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Modes of action of the current Priority Substances list under the Water Framework Directive and other substances of interest

Review of the Relevant Modes of Action

Dorota Napierska, Isabella Sanseverino, Robert Loos, Livia Gómez Cortés, Magdalena Niegowska and Teresa Lettieri

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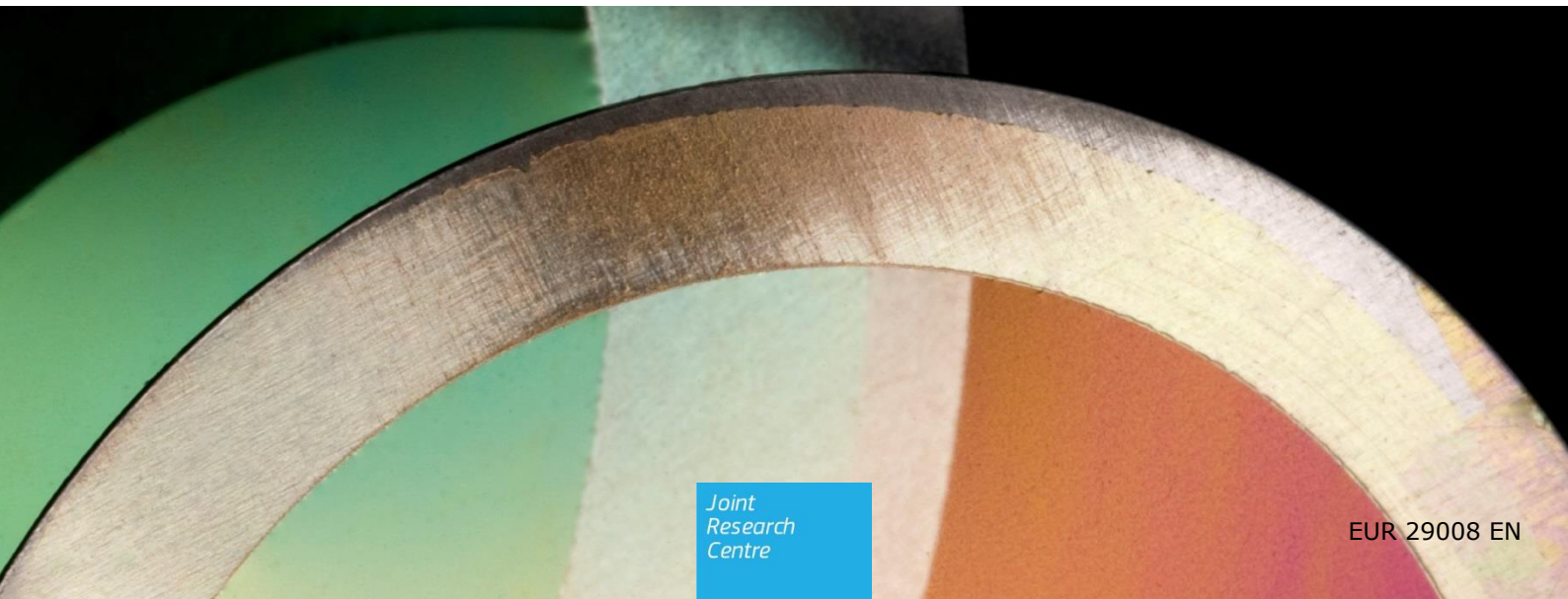

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Contents

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Executive summary

Aims of the report

The first main objective of this report is to review the current state of knowledge of the mode of action (MoA) and effects of the priority substances (PS) in the Water Framework Directive (WFD), and of other substances of concern included in the first Watch List (WL) and current exercise to prioritise candidates for the PS list. The second is to evaluate whether these substances can be analysed by effect-based methods (EBMs) in order to provide a more accurate assessment of the risks related to chemicals and their mixtures in the aquatic environment.

For this purpose, these substances were classified into group categories depending on their use/chemical structure classes, their relevant MoA and related potential to be analysed using EBMs.

Introduction

A better understanding of the MoAs of the substances present in water and their potential interactions is crucial for water quality assessments.

Depending on their MoA, these chemicals can exert additive, synergistic or antagonistic effects, which in the second case means that the toxicological effects of combinations of chemicals may be greater than the sum of the effects seen when they are present individually in the aquatic environment. Combination effects are not taken into account when individual environmental quality standards (EQS) are set. In this context, EBMs have been proposed as a way to explore the real effects caused by the sum of the chemicals present in the aquatic environment, and to capture the effects of related substances rather than having to list and monitor them individually. These methods, which include biomarkers and bioassays, could complement the analytical chemistry methods currently used, and constitute a useful tool for environmental risk assessments.

Findings

The analysis of the literature data identified different MoA, toxicological endpoints and effects with different specificity as well as non-specific effects.

Moreover, it was observed that bioassays were sensitive to combinations of pollutants that exert the same effect, at concentrations below their individual EQS. However, it was not possible to identify an EBM that could account for all the relevant effects (including on different organisms) of each PS, alone or in combination. Furthermore, certain factors (e.g. toxicokinetics and toxicodynamics) other than concentration may influence the toxicity of the substances, therefore even where an *in vitro* bioassay result might be expected to correlate with the results of field measurements (e.g. of biological quality elements contributing to ecological status), there may not be an exact correlation.

To predict the toxicity of a chemical mixture, data on the MoA of each component of the chemical mixture is required. Unfortunately, for some groups of chemicals the MoA remains unknown, highlighting the need for further investigation in this area.

Conclusions and Recommendations

A battery of bioassays is proposed that could be used to assess the chemical status of water environments more holistically (rather than with a limited but ever-growing list of individual EQS), and to try to overcome analytical difficulties and reduce monitoring costs.

For this purpose, a more systematic approach should be developed in order to define which panel of assays might be of greatest use for the specific circumstances (e.g. for the combination of substances that might be found). Besides, an intercalibration exercise will be required to ensure comparability among bioassays focussed on the same MoA.

Abstract

Hundreds of different substances may co-occur in the aquatic environment, and even if most are present at very small concentrations they could exert a toxic effect on aquatic organisms (Carvalho et al. 2014) exposed for their entire life cycle and indirectly on other organisms including humans (via prey/food and (drinking) water consumption). The Water Framework Directive 2000/60/EC (WFD) has established a strategy for water protection that includes specific measures for pollution control to achieve good chemical and ecological status at European level. Some of the substances in the current list of priority substances (PS) and in the first Watch List (WL) are considered in groups (e.g. brominated diphenylethers, neonicotinoid insecticides), but the overall approach to chemical pollution is otherwise based on the regulation of single substances, and inevitably doesn't cover as many as are probably relevant. It has become increasingly clear that the risks from the vast number of chemical substances present in the environment, including their metabolites and transformation products, cannot be adequately controlled on this basis. The Commission acknowledges the need to consider the potential toxic effects of mixtures of chemicals (EC COM(2012)252, 7th EAP). The challenge is to find a way to capture a more holistic picture of the chemical status of water bodies capable to reflect the cumulative or combined risk. One possible approach could be to use standards and methods that assess the presence of an adequate range of representative chemical effect types or modes of action (MoA).

Knowledge of the MoA allows exposure to chemicals to be linked to their effects in the aquatic environment, and the development and application of effect-based methods (EBMs) for assessing the combined effects of chemicals. The EBMs, including biomarkers of effects and bioassays, can target different levels of biological organisation in the aquatic environment, such as individual and/or sub-organism, community and population levels (Carvalho et al. 2014, Wernersson et al. 2014). It is however much less clear how these EBMs can be used to capture (predictively) the indirect effects that might occur in humans following long-term chronic exposure to pollutants via the aquatic environment.

The use of effect-based monitoring approaches, complementary to chemical analysis, could allow chemical status to be assessed more holistically (rather than with a limited but ever-growing list of individual substances). The use of EBMs offers also the advantage of overcoming some of the analytical difficulties (Kunz et al. 2015) and could reduce monitoring costs if employed for screening. EBMs could also help establish a link between chemical and ecological status. To become a credible complement to chemical monitoring information, however, a better understanding of the capabilities and deficits of available EBMs is needed.

This report, based on a comprehensive literature study, reviews the current PS list and other substances of interest, considering their MoAs. The review of data from the open sources clearly identified a few groups of toxicological endpoints, with the majority driven by non-specific mechanisms (e.g. oxidative stress, activation of metabolic/detoxification pathways, histopathology), and a few groups with more specific biochemical/physiological pathways (e.g. photosynthesis inhibition, acetylcholinesterase inhibition expression of metallothioneins).

The majority of current PS and other substances of interest can be grouped, based on a few common toxicological endpoints, and biomarkers are available for determining the concentrations and/or effects of some groups of substances. However, the identified biomarkers of effect seem not to be very specific. There is clearly no "one size fits all" EBM

that could determine the toxicological potency of every PS and other substance of interest or their mixture in relation to all aquatic organisms, but rather a battery of EBMs that should be selected as "fit for purpose".

The present report allowed identification of uncertainty and inconsistency in observations, and thus identified areas where future investigations can be best directed. The present knowledge about MoAs remains limited, especially for certain substances of emerging concern, such as pyrethroids and neonicotinoids.

1 Introduction

1.1 The Water Framework Directive, a brief description

Aquatic populations in lotic, lentic, wetland, and marine environments are annually exposed to waterborne chemical compounds at concentrations ranging from ng/L to µg/L (Carvalho et al. 2016). When pollutants enter aquatic habitats, direct (toxic) effects on aquatic biota and indirect effects on human health (via food and drinking water consumption) as well as other organisms are possible. Those effects vary with the intensity and duration of exposure to a toxicant, as well as with the species and the specific endpoints. Biota from a given habitat often exhibit a wide range of tolerance to specific toxicants with the consequence that a substance may exert lethal effects on some species, but cause no (observable) effects on others.

The Water Framework Directive 2000/60/EC (WFD)[1] has established a strategy for water protection that includes specific measures for pollution control to achieve good ecological and chemical status at European level. Good chemical status is defined in terms of compliance with European environmental quality standards (EQS) for priority substances (PS).

PS are substances identified as posing a significant risk to or via the aquatic environment at EU level, according to Article 16(2) of the WFD, The EQS are the environmental threshold concentrations in water, sediment or biota that should not be exceeded in order to protect the environment and human health. The PS are listed in Annex X to the WFD, which also identifies priority hazardous substances (PHS), i.e. the PS that are persistent, toxic and liable to bioaccumulate, or that give rise to an equivalent level of concern. Member States should take measures to progressively reduce the pollution from PS and to cease or phase-out discharges, emissions and losses of PHS.

The first list of PS in the field of water policy was published in Commission Decision 2455/2001/EC, and subsequently confirmed in Directive 2008/105/EC on environmental quality standards (EQS Directive)[2], which included EQS for the 33 PS or groups of PS and eight so-called "other pollutants". Annual average (AA) EQS and maximum allowable concentrations (MAC) EQS protect against long-term exposure and short-term peak concentrations, respectively, and are listed in Annex I to Directive 2008/105/EC.

Under Article 16(4) of the WFD, as amended by Directive 2013/39/EU[3], the Commission is required to review the list of substances designated as PS and PHS every six years. Each review comprises an assessment of existing PS and PHS, and also a review of candidate substances for consideration as new PS. The first review process was done between 2007 and 2011, resulting in 12 new PS or PS groups being added to the list, 6 of which are identified as PHS, as published in Directive 2013/39/EU amending Directive 2008/105/EC.

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(1) EU Water Framework Directive 2000/60/EC. "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy". http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32000L0060

(2) Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32008L0105

(3) DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF

During that review, the information (and EQS) on existing PS was also updated and the results of the hazard assessment led to the reclassification of two PS as PHS.

As it stands, the list of PS in Annex I to Directive 2013/39/EU[3] (replacing former Annex 10 of the WFD) contains 45 PS or PS groups, with 21 classified as PHS. Directive 2013/39/EU also set up the so-called Watch List (WL) mechanism for surface water, to gather more monitoring data for substances suspected of posing a risk at EU level. The first WL was established by Commission Decision 2015/495/EU and comprises 10 substances/groups of substances, including Diclofenac, 17-Alpha-ethinylestradiol and 17-Beta-estradiol.

The current review of the list of PS has also identified 10 candidate substances of potential interest at EU level (Carvalho et al. 2016, Lettieri et al. 2016), further considered in this report.

1.2 Taking into account mixtures in risk/effect assessment

There is an increasing awareness that chemicals occur in the environment as components of very complex mixtures (Beyer et al. 2014), and assessment criteria developed for each individual substance do not usually consider the consequence of simultaneous exposure to multiple chemicals (Carvalho et al. 2014; the 7th Environment Action Programme[4]). Environmental regulatory frameworks within the EU, such as the WFD and REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) focus mainly on toxicity assessment of individual chemicals. Only in a few instances does the assessment cover cumulative risks from exposure to multiple chemicals, for example, the European Food Safety Authority (EFSA) has developed approaches for taking account of cumulative and synergistic effects when setting maximum residue levels (MRLs) for pesticides with similar mode of action (MoA) (EFSA 2008 and 2009). We need to examine whether or not mixtures originating from different sources and through different pathways, in which each of the substances is present at very low concentrations, could have negative effects on the environment or human health (EC COM(2012)252).

The requirement set down in the WFD for water bodies to achieve good ecological status as well as good chemical status entails a focus not only on the risk posed by individual chemicals but also on their effects in combination. Assessing chemical status by chemically analysing a growing number of individual priority substances (PS) is presenting an increasing challenge. Chemical analysis generally requires a priori knowledge about the type of substances to be monitored whilst, for technical and economic reasons, it is not possible to analyse, detect and quantify all substances that are present in the aquatic environment. There is also a need to understand which effects are caused by the sum of the chemical substances in the aquatic environment (including emerging pollutants, metabolites and transformation products). Therefore, the use of effect-based methods (EBMs), i.e. scientific methodologies for the assessment of combined effects of chemicals has been mentioned in the context of the WFD in the Common Implementation Strategy (CIS) guidance no.19 (on water chemical monitoring), in the CIS guidance no. 25 (on sediment and biota monitoring), and (in relation to sediment assessment) in the CIS guidance no. 27 (on environmental quality standards, EQS). The Marine Strategy Framework Directive (MSFD) has foreseen the use of EBMs; in particular, the indicators

(4) Decision No 1386/2013/EU of the European Parliament and of the Council of 20 November 2013 on a General Union Environment Action Programme to 2020 'Living well, within the limits of our planet' http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32013D1386

related to descriptor 8 of the MSFD should also include effects from hazardous substances on ecosystem components. EBMs are sometimes required by national and regional authorities, for example in investigations of dredged sediment and at contaminated sites as well as within Whole Effluent Assessments (WEA); current use in some European countries is briefly described in the report by Wernersson et al. (2014).

1.3 Modes of action, biomarkers and bioassays: basic definitions and examples

The toxicity of mixtures will depend on the bioavailability and chemical reactivity of the compounds. To gain greater insight into the risks posed by environmental contaminants, it is beneficial to understand their **mode of action (MoA)**. According to the EC Scientific Committees, a MoA is a plausible hypothesis about measurable key events by which a chemical exerts its biological effects. The MoA is increasingly applied in computational models on prediction of the toxicity of mixtures. The joint action of different molecules with similar or different modes of action could result in a potentially unlimited number of additive, synergistic or antagonistic combinations. Since the large number of contaminants makes it impossible to perform ecotoxicity tests for each potential mixture, a robust approach for prospective environmental risk assessment of chemical mixtures is needed.

The MoA is basically the process initiated by the interaction of the toxicant with the receptor which progresses through molecular, biochemical, physiological and/or anatomical changes in the organism to result in sub-lethal and lethal effects. Identification of MoA can lead to an understanding of the biological receptor targeted by a particular chemical, and extrapolation to anticipated effects/production of a particular biological response (Borgert et al. 2004). Such effects/response can be detected by means of **biomarkers**, which are broadly defined as indicators signalling events at individual level or below. We have classified them in this report as markers of exposure and markers of effect.

A **biomarker of exposure** is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in body tissues and fluids. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposure to more than one source. Depending on the properties of the substance (e.g. biological half-life) and environmental conditions (e.g. duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are naturally found in body tissues and fluids (e.g. selenium).

Biomarkers of effect are defined as any measurable biochemical, physiological, or other alteration within an organism that, depending on magnitude, can be recognised as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g. increased tissue enzyme activity), as well as physiological signs of dysfunction such as, for example, decreased reproduction capacity.

In the recent "Technical Report on Aquatic Effect-based monitoring tools" (Wernersson et al. 2014), EBMs have been categorised as biomarkers when biological responses are observed in field-exposed organisms. EBMs, which measure the toxicity of environmental samples under defined laboratory conditions, at cellular and individual organism levels (*in*

vitro and *in vivo,* respectively), have been categorised as **bioassays**. Standardised bioassays rely on measuring responses in readily available model cell line/species, which may not be representative of other more vulnerable species, but which allow the quantification of chemical-caused toxic effects that are separate from those caused by other environmental stressors.

It is possible to capture exposure to similarly acting pollutants using EBMs (e.g. exposure to substances having acetylcholinesterase inhibitory activity, estrogenic activity). In particular, cause-effect relationships have already been established for several water monitoring methods (for details see: Wernersson et al. 2014), for example imposex biomarkers such as VSDI (response to TBT), the Micronucleus assay (genotoxic compounds, such as certain PAHs), Vtg in male fish (oestrogenic compounds, such as EE2), EROD (PAHs and dioxin-like compounds), DNA adducts (PAHs).

In some cases, it may however be appropriate to identify groups of substances that can be monitored by means of a biomarker of exposure, i.e. concentration of the chemical compound (of its specific metabolite) that can be quantified easily by analytical methods in biota. The factors leading to preference for such biomarkers can be the very low concentrations of some substances in the water column, especially those with very low water solubility, or their tendency to bioaccumulate through the food web. According to the Technical Guidance for Deriving EQS (TG- EQS 2011), if these substances pose a significant risk through indirect toxicity (i.e. secondary poisoning resulting from food-chain transfer) and their analysis is more feasible in other environmental matrices, such as biota and/or sediments, then a biota standard may be required. This is typically the case for hydrophobic substances, and biota standards (which are in this case equivalent to the biomarkers of exposure) have been proposed for hexachlorobenzene, hexachlorobutadiene and mercury and its compounds in the EQSD (2008/105/EC), establishing concentration limits in prey tissue (fish, molluscs, crustaceans and other biota) that may form the diet of top predators (including humans).

1.4 A major limitation of existing effect-based methods

The WFD requires quality standards to protect humans against two possible routes of exposure: fishery products and drinking water (TG EQS 2011). At present, environmental standards developed for priority substances (PS) and other substances of interest are designed to be sufficiently protective for humans (TG EQS 2011). Whilst scientific and data developments may allow us to assess risks of a chemical mixture to aquatic predators (including humans) by use of MoA and effect-based monitoring approaches in the future, at present it is not clear how the EBMs can be used to capture (predictively) the indirect effects that might occur in humans following long-term chronic exposure to pollutants via the aquatic environment. So far, some bioassays have been applied to investigate for example hormonal activity of chemicals in drinking water (Brand et al. 2013) and complex chemical mixtures in recycled water (Jia et al. 2015).

A major challenge in both human health and ecological risk assessment is extrapolation of chemical effects between species. Basic knowledge of the conservation of biological pathways across species is central to this extrapolation. Recently, many efforts have focused on adverse outcome pathways (AOPs), a toxicological pathway-based vision for human health assessments relying mainly on *in vitro* systems and predictive models, a vision equally applicable to ecological risk assessment (Villeneuve et al, 2014). A pathway-

based analysis of chemical effects opens numerous opportunities to apply non-traditional approaches for understanding the risks of chemical exposure. Conservation of initiating and key molecular events leading to adverse outcomes provides a framework for extrapolating chemical effects across species, and systematic organisation of this information has the potential to improve regulatory decision-making through greater integration and more meaningful use of mechanistic data.

This report examines the current state of knowledge on the MoAs and effects of the PS as well as other substances included in the first Watch List (WL) and those so far shortlisted in the current prioritisation exercise (Carvalho et al. 2016, Lettieri et al. 2016) in the aquatic environment. We consider also whether and how existing PS can be grouped together based on their MoA. The relevance of these different groups, as well as the relevance of the different substances included in each of the identified "effect" group, has been preliminarily assessed

2 Identification of relevant modes of action and related effect-based methods for priority substances and other substances of interest

As already mentioned in the Introduction, in the context of the WFD, effect-based methods (EBM) are mentioned in several Common Implementation Strategy (CIS) guidance documents, and in one technical report.

The following objectives for their use have been proposed (Wernersson et al. 2014):

- Early detection of biological imbalance

- Linking concentration with exposure and effects

- Early warning of changes in water quality at crucial sites

- Detecting and assessing significant pollutants to update risk assessments

- Detecting adverse biological effects to indicate where operational or investigative monitoring is required- Linking ecological and chemical status of the water quality.

EBMs may have an advantage in their ability to indicate effects of the total sum of all chemicals acting along a particular pathway. In this context, linking exposure to chemicals with effects in the aquatic environment requires knowledge of their mode of action (MoA). It facilitates understanding of the use and interpretation of biomarkers of effects in individual aquatic organisms, and their implementation as methods to assess exposure in the general population.

However, it is important to note that biomarkers of effect are not often substance-specific. Indeed the presence of a biomarker may not necessarily represent a risk, but can indicate potential health impairment (e.g., DNA adducts).

Several current priority substances (PS) are already monitored in the environment on the basis of biomarkers of exposure (i.e. the substance concentration is measured in biota): brominated diphenylethers (BDEs), fluoranthene, hexachlorobenzene, hexachlorobutadiene, mercury, benzo(a)pyrene, dicofol, perfluorooctane sulfonic acid (PFOS), dioxins and dioxin-like PCBs, hexabromocyclododecane (HBCDD), heptachlor and heptachlor epoxide (WFD monitoring programme for PS). The specific environmental quality standards (EQSs) have been established to ensure that the concentration of the PS does not exceed safe levels.

Grouping of substances could be an option to increase the efficiency of the chemical and ecological status assessment. Bunke et al. (2014) described and discussed approaches for the environmental risk assessment (aquatic compartment) of mixtures under REACH. Different criteria can be used to group substances for joint assessment:

• Chemical/structural similarity,

• Common use in specific sectors,

• Common MoA

• Common endpoint (effect)

Similarly, European authorities have developed methods for CRA (Combined Risk Assessment) to identify criteria to group active substances on the basis of their toxicological profile, with the creation of Cumulative Assessment Groups (CAG) (EFSA 2014a). In the combined ecotoxicological risk assessment in the frame of European authorization of

pesticides, the grouping is based on general criteria like chemical structure, mechanism of pesticide action and common toxic effect, or more refined criteria like mode or mechanism of action (Panizzi et al. 2017).

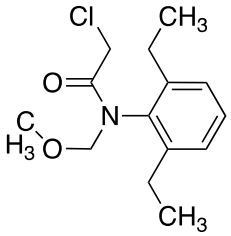
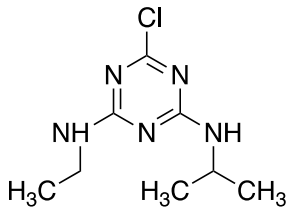
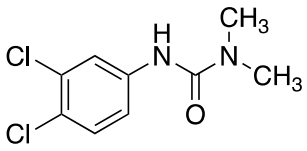
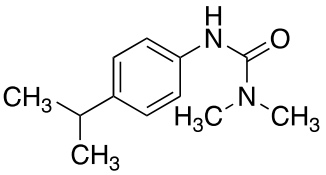
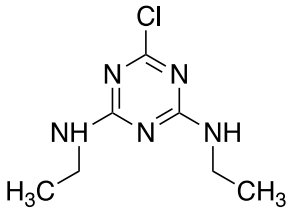
The PS and other substances included in the first Watch List (WL) and those so far shortlisted during the current prioritisation exercise (Carvalho et al. 2016, Lettieri et al. 2016) where first grouped into categories/chemical classes based on their common use and/or chemical structure in order to identify how they might be best considered in future monitoring programmes. Then the current state of knowledge on the MoA as well as the effects in the aquatic environment of each of the substances mentioned was examined.

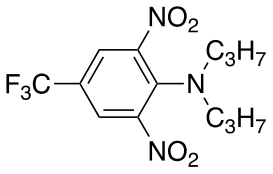
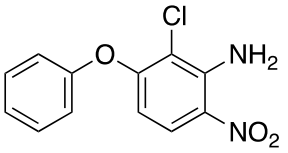
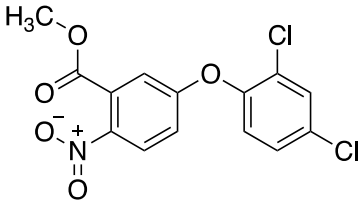
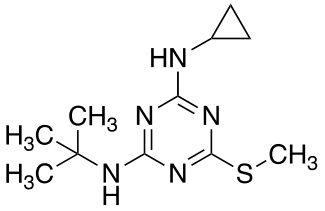
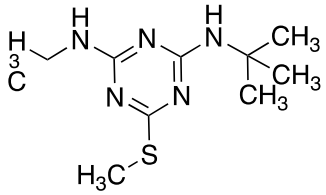
It should be noted that the purpose of this report is not to discuss in detail the results of all available toxicity tests, but to present an overview of the MoAs reported in ecotoxicological studies.

2.1 Grouping of the current priority substances and other substances of interest into use categories/chemical structure classes

2.1.1 Priority Substances (PS)

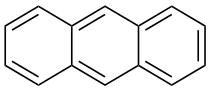
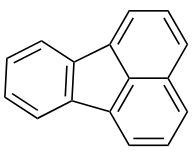
2.1.1.1 Herbicides

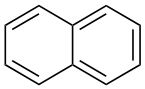
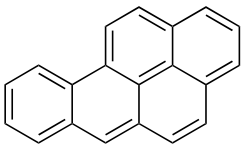
Substance	Use category/Substance type	Molecular structure	AA-EQS_{fw} (µg/L)
Alachlor (PS No. 1)	Herbicide of the chloroacetanilide family		0.3
Atrazine (PS No. 3)	Triazine herbicide		0.6
Diuron (PS No. 13)	Phenylurea herbicide It is also used in the field of material protection. Diuron-containing paints, for example, facade paints or ship floor paints (antifouling paints), remain free of algae growth.		0.2
Isoproturon (PS No. 19)	Urea herbicide		0.3
Simazine (PS No. 29)	Triazine herbicide		1

Trifluralin (PS No. 33)	Herbicide		0.03
Aclonifen (PS No. 38)	Herbicide (diphenylether)		0.12
Bifenox (PS No. 39)	Herbicide; (diphenylether)		0.012
Cybutryne (PS No. 40(*))	Triazine herbicidal biocide (or algicide)		0.0025
Terbutryn (PS No. 45)	Triazine herbicide or algicide		0.065

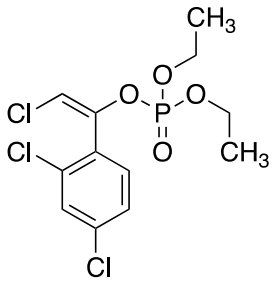
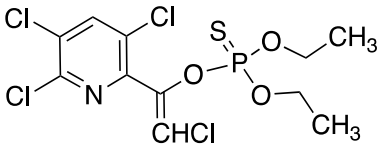
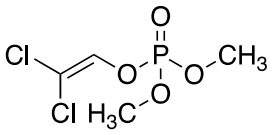
(*) Although cybutryne is used as antifouling biocide (group 2.1.10), in this report it is included in the herbicides group (2.1.1) due to its chemical structure similarity to these substances

2.1.1.2 Polyaromatic hydrocarbons (PAHs)

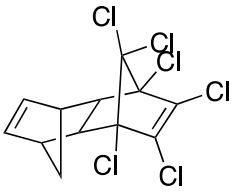
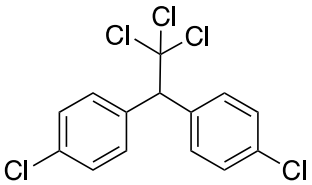
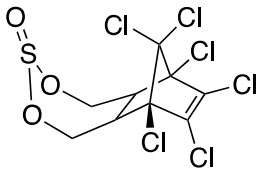
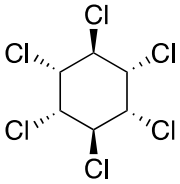
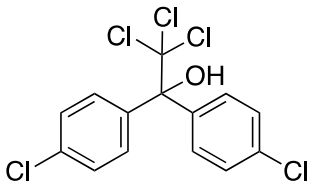
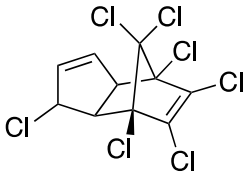
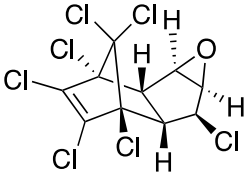
Substance	**Use category/Substance type**	**Molecular structure**	**AA-EQS$_{fw}$ (µg/L)**
Anthracene (PS No. 2)	Wood preservative; used in coating materials		0.1
Fluoranthene (PS No. 15)	Combustion by-product; coal tar and asphalt component; fluoranthene is found in many combustion products, along with other PAHs; its presence is		Biota: 30 µg/kg

	an indicator of less efficient or lower-temperature combustion.		
Naphthalene (PS No. 22)	Naphthalene is used mainly as a precursor to other chemicals.		2
Polyaromatic hydrocarbons (PAH) (PS No. 28): Benzo(a)pyrene Benzo(b)fluoranthene Benzo(k)fluoranthene Benzo(g,h,i)- perylene Indeno(1,2,3-cd)-pyrene	Combustion by-products		0.00017 Biota: 5 µg/kg

2.1.1.3 Organophosphorus insecticides

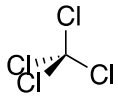
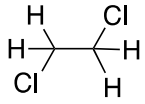
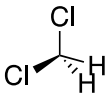
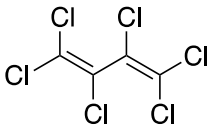
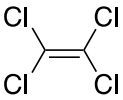
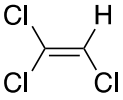
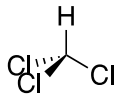
Substance	Use category/ Substance type	Molecular structure	EQS (µg/L)
Chlorfenvinphos (PS No. 8)	Organophosphorus insecticide		0.1
Chlorpyrifos (Chlorpyrifos- ethyl) (PS No. 9)	Organophosphorus insecticide		0.03
Dichlorvos (PS No. 42)	Organophosphorus insecticide		0.0006

2.1.1.4 Organochlorine insecticides

Substance	Use category/ Substance type	Molecular structure	EQS (µg/L)
Cyclodiene pesticides (PS No. 9a(*)): Aldrin Dieldrin Endrin Isodrin	Organochlorine insecticide		0.010
DDT total para-para- DDT (PS No. 9b(*))	Organochlorine insecticide		Total "DDT": 0.025 *p,p'*-DDT: 0.01
Endosulfan (PS No. 14)	Organochlorine insecticide		0.005
Hexachlorocyclohexane (PS No. 18)	Organochlorine insecticide		0.02
Dicofol (PS No. 34)	Organochlorine insecticide (acaricide; miticide) that is chemically related to DDT.		0.0013 Biota: 33 µg/kg
Heptachlor and Heptachlor epoxide (PS No. 44)	Organochlorine insecticide	 	0.0000007

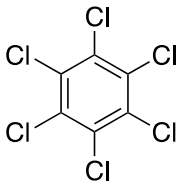
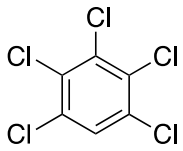
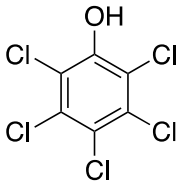
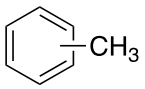
(*) Those substances are not priority substances (PS) but among the eight "other" pollutants for which the EQS are identical to those laid down in the legislation that applied prior to 13 January 2009 (Annex I of Directive 2008/105/EC).

2.1.1.5 Chlorinated solvents

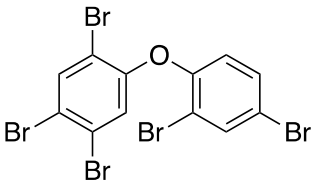
Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Carbon-tetrachloride (PS No. 6a(*))	Chlorinated solvent; formerly widely used in fire extinguishers, as a precursor to refrigerants and as a cleaning agent.		12
1,2-Dichloroethane (PS No. 10)	Chlorinated hydrocarbon; used for the production of vinyl chloride.		10
Dichloromethane (PS No. 11)	Chlorinated solvent		20
Hexachlorobutadiene (PS No. 17)	Solvent for other chlorine-containing compounds; industrial organic synthesis compound		0.1 Biota: 55 µg/kg
Tetrachloroethylene (PS No. 29a(*))	Excellent solvent for organic materials; used for dry cleaning of fabrics, hence it is sometimes called "dry-cleaning fluid".		10
Trichloroethylene (PS No. 29b(*))	Industrial solvent		10
Trichloromethane (Chloroform) (PS No. 32)	Chlorinated solvent; precursor to PTFE and refrigerants		2.5

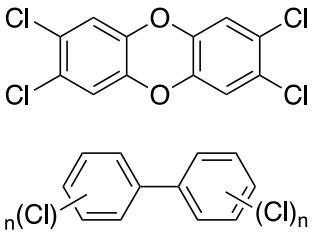
(*) Those substances are not priority substances (PS) but among the eight "other" pollutants for which the EQS are identical to those laid down in the legislation that applied prior to 13 January 2009 (Annex I of Directive 2008/105/EC).

