temperature and metal polluted soil on *D. veneta*. Better survival in polluted soil at 10 °C compared to 22 °C can be explained by the reduced tissue metal accumulation and mesophilic bacteria proliferation at the lower temperature. Regarding the pesticides, effects of freezing temperatures on toxicities of abamectin and carbendazim were investigated, but no interactions were determined (Bindesbøl et al. 2009b). De Silva et al. (2009) investigated influence of temperature and soil type on the toxicity of pesticides to *E. andrei*. They determined that effects of chlorpyrifos and carbofuran on earthworm survival, growth and reproduction in artificial soil may be higher at higher temperature, whereas carbendazim toxicity was lower at high temperatures. However, there was no clear trend of increased toxicity for sub-lethal endpoints with temperature and toxicity varied with the pesticide, endpoint, soil type and temperature. Using also *E. andrei*, Lima et al. (2015) showed a tendency to synergism when exposed to combined carbaryl and high temperatures, and antagonism when exposed to carbaryl and low temperatures. Another study performed by Garcia et al. (2008) assessed effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions and the results of the avoidance tests did not give a clear answer whether data from tests performed under temperate conditions can be used for pesticide risk assessment in tropical regions. In our recent study (Velki and Ečimović 2015) the toxicity of several insecticides, fungicides and herbicides on the mortality of earthworms at low and high temperature was assessed and an increase in exposure temperature generally led to an increase in toxicity, whereas a decrease in exposure temperature led to a decrease in toxicity. However, there were also some discrepancies (e.g. in case of herbicides fluzifop-p-butyl and glyphosate) in changes in toxicity. Besides metals and pesticides, the effect of temperature was investigated also on the toxicity of surfactant 4-nonylphenol and polycyclic aromatic hydrocarbons pyrene and phenanthrene (Bindesbøl et al. 2009b; Jensen et al. 2009).

### **8.3 The Need for Further Assessment of the Effect of Temperature on Pollutant Toxicity to Earthworms**

It must be emphasized that there is an urgent need for determination of effects of temperature on the strength of toxic effects of pollutants present in the environment. As evident from the above mentioned studies, some of the results obtained in the conducted investigations are not consistent and validation studies performed under environmental conditions are necessary. Due to climate changes, which include an increase in soil temperature and increased exposure to extreme weather conditions, organisms in the environment are more frequently exposed to temperature stress which can result in greater susceptibility to pollutants. So it is

important to explore effects of temperature stress on physiological status of earthworms, propensity to intoxication and strength of the toxic effects of pollutants. Forthcoming toxicity testing of pollutants should be conducted at adequate temperatures, taking into account possible changes in temperature by utilizing the predictions of climate change. Given that climate change projections indicate that the frequency, intensity and duration of extreme climate events will increase in the future, besides determination of pollutant effects under different exposure temperatures, it is also necessary to assess the effects of preexposure to the extreme temperatures. Finally, there is a need for determination of the response of different tissues to temperature stress. Because of the different functions and characteristics, different tissues may have different sensitivity and may be more or less exposed to temperature stress. In this context, they can manifest different stress responses to temperature change. Determination of the sensitivity of certain tissues will provide an insight into the mechanisms of the effects of temperature stress on the toxicity of pollutants.

## 9 Conclusions

In the field of soil ecotoxicological research there has been an apparent shift—from measurement of only mortality and usage of laboratory toxicity tests for assessment of pollutant effects to the application of model systems that enable obtaining environmentally more relevant data and assessment of effects at different levels of biological organization. However, some important issues in ecotoxicological investigations require more in-depth research. In order to make ecotoxicological testing more effective and the data obtained more applicable for environmental risk assessment, the following guidelines should be adopted in ecotoxicological investigations using earthworms:

1. Establish the link between responses of low levels of biological organization and changes observed at higher levels.

The success of utilization of biomarker responses in the environmental risk assessment depends on the identification of valid biomarkers and the establishment of process-level linkages between biomarkers and higher-level responses (Adams 2003). Understanding this link will enable the prediction of long-term pollutant effects based on measurements of early responses.

