Overview Report II:
An overview of current scientific knowledge on the life cycles, environmental exposures, and environmental effects of select endocrine disrupting chemicals (EDCs) and potential EDCs

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The International Panel on Chemical Pollution (IPCP)

DISCLAIMER

This is the second report within a series of three reports on EDCs that UN Environment has commissioned the International Panel on Chemical Pollution (IPCP) to prepare, in response to its commitment to the third and fourth sessions of the International Conference on Chemicals Management (ICCM 3 and 4) Resolutions that had called for international cooperative actions to provide up-to-date information and scientific expert advice to relevant stakeholders, raise awareness and facilitate science-based information exchange.

The series of reports include the following: (1) compilation of worldwide initiatives by various stakeholders to identify EDCs or potential EDCs based on the WHO/IPCS 2002 definitions; (2) a compilation of the current understanding of: the life cycle, environmental fate and distribution, environmental exposure in different regions, and evidence of adverse endocrine-related effects of EDCs and selected potential EDCs; and (3) a compilation of existing regulatory frameworks and policy initiatives on EDCs.

Given the complexity, breadth, and rapid ongoing development of this scientific field and in the regulatory frameworks, it is neither feasible nor possible for these three reports to include in-depth detail and discussion related to all the potentially relevant aspects or to predict future developments within the field. It instead provides a snapshot of the overall situation when the reports were prepared as well as references to further detailed and relevant information.

Executive Summary

Endocrine disrupting chemicals (EDCs) are chemicals that alter function(s) of the endocrine system and consequently cause adverse health effects. International research efforts to better understand the presence of EDCs and associated effects on the environment have been intensified over the past three decades and led to an increasing level of concern about and action on EDCs. In particular, at the 4th session of the International Conference on Chemicals Management (ICCM 4), a Resolution was adopted by the stakeholders inviting UN Environment to generate and disseminate information on EDCs. This report is the second within a set of three Overview Reports commissioned by UN Environment to the International Panel on Chemical Pollution (IPCP) on EDCs in response to its commitment to the ICCM 4 Resolutions.

The report serves as a repository of information with the aim to provide a concise and consistent overview of the environmental exposure and effects of a diverse set of EDCs or potential EDCs identified in Report I and to highlight critical knowledge and data gaps. A fact sheet style is used to efficiently present the relevant information for each chemical, including the chemicals' life cycles including production and use, emission sources, degradation pathways and kinetics, measured environmental concentrations in different regions, and evidence of (potential) adverse endocrine-related effects. The wide range of information included in the fact sheets is intended to serve as an information basis for a wide range of different stakeholders and applications.

Several general observations are made for these chemicals, including:

- Experimental data is lacking that measures the physical and chemical properties as well as degradation processes and rates in environmental compartments.
- Significant gaps in publicly available data exist describing their current and historical production, uses, and releases from use and disposal, particularly regarding quantitative information. Some of this information may have been generated, but have not been made publicly accessible.
- A substantial amount of knowledge has been generated regarding emission sources for some chemicals, however substantial gaps still exist for many others. The chemicals included are very diverse, and only a few commonalities can be identified. One of them is that wastewater treatment plant effluent is a primary point source for emissions of many of the chemicals into the environment. Information regarding emissions during manufacturing is very limited and available for only a few of these chemicals.
- Measured environmental concentrations exist globally with a majority of the sampling occurring after the year 2000. Field measurements are limited or non-existent in many developing and transition regions, especially in the Southern Hemisphere.

Readers are encouraged to find further, relevant information in Report I on worldwide initiatives to identify EDCs and potential EDCs, and in Report III on existing regulatory frameworks and policy initiatives on EDCs.

Contents

Abbreviations

AOP – Adverse Outcome Pathway
BCF – Bioconcentration Factor
CAS – Chemicals Abstracts Service
CoRAP – Community Rolling Action Plan
dw – Dry Weight
DWTP – Drinking Water Treatment Plant
EDC – Endocrine Disrupting Chemical
EPA – Environmental Protection Agency
EPI – Estimation Programs Interface
EU – European Union
ICCM – International Conference on Chemicals Management
IOMC – Inter-Organization Programme for the Sound Management of Chemicals
IPCP – International Panel on Chemical Pollution
IPCS – International Programme on Chemical Safety
LOD – Limit of Detection
LOQ – Limit of Quantification
LRL – Laboratory Reporting Level
MQL – Method Quantification Limit
ND – Not Detected
NR – Not Reported
OECD – Organisation for Economic Co-operation and Development
OH – Hydroxyl
ppbv – Parts per Billion by Volume
pptv – Parts per Trillion by Volume
QSAR – Quantitative Structure-Activity Relationship
REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals
RL – Reporting Level
SAICM – Strategic Approach to International Chemicals Management
SVHC – Substance of Very High Concern
UN – United Nations
US – United States
UN Environment – United Nations Environment Programme
VTG – Vitellogenin
WHO – World Health Organization
ww – Wet Weight
WWTP – Wastewater Treatment Plant

