# Mapping commonalities and differences in approaches for testing and assessment of endocrine disruptors within the EU and among relevant international trading partners - Final Report

Framework Contract ENV.A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters

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## EXECUTIVE SUMMARY

The objective of this study is to compare approaches for the regulatory screening, testing and assessments of substances for the endocrine disrupting properties within the European Union (EU) and among relevant international trading partners, as well as the results of these approaches, in order to establish commonalities and differences and assess the drivers for these differences.

To create a basis for the analyses of this study, an inventory of completed and on-going activities related the screening, priority setting, testing and assessment of chemicals for their endocrine disrupting properties in the EU (including within the Member States) and relevant international trading partners (US, Canada, Australia, Japan, China) was created. This was complemented by an expert consultation about ongoing activities, and resulted in a high level overview summary of the regulatory approaches for endocrine disrupting chemicals in the various legalities.

This overview summary showed a common concern about the harmful effects of endocrine disrupting properties across the legalities. However, significant differences relate to the question whether endocrine disrupting chemicals require dedicated regulatory systems and structures to capture their effects appropriately, or whether the adverse effects produced by endocrine disrupting chemicals can be dealt with adequately within the existing regulatory structures.

Case studies of eight chemicals, ethinylestradiol and estradiol, nonylphenol, bisphenol A, di-ethyl-hexyl phthalate (DEHP), mancozeb, prochloraz, procymidone and benzophenone-3, were conducted for a deep analysis of the commonalities and differences in screening, priority setting and testing of chemicals for their endocrine disrupting properties in the EU and among its international trading partners. The case studies served to address the following study questions:

- Are the differences and commonalities in the different legalities due to differences in scientific approaches, or are they an expression of the different features of the respective legal systems?
- What impact do differences in approach have on the final outcome of the derivation of regulatory values (e.g. water quality standards, acceptable daily intakes and similar)?

To facilitate systematic comparisons between the case studies, a common structure was adopted, covering: Discovery of endocrine disrupting properties of the chemical in question, its regulatory status across the different legalities, assessment endpoints used for hazard characterisation, the derivation of assessment values (acceptable daily intakes or water quality criteria), and exposure and risk assessments.

The comparative analysis of the case studies revealed that the endocrine disrupting properties of five of the chemicals were discovered in the context of scientific research activities; only three (two pesticides, prochloraz, procymidone and a cosmetic ingredient, benzophenone-3) were identified through regulatory testing efforts. This suggests that the framework of established regulatory testing is ill-equipped for identifying chemicals with endocrine disrupting properties. It also gives an impression of the potential benefits of international harmonisation in the regulatory domains dealing with pesticides and cosmetic ingredients.

While all the pesticides analysed in the case studies (mancozeb, prochloraz, and procymidone) are subject to regulations and restrictions in all the legalities considered in

this project, a more varied picture emerged for industrial chemicals such as bisphenol A and DEHP. For bisphenol A, restrictions apply in some jurisdictions (e.g. EU and China), while in others, it is essentially unregulated (USA, Canada, Japan, Australia). This is a reflection of differences in the regulatory regimes and of differences in the level of concern with which bisphenol A is regarded (higher in the EU than anywhere else). The use of DEHP is restricted in the EU, the USA and Australia, but not in Canada, Japan or China.

The environmental regulatory status of the industrial chemical nonylphenol varies considerably across the legalities considered, with water quality criteria implemented in the EU, the USA and Canada, but not in China, Japan and Australia. In contrast, the status of the pharmaceutical ethinylestradiol is rather uniform. With the exception of Canada, which has established water quality criteria, there are no environmental standards implemented in any of the other legislations.

The assessment values (acceptable daily intakes) that are applied for the pesticides considered in the case studies are rather uniform across all legislations, except for procymidone where the values vary by a factor of approximately 30. This appears to be a result of the internationally harmonised procedures of hazard characterisation that have evolved over the years in the regulatory domain of pesticides. It is of note that the assessment values derived for prochloraz are based on toxicities unrelated to endocrine disruption. A similar, rather uniform picture also emerged for DEHP where the assessment values utilised in the different countries and legislations do not differ much, with the exception of the EU where a range of values is applied.

Greater differences became obvious for the environmental standards used for ethinylestradiol and nonylphenol, and for bisphenol A. The water quality criteria (or equivalent) that are in use for risk assessments for ethinylestradiol vary by a factor of approximately 15. These differences are explained by the use of different experimental studies for the derivation of the values, and by the application of differing assessment factors.

In the case of nonylphenol, the differences between the various assessment values (factor of approximately 60) are due to the fact that their derivation was based on distinct chemical entities (nonylphenol with linear or branched side chain), with quite different toxic properties.

The greatest variations became apparent with bisphenol A where the assessment values in use internationally differ by no less than 10,000-fold. This is driven by the use of a variety of assessment endpoints, not all of which relate to endocrine disruption, and the application of widely differing assessment factors, reflecting differences in the evaluation of adversity, and a lack of scientific agreement about the basis for hazard characterisations.

In summary, the differences and commonalities in the different legalities in dealing with endocrine disruptors are mainly an expression of the features of the respective legal systems. In some cases, diverging scientific approaches also play a role. The impact of these differences on the final outcome of the derivation of regulatory values (e.g. water quality standards, acceptable daily intakes and similar) varies from compound to compound, but can be considerable.

The differences in the respective assessment values could diminish if more consistent methods of hazard assessment were applied across the various legalities, with uniform,

transparent and agreed criteria as to the selection of studies for hazard characterisations, and more transparency in the choice of assessment factors.

To discuss the commonalities and differences in screening, testing and evaluating endocrine disruptors across the EU and its international trading partners, an international workshop with risk assessment practitioners from competent authorities was held on 19-20 September 2016 in Brussels. Four working groups were set up, as follows:

**Working Group 1**: The scope for data sharing on ED hazards and exposures at the international level

**Working Group 2**: Setting priorities for screening and testing for ED properties – commonalities and differences and scope for common principles

**Working Group 3**: Research needs and horizon scanning in the ED arena – prospects for international cooperation?

**Working Group 4**: Harmonisation of hazard and risk assessment for endocrine disruptors at the international level – opportunities and limitations

There was widespread recognition among workshop participants of the need for international cooperation in promoting the chemical safety of chemicals with endocrine disrupting properties. There was also a willingness to move towards an international harmonisation of approaches. This found expression in four **recommendations** from workshop participants, which concerned the

- development of international guidance for harmonised hazard assessment of endocrine disruptors,
- development of a strategy for the testing of endocrine disruptors
- implementation of existing tests and assays for the identification of endocrine disruptors, as described in the OECD Conceptual Framework, Level 2 and 3, and
- creation of an institutional platform for international harmonisation of hazard and risk assessment for endocrine disruptors, and for the exchange of data and assessments

The implementation of the last of these recommendations would appear to be essential to initiate the process of international harmonisation in the assessment of endocrine disruptors. However, it is beyond the scope of this project to elaborate the finer organisational and institutional details of this process.