2.1.1.6 Aromatic organochlorine compounds

Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Hexachlorobenzene (HCB) (PS No. 16)	Fungicide;		0.01 Biota: 10 µg/kg
Pentachlorobenzene (PS No. 26)	Chlorinated aromatic hydrocarbon		0.007
Pentachlorophenol (PS No. 27)	Organochlorine compound used as a fungicide and a disinfectant		0.4
Trichlorobenzenes (PS No. 31)	Industrial solvent; intermediate for the production of other compounds		0.4

2.1.1.7 Dioxins, PCBs, BDEs

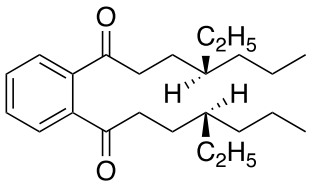
Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Brominated Diphenyl Ethers (BDEs) (PS No. 5)	Flame retardants; structurally similar to PCBs		(0.0005 µg/l) Biota: 0.0085 µg/kg

Dioxins and coplanar PCBs (PS No. 37)	Dioxins occur as by-products in the manufacture of some organochlorines, in the incineration of chlorine-containing substances such as PVC (polyvinyl chloride), in the chlorine bleaching of paper, and from natural sources such as volcanoes and forest fires		Sum of PCDD+PCDF+PCB-DL 8.0 10^{-3} $\mu g.kg^{-1}$ TEQ = 0.008 $\mu g.kg^{-1}$ TEQ (biota)

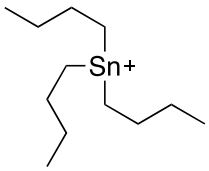
2.1.1.8 Metals

Substance	**Use category/Substance type**	**Molecular structure**	**EQS (µg/L)**
Cadmium and its compounds (PS No. 6)	By-product of zinc production. Cadmium was used for a long time as a corrosion-resistant plating on steel, and cadmium compounds are used as red, orange and yellow pigments, to colour glass, and to stabilise plastic.	Cd	0.08-0.25
Lead and its compounds (PS No. 20)	Used in lead-acid batteries and chemical industries	Pb	1.2
Mercury and its compounds (PS No. 21)	Mercury is used primarily for the manufacture of industrial chemicals or for electrical and electronic applications and in fluorescent lamps.	Hg	Biota: 20 µg/kg
Nickel and its compounds (PS No. 23)	Nickel is used in many specific and recognisable industrial and consumer products, including stainless steel, alnico magnets, rechargeable batteries, electric guitar strings, and special alloys.	Ni	4

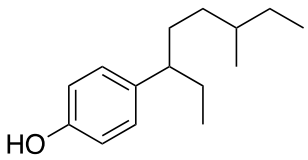
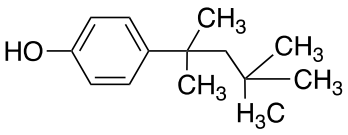
2.1.1.9 Phthalate

Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Di(2-ethylhexyl)-phthalate (DEHP) (PS No. 12)	Plasticiser; used for the production of PVC plastics, etc.		1.3

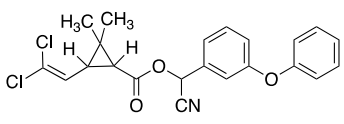
2.1.1.10 Anti-fouling biocide

Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Tributyltin compounds (PS No. 30)	Biocide in anti-fouling paint		0.0002

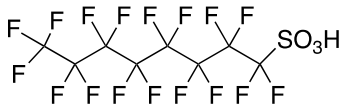
2.1.1.11 Alkylphenols (surfactant metabolites)

Substance	Use category/ Substance type	Molecular structure	EQS (µg/L)
Nonylphenols (PS No. 24)	Non-ionic surfactants (degradation product of alkylphenol ethoxylates)		0.3
Octylphenols (PS No. 25)	Phenolic surfactant (degradation product of alkylphenol ethoxylates)		0.1

2.1.1.12 Pyrethroid insecticides

Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Cypermethrin (PS No. 41)	Type II (cyano) Pyrethroid insecticide		0.00008

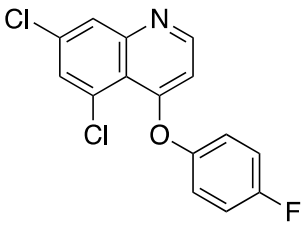
2.1.1.13 Perfluorinated surfactant

Substance	Use category/ Substance type	Molecular structure	EQS (µg/L)
Perfluorooctane-sulfonic acid (PFOS) (PS No. 35)	Surfactant		0.00064 Biota: 9.1 µg/kg

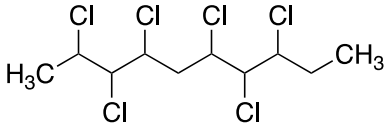
2.1.1.14 Benzene

Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Benzene (PS No. 4)	Constituent of crude oil; solvent		10

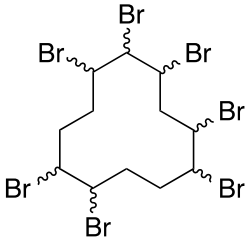
2.1.1.15 Quinoline fungicide

Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
Quinoxyfen (PS No. 36)	Quinoline fungicide often used to control powdery mildew infections on grapes and hops		0.15

2.1.1.16 Chloroalkanes

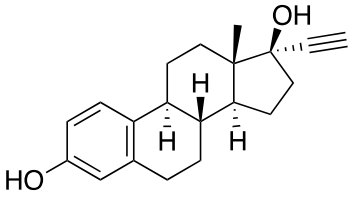
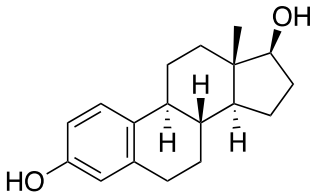
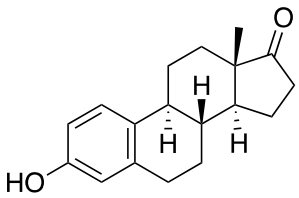
Substance	Use category/Substance type	Molecular structure	EQS (µg/L)
C10-13 chloroalkanes (PS No. 7)	Complex mixtures of polychlorinated n-alkanes		0.4

2.1.1.17 Hexabromocyclododecane (HBCDD)

Substance	Use category/ Substance type	Molecular structure	EQS (µg/L)
Hexabromocyclo-dodecane (HBCDD) (PS No. 43)	High production volume chemical used as a flame retardant, mainly within the polymer and textile industry (16 stereoisomers)		0.0016 Biota: 167 µg/kg

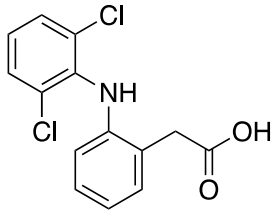
2.1.2 Watch List substances

2.1.2.1 Hormones

Substance	Use category/Substance type	Molecular structure	PNEC(*) (µg/L)
17-Alpha-ethinylestradiol (EE2)	Synthetic estradiol used in contraceptive pills and for the treatment of menopausal and post-menopausal symptoms		0.000035
17-Beta-estradiol (E2)	Natural female sex hormone; estrogenic compound		0.0004
Estrone (E1)	Estrogenic hormone and oxidation product of estradiol		0.0004

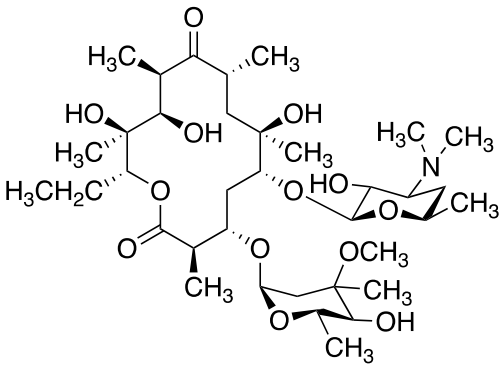
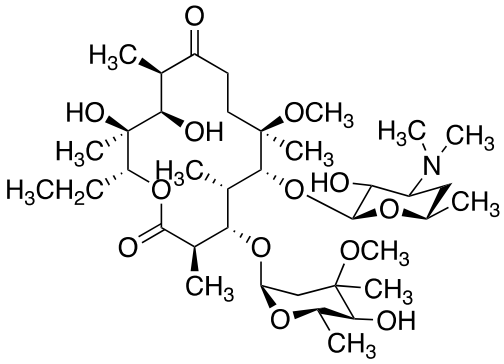
(*) Predicted No Effect Concentration (PNEC). Commission's Priority Substances proposal from the year 2012 (EU, 2012)

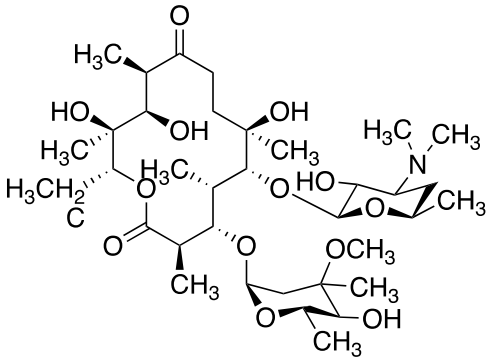
2.1.2.2 Pharmaceuticals

Substance	Use category/Substance type	Molecular structure	PNEC(*) (µg/L)
Diclofenac	Non-steroidal anti-inflammatory drug (NSAID)		0.1

(*) Commission's Priority Substances proposal from the year 2012 (EU, 2012)

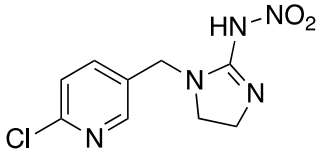
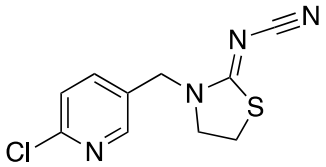
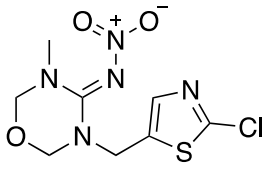
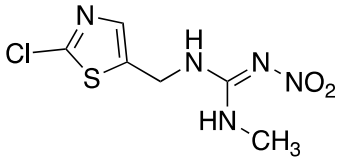
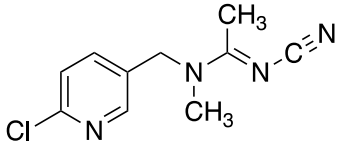
2.1.2.3 Antibiotics

Substance	Use category/ Substance type	Molecular structure	PNEC(*) (µg/L)
Erythromycin	Macrolide antibiotic		0.20
Clarithromycin	Macrolide antibiotic		0.13

Azithromycin	Macrolide antibiotic		0.090

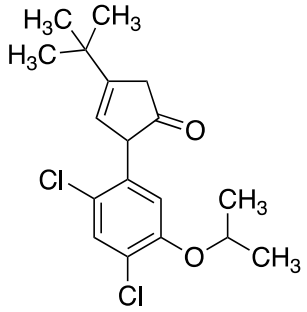
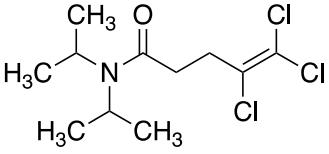
(*) Carvalho et al. 2015

2.1.2.4 Neonicotinoid insecticides

Substance	Use category/ Substance type	Molecular structure	PNEC(*) (µg/L)
Imidacloprid	Neonicotinoid insecticide		0.009
Thiacloprid	Neonicotinoid insecticide		0.050
Thiamethoxam	Neonicotinoid insecticide		0.14
Clothianidin	Neonicotinoid insecticide		0.13
Acetamiprid	Neonicotinoid insecticide		0.5

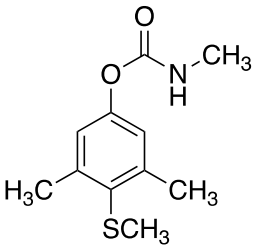
(*) Carvalho et al. 2015

2.1.2.5 Herbicides

Substance	Use category/ Substance type	Molecular structure	PNEC(*) (µg/L)
Oxadiazon	Herbicide		0.088
Triallate (Trial-late)	Herbicide		0.67

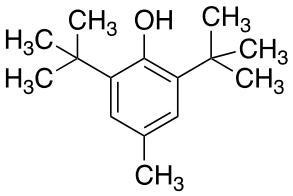
(*) Carvalho et al. 2015

2.1.2.6 Carbamate insecticide

Substance	Use category/Substance type	Molecular structure	PNEC(*) (µg/L)
Methiocarb	Carbamate insecticide and herbicide		0.01

(*) Carvalho et al. 2015

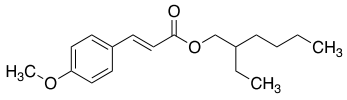
2.1.2.7 Antioxidant

Substance	Use category/Substance type	Molecular structure	PNEC(*) (µg/L)
2,6-Di-tert-butyl-4-methylphenol	Antioxidant used in many materials such as packaging materials, adhesives that come in contact with food and also in cosmetics, personal care products and		3.16

	pharmaceuticals; food additive		

(*) Carvalho et al. 2015

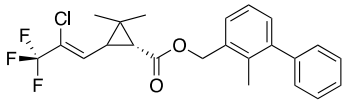
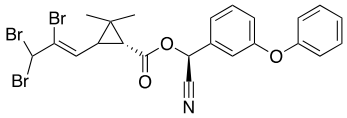
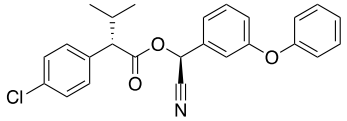
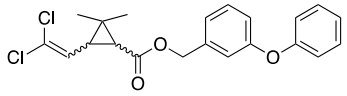
2.1.2.8 Sunscreen agent

Substance	Use category/Substance type	Molecular structure	PNEC(*) (µg/L)
2-Ethylhexyl 4-methoxycinnamate	Sunscreen agent		6.0 $PNEC_{sed}$ = 0.2 mg/kg

(*) Carvalho et al. 2015

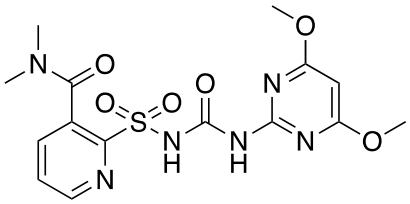
2.1.3 Candidate substances identified through the monitoring- and modelling-based prioritisation exercises in 2015-2017

2.1.3.1 Pyrethroid insecticides

Substance	Use category/ Substance type	Molecular structure	PNEC(*) (µg/L)
Bifenthrin	Type I (noncyano) Pyrethroid insecticide and acaricide		0.00002
Deltamethrin	Type II (cyano) Pyrethroid insecticide		3.1E-06
Esfenvalerate	Type II (cyano) Pyrethroid insecticide		0.0001
Permethrin	Type I (noncyano) Pyrethroid insecticide		0.00047

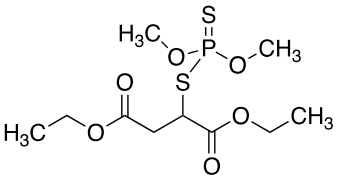
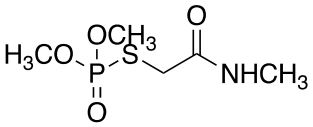
(*) PNEC value proposed (Directory listing for /CircaBC/env/wfd/Library/working_groups/priority_substances /2a - Sub-Group on Review of Priority Substances 2014 start/Dossier Draft of substances identified in the second prioritisation process (2016))

2.1.3.2 Sulfonylurea herbicide

Substance	Use category/ Substance type	Molecular structure	PNEC(*) (µg/L)
Nicosulfuron	Sulfonylurea herbicide		0.0087

(*) PNEC value proposed (Directory listing for /CircaBC/env/wfd/Library/working_groups/priority_substances /2a - Sub-Group on Review of Priority Substances 2014 start/Dossier Draft of substances identified in the second prioritisation process (2016))

2.1.3.3 Organophosphorus insecticides

Substance	Use category/ Substance type	Molecular structure	PNEC(*) (µg/L)
Malathion	Organophosphorus insecticide		0.0012
Omethoate	Organophosphorus insecticide		0.00084

(*) PNEC value proposed (Directory listing for /CircaBC/env/wfd/Library/working_groups/priority_substances /2a - Sub-Group on Review of Priority Substances 2014 start/Dossier Draft of substances identified in the second prioritisation process (2016))

2.1.3.4 Metals and non-metal trace elements

Substance	Use category/ Substance type	Molecular structure	PNEC(*)
Silver	Metal	Ag	0.01
Uranium	Metal	U	0.5
Selenium	Metal	Se	0.73

(*) PNEC value proposed (Directory listing for /CircaBC/env/wfd/Library/working_groups/priority_substances /2a - Sub-Group on Review of Priority Substances 2014 start/Dossier Draft of substances identified in the second prioritisation process (2016))

2.2 State-of-the-art review/identification of the relevant mode of action

A review of the relevant mode(s) of action (MoA) and effects has been done for each of the priority substances (PS), the substances included in the Watch List (WL) and those so far shortlisted during the prioritisation exercise (Carvalho et al. 2016, Lettieri et al. 2016). The review was based on a literature survey.

2.2.1 Priority Substances (PS)

2.2.1.1 Herbicides

Alachlor (PS No 1)

Table 1. Overview of the available data on mode of action (MoA) for alachlor (acetanilide herbicide; CAS-number: 15972-60-8)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** inhibits elongase and geranylgeranyl pyrophosphate (GGPP) cyclisation enzymes, part of the gibberellin pathway.	Interfering with a plant's ability to produce protein and interfering with root growth	Jurado et al. 2011
African clawed frog *Xenopus laevis*		Embryotoxicity and teratogenicity Alachlor more embryotoxic but less teratogenic than its degradation product (2, 6-diethylaniline)	Osano et al. 2002
Crucian carp *Carassius auratus*	Induction of hepatic detoxifying enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST); reduction of reduced glutathione (GSH) content	Decreased Hepatic and Gonadosomatic Indices	Yi et al. 2007
Freshwater fish *Channa punctatus*	Decrease of glycogen, total proteins, DNA, RNA; increase of activity of the enzymes (aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH)		Tilak et al. 2009
Freshwater fish *Clarias batrachus*	Changes in biochemical parameters (total protein, triglycerides, ALT and AST and alkaline phosphatase (ALP)		Rajini et al. 2014

Atrazine (PS No 3)

Table 2. Overview of the available data on mode of action (MoA) for atrazine (triazine herbicide; CAS-number: 1912-24-9)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** blocks photosystem II through binding to the Q_B-site of the D1 subunit in PS II (this causes a block of the formation of plastoquinol (QH_2) which leads to inhibited electron transport and ultimately prevents the formation of ATP and NADPH).	Inhibition of photosynthesis	Jurado et al. 2011
11 freshwater snail species from five families	Changes at subcellular and cellular levels (e.g. reduced activities of several detoxification enzymes including glycogen phosphorylase, SOD, CAT, glutathion reductase (GR), succinin dehydrogenase, AChE, LDH; increased activity of lipid peroxide and transaminases)	No effect reported at population-level in the snail species studied	Summarised by Gustafson et al. 2015
African clawed frog *Xenopus laevis*	Decrease in testosterone levels (hypothesised that atrazine induces aromatase and promotes the conversion of testosterone to estrogen)	Demasculinisation of the males and production of hermaphrodites	Hayes et al. 2002
African clawed frog *Xenopus laevis*	Depressed testosterone	Demasculinisation and complete feminisation in males (suppressed mating behaviour, reduced fertilisation)	Hayes et al. 2010
Bluegill sunfish *Lepomis macrochirus* Adult female zebrafish *Danio rerio* Neotropical freshwater fish *Prochilodus lineatus* Juvenile zebrafish *Danio rerio*	Induction of antioxidant defenses causing an unbalance between ROS production and elimination (effects on the oxidative stress markers and detoxifying enzymes)		Elia et al. 2002 Jin et al. 2010 Paulino et al. 2012 Blahová et al. 2013

Aquatic organism	MoA	Effect	Ref
Atlantic salmon *Salmo salar*	Reduction in gill Na^+K^+ATPase activity (in smolts in fresh water) Elevated plasma cortisol, thyroxine, osmolality, and monovalent ion concentrations (in smolts transferred to sea water)	Data suggest that exposure of salmon smolts to atrazine in fresh water may compromise their physiological capabilities to survive in saline conditions	Waring & Moore 2004
Fish mummichog *Fundulus heteroclitus*		Capacity of mummichog larvae to osmoregulate (with higher prevalence of dehydrated larvae (at salinity 15 and 35 PSU) or hyperhydrated larvae (at salinity 3 PSU)	Fortin et al. 2008
Zebrafish *Danio rerio*	Induced aromatase expression (Cyp19A1) not directly via the estrogen receptor but a complex mechanism involving NR5A receptor activation, as well as receptor phosphorylation, amplification of cAMP, and PI3K signalling	Increased ratio of female to male fish	Suzawa & Ingraham 2008
Fish (Fathead minnow *Pimephales promelas*)	No significant effects in steroid hormone concentrations, in gonad and brain aromatase activity (CYP19 isoforms) or in gonad–somatic indices	Reduced egg production through alteration of final maturation of oocytes; histological abnormalities in gonads were also observed in males	Tillitt et al. 2010
Japanese medaka *Oryzias latipes*	Chromosomal abnormalities in spermatogonia	Reduced egg production (through alteration of final maturation of oocytes); abnormal germ cells in males No effect of on gonadosomatic index (GSI), aromatase protein, or whole body 17 β-estradiol or testosterone	Papoulias et al. 2014
Guppy *Poecilia reticulata*		Disrupted mating signals	Shenoy 2012

Aquatic organism	MoA	Effect	Ref
Zebrafish fibroblast cell line	Copy number alterations (CNAs) in the zebrafish genome	CNAs were associated with previously reported gene expression alterations in adult male and female zebrafish, exposed to atrazine during embryogenesis	Wirbisky & Freeman 2017
Amphibian and fish	Acceleration or delay metamorphosis; reduction of immune function; reduction of sex hormone concentrations	Effects on fish and amphibian reproductive success, sex ratios, gene frequencies, populations, and communities remain uncertain	Rohr & McCoy 2010
Male vertebrate (different classes: teleost fish, amphibians, reptiles, and mammals)	Reductions in androgen levels and the induction of estrogen synthesis	Demasculinisation and feminisation of male gonads These effects are strong, consistent across vertebrate classes, and specific	Hayes et al. 2011
Fish, amphibians and reptiles	Different expression of genes and/or associated proteins, concentrations of hormones; induction of detoxification responses)	No clear adverse outcomes in terms of apical endpoints	Van Der Kraak et al. 2014

Diuron (PS No 13)

Table 3. Overview of the available data on mode of action (MoA) for Diuron (phenylurea herbicide; CAS-number: 330-54-1)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** binds to the Q_B-site in the D1 subunit of PSII.	Inhibition of photosynthesis	Berg et al. 2007
Oyster	Genotoxicity in oyster haemocytes and spermatozoa (at 0.25 µg/L and 0.50 µg/L upwards)	Embryotoxic effects on oyster embryo (Diuron embryotoxicity was confirmed after exposure to concentrations of 0.05 µg/L upwards, and a significant increase in the number of abnormal D-larvae was observed at	Akcha et al. 2012; Barranger et al. 2014

Aquatic organism	MoA	Effect	Ref
		concentrations of between 0.05 and 0.25 µg/L)	
Oyster *Crassostrea gigas*	Oxidative stress resulting in DNA oxidation (the formation of 8-oxodGuo), in both early germ cells and gametes, such as spermatozoa	Transmission of diuron-induced DNA damage to offspring	Barranger et al. 2016
Oyster *Crassostrea gigas* (diuron and its metabolites)	Genotoxicity in early life stages mediated at least partially by ROS production (no significant difference between effect of diuron and all of its metabolites)	Embryotoxicity in early life stages of the offspring (diuron more embryotoxic than its metabolites)	Behrens et al. 2016
Oyster *Crassostrea gigas*	Transcriptional changes occurring in oyster spats (non exposed) originating from genitors exposed (different molecular pathways involved in energy production, translation and cell proliferation particularly disturbed); decreased activity of antioxidant enzyme CAT	Exposure to an ecologically realistic concentration of diuron during oyster gametogenesis stage can impact the next generation (link made between the transcriptional changes and oxidative stress and mitochondrial damage in offspring, as well as a growth delay)	Rondon et al. 2016
Tilapia *Oreochromis niloticus*	Testosterone levels decreased by diuron Significant decreases in testosterone and in 11-ketotestosterone (11-KT) by diuron metabolites	Limited effects of diuron on gonadal histology in males Significant decreases in gonadosomatic index, diameter of seminiferous tubules and in the mean percentages of germ cells (spermatids and spermatozoa) by diuron metabolites	Pereira et al. 2015
Tilapia *Oreochromis niloticus*	Increases in estradiol plasma levels	Increases in GSI in females Increase in the percentage of final vitellogenic oocytes and a decrease in germinative cells	Pereira et al. 2016
Fish and amphibians	Cytochrome P450 stimulation, Ubiquinol-cytochrome-c reductase	Steroid biosynthesis, cholesterol metabolism	Marlatt & and

Aquatic organism	MoA	Effect	Ref
	inhibition, Phospholipid translocating ATPase and NADPH peroxidase inhibition, pregnane X receptor activation		Martyniuk 2017

Isoproturon (PS No 19)

Table 4. Overview of the available data on mode of action (MoA) for isoproturon (phenylurea herbicide; CAS-number: 34123-59-6)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** binds to the Q_B-site in the D1 subunit of PSII.	Inhibition of photosynthesis	Jurado et al. 2011
Tadpoles of *Bombina bombina* and *Bombina variegata*	Increased activity of microsomal and soluble glutathione-*S*-transferase (sGSTs) (Uptake through the protective jelly matrix surrounding the egg leading to a direct exposure of the embryos)	Physical and behavioural abnormalities (reduced mobility and developmental deformities)	Greulich et al. 2002

Simazine (PS No 29)

Table 5. Overview of the available data on mode of action (MoA) for Simazine (triazine herbicide; CAS-number: 122-34-9)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** binds to the Q_B-site in the D1 subunit of PSII.	Inhibition of photosynthesis	Jurado et al. 2011
Common carp *Cyprinus carpio*	Increased production of ROS leading to oxidative damage to lipids and proteins	Inhibited antioxidant capacities in common carp tissue	Stara et al. 2012
Common carp *Cyprinus carpio*	Increased activity of alkaline phosphatase, alanine aminotransferase; increase in total protein and albumins	Increase of hepatosomatic indices (HSI) relative to controls; decrease in leukocyte count Severe hyaline degeneration of the	Velisek et al. 2012

Aquatic organism	MoA	Effect	Ref
		epithelial cells of caudal kidney tubules	
Fish (barramundi *Lates calcarifer*)	Increased vitellogenin (Vtg) transcription levels		Kroon et al. 2015
Tadpoles of *Xenopus laevis*		Inhibition of percent of frogs completing metamorphosis Damaged liver tissues but no significant effects neither on liver weight nor on hepatosomatic index (HSI)	Sai et al. 2016

Trifluralin (PS No 33)

Table 6. Overview of the available data on mode of action (MoA) for trifluralin (dinitroaniline herbicide; CAS-number: 1582-09-8)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** inhibits mitotic cell division (due to either microtubule depolymerisation or alteration in the concentration of calcium ions within the cell) and early developmental cell division in roots.	Inhibition of root development	Grover et al. 1997
Carp *Cyprinus carpio*	Increase of functional enzymes (ALP,ALT, AST) activities in blood serum and the organs	A decrease in relative growth rate was found	Poleksić & Karan 1999
Tilapia *Oreochromis niloticus*	Genotoxicity	Higher micronucleus (MN) frequencies in peripheral erythrocytes	Könen & Çavaş 2008

Aclonifen (PS No 38)

Table 7. Overview of the available data on mode of action (MoA) for aclonifen (diphenylether herbicide; CAS-number: 74070-46-5)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** interferes with plant-specific processes such as photosynthesis and carotenoid biosynthesis. Acts on two different biochemical pathways: 1) carotenoid synthesis, and 2) protoporphyrinogen oxidase (in the chlorophyll synthesis pathway, causing a phytotoxic protoporphyrin IX accumulation).	Inhibition of chlorophyll synthesis	Kilinc et al. 2009
No information about the MoA in non-target aquatic organisms (tested within environmentally relevant concentrations) has been found in the scientific literature			

Bifenox (PS No 39)

Table 8. Overview of the available data on mode of action (MoA) for bifenox (diphenylether herbicide; CAS-number: 42576-02-3)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** acts through a phytotoxic protoporphyrin IX accumulation.	Release of peroxides that destroy the cell membranes of plants and lead to tissue death. bifenox also inhibits photosynthesis. The effect is enhanced by high light intensity	Kilinc et al. 2011
Non-target aquatic organisms	No information about the MoA in non-target aquatic organisms (tested within environmentally relevant concentrations) has been		

Aquatic organism	MoA	Effect	Ref
	found in the scientific literature.		

Cybutryne (PS No 40)

Table 9. Overview of the available data on mode of action (MoA) for cybutryne (triazine herbicidal biocide (or algicide); CAS-number: 28159-98-0)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** binds to the plastoquinone binding-niche at the D1 protein in PSII.	Inhibition of photosynthesis	Tietjen et al. 1991 Jansen et al. 1993 (in Wendt et al. 2013)
In vitro: Rabbitfish *Siganus fuscessens* livers	Possible double action: - binding to coenzyme Q (CoQ)-binding site in the mitochondrial respiratory chain blocking the electron transport causing ROS production - opening of small-size pores causing ROS inhibition	Inhibition of the mitochondrial reactive oxygen species (ROS) production at low doses (0.004 mg/L) Production of ROS increase with the doses (balance between inhibition and stimulation effects)	Liang et al. 2013
In vitro: gametes and embryos of the Pacific oyster *Crassostrea gigas*	Spermiotoxicity and embryotoxicity	Affected fertilising capacity and offspring quality	Mai et al. 2013

Terbutryn (PS No 45)

Table 10. Overview of the available data on mode of action (MoA) for terbutryn (triazine herbicide (algicide); CAS-number: 886-50-0)

Aquatic organism	MoA	Effect	Ref
Plants (including algae)	**A specific MoA:** inhibits PSII	Inhibition of photosynthesis	Jurado et al. 2011
Non-target aquatic organisms	No information about the MoA in non-target aquatic organisms (tested within environmentally relevant concentrations) has been		

Aquatic organism	MoA	Effect	Ref
	found in the scientific literature.		

2.2.1.2 Polyaromatic hydrocarbons (PAHs)

Anthracene (PS No 2)

Table 11. Overview of the available data on mode of action (MoA) for anthracene (polyaromatic hydrocarbon; CAS-number: 120-12-7)

Aquatic organism	MoA	Effect	Ref
General	There are two major mechanisms involved in photoinduced toxicity of PAHs(1): photosensitisation and photomodification. In the former, production of singled oxygen ($^1O_2$) leads to cellular damage. In the later, photooxidation of PAHs results in a variety of products/new compounds that are often more toxic then their parent PAHs. In the case of photosensitisation reactions induced by PAHs, the physiology of cellular damage via ($^1O_2$) is reasonably well understood. For example, nonspecific peroxidation of lipids and proteins in membranes occurs in its presence.		EU Risk Assessment Report: Anthracene 2004
Duckweed *Lemna gibba*	The primary site of action of photomodified anthracene was found to be electron transport at or near photosystem I (PSI). This was followed by inhibition of PSII, probably due to excitation pressure on PSII once the downstream electron transport through PSI was blocked. Net photosynthesis (carbon fixation) was also inhibited	A linkage between inhibition of photosynthesis and inhibition of plant growth established	Huang et al. 1997
Duckweed *Lemna gibba*	Photooxidation products of anthracene: inhibition of photosynthetic activity and electron transport (inhibition of PSI) or the cytochrome-b6/f complex, followed by photooxidative damage to PSII		Mallakin et al. 2002

Aquatic organism	MoA	Effect	Ref
Juvenile sunfish *Lepomis spp.* (in the presence of simulated sunlight)		Opercular ventilation rate significantly increased, and histological evidence indicated major structural changes occurred in the gills	Oris & Giesy 1985
Common goby *Pomatoschistus microps*	Inhibition of AChE and GST activity, induction of LDH and CAT activity; increased SOD, GR and glutathione peroxidase (GPx) activities (oxidative stress)		Vieira et al. 2008
Milkfish *Chanos chanos*	Increased lipid peroxidation (LPO) and CAT activity, inhibited AChE and GST activity		Palanikumar et al. 2012
Milkfish *Chanos chanos*		Reduction in feeding and growth rate	Palanikumar et al. 2013

([1]) The toxicity of most PAHs can be greatly enhanced on exposure of a living organism and/or the chemicals to ultraviolet radiation. The mechanisms of photoenhanced toxicity are not fully understood.