1. Background, Aims, and Scope

Endocrine disrupting chemicals (EDCs)[A] are chemicals that alter function(s) of the endocrine system and consequently cause adverse health effects. Potential EDCs[B] are chemicals that possess properties that might be expected to lead to endocrine disruption. The endocrine system consists of many interacting tissues that communicate with one another and the rest of the body by means of hormones. This system is responsible for controlling a large number of processes in the body from gamete formation, to conception and early developmental processes such as organ formation, and to most tissue and organ functions throughout adulthood. EDCs interfere in some way with hormone action and in doing so can alter endocrine function and lead to adverse effects on the health of humans and wildlife. Some of the observed health effects associated with EDCs include, but are not limited to, cancer as well as reproductive, developmental, immunological, and neurological disorders. For more background information on endocrine disruption including the makeup of the endocrine system and how EDCs act, see the report "State of the Science of Endocrine Disrupting Chemicals – 2012" [1].

Over the past three decades, international research efforts to better understand EDCs have been intensified [1]. This has resulted in growing global concern regarding EDCs. In 2012, the third session of the International Conference on Chemicals Management (ICCM 3) recognised EDCs as one of the Emerging Policy Issues[C] under the UN Strategic Approach to International Chemicals Management (SAICM) [2]. The fourth session (ICCM 4) in 2015 [3] affirmed to support further research and develop cooperative actions regarding EDCs. The ICCM 4 Resolution further requested all interested stakeholders to support cooperative actions led by the Inter-Organization Programme for the Sound Management of Chemicals (IOMC), including to address the needs identified by developing countries and countries with economies in transition[D] by generating and disseminating information on EDCs.

As part of its commitment to the IOMC's work plan, the United Nations Environment Programme (UN Environment) initiated the project "Provision of Information on EDCs" in August 2015 to increase and improve intergovernmental and intersectoral understanding, coordination and cooperation as well as awareness of EDCs. Among other activities under the project framework, UN Environment commissioned the International Panel on Chemical Pollution (IPCP) to develop a set of three overview reports that focus on existing scientific knowledge of environmental exposure and effects as well as regulatory frameworks and policy initiatives regarding EDCs.

[A] According to the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) 2002 definition, an endocrine disruptor is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations*" [63].

[B] According to the WHO/IPCS 2002 definition, a potential endocrine disruptor is "*an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations*" [63].

[C] All SAICM Emerging Policy Issues can be found at http://www.saicm.org [64].

[D] Regional resolutions on endocrine-disrupting chemicals from Africa (SAICM/RM/Afr.5/7), Asia-Pacific (SAICM/RM/AP.4/7), and Latin America and the Caribbean (SAICM/RM/LAC.4/11). See the SAICM website at www.old.saicm.org.

This report is the second within the set of three overview reports. It aims to provide a state-of-the-science summary of the environmental exposure and effects of select EDCs and potential EDCs from those identified in Report I. It should be noted that this set of chemicals is not exhaustive of all EDCs and potential EDCs, but reflects those that have been previously assessed and identified by stakeholders using the WHO/IPCS 2002 definitions[A,B]. For a detailed description of existing initiatives in identifying EDCs and potential EDCs and of the chemicals identified, see Report I. The present report synthesizes and presents existing scientific information on the following topics regarding EDCs:

- life cycles including production and use, possible emission sources, and environmental fate and distribution;
- environmental exposure in different regions;
- evidence of (potential) adverse endocrine-related effects.