**List of abbreviations**

ADI Acceptable Daily Intake

AOEL Acceptable Operator Exposure Level

AOP Adverse Outcome Pathways

APVMA Australian Pesticides and Veterinary Medicines Authority

aPAD acute Population Adjusted Dose

ARfDs Acute Reference Doses

BMD Benchmark Dose

BMDL Benchmark Dose Lower limit

CDER Center for Drug Evaluation and Research

CEPA Canadian Environmental Protection Act

CMR Carcinogen Mutagen Reproductive toxicant

cPAD chronic Population Adjusted Dose

DEHP Diethyl-hexyl phthalate

DNEL Derived No Effect Level

E2 17β-estradiol

EBDCs Ethylene(bis)dithiocarbamates

ED Endocrine Disruption

EDC Endocrine Disrupting Chemical

EDTA AG Endocrine Disrupters Testing and Assessment

EDSP Endocrine Disrupter Screening Programme (USA)

EE2 17α-ethinylestradiol

EPA Environmental Protection Agency

EQS Environmental Quality Standards

EQSD Environmental Quality Standards Directive

ER Estrogen Receptor

ETU Ethylenethiourea

EU European Union

FAO Food and Agriculture Organisation

FDA Food and Drug Administration

FQPA Food Quality Protection Act (USA)

FSANZ Food Standards Australia and New Zealand

FSCJ Food Safety Commission of Japan

HC Hazardous Concentration

JMPR Joint FAO WHO Meeting on Pesticide Residues

NICNAS National Industrial Chemicals Notification and Assessment Scheme

NIEHS National Institute for Environment and Health Studies

METI Ministry of Economy, Trade and Industry (Japan)

MHLW Ministry of Health, Labour and Welfare (Japan)

MOE Ministry of the Environment (Japan)

MoE Margin of Exposure

MRL Maximum Residue Limit

NAFTA North American Free Trade Agreement

NGO Non-Governmental Organisations

NOAEL No-Observed-Adverse-Effect-Level

NOEC No-Observed-Effect-Concentration

NTP National Toxicology Programme (USA)

OECD Organisation for Economic Cooperation and Development

OHAT Office of Health Assessment and Translation (USA)

PBT Persistent Bioaccumulative Toxic

PNEC Predicted No-Effect Concentration

QSAR Quantitative Structure-Activity Relationships

SAICM Strategic Approach to International Chemicals Management

SDWA Safe Drinking Water Act (USA)

SSD Species Sensitivity Distribution

STMR Supervised Trials Median Residue value

STW Sewage Treatment Works

TSCA Toxic Substances Control Act (USA)

TGA Therapeutic Goods Administration

UNEP United Nations Environment Programme

US United States

USA United States of America

WHO World Health Organisation

WNT Working Group of National Coordinators of the Test Guidelines Programme (OECD)

WQC Water Quality Control

# Contents

# 1. INTRODUCTION

This document is the Final Report for the project Mapping commonalities and differences in approaches for testing and assessment of endocrine disruptors within the EU and among relevant international trading partners. It incorporates the 2nd interim report for this project.

The objective of this project is to compare approaches for the regulatory screening, testing and assessments of substances for the endocrine disrupting properties within the European Union (EU) and among relevant international trading partners, as well as the results of said approaches, in order to establish commonalities and differences and assess the drivers for these differences.

Accordingly, the project addresses the following specific objectives:

1. To gain an overview of regulatory screening, priority setting, testing and assessment approaches applied to identify and assess endocrine disruptors in EU Member State, at EU level and by relevant international trading partners (US, Canada, Australia, Japan, China) focusing in particular on case studies of application;

2. To map out commonalities and differences in the screening, priority setting, testing and assessments approaches addressing the used methodologies, type of data considered, technical assessments of specific cases and interpretation of results of specific cases;

3. To ascertain the extent to which differences are due to variations in scientific approaches or in different legislative frameworks and regulatory culture;

4. To identify opportunities to foster international cooperation on scientific issues related to promoting chemical safety in regards to potential for endocrine disruption.

## *1.1 Tasks*

In line with the technical specifications for this project, and on the basis of the above specific objectives, the study includes the following tasks:

Task 1: Collate an inventory of completed and on-going activities related the screening, priority setting, testing and assessment of chemicals for their endocrine disrupting properties in the EU (including within the Member States) and relevant international trading partners (US, Canada, Australia, Japan, China);

Task 2: Elaborate a descriptive overview of results of regulatory screening, priority setting, testing and assessments as regards endocrine disruptors in the EU (including within the Member States) and relevant international trading partners (US, Canada, Australia, Japan, China);

Task 3: Identify, describe and assess commonalities and differences in screening approaches, priority setting approaches, testing approaches and assessments of chemicals as regard their endocrine disrupting properties;

Task 4: Identify opportunities to foster international cooperation on scientific issues related to promoting chemical safety in regards to potential for endocrine disruption;

Task 5: Organise an international workshop on commonalities and differences in approaches for testing and assessment of endocrine disruptors within the EU and among relevant international trading partners (US, Canada, Australia, Japan, China).

### *1.2 Contents of this report*

This report describes all the results obtained in relation to all tasks of the project, under the following headings:

- Inventory of concluded and on-going activities regarding the screening, priority setting, testing and assessment of chemicals for their endocrine disrupting properties in the EU and relevant international trading partners
- Descriptive overview of results of regulatory screening, priority setting, testing and assessment in the EU and relevant international trading partners
- Identification and assessment of commonalities and differences in screening approaches, priority setting approaches, testing approaches and assessments of chemicals regarding their endocrine disrupting properties
- The international workshop on commonalities and differences in approaches for testing and assessment of endocrine disruptors within the EU and among relevant trading partners
- Possibilities for fostering international cooperation on scientific issues related to promoting chemical safety in regards to the potential for endocrine disruption.

## 2. INVENTORY OF CONCLUDED AND ON-GOING ACTIVITIES REGARDING THE SCREENING, PRIORITY SETTING, TESTING AND ASSESSMENT OF CHEMICALS FOR THEIR ENDOCRINE DISRUPTING PROPERTIES IN THE EU AND RELEVANT INTERNATIONAL TRADING PARTNERS

In this section of the report we present an inventory of completed and on-going activities related the screening, priority setting, testing and assessment of chemicals for their endocrine disrupting properties in the EU (including within the Member States) and relevant international trading partners (US, Canada, Australia, Japan, China). This work was subdivided into 4 separate work steps:

Step 1: Literature searches

Step 2: Expert consultation

Step 3: Overview summaries

Step 4: Summary of commonalities and differences in the various legislations

### *2.1 Step 1: Literature searches*

Web-based searches via search engines yielded more than 200 relevant documents from EU and non-EU countries, as well as relevant authorities. In addition to these documents, the expert consultation (see below) produced more than 60 documents which have been added to a document inventory as Annex 1 "EDC assessment documents" to this report.

### *2.2 Step 2: Expert consultation*

Experts from EU and non-EU countries were consulted in order to prepare for the project workshop and to develop an overall picture of the legislative frameworks and regulatory activities related to endocrine disruption of selected member states and international trading partners.

The aims of this consultation exercise were

- to ensure that the international workshop takes note of all relevant current, ongoing or planned activities and approaches regarding identification, testing and assessment of EDs, and

- to ensure that all points considered critical and important by experts are properly discussed during the workshop and reflected in the workshop report.
- The consultation took the form of collection of expert views via structured written responses to questions. To this end an expert consultation guide was developed which covered the list of topics below:
  - Definition of Endocrine Disruptors (EDs) – requirements for regulatory purposes
  - Identification of EDs – overview of regulatory activities related to the screening, priority setting, testing and assessment
  - Completeness and fitness-for-purpose of different approaches
  - Regulatory assessment of EDs
  - Evidence based assessment of EDs
  - Research and regulatory needs
  - Data sharing
  - Comparative case studies

The expert consultation guide can be found in Annex 2 "Expert consultation guide".