2. Assess the effects of pollutant mixtures.

Since pollutants in the environment are most commonly present as mixtures, their effects have to be assessed. Prerequisite of such investigations is to assess the effects of realistic mixtures (combinations and concentrations of pollutants that can realistically be found in the environment) in order to reduce the needless testing. The changes in toxicity that can arise from interaction between compounds present in the pollutant mixture must be taken into account in the risk assessment.

3. Consider the possibility of occurrence of hormetic effects under environmental conditions.

Pollutants in the environment are most commonly present at very low concentrations, but are usually assessed only at the effect levels. Effects of such low (sub-effective) concentrations often remain uninvestigated although it is known that they can cause effects opposite to the effects caused by the higher concentrations, i.e. the hormetic effect. Due to the incidence of hormesis and its potential significance in ecological risk assessment, in order to adequately assess the risk posed to earthworms, it is crucial to consider and investigate the occurrence of hormetic effects.

4. Use of soil microcosmic systems in ecotoxicological investigations.

Soil microcosmic systems provide significant advantages compared to usage of standardized toxicity tests. Although these are artificial systems, they share some common features with real ecosystems and therefore provide more realistic data compared to laboratory toxicity tests. Since application of soil microcosms can contribute to the quantification of the impact of pollutants, its utilization should be an integral part of the soil ecotoxicological studies.

5. Explore how temperature affects the toxicity of pollutants and earthworm responses in order to determine possible changes in pollutant toxicity.

Temperature is an important factor in ecotoxicological investigations since it affects physiological and biochemical processes in organisms. Due to climate changes, organisms in the environment are more frequently affected by temperature stress and can therefore be more susceptible to pollutants. Having regard to the prediction of climate change and exploring the effects of pollutants on earthworms under temperature stress, a better insight and prediction of the effects of pollutants in the environment can be obtained.

The inclusion of these guidelines in ecotoxicological studies will contribute to the better quantification of impacts of pollutants in the sense that it will support a more realistic approach in monitoring the adverse effects of pollutants on earthworms. Addressing these issues and promoting the assessment of pollutant effects under conditions mimicking those that occur in the environment will provide insight in the pollutant adverse effects on the soil ecosystem as a whole and will allow more accurate prediction of the real field effects of pollutants.

## 10 Summary

The role and importance of earthworms in the functioning of soil ecosystems is well-established. Due to their feeding and burrowing activities, earthworms beneficially affect the soil structure and quality. Also, earthworms have proved to be important model organisms for investigation of pollutant effects on soil ecosystems. Assessment of effects of various pollutants on earthworms was often a subject of research and valuable data was obtained. However, in future soil ecotoxicological studies it is necessary to address some important issues regarding the effects of

pollutants on earthworms which require further in-depth investigation. The aim of this review is to emphasize and discuss several issues in soil ecotoxicological investigations using earthworms that are of essential importance for risk assessment but are poorly explored and understood to date.

In order to enable an early detection of pollutant effects and prediction of subsequent adverse effects at population and ecosystem levels, it is necessary to establish links between biomarker responses at different levels of biological complexity. Gaining such information will help in developing protocols for predicting effects of pollutants on earthworm populations based on measurements of early earthworm responses.

Pollutants in the environment are commonly present as mixtures and the results of previous studies have provided clear evidence that the interactions between compounds of the mixtures could lead to changes in the intensity of the toxic effect. Therefore it is crucial to assess mixture effects to earthworms under environmentally relevant conditions and to develop methods for assessing the risks of pollutant mixtures. Also, since pollutants in the environment are often present at low concentrations, the possibility of occurrence of hormesis has to be considered and the understanding of hormesis mechanisms has to be addressed in detail.

One of the major future research goals should be to improve the understanding of how experimental conditions affect the responses of earthworms to pollutants. In this sense, the application of environmentally more relevant experimental conditions and inclusion of microcosmic systems in the assessment of pollutant effects is discussed. Also, consideration of effects of climate change to the strength of toxic effects of pollutants and biomarker responses of earthworms is taken into account.

Inclusion of these issues in future investigations will enable obtaining comprehensive data which will facilitate development of new assessment protocols and improved guidelines for better quantification of impacts of pollutants on earthworms and soil ecosystems.

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