This report aims to provide a concise and consistent overview for a diverse set of chemicals while highlighting critical knowledge and data gaps. In particular, a fact-sheet style is used to efficiently present the relevant information for each chemical, and references are included to the original data and to sources where further details can be obtained by readers. The report serves as a repository of information for the chemicals considered and within the scope of this project. It offers a wide-ranging summary of relevant information, however it is not exhaustive and does not include reference to all published information that may exist. All information was retrieved from publicly accessible data sources, and not every aspect within the scope of this report can be described in the same level of detail for each included chemical. For an efficient presentation of the available information within this report, chemicals that have similar chemical structures or uses are often grouped together and presented in the same factsheet. It should be noted that such grouping does not reflect any recommendations on grouping or read-across under any regulatory context. For example, in some cases, information was found, and is presented, that describes rather entire groups of chemicals that have similar chemical structures or uses. It is not necessarily the case that single, overarching descriptions can be applied to all chemicals within a group of similar chemicals, and deviations are highlighted in the respective fact sheet whenever possible. The wide range of information included in the fact sheets is intended to serve as an information basis for a wide range of different stakeholders and applications. No standardized or specific uses for the information presented in the fact sheets is recommended by the authors.

2. Methodology

Data in this report were gathered and synthesized through a desk review of publicly available documents including peer-reviewed literature, industry-reported data, and technical reports by other stakeholders. Where no experimentally derived data were identified, available estimated values from computer modeling are presented. No new experimental data were generated in the process of collecting and synthesizing information. Some chemicals have been grouped together based on the similarities in their chemical structure or known uses. The information collected for each chemical or each group of chemicals is structured into a fact sheet with the following labeled sections that together cover the scope of the report:

Key References – One to three references that offer a broad overview of the target chemical(s) are provided. They aim to serve as starting points of reference for additional research. These references include highly-

cited, peer-reviewed critical review articles, encyclopedias, and comprehensive assessment documents that have been provided by regulators for regulatory purposes.

Chemical Identification – The chemical names are presented along with the Chemical Abstracts Number(s) (CAS number). Some chemicals may have multiple CAS numbers, and some represent groups of chemicals that may have additional CAS numbers not specified here; this is noted where applicable. An abbreviation is defined for each chemical and used throughout the fact sheet. A two-dimensional drawing of the chemical structure is also provided and was created using the ChemDraw software package [4]. It does not reflect all structural isomers that can exist for a chemical, but rather the most common/prevalent ones. For chemicals that exist in branched forms, an isomer that is minimally branched and one that is highly branched were chosen and are described separately throughout the fact sheet for the chemical. This section of the fact sheets also notes which stakeholder initiatives have identified the chemical as an EDC or potential EDC as the basis for inclusion in this report.

Physical and Physicochemical Properties – A set of physical and physicochemical properties are included that are important for describing the expected environmental fate. These include: molecular formula, molecular weight, physical state, melting point, density, vapor pressure, water solubility, partition coefficients (for octanol/water, organic carbon/water, and air/water), and the dissociation constant. Where available, experimentally determined values from previous studies are presented and referenced. If multiple sources were found to report different values for the same property, all values are provided. Where no experimental value was found, the EPI (Estimation Programs Interface) Suite software package (v4.11) from the US EPA [5] was used to estimate the value where possible, and values determined from multiple estimation methods within EPI Suite are provided. Documentation on the calculation methods used within EPI Suite can be found within the software's help menu, and a review of the program is also available [6].

Degradation Pathways and Kinetics – Information on four potential degradation mechanisms (hydrolysis, photolysis, hydroxyl (OH) radical reactions, and biodegradation) are presented along with where they are expected to occur (within the technosphere and/or natural environment) as well as reported empirical or estimated values for these processes.

Intentional Uses and Production Levels – Information on the reported production levels and uses is presented. Depending on the information available, production levels may be reported across different time periods and for specific regions or applications.

Emission Sources into the Environment – Reported sources of emissions into the environment are presented by life cycle phase (production, use disposal) and include other emission sources when relevant. Where available, reported quantitative release rates or historical release amounts are presented. Where no concrete data were identified, potential emission sources are qualitatively inferred based on the chemical's known (or expected) uses.

Distribution in the Abiotic Environment and Biota – When possible, depending on the availability of data describing emissions pathways, the expected distribution of the chemical in the four environmental compartments of air, water, soil, and sediment is presented. This has been calculated using the level III distribution model in the EPI Suite software package (v4.11) [5]. Documentation on the calculation methods used within the EPI Suite model can be found within the software's help menu (LEV3EPI model). Experimentally derived or estimated bioconcentration factors (BCFs) are also presented and describe a chemical's potential to bioaccumulate in organisms.