Fluoranthene (PS No 15)

Table 12. Overview of the available data on mode of action (MoA) for fluoranthene[(1)] (polyaromatic hydrocarbon; CAS-number: 206-44-0)

Aquatic organism	MoA	Effect	Ref
General	There are two major mechanisms involved in photoinduced toxicity of PAHs[(2)]: photosensitisation and photomodification. PAHs and their metabolites can affect structures and functions at cellular and subcellular levels. The first target of these lipophilic substances at a cellular level is the plasma membrane, where membrane lipids could be oxidised. The disturbance of this membrane and of the inner subcellular membranes and changes in enzyme activities (partly due to the change of structure of nucleic acids and proteins) may cause inhibition of		EC 2008 Kolb & Harms 2000 Chiang et al. 1996 Duxbury et al. 1997

Aquatic organism	MoA	Effect	Ref
	photosynthetic and respiration processes.		
Duckweed *Lemna minor*	Higher occurrence of reactive oxygen species (ROS) reflected in an increase in the activities of antioxidant enzymes (SOD, CAT, ascorbate peroxidase, guaiacol peroxidase) Increased content of antioxidant compounds like ascorbate or total thiols Increased in malondialdehyde (MDA) content	Microscopic observations of duckweed roots also confirmed the presence of ROS and related histochemical changes at the cellular and tissue levels. Non significant changes in number of plants, biomass production, leaf area size, content of chlorophylls a and b and carotenoids and parameters of chlorophyll fluorescence were in contrast with considerable changes at biochemical and histochemical levels.	Zezulka et al. 2013
Daphnia magna		Egg survival during development and production equally affected at concentrations which affected adult survival	Barata & Baird 2000
Benthic copepods (mature female *Schizopera knabeni and Coullana sp.*)		Reproduction of *S. knabeni* was significantly impaired (this decrease in offspring production was likely related to specific modes of action e.g. impairment of embryonic development). Decrease in grazing rates	Lotufo 1998
Fish (fathead minnow	A disruption of mucosal cell membrane function and integrity (as a result of rapid LPO reactions)		Weinstein et al. 1997

Aquatic organism	MoA	Effect	Ref
Pimephales promelas) (in the presence of solar ultraviolet radiation)			
Nile tilapia *Oreochromis niloticus*	Inhibition of hepatic CYP1A dependent Ethoxyresorufin-O-deethylase (EROD)		Pathiratne & Hemachandra 2010
Native (Lahontan redside minnow *Richardsonius egregious)* and nonnative (bluegill sunfish *Lepomis macrochirus*) fish species		The addition of fluoranthene to the ultraviolet radiation (UVR) elicited an increase in mortality in both species. The native redside minnow was more tolerant to UVR and fluoranthene exposure when compared to the non-native bluegill. In addition, increased pigmentation (mechanisms of protection) was exhibited to the greater extent in the native redside.	Gevertz et al. 2012
Native (Lahontan redside minnow *Richardsonius egregious)* and nonnative (bluegill sunfish *Lepomis macrochirus*) fish species		The accumulation of damage to bluegill skin was rapid and widespread (damage to the dermis as well as to nuclei, indicating impairment to respiratory processes and potential DNA damage).	Gevertz et al. 2014

([1]) In contrast to fluoranthene, data on the toxic effects of its stable biodegradation products on various aquatic species are largely unknown

([2]) The toxicity of most PAHs can be greatly enhanced on exposure of a living organism and/or the chemicals to ultraviolet radiation. The mechanisms of photoenhanced toxicity are not fully understood

Naphthalene (PS No 22)

Table 13. Overview of the available data on mode of action (MoA) for naphthalene (polyaromatic hydrocarbon; CAS-number: 91-20-3)

Aquatic organism	MoA	Effect	Ref
General	The high degree of localisation of naphthalene metabolic enzymes, the production of metabolites capable of causing toxicity, GSH depletion in tissues where cytotoxicity occurs, the cytotoxic reactions of the tissues themselves, and the local production of tumours — all along with the lack of tumours in tissues not having these phenomena— suggest that these processes are involved in the mode of naphthalene's carcinogenic action in rodents, and that the balance of the activities of the enzymes responsible for these cellular processes is ultimately what determines the potential for naphthalene to cause tissue injury, and this balance likely varies across tissues and species. Lots of experiments have indicated that its toxicity are closely related to ROS and oxidative stress.	High level exposure to naphthalene may lead to the destruction of red blood cells. Animal studies have shown that naphthalene itself is not carcinogenic, but the carcinogenicity rise after metabolism by the cytochrome P450 monooxygenase system. Naphthalene exposure is associated with several toxic manifestations in humans and laboratory animals.	HSDB Rhomberg et al. 2010 Shi et al. 2005
Freshwater dipteran *Chironomus attenuates*	Elevated haemolymph Na^+, K^+ and Cl^- concentrations Data support the hypothesis that loss of ionic regulation in aquatic organisms is due to inhibition of specific enzyme systems and not to a general alteration of membrane integrity.		Harmon et al. 1983
Daphnia magna	Decreased haemoglobin concentration and inhibition of oxygen uptake	Behavioural changes	Crider et al. 1982
Marine crab *Scylla serrata*	Overall increase in LPO activity; in contrast, the enzymatic (CAT, GPx, SOD) and non-enzymatic antioxidants (vitamins C, E and GSH) showed decreased activities for hepatopancreas, haemolymph and ovary.		Vijayavel et al. 2004

Aquatic organism	MoA	Effect	Ref
Freshwater goldfish *Carassius auratus*	Induced hydroxyl radical (·OH) production, increased LPO content and protein carbonyl (PCO) content. Either LPO or PCO content showed significant relation with·OH production.		Shi et al. 2005

Polyaromatic hydrocarbons (PAH) (PS No 28)

Benzo(a)pyrene (CAS: 50-32-8)

Benzo(b)fluoranthene (CAS: 205-99-2)

Benzo(k)fluoranthene (CAS: 207-08-9)

Benzo(g,h,i)- perylene (CAS: 191-24-2)

Indeno(1,2,3- cd)-pyrene (CAS: 193-39-5)

For the group of priority substances (PS) of polyaromatic hydrocarbons (PAH), the biota EQS and corresponding AA-EQS in water refer to the concentration of **benzo(a)pyrene**, on the toxicity of which they are based. Benzo(a)pyrene can be considered as a marker for the other PAHs, hence only benzo(a)pyrene needs to be monitored for comparison with the biota EQS or the corresponding AA-EQS in water.

Table 14. Overview of the available data on mode of action (MoA) for polyaromatic hydrocarbons (PAHs; CAS-number: not applicable)

Aquatic organism	MoA	Effect	Ref
General	Photosensitisation generally has been considered to be the major mechanism of PAH phototoxicity, although the role of photomodification has become recognised as a key mechanism of toxicity	Cardiotoxicity The oncogenic effect of the compound was associated with the degree of response. The degree of response was different for five tested PAHs and ranked as follows (greatest response first): Benzo(a)pyrene > Benzo(a)anthracene > Indeno(1,2,3-cd)pyrene > Benzo(b)fluoranthene > Fluoranthene > Benzo(ghi)perylene	Incardona et al. 2011 Lampi et al. 2005
	For aquatic species, PAHs are generally accepted as acting through either of two modes of action: (1) "dioxin-like" toxicity mediated by activation of the aryl hydrocarbon receptor		Incardona et al. 2006

Aquatic organism	MoA	Effect	Ref
	(AhR), which controls a battery of genes involved in PAH metabolism, such as cytochrome P4501A (CYP1A) and (2) "non polar narcosis", in which tissue uptake is dependent solely on hydrophobicity and toxicity is mediated through non-specific partitioning into lipid bilayers.		
Burrowing clam *Ruditapes decussatus*	Benzo(b)fluoranthene: genotoxic effect in the gills; GST activity and GSH biosynthesis appear to be associated with limited lipid peroxidation even though they were insufficient to prevent induced genotoxicity		Martins et al. 2013
Juvenile white shrimp *Litopenaeus vannamei*	Benzo(a)pyrene: induced mRNA expression levels of SOD, cytochrome P450 (CYP) 1A1, GST in hepatopancreas; induced 7-Ethoxyresorufin O-deethylase (EROD), GST and SOD activities in gill and hepatopancreas; reduced GSH contents		Ren et al. 2015
Fish (review)	Benzo(k)-fluoranthene and indeno[1,2,3-cd]pyrene were consistently the most potent considering CYP1A induction or AhR binding		Barron et al. 2004
Fish: Larval and Juvenile zebrafish	Benzo(a)pyrene: AhR2 dependent	Developmental exposures are associated with cardiac toxicity. Anxiety-like behaviour in developmentally exposed fish Decreased learning and memory	Summarised in Knecht et al. 2017

Aquatic organism	MoA	Effect	Ref
Japanese flounder *Paralichthys olivaceus*, red sea bream *Pagrus major*, and Java medaka *Oryzias javanicus*	Benzo(a)pyrene: P450 enzyme induction by EROD activity		Cheikyula et al. 2008
Brown Trout *Salmo trutta fario* erythrocytes (*in vitro*)	Benzo(a)pyrene: induced the co-expression of mini-P-gp and P-gp		Valton et al. 2015
Zebrafish embryos *Danio rerio*	Benzo(a)pyrene: AhR2-dependent Benzo(k)fluoranthene: AhR2-independent (i.e. absent myocardial or endocardial CYP1A induction)	Bradycardia, pericardial edema, and myocardial CYP1A immunofluorescence More severe pericardial edema, looping defects, and erythrocyte regurgitation through the atrioventricular valve	Incardona et al. 2011
Zebrafish *Danio rerio*	Benzo(a)pyrene: altered antioxidant activity	Induced anxiolytic-like behavioural response	Mohanty et al. 2017
Zebrafish embryos *Danio rerio*	Benzo(a)pyrene: a decrease in larval photomotor response (LPR) activity, suggesting that the aryl hydrocarbon receptor (AhR2) plays a role in B[a]P induced larval hyperactivity.	Adult zebrafish (exposed as embryos to B[a]P) exhibited decreased learning and memory. Together this data demonstrates that developmental B[a]P exposure adversely impacts larval behaviour, and learning in adult zebrafish.	Knecht et al. 2017
Marine fish (the sea bass *Dicentrarchus labrax*)	Benzo(b)fluoranthen: disrupted metabolic responses and defences to toxicological challenge	Hepatic histopathological changes that indicate metabolic failure and inflammation.	Martins et al. 2015
Chinese rare minnow *Gobiocypris rarus*	Benzo(a)pyrene: significantly upregulated mRNA levels of p53 network genes (p53, p21, mdm2, gadd45a, and bax mRNA) in	Microphotographs revealed enlargement of the cell nuclei and cellular degeneration in males, while atrophy and vacuolization of hepatocytes were observed in females.	Yuan et al. 2017

Aquatic organism	MoA	Effect	Ref
	the livers from males and females	These results suggested that BaP induced liver DNA repair and apoptosis pathways and caused adverse pathological changes in rare minnow.	
Scallop *Chlamys farreri*	Benzo(a)pyrene: increased mRNA expression level of glutathione S-transferase isoform GST-theta		Yao et al. 2017

2.2.1.3 Organophosphorus insecticides

Chlorfenvinphos (PS No 8)

Table 15. Overview of the available data on mode of action (MoA) for Chlorfenvinphos (organophosphorus insecticide; CAS-number: 470-90-6)

Aquatic organism	MoA	Effect	Ref
General	**A specific MoA:** cholinesterase (ChE) inhibition. The inhibition of the enzyme acetylcholinesterase (AChE) results in the buildup of acetylcholine (ACh) at choline receptors. CAT, SOD, GPx has been suggested to be the cause of the oxidative status alteration.	Neurotoxicity (impaired neuromuscular control)	Hart 1993 Lukaszewicz-Hussain 2008
Blue mussel *Mytilus edulis*	AChE activity in haemolymph highly variable (no relationship to either sublethal effects or lethality) Evident concentration dependent inhibition for each of the remaining biomarkers (phagocytic activity, spontaneous cytotoxicity, neutral red retention time, total haemolymph protein)	The immune function and well-being of the mussels was significantly impacted in the absence of measurable inhibition of haemolymph AChE. Impaired neuromuscular control (at the highest exposure concentration)	Rickwood and Galloway 2004
Mosquitofish *Gambusia holbrooki*	AChE inhibition	Noticeable decrease of normal responses (observed for concentrations above 1.56 µg/L)	Sismeiro-Vivas et al. 2007

Aquatic organism	MoA	Effect	Ref
		A statistically significant correlation was found between AChE inhibition and all behavioural endpoints, but not with survival. Behavioural impairment was registered in fish with >40% AChE inhibition levels, while mortality was only observable in fish exhibiting AChE inhibition levels >80%.	
African sharptooth catfish *Clarias gariepinus*	Inhibition of AChE activities in plasma and eye homogenate		Mdegela et al. 2010
Pumpkinseed sunfish *Lepomis gibbosus*	Inhibition of AChE activity *in vivo* and *in vitro*		Rodrigues et al. 2011

Chlorpyrifos (Chlorpyrifos-ethyl) (PS No 9)

Table 16. Overview of the available data on mode of action (MoA) for Chlorpyrifos (chlorpyrifos-ethyl) (organophosphorus insecticide; CAS-number: 2921-88-2)

Aquatic organism	MoA	Effect	Ref
General	**A specific MoA:** inhibition of AChE by the active metabolite, chlorpyrifos oxon (CPYO) Inhibition of AChE by CPYO is reversible and, in the case of sub-lethal exposures, recovery of AChE can occur.	Neurotoxicity (because of continual nerve stimulation) Also suspected to be an endocrine disruptor.	Giesy et al 1999; Giddings et al. 2014 Mandal & Das 2011, El-Bendary et al. 2014) The specifics of the mode of action are discussed in greater detail in Solomon et al. 2013
Daphnia carinata (three successive generations)		Affected survival and fecundity of animals in the first generation	Zalizniak & Nugegoda 2006

Aquatic organism	MoA	Effect	Ref
		In the second generation, the most affected endpoint was time to the first brood with an indication of hormesis. (the study demonstrated a negative effect on reproduction of *D. carinata* during prolonged exposure to 0.005 µg/L)	
Daphnia magna		Promoted decrease of offspring but not increased appearance of male offspring	Palma et al. 2009
Shrimp *Palaemonetes argentinus*	Inhibited AChE Evident oxidative stress (increased H_2O_2content and increased levels of TBARs and carbonyl groups in proteins) The mobilisation of α-tocopherol from abdomen to cephalothorax A strong decrease of metallothioneins (MT) level occurred in cephalothorax (as an oxidative stress response).	The cephalothorax showed a more sensitive and enhanced oxidative stress response, compared with the abdomen. The induction of antioxidant enzymes like CAT, GST and GPx seems not be sufficient to prevent oxidative damages. Significant correlation between Integrated Biomarker Response values and exposure (significant effects observed at 3.5 ng/L)	Bertrand et al. 2016
Freshwater burrowing crab *Zilchiopsis collastinensis*		No differences in effective hatching but decreased survival of neonates, i.e. when crabs are outside the egg and not protected by chorion. (significant effects observed at 48 ng/L which is below the median LC50 values for embryos)	Negro et al. 2015
Damselfly *Coenagrion scitulum*	Reduced key component of the adult immune response (as measured by reductions	Exposure during the larval stage did not affect larval traits but	Van Dinh et al. 2016

Aquatic organism	MoA	Effect	Ref
larval stage	in the encapsulation response and in phenoloxidase activity)	caused delayed effects across metamorphosis by increasing the incidence of wing malformations.	
Coho salmon *Oncorhynchus kisutch*	Inhibition of AChE activity	Impaired cholinergic nervous system that lead to a proportional increase in the use of white muscle that may differentially impair swimming behaviours, such as predator avoidance.	Tierney et al. 2007
Juvenile Coho salmon *Oncorhynchus kisutch*	Inhibition of AChE activity in the brain	Reductions in spontaneous swimming and feeding activity were significantly correlated to AChE inhibition.	Sandahl et al. 2005
Guppy *Poecilia reticulata*		Concentration-related reductions in the frequency of reproductive behaviour (gonopodial thrusts) in males (chronic exposure for 14 d at nominal concentrations of 0.002 and 2 µg/L)	De Silva and Samayawardhena 2005
Common carp *Cyprinus carpio*	Significant changes in antioxidant enzyme (SOD, CAT and GPx) activities and MDA content in the brain and kidney	Pathological changes in tissue	Xing et al. 2012
Common carp *Cyprinus carpio*	Increase the activity of biotransformation enzymes (EROD and pentoxyresorufin-O-deethylase (PROD), and mRNA expression of CYP and CYP1A in the liver		Xing et al. 2014
Common carp *Cyprinus carpio*	Disrupted genomic DNA (altered DNA methylation)		Wang et al. 2014

Aquatic organism	MoA	Effect	Ref
Common carp *Cyprinus carpio*	Upregulated mRNA expression of cytokines IL-6, IL-8 and TNF-α in the head kidney and spleen Inhibited expression of IL-10 and TGF-β mRNA in both head kidney and spleen	Suggested immunotoxicity and immune organ inflammation	Chen et al. 2014
Fish and amphibian	Inhibition of AChE	Although effects on behaviour due to inhibition of AChE can be observed in vertebrates, these have not been experimentally related to effects on survival, development, growth, and reproduction of individuals in a quantitative manner.	Summarised in Giddings et al. 2014
Lake Sturgeon *Acipenser fulvescens* (larvae; testicular and ovarian tissue)	Inhibitory effect on testosterone synthesis in both testicular and ovarian tissue (*in vitro* bioassay)	No effect on the measured indicators of thyroid follicular development in larvae (thyroid gland histology)	Brand et al. 2015
California killifish *Fundulus parvipinnis*	Reduction in brain and muscle AChE Suppressed rate of cortisol release	Reduced activity and a decrease in mean swimming speed Evidence of the linkages between reduced swimming activity and AChE activity, indicating the importance of this physiological mechanism in regulating behavioural outcomes	Renick et al. 2016

Dichlorvos (PS No 42)

Table 17. Overview of the available data on mode of action (MoA) for dichlorvos (organophoshorous insecticide and acaricide; CAS-number: 62-73-7)

Aquatic organism	MoA	Effect	Ref
General	**A specific MoA:** inhibitor of AChE	Neurotoxicity	
Daphnia magna	AChE activity inhibition	The correlation analysis between swimming behaviour and AChE activity	Ren et al. 2015
Pacific oyster *Crassostrea gigas*	Decrease in AChE activity in gill tissues		Anguiano et al. 2010
African Clawed Frogs *X. Laevis* Zebrafish *Danio rerio*		A dose-dependent decrease in heart rate and free-swimming larval activity Kyphosis and decreased spine length	Watson et al. 2014
Loach *Misgurnus anguillicaudatus*	The glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) activity in liver decreased; the GPT and GOT activity in serum, the MN rate (‰) and three comet parameters increased		Nan et al. 2015

2.2.1.4 Organochlorine insecticides

Cyclodiene pesticides (No 9a: Aldrin, Dieldrin, Endrin, Isodrin)

Table 18. Overview of the available data on mode of action (MoA) for cyclodiene pesticides (organochlorine insecticides; CAS-numbers: 309-00-2, 60-57-1, 72-20-8, 465-73-6)

Aquatic organism	MoA	Effect	Ref
Insects	**A specific MoA:** interrupt normal synaptic transmission of nerve signals: first, it may increase pre-	Neurotoxicity	

Aquatic organism	MoA	Effect	Ref
General	synaptic neurotransmitter (acetylcholine) release One cause of toxicity may be the inhibition in oxidative phosphorylation Acting directly on mitochondria membrane and/or membrane proteins, they have been shown to uncouple electron transport and cause calcium ions move across the membrane in rat mitochondria In addition, aldrin and dieldrin may inhibit GABA (gamma aminobutyric acid) mediated neuroinhibition. Dieldrin interact with the mammalian constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) to induce cytochrome P450 (CYP) 2B or CYP3A family enzymes. Dieldrin could act as endocrine disrupter preventing the connection of 5-Alpha-dihydrotestosterone and E2 with the androgenic and estrogenic receivers.	Connection between Parkinson's disease mortality and pesticide use Aldrin and Dieldrin are potential endocrine disruptors. Dieldrin associated with the incidence of cancer and the dysfunctions of reproductive, endocrine and immune systems	Nelson 1975 Ritz & Yu 1999 Goodwin et al. 2002 Sueyoshi & Negishi 2001 Jorgenson 2001 Briz et al. 2011
Frogs *Xenopus laevis* (African clawed frog) (various life stages: (embryos, tadpoles, juveniles, adults)		In tadpoles, Dieldrin had been pointed out as an endocrine disruptor and teratogenic agent.	Schuytema et al. 1991

Aquatic organism	MoA	Effect	Ref
Neotropical anuran (amphibian) *Physalaemus cuvieri*		Dieldrin may be responsible for the high frequency of *P. cuvieri* with intersexual gonads.	Moresco et al. 2014
Female and male largemouth bass *Micropterus salmoides* (dieldrin-fed)	Cell pathways identified by the gene set enrichment were significantly increased in female, while the majority of cell pathways were significantly decreased in male fish.	Suggested that brain sexual dimorphic responses and neurotransmitter systems are targeted by dieldrin.	Martyniuk et al. 2013
Flathead mullet *Mugil cephalus* (fish Liver microsomes)	The contribution of CYP1A to the aldrin metabolism (inhibition of EROD). The results indicate that CYP1A and CYP3A are the cytochrome P450s involved in aldrin epoxidase activity.		Bozcaarmutlu et al. 2014
Tilapia guineensis (sampled from a municipal domestic water supply lake)	The PCA biplot analysis revealed a positive correlation between dieldrin sediment concentration and Vtg, Zrp and E2 in male fish.	A possible dieldrin role in male fish feminisation	Adeogun et al. 2016

Dichlorodiphenyltrichloroethane (No 9b: DDT) total and Para-para-DDT

Table 19. Overview of the available data on mode of action (MoA) for Dichlorodiphenyltrichloroethane (DDT) total[(1)] (organochlorine insecticides; CAS-number: not applicable) and Para-para-DDT[(2)] (CAS-number: 50-29-3)

Aquatic organism	MoA	Effect	Ref
Insects	**A specific MoA:** DDT opens sodium channels in insect neurons and has been reported to be an endocrine disruptor	Neurotoxicity	
			IARC
General	The most well accepted mechanism is interference with membrane ion fluxes, which leads to prolongation of the	Possible carcinogen to humans	ATSDR 2002
		The primary target organs for DDT toxicity include the	Newman and Unger 2003

Aquatic organism	MoA	Effect	Ref
	action potential and repetitive firing Other contributory mechanisms (that may be secondary to inferences with ion fluxes) may include decreases in brain serotonin and increases in levels of aspartate and glutamate	nervous system, the reproductive system and the liver.	
p,p'-DDT Zebra mussel *Dreissena polymorpha*	The MN frequency analysis confirmed the genotoxicity potential of the three homologues and p,p'-DDE showed the highest irreversible DNA damage.		Binelli et al. 2008
(DDT metabolites) *In vitro* (four cell-based assays)	p,p'-DDT displayed a similar spectrum of estrogenic activities similar to E2, however, with a lower potency.		Wetterauer et al. 2012
(p,p'-DDE, persistent metabolite of p,p'-DDT) Wild seals Male Japanese medaka *Oryzias latipes* Male zebrafish	An androgen receptor antagonist Induced Vtg Estrogen related genes significantly up-regulated	Reduced sperm count Gonadal intersex	Summarised in Sun et al. 2015
Carp *Cyprinus carpio* (microcosms, exposure via food with *Tubifex tubifex* (Oligochaeta, Tubificidae) as a prey)	Significant changes observed in some biomarkers, including SOD, CAT, GST, GSH, and carboxylesterase,		Di et al. 2017

Aquatic organism	MoA	Effect	Ref
	in tissues of both organisms		

(1) DDT total comprises the sum of the isomers 1,1,1-trichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 50-29-3; EU number 200-024-3); 1,1,1-trichloro-2 (o-chlorophenyl)-2-(p-chlorophenyl) ethane (CAS number 789-02-6; EU Number 212-332-5); 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (CAS number 72-55-9; EU Number 200-784-6); and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 72-54-8; EU Number 200-783-0)

(2) Para-para DDT is abundant to approximately 77% of all DDT congeners (Snedeker 2001)

Endosulfan (PS No 14)

Table 20. Overview of the available data on mode of action (MoA) for endosulfan (organochlorine insecticide; CAS-number: 115-29-7)

Aquatic organism	MoA	Effect	Ref
	A specific neurotoxic MoA via the GABA receptor system (opening the chloride transport, increasing glutamate level). It penetrates into the insect via the tracheas, by ingestion, and has some contact activity.	Neurotoxicity	Hassall 1990
In vitro: Dispersed head kidney cells of Rainbow Trout *Oncorhynchus mykiss*	Decreased adrenocorticotropin (ACTH)- or 2'-o-dibutyryladenosine 3':5'-cyclic monophosphate (dbcAMP)-stimulated cortisol secretion and cellviability in a concentration-dependent pattern (the doses required to disrupt cortisol secretion were significantly lower than doses lethal to the head kidney cells).	The study identified endosulfan as an adrenotoxicant in rainbow trout.	Leblond et al. 2001
In vitro: Dispersed head kidney cells of Rainbow Trout *Oncorhynchus mykiss*	Alterations in biochemical parameters known to be involved in oxidative stress (the activity of enzymes: CAT, GPx, GST), reduced levels of GSH and increase in LPO levels.		Dorval et al. 2003
Freshwater fish *Channa punctatus* Bloch	Modulation of antioxidant systems in liver (significant induction of GPx, GST activity), and GSH levels in all the organs. CAT activity significantly decreased; LPO values significantly increased in all the organs		Pandey et al. 2001
(dietary exposure to endosulfan) Nile tilapia	Increased hepatic EROD activity; decreased T4 plasma levels	Liver morphological changes; morphological alterations of	Summarized in EFSA 2011

Aquatic organism	MoA	Effect	Ref
Atlantic salmon	Increased EROD activity; decreased Na^+, K^+-ATPase activity in the intestine Glycogen store depletion coupled with lipidosis	lymphocytes in blood Vacuolation of intestinal villi in hind gut; signs of atrophy and necrosis in liver cells	
Mature zebrafish *Danio rerio*	Increased Vtg levels in males	Decreased hatching rate, together with pathological alterations in testes	Han et al. 2011
Juvenile catfish *Clarias batrachus*	Decrease of gonadotropin-releasing hormone mRNA levels in brain, together with increased ovarian aromatase activity		Chakrabarty et al. 2012
Catfish *Clarias batrachus*	Decreased expression of steroidogenic enzymes in testes		Rajakumar et al. 2012
Freshwater cichlid fish *Cichlasoma dimerus*	Decreased βFsh pituitary content and altered GnRH in larvae and juveniles The steps of the endocrine regulation of steroidogenesis affected by endosulfan in both testes and ovaries appear to be located downstream of adenylate cyclase activation and upstream of pregnenolone conversion to progesterone and/or dehydroepiandrostenedione	Altering testes tissue structure in adults Altered Fsh producing cells (higher nuclear area and mean nuclear diameter) in larvae and juveniles	Da Cuña et al. 2011, 2013 Piazza et al. 2011, 2015 Da Cuña et al. 2016

Hexachlorocyclohexane (PS No 18)

Table 21. Overview of the available data on mode of action (MoA) for Hexachlorocyclohexane (HCH) (organochlorine insecticide; CAS-number: 608-73-1 - mixture of hexachlorocyclohexanes including Lindane)

Aquatic organism	MoA	Effect	Ref
	A specific MoA: different isomers action on GABA receptor chloride channel complex. The isomers binds to the picrotoxin site of the receptor and decrease Cl- flux which results in neuronal hyperexcitability (stimulated transmitter release). The same action could be accomplished by binding to glycine receptors. Biochemical effects induced in rodents and human: calcium homeostasis disturbance; phosphoinositide turnover; activation of phospholipases; oxidative stress; effects on DNA Integrity; estrogenicity	Alfa- and gamma- isomers: activation of the central nervous system Beta- and delta- isomers: depression of central nervous system	Matsumura and Ghiasuddin 1983 Vale et al. 2003 Olivero-Verbel et al. 2011
Daphnia magna	Triggers an increase in the Krebs cycle activity.		De Coen et al. 2001
Fish gill cell line-based (RTgill-W1) assay	Inhibition of metabolic activity	A very good agreement between *in vivo* and *in vitro* effective concentrations	Tanneberger et al. 2013
Fish *Etroplus maculatus*	Reduced red blood cell count (RBC), haemoglobin (Hb), hematocrit (Ht), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) The white cell count (WBC) was significantly higher.	Destructive effects on the gills (proliferation of the lamellar epithelium and lamellar fusion), liver (necrosis) and kidney (constriction of the tubular lumen)	Nandan and Nimila 2012
Tilapias *O. mossambicus*	Increase of heat shock proteins (HSP70 and HSP60) gene expression; increase in		Daiwile et al. 2015

Aquatic organism	MoA	Effect	Ref
	melanin concentrating hormone levels and decrease in melanophore index		
Wild Atlantic eels (the European eel *Anguilla Anguilla* and the American eel *Anguilla rostrata*)	Identified genes involved in lipolysis and cell growth (in the global hepatic transcriptome determined by RNA-Seq)	Established significant relationships between the hepatic expression levels of specific transcripts and the concentrations of individual contaminants measured in fish	Baillon et al. 2015

Dicofol (PS No 34)

Table 22. Overview of the available data on mode of action (MoA) for Dicofol (organochlorine insecticide (acaricide; miticide); CAS-number: 115-32-2). Chemically related to DDT.

Aquatic organism	MoA	Effect	Ref
General	The exact MoA is not known (it is thought to be related to the inhibition of certain enzymes in the central nervous system).	Hyperstimulation of nerve transmission along nerve axons (cells)	
In vitro system	Inhibits aromatase activity (CYP19), an important steroidogenic enzyme catalysing conversion of androgens to oestrogens, acting thus as an anti-oestrogen.		Vinggaard et al. 2000
In vitro system	Anti-androgenic activity in reporter gene		Thiel et al. 2011
Xenopus laevis cell line	3,3',5-L-triiodothyronine-T(3)- antagonist activity		Sugiyama et al. 2005
Daphnia magna		Significantly increased number of male neonates during the sub-chronic assay (4–6 day experiments) but not apparent during the prolonged 21-day assay	Haeba et al. 2008

Heptachlor and heptachlor epoxide (PS No 44)

Table 23. Overview of the available data on mode of action (MoA) for heptachlor and heptachlor epoxide (organochlorinated insecticide; CAS-number: 76-44- 8 / 1024-57-3)

Aquatic organism	MoA	Effect	Ref
General	The MoA of heptachlor or heptachlor epoxide is uncertain. Activation of protein kinase C (MAPKs) Reduced cellular levels of MAPK cascade proteins, which are important intermediates in the signal transduction pathway of immune cells. Stimulation of apoptosis protease CPP32	The neurological and hepatic effects seen from exposure to heptachlor and heptachlor epoxide are typical of exposure to other chlorinated pesticides. Carcinogenicity	US ATSDR 2007 and WHO 2006
Amphibian (tadpoles and adults of *Rana kl. Esculenta*)	Changes in enzyme activities, particularly those involved in the protective response to xenobiotic injury in the cell epidermis (keratinocytes and mitochondria-rich cells) of adults	Severe morphological alterations in the larval epidermal cells (apical and skin cells)	Fenoglio et al. 2009

2.2.1.5 Chlorinated solvents

Carbon-tetrachloride (No 6a)

Table 24. Overview of the available data on mode of action (MoA) for Carbon tetrachloride (chlorinated solvent; CAS-number: 56-23-5)

Aquatic organism	MoA	Effect	Ref
General	Metabolised by the CYP2E1 pathway producing trichloromethyl peroxy free radicals. The free radicals cause LPO making the membrane more permeable to ions and molecules. Calcium and enzymes leak out of the membrane disrupting the membrane potential. When lipids are exposed to peroxidation, GSH works in a preventative manner as an antioxidant. When the	Cytotoxic (apoptosis or necrosis) Hepatotoxin (toxic to the liver)(1) Exposure to high concentrations can affect the central nervous system.	Liu et al. 1993 Rood et al. 2001 Manibusan et al. 2007

Aquatic organism	MoA	Effect	Ref
	oxidation continues, GSH becomes depleted and oxidation is accelerated.	Chronic exposure to carbon tetrachloride can cause liver and kidney damage and could result in cancer.	
Midge *Chironomus tentans*	Expression of HSP70 and haemoglobin genes (mRNA) increased		Lee et al. 2006
Juvenile brown trout *Salmo trutta lacustris*	The expression of 1,273 genes was monitored by abundance of transcripts. No genes found that reacted exclusively to CCl4. Four haemoglobins and two metallothioneins showed dose responses.		Krasnov et al. 2007
Common carp *Cyprinus carpio* (via injection)	In the serum: elevated activities of GPT, GOT, LDH, and increased the reduced levels of total protein and albumin In the liver: reduced levels of SOD, GPx, CAT, GSH, total antioxidant capacity and MDA formation	Reduced levels of liver index Hepatotoxicity	Jia et al. 2013

(1) Carbon tetrachloride has been widely used in experimental toxicology as a model solvent causing ''classical hepatotoxicity''

1,2-Dichlorethane (PS No 10)

Table 25. Overview of the available data on mode of action (MoA) for 1,2-dichlorethane (DCE, Ethylene dichloride) (chlorinated hydrocarbon; CAS-number: 107-06-2)

Aquatic organism	MoA	Effect	Ref
General	Binds to DNA and might cause genotoxicity Reactive intermediates capable of binding covalently to cellular macromolecules and induce toxic and carcinogenic effects. In addition, DCE can promote lipid peroxidation *in*	Carcinogenic In acute toxicity studies, DCE mainly affects respiratory systems, liver and kidneys. Evidence that the toxicity and carcinogenicity of DCE are associated with its	IARC, EU ECHA Nagano et al. 2006 ATSDR 2001

Aquatic organism	MoA	Effect	Ref
	vitro is also associated with tissue damage. The level of glutathione present in the liver appears to modulate effects of DCE in animals.	metabolism to active intermediates.	
Channel catfish *Ictalurus punctatus*	Detection of DNA adducts: S-[2-(N7-guanyl)ethyl] glutathione adducts in liver tissue after 2 h of exposure which were still detectable three weeks after a single pulse exposure.	Formation of DNA adducts by reactive chemicals or their metabolites are often a precursor of mutagenesis and other adverse effects.	Jemal et al. 2010

Dichloromethane (PS No 11)

Table 26. Overview of the available data on mode of action (MoA) for Dichloromethane (DCM, Methylene chloride) (chlorinated solvent; CAS-number: 75-09-2)

Aquatic organism	MoA	Effect	Ref
General	Dichloromethane (DCM) is metabolised via a GST-dependent pathway to formaldehyde (HCHO), a mutagenic compound that could play an important role in the carcinogenic effects of DCM. HCHO can form DNA-protein cross-links in the liver of mouse. CO–induced inhibition of cytochrome c oxidase (COX) could also be a mechanism of toxicity of DCM which liberates CO as a product of its metabolism by cytochrome P4502E1.	Carcinogenic	EU ECHA Trotsenko & Torgonskaya 2009 Casanova et al. 1992 Lehnebach et al. 1995
No information about the MoA in non-target aquatic organisms (tested within environmentally relevant concentrations)		No information about the aquatic acute and/or chronic toxicity could be found under the harmonised classification and labelling (CLP00) approved by the European Union.	ECHA

Aquatic organism	MoA	Effect	Ref
has been found in the scientific literature.			