Among international trading partners (non-EU countries), we contacted 22 experts (from 5 countries), of which:

- 6 completed interviews: 1 from Australia, 1 Canada, 2 Japan, 2 US
- 2 promised they would complete the questionnaire but did not have sufficient time before the extended deadline (end of May 2016) (one submitted some comments by email, Australia)
- 4 were undeliverable to given email addresses
- 8 did not reply despite 3 attempts of contact (one because he had retired)
- 2 refused because questions were not pertinent to their organisations

From EU Member States 16 experts from 8 MS were contacted, of which:

- 7 completed interviews from Austria (2), Belgium (1), Denmark (1), Germany (2), Italy (1)
- 4 promised they would complete the questionnaire but did not have sufficient time before the extended deadline (2 submitted some comments by email, France and UK)
- 3 did not reply despite 3 attempts of contact
- 2 refused altogether (UK)

Among EU Institutions 2 experts were contacted, of which:

- ECHA replied
- EFSA promised to send completed questionnaire but then failed to respond to reminders

Altogether, we obtained 14 responses. An anonymised compilation of these responses can be found in Annex 3 "Compilation of anonymised expert responses".

### *2.3 Step 3: Overview summaries*

The outcomes of Steps 1 and 2 were distilled into overview summaries. These summaries have served as background material for the project workshop.

United States

*Legislative framework*

The Toxic Substances Control Act 1976 (TSCA) authorises the US Environmental Protection Agency (USEPA) to require reporting, record-keeping and testing, and to enforce restrictions related to the importation, use, and disposal of specific chemicals and mixtures. Until recently, efforts have been focused on making basic screening level information on the toxicity of existing chemicals publicly available by maintaining the TSCA Chemical Substance Inventory which compiles information about more than 84,000 chemicals. There are no fixed data requirements for industrial chemicals, and accordingly, no requirements for reporting endocrine disrupting properties of chemicals. New chemicals can be added to the TSCA Chemical Substance Inventory through the submission of a Pre-manufacture Notice, in which all currently available data should be included. After submission, USEPA can request further information (EPA, 2016a). In April 2010, Senator Lautenberg introduced new legislation aiming to reform the TSCA by placing the burden of proof on chemical manufacturers. The ''Frank R. Lautenberg Chemical Safety for the 21st Century Act'' was approved by the US Senate on the 8th June 2016.

Food, drugs, cosmetics and pesticides, amongst others, are excluded from TSCA. The Federal Food, Drug, and Cosmetic Act and Federal Insecticide, Fungicide, and Rodenticide Act was amended by the passage of the Food Quality Protection Act (FQPA) in 1996 that allows USEPA to use and require data on endocrine disrupting properties of pesticides. In addition, the Safe Drinking Water Act (SDWA) provides USEPA with the authority for testing any substance that may be found in sources of drinking water.

The US has not yet taken any legal action to restrict use of a chemical on the basis of its endocrine disrupting properties, but authorities have used 'soft' regulatory action such as voluntary programmes with industry and recommendations to consumers (e.g. the Food and Drug Administration (FDA) is supporting industry's action to replace or minimise exposure to bisphenol A (BPA)).

*Activities related to the screening, testing and assessment of endocrine disrupters*

Passage of the FQPA required the EPA to develop a screening and testing program to determine human health effects of endocrine disrupting chemicals. The Endocrine Disruptor Screening and Testing Advisory Committee was established to make recommendations on how to develop the Endocrine Disruptor Screening Program (EDSP). The EDSP is a two-tiered screening and testing process that addresses both potential human and environmental effects. Tier 1 screening aims to identify chemicals with the potential to interact with the estrogen, androgen and thyroid hormone systems. The purpose of Tier 2 testing is to identify and establish a quantitative dose-response relationship for any resulting adverse effects, on the basis of which risk can be assessed and risk mitigation measures developed for the protection against adverse effects in humans and wildlife. The EPA began issuing test orders for the first list of 52 chemicals for Tier 1 screening in October 2009, mainly focused on active substances used in plant protection products. Eighteen of those will now undergo Tier 2 testing. The EPA published a second list of 109 chemicals for Tier 1 Screening in May 2013, focussing on possible water pollutants.

The National Toxicology Program (NTP) Tox21 is a federal collaboration among the National Institute of Health, USEPA and the FDA with the aim of applying high-throughput methods in the hazard identification for chemicals. The EPA's contribution to Tox21 is ToxCast, is a battery of in vitro endocrine disruption assays used to develop activity signatures of chemicals. It is used for prioritisation for testing under EDSP (Reif et al. 2010). The Interagency Centre for the Evaluation of Alternative Toxicological Methods (ICCVAM) is another NTP program that supports the scientific development and evaluation of new, revised and alternative methods to replace, reduce or refine animal

use in chemical testing. For example, the ToxCast estrogen receptor (ER) model for bioactivity integrates the results of 18 high-throughput in vitro screening assays with a computational model to predict the potential of a chemical to interact with the estrogen receptor that has been accepted by the USEPA as an alternative to the three test methods currently used in the EDSP Tier 1 battery.

The NTP's Office of Health Assessment and Translation (OHAT) has also led the development of a systematic review process to standardise the collection, assessment and synthesis of scientific evidence of hazardous properties to support evidence-based hazard assessments.

Finally, the National Institute for Environment and Health Studies (NIEHS) offers financial support to studies investigating the potential human health effects related to exposure to endocrine disrupters.

Canada

*Legislative Framework*

The Government of Canada's legal tool for assessing and managing chemical substances in the environment is the Canadian Environmental Protection Act, 1999 (CEPA 1999), jointly administered by Environment and Climate Change Canada and Health Canada.

Health Canada is responsible for the assessment of potential risks to human health posed by existing substances, i.e. those on the Domestic Substances List, a compilation of about 23,000 substances used, imported or manufactured in Canada for commercial purposes between January 1, 1984, and December 31, 1986, at a quantity of greater than 100 kg per year. The Existing Substances Division conducts this work within Health Canada, jointly with Environment and Climate Change Canada. If a substance is found to be CEPA-toxic as defined in Section 64 of the Act, it is added to Schedule 1 (the List of Toxic Substances) of the Act and options for controlling risks to human health and/or the environment are reviewed.

Environment and Climate Change Canada oversees the New Substances Notification Regulations created to ensure that no new chemicals are introduced into the Canadian marketplace before completion of an assessment of their potential toxicity has been completed, and before any appropriate or required control measures have been taken. Any company or individual who plans to import or manufacture a substance subject to notification under the Regulations must provide a New Substances Notification (NSN) package containing all information prescribed in the Regulations prior to import or manufacture. This NSN Package is jointly assessed with Health Canada to determine whether there is a potential for adverse effects of the substance on the environment and human health. When this process identifies a new substance that may pose a risk to human health or the environment, CEPA 1999 empowers Environment and Climate Change Canada to intervene prior to or during the earliest stages of its introduction into Canada.

CEPA 1999 not only defines 'hormone disrupting substances' but also requires that research is carried out on 'methods related to their detection, methods to determine their actual or likely short-term or long-term effects on the environment and human health, and preventive, control or abatement measures to deal with those substances to protect the environment and human health'.

*Activities related to the screening, testing and assessment of endocrine disrupters*

Since 2001, Health Canada maintains an active research program related to exposure and biomonitoring, toxicological and epidemiological studies of substances suspected of having effects on the endocrine system, with the aim of supporting the risk assessment and risk management activities of the Department. Examples include the Maternal-Infant

Research on Environmental Chemicals (MIREC) and Plastics and Personal-care Product use in Pregnancy (P4).

Environment and Climate Change Canada has also been investigating hormone-disrupting substances for over 15 years. Research has included work on individual priority substances, wildlife toxicity studies and method development to improve the detection of substances, targeted at pulp mill and municipal wastewater effluents, as well as research in priority ecosystems such as the Great Lakes Areas of Concern.

However, there are currently no screening and testing activities comparable with the US EDSP.