Measured Environmental Concentrations – Reported environmental concentrations across different regions, environmental compartments, and sampling dates are presented. The presented set of reported measurements is not exhaustive and aims instead to provide a snapshot of existing measurements. Preference was given to presenting the most recent measurements available for a mix of different regions and environmental compartments. Information regarding the sampling location, year, number of samples taken, concentration range, median concentration, and numbers of samples greater than the limit of detection (LOD) is also presented, depending on the data availability. Additional references are highlighted for any studies that offer an overview of many measured environmental concentrations.

Scientific Evidence of Adverse Endocrine-Related Environmental Effects – (Potential) endocrine-related adverse effects on wildlife after exposure to the chemical(s) reported in the peer-reviewed literature are presented. A qualitative, summarized description of the reported observation(s) from each study is provided, and the observations are separated on the in vitro level, in vivo level, and population level. The presented set of reported observations is not exhaustive and aims instead to provide a snapshot of existing observations across a diverse set of test organisms and endpoints. As the focus is on environmental effects, preference within the current report was given to presenting observations from in vitro studies that do not use human cell lines or tissues and from in vivo studies that focus on fish and water-dwelling organisms. Studies related to mammals were also included, especially where limited information was found to be available for other test systems. In general, preference was given to presenting observations from studies that are highly cited, are the first study describing a certain observation, are representative for the observation, and/or have investigated different types of organisms. Useful references that offer an overview of observed effects are often provided at the beginning of this section in most fact sheets. Additional supporting information regarding the development and use of adverse outcome pathways in ecotoxicology as well as a description of the different testing levels (in vitro, in vivo, and population level) are provided in the Annex. It is worth noting that in the interpretation of these reported observations caution needs to be taken, because it was not investigated if these reported effects have been caused by primary or secondary endocrine mode(s)-of-action.

References – Fact sheet-specific references are numerically cited and linked to a list of references at the end of the respective fact sheet.

3. General Observations

Several general observations arise from the collection and synthesis of the data for the selected chemicals regarding their environmental life cycle, exposure, and effects. EDCs and potential EDCs are a very diverse mix of chemicals used across a wide range of industries and products with varying life cycles and environmental exposures. Overall, a substantive body of knowledge has been developed, however substantial gaps still exist. Some general observations for each of the main fact sheet sections are provided below.

- **Physical and Physicochemical Properties**: While many chemical databases and published studies exist, some experimental data measuring the physical and chemical properties for many of these chemicals were found to be lacking, especially regarding partition coefficients. Much of the reported information is instead based on computer estimates for these values.
- **Degradation Pathways and Kinetics**: Measured degradation rates have been reported for many chemicals, particularly in wastewater treatment processes. There are, however, still a limited number

of experimental studies reporting on the degradation processes and rates in environmental compartments under natural conditions.

- **Intentional Uses and Production Levels**: While many chemicals management regulations require the registration of chemical import/production levels and uses, there are significant gaps in publicly available data, particularly those describing the quantitative production amounts, their uses, and their distributions among reported uses. Quantities were found to have been collected, but often the actual values are marked as confidential business information and are consequently not published. The only identified, publicly available production estimates for some chemicals are regulatory, registered production level ranges that may span an order(s) of magnitude.
- **Emission Sources into the Environment**: Wastewater treatment plant effluent has been identified as a primary point source for emissions of many of these chemicals into the environment. Additional emission sources (such as from disposal) are often unclear and can only be partially inferred based on the chemicals' reported uses (which are also often unclear). Limited information regarding emissions during manufacturing has been identified for only few of these chemicals (e.g. 4-nonylphenols); such information may not necessarily reflect the situation in all countries or regions. This lack of information on emission sources may lead to substantial difficulties in obtaining a comprehensive picture of environmental and human exposure to the target chemical.
- **Distribution in the Abiotic Environment and Biota**: The environmental distribution of emitted chemicals can be modeled based on their physicochemical properties, provided there is available information regarding their emission patterns. There is a limited availability of experimental studies investigating the bioaccumulation potential of many chemicals, and estimated values are often provided instead.
- **Measured Environmental Concentrations**: Measured environmental concentrations for many of these chemicals were identified to exist globally with more sampling occurring after the year 2000. Concentrations in water and sediment were found to be reported most often. Field measurements were found to be more limited or non-existent in some regions, especially in Africa.
- **Scientific Evidence of Adverse Endocrine-Related Environmental Effects**: Reported observations can be found in the literature across many different species. Many observations have been reported as the result of both in vitro and in vivo studies. No studies were identified that focus on investigating effects at the population level.