Hexachlorobutadiene (PS No 17)

Table 27. Overview of the available data on mode of action (MoA) for hexachlorobutadiene (HCBD) (chlorinated solvent; CAS-number: 87-68-3)

Aquatic organism	MoA	Effect	Ref
General	GSH/mercapturate/β-lyase pathway is likely to be the predominant means of bioactivation of HCBD to a DNA reactive species, while oxidative metabolism to one or more DNA reactive metabolites may occur.	Possible carcinogen in rodents Genotoxic Associated with human renal tubular dysfunction, hypotension, cardiac disease and neurological disorders Fatty liver degeneration, epithelial necrotising nephritis, central nervous system depression and cyanosis	HSDB Jaffe et al. 1983 Davis 1984 IARC ATSDR US EPA 2003
Goldfish *Carassius auratus* (via injection)	Gamma glutamyl transpeptidase (GGT), a histochemical marker of proximal tubule brush border in mammals, was demonstrated in the goldfish kidney.	Cytoplasmic vacuolation and necrosis in the renal tubules Greater ratio of kidney to body weight	Reimschuessel et al. 1989
Goldfish *Carassius auratus* (via injection)	Cell proliferation and regeneration of the epithelium following injury	The presence of large numbers of developing nephrons may provide a marker for renal injury.	Reimschuessel et al. 1990a, 1990b

Tetrachloroethylene (No 29a)

Table 28. Overview of the available data on mode of action (MoA) for tetrachloroethylene (chlorinated solvent; CAS-number: 127-18-4)

Aquatic organism	MoA[1]	Effect	Ref
General	Considerable evidence supports the fact that oxidative and conjugative metabolites are involved in both genotoxic and non-genotoxic mechanisms of toxicity.	A probable human carcinogen Reduced central nervous system activity	IARC Cichocki et al. 2016
Brine shrimp *Artemia salina* nauplii		Teratogenic	Kerster et al. 1983
Japanese medaka *Oryzias latipes* embryos		Reduced hatchability and larval survival	Spencer et al. 2002
Japanese medaka *Oryzias latipes* larvae	A higher protein concentration (protein synthesis) in treated fish	Significantly reduced length and weight	Spencer et al. 2006

([1]) The mechanisms of action for cancer and non cancer toxicity dependent on multiple factors, including tissue and species (Cichocki et al. 2016)

Although Trichloroethylene (TCE) is a widely studied chemical, considerably less experimental and epidemiologic evidence is available for Tetrachloroethylene, one of the most widely used chlorinated solvents.

Trichloroethylene (No 29b)

Table 29. Overview of the available data on mode of action (MoA) for Trichloroethylene (TCE) (chlorinated solvent; CAS-number: 79-01-6)

Aquatic organism	MoA[1]	Effect	Ref
General	Metabolites such as TCA or DCA are known to induce oxidative stress in mammals (including lipid peroxidation, excess free radical production and peroxisomal proliferation). Induction but also inactivation of P450 level reported in mammals. The second and the minor pathway involves conjugation	Human carcinogen, with the kidney being the target tissue Central nervous system, kidney, liver, immune system, male reproductive system, and developmental toxicity	IARC Summarized in Vidal et al. 2001, Houde et al. 2015 and Cichocki et al. 2016

Aquatic organism	MoA[1]	Effect	Ref
	of TCE with glutathione and further metabolism by the mercapturic acid pathway.	Exposure of avian cells and embryos have shown toxicity on cardiac output.	
Daphnia magna	Effects on genes and proteins related to metabolism, reproduction, and growth (at concentrations of 0.1–10 µg/L)		Houde et al. 2015
Freshwater clams *Corbicula fluminea*	Biochemical effects (cytochromes P450 and P418, NADH-cytochrome c reductase, CAT, peroxided and peroxidisable lipids and net peroxidation as biomarkers).		Vidal et al. 2001
Aquatic organisms: Alage, clams fish	Oxidative stress in freshwater clams. Metabolic perturbations during fish embryogenesis and cellular changes in rainbow trout (chronically exposed)	Impacts on algal growth and on the density and chlorophyll content of phytoplankton	Summarised in Houde et al. 2015

([1]) The mechanisms of action for cancer and non cancer toxicity dependent on multiple factors, including tissue and species (Cichocki et al. 2016)

Trichloromethane (PS No 32)

Table 30. Overview of the available data on mode of action (MoA) for Trichloromethane (Chloroform) (organic compound; CAS-number: 67-66-3)

Aquatic organism	MoA	Effect	Ref
General	Cytotoxicity and compensatory cell proliferation Inhibition of multiple ionic currents, including L-type Ca^{2+} current (ICa.L), transient outward K^{+} current (Ito), voltagegated sodium current (INa), HCN2 current, and hERG current, but not the	Carcinogenic Cardiac toxicity	Golden et al. 1997 Zhou et al. 2011

Aquatic organism	MoA	Effect	Ref
	inward rectifier K^+ current IK1		
Daphnia magna	The mechanisms are unknown but potential MoA include enzymes inhibition, disruption of membrane permeability and structural DNA damage.		Bernot et al. 2005
Fish		Effect on, behaviour, biochemistry, development, growth, histology, physiology; injury, intoxication	www.pesticideinfo.org

2.2.1.6 Aromatic organochlorine compounds

Hexachlorobenzene (PS No 16)

Table 31. Overview of the available data on mode of action (MoA) for Hexachlorobenzene (HCB) (aromatic organochlorine compound - fungicide; CAS-number: 118-74-1)

Aquatic organism	MoA	Effect	Ref
General	Induction of rodent liver cancer by porphyrinogenic compounds followed a cytotoxic MoA. Binding to the Ah-receptor Can lead to an uncoupling of oxidative phosphorylation after metabolism to pentachlorophenol. Inhibition in phospholipid synthesis	Carcinogenic and reproductive toxin	Carthew and Smith 1994 HSDB Masini et al. 1985 Cantoni et al. 1987
Green alga *Chlorella kessleri* Crab *Chasmagnathus granulatus*	Decrease in uroporphyrinogen decarboxylase activity in both organisms Oxidative stress (high MDA levels in crab hepatopancreas, probably due to induced LPO) Antioxidant defenses such as SOD activity and reduced GSH content below normal values	Reduced HSI Epithelium disorganisation in hepatopancreas tubules	Chaufan et al. 2006

Aquatic organism	MoA	Effect	Ref
Juvenile common carps *Cyprinus carpio*	Oxidative stress in brain: decreases of GSH content and SOD activity, elevated contents of reactive oxygen species (ROS), thiobarbituric acid-reactive substances (TBARS, as an indicator of lipid peroxidation products), glutathione disulfide (GSSG), and activities of nitric oxide synthase (NOS), GPx, and GR. Inhibited activities of AChE and GST. No significant changes of GSH content and SOD activity in liver	Brain and not the liver was a sensitive target organ.	Song et al. 2006
Chub *Leuciscus cephalus L.*	Significant positive correlations found between GST and EROD activity with HCB concentration in muscle		Blahová et al. 2010
Grass goby fish *Zosterisessor ophiocephalus*	Significant negative correlations were found between HCB body burden with AChE activity in muscle.		Barhoumi et al. 2014

Pentachlorobenzene (PS No 26)

Table 32. Overview of the available data on mode of action (MoA) for Pentachlorobenzene (chlorinated aromatic hydrocarbon; CAS-number: 608-93-5)

Aquatic organism	MoA	Effect	Ref
General		High toxicity (rodents) Histopathological damage to liver, kidney and forestomach; depletion of thymic lymphocytes	HSDB
Zebrafish *Danio rerio* embryo	Expression of potential marker genes: the aryl hydrocarbon receptor 2, cytochrome P450 1A (CYP1a), HSP70, the transcription factors musculoaponeurotic fibrosarcoma oncogene family protein g (avian) 1 and NF-E2-p45- related factor, and heme oxygenase 1 (HMOX1).		Weil et al. 2009

Aquatic organism	MoA	Effect	Ref
	HMOX1 and CYP1a proved to be the most sensitive genes.		

Pentachlorophenol (PS No 27)

Table 33. Overview of the available data on mode of action (MoA) for pentachlorophenol (PCP) (organochlorine compound; CAS-number: 87-86-5)

Aquatic organism	MoA	Effect	Ref
General	The molecule easily traverses biological membranes. The mechanism of action is to uncouple oxidative phosphorylation through binding mitochondrial proteins which inhibit ATP-ase. Oxidative stress The pentachlorophenol metabolite tetrachlorohydroquinone induces massive ROS and prolonged p-ERK expression in splenocytes, leading to inhibition of apoptosis and necrotic cell death.	Tumour promotion effects Liver toxicity in rats Immunotoxicity, carcinogenicity, oxidative stress and metabolic disorders	HSDB Wang et al. 2001 Chen et al. 2014 Summarized in Yang et al. 2017
Adult female *Xenopus* frog	Altered levels of plasma hormones (progesterone, testosterone, and estradiol)	Ovary injuries	Orton et al. 2009
Carp	Changes of serum testosterone level and hepatic microsome enzyme activity		Zhang et al. 2008
Zebrafish	Chronic exposure alters thyroid hormones and thyroid hormone pathway mRNAs		Yu et al. 2014
Rare minnow *Gobiocypris rarus*	A significant decrease in the mRNA level of hepatic estrogen receptor-α (ERα) in male or juvenile Increased mRNA levels of ERβ1, ERβ2, VtgI, and VtgII in male or juvenile		Zhang et al. 2014
Matured rare minnow *Gobiocypris rarus*	In male fish: 14-d exposure caused up-regulation of mRNA levels of hepatic erα, erβ, ar, gr, vtg and gonadal erα, vtg, ar,	Overall, PCP interfere with steroid receptors, evoke responses of HPG/I axis, and result in	Yang et al. 2017

Aquatic organism	MoA	Effect	Ref
	dmrt1, providing evidence for agonistic activities for steroid receptor The up-regulated mRNA of gnrh, crf, pomc in the brain	adverse effects on reproductive and interrenal system in rare minnow at environmental relevant concentrations	

Trichlorobenzenes (PS No 31)

Table 34. Overview of the available data on mode of action (MoA) for trichlorobenzenes (industrial solvent; CAS-number: 12002-48-1)

Aquatic organism	MoA	Effect	Ref
General		Baseline toxicant	
Tetrahymena (ciliated protozoon)		The order of toxicity was: 1,2,4-trichlorobenzene > o-dichlorobenzene > p-dichlorobenzene > m-dichlorobenzene > chlorobenzene	Zhang et al. 2012
Invertebrates		Not biotransformed	Ashauer et al. 2012
Marine risk assessment for 1,2,4-trichlorobenzene		It was concluded that no risks are expected for aquatic organisms.	van Wijk et al. 2006

2.2.1.7 Dioxins, PCBs, BDEs

Brominated diphenylethers (PS No 5)

Table 35. Overview of the available data on mode of action (MoA) for brominated diphenylethers(1) (P)BDEs (flame retardants; CAS-number: 32534-81-9)

Aquatic organism	MoA	Effect	Ref
General	Studies indicate that the substances could both inhibit and activate the AhR.	The congeners can have different mechanisms of action but the general actions of BDEs are: developmental neurotoxicity, carcinogenicity (a prenatal developmental toxin) and endocrine disruption (also tend to deposit in human adipose tissue).	US EPA 2014 Wahl et al. 2010 Chevrier et al. 2010 PolyBDEs EQS

Aquatic organism	MoA	Effect	Ref
		The data set shows that PBDEs can cause a wide range of effects, in particular on mammals. The data set available however did not allow for the identification of one congener that would be systematically more toxic, or a MoA that would be specific of one congener.	dossier 2011
Wildlife	The competitive binding seen with thyroxin indicates that metabolites of some of the lower brominated diphenyl ethers may have a potential to cause endocrine disturbing effects in wildlife.	Potential for endocrine disruption. *In vitro* data indicating potential for endocrine disruption in intact organisms. Also includes effects *in vivo* that may, or may not, be ED-mediated. May include structural analyses and metabolic considerations.	EQS Substance data sheet 2005 (citing COM(2001) 262 final) PolyBDEs EQS dossier 2011
Aquatic organisms		PentaBDE has effects on: 1) reproduction 2) cerebral development 3) thyroid hormones (TH)	The Norwegian Pollution Control Authority 2009
Fathead minnows *Pimephales promelas*		BDE-47: alterations in reproductive output (inhibited egg production and reduction in mature sperm) The condition index of males was significantly reduced compared with control males.	Muirhead et al. 2006
Fathead minnows *Pimephales promelas*	BDE-47: depressed plasma thyroxine (T4), but not 3,5,3´-triiodothyronine (T3). Decline in T4 was accompanied by elevated mRNA levels for TSHβ (low dose only) in the pituitary. PBDE-47 intake elevated transcript for TH receptor in the brain of females and decreased mRNA for TH receptor β in the brain of both sexes, without		Lema et al. 2009

Aquatic organism	MoA	Effect	Ref
	altering these transcripts in the liver.		
Fathead minnows *Pimephales promelas*	BDE-47: several alterations in gene expression (decrease in hepatic estrogen receptor and in ovarian aromatase) Increase in deiodinase 2 expression in brain tissue and decrease in hepatic transthyretin expression in males. No significant differences in plasma hormone levels	No significant differences in GSI, secondary sexual characteristics, or reproductive success Overall, exposure to BDE-47 is capable of altering both sex steroid–related and thyroid-related transcripts but that these observed alterations do not necessarily manifest themselves at higher levels of biological organisation for the endpoints selected.	Thornton et al. 2016a
Fathead minnows *Pimephales promelas* (dietary exposure)		BDE-47: reduced fecundity and sex ratio was biased towards females; fewer tubercles in males	Thornton et al. 2016b
Mangrove killifish *Kryptolebias marmoratus* larvae	BDE-47: upregulated expression of TH metabolism-related genes (e.g. deiodinases, UGT1ab) and HPT axis-related genes and significant changes in TH levels	Impacts on the thyroid endocrine system	Kang et al. 2017
Juvenile Chinook salmon *Oncorhynchus tshawytscha* (dietary exposure starting with 0.3 ng total PBDEs/g food)	BDE-47 and BDE-99: the concentrations of both circulating T4 and T3 were altered in juvenile salmon by BDE-99 but not by BDE-47.	The disruption of circulating thyroid hormone concentrations has the potential to impact a number of critical functions in juvenile salmon including growth, parr-smolt transformation, and immunological processes.	Arkoosh et al. 2017
Carp (juvenile) *Carassius auratus* Zebrafish (embryos to adult) *Danio rerio* Rainbow trout (juvenile) *Oncorhynchus mykiss*	Different congeners: altered concentrations of both circulating T4 and T3 (down- or up regulation, depending on the PBDE congener and the species studied)	PBDEs have the potential to act as endocrine disrupting compounds capable of altering the concentration of thyroid hormones in fish by a number of mechanisms.	Summarised in Arkoosh et al. 2017

Aquatic organism	MoA	Effect	Ref
European flounder (adult) *Platichthys flesus* Lake trout (juvenile) *Salvelinus namaycush*			

([1]) For the group of priority substances (PS) covered by brominated diphenylethers, the environmental quality standards (EQS) refers to the sum of the concentrations of congener numbers 28, 47, 99, 100, 153 and 154 (triBDE 28, tetraBDE 47, pentaBDE 99 and 100, hexaBDE 153 and 154). Structurally similar to PCBs.

Dioxins and dioxin-like compounds (PS No 37)

Table 36. Overview of the available data on mode of action (MoA) for Dioxins and dioxin-like compounds (CAS-number: see footnote 10 in Annex X to Directive 2000/60/EC)

Aquatic organism	MoA	Effect	Ref
General	**Common MoA:** interaction with the AhR		
Fish	Responses linked to cytochrome P4501A (CYP1A; i.e. gene expression, protein or catalytic activity inductions). CYP1A induction is due to activation of AhR.	Apoptosis Embrionic mortality	Reviewed in Whyte et al. 2000 Dioxin and Dioxin-Like PCBs EQS dossier 2011

2.2.1.8 Metals

Cadmium and its compounds (PS No 6)

Table 37. Overview of the available data on mode of action (MoA) for cadmium and its compounds[(1)] (metal; CAS-number: 7440-43-9)

Aquatic organism	MoA	Effect	Ref
General	Studies of cadmium toxicity in animal cells unveiled a huge set of cellular targets for the deleterious action.[(2)] Thiol-containing proteins are most likely to bind cadmium and	Chronic exposure leads to adverse effects on growth, reproduction, immune and endocrine systems, development, and	US EPA 2016

Aquatic organism	MoA	Effect	Ref
	to be affected by the presence of the metal, thus perturbing redox homeostasis and signalling events. Consequences include increased production of reactive oxygen species and changes in the expression of different genes that trigger cell cycle arrest, differentiation, immortalisation or apoptosis. Perturbations of calcium, zinc, or iron homeostasis, individually or most probably collectively, play a key role in the toxicological action of cadmium.	behaviour in aquatic organisms. Other toxic effects include histopathologies of the gill, liver and kidney in fish, renal tubular damage, immunosuppression, and structural effects on invertebrate gills.	Bertin & Averbeck 2006 Martelli et al. 2006
Freshwater and marine organisms	Cadmium competes with calcium at high affinity binding sites in the gill membrane and blocks the uptake of calcium from water by interfering with ion uptake in specialised calcium channels that are located in the mitochondria-rich chloride cells. Cadmium exposure is also associated with the disruption of sodium balance and accompanying Na+/K+-ATPase activity. Once inside the cell, cadmium can disrupt enzymatic function by either directly affecting Ca-ATPase activity or inhibiting antioxidant processes. Cadmium also inhibits enzymes such as catalase, glutathione reductase, and superoxide dismutase and reducing agents such as GSH, ascorbate, b-carotene and a-tocopherol, all of which can lead to the generation of excess reactive oxygen species and reduced ATP production.	The combined effect of competition for the binding sites and blockage of calcium uptake on the gill membrane results in acute hypocalcaemia in freshwater fish, which is characterised by cadmium accumulation in tissues as well as decreased calcium concentrations in plasma.	Summarised in US EPA 2016
Various trophic saltwater organisms	Cadmium exerts harmful effects on aquatic organisms in many ways, although all the major mechanisms of toxicity are a consequence of the strong coordinating properties of		Wang et al. 2010 (review)

Aquatic organism	MoA	Effect	Ref
	cadmium cations (Cd^{2+}) that affect the properties of many biological molecules (enzymes, etc.), often by blocking and reducing the thiol sites on proteins		
Gammarus fossarum	Cellular and molecular osmoregulatory responses: decreased haemolymph osmolality (HO) In slightly impacted individuals: a lower Na+/K+-ATPase (NKA) fluorescence In impacted individuals: a very limited NKA fluorescence	A thinner epithelium and a slight collapse of the gill Dramatic alterations of the gill structure, including hyperplasia and alteration of the pillars	Issartel et al. 2010
Marine mussel *Mytilus galloprovincialis*	Increase in CAT activity (oxidative stress)		Rocha et al. 2015
Fish (different species)	The presence of cadmium-binding molecules called metallothioneins	Cadmium accumulation in the kidney, liver, and gills of freshwater fish	Levit 2010 (literature review)
Prussian carp *Carassius auratus gibelio*	Inhibition of steroid formation	Inhibits ovarian maturation.	Szczerbik et al. 2006
Juvenile rainbow trout		Delayed egg formation and inhibited egg development into the fry stage	Vetillard and Bailhache 2005
Yellow perch *Perca flavescens*	Identified transcriptional signatures specific to Cd exposure: 176 genes were differentially transcribed (mainly involved in iron metabolism, transcriptional and translational processes, vitamin metabolism, blood coagulation, and calcium transport).		Bougas et al. 2013

([1]) For Cadmium and its compounds, toxicity and therefore the EQS values vary depending on the hardness of the water as specified in five class categories (Class 1: < 40 mg $CaCO_3$/L, Class 2: 40 to < 50 mg $CaCO_3$/L, Class 3: 50 to < 100 mg $CaCO_3$/L, Class 4: 100 to < 200 mg $CaCO_3$/L and Class 5: ≥ 200 mg $CaCO_3$/L).

([2]) Probably, because so many processes have to be simultaneously studied, relatively few mechanisms have been fully elucidated in a cellular context.

Lead and its compounds (PS No 20)

Table 38. Overview of the available data on mode of action (MoA) for lead and its compounds (metal; CAS-number: 7439-92-1)

Aquatic organism	MoA	Effect	Ref
General	Lead binds to sulfhydryl group which is prevalent in many enzymes. It can also mimic different metals in biological systems, especially calcium, magnesium and zinc, leading to e.g. inhibition of enzymes or altered enzyme activity, altered metal transports, apoptosis, genetic regulation. The activity of delta-aminolevulinic acid dehydratase (δ-ALAD), enzyme functioning in the production of haem groups, can be depressed by lead leading to anemia. This is because lead has a higher affinity for the polycysteine arrays than zinc. When binding to the arrays, lead distorts the protein conformation, disabling the functionality of the protein.	Developing stages are generally more sensible than adult organisms. Lead can damage the proximal renal tubules (and cause renal tumours). Lead toxicity affects CNS in different ways where membrane associated ion channels and signalling molecules appears to be the primary route for lead toxicity/	Garza et al. 2006 Newman and Unger 2003
General	Various molecular, cellular and intracellular mechanisms have been proposed to explain the toxicological profile of lead that includes generation of oxidative stress, ionic mechanism and apoptosis. Of these, oxidative stress has been found to be more pronounced and much more severe. Lead causes generation of ROS which results in critical damage to various biomolecules like DNA, enzymes, proteins and membrane based lipids, while simultaneously it impairs the antioxidant defense system		Flora et al. 2012 (a review with recent updates)
Freshwater crab *Dilocarcinus pagei*	Affected osmolality and ion concentrations (both in the whole animal and in the isolated tissue)	Lost weight *in vivo* Muscle weight decreased *in vitro*	Amado et al. 2006

Aquatic organism	MoA	Effect	Ref
Rainbow trout	Ionoregulatory disruption rather than respiratory or acid/base distress		Rogers et al. 2003
Juvenile rainbow trout (diet exposure)	Mild disruptions in plasma Na^+ and Ca^{2+} level, and a significant up-regulation in Na^+, K^+-ATPase activity at the anterior intestine in fish		Alves & Wood 2006

Mercury and its compounds (PS No 21)

Table 39. Overview of the available data on mode of action (MoA) for mercury and its compounds (Metal; CAS-number: 7439-97-6)

Aquatic organism	MoA	Effect	Ref
General	Mercury(1) can form covalent bonds to sulfhydryl groups and impair with enzymes and their cellular function. Increased enzyme production, decreased cardiovascular function, blood parameter changes, immune response	Methylmercury (MeHg) has been linked to neurological damage (Minamata disease) and increased risk of myocardial infarction. Birds fed inorganic mercury show a reduction in food intake and consequent poor growth. Other (more subtle) effects include kidney function and structure and behavioural changes. In controlled feeding studies, the consumption of diets that contained Hg (as methylmercury) at environmentally realistic concentrations resulted in a range of toxic effects in fish, birds, and mammals, including behavioural, neurochemical, hormonal, and reproductive changes. Limited field-based studies, corroborated laboratory-based results, demonstrating significant relations between methylmercury exposure	Goyer et al. 2000 Guallar et al. 2002 Branco et al. 2017(2) Boening 2000 Scheuhammer et al. 2007

Aquatic organism	MoA	Effect	Ref
		and various indicators of methylmercury toxicity, including reproductive impairment.	
Copepod *Tigriopus japonicus*	Increased antioxidant enzymes activities (GPx and GR) Increased intracellular ROS level and decreased GSH level Activation of different patterns of mitogen-activated protein kinase (MAPK) pathways		Lee et al. 2017
Fish (grayling *Thymallus thymallus*) embryos		Impaired feeding efficiencies and reduced competitive abilities in grayling from the exposed groups	Fjeld et al. 1998
Zebrafish *Danio rerio* (dietary exposed for two months)	Methylmercury (MeHg) and inorganic mercury (iHg): differences in genetic pattern were observed for both Hg species, (an early genetic response after 7 days for both species in the three organs (muscle, liver, and brain) and a late genetic response (62 days) for iHg). Among the 18 studied genes involved in key metabolic pathways of the cell, major genetic responses were observed in muscle. In brain, high MeHg and iHg concentrations induced metallothionein production.	A dissimilar metabolisation of both Hg species. Preferential bioaccumulation of MeHg in brain and iHg in liver. Damage mainly because of MeHg in muscle and also in liver tissue	Gentès et al. 2015
Yellow perch *Perca flavescens* (from a mercury hotspot)		No negative relationships between fish condition or liver somatic index (LSI) and Hg were found. However, within the liver, kidney, and spleen tissues of females, the relative area occupied by macrophage aggregates (MAs; indicators of oxidative stress and	Batchelar et al. 2013

Aquatic organism	MoA	Effect	Ref
		tissue damage) was positively related to both muscle and liver Hg concentrations.	
Yellow perch *Perca flavescens* (from a mercury hotspot)		Morphological alterations in the liver (increased relative area of MAs, enlarged hepatic lysosomes. Analysis revealed that the MAs and hepatic lysosomes contained Hg) No relation between general health indicators (Fulton's condition index) and total Hg was observed.	Müller et al. 2015
Fish *Sparus aurata L*. and *Dicentrarchus labrax L.* (*in vitro*: isolated head-kidney and blood leucocytes)	(Methylmercury): a dose-dependent reduction in the viability of leucocytes; alterations in gene expression profiles (genes related to cellular protection (metallothionein), stress (HSP70) and oxidative stress (SOD, CAT and GR), apoptosis (Bcl2 associated X protein and caspase 3), immunity (interleukin-1β and immunoglobulin M)		Morcillo et al. 2016
Zebrafish *Danio rerio*	Mercury Chloride: induction of MT	Liver morphology and ultrastructure alterations (cytoplasm vacuolisation, decrease in both lipid droplets and glycogen granules, increase in number of mitochondria, increase of rough endoplasmic reticulum and pyknotic nuclei)	Macirella et al. 2016
Zebrafish *Danio rerio*	Mercury Chloride: modifications of Na^+/K^+-	Gill morphology alterations (hyperplasia and ectopia of chloride cells, lamellar	Macirella & Brunelli 2017

Aquatic organism	MoA	Effect	Ref
	ATPase and MTs expression pattern	fusion, increased mucous secretion, alteration of pavement cells, detachment of the secondary epithelium, pillar cell degeneration, degeneration, and apoptosis)	
Zebrafish *Danio rerio* (fed diets containing elevated levels of methylmercury MeHg and/or selenomethionine SeMet)	The expression levels of proteins associated with gap junction signalling, oxidative phosphorylation, and mitochondrial dysfunction were significantly altered in the brain of zebrafish after exposure to MeHg and SeMet alone or in combination. Analysis of upstream regulators indicated that these changes were linked to the mammalian target of rapamycin (mTOR) pathways, which were activated by MeHg and inhibited by SeMet, possibly through a reactive oxygen species mediated differential activation of RICTOR, the rapamycin-insensitive binding partner of mTOR.		Rasinger et al. 2017
Wild yellow perch *Perca flavescens* (exposed to an environmental gradient of methylmercury)	Catalase mRNA levels significantly lower in brains of perch collected from lakes with high Hg when compared to those individuals from lakes with relatively lower Hg. Other transcripts (COX, GPx, GST, HSP70, protein disulfide isomerase, and SOD) did not show differential expression.		Graves et al. 2017
Humans and wildlife (review)	Concluded that there are five main endocrine-related mechanisms of Hg across the thyroid and adrenal systems: (1) accumulation in the endocrine system; (2) specific cytotoxicity in endocrine tissues; (3) changes in hormone concentrations; (4) interactions with sex		Tan et al. 2009

Aquatic organism	MoA	Effect	Ref
	hormones; and (5) up-regulation or down-regulation of enzymes within the steroidogenesis pathway.		
A review of mechanisms of Hg in the environment	Essential mechanism associated with the toxicity of Hg is oxidative stress: damage to mitochondria as a result of the depletion of GSH enhances the generation of free radicals. They may induce DNA damage, protein modification, lipid peroxidation.		Wu et al. 2016

([1]) Mercury is one of the most hazardous contaminants that may be present in the aquatic environment, but its ecological and toxicological effects are strongly dependent on the chemical species present. Depending on the prevailing environmental conditions, inorganic mercury species may be converted to many times more toxic methylated forms such as methylmercury, a potent neurotoxin that is readily accumulated by aquatic biota.

([2]) This review describes the predominant biomarkers used by toxicologists and epidemiologists to evaluate exposure, effect and susceptibility to Hg compounds, weighing up their advantages and disadvantages. Most importantly, and in the light of recent findings on the molecular mechanisms underlying Hg-mediated toxicity, potential novel biomarkers that might be predictive of toxic effect are presented, and the applicability of these parameters in risk assessment is examined.