Australia

*Legislative Framework*

Since the early 1990s, new chemicals have undergone an assessment of their potential environmental and health impacts, and many of the older chemicals have been revised and, in some cases, phased out of production. At the Commonwealth level, chemicals are regulated according to four categories;

- The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is responsible for the regulation of non-agricultural chemicals such as industrial chemicals regulated by the Industrial Chemicals (Notification & Assessment) Act 1989,
- The Australian Pesticides and Veterinary Medicines Authority (APVMA) oversees the permit process of agricultural and veterinary chemicals (such as pesticides) regulated by the Agricultural and Veterinary Chemicals (Administration) Act 1992, Agricultural and Veterinary Chemicals Act 1994, Agricultural and Veterinary Chemicals Code Act 1994, and Agricultural and Veterinary Chemical Products (Collection of Levy) Act 1994
- The Therapeutic Goods Administration (TGA) is responsible for registering pharmaceuticals and medicinal products regulated by the Therapeutic Goods Act 1989, and
- The Food Standards Australia and New Zealand (FSANZ) is responsible for developing standards for food related issues including food additives regulated by Food Standards Australia New Zealand Act 1991, Australia New Zealand Food Standards Code.

From a regulatory perspective, the current Australian position is that although endocrine disrupters present concerns equivalent to carcinogens, mutagens, reproductive toxicants or persistent and bio-accumulative agents, endocrine disruption is not an adverse end-point per se, but rather a mode or mechanism of action of a chemical that can potentially lead to adverse toxicological or eco-toxicological outcomes. The existing legislation is considered to provide adequate protection and approaches devoted to the explicit screening and testing of EDCs are not currently proposed.

NICNAS is currently going through reforms. Known EDCs are proposed to receive a similar regulatory treatment to that proposed for CMR and PBT chemicals and require a pre-market assessment. NICNAS is not requiring the generation of new data and information, but rather that applicants confirm whether their chemical is known to be an EDC by checking specified authoritative lists. It is anticipated that such lists can be developed by the time the reforms are due to be implemented (July 2018) with the assistance of projects such as the US EDSP.

*Activities related to the screening, testing and assessment of endocrine disrupters*

Australia contributes to ongoing international work, particularly through the OECD Test Guidelines program, to further refine the methods used to identify the risks and to develop even more sensitive assessment methods.

Additionally, NICNAS monitors the scientific literature and liaises with other regulators nationally and internationally to maintain an up-to-date understanding of research. This includes incorporation of new tools for characterising the hazard and exposure to endocrine disrupters, as these are progressively validated and gain international regulatory acceptance.

Furthermore, organisations such as the CSIRO, universities, and regional water authorities have research and monitoring projects aimed at better understanding the presence, behaviour and fate of EDCs in aquatic environments.

China

*Legislative Framework*

Industrial chemicals are mainly regulated by the following regulations:

- Decree 591 - Regulations on Safe Management of Hazardous Chemicals entered into force on 1 Dec 2011. It implements the Global Harmonised System for Classification and Labelling of Chemicals and regulates hazardous chemicals through the entire supply chain by operating a license system and HazChem registrations.
- MEP Order 7 - The Measures for Environmental Management of New Substances came into force on 15 Oct 2010. This regulation is similar to EU REACH and requires that manufacturers and importers of new substances notify new substances and obtain approval.
- SAWS Order 53 - The Measures for the Administration of Registration of Hazardous Chemicals came into force on 1 Aug 2012 and sets out detailed requirements on HazChem registrations with the State Administration of Work Safety (SAWS).
- MEP Order 22 - The Measures for Environmental Administration Registration of Hazardous Chemicals came into force on 1 March 2013. This regulation requires that manufacturers and companies who use hazardous chemicals to manufacture products ("user") in China shall register hazardous chemicals listed in the Catalogue of Hazardous Chemicals with local environmental protection authorities and obtain environmental administration registration certificates.

There is no dedicated regulation focusing on endocrine disruptors in China. The Ministry of Environmental Protection and the Ministry of Agriculture are taking the lead for controlling endocrine disruptors within their own jurisdictions. According to China's action plan for water pollution prevention issued by the state council in 2015, the Chinese government plans to organize a national survey on the production and uses of Environmental Endocrine Disruptors before the end of 2017. Measures will be taken to eliminate, restrict or substitute endocrine disrupters. Both industrial chemicals and pesticides are affected.

In Dec 2015, the Ministry of Agriculture announced the publication of industry standard NY/T2873-2015 Evaluation Method of the Endocrine Disruption Effects of Pesticides. The standard will be implemented from 1 April 2016. NY/T2873-2015 consists of 7 testing methods given in two tiers (in vitro and in vivo) to screen pesticides for endocrine disrupting properties. The methods are said to be similar to those developed by US EPA.

Japan

*Legislative Framework*

The Ministry of Economy, Trade and Industry (METI) has overall responsibility for the management of chemicals. The Chemical Substances Control Law regulates the manufacture and import of bioaccumulative, persistent and toxic chemical substances. It was first enacted in 1973, and Japan pioneered the introduction of a pre-manufacturing evaluation and regulation system for new chemical substances.

Under the Act on Confirmation, etc. of Release Amounts of Specific Chemical Substances in the Environment and Promotion of Improvements to the Management Thereof, business operators are required to provide the Material Safety Data Sheet when transactions of Class I and II designated chemical substances (and products containing them) occur. The list of Class I Designated Chemical Substances is determined based on advice given by the Pharmaceutical Affairs and Food Sanitation Council (Ministry of Health, Labour and Welfare), the Chemical Substances Council (METI), and the Central Environment Council (Japan Environment Agency). Hazardous substances are selected based on their degree of hazard and the possibility of exposure.

Other relevant laws applicable to the control of chemical substances include Food Sanitation Law, Agricultural Chemicals Regulation Law, Water Pollution Control Law, none of which specifically mentions endocrine disruption.

*Activities related to the screening, testing and assessment of endocrine disrupters*

The Ministry of Health, Labour and Welfare (MHLW) established a Committee on Health effects of Endocrine Disruptors in 1998. The Committee developed a framework for testing of possible endocrine disrupters consisting of two tiers; screening and definitive tests. Screening tests were carried out on a number of chemicals and a priority list for future definitive testing was established based on those results. MHLW is widening its research interest to signal toxicity, to encompass disruption of signalling of any neuro-immuno-endocrine system suggesting a wider range of molecular mechanisms not limited to nuclear receptor systems and including epigenetic alterations.

The Ministry of Economy, Trade and Industry (METI) established an advisory body – the Endocrine Disruptive Effect Subcommittee and by 2009 had funded 15 studies on hazard assessment of 15 chemicals of potential concern as endocrine disrupters which did not identify significant risks to human health.

The Ministry of the Environment (MOE) established the Strategic Programme on Environmental Endocrine Disruptors 98 (SPEED 98). Simultaneously, the MOE carried out an Environmental Monitoring Survey of suspected endocrine disrupters as listed in the SPEED ’98 report and in air, water and wildlife. In addition, the ExtEND 2005 and later ExTEND 2010 were established to research mechanisms of endocrine disruption, environmental monitoring, development of test methods, hazard and risk assessment, risk management and communication. Within the scope of the OECD Conceptual Framework for testing and assessment, they contributed substantially to development of test protocols such as the Medaka Extended One Generation Reproductive Toxicity assay (TG 240) and the Larval Amphibian Growth and Development Assay (TG 241). MOE is now preparing a new program titled ‘ExTEND 2016’ to be published in June 2016. Moreover, the MOE has established a Joint Research Program on Endocrine Disrupters with the United Kingdom.

<u>OECD</u>

Endocrine disruption has figured highly on the chemical regulation agenda of regulatory authorities in most OECD countries, and it has been proposed by UNEP as a SAICM policy emerging issue. In turn, this is reflected in the OECD Test Guidelines Programme that since 1996 has spent approximately half of its resources on the development of test

guidelines and other tools, to support member countries' needs in relation to the testing and screening of chemicals for endocrine disrupting properties.