4. Chemical Fact Sheets

Table 1 outlines the chemicals that have been reviewed and the location in the report of their corresponding fact sheet. This set of chemicals is introduced and discussed in more detail in section 4 in Report I. In brief, each of the chemicals included within the fact sheets has been previously assessed and identified by at least one stakeholder as an EDC or potential EDC using the WHO/IPCS 2002 definitions. EDCs or potential EDCs identified in the European Union's Candidate List of Substances of Very High Concern (SVHC), the

Substitute-It-Now (SIN) List by ChemSec, and an evaluation using the proposed Danish criteria were used as the basis for inclusion within the fact sheets. Each fact sheet notes which of these three stakeholder initiative(s) has identified the chemical(s) as an EDC or potential EDC.

Table 1. The chemical name, CAS number, and page number of the corresponding fact sheet for chemicals previously identified as EDCs or potential EDCs that have been reviewed in this report.

Chemical name	CAS number(s)	Fact sheet location
4-NONYLPHENOLS		
4-Nonylphenol, branched and linear	84852-15-3/ 26543-97-5/ 104-40-5/ 17404-66-9/ 30784-30-6/ 52427-13-1/ 186825-36-5/ 142731-63-3/ 90481-04-2*/ 25154-52-3*/ Others not identified	Pages 10-22
4-Nonylphenol, branched and linear, ethoxylated	104-35-8/7311-27-5/ 14409-72-4/ 20427-84-3/ 26027-38-3/ 27942-27-4/ 34166-38-6/ 37205-87-1/ 127087-87-0/ 156609-10-8/ 68412-54-4*/ 9016-45-9*/ Others not identified	
4-TERT-OCYLPHENOLS		
4-(1,1,3,3-tetramethylbutyl)phenol	140-66-9	Pages 23-34
4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated	2315-67-5/ 2315-61-9/ 9002-93-1/ 2497-59-8	
4-HEPTYLPHENOL AND 4-TERT-PENTYLPHENOL		
4-Heptylphenol, branched and linear	6465-71-0/ 6465-74-3/ 6863-24-7/ 1987-50-4/72624-02-3/ 1824346-00-0/ 1139800-98-8/ 911371-07-8 / 911371-06-7 /911370-98-4/ 861011-60-1/ 861010-65-3/ 857629-71-1/ 854904-93-1/ 854904-92-0/ 102570-52-5/ 100532-36-3/ 72861-06-4/ 71945-81-8/ 37872-24-5/ 33104-11-9/ 30784-32-8/ 30784-31-7/ 30784-27-1	Pages 35-42
p-(1,1-dimethylpropyl) phenol	80-46-6	
PHTHALATES (EU REACH SVHCs)		
Bis(2-ethylhexyl) phthalate; DEHP	117-81-7	Pages 43-55
Diisobutyl phthalate; DIBP	84-69-5	
Dibutyl phthalate; DBP	84-74-2	
Benzyl butyl phthalate; BBP	85-68-7	
BENZOPHENONES		
Benzophenone-1; 2,4-Dihydroxybenzophenone;	131-56-6	Page 56-64

Resbenzophenone		
Benzophenone-2; 2,2',4,4'-tetrahydroxybenzophenone	131-55-5	
Benzophenone-3; Oxybenzone	131-57-7	
4,4'-dihydroxybenzophenone	611-99-4	
3-BC, MBC, EHMC		
3-Benzylidene camphor (3-BC); 1,7,7-trimethyl-3-(phenylmethylene)bicyclo[2.2.1]heptan-2-one	15087-24-8	Pages 65-77
3-(4-Methylbenzylidene) camphor; 1,7,7-trimethyl-3-[(4-methylphenyl) methylene]bicyclo[2.2.1] heptan-2-one	36861-47-9	
2-ethylhexyl 4-methoxycinnamate	5466-77-3 / 83834-59-7	
BISPHENOLS F AND S		
Bisphenol F	620-92-8	Pages 78-86
Bisphenol S	80-09-1	
BHT AND BHA		
Butylated hydroxytoluene	128-37-0	Page 87-96
Tert.-Butylhydroxyanisole (BHA); tert-butyl-4-methoxyphenol	25013-16-5	
CARBON DISULPHIDE	75-15-0	Pages 97-102
DITHIOCARBAMATES		
Metam-sodium	137-42-8	Pages 103-113
Zineb	12122-67-7	
Ziram	137-30-4	
Thiram	137-26-8	
MTBE Tert-butyl methyl ether; MTBE; 2-methoxy-2-methylpropane	1634-04-4	Pages 114-120
PARABENS		
Methylparaben	99-76-3	Pages 121-131
Ethylparaben	120-47-8	