Nickel and its compounds (PS No 23)

Table 40. Overview of the available data on mode of action (MoA) for nickel and its compounds (metal; CAS-number: 7440-02-0)

Aquatic organism	MoA	Effect	Ref
General	(1) Disturbs established cellular homeostasis via changes of intracellular calcium levels and also producing oxidative stress. (2) The appearance of nickel-bound abnormal proteins or poisoning of an oxygen sensor is another important aspect of nickel toxicity. These changes may lead to the activation of some signalling pathways and subsequent transcription factors and eventually to alterations in gene expression and cellular metabolism. The induction of DNA damage, DNA methylation or suppression of histone acetylation by nickel allows inherent changes in gene expression to take place.		Summarised in Denkhaus & Salnikow 2002

Aquatic organism	MoA	Effect	Ref
Fathead minnows *Pimephales promelas*	The major competitor with Ca^{2+} and Cu^{2+} for binding to the gill		Meyer et al. 1999
Lake whitefish *Coregonus clupeaformis*	(chronic dietary exposure): Hematological parameters not different	Histopathological lesions in kidney and liver. Organ and whole organism parameters, including LSI, growth, and condition factor unaffected	Ptashynski et al. 2002
Freshwater pulmonate snail *Lymnaea stagnalis*	Calcium homeostasis significantly disrupted (reductions in net Ca^{2+} uptake, and reductions in Ca^{2+} concentrations in the haemolymph and soft tissues. Also, observed reduced soft tissue Mg^{2+}). Pharmacological inhibitors that block Ca^{2+} uptake pathways in snails did not inhibit. Ni uptake, suggesting that the uptake of Ni does not occur via Ca^{2+} uptake pathways.	Juvenile snail growth significantly reduced	Niyogi et al. 2014
Marine and estuarine invertebrates and fish	In freshwater, three main mechanisms of Ni toxicity exist: 1) ionoregulatory impairment 2) inhibition of respiration 3) promotion of oxidative stress. Current knowledge suggests that the mechanisms of Ni toxicity in freshwater differ between fish and invertebrates. In teleost fish, Ni acts as a respiratory toxicant significantly increasing ventilation rate, ventilatory stroke volume and oxygen consumption. In invertebrates, Ni appears to be an ionoregulatory toxicant, disrupting Mg homeostasis. In marine invertebrates Ni causes ionoregulatory disruption:	Swelling of the gill lamellae The skeletal malformations Affected reproduction (decreases fecundity and/or viability of eggs) in copepods	Summarised in Blewett & Leonard 2017

Aquatic organism	MoA	Effect	Ref
	- inhibition of calcium influx in developing sea urchin embryos - mechanism may relate to Ni impacts on Ca^{2+} metabolism - altered levels of haemolymph Na^{+}, Mg^{2+} and Ca^{2+} in the green shore crab. Gene expression in the mussel *Mytilus galloprovincialis* identified pathways such as proteolysis, catabolism, and cellular metabolic processes.		
Summary review of the extent literature on mechanisms of Ni toxicity	5 potential pathways by which Ni may exert toxicity on aquatic organisms were identified: 1) disruption of Ca^{2+} homeostasis 2) disruption of Mg^{2+} homeostasis 3) disruption of $Fe^{2+}/^{3+}$ homeostasis 4) an allergic reaction at respiratory epithelia 5) generation of reactive oxygen species (ROS).	At the level of the whole organism, the organ-level responses contribute to potential reductions in growth and reproduction and/or alterations in energy metabolism, with several potential feedback loops between each of the pathways.	Brix et al. 2017

2.2.1.9 Phthalate

Di(2-etylhexyl)phthalate (DEHP) (PS No 12)

Table 41. Overview of the available data on mode of action (MoA) for di(2-etylhexyl)phthalate (DEHP) (plasticiser; CAS-number: 117-81-7)

Aquatic organism	MoA	Effect	Ref
General	Activation of peroxisome proliferator-activated receptor alpha (PPAR alpha) Acting through its metabolite monoethylhexyl phthalate (MEHP) through a receptor-mediated signalling pathway to suppress estradiol production in the ovary Increased expression of Ah-receptor transcription	(in rodents) Teratogenesis Liver toxicity Effects on reproduction, damage to sperm, early onset of puberty in females, anomalies of reproductive tract, infertility and adverse outcomes of pregnancy	HSDB Adams et al. 1995 Lovekamp-Swan & Davis 2003 Zhang et al. 2006 Lyche et al. 2009

Aquatic organism	MoA	Effect	Ref
	MEHP has been shown to be able to and been shown to decrease estrogen production by acting on granulosa cells through peroxisome proliferator activity.		Summarised in Mankidy et al. 2013
The midge *Chironomus riparius* larvae	Rapid and differential changes in expression of genes that encode proteins belonging to tow different metabolic pathways: stress-related (HSP70) and endocrine-related (EcR/usp)	Potential capacity to alter the ecdysone signalling pathway	Planelló et al. 2011
Chironomus riparius larvae (concentrations staring from (10^{-3} µg/L)	Significant changes detected in almost all the studied biomarkers: e.g. strong repression of the cell stress response HSP70; general inhibition of the ecdysone hormone pathway EcR; the energy metabolism GAPDH activity loss in long exposures	No mortality observed	Herrero et al. 2017
Male and female zebrafish, *Danio rerio* hepatocyte cultures	The increase of Vtg levels and peroxisome proliferators activated receptors mRNA levels	Estrogenic potency in both	Maradonna et al. 2013
Female Japanese medaka *Oryzias latipes* Female zebrafish *Danio rerio* Chinese rare minnow *Gobiocypris rarus*, carp *Cyprinus carpio*, fathead minnows	Modulated transcription profiles of genes involved in steroidogenesis and altered plasma sex hormone levels	Retards the oocyte development. Exposure during the fry stage affected the normal maturation of with a reduction in the gonadal somatic index (GSI) and body weight. Negatively affected embryos: body weight was reduced and the sex ratio was distorted. Significantly impairs oogenesis and embryo production.	Summarised in Ye et al. 2014

Aquatic organism	MoA	Effect	Ref
Pimephales promelas			
Guppy *Poecilia reticulate* (DEHP 10 µg/L applied continuously for 91 days)		Inhibition of the growth of in terms of body weight and body length observed as early as 14 days after the start of exposure	Zanotelli et al. 2010
Zebrafish *Danio rerio* (via intraperitoneal injection)	Likely via peroxisome proliferator activated receptor (PPAR) signalling pathways in the testis and oestrogen signalling pathways in the liver	Disrupts spermatogenesis in adults with a consequent decrease in their ability to fertilise oocytes spawned by untreated females. (only after exposure to high concentrations of DEHP)	Uren-Webster et al. 2010
Zebrafish *Danio rerio* (via food intake)	Modification at transcription levels of gene critical for lipid metabolism (PPARa, SREBP and Cb1)	Effect on food intake	Migliarini et al. 2011
Fathead minnows *Pimephales promelis* embryos	Oxidative stress identified as the critical mechanism of toxicity Weak potency as agonists of the AhR	DEHP targeted steroid biosynthesis pathways resulting in greater production of E2 with a concurrent reduction in concentration of testosterone	Mankidy et al. 2013
Marine medaka *Oryzias melastigma* (exposure to DEHP, and MEHP from hatching to adulthood)	Increase in plasma 17β-estradiol (E2) along with a significant decrease in testosterone (T)/E2 ratios was observed in males. Upregulated expression of brain steroid hormone receptor genes (estrogen receptor isoforms α, β and γ, cytochrome P450 19b (CYP19b),gonadotropin-releasing hormone receptor 2 (gnrhr2) and follicle-stimulating hormone β (fshβ) The liver Vtg level significantly increased after DEHP and MEHP exposure in males.	Exposure to DEHP, but not MEHP, accelerated the start of spawning and decreased the egg production of exposed females. Exposure to both DEHP and MEHP resulted in a reduction in the fertilisation rate of oocytes spawned by untreated females paired with treated males.	Ye et al. 2014
Zebrafish *Danio rerio* embryos	Exposure to MEHP: decreased whole-body T4 contents and increased whole-body T3	Only acute exposure to MEHP alters whole-body contents of thyroid	Zhai et al. 2014

Aquatic organism	MoA	Effect	Ref
	contents. Upregulated genes related to thyroid hormone metabolism (Dio2 and UGT1ab) and genes involved in thyroid development (Nkx2.1 and Pax8) and thyroid hormone synthesis However, the genes encoding proteins involved in TH transport (transthyretin, TTR) was transcriptionally significantly down-regulated	hormones in zebrafish embryos and changes the transcription of genes involved in the HPT axis, thus exerting thyroid endocrine toxicity	
Goldfish *Carassius auratus*	Decreased levels of 11-ketotestosterone (11-KT), luteinising hormone and StAR mRNA levels encoding regulator of cholesterol transfer to steroidogenesis E2 levels remained unchanged Vtg production was not induced in and mRNA levels of genes with products mediating estrogenic effects remained unchanged or decreased.	DEHP interferes with testis and pituitary hormonal functions to reduce sperm quality (sperm motility and velocity) but does not exhibit estrogenic activity.	Golshan et al. 2015

2.2.1.10 Anti-fouling biocide

Tributyltin compounds (Tributyltincation) (PS No 30)

Table 42. Overview of the available data on mode of action (MoA) for tributyltin compounds (anti-foulant biocide; CAS-number: 36643-28-4)

Aquatic organism	MoA	Effect	Ref
General	**Two MoAs have been proposed**: first, the compounds could lead to inhibition of ATPase, and secondly, it can prevent phosphate incorporation of ATP. The toxicity is thought to be a result of high lipid solubility and a high stability in biological pH levels. This gives the compound the ability to cross the blood brain barrier and impair neuronal functions. Another MoA is	Immunosuppression, endocrine effects, neurotoxic effects, and effects on enzymatic activity. In addition to being bioaccumulative, exposure to organotins may also produce the following types of damage: ocular, dermal, cardiovascular, pulmonary, gastrointestinal, blood dyscrasias, reproductive developmental, liver,	HSDB

Aquatic organism	MoA	Effect	Ref
	inhibition of oxidative phosphorylation.	kidney, and possibly carcinogenic effects. Imposex has been shown in gastropods.	
Aquatic organisms	MT induction, AChE inhibition, imposex, lysosomal enlargement, lysosomal membrane destabilisation, peroxisome proliferation, lysosomal activity, genetic or molecular biomarkers, TBT sensitive immunological biomarkers, apoptosis induction, phagocytic index, and amoebocytic index	In many marine species: larval mortality and impairment in growth, development, reproduction, and survival	Summarised in Okoro 2011
Zebrafish	Effect on antioxidant ability and immune responses		Zhang et al. 2017

2.2.1.11 Alkylphenols

Nonylphenols (4-Nonylphenol) (PS No 24)

Table 43. Overview of the available data on mode of action (MoA) for nonylphenols (4-Nonylphenol) (degradation product of alkylphenol ethoxylates (nonionic surfactants); CAS-number: 84852-15-3)

Aquatic organism	MoA	Effect	Ref
General	Estrogen agonist activities both *in vivo* and *in vitro* NP could inhibit the activity of 17 α-hydroxylase enzymes, which are involved in testosterone synthesis. Vtg induction in male and immature fish	Endocrine disruption (e.g. can affect reproduction)	Laurenzana et al. 2002 Naderi et al. 2012
Pacific oyster *Crassostrea gigas*	Altered both cellular and humoral elements of the innate immune response (total haemocyte counts, differentially expressed genes (bpi in the haemocytes, transglutaminase in the mantle), altered mRNA transcript abundance of several genes (bpi,		Hart et al. 2016

Aquatic organism	MoA	Effect	Ref
	galectin, C-type lectin 2), plasma lysozyme activity levels)		
Invertebrates Marine bivalves	Changes in variety of innate immune components: decreased haemocyte lysosomal membrane stability in mussels and cockles, altered phagocytosis in mussels, affected haemocyte counts and size distribution frequency, decreased haemocyte membrane stability and lysozyme activity, while increased haemocyte apoptosis in clams	Reduced fecundity in freshwater snails and inhibited development in euryhaline copepods Decreased sperm motility, altered sex ratios, increased percent hermaphroditism, delayed spermatogenesis, and increased developmental abnormalities in oysters	Summarised in Hart et al. 2016
Salmo salar	Disturbed balance between levels of thyroid hormone, growth hormone, cortisol, insulin-like growth factor-I and sex steroids	May affect smoltification and osmoregulation.	Moore et al. 2003
Zebrafish *Danio rerio*		NP exposure showed marked influence on locomotor activity of the male zebrafish, whereas that of the female was not significantly affected.	Xia et al. 2010
Rainbow trout *Oncorhynchus mykiss*		Significantly reduced semen production at concentration of 280 and 130 ng/L Semen production completely inhibited at concentration of 750 ng/L The percentage of eyed stage embryos was slightly but significantly lower. 4-nonylphenol was taken up by the larvae as estimated exposure levels of ≥280 ng/L (LOEC: 280 ng/L) were toxic and caused a severe decrease in the percentage of viable larvae.	Lahnsteiner et al. 2005
Zebrafish Oocytes	A novel nongenomic estrogenic mechanism involving activation	Inhibition of meiotic maturation of oocytes	Fitzgerald et al. 2015

Aquatic organism	MoA	Effect	Ref
	of the Gper/Egfr/Mapk3/1 pathway		
Fish *Clarias gariepinus*	Effects on haematological, biochemical, enzymes and hormones	Altered hepatic (HSI) and gonad (GSI) somatic indices and even organ damage (liver, testis and kidney)	Summarized in Sayed et al. 2012
Catfish *Silurus meridionalis*	(diet including annelid worm collected in contaminated streams): fed fish displayed similar serum estradiol-17β and Vtg levels and gonadal Sf1, Dmrt1, Foxl2, Cyp19a1a expression levels to those of female control.	Fish feminisation (by affecting aromatase expression and endogenous estrogen level)	Dong et al. 2014
Catfish *Heteropneustes fossilis*		Various body malformations in larvae, such as vertebral deformations, e.g. fin blistering/necrosis, axial deformities (lordosis, kyphosis, and scoliosis) of the spine in the abdominal and caudal region, tail curved completely backward, shortened body, severe spinal and yolk sac malformations, C-shaped severe spinal curvature, cranial malformation with undeveloped head, and failure of eye development (0.1 and 1.0 µg/L)	Chaube et al. 2013
Catfish *Silurus asotus*	(4 weeks of oral administration): Depletion of the endogenous anti-oxidant molecule GSH and temporal inhibition of GSH-related anti-oxidant enzymes. Such declines in anti-oxidant capacity and elevated oxidative stress seem to be compensated eventually by subsequent activation of various anti-oxidant enzyme systems.		Park 2015

Aquatic organism	MoA	Effect	Ref
Embryonic zebrafish	Spatiotemporal expression profiles of estrogen, androgen, and thyroid hormone receptors (to demonstrate that localization of these receptors might be mediating contaminant effects on development)	Exposure to nanomolar contaminant concentrations resulted in abnormal morphological development, including changes to body length, pericardia (heart), and the head.	Kinch et al. 2016
Brown trout *Salmo trutta caspius*	The male plasma T3 level decreased while the female T3 level increased.	Histopathological lesions were observed in gill and intestine tissues.	Shirdel and Kalbassi 2016

Octylphenols ((4-(1,1',3,3'-tetramethylbutyl)-phenol)) (PS No 25)

Table 44. Overview of the available data on mode of action (MoA) for octylphenols ((4-(1,1',3,3'-tetramethylbutyl)-phenol) (OP) (phenolic surfactant - degradation product of alkylphenol ethoxylates; CAS-number: 140-66-9)

Aquatic organism	MoA	Effect	Ref
General	Estrogen receptor agonist Inhibitory effects on cytochrome P450 activities and decrease of testosterone hydroxylating CYP activities in rat liver		Servos et al. 1999 Ackermann et al. 2002 OECD 2011
American bullfrog *Rana (Lithobates) catesbeiana*	Estradiol and octylphenol affect CYP19a1 and nr5a1mRNA levels differently.	OP affects the estrogen-dependent signalling required for normal reproductive development in vertebrates.	Wolff et al. 2015
Amphibians	Induced Vtg synthesis in hepatocyte of males	Alterations in sex ratio, abnormal testicular development, affected male sexual behaviour	Summarized in Li et al. 2016
Japanese medaka *Oryzias latipes*		Developmental disturbances ranged from circulatory problems to difficulties of inflating swim bladders.	Gray & Metcalfe 1999
Sand goby *Pomatoschistus minutus*	Induced Vtg mRNA expression	Inhibited development of sperm duct glands	Robinson et al. 2004

Aquatic organism	MoA	Effect	Ref
Tilapia species (*Tilaipia guineensis, Sarotherodon galileaus* and *Oreochromis niloticus*)	Vtg, Zrp and cyp19a1 mRNA was significantly higher in males.	Gonado-histopathological changes, intersex and endocrine disruptor responses in relation to contaminant burden	Ibor et al. 2016
Mangrove killifish *Kryptolebias marmoratus*	Induced CYP2AD12 (cytochrome P450)		Puthumana et al. 2017

2.2.1.12 Pyrethroid insecticides

Cypermethrin (PS No 41)

Table 45. Overview of the available data on mode of action (MoA) for cypermethrin (pyrethroid insecticide; CAS-number: 52315-07-8)

Aquatic organism	MoA	Effect	Ref
General	The primary MoA is interference with ion channels in the nerve axon. Cypermethrin prolongs the opening of sodium channel, a major site of its action, leading to hyper-excitation of the central nervous system. In addition to sodium channel, cypermethrin can modulate chloride, voltage-gated calcium and potassium channels, alter the activity of glutamate and acetylcholine receptors and adenosine triphosphatases, and induce DNA damage and oxidative stress in the neuronal cells.	Hyperactivity of the nervous system Neurotoxicity	Singh et al. 2012
Daphnia magna	Cytochrome P450 activity	Negatively affected adult growth and number and size of neonates	Gottardi et al. 2017
Marbled crayfish *Procambarus fallax f. virginalis*	Oxidative stress and disruption of antioxidant system in the juvenile crayfish (decreased in levels of TBARS, changes in CAT, SOD, GR and GST activity)		Lidova et al. 2016

Aquatic organism	MoA	Effect	Ref
Common carp *Cyprinus carpio L.*	Immunopositive reactions of 8-OHdG observed in the nuclei and cytoplasm of neurons, and positive reactions for iNOS detected in the cytoplasm of neurons and in the glial cells of the experimental groups. Up-regulated caspase 3, capsase 8, iNOS, and MT1 genes in the brain. Findings revealed that Cyp toxication harms the organs of common carp, particularly the brain, and also gives rise to inflammation, DNA damage, and apoptosis.	Histopathological changes, including hyperplasia of lamellar cells, telangiectasia of lamellae and thickening Cellular infiltration in gills, haemorrhage, diffuse hydropic degeneration, and focal necrosis in the liver	Arslan et al. 2017
Atlantic salmon *Salmo salar*	Inhibits ability of male salmon parr to detect and respond to the female salmon priming pheromone PGF2α.	Reduced fertilisation success	Richterova & Svobodová 2012
Fish (different species)	Marked decrease in protein and glycogen levels of different organs Dose- and time-dependent biochemical, haematological alterations have been reported in several fishes: decline in the calcium and phosphorus; increase in levels of free amino acids coupled with marked decline in protein level; alterations in major metabolites and enzymes of protein and carbohydrate metabolism in liver and gill tissues; increase in levels of serum GOT, GPT, pyruvic-acid-transaminase, glucose, and ALP, ALT, AST and a decrease in the concentration of plasma total protein, albumin, cholesterol and lysozyme; significant alteration in the levels of ammonia and urea in freshwater fish; increase in WBC, MCV, MCH, monophils and heterophils with marked reduction in RBC and lymphocytes	Cypermethrin can affect early stages of fish more potentially; It can also result in growth retardation and protein deposition in fish body Histopathological alterations (hyperplasia, disintegration of hepatic mass and focal coagulative necrosis) Behavioural response of fishes: gill flailing, hyperactivity, loss of buoyancy, loss of equilibrium and inability to remain, and swimming alteration	Summarised in Prusty et al. 2015

Aquatic organism	MoA	Effect	Ref
Embryo-larval zebrafish *Danio rerio*	Expression of genes in the hypothalamic-pituitary-gonadal axis; the transcription patterns of many key genes (Vtg1, Vtg2, ERa, ERβ1, ERβ2, CYP19a1a and CYP19a1b) affected		Guo et al. 2017

2.2.1.13 Perfluorinated surfactant

Perfluorooctane sulfonic acid and its derivatives (PFOS) (PS No 35)

Table 46. Overview of the available data on mode of action (MoA) for Perfluorooctane sulfonic acid and its derivatives (PFOS) (synthetic perfluorinated carboxylic acid and fluorosurfactant; CAS-number: 1763-23-1)

Aquatic organism	MoA	Effect	Ref
General	One major pathway affected by PFOS is peroxisomal fatty acid–oxidation (which could be explained by the structural similarity between PFOS and endogenous fatty acids)	Carcinogen, liver toxicant, developmental toxicant, immune system toxicant; also exerts hormonal effects including alteration of thyroid hormone levels.	Lau et al. 2007
Fish (review)		Abnormal development, reduced offspring survival, and endocrine disruption	Summarised in Ahrens and Bundschuh 2014,
	Reactive oxygen species production	Lordosis and pericardial oedemas	Shi et al. 2008, Shi and Zhou 2010, Zheng et al. 2012
	Interference with lipid metabolism	Hepatic steatosis	Cheng et al. 2016
	Sexual and thyroid hormone synthesis	Endocrine disrupting properties	Oakes et al. 2005 Du et al. 2009
Zebrafish *Danio rerio*		Chronic zebrafish PFOS exposure alters sex ratio and maternal	Wang et al. 2011

Aquatic organism	MoA	Effect	Ref
		related effects in F1 offspring (adversely impacts embryonic growth).	
Zebrafish *Danio rerio*	Lowest tested PFOS concentration (0.6 µg/L) showed an estrogenic potential in terms of significant Vtg induction, Vtg levels were generally found to decrease with increasing PFOS-exposure in F1 and F2 generations.	Histological analyses of F1 and F2 fish revealed hepatocellular vacuolisation, predominantly in males (hepatotoxicity might explain the suppressed Vtg response seen in PFOS-exposed F1 and F2 males). Granulomas, mainly in the liver (could be a consequence of a PFOS-induced reduction of the immune response potential).	Keiter et al. 2012
Adult zebrafish *Danio rerio*		Exposure during different life stages adversely affects adult behaviour and F1 offspring morphology, behaviour, and survival.	Chen et al. 2013a
Adult zebrafish *Danio rerio*	Change in gene expression (decrease of slco1d1 for females and males; increase of tgfb1a in males)	Reduced aggression behaviour	Jantzen et al. 2016
Zebrafish larvae	Altered expression of genes related to the stress response, GABAergic, dopaminergic, histaminergic, serotoninergic, cholinergic systems and neuronal maintenance	Increases in the swimming speed	Khezri et al. 2017

2.2.1.14 Benzene (PS No 4)

Table 47. Overview of the available data on mode of action (MoA) for benzene (organic chemical compound; CAS-number: 71-43-2)

Aquatic organism	MoA	Effect	Ref
General	The cause of benzenes high potential for carcinogenicity is suspected to be a result of formation of DNA adducts, cross-linking, oxidative damage or inhibition of topoisomerase II. Induces DT-Diaphorase, an enzyme protecting the cell from oxidative damage.	Very potent carcinogen to humans acting on liver, kidneys, lungs, heart and brain Hematotoxic and leukemogenic effects Exposure can lead to DNA-strand breaks and chromosome damages. Immune dysfunction	Whysner et al. 2004 ATSDR 2007 Zhang et al. 2010
Pacific herring *Clupea harengus*		Significant reduction in survival of ovarian eggs and resultant embryos and larvae through yolk absorption Induced premature spawning and aberrant swimming behaviour and disequilibrium in adults of both sexes	Struhsaker 1977
Medaka *Oryzias latipes* embryos		Change in mean heart rate	Teuschler et al. 2005

2.2.1.15 Quinoline fungicide

Quinoxyfen (PS No 36)

Table 48. Overview of the available data on mode of action (MoA) for quinoxyfen (quinoline fungicide; CAS-number: 124495-18-7)

Aquatic organism	MoA	Effect	Ref
General	Systemic with protective properties, translocates and inhibits appressoria development stopping infections. Signal transduction The actual MoA is yet to be fully understood, but quinoxyfen is believed to inhibit infection through disruption of early cell signalling events in the fungus that control	Quinoxyfen provides a new multi-site MoA to control powdery mildew that is different from the demethylation inhibitors and the strobilurins that act on a single site.	Lee 2006

Aquatic organism	MoA	Effect	Ref
	the morphological changes that lead to infection. The main MoA at the cellular level is the inhibition of primary appressorial formation. Quinoxyfen, has virtually no effect on spore germination, primary germ tube development, secondary appressoria or haustoria.		Longhurst 1995
In vitro assay	Antiandrogenic	Endocrine activity	Orton et al. 2011
No information about the MoA in non-target aquatic organisms has been found in the scientific literature			

The data indicate quinoxyfen is relatively non-toxic to terrestrial wildlife, but highly toxic to freshwater fish and extremely toxic to aquatic invertebrates.

2.2.1.16 Chloroalkans

C10-13 Chloroalkanes (PS No 7)

Table 49. Overview of the available data on mode of action (MoA) for C10-13 chloroalkanes(1) (also called short chain chlorinated paraffins (SCCP)) (a complex mixture of polychlorinated n-alkanes-hydrocarbons having 10 to 13 carbon atoms arranged in chains and containing 50-70% by weight of chlorine; CAS-number: 85535-84-8)

Aquatic organism	MoA	Effect	Ref
General	The liver damage is associated with peroxisome proliferation, whereas thyroid effects are correlated to altered thyroid hormone status and glucuronyl transferase induction.	May affect the liver, the thyroid hormone system, and the kidneys in mammals, e.g., by causing hepatic enzyme induction and thyroid hyperactivity, which in the long-term can lead to carcinogenicity in these organs.	ECHA SVHC Support Document Swedish Pollutant Release and Transfer Register Nielsen & Ladefoged 2013

Aquatic organism	MoA	Effect	Ref
Frog *Xenopus laevis* embryos	C12 group: induction of phase II detoxification enzyme GST	Developmental malformations and reduced embryo growth	Burýšková et al. 2006
Juvenile rainbow trout *Oncorhynchus mykiss* (dietary exposure at high concentration)	C10, C11 and C12 groups	Behavioural effect: diminished or no startle response, loss of equilibrium Histopathological lesions in the livers (hepatocyte necrosis, sites of inflammation, and glycogen/lipid depletion). The most severe pathologies were observed for C10 and C11). No lesions were present in the thyroid, although trout exposed to C10 had slightly more active thyroids, as indicated by an increased mean thyroid epithelium cell height relative to controls.	Cooley et al. 2001
Zebrafish *Danio rerio* embryos	C10 and C12 groups: altered gene expression in the hypothalamic-pituitary-thyroid (HPT) axis (decreased expression of tyr, ttr, dio2 and dio3) and thyroid hormone levels (inhibited the production of T3) Specific modes of action differ with different congeners.	C10-groups induced stronger effects than C12- groups, including teratogenic effect, survival rate decrease, hatching delay effect and growth inhibition. C10-groups also showed more potential to disrupt thyroid hormone homeostasis than C12-groups.	Liu et al. 2016

([1]) No indicative parameter is provided for this group of substances

2.2.1.17 Hexabromocyclododecane (HBCDD)

Hexabromocyclododecane (HBCDD) (PS No 43)

Table 50. Overview of the available data on mode of action (MoA) for hexabromocyclodo-decane (HBCDD) (cycloaliphatic brominated flame retardant; CAS-number: See footnote 12 in Annex X to Directive 2000/60/EC)

Aquatic organism	MoA	Effect	Ref
General	A high affinity of HBCD for the thyroid hormone receptor was recently described for HeLaTR human cervical carcinoma cells. Antagonistic activity with the androgen (AR), estrogen (ER), progesterone (PR), and aryl hydrocarbon (Ah) receptors Inhibition of plasma membrane uptake of neurotransmitters (dopamine, glutamate) Genetic alterations in mammalian cells (e.g. cell cycle or proliferative changes, particularly in relation to carcinogenesis) Importance of oxidative stress and initiation of apoptotic cell death for mediating cellular toxicity It appears likely that HBCD affects the thyroid axis by altering expression of biotransformation enzymes.	From a toxicological point of view, HBCD exerts effects on different endpoints in both *in vitro* and *in vivo* systems. Studies have confirmed HBCDs potential to disrupt the thyroid axis in *in vivo* and *in vitro* animal models, including mammals, fish and birds. Interferes with thyroid homeostasis by decreasing total thyroxin levels and increasing thyroid weight. Neurotoxic	Summarised in Cantón et al. 2008 Marvin et al. 2011
Zebrafish liver cells	Proteomic responses related to decreased protein metabolism		Kling and Förlin 2009
Marine copepod *Tigriopus japonicus*	Induced the transcription of oxidative stress response genes and apoptotic genes (e.g. SOD, CAT, GST, OGG1, P53 and Caspase-3) in adults.	Significant growth delay in nauplii	Shi et al. 2017

Aquatic organism	MoA	Effect	Ref
Marine medaka *Oryzias melastigma*	Oxidative stress and apoptosis, suppressed nucleotide and protein synthesis (technical HBCD,tHBCD, 0, 5, 20 and 50µg/L)	Developmental toxicity, particularly in the cardiovascular system of the embryos	Hong et al. 2014

2.2.2 Watch List substances

2.2.2.1 Hormones

17-Alpha-ethinylestradiol (EE2), 17-Beta-estradiol (E2) and Estrone (E1)

Table 51. Overview of the available data on mode of action (MoA) for 17-Alpha-ethinylestradiol (EE2), 17-Beta-estradiol (E2) and Estrone (E1) (steroid hormones; CAS-number: 57-63-6, 50-28-2 and 53-16-7 respectively)

Aquatic organism	MoA	Effect	REF
General: all three substances have estrogenic activity	**A specific MoA**: act via the estrogen receptor (ER)	Endocrine disruption Human: link to breast cancer in women and prostate cancer in men	Moore et al. 2016 Nelles et al. 2011 Adeel et al. 2017
Fish Amphibians Reptiles (turtles)		Can perturb physiology and affect reproductive development (reduced testes size, lower sperm count, induced VTG, affect reproductive fitness, alter other reproductive characteristics). Affect developing eggs Gonadal differentiation	Reviewed by Bhandari et al. 2015 Adeel et al. 2017

2.2.2.2 Pharmaceuticals

Diclofenac

Table 52. Overview of the available data on mode of action (MoA) for diclofenac (phenylacetic acid derivatives; CAS-number: 15307-86-5)

Aquatic organism	MoA	Effect	REF
General	**A specific MoA**: the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis. Research suggests diclofenac can inhibit the thromboxane-prostanoid receptor, affect arachidonic acid	Reduced inflammation (used to relieve pain, swelling, and joint stiffness caused by arthritis)	Gan 2010

Aquatic organism	MoA	Effect	REF
	release and uptake, inhibit lipoxygenase enzymes, and activate the nitric oxide-cGMP antinociceptive pathway. Other novel MOAs may include the inhibition of substrate P, inhibition of peroxisome proliferator activated receptor gamma (PPARgamma), blockage of acid-sensing ion channels, alteration of interleukin-6 production, and inhibition of N-methyl-D-aspartate (NMDA) receptor hyperalgesia.		
Invertebrates		Negative impacts on invertebrate reproductive success Impaired osmoregulation ability of crabs	Zanuri et al. 2017 Eades & Waring 2010
Fish	Increased lipid peroxidation (LPX); changes in activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) Differential gene expression (including Vtg); induction of detoxification enzymes Increase of hepatic mRNA levels of c7 (complement component 7), a gene involved in the innate immune system	Oxidative stress in liver and in gill; reduced testosterone levels Induced cytological and histological effects Toxic effects on kidneys Renal hematopoietic hyperplasia Reduced feeding rate and/or activity	Islas-Flores et al. 2013 Summarised in Guiloski et al. 2017 and Gröner et al. 2017 Schwaiger et al. 2004 Näslund et al. 2017 Nassef et al. 2010
Nile tilapia *Oreochromis niloticus* Chronic exposure	Vtg gene expression induced and luteinising hormone gene expression reduced	Estrogenic effects (biomarkers associated with reproduction and HPG axis)	Gröner et al. 2017

2.2.2.3 Antibiotics

Erythromycin, Clarithromycin and Azitromycin

Table 53. Overview of the available data on mode of action (MoA) for erythromycin, clarithromycin and azitromycin (macrolides; CAS-number: 114-07-8, 81103-11-9 and 83905-01-5 respectively)

Aquatic organism	MoA	Effect	REF
General	**A specific MoA**: inhibit the synthesis of proteins/enzymes (inhibition by binding to bacterial 50S ribosomal subunits; binding inhibits peptidyl transferase activity and interferes with translocation of amino acids during translation and assembly of proteins).	Inhibit the synthesis of proteins/enzymes vital for the normal functioning of microbial cells.	Carvalho et al. 2015
Algae *Pseudokirchneriella subcapitata* Azithromycin	Electrophilic mechanism of action (involves the inhibition or regulation of enzymes involved in cell wall biosynthesis, nucleic acid metabolism and repair, or protein synthesis, respectively, or disruption of membrane structure)	ROS content and photosynthetic activity changes.	Wan et al. 2015 Paíga et al. 2016 Fu et al. 2017
Alga *Desmodesmus subspicatus* and the cyanobacterium *Anabaena flosaqua* Clarithromycin and its major metabolite 14-hydroxy(R)-clarithromycin		Strong toxicity No toxic effects on fish (*Danio rerio* embryo) and the crustacean (*Daphnia magna*)	Baumann et al. 2015
Crucian carp *Carassius auratus* Erythromycin	Biochemical disturbance: enzymes involved in processes such as phase I biotransformation (EROD activity) and antioxidant defense (SOD activity) in liver		Liu et al. 2014

Aquatic organism	MoA	Effect	REF
Rainbow trout *Oncorhynchus mykiss*		DNA damaging effects related to the oxidative damage	Rodrigues et al. 2016
Cyanobacteria, algae, rotifers and fish		Decrease in growth rate, reproduction and survival	Summarised in Rodrigues et al. 2016

2.2.2.4 Neonicotinoid insecticides

Imidacoprid, Thiacoprid, Thiamethoxam, Clothianidin and Acetamiprid

Table 54**.** Overview of the available data on mode of action (MoA) for imidacoprid, thiacoprid, thiamethoxam, clothianidin and acetamiprid (neonicotinoids; CAS-number: 138261-41-3, 11988-49-9, 153719-23-4, 210880-92-5 and 135410-20-7, respectively)

Aquatic organism	MoA	Effect	REF
Insects	**A specific MoA:** taken up by insects via contact and ingestion and bind agonistically to the post-synaptic nicotinic acetylcholine receptors (nAChR) in the invertebrate central nervous system, thus competing with the natural neurotransmitter acetylcholine (ACh) and thereby disrupting nerve impulses.	Neurotoxic Selective for insect nAcChR receptors and consequently has much less pronounced effects in mammals. Toxicity studies with the arthropods suggest that binding to these receptors is long-lasting and lethal effects are typically delayed such that repeated or chronic exposure can lead to cumulative effects over time.	Roessink et al. 2013
Aquatic invertebrates Mussels (imidacloprid, clothianidin, and thiamethoxam)	In mussels, imidacloprid exposure caused a decrease in AChE activity while thiacloprid induced an opposite effect with mixture of neonicotinoids increasing the activity of AChE.	Effects on survival, growth, emergence, mobility, and behaviour Potential for aquatic invertebrates to be negatively impacted	Dondero et al. 2010 Reviewed by Morrissey et al. 2015 Anderson et al. 2015
Amphibians	Genotoxic effects measured through micronucleus assay		Summarised in Iturburu et al. 2017

Aquatic organism	MoA	Effect	REF
Fish Zebrafish	Changes in gene transcription, erythrocyte damage Affect the protective capability of GST against DNA damage and decrease of GST activity, while increasing CAT activity.	Disintegration of gonadal tissue, impaired swimming, notochord degeneration and locomotor defects in embryos and larvae	Gibbons et al. 2015 Ge et al. 2015
Fish, algae, amphibians, and molluscs are relatively insensitive to imidacloprid.			Anderson et al. 2015

Overall, the available published studies indicate that fish are relatively insensitive to neonicotinoid insecticides, as would be expected from properties of the vertebrate nAChR, while neonicotinoids showed the highest ecological relevance for the composition of invertebrate communities (Van Dijk et al. 2013, Münze et al. 2017, Miles et al. 2017). However, the majority of this research has focused on imidacloprid, which was the first widely applied neonicotinoid and is rarely used in modern row crop agriculture production systems. There is a dearth of information on the toxicological effects of the neonicotinoids that are most commonly used presently, including thiamethoxam and its metabolite clothianidin (Miles et al. 2017). Although modern insecticides such as neonicotinoids previously were expected to exert only low toxicity on mammals, birds, and fish, because these compounds have a low affinity for vertebrates relative to insect nicotinic receptors, current research has provided evidence for respiratory, cardiovascular, neurological, and immunological toxicity in rats and humans (Köhler and Triebskorn 2013).

2.2.2.5 Herbicides

Oxadiazon

Table 55. Overview of the available data on mode of action (MoA) for oxadiazon (herbicide; CAS-number: 19666-30-9)

Aquatic organism	MoA	Effect	REF
Plants	**A specific MoA:** oxadiazon exibits contact action (similarly as aclinifen) inhibits protoporphyrinogen oxidase (causing a phytotoxic protoporphyrin IX accumulation).	Irreversible cell membrane damage	Iriti et al. 2009

Aquatic organism	MoA	Effect	REF
Aquatic macrophyte *Callitriche obtusangula*	Increase in oxidative stress		Iriti et al. 2009
Algae and fish		The most sensitive endpoint: reproduction	Carvalho et al. 2015

The study of Silva et al. (2015) estimated the impact of measured pesticide mixtures in surface waters from 2002 and 2008 within three important Portuguese river basins on primary producers, arthropods and fish by toxic pressure calculation and identified oxadiazon as one having the relatively largest toxic effects on primary producers.