The work on endocrine disrupters testing and assessment is overseen by the Working Group of National Coordinators of the Test Guidelines Programme (WNT) and managed by four main expert groups:

- An advisory group on endocrine disrupters testing and assessment (EDTA AG)
- A validation management group (VMG) on ecotoxicity testing
- A VMG on non-animal testing
- A VMG for mammalian testing (Expert group on development and reproductive toxicity)

The EDTA AG is an advisory group to the WNT and to the VMGs. National experts nominated by the National coordinators and the European Commission, and representatives from the Business and Industry Advisory Committee, Environmental NGOs, and International Council on Animal Protection in OECD Programmes participate in the work.

Between 2007 and 2012, the OECD has published 12 Test guidelines specifically developed or updated for the screening or testing of chemicals for endocrine disruption. A further 11 adopted test guidelines were reviewed and found to provide useful information, although not specifically developed for screening/testing chemicals for endocrine disruption. Additionally, there are currently 16 projects for the screening or testing of chemicals for endocrine disruption, on the work plan.

Following the Workshop on OECD Countries' Activities Regarding Testing, Assessment and Management of Endocrine Disrupters, held in September 2009 in Copenhagen, to take stock of over 10 years of activity, further work was recommended and completed, namely:

- The revision of the 2002 Conceptual Framework for Testing and Assessment of Endocrine Disrupters, approved by the WNT in April 2012. It includes all published Test Guidelines; test methods for which inclusion in the Test Guidelines work plan has been approved by the WNT; some existing Test Guidelines not specifically developed for screening/testing of chemicals for endocrine disruption, and a few non OECD test methods. The CF is not a testing strategy and simply reflects the type of information the tests provide at the different levels.
- The development of a guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption (GD 150) to support regulatory authorities' decisions when they receive test results from a Test Guideline or draft Test Guideline for the screening/testing of chemicals for endocrine disruption. The guidance is worded to permit flexible interpretation in the context of different domestic legislation, policies and practice and provides guidance on how to interpret the outcome of individual tests, taking into account existing information, and how to increase evidence on whether or not a substance may be an endocrine disrupter.
- A detailed review paper on endpoints not included in existing Test Guidelines (DRP 178). To date, OECD work related to endocrine disrupters focused on oestrogen/androgen thyroid signalling processes and steroidogenesis. However, other endocrine and neuro-endocrine pathways may also have adverse outcomes, such as symptoms of metabolic syndrome, reproductive dysfunction, altered foetal development.

Other relevant OECD activities not solely concerned with endocrine disruption include a new programme on the development of Adverse Outcome Pathways (AOP) initiated in 2012. Several projects on the work programme are related to endocrine disrupters' assessment, such as;

- the AOP for Embryonic Vascular Disruption and Developmental Defects
- the AOP on Xenobiotic Induced Inhibition of Thyroperoxidase and Depressed Thyroid Hormones Synthesis and Subsequent Adverse Neurodevelopmental Outcomes in Mammals
- the AOPs linking Aromatase Inhibition, Androgen Receptor Agonism, Estrogen Receptor Agonism, or Steroidogenesis Inhibition, to Impaired Reproduction in Small Repeat-Spawning Fish Species

Quantitative Structure-Activity Relationships (QSARs) are methods for estimating properties of a chemical from its molecular structure. They have the potential to provide information on hazards of chemicals, while reducing time, monetary cost and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox including some relevant to endocrine mechanisms of toxicity.

Europe

*Legislative Framework*

Due to growing concerns about the potential detrimental effects of endocrine disruptors (EDs) on human and animal health, and the environment, the Commission adopted the 'Community strategy for endocrine disruptors' in 1999, to identify the risks posed by endocrine disruptors.

Legislative actions of the Strategy introduced rules relevant to EDs in major pieces of EU chemical legislation regarding industrial chemicals (REACH), pesticides (PPPR), biocides (BPR), cosmetics and media-oriented regulations such as the Water Framework Directive (WFD). Several industrial substances and pesticides have been assessed for their endocrine disrupting properties.

Under REACH, endocrine disrupters may be addressed under processes for dossier and substance evaluation and identified as Substances of Very High Concern (SVHC) based on article 57(f) (substances of equivalent concern to CMR or PBT/vPvB substances). The 'SVHC Roadmap' gives an EU-wide commitment for having all relevant currently known SVHCs included in the candidate list by 2020. As of May 2016, five substances or substances group had been placed on the candidate list for authorisation due to endocrine disrupting properties. Following a review, the Commission concluded that companies applying for authorisation of endocrine disrupters will only be able to go via the adequate control (risk assessment) route if they can demonstrate that a safe threshold exists. When a threshold cannot be determined, a socio-economic analysis of the substance and any alternative must be carried out.

Under the Biocidal Products Regulation and the Plant Protection Product Regulation, endocrine disrupting properties are explicitly cited as an exclusion criterion for approval of active substances. However, a number of derogations, where an approval may be granted for five years, do exist in case of public health concerns, negligible exposure or socio-economic consequences. Until the criteria for the identification of endocrine disrupters are adopted, interim criteria are in place.

*Activities related to the screening, testing and assessment of endocrine disrupters*

*European Chemical Agency (ECHA)*

Under REACH, criteria for the prioritisation of substances for evaluation include suspected endocrine disruption. Currently, the databases for registration under REACH and for CLP (Classification, Labelling and Packaging) are screened for alerts for endocrine disruption such as structural similarity and/or reproductive effects (IT screening). To date, over 50

substances have been included in the Community Rolling Action Plan (CoRAP) on such as basis for initial concern. For a further 15 substances whose evaluation had been initiated by other concerns, endocrine disruption was identified as an additional concern. Evaluations (manual screening) are carried out by Member States and the substances can be identified case-by-case as SVHC on the basis of equivalent concern. In the absence of regulatory criteria, the IPCS definition for endocrine disrupters has been used and interpreted in the light of expert judgment.

Since February 2014, ECHA also coordinates an Endocrine Disrupter Expert Group (EDEG) to provide informal, non-binding scientific advice on questions related to the identification of endocrine disrupters. It consists of experts nominated by Member State competent authorities, the European Commission and accredited stakeholders. The expert group focuses on;

- Screening methods and activities to identify potential endocrine disrupters
- Integrated approaches to testing and assessment of endocrine disrupting properties
- Complex scientific issues related to information and testing needs
- Interpretation of test data and other relevant information

*European Food Safety Agency (EFSA)*

EFSA collected any indication for ED (both human and other organisms) in the Conclusions of the risk assessment of 41 active substances since 2014. From the available information, hazard or risk-based concerns were identified for 15 substances. For some substances, the interim criteria were not met, but EFSA highlighted evidence suggesting possible concerns and recommended additional studies to finalise their assessment of potential endocrine-mediated adverse effects.

*DG Joint Research Centre (JRC)*

The Commission's Joint Research Centre (JRC) is engaged in activities for establishing the technical basis for the large-scale in vitro screening of substances for endocrine disrupting properties.

*EU Member States*

Member States (MS) experts and competent authorities play an active role in regulatory and scientific activities at the EU and international level under the auspices of the OECD.

Several MS have also taken unilateral national legislative measures to restrict the use of some chemicals, despite the impact of such measures on the functioning of the internal market. Their major motivation for doing so is borne out of frustration with progress at EU level. Bisphenol A is undoubtedly the most pertinent example; its use has been banned in specific consumer products aimed at babies or young children in France, Denmark, Austria, Belgium and Sweden. Some Member States, such as Italy and Denmark, have also taken 'soft' measures at the national level such as providing advice to the public, creating incentives for industry for the development of safer alternatives or the promotion of voluntary agreements.