Propylparaben; propyl 4-hydroxybenzoate	94-13-3	
Butylparaben; butyl 4-hydroxybenzoate	94-26-8	
OTHER PHENOL DERIVATIVES		
4-nitrophenol	100-02-7	Pages 132-146
2,4,6-tribromophenol	118-79-6	
Resorcinol	108-46-3	
PCP, TEBUCONAZOLE, AND TRICLOSAN		
Pentachlorophenol (PCP)	87-86-5	Pages 147-161
Tebuconazole	107534-96-3	
Triclosan	3380-34-5	
PHTHALATES (NON-EU REACH SVHCs) 1		
Diethyl phthalate (DEP)	84-66-2	Page 162-176
Dihexyl phthalate (DHP)	84-75-3	
Dicyclohexyl phthalate (DCHP)	84-61-7	
PHTHALATES (NON-EU REACH SVHCs) 2		
Dioctyl phthalate (DOP)	117-84-0	Page 177-193
Diisodecyl phthalate (DiDP)	68515-49-1 / 26761-40-0	
Diundecyl phthalate (DuDP), branched and linear	3648-20-2	
QUADROSILAN Quadrosilan; 2,6-cis-Diphenylhexamethylcyclotetrasiloxane	33204-76-1	Pages 194-197
TRIPHENYL PHOSPHATE	115-86-6	Pages 198-203

* Identified as additional CAS numbers by ChemSec for these compounds on the SIN List and are not originally on the EU REACH SVHC list.

[Authors: All of the fact sheets are provided together in a separate pdf document. Please visit this **separate document** in order to review the fact sheets.]

5. Acknowledgement

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6. General References

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Annex: Additional Detailed Information

A.1 Adverse Outcome Pathways

A.1.1 Introduction

Endocrine disrupting chemicals (EDCs) are extraordinarily diverse in their structure and can affect growth, development, and reproduction in diverse ways. Linking apical (or adverse) effects to an endocrine mechanism can be challenging, as typically mechanistic data are characterized by in vitro assays and adverse effects on individuals or populations are measured in in vivo studies. Adverse outcome pathways (AOPs) were first introduced by Ankley et al. [7] and provide a framework for organising diverse data collected at different levels of biological organisation to established scientifically plausible links. AOPs share a common structure consisting of a molecular initiating event leading to a series of key events in the cells and organs, with an adverse outcome observed in an individual organism or at the population level [7–10]. AOPs provide a framework for considering data generated in varied experimental designs methodologies, to examine the probable relationships between a chemical with an endocrine mode of action and the resulting downstream effect.

A.1.2 Building Blocks of AOPs

An AOP begins with interaction at a molecular target. This then signals events within a cell, is followed by events within an organ, and later leads to an effect on the individual level. Ultimately an effect then occurs at the population level.[7–10] Interactions among the levels within an AOP may be causal, mechanistic, inferential, or correlation-based [7].

Information on how these biological levels interact may be derived from in vitro, in vivo, in-silico, or -omics studies [11,12]. Following the conceptual framework, the first steps of an AOP are a molecular interaction followed by a cellular response. These processes are investigated by using in vitro bioassays, which are commonly used tools to measure the activation of pathways by chemical contaminants. Typically, these assays utilize cell cultures or tissues to assess specific cellular effects in a rapid and cost-effective way. In vivo bioassays can be used to investigate processes on the organ and individual levels. They cover more integrative responses of bioactive compounds on parameters such as growth, development, reproduction, feeding activity, cancer development, and mortality in test species from a broad selection of taxonomic groups (e.g. algae, macrophytes, invertebrates, rodents and fish). The final in vivo response level in the AOP framework covers effects on populations.

A.1.3 Applications and Limitations

The development and use of AOPs in investigating the effects of chemicals can offer a number of potential benefits. However, limitations and uncertainties have also been noted. Some identified benefits of the AOP approach include the facilitation of hazard evaluation and predicting potential population risks without extensive use of test organisms and field studies. In addition, it is seen to also provide a platform for greater use of predictive approaches in ecotoxicology and ecological risk assessment. The development and application of AOPs have been suggested by multiple stakeholders as a way to support the organization of information during the investigation of chemical effects.