Triallate (Trial-late)

Table 56. Overview of the available data on mode of action (MoA) for triallate (Thiocarbamate herbicide; CAS-number: 2303-17-5)

Aquatic organism	MoA	Effect	REF
Plants	**A specific MoA**: acts as inhibitor of very long-chain fatty acids (VLCFAs). VLCFAs are used by plants for synthesis of the waxes, cutins, and suberins that are necessary to keep moisture in plant cells and tissues while keeping other substances out.	Arrested cell division and growth	
Microcosm (macrophyts and algae, planktonic and bentic invertebraes)		Mortality Reduced algal growth	Johnson 1986

Triallate is unlikely to be genotoxic or carcinogenic; no classification has been proposed for reproductive toxicity (Carvalho et al. 2015).

2.2.2.6 Carbamate insecticide

Methiocarb

Table 57. Overview of the available data **on mode of action (MoA)** for methiocarb (carbamate herbicide; CAS-number: 2032-65-7)

Aquatic organism	MoA	Effect	REF
Insects	**A specific MoA**: inhibits reversibly acetylcholinesterase (AChE) activity resulting in a cholinergic stimulation.	Neurotoxin; contact and stomach action on mites	
Snail *Eobania vermiculata* Molluscs	Biochemical changes in the digestive gland (decrease in carbohydrate, lipid and protein contents) AChE inhibition	Histochemical alterations of the digestive gland Neurotoxic effects	Radwan et al. 2008 Ozden et al. 2009
Juvenile rainbow trout *Oncorhynchus mykiss*		Some of lesions in gills	Altinok & Capkin 2007

There was no evidence of genotoxicity or carcinogenicity, and Methiocarb did not affect reproductive and developmental parameters (Carvalho et al. 2015). Methiocarb is a known poison to water organisms (pesticideinfo.org). There is little information on mechanisms of its action other than AChE inhibition; in rats, biochemical and histological evaluations demonstrated that exposure of methiocarb resulted in the induction of lipid peroxidation and changes in antioxidant system (decreased levels of GSH and activities of SOD, CAT and GSH-Px) in liver and kidney (Ozden et al. 2009).

2.2.2.7 Antioxidant

2,6-Di-tert-butyl-4-methylphenol

Table 58. Overview of the available data on mode of action (MoA) for 2,6-Di-tert-butyl-4-methylphenol (antioxidant; CAS-number: 128-37-0)

Aquatic organism	MoA	Effect	REF
General	**A specific MoA**: antioxidant properties		

Aquatic organism	MoA	Effect	REF
	No studies have been found on the possible mechanism explaining observed toxicity in aquatic organisms.		

2,6-Di-tert-butyl-4-methylphenol (called butylated hydroxyl toluene, BHT, in English language literature) is the most widely used commercial antioxidant. The MoA has been studied rather in the context of its BHT antioxidant properties (radical scavenging efficacy), e.g. its ability/potential to inhibit lipid peroxidation.

2.2.2.8 Sunscreen agent

2-Ethylhexyl 4-methoxycinnamate

Table 59. Overview of the available data on mode of action (MoA) for 2-ethylhexyl 4-methoxycinnamate (sunscreen ingredient/UV filter; CAS-number: 5466-77-3)

Aquatic organism	MoA	Effect	REF
General	**A specific MoA:** endocrine disruption	Listed as Endocrine disruptor-Category 1 both for human health and aquatic organisms	Carvalho et al. 2015 and references therein
In larvae (but not in embryos) of *Chironomus riparius*	Increased ecdysone receptor (EcR) and heat shock protein (Hsp70) mRNA levels		Ozáez et al. 2016
Snails		Toxic effects on reproduction	Carvalho et al. 2015
Fish (fathead minnows)	Affected expression of genes involved in different hormonal pathways	Low but multiple hormonal activities in fish including vitellogenin induction, histological changes in gonads	Christen et al. 2011

The results obtained by Paredes et al. (2014) show that, using marine organisms from different trophic levels, according to their EC50 values for the same test species using the same standard bioassays, the toxicity of EHMC is similar to that of the most toxic trace metals copper, mercury, cadmium, lead or zinc.

2.2.3 Candidate substances identified through the monitoring- and modelling-based prioritisation exercises in 2015-2017

2.2.3.1 Pyrethroid insecticides

Bifenthrin

Table 60. Overview of the available data on mode of action (MoA) for bifenthrin (Pyrethroid; CAS-number: 82657-04-3)

Aquatic organism	MoA	Effect	Ref
Insects General	Acts in the nervous system of insects by interacting with the sodium channel and disrupting the normal transmission of nerve impulses.	 Neurotoxicity	https://circabc.europa.eu/sd/a/e6e9d673-d2fd-4913-83b6-af317551ee73/Bifenthrin_JRC_2016_DRAFT_DOSSIER%26%20ANNEX_30112016.zip

Deltamethrin

Table 61. Overview of the available data on mode of action (MoA) for deltamethrin (pyrethroid; CAS-number: 52918-63-5)

Aquatic organism	MoA	Effect	Ref
Insects and mammals	The primary MoA in both insects and mammals is the reversible disruption of voltage-sensitive sodium channels' (VSSCs) activity (or function)	Neurotoxicity	https://circabc.europa.eu/sd/a/7f9d9d16-adb8-47d4-baf0-424911e8ffd0/Deltamethrin_JRC_2016_DRAFT_DOSSIER%20%26%20ANNEX_30.11.2016.zip

Esfenvalerate

Table 62. Overview of the available data on mode of action (MoA) for esfenvalerate (pyrethrorid; CAS-number: 66230-04-4)

Aquatic organism	MoA	Effect	Ref
General	It acts as an agonist of the pre-synaptic voltage-gated sodium channels	Neurotoxicity	https://circabc.europa.eu/sd/a/a72d3901-5bb5-4656-9a74-e81e594eea0b/Esfenvalerate_JRC_2016_DRAFT_DOSSIER%20%26%20ANNEX_30112016.zip

Permethrin

Table 63. Overview of the available data on mode of action (MoA) for permethrin (pyrethroid; CAS-number: 52645-53-1)

Aquatic organism	MoA	Effect	Ref
Insects General	Acts on the insect nervous system by reducing the kinetics of opening and closing of Na channels. Permethrin also induces hepatic microsomal enzymes.	Convulsions, paralysis and mortality Neurotoxic effects	https://circabc.europa.eu/sd/a/5fef29a7-5bab-4472-ad5f-0d6c5bc2d76d/Permethrin_JRC_2016_DRAFT%20%26%20ANNEX_30112016.zip

Pyrethroid insecticides cross the blood-brain barrier (Singh et al., 2012) and induce neurotoxicity by prolonging the opening of VGSC. Recently, attention has focused on the potential human health risks associated with pyrethroid exposure as use of these pesticides has significantly increased (DeMicco et al. 2010, Domingues et al. 2016, Viel et al. 2015). There is also growing evidence that long-term/low-dose pyrethroid exposure may have significant neurotoxic effects (Baltazar et al. 2014). Pyrethroid insecticides can be toxic to many marine and freshwater forms including aquatic invertebrates, insects and fishes (Prusty et al. 2015). The pyrethroid insecticides have been shown to affect mechanisms involved in fish reproduction; though a lot of advance has been made in understanding the MoA and toxic effect of these pesticides on different fish species, concise information on the toxic impact of pyrethroids on various physiochemical, biological and metabolic processes is lacking (Prusty et al. 2015). Review of relevant literature suggests that Type II synthetic pyrethroid insecticides are in general more toxic and cause alterations in the metabolic processes, hematology, enzymatic activity and reproductive physiology of fish, providing evidence for ecological disturbance in the natural environment due to unintentional dispersal of insecticides (Murthy et al. 2013). It can also be concluded that young animals and animals at the embryonic stage are more susceptible to the effects of these pesticides.

2.2.3.2 Sulfonylurea herbicide

Nicosulfuron

Table 64. Overview of the available data on mode of action (MoA) for nicosulfuron (sulfonylurea; CAS-number: 111991-09-4)

Aquatic organism	MoA	Effect	Ref
Plants	Acts by inhibition of the synthesis of acetolactate synthatase, the first enzyme in a pathway synthesising essential amino acids made only by plants.		https://circabc.europa.eu/sd/a/2621878e-4364-4eae-880f-44af596eccd1/Nicosulfuron_JRC-2016-DRAFT%20%26%20ANNEX_30112016.zip

Seguin et al. (2001) tested the sensitivity of phytoplankton to the herbicides atrazine and nicosulfuron in experiments conducted in increasingly complex systems, from single strain phytoplankton cultures to mesocosms mimicking whole ecosystems. The endpoints used to assess sensitivity to atrazine and nicosulfuron were total biomass increase, photosynthetic efficiency, and community diversity, depending on the system considered. Nicosulfuron appeared to be very much less toxic to phytoplankton than atrazine, in accord with the planned changes in agricultural practices to reduce the effects of surface water contamination on aquatic biota. Nevertheless, nicosulfuron had significant effects in some systems (principally microcosms), whereas the single monocultures were almost insensitive to it. This points out the inaccuracy of using the standardised toxicity test on phytoplanktonic algae alone for predicting the effects of xenobiotics on natural communities and the need for tests in microcosms and mesocosms to obtain reliable evidence about the toxicity of a given chemical on freshwater aquatic ecosystems.

2.2.3.3 Organophosphorus insecticides

Malathion

Table 65. Overview of the available data on mode of action (MoA) for malathion (organothiophosphate; CAS-number: 121-75-5)

Aquatic organism	MoA	Effect	Ref
General	Works by contact and ingestion action and acts as a cholinesterase inhibitor.	According to the recent IARC evaluation (IARC, 2016), the overall evidence for receptor-mediated effects of malathion is strong. There is a compelling evidence for the activity of malathion on thyroid-hormone receptor-mediated pathways. The evidence for this activity was found in *in vivo* studies with experimental animals, and in some supporting studies in	https://circabc.europa.eu/sd/a/0ee96946-2634-4f2a-b011-1be991117859/Malathion_JRC-2016-DRAFT_DOSSIER%20%26%20ANNEXES_%209112016.zip

Aquatic organism	MoA	Effect	Ref
		human and rodent cells in vitro. Evidence for the disruption of sex hormones, primarily for the androgen pathway, was observed in studies in rodents *in vivo* and studies in fish (IARC, 2016).	

Omethoate

Table 66. Overview of the available data on mode of action (MoA) for omethoate (organophosphorus insecticide/acaricide; CAS-number: 1113-02-6)

Aquatic organism	MoA	Effect	Ref
General	Works by contact and ingestion action and acts as a cholinesterase inhibitor.	Endocrine disrupting effects	https://circabc.europa.eu/sd/a/0ee96946-2634-4f2a-b011-1be991117859/Malathion_JRC-2016-DRAFT_DOSSIER%20%26%20ANNEXES_%2029112016.zip

2.2.3.4 Metals and non-metal trace elements

Silver

Table 67. Overview of the available data on mode of action (MoA) for silver (transition metal; CAS-number: 7440-22-4)

Aquatic organism	MoA	Effect	Ref
General	Overall, the mechanism of action has been linked to Ag ability to generate ionic silver and to increase the production of reactive oxygen species (oxidative damage). Disinfectant and microbiocide: ionic silver is generally considered to interact with multiple microbial target sites. One of the major target sites for ionic silver is at the bacterial cell membrane level, where it can inhibit the proton motive force and	Silver is one of the most toxic of the heavy metals to freshwater microorganisms. Silver is most toxic to microscopic organisms or larval forms of aquatic animals - invertebrates and embryos of fish are generally much more sensitive than juvenile and adult fish. Ionic silver is more toxic to aquatic organisms than silver compounds. Water hardness, length of exposure, size of the organism and life stage of	https://circabc.europa.eu/sd/a/ff3427f0-257f-449c-8b18-9afe44d619d0/Silver_JRC_2016_DRAFT_DOSSIER%20%26%20ANNEXES_12December%202016.zip

Aquatic organism	MoA	Effect	Ref
	the respiratory electron transport chain, and affect membrane permeability resulting in bacterial cell death. Silver nanoparticles have been reported to act as endocrine-disruptors in amphibians.	the organism all affect the toxicity values. There is much debate in the literature regarding the adverse effects caused by silver nanoparticles (Ag-NP) exposure on environmental species. In several animal toxicity studies an increase of various liver enzymes was observed, indicating liver toxicity after a silver nanoparticle administration (SCENIHR 2014)	

Uranium

Table 68. Overview of the available data on mode of action (MoA) for uranium (metal; CAS-number: 7440-61-1)

Aquatic organism	MoA	Effect	Ref
General	Uranium builds up in living systems inter alia due to its high affinity to phosphorus containing components such as DNA causing its damage followed by mutations (once attached to the DNA, uranium amplifies natural background radiation and causes through photoelectron enhancement effects damages to the DNA)	The main risk of exposure to depleted uranium is chemical poisoning by uranium oxide rather than the potential environmental impact through its radioactivity. Although the different uranium isotopes are naturally radioactive, uranium's chemical toxicity is 6 orders of magnitude more harmful than its radioactivity. The uranium compounds may cause damage to organs through prolonged or repeated exposure - the main chemical effect associated with exposure to uranium and its compounds is kidney toxicity. The most remarkable damage of uranium coming along with low and medium contaminations is cancer.	https://circabc.europa.eu/sd/a/47ea37a9-33ed-45da-ac62-2064abffd775/Uranium_JRC-2016-DRAFT_%20DOSSIER%20%20AND%20ANNEX_7112016%20updated%20_12%20December2016.zip

Selenium

Table 69. Overview of the available data on mode of action (MoA) for selenium (non-metal trace element; CAS-number: 7782-49-2)

Aquatic organism	MoA	Effect	Ref
General	Essential micronutrient at low levels: protects intracellular structures against oxidative damage and it is an essential component of glutathione peroxidase and thioredoxin reductase, enzymes on charge of detoxification. Toxic at concentrations only slightly higher concentrations: selenium is erroneously substituted for sulfur, resulting in the formation of a triselenium linkage or a selenotrisulfide linkage (both prevent the formation of the necessary disulfide chemical bonds). The end result is distorted, dysfunctional enzymes and protein molecules, which impairs normal cellular biochemistry.	Although the biomagnification potential of selenium is variable depending on the trophic network studied, its bioaccumulation potential has been clearly demonstrated. There is strong evidence that selenium causes harm to fish following long-term (chronic) exposure at concentrations only slightly above essentiality. The primary concern related to selenium toxicity for water birds (as for fish) is accumulation of selenium in eggs from maternal transfer, resulting in deformities or death of the developing fish/bird.	https://circabc.europa.eu/sd/a/75adecaa-4077-494c-9ddd-f69b5cacde92/Selenium_JRC-2016-DRAFT_v2_12dec2016(0).doc

2.3 Final grouping of the current priority substances included in the Watch List and those so far shortlisted during the prioritisation exercise according to the identified mode of action and effect

Grouping of substances according to common mode of action (MoA) or/and common effect seems to be a good option to increase the efficiency of the chemical status assessment, i.e. to assess chemical status more holistically using selected effect-based methods (EBMs) rather than using chemical analyses with a limited but ever-growing list of individual substances.

The literature data review identified the following groups of common toxicological endpoints:

2.3.1 Photosynthesis inhibition

Effect-based methods (EBMs) for PSII inhibition and algal growth reflect very well the aquatic contamination with PSII-inhibiting herbicides and are well suited for effect-based detection and quantification of this group of chemicals independent of the exact composition of the herbicide mixture. Examined parameters like photosynthetic pigment content, induced chlorophyll fluorescence and Hill reaction activity can be used as markers.

2.3.2 Endocrine disruption

Endocrine disrupters interfere with the functioning of the endocrine system mainly through three different ways: (a) by mimicking the action of endogenous hormones, (b) by blocking hormone receptors and (c) by affecting the synthesis, transport, metabolism and excretion of hormones, thus altering their levels.

In aquatic species endocrine disruption can be exert principally on the reproductive, thyroid and adrenal systems. Specific receptor (e.g. ER, AhR) activation and/or inhibition responses, as well as Vtg expression, are typically studied and reported.

2.3.3 Oxidative stress

Oxidative stress, defined as a disruption of the prooxidant/antioxidant balance in favour of the former, causes damage to cell components by reactive oxygen species (ROS) or other reactive products originating from the effect of various chemical pollutants. Oxidative stress represents therefore an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. The main targets of ROS compounds in the cell are DNA, lipids, and membrane proteins, and interactions with these targets lead to lipid peroxidation and membrane breakdown, affecting plasma ion balance. The main antioxidative enzymes are catalase (CAT) which converts hydrogen peroxide (H_2O_2) to O_2 and H_2O, glutathione peroxidase (GPx) which converts H_2O_2 to H_2O, coupled to the oxidation of reduced glutathione (GSH) to oxidised glutathione (GSSG), and superoxide dismutase, which converts O_2 to H_2O_2. Moreover, the changes in lipid hydroperoxides levels provide further evidence for substance(s)-induced oxidative stress (Dorval et al 2003).

2.3.4 Activation of metabolising/detoxifying pathways

Cytotoxicity can also result from the conversion of aromatic chemicals to more toxic metabolites by the inducible cytochrome P4501A (CYP1A) enzyme complex. For aquatic species, as in higher vertebrates, CYP1A activity in fish is induced via the aryl hydrocarbon receptor (AhR) which binds planar aromatic hydrocarbons, including dioxins and many PAHs, with high affinity. The main role of the CYP1A and related enzyme systems is to aid detoxification, through the activation, conjugation, and elimination of potentially harmful aromatic chemicals. However, some chemicals, such as the PAH benzo(a)pyrene (BaP), can be converted into reactive metabolites that cause cytotoxicity rather than prevent it. Ethoxyresorufin O-deethylase (EROD), a specific cytochrome P450-dependent monooxygenase, is often used as an indicator of polycyclic aromatic hydrocarbon pollution and was consistently observed in livers of rainbow trout (*Oncorhynchus mykiss*), and in liver cell lines from fathead minnow (*Pimephales promelas*, PLHC-1).

Available evidence suggests that glutathione (γ-glutamyl-l-cysteinylglycine; GSH) conjugation plays an important role in the formation of toxic metabolites from a variety of chemicals. GSH is present in high concentrations in most living cells and participates in a variety of vital cellular reactions. In particular, GSH protects cells from potentially toxic electrophiles formed via the metabolism of xenobiotics, and such reactions have long been associated with the process of detoxification. However, several classes of compounds are converted, via conjugation with GSH, into either cytotoxic, genotoxic, or mutagenic metabolites. Glutathione-S-transferase (GST)-dependent pathway plays also role in those transformations, and GST enzymatic activity is often used as a biomarker of phase II.

2.3.5 Genotoxicity

Polycyclic aromatic hydrocarbons (PAHs) are priority environmental mutagens and carcinogens that occur in the aquatic environment as mixtures rather than the individual compounds for which guidelines are issued. As a consequence of their acknowledged toxicity and pro-mutagenic and/or carcinogenic potential, PAHs are deemed priority in biomonitoring programmes. Still, the differences between the toxicity of carcinogenic and non-carcinogenic PAHs are poorly known especially, when aquatic organisms are exposed to ecologically relevant concentrations of these compounds in sediments. Nickel and its compounds are also highly carcinogenic because exposure leads to protein-DNA crosslinking. Increased micronucleus (MN) frequency test and Comet assay are often used to conclude on the genotoxic mode of action (MoA) in the aquatic organisms.

2.3.6 Histopathology

Considerable interest has been shown in recent years in histopathological studies while conducting sub-lethal tests in aquatic organisms, especially in fish. Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant are a functional response of organisms, which provides information on the nature of the toxicant. Considering that the liver is the organ primarily involved in the regulation of metabolic pathways, homeostasis and detoxification can be investigated on the level of the morphological and ultrastructural effects in this organ. Histopathological examination of different organs (gonads, gills, skin) may be associated especially with the exposure to PAHs, metals and pesticides, but may also indicate the overall contamination.

2.3.7 Stress proteins: SfG and LMS

Through those assays, a general physiological stress manifested in the aquatic species after exposure to many compounds can be captured.

2.3.8 Unique pathway of toxicity

Some very specific toxicity pathways, bioassays and biomarkers of effect have been also identified: acetylcholinesterase (AChE) inhibition, which is associated with exposure to organophosphate and carbamate insecticides as well as other neurotoxic xenobiotics; delta-aminolevulinic acid dehydratase (δ-ALAD) activity that can be depressed by lead; PAHs metabolites which constitute biomarkers of exposure based on chemical analysis of biota (especially in bile); presence of heavy metal-binding molecules called metallothioneins (MT) related to the accumulation of heavy metals; bioassay using the strains of bioluminescent *E. coli* reporters (zntA and arsR gene promoters) proved to be a sensitive test for the presence of heavy metals in the mixtures (Carvalho et al. 2014); and imposex, which is the most sensitive indicator of exposure to TBT of all known non-target pathological conditions (Okoro 2011).

2.4 Inventory of modes of action identified in the classes of priority substances and preliminary identification of potential effect-based methods

Based on the information collected (see Section 2.2), an inventory of all relevant modes of action (MoAs) has been performed for the classes of current priority substances (PS), A preliminary proposal for potential effect-based methods (EBMs) has been made, focusing on the detection of biomarkers of exposure and/or biomarkers of effects. The EBM have been chosen from the EU Report 2014 (Carvalho et al. 2014), and based on a literature review (e.g. ROS, oxidative stress biomarkers like depletion of GSH, enzymatic activity of SOD, CAT which are relatively fast, cheap and informative).

Few very general EBMs, referring to overall organism health, e.g. externally visible fish disease or benthic diatom malformations, have been listed in any of tables (as we cannot really link them to the known MoA and/or effects), however, they are recommended as EBMs that could be used to assess/monitor the general health status of aquatic organisms/population, especially at sampling sites exposed to severe anthropogenic pressures.

2.4.1 Herbicides

Substance	**Target species** (target species)	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint/ effect at the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
Alachlor	Plant	Elongase and GGPP inhibition	Algal growth inhibition	Fish Amphibians	Changes in biochemical /detoxification parameters (total protein, SOD, CAT, GST, ALT, AST; GSH)	Decreased HSI and GSI Embryotoxicity	Oxidative stress markers/ Xenobiotic-metabolising enzymes activity FETAX
Atrazine	Plant	Photosystem II inhibition	PSII inhibition Chlorophyll concentration Algal growth inhibition ROS production/ lipid peroxidation(1)	Snail Fish Amphibians	Changes in biochemical/ detoxification parameters (SOD, CAT, GR, LDH) Induction of aromatase (CYP19A1) - adrenotoxicant Changes in sex hormones concentration (decrease of testosterone and induction of estrogen)	Reduced egg production Demasculinisation and feminisation	Oxidative stress markers/ Xenobiotic-metabolising enzymes activity Zebrafish reproduction CYP19A1/aromatase induction (gene expression) T/E2 assay Histological examination of reproductive organs
Diuron	Plant	Photosystem II inhibition	PSII inhibition Chlorophyll concentration	Oyster	ROS production/ genotoxicity (CAT activity, formation of 8-oxodGuo)	Embryotoxicity	Oxidative stress markers

Substance	**Target species** (target species)	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint/ effect at the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
			Algal growth inhibition ROS production/ lipid peroxidation(1)	Fish Amphibians	Changes in sex hormones concentration (decrease of testosterone) Cytochrome P450 stimulation Steroid biosynthesis, cholesterol metabolism and pregnane X receptor activation	Changes in GSI and germ cells/oocytes quantity	T/E2 assay CYP19A1/aromatase induction (gene expression) Cytochrome P4501A activity /EROD
Isoproturon	Plant	Photosystem II inhibition	PSII inhibition Chlorophyll concentration Algal growth inhibition ROS production/ lipid peroxidation(1)	Lack of studies(2)			
Simazine	Plant	Photosystem II inhibition	PSII inhibition Chlorophyll concentration	Fish	ROS production/ changes in detoxification parameters (total protein, AP, ALT)	Changes in HSI	Oxidative stress markers/ Hepatotoxicity enzymatic markers

Substance	**Target species** (target species)	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint/ effect at the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
			Algal growth inhibition ROS production/ lipid peroxidation(1)	Amphibians		Inhibition of metamorphosis	FETAX
Trifluralin	Plant	Cell mitosis inhibition	Cell count	Fish	Changes in detoxification parameters (AP, AST, ALT) Genotoxicity	Inhibition of growth Higher MN frequencies in peripheral erythrocytes	Hepatotoxicity enzymatic markers MN frequencies
Aclonifen	Plant	Protoporphyri-nogen oxidase inhibition/ carotenoid biosynthesis inhibition	Chlorophyll concentration (Chlorophyll formation is not inhibited directly, but the pigment is destroyed in the presence of light because of the missing photo-oxidative carotene shield)	Lack of studies			
Bifenox	Plant	Protoporphyri-nogen oxidase inhibition	Chlorophyll concentration	Lack of studies			

Substance	**Target species** (target species)	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint/ effect at the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
Cybutryne	Algae	Photosystem II inhibition	PSII inhibition Chlorophyll concentration Algal growth inhibition ROS production/ lipid peroxidation(1)	Lack of studies(3)			
Terbutryn	Algae	Photosystem II inhibition	PSII inhibition Chlorophyll concentration Algal growth inhibition ROS production/ lipid peroxidation(1)	Lack of studies			

(1) PSII inhibiting herbicides cause oxidative stress through production of reactive oxygen species (ROS), and it is this production of radicals, rather than starvation following the photosystem blockage, that causes cell death in exposed organisms (Rutherford and Krieger-Liszkay 2001; Fufezan et al. 2002; Wendt et al. 2013).

(2) One study with frog tadpoles reports increased activity of glutathione-*S*-transferase.

(3) One study on *in vitro* exposures of oyster's gametes and embryos suggest spermiotoxicity and embryotoxicity.

2.4.2 Polyaromatic hydrocarbons (PAHs)

Substance	Organism	MoA		Biological endpoint/ effect on the organism level	Potential EBM
Anthracene	Plant (Duckweed)	In general, there are two major mechanisms involved in photoinduced toxicity of PAHs: **photosensiti-zation** and **photomodifi-cation** ROS and oxidative stress	Photosystem I and II inhibition	Inhibition of photosynthesis	PSII inhibition
	Fish		Changes in biochemical parameters linked to oxidative stress/ detoxification (LPO, CAT, SOD, GST, GR, GPx, AChE, LDH)	Histological /structural changes in the gills	Oxidative stress markers/Xenobiotic-metabolising enzymes activity Histological examination of gills
Fluoranthene	Plant (Duckweed)		Changes in biochemical parameters linked to oxidative stress (SOD, CAT, MDA)	Histochemical changes at the cellular and tissue levels	Oxidative stress markers
	Benthic copepods			Decrease in offspring production	Amphipod embryo alterations
	Fish		Lipid peroxidation Changes in biochemical parameters linked to detoxification (EROD)	Histopathological changes in the skin	Oxidative stress markers Cytochrome P4501A/EROD activity Histological examination of skin
Naphthalene	*Daphnia magna*		Elevated Na^+, K^+ and Cl^- concentrations and decreased haemoglobin concentration in haemolymph		

Substance	Organism	MoA		Biological endpoint/ effect on the organism level	Potential EBM
	Fish		ROS production & lipid peroxidation		Oxidative stress markers
Polyaromatic hydrocarbons (PAH) Benzo(a)pyrene Benzo(b)fluoranthene Benzo(k)fluoranthene Benzo(g,h,i)-perylene Indeno(1,2,3-cd)-pyrene	Clam Shrimp		Changes in biochemical parameters linked to oxidative stress/ detoxification (GSH; LPO, SOD, GST, EROD)		Oxidative stress markers Cytochrome P4501A /EROD activity Mussel histopathology
	Fish		CYP1A induction/ AhR binding Changes in biochemical parameters linked to oxidative stress/ detoxification (P450/EROD)	Hepatic histopathological changes that indicate metabolic failure and inflammation	Oxidative stress markers Cytochrome P4501A/EROD activity LH and MLN DNA adducts PAH bile metabolites

2.4.3 Organophosphorus insecticides

Substance	Target species	Specific MoA (target species)	Potential EBM (target species)	Non-target organism	Non-specific MoA (reported for non-target species)	Biological endpoint/ effect on the organism level (reported for non-target species)	Potential EBM (non-target species)
Chlorfenvinphos	Insects	AChE inhibition	AChE activity	Mussel	Inhibited phagocytic activity, spontaneous cytotoxicity, neutral red	Impacted immune function	LMS

Substance	**Target species**	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint/ effect on the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
			Oxidative stress markers (CAT, SOD and GPx)		retention time, total haemolymph protein		
				Fish	AChE inhibition		AChE activity
Chlorpyrifos (Chlorpyrifos-ethyl)				Daphnia		Affected reproduction/ decrease of offspring	
				Shrimp	AChE inhibition ROS/Changes in biochemical parameters linked to oxidative stress (TBAR)		AChE activity Oxidative stress markers
				Fish	AChE inhibition Changes in biochemical parameters linked to oxidative stress (CAT, SOD and GPx; MDA) and biotransformation enzymes (EROD)	Pathological changes in tissue	AChE activity Oxidative stress markers/ Xenobiotic-metabolising enzymes activity
				Amphibian	AChE inhibition		AChE activity
Dichlorvos				Daphnia	AChE inhibition		AChE activity
				Oyster	AChE inhibition		AChE activity

Substance	Target species	Specific MoA (target species)	Potential EBM (target species)	Non-target organism	Non-specific MoA (reported for non-target species)	Biological endpoint/ effect on the organism level (reported for non-target species)	Potential EBM (non-target species)
				Fish	Increased frequencies of MN and positive Comet assay	Genotoxicity	MN assay and Comet assay

2.4.4 Organochlorine insecticides

Substance	Target species	Specific MoA (target species)	Potential EBM (target species)	Non-target organism	Non-specific MoA (reported for non-target species)	Biological endpoint /effect on the organism level (reported for non-target species)	Potential EBM (non-target species)
Endosulfan	Insects	Via gamma aminobutyric acid (GABA) receptor system (opening the		Fish	Adrenotoxicant (decreased T4, increased Vtg and aromatase level) Disrupts cortisol secretion Alterations in biochemical parameters linked to oxidative stress (CAT, GPx, GST, GSH and LPO) and biotransformation (EROD, GST) Decreased Na^+, K^+-ATPase	Morphological changes in liver, testes and blood lymphocytes	Vtg CYP19A1/aromatase induction (gene expression) LH Histological examination of testes Oxidative stress markers/Xenobiotic-metabolising enzymes activity

Substance	**Target species**	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint /effect on the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
Hexachloro-cyclohexane		chloride transport, increasing glutamate level)		Fish	Alterations in blood parameters (RBC, Hb, Ht, WBC) Increase in HSP60 and 70 genes	Histophatological changes in gills, liver and kidney	Blood parameters LH Histological examination of gills and kidney Stress proteins
Dicofol		The exact MoA is not known		*In vitro* system	Anti-oestrogen (inhibits aromatase activity, CYP19)		CYP19A1/aromatase gene expression
Heptachlor and Heptachlor epoxide		The exact MoA is uncertain		Amphibian	Changes in activities of enzyme involved in the protective response to xenobiotic (acid and alkaline phosphatases)	Morphological alterations in the larval epidermal cells	FETAX

2.4.5 Chlorinated solvents

Substance	**Organism**	**MoA**	**Biological endpoint/effect on the organism level**	**Potential EBM**
1,2-Dichloroethane	Fish	Formation of DNA adducts (mutagenesis)		DNA adducts
Dichloromethane	Lack of studies			

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Hexachlorobutadiene	Fish	GGT (a histochemical marker) in the kidney Cytotoxicity and compensatory cell proliferation	Kidney histology/efefcts on nephrons	Histological examination of kidney
Trichloromethane (Chloroform)	Lack of studies			

2.4.6 Aromatic organochlorine compounds

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Hexachlorobenzene (HCB)	Crab	Alterations in biochemical parameters linked to oxidative stress (LPO, MDA, GSH, SOD)	Reduced HSI/histological changes in hepatopancreas	Oxidative stress markers Histological examination of hepatopancreas
	Fish	Elevated ROS/alterations in biochemical parameters linked to oxidative stress (GSH, TBARS, SOD, NOS, GPx, GR) and detoxification (GST and EROD) Inhibited AChE		Oxidative stress markers/xenobiotic-metabolising enzymes activity AChE activity

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Pentachlorobenzene	Fish (zebrafish)	Marker genes (HMOX1 and CYP1a proved to be the most sensitive genes)		Oxidative stress markers/Cytochrome P4501A /EROD activity
Pentachlorophenol	Amphibian (frog)	Altered (minor) levels of hormones in plasma	Ovary injuries	Histological examination of ovaries
	Fish	Interfere (agonistic) with steroid receptors (ER, AR) and hepatic microsome enzyme activity		CYP19A1/aromatase induction ER induction (gene expression) Xenobiotic-metabolising enzymes activity
Trichlorobenzenes	Lack of studies			

2.4.7 Dioxins, PCBs, BDEs

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Brominated Diphenyl Ethers (BDEs)	Fish	Impacts on the thyroid endocrine system Altered genes expression (hepatic ER and ovarian AR)	Altered reproductive output (inhibited egg production and reduction in mature sperm)	CYP19A1/aromatase induction ER induction (gene expression) Fish reproduction

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
				Thyroid endocrine system (hormons level, genes expression)
Dioxins and coplanar PCBs	Fish	Activation of Ah receptor Most potent inducers CYP1A/EROD activity		Cytochrome P4501A/EROD activity

2.4.8 Metals

Substance	Organism	MoA		Biological endpoint/effect on the organism level	Potential EBM
Cadmium and its compounds	Crustacean	In general, cadmium exposure is associated with the disruption of calcium, sodium balance, generation of ROS what affect the properties of many biological molecules (enzymes, etc.)	Lower Na^+/K^+-ATPase/ decreased haemolymph osmolality	Alterations of the gill structure	Histological examination of gills LMS
	Mussel		Oxidative stress (CAT activity)		Oxidative stress markers
	Fish		Induction of MTs	Cadmium accumulation	MT
Lead and its compounds	Crab	Lead exposure is associated with decreased activity of ð-ALAD enzyme Lead binds to sulfhydryl groups which are prevalent in many enzymes	Affected osmolality and ion concentrations	Lost weight	LMS
	Fish		Ionoregulatory disruption		ALA-D activity Oxidative stress markers/xenobiotic-