Several Member States provide national funding for research and have established specific research programmes and collaboration. For example, The Danish Centre for Endocrine Disrupters formed in 2008 as a network of scientists and relevant institutions.

Together with the results of Task 2, the material gathered in Task 1 will form the basis of the core of this project, the case studies in Task 3.

### *2.4 Summary of commonalities and differences in the various legislations*

In order to map the commonalities and differences in approaches to testing and assessment of endocrine disrupting substances within the European Union and with international trading partners, an expert consultation exercise was carried out and completed by web searches. Overviews of the different legislative frameworks and activities relevant to endocrine disruption are described in the rest of this document.

Preliminary findings suggest that;

- Most jurisdictions have had to address chemical hazards retrospectively. There are generally separate provisions to deal with existing chemicals (already placed on the market) and new substances. In some instances, this results in different information requirements for new and existing chemicals.
- It is also common for certain uses of chemicals, e.g. as pesticides, food additives, medicinal drugs, cosmetics, or chemical pollution in a given environmental media to be regulated by specific chemical legislation in addition to general chemical legislation. This is generally translated by more stringent pre-market information requirements or post-market monitoring.
- A notable difference between trading partners is related to whether the burden of proof resides mainly with the regulator or the manufacturer/applicant. There are however indications of a tendency to shift that burden to the latter. This has potential implications for sharing data along the supply chain and with the public.
- There appears to be a general consensus that endocrine disrupters raise a level of concern equivalent to that of Carcinogens, Mutagens and Reproductive toxicants (CMRs) or Persistent, Bioaccumulative and Toxic chemicals (PBT).
- There are however different stances on whether the concerns above require additional or complementary regulatory approaches or whether these concerns are adequately addressed by current legislation.

When legal action has been taken, approaches also vary widely from soft voluntary programmes with industry or recommendations to consumers, to implementation of testing and screening programmes through to actual restriction measures based on hazard criteria.

## 3. DESCRIPTIVE OVERVIEW OF RESULTS OF REGULATORY SCREENING, PRIORITY SETTING, TESTING AND ASSESSMENT IN THE EU AND RELEVANT INTERNATIONAL TRADING PARTNERS

In this section we give a descriptive overview of results of regulatory screening, priority setting, testing and assessments as regards endocrine disruptors in the EU (including within the Member States) and relevant international trading partners (US, Canada, Australia, Japan, China).

Additionally, some non-governmental organisations (NGO) such as the Endocrine Disruption Exchange (TEDX) and ChemSec have published their own lists of endocrine disruptors (e.g. SINList 2.1) that may be used to prioritise or include chemicals in the candidate list of Substances of Very High Concern under REACh (Annex XIV). These may therefore also be of relevance and are also been considered.

To develop a descriptive overview of the different approaches, the OECD conceptual framework and related guidance documents will be used as a frame of reference, whereby the activities ongoing in non-EU and EU countries will be mapped onto the Framework. Particular attention will be given to the following aspects as they may drive the outcomes of different approaches to testing, prioritisation, etc.:

- The selection criteria for chemicals to be screened, whether related to specific legislative imperatives or existing information such as in silico data, peer-reviewed literature, or a measure of potential exposure.
- The test or battery of assays employed and the endocrine modalities and taxonomic groups covered. This will include consideration of whether results for human health and environmental receptors are integrated.
- The interpretation of results of individual tests and integration of evidence from different tests and whether expert judgment is used on a case-by-case basis or other criteria-based methods such as decision trees or weight-of-evidence approaches are recommended.

The following insights are emerging from the material gathered:

### *3.1 Selection criteria for the screening of chemicals for ED properties*

With the Endocrine Disrupter Screening Programme (EDSP), the USA seems to have the most advanced and elaborated screening programme for endocrine disrupting properties. Prioritisation for screening is derived on the basis of biological activity and monitoring data which includes quantitative structure-activity relationships (QSAR) and test outcomes of the ToxCast programme.

A similar approach, with biological activity as the driver for prioritisation and screening is taken in the Japanese ExTEND 2010 programme.

Several other efforts, particularly in the EU, have used information available from the literature. Examples are the RPS BKH Endocrine Disrupter Database for the European Commission and the SIN list.

### *3.2 Assays, endocrine modalities and taxonomic groups*

The assay systems used in the various screening and testing programmes will be mapped onto the OECD Framework, as shown in the Table below.

Most testing and screening activities do not go beyond the familiar estrogen, (anti)androgen and thyroid modalities (EAT); some also encompass steroidogenesis. It is notable that the US activities encompass a large number of in vitro assays for these modalities, more than are currently validated by OECD activities.

| OECD Framework | REACH, BPR, PPPR | US, Toxcast, EDSP | EXTEND 2010 |
|---|---|---|---|
| **Level 1**<br><br>Existing Data and Non-Test Information | QSAR, read across and *in vitro* testing with different ED modalities are used under REACH | QSAR, HTP assays | QSAR, HTP assays |
| **Level 2**<br><br>*In vitro* assays providing data about selected endocrine mechanism(s) / pathways(s) | | yes | yes |
| **Level 3**<br><br>*In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s) | | yes | yes |
| **Level 4**<br><br>*In vivo* assays providing data on adverse effects on endocrine relevant endpoints | yes | yes | |
| **Level 5**<br><br>*In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism | yes | yes | |

### *3.3 Interpretation of results, decision trees*

The interpretation of test results has been aided by the use of decision trees, such as the one developed by the German BfR in the context of EU pesticide legislation[1]. The development of weight-of-evidence approaches is making progress with the SYRINA method[2] .

[1] Described in Kortenkamp A, Martin OV, Faust M, Evans R, McKinlay R, Orton F and Rosivatz E 2012, State of the art assessment of endocrine disrupters. Final Report.[Online] Available at: http://ec. europa. eu/environment/endocrine/documents/4_SOTA% 20EDC% 20Final% 20Report

[2] Vandenberg LN, Ågerstrand M, Beronius A, Beausoleil C, Bergman Å, Bero LA, Bornehag CG, Boyer CS, Cooper GS, Cotgreave I, Gee D, Grandjean P, Guyton KZ, Hass U, Heindel JJ, Jobling S, Kidd KA, Kortenkamp A, Macleod MR, Martin OV, Norinder U, Scheringer M, Thayer KA, Toppari J, Whaley P, Woodruff TJ, Rudén C. A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals. Environ Health. 2016 Jul 14;15(1):74. doi: 10.1186/s12940-016-0156-6

## 4. IDENTIFICATION AND ASSESSMENT OF COMMONALITIES AND DIFFERENCES IN SCREENING APPROACHES, PRIORITY SETTING APPROACHES, TESTING APPROACHES AND ASSESSMENTS OF CHEMICALS REGARDING THEIR ENDOCRINE DISRUPTING PROPERTIES

This section reports the results of a deep analysis of the commonalities and differences in screening, priority setting and testing of chemicals for their endocrine disrupting properties in the EU and among EU trading partners. In accordance with the technical specifications for this project, we have chosen the method of case studies for the analysis.

The goals of this work were to address the following issues:

- Are the differences and commonalities in the different legalities due to differences in scientific approaches, or are they an expression of the different features of the respective legal systems?
- What impact do differences in approach have on the final outcome of the derivation of regulatory values (e.g. water quality standards, acceptable daily intakes and similar)?