Limitations in the development and use of AOPs have also been identified. The current single AOPs and AOP networks may not capture all relevant events and characteristics (e.g., including non-monotonic and low-dose responses, differences in sex, age, and developmental sensitivities, rapid signaling and other "non-classical" hormone actions, and epigenetic or transgenerational consequences; [13]). The current understanding is limited regarding these signaling pathways, how they may interact, and which pathways are most sensitive during different life stages. It is acknowledged that many challenges and open questions for AOPs remain to be addressed.

Information Box: Example of using AOPs – Steroidal Estrogens and Wild Fish

One well-characterized example of EDC AOPs involves the impact of steroidal estrogens on the reproductive health of wild fish. Estrogenic chemicals in wastewater treatment plant discharges typically include natural and synthetic steroids that mimic the female sex hormone, estrogen. Examples include 17β-estradiol (E2) and 17α-ethinylestradiol (EE2, used in birth-control pills), and weaker estrogen mimics, such as octylphenol, nonylphenol, and their ethoxylates and carboxylates [14]. For most of these compounds, the AOPs begin with the binding of an estrogenic chemical to the estrogen receptor (known as the 'molecular initiating event') leading to more specific biological responses or biomarkers associated with the receptor activation at intermediate levels of the biological organization (known as 'key events'). These can include, for instance, the changes in expression of estrogen-responsive genes in plasma sex steroid and vitellogenin concentrations (VTG; a precursor protein normally synthesized by females during oocyte maturation), and gonad abnormalities leading to adverse outcomes such as changes in secondary sex characteristics and altered reproduction (e.g., decreased spawning or fecundity) at the whole-organism level, with consequent effects on populations [7,9,14–16]. In an EE2 whole-lake dosing study, it was demonstrated that the last AOP response level was achieved, namely, a collapse of the native fathead minnow population [15].

A.2 Testing Levels

In the fact sheets included in this report, observed scientific evidence of endocrine-related adverse effects reported in peer-reviewed literature has been summarized and presented. These observations result from different categories, or levels, of empirical studies (experiments), namely in vitro, in vivo, and population level studies. Each of these study levels has its own unique characteristics including conveniences and liabilities, which are important to understand when considering study observations. In this section, each of these study levels are briefly introduced and some main limitations are discussed.

A.2.1 In vitro level

In vitro (from Latin meaning ‘within the glass’) refers to performing an experiment not within a living organism (i.e. often done using e.g. single cells in a petri dish). Many experiments are performed in this way, and key conveniences of this level include the often lower costs needed to complete an experiment, the ability to control the experimental conditions, and the ability to avoid or minimize harm to living animals. They are also able to help specifically identify the mode-of-action of an EDC. However, there are a number of identified limitations for the use of in vitro studies. These include:

- Limited ability to metabolize substances during testing and represent metabolisms in the integrated whole animal [17]. In the context of EDCs, if a metabolite is the endocrine active compound, it may not be detected [18].
- Lack of other, potentially important toxicokinetic considerations during testing (such as absorption, distribution, and excretion) [18].
- Inability to test many relevant microbes [and cell lines] in vitro. This can result in instead focusing testing on certain microbes [and cell lines] because they are easy to culture. [19]

A.2.2 In vivo level

In vivo (from Latin meaning ‘within the living’) refers to performing an experiment using a whole, living organism. These experiments are better able to examine overall effects of a chemical on a living subject and can consider the toxicokinetics that exist. However, there are a number of identified limitations for the use of in vivo studies. These include:

- High costs and ethical concerns in regards to the use of (many) living organisms for testing [20].
- In the context of EDCs, they cannot necessarily specifically identify the mode(s)-of-action since overall effects are observed.
- Inability to test many relevant organisms in vivo. This can result in instead on focusing testing on certain organisms because they are easy to breed in the laboratory. [19]

A.2.3 Population level

Population level studies refer to performing an experiment using an entire community of (multiple) living organisms. These experiments are able to examine overall effects of a chemical on an entire population, and they may most closely represent realistic effects that could occur in the natural environment. However, there are a number of identified limitations for the use of population level studies. These include:

- High costs and ethical concerns in regards to the use of (many) living organisms for testing.
- In the context of EDCs, they cannot necessarily specifically identify the mode(s)-of-action since overall effects of the population are observed.