Substance	Organism	MoA		Biological endpoint/effect on the organism level	Potential EBM
		Causes oxidative stress			metabolising enzymes activity
Mercury and its compounds	Copepod	Mercury can form covalent bonds to sulfhydryl groups and impair with enzymes and their cellular function Essential mechanism associated with the toxicity of Hg is oxidatative stress Non-specific endocrine-related mechanisms	ROS/alterations in biochemical parameters linked to oxidative stress (GPx, GR, GSH and LPO) and MAPK pathways		Oxidative stress markers Amphipod embryo alterations
	Fish		Induction of MT Different genetic pattern observed for MeHg and iHg Alterations in expression profiles in genes related to cellular protection (MT), stress (HSP70), oxidative stress (SOD, CAT, GR) and apoptosis	Morphological alterations in the liver and gill Macrophage aggregates Reduction in the viability of leucocytes	LH Histological examination of gill MT Oxidative stress markers/xenobiotic-metabolising enzymes activity
Nickel and its compounds	Snail	5 potential pathways by which Ni may exert toxicity on aquatic organisms: 1) disruption of Ca^{2+} homeostasis, 2) disruption of Mg^{2+} homeostasis, 3) disruption of $Fe^{2+}/^{3+}$ homeostasis, 4) an allergic	Disrupted calcium homeostasis	Histopathological lesions in kidney and liver	LMS LH Histological examination of kidney
	Freshwater invertebrates		Ionoregulatory toxicant, disrupting Mg homeostasis		LMS

Substance	Organism	MoA		Biological endpoint/effect on the organism level	Potential EBM
	Freshwater fish	reaction at respiratory epithelia, and 5) generation of ROS	Respiratory toxicant significantly increasing ventilation rate, ventilatory stroke volume and oxygen consumption	Swelling of the gill lamellae	Oxidative stress markers/xenobiotic-metabolising enzymes activity Histological examination of gills
	In marine invertebrates		Ionoregulatory disruption (mainly Ca^{2+} metabolism)		LMS

2.4.9 Phthalate

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Di(2-ethylhexyl)-phthalate (DEHP)	Fish	The increase of Vtg levels and peroxisome proliferators activated receptors mRNA levels Increased liver Vtg level in males Modulated transcription profiles of genes involved in steroidogenesis Increases plasma 17β-estradiol (E2) along with decrease in testosterone (T)/E2 ratios in males Oxidative stress	Estrogenic potency Reduced GSI Retarded oocyte development Impaired embryo production Disrupted spermatogenesis Reduction in the fertilisation rate	SfG in mussels (Wernersson et al. 2014) Vtg T/E2 assay Oxidative stress markers Intersex in male fish

2.4.10 Anti-fouling biocide

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Tributyltin compounds	Aquatic organisms	Imposex MT induction Lisosomal enlargement, lysosomal membrane destabilisation, peroxisome proliferation, lysosomal activity, genetic or molecular biomarkers, apoptosis induction, phagocytic index, and amoebocytic index		VDSI and/or RPSI MT LMS

2.4.11 Alkylphenols

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Nonylphenols	Invertrebrates	Decreased haemocyte lysosomal membrane stability in mussels Altered phagocytosis in mussels Affected haemocyte counts and size distribution frequency, decreased haemocyte membrane stability and lysozyme activity in clams	Reduced fecundity in freshwater snails Inhibited development in euryhaline copepods Decreased sperm motility, altered sex ratios, increased percent hermaphroditism, delayed spermatogenesis, and increased developmental abnormalities in oysters	LMS SfG Histological examination of with focus on reproductive organs
	Fish	Vtg induction in male and immature fish	Reduced semen production	FET

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
		Affecting AR expression and endogenous estrogen level Oxidative stress (GSH depletion and inhibition of GSH-related anti-oxidant enzymes)	Fish feminisation Altered HSI and GSI Various body malformations in larvae	Vtg CYP19A1/aromatase gene expression Oxidative stress markers Intersex in male fish
Octylphenols	Amphibians	Induced Vtg synthesis in hepatocyte of males	Alterations in sex ratio, abnormal testicular development, affected male sexual behaviour	Vtg Histological examination of with focus on reproductive organs
	Fish	Higher Vtg, Zrp and cyp19a1 mRNA levels in males	Histopathological changes in gonads, intersex	Vtg Histological examination of with focus on reproductive organs Intersex in male fish

2.4.12 Pyrethroid insecticides

Substance	**Target species** (target species)	**Specific MoA** (target species)	**Potential EBM** (target species)	**Non-target organism**	**Non-specific MoA** (reported for non-target species)	**Biological endpoint/ effect on the organism level** (reported for non-target species)	**Potential EBM** (non-target species)
Cypermethrin	Insects	Prolongs the opening of sodium channel Modulates chloride, voltage-gated calcium and potassium channels	No information available	Daphnia magna	Cytochrome P450 activity		Cytochrome P4501A activity /EROD
				Crayfish	Oxidative stress and disruption of antioxidant system (decreased in levels of TBARS, changes in catalase, SOD, GR and GST activity)		Oxidative stress markers/xenobiotic-metabolising enzymes activity
				Fish	Dose- and time-dependent biochemical and hematological alterations 8-OHdG expression in the nuclei and cytoplasm of neurons Affected transcription patterns of many key genes (Vtg, ER, CYP19a)	Behavioural response/ swimming alteration Histopathological changes (hyperplasia of lamellar cells, telangiectasia of lamellae and thickening) Cellular infiltration in gills, haemorrhage, diffuse hydropic degeneration, and focal necrosis in the liver Reduced fertilisation success	Oxidative stress markers/xenobiotic-metabolising enzymes activity Histopathological examination (liver, gills)

Substance	Target species	Specific MoA (target species)	Potential EBM (target species)	Non-target organism	Non-specific MoA (reported for non-target species)	Biological endpoint/ effect on the organism level (reported for non-target species)	Potential EBM (non-target species)
							Zebrafish reproduction

2.4.13 Perfluorinated surfactant

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Perfluorooctan-sulfonic acid (PFOS)	Fish	Interfere with lipid metabolism Vtg induction/depression Disturbance of sexual and thyroid hormone synthesis Reactive oxygen species production	Abnormal development/impact on embryonic growth and offspring morphology Reduced offspring survival	FET Vtg T/E2 assay Oxidative stress markers/Xenobiotic-metabolising enzymes activity

2.4.14 Benzene

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Benzene	Fish	Formation of DNA adducts		DNA adducts

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
		Oxidative damage		Oxidative stress markers/Xenobiotic-metabolising enzymes activity

2.4.15 Quinoline fungicide

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Quinoxyfen	Lack of studies			

2.4.16 Chloroalkans

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
C10-13 chloroalkanes	Amphibian (frog)	Induction of phase II detoxification enzyme GST	Developmental malformations and reduced embryo growth	FETAX Xenobiotic-metabolising enzymes activity
	Fish	Altered gene expression in HPT axis (tyr, ttr, dio2 and dio3) Inhibition of thyroid hormone levels (T3)	Histopathological lesions in the livers Disrupted thyroid hormone homeostasis	LH T3 assay

2.4.17 Hexabromocyclododecane (HBCDD)

Substance	Organism	MoA	Biological endpoint/effect on the organism level	Potential EBM
Hexabromocy-clo-dodecane (HBCDD)	Copepod	Induced the transcription of oxidative stress response genes and apoptotic genes (SOD,CAT, GST, OGG1, P53 and Caspase-3) in adults	Significant growth delay in nauplii	Oxidative stress markers LMS
	Fish	Oxidative stress and apoptosis	Embryotoxicity (cardiovascular system)	FET Oxidative stress markers

2.5 Potential effect-based method linked to mode of action and effect for each of the current priority substances and other substances of interest

Based on all the information collected and presented in Sections 2.2, 2.3 and 2.4, the current priority substances (PS) as well as other substances of interest/concern have been grouped according to their mode of action (MoA) and/or common observed effects, together with preliminary linked potential effect-based method (EBM). For number of the chemicals, no EBM could be proposed due to the lack of information about MoA relevant to the aquatic organism(s) and/or no availability of relevant EBMs.

It should be noted here that bioassays addressing for example estrogen receptor (ER) or androgen receptor (AR) activation/inhibition may be sensitive enough to detect the endocrine-related chemicals as a group in surface water, and provide this way a cost-efficient and feasible monitoring alternative to chemical analysis. However, even if these bioassays may be seen as a good measure for the contamination with the ED compounds, they cannot be directly linked to the effects and distinguish between agonist and antagonist effects. The biological effects (e.g. female-protein like Vtg induction in males, inhibition or induction of the steroidal hormones) can only be measured *in vivo*, in the chosen sentinel species.

Below the summarising Table 70, and the Figure 1 with the corresponding Venn diagrams.

Table 70. Summary of already existing effect-based methods (EBMs) which can be used to detect/monitor the mode of action (MoA)/effects reported in the literature for the priority substances (PS), Watch List (WL) and emerging substances (for details see Sections 2.2, 2.3 and 2.4). For number of the chemicals no EBM could be linked/proposed due to the lack of information about MoA relevant to the aquatic organisms and/or the lack of relevant EBM(s) available.

Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/Oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Herbicides																								
Alachlor		■												■						■	■			
Atrazine	■	■				■			■											■	■			
Diuron	■	■			■	■			■											■				
Isoproturon	■	■																		■				
Simazine	■	■												■						■	■			
Trifluralin												■									■			
Aclonifen		■																						
Bifenox		■																						
Cybutryne	■	■																		■				
Terbutryn	■	■																		■				
Oxadiazon		■																		■				

Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/Oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Triallate																								
Polyaromatic hydrocarbons (PAHs)																								
Anthracene																								
Fluoranthene																								
Naphthalene																								
Polyaromatic hydrocarbons (PAH)																								
Organophosphorus insecticides																								
Chlorfenvinphos																								
Chlorpyrifos-ethyl																								
Dichlorvos																								
Malathion																								
Omethoate																								
Organochlorine insecticides																								
Cyclodiene pesticides																								

Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
DDT total and para-para-DDT																								
Endosulfan																								
Hexachloro-cyclohexane																								
Dicofol																								
Heptachlor and Heptachlor epoxide																								
Chlorinated solvents																								
Carbon tetrachloride																								
Tetrachloroethylene																								
Trichloroethylene																								
1,2-Dichloroethane																								
Dichloromethane																								
Hexachlorobutadiene																								
Trichloromethane (Chloroform)																								

Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Aromatic organochlorine compounds																								
Hexachlorobenzene (HCB)																								
Pentachlorobenzene																								
Pentachlorophenol																								
Trichlorobenzenes																								
Dioxins, PCBs, BDEs																								
Brominated Diphenyl Ethers (BDEs)																								
Dioxins and coplanar PCBs																								
Metals																								
Cadmium and its compounds																								
Lead and its compounds																								
Mercury and its compounds																								
Nickel and its compounds																								

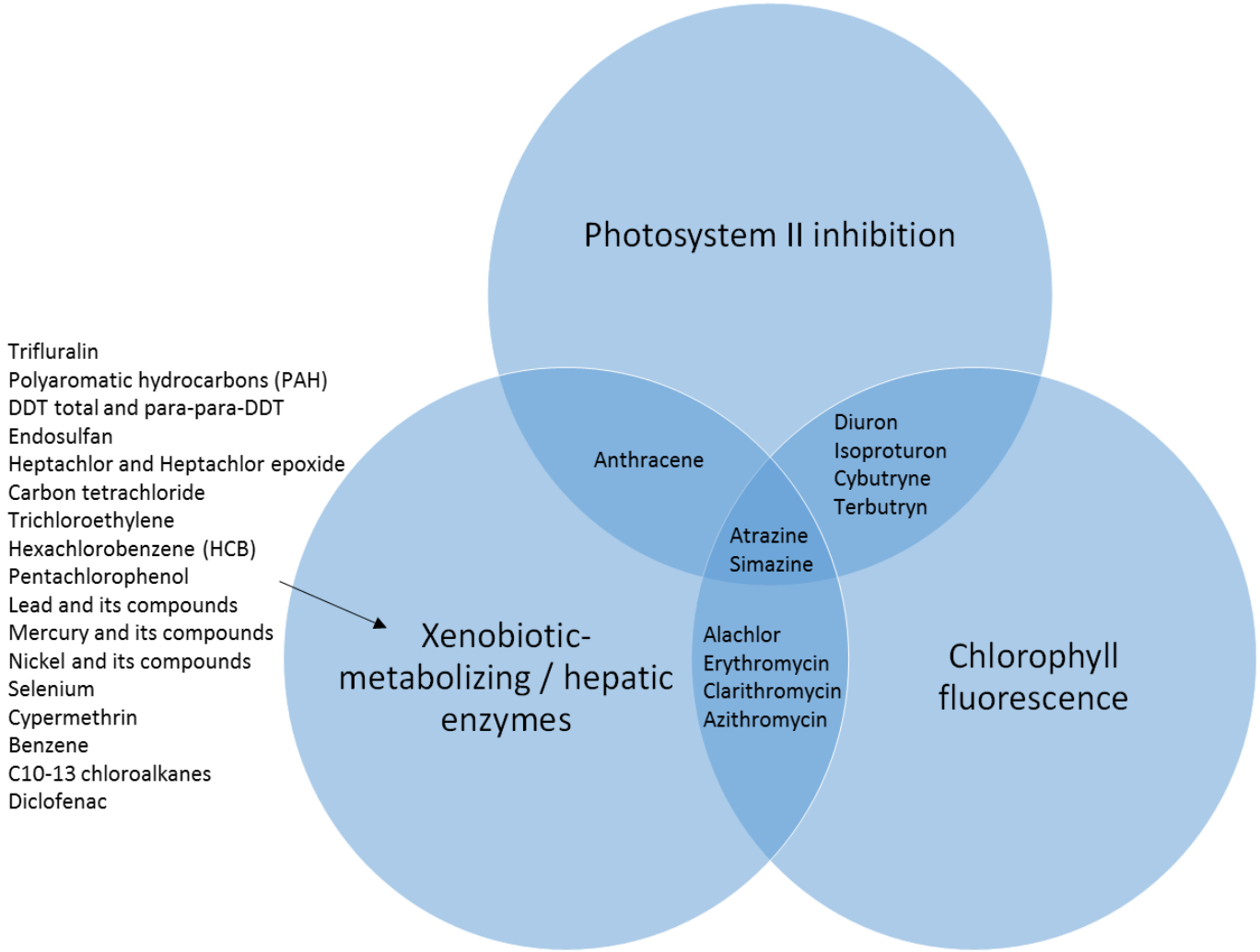
Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/Oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Silver																								
Uranium																								
Selenium																								
Endocrine disrupters																								
Di(2- ethylhexyl)-phthalate (DEHP)																								
Nonylphenols																								
Octylphenols																								
Tributyltin compounds																								
17-Alpha-ethinylestradiol (EE2)																								
17-Beta-estradiol (E2)																								
Estrone (E1)																								
Pyrethroid insecticides																								
Cypermethrin																								
Bifenthrin																								

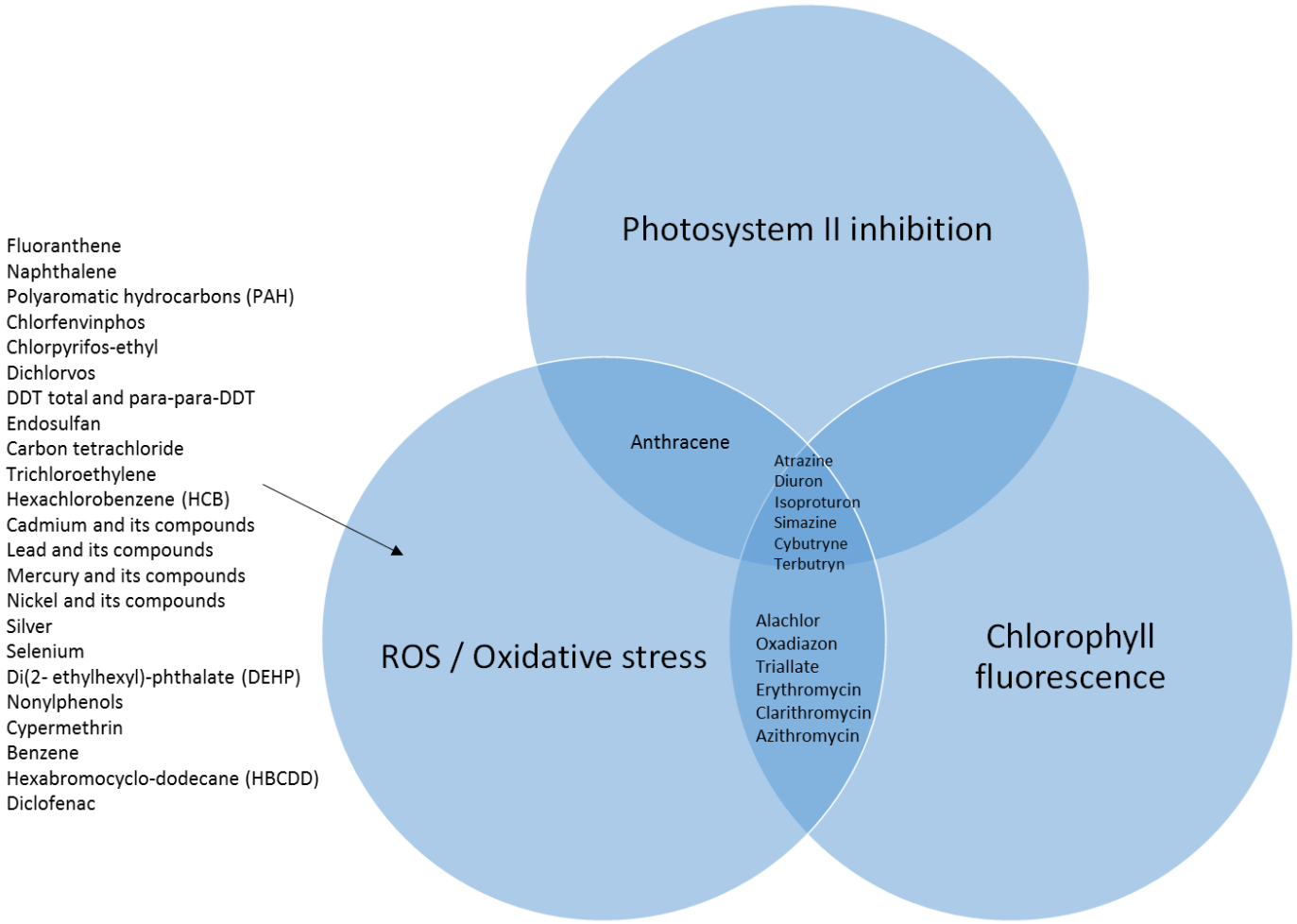
Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Deltamethrin																								
Esfenvalerate																								
Permethrin																								
Perfluorinated surfactant																								
Perfluorooctan-sulfonic acid (PFOS)																								
Benzene																								
Quinoline fungicide																								
Quinoxyfen																								
C10-13 chloroalkanes																								
Hexabromocyclo-dodecane (HBCDD)																								
Antibiotics																								
Erythromycin																								
Clarithromycin																								
Azithromycin																								

Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/Oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Neonicotinoid insecticides																								
Imidacloprid																								
Thiacloprid																								
Thiamethoxam																								
Clothianidin																								
Acetamiprid																								
Anti inflammatory drug																								
Diclofenac																								
Antioxidant																								
2,6-Di-tert-butyl-4-methylphenol																								
Sunscreen agent / UV filter																								
2-Ethylhexyl 4-methoxycinnamate																								
Carbamate insecticide and herbicide																								
Methiocarb																								

Substance	Photosystem II inhibition/algal	Chlorophyll	AChE inhibition	ALA-D activity	Cytochrome P4501a/EROD	CYP19A1/ aromatase	Vtg induction	Intersex in male	T/E2 assay	MT induction	Genotoxicity (DNA	Genotoxicity (MN frequency, Comet	Fish Embryotoxicity (FET)	Amphibian Embryotoxicity	SfG	LMS	HSP	Thyroid hormone(s) assay	Imposex in gastropoda	ROS/oxidative	Xenobiotic-metabolising/	Liver histopathology	Histopathology of organs other than	PAH metabolites
Sulfonylurea herbicide																								
Nicosulfuron																								

Based on the information summarized in Table 70, different groups of PS sharing the common MoA can be proposed. As example, three diagrams have been created (Figure 1).


Trifluralin
Polyaromatic hydrocarbons (PAH)
DDT total and para-para-DDT
Endosulfan
Heptachlor and Heptachlor epoxide
Carbon tetrachloride
Trichloroethylene
Hexachlorobenzene (HCB)
Pentachlorophenol
Lead and its compounds
Mercury and its compounds
Nickel and its compounds
Selenium
Cypermethrin
Benzene
C10-13 chloroalkanes
Diclofenac
Photosystem II inhibition
Anthracene
Diuron
Isoproturon
Cybutryne
Terbutryn
Atrazine
Simazine
Xenobiotic-metabolizing / hepatic enzymes
Alachlor
Erythromycin
Clarithromycin
Azithromycin
Chlorophyll fluorescence


Fluoranthene
Naphthalene
Polyaromatic hydrocarbons (PAH)
Chlorfenvinphos
Chlorpyrifos-ethyl
Dichlorvos
DDT total and para-para-DDT
Endosulfan
Carbon tetrachloride
Trichloroethylene
Hexachlorobenzene (HCB)
Cadmium and its compounds
Lead and its compounds
Mercury and its compounds
Nickel and its compounds
Silver
Selenium
Di(2- ethylhexyl)-phthalate (DEHP)
Nonylphenols
Cypermethrin
Benzene
Hexabromocyclo-dodecane (HBCDD)
Diclofenac
Photosystem II inhibition
ROS / Oxidative stress
Chlorophyll fluorescence
Anthracene
Atrazine
Diuron
Isoproturon
Simazine
Cybutryne
Terbutryn
Alachlor
Oxadiazon
Triallate
Erythromycin
Clarithromycin
Azithromycin

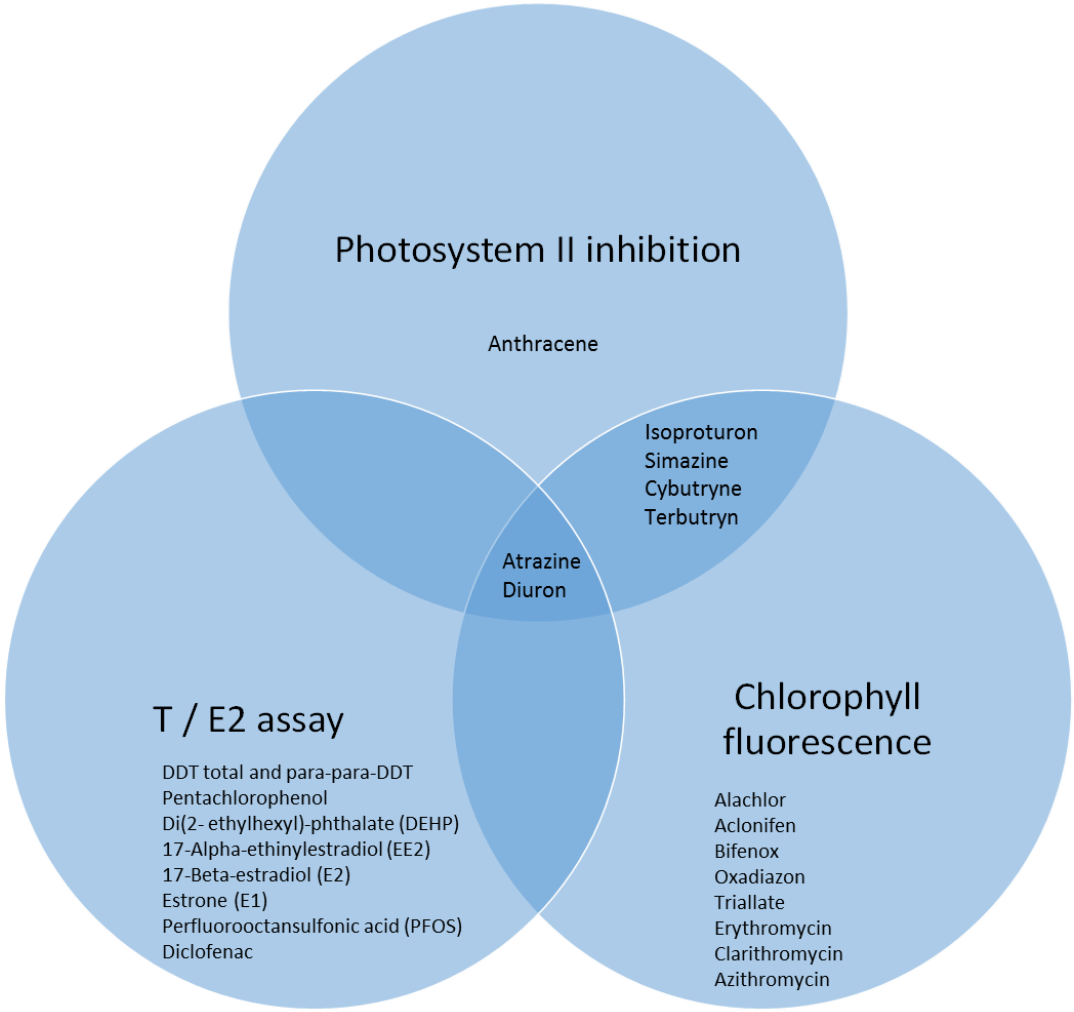


Figure 1. Diagrams representing common mode of action (MoA) of priority substances (PS).

The literature data review clearly identified a few groups of toxicological endpoints. It is clear that there is no "one size fits all" bioassay/EBM that could provide the toxicological potency of every PS and their mixture toward all aquatic organisms in all water bodies, but rather a battery of bioassays that should be selected as "fit for purpose". For example, while Photosystem II inhibition, algae growth inhibition and chlorophyll fluorescence measurements will detect the biological effects caused by the herbicides in plants, applying certain bioassays (e.g. testosterone/estrogen levels *in vivo*) would indicate their endocrine disrupting properties in the higher organisms.

It could be here also noted, that two *in vivo* tests, fish embryo toxicity (FET) and impaired frog embryo development (FETAX), showed high sensitivity while testing two mixtures of 14 or 19 substances of concern (pesticides, pharmaceuticals, heavy metals, polyaromatic hydrocarbons, a surfactant, and a plasticiser) (Carvalho et al. 2014). Both tests were designed to detect xenobiotics that selectively impair embryo development, growth and survival, at concentrations far less than those required to in adults. The use of fish embryos in wastewater effluent acute testing is already established (DIN, 2001; ISO, 2007).

3 Discussion

In line with the 7th EAP and the Commission Communication on mixtures, it is relevant to consider how to better take into account the risk coming from mixtures. Scientific evidence of the toxicological importance of chemical mixtures is certainly not new and many of the concepts of mixture toxicology pertinent to environmental species have their origins in medical toxicology research. This implies a need to better understand the modes of action (MoAs) of the current priority substances (PS) under the WFD, and of other substances of interest in the aquatic environment, and how they could be monitored using EBM (Wernersson et al. 2014). EBM might also help in answering the question of whether exposure to mixtures of PS (and other substances including emerging pollutants), at the level assumed to be safe for each compound (environmental quality standard, EQS or predicted non-effect concentration, PNEC), may produce adverse effects (Carvalho et al. 2014).

3.1 Main modes of action identified

The review of literature data clearly identified a number of more or less specific modes of action (MoAs)/common toxicological endpoints, such as photosynthesis inhibition, endocrine disruption, oxidative stress, activation of metabolising/detoxifying pathways, induction of stress proteins, inhibition of growth, perturbed lysosomal stability, genotoxicity, histopathological changes. Some very specific pathways of toxicity could also be determined, i.e. inhibition of AChE activity, inhibition of ALAD, induction of metallothioneins and imposex (for more details see Section 2.5).

Non-specific effects such as oxidative stress, activation of pathways involved in metabolism/detoxification of xenobiotics, histopathological changes etc., may only indicate the overall level contamination but still provide the basis for good methods complementing specific assays and current chemical analysis. On the other hand, plenty of studies suggest that genetic predispositions, including variations in metabolism and antioxidant capacities of different species, may also play an important role in response/degree of response to the studied compound.

Altogether, data reviewed in this report suggest that the mechanism of action of individual compounds may be different in different species (e.g. atrazine can inhibit photosynthesis in algae but can also induce estrogenic effects in teleost fish, amphibians and reptiles; Hayes et al. 2011). Moreover, the toxicity test response is strongly dependent on the sensitivity of the species used (e.g. the insecticides were more toxic than herbicides to the aquatic species tested; Palma et al 2008).

The biochemical/physiological response relative to a chemical compound often strongly depends on the test system and the biological endpoint. For aquatic species, PAHs (apart from acting through AhR), can also be toxic via tissue uptake which is dependent solely on hydrophobicity, and toxicity is mediated through non-specific partitioning into lipid bilayers. The metabolic pathways of the PAHs have not been fully studied and biotransformation/degradation is considered as a non-specific process with multiple pathways (van Herwijnen et al. 2003). Roberts (2017) reviewed models predicting PAH phototoxicity and concluded that they generally express toxic effect as a function of UV exposure (intensity and time) and PAH concentration, either as a waterborne concentration or body burden. In other words, an exposure scenario with low UV exposure and high PAH body burden would be expected to yield the same results as a high UV exposure with low

PAH body burden. For ecological risk assessment, this is an important concept to consider. Typically, an ecological risk assessment of a scenario involving PAHs would consider primarily the concentration of the PAHs. However, reciprocity dictates that the UV exposure is equally important and thus careful measures of it are necessary for an accurate assessment of the potential for phototoxicity to occur in a given area. It should be noted that the availability of toxicity data for individual PAH photomodification products is limited to few compounds and toxicological endpoints (Bicho et al. 2013, Willis & Oris 2014).

There is a growing body of literature showing that the sensitivity of organisms to toxicants is independent of their geographic origin. From a global perspective, Maltby et al. (2003) and Dyer et al. (1997) showed similar sensitivities among North American and European taxa with different geographic distributions. The study of Hose and Van den Brink (2004) confirmed the species-sensitivity distribution concept for endosulfan using laboratory, mesocosm, and field data. The difference in the sensitivities of different taxonomic groups might be expected for toxicants, such as endosulfan, that have a specific toxic MoA (Van den Brink et al. 2002, Maltby et al. 2003). Maltby et al. (2003) showed that for numerous pyrethroid and organophosphate insecticides, there was a significant difference in the sensitivity of vertebrate (predominantly fish) and arthropod groups. Even though for the organochlorine pesticide lindane, there was no significant difference in sensitivity of arthropods and fish, both groups were significantly more sensitive than non-arthropod invertebrates (Maltby et al. 2003).

Studies with invertebrates or fish generally provide a good indication of the effects at the population level, but within those groups of organisms, large differences in effect may exist, even between related species. Finally, the chemical form can play crucial role when considering the MoA/effects of certain pollutants. Mercury for example is one of the most hazardous contaminants that may occur in the aquatic environment, but its ecological and toxicological effects are strongly dependent on the chemical species present. Species distribution and transformation processes in natural aquatic systems are controlled by various physical, chemical, and biological factors. Depending on the prevailing environmental conditions, inorganic mercury species may be converted to many times more toxic methylated forms such as methylmercury, a potent neurotoxin that is readily accumulated by aquatic biota. Despite a considerable amount of literature on the subject, the behaviour of mercury and many of the transformation and distribution mechanisms operating in the natural aquatic environment are still poorly understood.

3.2 Grouping of the substances and weight-of-evidence

As shown in the previous sections of this report, contaminants representing the same mode(s) of action can be preliminarily grouped, for example as proposed in the Figure 1. It is clear that in some cases, priority substances (PS) can act via different mode of action (MoA), depending on the species being exposed. Although herbicides are designed to control plants, they can also affect the fitness of aquatic animals like mussels and fish, clearly targeting other biological sites of action pre-empting a cascade of biochemical and physiological impairments.

Aquatic toxicity is one of a batch of tests used in environmental risk assessment to determine the safe use and disposal of chemicals. Standard test methods for determining aquatic toxicity are time consuming and expensive, and largely for this reason, reliable toxicity data for many compounds are unavailable.

The final opinion of the three EU Scientific Committees (SCHER, SCENIHR and SCCS)[5] on the Toxicity and Assessment of Chemical Mixtures summarised that, for ecological effects, the exposure to mixtures of dissimilarly acting substances at low, but potentially relevant concentrations should be considered as a possible concern, even if all substances are below the individual EQS/PNECs. Consequently, there is a need to improve the current knowledge and methodologies, and to develop holistic approaches for the (ecological) risk assessment of chemicals under realistic conditions.

Moreover, the above mentioned opinion of the scientific committees noted that the REACH Regulation is generating the largest database on chemicals in history, and that this information could be used to reduce some of the current uncertainties. The information is primarily generated by industry and may require a peer-review assessment in some cases, but could nevertheless be highly valuable for moving forward with a scientifically based evaluation of the combined effects exerted by chemical mixtures.

Unfortunately, ecotoxicological data from studies used for regulatory purposes are not always available; we therefore focused in the report on studies we could find in the open source literature. We have been as inclusive as possible and have only excluded studies, such as those on mixtures, where it is not possible to assign MoA/causality. The process allowed identification of gaps, uncertainty and inconsistency in observations, and thus identified areas where future investigations can be best directed.