The case studies were chosen to represent both human health and the environment and to cover several regulatory domains, including media-oriented regulation (e.g. water) and several chemical-oriented regulations (pesticides, chemicals in consumer items, general chemicals). With this in mind, the following substances were selected for the case studies:

- Ethinylestradiol and estradiol (environmental risk assessment)
- Nonylphenol (environmental risk assessment)
- Bisphenol A (human health)
- Di-ethylhexyl phthalate (human health)
- Mancozeb (human health)
- Prochloraz (human health)
- Procymidone (human health)
- Benzophenone-3 (human health)

To facilitate a systematic comparison between the case studies, which would help identifying commonalities and differences in the assessments, a common structure was developed, with the following subheadings:

- Discovery as an endocrine disruptor
- Regulatory framework in selected jurisdictions
- Assessment and measurement endpoints for hazard characterisations
- Assessment values and their derivation
- Exposure assessments
- Risk assessments

The presentation of the eight case studies is followed by an analysis of commonalities and differences in the regulatory status and the assessment values used in the various legalities.

### *4.1 Case Study: 17α-Ethinylestradiol and 17β-Estradiol in the Aquatic Environment*

Summary

The endocrine disrupting properties of 17α-ethinylestradiol (EE2) and 17β-estradiol (E2) on fish have been discovered not through screening exercises, but through chance observations by anglers of hermaphroditism in fish in the United Kingdom. Today, the endocrine disrupting effects of EE2 and E2 at low ng/L concentrations on individual fish are well established and are not controversial. What is unresolved is whether there are negative impacts on the ability of fish to reproduce at environmental exposures to EE2 and E2. For the purpose of environmental risk assessments, various assessment values for EE2 and E2, so-called Predicted No-Effect Concentrations (PNECs), have been derived in Canada, the USA, China and Europe by using deterministic approaches and by constructing Species Sensitivity Distributions (SSDs). For EE2, these values range from 0.016 – 0.5 ng/L and for E2 from 0.1 – 2 ng/L. The differences are explained mainly by the choice of assessment factors that were used to convert experimental values into PNECs. Although of lesser importance in the case of EE2, the choice of experimental studies and fish species also had an impact on the different PNECs for E2. While non-endemic, sometimes very sensitive, species are used to construct SSDs for European PNECs, only resident species are considered in the USA, Canada and Australia. Due to the smaller water flow available for dilution of sewage treatment discharges in Europe, considerable proportions of river stretches cannot comply with various EE2 and E2 PNECs. In the USA, the proportion of river segments that exceed PNECs is considerably smaller. Risk management options for reducing the discharge of EE2 and E2 are limited to upgrading sewage treatment technologies, at considerable cost. This is currently opposed by the pharmaceutical and water industries, with the argument that detrimental effects on the ability of fish populations to reproduce have not been shown, despite evidence for signs of endocrine disruption at current exposure levels.

Scope of this case study

Most of the studies addressing 17α-ethinylestradiol (EE2) or 17β-estradiol (E2) as environmental endocrine disruptors have focused on effects on aquatic wildlife. The risks to human health e.g. via drinking water are generally considered negligible (Laurenson et al. 2014). Accordingly, this case study focuses on the effects of EE2 and E2 on wildlife, especially fish. The considerable literature on cancer risks associated with the therapeutic use of steroidal estrogens e.g. in hormone replacement therapy is deemed out of scope.

Discovery as an endocrine disruptor

The discovery of EE2, E2 and other steroidal estrogens as endocrine disruptors in fish was accidental; it was not the result of systematic screening, nor were the endocrine disrupting properties of EE2 or E2 in fish predicted on the basis of their biological activity in humans.

Rather, it was the accidental observation in the 1980s by anglers of hermaphrodite fish in the settlement lagoons of some sewage treatment works (STWs) in south-east England that triggered research into the effects of STW effluents on fish. In subsequent studies with caged fish in UK rivers near STW outlets, the observation was made that male fish exhibited feminised phenotypes, characterised by the production of the female yolk protein precursor vitellogenin and an intersex state where the reproductive tracts contains male and female gonads (Purdom et al. 1994).

Systematic field studies in the UK then revealed that this kind of endocrine disruption was widespread in wild fish caught near STW outlets (Jobling et al. 1998). The effect could be traced to steroidal estrogens in STW effluents. The most potent components of these effluents were identified as EE2 and E2 (Desbrow et al. 1998), present at concentrations in the low ng/L range in UK rivers.

Experimental exposures under controlled laboratory conditions demonstrated that EE2 and E2 could induce the intersex state with sometimes near complete reproductive failure (Lange et al. 2001, 2009, Nash et al 2004). Exposure to EE2 for 3 years in a Canadian Lake led to the collapse of the residing fathead minnow population (Kidd et al. 2007). Similar observations have since been repeated in numerous studies throughout the world. Accordingly, the endocrine disrupting effects of EE2 and E2 at low ng/L exposure concentrations on individual fish are not controversial. What is currently under investigation is the impact of feminisation on the ability of fish to reproduce at environmental exposures to EE2 and E2.

The consequences of feminisation on the reproductive capability of breeding groups of wild fish were shown to be significant in wild caught roach with relatively severe intersex. However, mild intersex had little impact on the ability of the fish to reproduce (Harris et al. 2011). Essentially the same results were obtained in a study where roach were exposed in the lab to STW effluent for 3 years (Hamilton et al. 2015). A field study investigating the impact of endocrine disruption in wild roach in the UK found that fish populations in river stretches exposed to EE2 and E2 were able to reproduce, despite being affected by intersex (Hamilton et al. 2014). However, the authors of that study highlight several factors that might have obscured the detection of reproductive impacts in the wild, including migration of fish and the introduction of populations to certain river stretches.

Regulatory framework in selected jurisdictions

With the exception of Canada (British Columbia), no environmental quality standards for EE2 or E2 exist in any jurisdiction in the world, although various risk assessment exercises have been conducted on the basis of draft or provisional reference values.

*European Union*

The Water Framework Directive and its daughter directives, the 2008/105/EC Environmental Quality Standards Directive (EQSD) and the 2013/39/EU Directive with the latest amendment of the list of Priority Substances (PS), are the existing body of legislation for the protection and sustainable use of European water resources in which chemicals in the aquatic environment are regulated. The Water Framework Directive articulates a holistic 'response' principle – Good Ecological Status – and a chemical-related assessment - Chemical Status. The latter is based on Environmental Quality Standards for selected PS which all Member States need to adhere to. The European Commission had developed draft Environmental Quality Standards (EQS) for EE2 and E2 (European Commission 2011 a, b), but this was replaced by a watch list mechanism established for emerging pollutants. The 1st EU Watch List was launched in 2015; it encompasses EE2 and E2. For chemicals on the Watch List, monitoring data will be acquired at European Union-wide level and these data will be used to support future chemical prioritization initiatives.

*United States of America*

The National Environmental Policy Act requires the US Food and Drug Administration (FDA) to assess environmental impacts that result from the approval of individual drug applications. An exemption from the requirement for an environmental assessment is possible when the estimated concentration of the substance at the point of entry into the aquatic environment is below 1 part per billion (i.e. 1 µg/L, which is higher than the derived PNECs in the table below) or when the application does not increase the use of the active substance. Most drug applications for pharmaceutical estrogens, including EE2, have qualified for this exemption. Nevertheless, the Center for Drug Evaluation and Research (CDER) at the FDA has conducted a risk assessment exercise for EE2 in US aquatic environments which showed that approximately 1% of river stretches exceed a PNEC of 0.1 ng/L (Laurenson et al. 2014).

*Canada (British Columbia)*

The Ministry of the Environment of British Columbia has established ambient water quality guidelines for EE2 (Nagpal and Meays 2009). To protect freshwater aquatic life from adverse effects, these guidelines recommended that the 30-d average concentration of EE2 in water should not exceed 0.5 ng/L, with no single value above 0.75 ng/L.