Overall, the review of available data showed that PS might affect common biomarker-type responses, such as expression of genes and/or associated proteins, concentrations of hormones, and biochemical processes (e.g. induction of detoxification responses), at concentrations that can be found in the environment. However, even in the light of the available studies, the exact mechanisms by which the PS induce different effects observed in different species are not always clear, and may involve interaction with multiple receptor systems (Mankidy et al. 2013). Often, the studies were designed to evaluate a population endpoint (e.g. egg production) in conjunction with histological (e.g. gonad development) and biochemical (e.g. hormone production) markers, so it was possible to assign causality. For many other studies it was not feasible to assess the relevance of the responses observed at the biochemical/physiological level to endpoints directly related to survival, growth, development, or reproduction.

What is more, the existing weight of evidence is not very well balanced; while there are many studies and relatively strong evidence available for some compounds - almost no data could be found for others. For the pyrethroids and neonicotinoids (insecticides with well-characterised MoA in their target organisms), information regarding the potential MoA that causes toxicity (reported mainly as mortality) observed in aquatic (including non-target) species is largely missing. Is it therefore difficult to choose the EBM that might detect (specifically) the presence of those substances in the monitored water. For many aquatic invertebrates (especially aquatic insects) with long larval aquatic stages, exposure to pyrethroids and neonicotinoids is expected to be prolonged due to either repeated pulse events and/or low-level chronic exposures.

Moreover, several studies report sometimes ambiguous and/or conflicting results. A very good example here is a relatively well studied compound atrazine, which induces

[5] SCHER (Scientific Committee on Health and Environmental Risks), SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) and SCCS (Scientific Committee on Consumer Safety). Toxicity and assessment of chemical mixtures. 2012.
https://ec.europa.eu/health/sites/health/files/scientific_committees/environmental_risks/docs/scher_o_155.pdf

aromatisation of testosterone to estradiol, thereby causing an estrogenic effect in exposed individuals; however, this mechanism has been debated (Shenoy 2012). Reviews performed for atrazine have described both effects and the absence of effects at multiple levels of biological organisation on aquatic organisms after exposure to environmentally relevant concentrations (Solomon et al. 2008, Rohr and McCoy 2010, Hayes et al. 2011, Van Der Kraak et al. 2014). Ambiguity among study results has led to controversy regarding the risk atrazine presents with respect to health of aquatic populations. A lack of clearly defined mechanisms for atrazine's effects contributes to on-going debates.

Another good example is nickel (Ni). Current ecological risk assessment and water quality regulations for Ni use mechanistically based, predictive methods such as biotic ligand models (BLMs). However, despite many detailed studies, the precise mechanism(s) of Ni toxicity to aquatic organisms remains elusive (Brix et al. 2017).

Generally, the evidence for adverse effects caused by the reviewed PS and other substances is apparent but a clear mechanistic understanding is often lacking, which highlights the fact that the molecular basis of observed effects/toxicity/mortality is not adequately studied.

3.3 Efforts to identify the most toxic compound in the class/group

Experimental designs for evaluating complex mixture toxicity in aquatic environments can be highly variable and, if not appropriate, can produce (and have already produced) data that are difficult or impossible to interpret accurately (Landrum et al. 2012). Specifically, it was recognised that toxicity is controlled by toxicokinetics that governs the bioaccumulation and distribution of the chemical and/or chemicals in tissues (based on their physical and chemical properties and facility for biotransformation) and by toxicodynamics, which governs the biochemical and physiological response of the organism. The closer the relationship between the concentration of the toxicant in whole tissues and the concentration at the site of toxic action, the better the interpretation of the dose-response relationship is (McCarty et al. 2011). Without adequate thought and attention to the basic requirements for establishing a dose-response relationship and determining the causative agent(s) for any observed toxicity, studies may not produce results that are environmentally relevant.

In general, mixture toxicity can be predicted only if enough information is available on the single toxicity of the mixture components. Identifying the toxic thresholds of the priority substances (PS) and other substances including emerging pollutants that might be components of mixtures will be critical to interpreting interactions in such mixtures. However, a few very general conclusions can be drawn. For example, in the case of the herbicides, the weight of available evidence suggests that the toxicity towards the target species, algae, is greatest for diuron (Carvalho et al. 2014 and Napierska et al. *data not shown*). The herbicides atrazine and simazine are inhibitors of photosynthesis at photosystem II, and there are widespread reports of endocrine disruption in fish and amphibians from a chemical like atrazine at low doses (Marlatt & Martyniuk 2017). Phenylurea herbicides are inhibitors of photosynthesis at photosystem II Site B (cf. Site A inhibition by atrazine), and diuron has already been shown to be anti-androgenic (it appeared particularly toxic for the development of oysters, at environmentally realistic concentrations; these effects were observed from 0.05 µg/L upwards; Akcha et al. 2012, Barranger et al. 2014, Behrens et al. 2016). Almost no information was found on the MoA/effects in the non-target species of other prioritised herbicides. Therefore, one

hypothesis to be tested is whether or not other herbicides have similar (or different) endocrine disrupting effects in vertebrates.

Larras et al. (2012), using bioassays and species sensitivity distributions to assess herbicide toxicity towards benthic diatoms, provided the following ranking of toxicity: diuron > terbutryn > isoproturon > atrazine > metolachlor. The hazardous concentration (HC) that affected 5% of the species revealed that, even at the usual environmental concentrations of herbicides, diatom assemblages could be affected, especially by isoproturon, terbutryn, and diuron. In a study of diuron and other substances in an artificial mixture, diuron was used as a reference compound and the data from bioassays' tests were expressed as diuron equivalent concentrations (DEQ) (Carvalho et al. 2014).

A concern related to herbicide and pesticide impacts on nontarget organisms arises when dealing with formulated products. The addition of adjuvants to pesticide formulations, due to their biological and chemically active nature, may enhance the toxicity of the active ingredient (a.i.) to non-target organisms (Marques et al. 2012). Apparently, the ecotoxicological data from the testing of specific formulations are not published.

For the PS group of polyaromatic hydrocarbons (PAHs), the biota EQS and corresponding AA-EQS in water are based on the toxicity of benzo(a)pyrene. To properly interpret the toxicity of the PAHs, it is critical to understand their biotransformation and to account for the toxicity of their metabolites. 5-ring PAHs such benzo(a)pyrene (BaP), benzo(e)pyrene (BeP), and benzo(k)fluoranthene (BkF) are all relatively potent CYP1A inducers in fish (Barron et al. 2004), and might therefore be expected to cause AhR-dependent cardiotoxicity similar to that previously described for benzo(a)anthracene (BaA; Incardona et al. 2006). Recent studies using the zebrafish experimental model have shown that PAHs are toxic to the embryonic cardiovascular system, and that the severity and nature of this developmental cardiotoxicity varies by individual PAH (Barron et al. 2004).

For PolyBDEs, so far there is not enough scientific background on these heterogeneous compounds as regards their effects assessment, even if historically, pentaBDE 99 came up as a representative of BDE toxicity. It is recognised that the consideration of only 6 congeners for monitoring may be underprotective if an additive MoA is assumed for all 209 BDE congeners. Arkoosh et al. (2017) concluded that studies have demonstrated a potential action of PBDEs as endocrine disrupting compounds capable of altering the concentration of thyroid hormones in fishes by a number of mechanisms. However, their effect on the thyroid system of fishes is still unclear, despite the fact that several studies have been conducted to understand the endocrine disrupting effect of BDE-47 in fishes. In this case there are not enough data to derive an EQS for the individual components of the commercial products, which makes it difficult to predict the toxicity of the mixture. An integrative approach such as the toxic equivalent (TEQ) approach is not a way out since the representativeness of BDE congeners within the group is not scientifically defined yet at this stage (EU dossier 2011).

Finally, despite numerous studies on heavy metals, it is not feasible to provide a ranking of their toxicity based on their MoA and observed effects in aquatic organisms, due to the huge number of different pathways involved. Wu et al. (2016) performed a literature review on the toxicity and corresponding mechanisms associated with lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As), individually and as mixtures, in the environment. Heavy metals are ubiquitous and generally persist in the environment, enabling them to biomagnify in the food chain. Heavy metal exposure to biological systems may lead to oxidative stress which may induce DNA damage, protein modification, lipid peroxidation, and other effects. Interestingly, a metal like Pb becomes toxic to organisms through the

depletion of antioxidants while Cd indirectly generates reactive oxygen species (ROS) by its ability to replace iron and copper. ROS generated through exposure to arsenic were associated with many modes of action, and heavy metal mixtures were found to have varied effects on organisms. The study by Spehar and Fiandt (1986) showed that acute adverse effects observed with mixture of metals were higher than additive for fish and nearly strictly additive for daphnids; while chronic tests showed that the joint action was less than additive for fishes but still nearly strictly additive for daphnids. These results indicate that the long-term metal interactions might be different among different organisms, in this case between fish and lower invertebrates. Moreover adverse effects of mixtures were observed when the metals were present at concentrations below the maximum acceptable toxicant concentration (MATC), suggesting that the components at or below no-effect concentrations may contribute significantly to the toxicity of a mixture on a chronic basis.

Madoni (2000), by examining data from literature concerning the acute toxicity of heavy metals in ciliates, concluded on the following order of toxicity: Cu>Hg>Cd>Ni>Pb>Cr>Zn. In aquatic invertebrates the order of toxicity was generally: Hg>Cd>Cu>Cr>Zn>Ni>Pb. Such variations discourage any attempt to conclude on an absolute scale of heavy metal toxicity on aquatic organisms, but point to higher sensitivity in ciliated protozoa than in invertebrate metazoans to nickel ions that could be a convenient bioindicator for evaluating the toxicity of waters polluted by heavy metals.

3.4 Added value resulting from the application of a battery of bioassays

Considering that it is not practical to perform the chemical analysis of all xenobiotic compounds entering the environment nor to test all possible mixture combinations occurring in the environment, more integrated approaches should be used to predict mixture hazard.

It is evident that contamination of European waters with chemicals is not limited to a small number of PS but that contamination patterns are rather diverse and complex. Current chemical analytical efforts could be complemented (or in some cases even replaced by) with effect-based methods (EBM), which offer to capture groups of compounds (as well as their transformation products) considering their biological effects. The EBM are designed to capture effects at different levels of complexity and specificity; that is, they measure either a specific response, a physiological response, or an unspecific response at the molecular, cellular, organ, organism, or population level (EU EBM Report 2014). A specific effect is understood as the consequence of an interaction of a chemical with a specific group of biomolecules. This could be measured as an enzyme activity, an agonistic or antagonistic response indicating receptor binding of a chemical, an alteration of protein or gene expression, a protein or DNA adduct formation, or an alteration of membrane integrity.

Using biological effects (biomarkers of effects) to detect contamination allows several chemical structures that produce the same effect to be aggregated, irrespective of whether or not their identities (as required for biomarkers of exposure) and concentrations are known. This means one would always accommodate for the totality of mixture components producing a certain effect irrespective of whether or not we know the exact composition. Moreover, the bioavailability of the contaminants and its relevance for elucidating subsequent adverse biological effects may also be informed applying EBMs.

EBMs have proven ability to detect responses of organisms at concentrations approaching, but below, exposure concentrations that elicit reproductive toxicity (e.g. nonylphenol gives an estrogenic response well below the lethal concentration).

The study of Brack et al. (2003) already demonstrated the strength of combining chemical and biological techniques to identify toxic metabolites even if their absolute amounts are small as compared to other components in the mixture. The results of this study also stressed the significance of the application of a battery of test systems and of including computational chemistry as an additional tool for the identification of chemical substances.

The combined testing approach reduces the probability of overlooking minor components that may still contribute significantly to the overall toxic potential associated with environmental samples. Moreover, it provides the possibility to test whether the established safety thresholds are truly protecting the aquatic organisms. A very good example here is an EU-coordinated robust study on an artificial mixture of PS which provided evidence that effects may occur at concentrations of individual components that EU legislation would consider not to pose significant risk (Carvalho et al. 2014). These results point to the need for additional studies to determine the type and degree of interaction of toxicants because single-chemical water quality criteria may not sufficiently protect some species when other toxicants are also present and suggest that bioassays could fill the gap between chemical and ecological assessments.

3.5 Detection of related pollutants

To propose effect-based methods (EBMs) for effect-based water quality monitoring it should be considered up front what type of effects may be expected from the present contamination of freshwaters. One approach would be to examine all chemicals that potentially occur in freshwaters due to anthropogenic activities, which would include all compounds undergoing environmental risk assessments for their aquatic exposure potential, i.e. industrial chemicals, pesticides, biocides, pharmaceuticals, detergents, personal care products and the like. Alternatively, perhaps only compounds that have actually been identified and quantified in freshwaters (e.g. for which data have been gathered during the prioritisation of candidates for the PS list) should be considered.

As mentioned previously, in order to predict the adverse effects of chemical mixtures, toxicity data for their individual components are required. Unfortunately, for different groups of priority substances (PS) and other substances proposed in this report, the type of information and toxicological studies available varies considerably in level of detail and precision. Ideally, such information would cover well-characterised target molecules (e.g. AChE), the identification of an affected pathway (e.g. inhibition of enzymatic activity) or functional disturbance (such as disrupted nerve signalling), not only in the organism recognised as a target but also in the non-target species representing different trophic levels. A molecular target (the most specific type of information in this context) could be established only for some compounds (e.g. AChE inhibition in the case of organophosphorus insecticides).

Even though the existing/available information on MoA and related effects for PS is not structured/strong enough to build simple effect categories readily translatable into assays relying on cause-effect and concentration-dependent response, using a battery of test systems would provide information on which category of effects seems to be problematic and which could be attributed to a broader class of compounds. For example, Wolff et al. (2015) showed perturbation in the biological status of the gonad of wild frog tadpoles

residing in a municipal waste-water effluent receiving environment. In the absence of detectable levels of E2 or OP, the observations suggested an estrogenic or anti-androgenic capability of this complex anthropogenic mixture.

3.6 Additive/synergistic mode of action of chemical compounds

Organisms in the environment are generally exposed to mixtures of pollutants. These exposures may sometimes be detrimental to the organism, even though chemical substances may be at concentrations lower than the no observed effect concentration (NOEC).

In the estimation of the toxicity of chemical mixtures, a critical parameter to be always considered is the mode of action (MoA) of the individual compounds (Balistrieri and Mebane 2014; Charles et al. 2014). Many models have been introduced to help predict toxicities of chemicals mixtures to organisms. Most of these models are based on the concepts of concentration addition (CA) and independent action (IA). The concept of CA assumes that components of mixtures exhibit similar modes of action in sub-lethal or lethal effects. Consequently since they act on the same target and biochemical pathway the components can be regarded as dilutions of one another (Chen et al. 2013b). Various studies show that CA applies in circumstances where components of the mixture have the same MoA (Altenburger et al. 2000). In CA, all chemicals of concern in the mixture act on one biological site and differ only in their potencies. The next important concept is the IA, which assumes that completely different and independent MoAs are presented by components in a mixture (Backhaus et al. 2000). In this, the toxic effect of each chemical in the mixture is not affected by other chemicals. In IA, the MoA of mixture constituents always differs and the nature and site of action may also differ.

To give an example, as concluded by Mayer & Reichenberg (2006) for hydrophobic organic substances, because baseline toxicity is concentration additive – the substances that do not exert toxicity as individual compounds can still contribute to the toxicity of a mixture. Hexachlorobenzene can, for example, be expected to contribute to the baseline toxicity of chlorobenzene mixtures. Another example would be anthracene and phenanthrene, which at less than aqueous solubility can be expected to make very similar contributions to baseline toxicity.

However, in cases where there are chemical interactions, deviation from both the CA and IA concepts can be expected: the presence of one chemical affects the toxicity of the other present in the mixture and the combined toxicity is not necessarily the sum of the individual toxicants. It also implies that competition among multiple substances for organic and inorganic ligands during accumulation and uptake by organisms is not considered (Balistrieri and Mebane 2014). This is explained as the antagonistic and synergistic effects. An effect is said to be antagonistic when the effect of the two chemicals is lower than the summed effect of each chemical alone. Synergistic effects are said to have occurred when the combined effect of two chemicals is greater than the sum of the effect of each chemical alone.

Even for a mixture of well-studied substances, the prediction of the chemical interactions between them is not straightforward.

Sjollema et al. (2014) investigated the toxic effects of four ubiquitous herbicides (atrazine, diuron, Irgarol(®)1051 and isoproturon) and herbicide mixtures on marine microalgae. Using a pulse amplitude modulation (PAM) fluorometry-based bioassay they demonstrated

a clear species and herbicide specific toxicity and showed that the current environmental legislation does not protect algae sufficiently against diuron and isoproturon.

Systematic analysis of mechanisms of PAH developmental toxicity in zebrafish showed that biological effects of PAHs cannot be predicted simply by quantitative measures of AhR activity or a compound's hydrophobicity (Incardona 2006). These results indicate that current models of PAH toxicity in fish are greatly oversimplified and that individual PAHs are pharmacologically active compounds with distinct and specific cellular targets.

Willis & Oris (2014) examined the hypothesis that phototoxic (anthracene and pyrene) and non-phototoxic (carbazole and phenanthrene) pathways of mixtures could be predicted from single exposures. Anthracene and pyrene were phototoxic as predicted; however, carbazole exhibited moderate photoinduced toxicity and phenanthrene exhibited weak photoinduced toxicity. The toxicity of each chemical alone was used to compare the toxicity of mixtures in binary, tertiary, and quaternary combinations of these PAHs, and a predictive model for environmental mixtures was generated. The results indicated that the acute toxicity of PAH mixtures was additive in phototoxic scenarios, regardless of the magnitude of photo-enhancement.

In laboratory bioassays with sediments spiked with phenanthrene (Phe) and benzo(b)fluoranthene (B(b)F), non-carcinogenic and carcinogenic PAHs, respectively, the effects of exposure (related to DNA damage and oxidative stress) were analysed in the gills of a burrowing clam, *Ruditapes decussatus* (Martins et al. 2013). Overall, the findings indicated that low concentrations of sediment-bound PAHs, carcinogenic or not, may be rendered significantly bioavailable to benthic filter-feeders and induce genotoxicity, revealing that even PAHs considered non-carcinogenic to humans present a pro-mutagenic hazard to bivalve molluscs.

Recently, it has been observed that environmentally relevant mixtures of metals do not follow strictly the similar or dissimilar MoA. Proposals for a novel model, which integrates CA with IA in predicting toxicities of non-interactive mixtures have been made (Beyer et al. 2013).

As already mentioned, when pollutants enter aquatic habitats, indirect effects on human health (via food and drinking water consumption) are possible. Harmonisation of the principles for grouping substances relevant to human and ecological risk assessment is currently discussed, although protection goals are different and the definition and handling of similar/dissimilar MoA is still debated between the two fields (EFSA 2014b, Panizzi et al. 2017). A major challenge in both human health and ecological risk assessment is extrapolation of chemical effects between species.

The three EU Scientific Committees (SCHER, SCENIHR and SCCS)[6] concluded that for chemicals with different MoA (i.e. acting independently), no robust evidence is available that exposure to a mixture of such substances is of human health concern if each individual chemical is present at or below their no-effect level. They recommend an approach for the assessment of chemical mixtures that emphasises the need for information on the MoA, but the decision algorithm comes to a stop if the threshold of toxicological concern for single substances is not exceeded. So far, some bioassays have been applied to investigate

[6] SCHER (Scientific Committee on Health and Environmental Risks), SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) and SCCS (Scientific Committee on Consumer Safety). Toxicity and assessment of chemical mixtures. 2012.
https://ec.europa.eu/health/sites/health/files/scientific_committees/environmental_risks/docs/scher_o_155.pdf

for example hormonal activity of chemicals in drinking water (Brand et al. 2013) and complex chemical mixtures in recycled water (Jia et al. 2015).

However, in relation to ecological effects, the EC Scientific Committees admit that the situation is less clear in relation to ecological effects (EC COM(2012) 252).

Future testing for mixture effects will take advantage of the ongoing revolution in biology and biotechnology. Studies on the potential for using omics in the assessment of chemical mixtures were initiated by use of marine animals, e.g. by Dondero et al. (2010) and Dorne (2010). Within human biology, new methods that use biological data in order to find biochemical pathways relevant to the different responses of an organism to different conditions are being developed. Biochemical pathways, instead of being treated as just sets of genes, are viewed as a network of interactions between proteins or metabolites. Such novel methods and approaches are anticipated to play a major role in future risk analyses of multiple stressors by multiple routes using a receptor-oriented approach (Løkke et al. 2013). Many efforts have also focused on adverse outcome pathways (AOPs), to create a framework for extrapolating chemical effects across species and improve regulatory decision-making through greater integration and more meaningful use of mechanistic data.

3.7 Use of effect-based methods for detection of long-term (chronic) exposure to low levels of the pollutants

As to the duration of the exposure, short exposure periods (24h–96h) were generally tested for the majority of priority substances (PS). Data demonstrate the paucity of knowledge about the effects that longer exposure periods to PS may trigger in freshwater and marine organisms.

Predicting chronic toxicity levels from the results of acute toxicity tests in fish species seems to be unreliable. Because of the scarcity of studies, a good estimate of the reliability of the extrapolation factors currently used in risk assessment is not possible. Performance of complete life-cycle tests, despite their time- and cost-consuming aspects, is the only way to estimate the real chronic toxicity of chemicals to fish (Roex et al. 2000).

Even if there are substantial numbers of effect-based methods (EBMs) available, the need to provide and improve systematic links between contaminant exposure with biological adverse effects calls for mechanistic principles (Hendriks 2013) as it is neither technical nor logistically feasible to investigate every exposure situation for all potentially relevant endpoints. The literature offers conceptual frameworks to address the relation between observation of specific biological effects and adverse outcomes (Ankley et al. 2010) and for addressing combined effects from mixtures of pollutants (Altenburger et al. 2015). Operationalisation of EBMs for water monitoring purposes can be already found.

Biological responses and contaminant levels in biological tissues were investigated in fish specimens collected from five stations in a moderately polluted ecosystem on the north coast of Tunisia (Barhoumi et al. 2014.). Elevated EROD, GST and CAT activities, as well as TBARS levels in liver were positively correlated with tissue contaminant levels at station S1. Significant negative correlations were also found between the hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT) body burdens with AChE activity in muscle at station S2. The integration of biological responses and contaminant tissue content indicated that certain areas of the Bizerte lagoon, notably station S1, are

significantly impacted by various human activities, which likely represent a threat to aquatic wildlife.

Blahová et al. (2010) assessed the contamination of two rivers in the Czech Republic using selected biochemical markers. Significant positive correlations were found between EROD activity and HCH concentration, and also between GST and EROD activity with HCB concentration in muscle, after adjusting for age.

Faria et al. (2010) reported contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Dreissena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). Effects of these contaminants on aquatic river invertebrates were assessed by integrating analyses of metals and organochlorine residues in both species with a wide range of biomarkers. The results obtained evidenced similar response patterns in mussels and crayfish with increasing toxic stress levels from upper parts of the river towards the meander located immediately downstream from the most polluted site, close to the waste dumps. The aforementioned stress levels could be related with concentrations of mercury, cadmium, hexachlorobenzene, polychlorobiphenyls and dichlorodiphenyltrichloroethanes from 4- to 195-fold greater than local background levels. The response of biomarkers to these pollutant concentrations differences was reflected in high activities and levels of antioxidant enzymes, metallothioneins, lipid peroxidation and DNA strand breaks and decreased levels of glutathione.

Baillon et al. (2015) performed transcriptome profile analysis which revealed specific signatures of pollutants in Atlantic eels. Among the variables analysed, arsenic (As), cadmium (Cd), lindane (γ-HCH) and the hepato-somatic index (HSI) were found to be the main factors affecting the eel's transcriptome. The study proposed specific gene signatures of pollutants and their impacts in fish exposed to multi-stress conditions.

Capolupo et al. (2017) reported a comprehensive evaluation of the environmental quality of a coastal lagoon in Italy. Overall, the use of physiological and chemical analyses detected chronic alterations in mussel health status induced by specific toxicological pathways, proving a suitable approach in the framework of biomonitoring programs of coastal lagoons.

The technical report on EBMs (Wernersson et al. 2014) includes a dedicated section on the use of EBMs in the different Member States and in the context of the Regional Seas Conventions. However, as with any investigative monitoring, the optimum set of methods to use varies on a case-to-case basis. The report also includes descriptions of tools and methodologies that are considered promising in the near future because of the fast development in this area. There is a specific section related to recent research development in OMICs technologies that could have a potentially wide future application in the monitoring and assessment of aquatic environments.

In summary, we find that aquatic chronic exposure and effect assessment might benefit from complementary effect-based characterisations. It should be noted that many EBMs are being applied/implemented already on a regular basis (e.g. in the OSPAR monitoring programme) or potentially available for such efforts. Probably, a systematic approach should be developed to define which panel of assays could be of greatest use for the specific circumstances (e.g. for the combination of pollutants that might be found).

It should be considered that despite the large numbers of assays/methods established and used for chemical bioactivity screening major questions remain regarding their utility. They include questions as to how the translation of molecular interaction between chemicals and

biomolecules into adverse effects can be made 'operational' to be used for extrapolation between the different organisms and application in ecological risk assessment. For example, although effects on behaviour due to inhibition of AChE can be observed in vertebrates, these have usually not been experimentally related to effects on survival, development, growth, and reproduction of individuals or ecosystem stability or function in a quantitative manner. A good example of a 'quantitative' study is one by Sismeiro-Vivas et al. (2007) who evaluated the effects of sublethal concentrations of chlorfenvinphos on several behavioural parameters of the mosquitofish, *Gambusia holbrooki*. Behavioural impairment was registered in fish with >40% AChE inhibition levels, while mortality was only observable in fish exhibiting AChE inhibition levels >80%. Additionally, significant correlations were found between behavioural impairment and AChE inhibition, suggesting a mechanistic link.

Driven by major scientific advances in (bio)analytical methods, computation, and a need for more relevant approaches to chemical screening, the EBMs have potential to undergo transition from a tool of observation to a tool of prediction.

4 Recommendations

The direct effects of toxicants typically reduce organism abundance (by increased mortality or reduced fecundity). While progress has been made in the study of those direct effects for species representing different trophic levels, little is known about the relationship between effective tissue dose and ultimate toxic effect at the level of the mechanism/mode of action (MoA).

The new high-throughput technologies (e.g. gene and protein expression, enzyme, and cell-based bioassays) available nowadays in the laboratories could significantly facilitate the study of the mechanisms of toxic action and develop new quantitative methodologies in support of risk assessment. The technical report on the effect-based methods (EBMs) (Wernersson et al. 2014) includes descriptions of methods and approaches that are considered promising in the near future because of the fast development in this area. There is a specific section related to recent research development in OMICs technologies that could have a potentially wide future application in the monitoring and assessment of aquatic environments.

To improve our ability to link specific compounds with specific EBMs we need a better understanding of how MoA knowledge for compounds and experimental effect detection can be linked. Recently, the concept of an adverse outcome pathway (AOP) has received a lot of attention. The AOP concept can be used to guide research aimed at improving both our understanding of chronic toxicity, including delayed toxicity as well as epigenetic and transgenerational effects of chemicals, and our ability to predict adverse outcomes (Groh et al. 2015). The AOP concept provides conceptual guidance but has been of little help in practice. Studies such as that performed by Brix et al. (2017), where the authors used AOP analysis to identify multiple potential mechanisms of Ni toxicity and their interactions with freshwater aquatic organisms, could contribute to a robust framework for future development of AOPs for other compounds.

Standardisation and intercalibration aspects are of particular importance if EBM results are to be used in a regulatory context. However, for investigative purposes, such as screening and operational monitoring, non-standardised methods could still be very valuable. Intercalibration is required to insure the comparability of the data.

Furthermore, more investigations of population, community, and ecosystem-level effects and pathways of poorly studied compounds are needed. Risk assessments of the chemicals should include investigations of *in situ* or field community condition under the umbrella of the EBMs application.

5 Conclusions

In this report the ecotoxicological data collection for priority substances (PS) and other substances of concern allowed the identification of common toxicological endpoints, modes of action (MoAs) and effects with different degrees of specificity.

Substances sharing the same MoA can be preliminarily grouped taking into account that they may act via different MoAs in different species.

However, for some classes of chemicals, such as the neonicotinoids and pyrethroids, the MoA remains unknown, and more efforts are needed to investigate the mechanisms behind their toxicity.

At the current stage, although there are still some major issues such as the ecological risk assessment and its link to the MoA and adverse effects on different organisms, selected biosassays and biomarkers could complement the current chemical monitoring based on their MoAs.

There is no "one-size-fits-all" bioassay that can assess the toxicological potency of every PS or mixture in relation to all aquatic organisms, but rather a battery of bioassays that could be selected as “fit for purpose”. Furthermore a systematic approach should be developed to define which panel of assays could be of greatest use for the specific circumstances (e.g. for the combination of pollutants that might be found). In addition, an intercalibration exercise will be required to ensure comparability among bioassays based on the same MoA.

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List of abbreviations and definitions

AA	Annual average
ACh	Acetylcholine
AChE	Acetylcholinesterase
ACTH	Adrenocorticotropin
AhR	Aryl hydrocarbon receptor
ALA-D	Aminolevulinic acid dehydratase
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
AR	Androgen
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
AOP	Adverse outcome pathway
BaP	Benzo[a]pyrene
BeP	Benzo[e]pyrene
BbF	Benzo[b]fluoranthene
BDE	Brominated diphenyl ether
BkF	Benzo[k]fluoranthene
BLMs	Biotic ligand models
CA	Concentration addition
CAR	Consititutive androstane receptor
CAT	Catalase
Cb1	Cannabinoid receptor 1
ChE	Cholinesterase
CIS	Common Implementation Strategy
CNA	Copy number alteration
CNS	Central nervous system
COX	Cytochrome c oxidase
CPYO	Chlorpyrifos oxon
CYP(s)	Cytochrome(s) P450
dbcAMP	2’-o-dibutyryladenosine 3’:5’-cyclic monophosphate
DCA	Dichloroacetate
DCE	1,2-Dichlorethane
DCM	Dichloromethane

DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEHP	Di(2-ethylhexyl)-phthalate
DEQ	Diuron equivalent concentration
DNA	Deoxyribonucleic acid
EBM	Effect-based method
E1	Estrone
E2	17β-estradiol
EcR	Endocrine-related
EE2	17α-ethinylestradiol
Egfr	Epidermal growth factor receptor
EHMC	2-Ethylhexyl 4-methoxycinnamate
EQS	Environmental quality standard
ER	Estrogen receptor
EROD	Ethoxyresorufin O-deethylase
FET	Fish embryo toxicity test
FETAX	Frog Embryo Teratogenesis Assay Xenopus
Fshβ	Follicle-stimulating hormone beta
GABA	Gamma aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GGPP	Geranylgeranyl pyrophosphate
GGT	Gamma glutamyl transpeptidase
Gnrhr2	Gonadotropin-releasing hormone receptor 2
GOT	Glutamic-oxalacetic transaminase
Gper	G protein-coupled estrogen receptor 1
GPT	Glutamic-pyruvic transaminase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSI	Gonadal somatic index
GSSG	Glutathione disulfide
GST	Glutathione S-transferase
Hb	Haemoglobin
HBCDD	Hexabromocyclo-dodecane
HC	Hazardous concentration

HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HCHO	Formaldehyde
HMOX1	Heme oxygenase (decycling) 1
HO	Haemolymph osmolality
HPT axis	Hypothalamic–pituitary–thyroid axis
HSI (LSI)	Hepato-somatic index (Liver somatic index)
HSP	Heat shock protein
Ht	Haematocrit
IA	Independent action
iNOS	Inducible nitric oxide synthase
KT	Ketotestosterone
LDH	Lactate dehydrogenase
LH	Liver histopathology
LMS	Lysosomal stability
LPO	Lipid peroxidation
LPR	Larval photomotor response
MA	macrophage aggregate
MAC	Maximum allowable concentrations
MAPK	Mitogen-activated protein kinase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
MEHP	Monoethylhexyl phthalate
MoA	Mode of action
mRNA	Messenger ribonucleic acid
MN	Micronucleus
MSFD	Marine Strategy Framework Directive
MT	Metallothionein
NADH	Nicotinamide adenine dinucleotide (reduced form)
NOEC	No observable effect concentration
NKA	Na+/K+-ATPase
NOS	Nitric oxide synthase
8-OHdG	8-hydroxy-2-deoxyguanosine (8-oxodGuo)

OP	Octylphenol
PAH	Polyaromatic hydrocarbon
PBDE	Polybrominated diphenylethers
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCO	Protein carbonyl
PCP	Pentachlorophenol
PFOS	Perfluorooctane sulfonic acid
Phe	Phenanthrene
PHS	Priority hazardous substance
PLHC	Fish hepatoma cell line
PNEC	Predicted No-Effect Concentration
PPARα	Peroxisome proliferator- activated receptor alpha
PR	Progesterone
PROD	Pentoxyresorufin-O-deethylase
PS	Priority substance
PSI	Photosystem I
PSII	Photosystem II
PXR	Pregnane X receptor
RBC	Red blood cell count
ROS	Reactive oxygen species
RPSI	Relative Penis Size Index
SCHER	Scientific Committee on Health and Environmental Risks
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCCS	Scientific Committee on Consumer Safety
SfG	Scope for growth
sGST	Soluble glutathione-S-transferase
SOD	Superoxide dismutase
SREBP	Sterol regulatory binding protein
StAR	Steroidogenic acute regulatory protein
T	Testosterone
T3	Triiodothyronine
T4	Thyroxine
TBARs	Thiobarbituric acid reactive substances
TBT	Tributyltin

TCA	Trichloroacetate
TCE	Trichloroethylene
TEQ	Toxic Equivalent
TH	Thyroid hormone
TSHβ	Thyroid stimulating hormone beta
TTR	Transthyretin
UVR	ultraviolet radiation
VDSI	Vas deferens sequence index
VLCFAs	Very long-chain fatty acids
Vtg	Vitellogenin
WBC	White blood cell count
WEA	Whole Effluent Assessments
WFD	Water Framework Directive
WL	Watch List
Zrp	Zona radiata protein

List of figures

List of tables

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