*Japan*

Japan's basic Environmental Law establishes two kinds of Environmental Quality Standards relevant to water pollution – standards for the protection of human health, and standards for protecting the living environment. Environmental Quality Standards have been set for many water-borne pollutants, but there are currently no such provisions for EE2 or E2.

*China*

In China, a "black" list of 68 priority pollutants was developed for regulatory purposes, mainly based on existing priority lists in the USA and the EU. Based on this list, regional priority pollutants were selected for Beijing, Tianjin, and Zhejiang (Jin et al. 2014). There are currently no binding standards for EE2 or E2.

Assessment and measurement endpoints for hazard characterisations

In aquatic communities exposed to EE2 and E2, fish are generally considered to be the most sensitive taxa. The assessment endpoint in hazard characterisations is fish reproduction, with the aim of ensuring exposure levels low enough to maintain self-sustaining fish populations. Widely selected measurement endpoints for this assessment endpoint include the induction of vitellogenin in male fish, changes in the presence of secondary sexual features, intersex status, skewed sex ratios from male to female, and endpoints relevant to reproductive success, such as sperm quality. Of these, vitellogenin induction is generally considered as a biomarker of the effects of estrogenic chemicals on fish, rather than a measurement endpoint for adverse effects for the derivation of regulatory standards.

Assessment values and their derivation

Ecotoxicological hazard characterisations aim to establish Predicted No-effect Concentrations (PNECs). For EE2 and E2, two different approaches have been used for this purpose: The "deterministic" approach focuses on a single experimental study considered to be "critical", which is then used to derive a No-Observed-Effect-Concentration (NOEC). Alternatively, a Species Sensitivity Distribution (SSD) is used to establish a hazardous concentration (HC) that affects 5% of relevant taxa (HC05). NOECs and HC05 values are then combined with assessment factors (AF) to yield a PNEC. Often, both approaches are used side-by-side.

The deterministic and the SSD approach have been used widely, and have produced PNECs ranging from 0.016 to 0.5 ng/L for EE2 and 0.1 to 2 ng/L for E2 (Table 1). The PNECs that formed the basis for the draft European Commission EQS are highlighted in bold.

| Table 1: EE2 PNECs | | | | |
|---|---|---|---|---|
| **PNEC (ng/L)** | **Approach** | **Basis (points of departure)** | **AF** | **Source and year** |
| 0.5 | Deterministic | LOEC of 1.0 ng/L from 3 experimental studies in fish for chronic reproductive effects and egg production | 2 | British Columbia, Nagpal and Meays (2009) |
| 0.35 | SSD | Based on chronic NOECs for 26 species; HC05 = 0.35 ng/L, but see update by Caldwell et al. (2012) below | none | Caldwell et al. (2008) |
| 0.1 | Deterministic | NOEC = 0.57 ng/L from a full life cycle study in zebrafish (Wenzel et al. 2001) | 5 | UK Environment Agency (2004) |
| 0.1 | Deterministic | LOEC = 0.19 ng/L for mortality in fertilised zebrafish eggs (Soares et al. 2009) | 2 | CDER commissioned study, see Laurenson et al. (2014) |
| 0.1 | SSD | As Caldwell et al. (2008), but updated taking account of new data in Chinese rare minnows, Zha et al. (2008), with an extrapolated NOEC of 0.1 ng/L (from a LOEC of 0.2 ng/L); HC05 = 0.06 – 0.08 ng/L | < 1 | Caldwell et al. (2012) |
| **0.035** | **SSD** | **Based on chronic reproductive NOECs, HC05 = 0.07 ng/L** | **2** | **European Commission (2011 a), draft EQS** |
| 0.016 | Deterministic | Based on a LOEC = 0.32 ng/L for decreased egg fertilisation and skewed sex ratios in the fathead minnow (Parrot and Blunt 2005) | 20 | European Commission (2011 a) |
| | | **E2 PNECs** | | |
| 2 | SSD | Based on 21 NOECs for chronic reproductive toxicity, HC05 = 4.3 ng/L | 2 | Caldwell et al. (2012) |
| 1 | Deterministic | LOEC = 10 ng/L, toxicity on early life stages of the medaka (Nimrod and Benson 1998) | 10 | UK Environment Agency (2004) |
| 0.73 | SSD | Based on 31 NOECs for chronic reproductive toxicity and other endpoints, HC05 = 1.46 ng/L | 2 | Wu et al. (2014) |
| **0.4** | **SSD** | **Based on NOECs for chronic reproductive toxicity, HC05 = 0.8 ng/L** | **2** | **European Commission (2011 b), draft EQS** |
| 0.1 | Deterministic | Based on extrapolated NOEC = 0.5 ng/L for semen quality in rainbow trout (Lahnsteiner et al. 2006) | 5 | European Commission (2011 b) |

The following issues, which all impact to varying degrees on the numerical value of these PNECs, have been debated in the literature:

*The choice of critical experimental studies and selection criteria*

Different experimental studies were chosen for the various PNECs listed in Table 1, and criteria for the selection of these studies were not always made explicit, nor were they consistent. For EE2, Caldwell et al. (2008, 2012), a publication by the pharmaceutical industry, selected studies according to Klimisch scores, with a requirement for a score of 1. The quality criteria for the studies that underpinned the derivation of all other EE2 PNECs are unclear. For E2, Wu et al. (2014) and the European Commission (2011 b) used studies with Klimisch scores of 1 and 2, but which quality criteria were used to select studies for all the other E2 PNECs is unclear.

Despite the fact that different sets of NOECs were used to construct the EE2 SSDs by Caldwell et al. (2012) and by the European Commission (2011 a), the respective HC05 values were very similar (0.06-0.08 ng/L in Caldwell and 0.07 ng/L in European Commission). The lowest NOEC in Caldwell was 0.1 ng/L, an extrapolated NOEC from Zha et al. (2008) (LOEC = 0.2 ng/L). The lowest NOEC in the European Commission SSD was 0.16 ng/L, extrapolated from the LOEC of 0.32 ng/L by Parrot and Blunt (2005), which was also used by Caldwell et al. (2012). The study by Soares et al. (2009), with a LOEC of 0.19ng/L, was critical for the EE2 PNEC derived by CDER, but this value was not used for constructing the European Commission SSD. The Soares study was erroneously assigned a NOEC of 1 ng/L in the SSD by Caldwell et al. (2012), but use of the correct value would have had little impact on the Caldwell HC05, because here the Zha study with an extrapolated NOEC of 0.1 ng/L was used.

Different studies were also chosen to build SSDs for E2, but here, this had a greater impact on the respective HC05 values. The lowest value in Caldwell et al. (2012) was 5 ng/L, from a study by Nash et al. (2004), which in fact is not a NOEC. The smallest NOEC used by the European Commission (2011 b) for constructing their SSD was 0.5 ng/L from Lahnsteiner et al. (2006), not used by Caldwell et al. (2012). The Lahnsteiner study was also the lowest NOEC in Wu et al. (2014), but assigned a NOEC of 1 ng/L. Furthermore, Wu et al. used a variety of endpoints, not strictly limited to reproductive toxicity, including vitellogenin induction. These differences and inconsistencies readily explain why the SSDs from Caldwell, Wu and the European Commission produced HC05 values of 4.3, 1.46 and 0.8 ng/L, respectively.

*The choice between the deterministic and the SSD approach*

In general, the NOECs derived by the deterministic approach tended to be higher than the HC05 derived from SSDs, with the exception of the 0.5 ng/L E2 NOEC from the Lahnsteiner study. However, since the SSD approach is also sensitive to the choice of NOECs, this does not necessarily mean that it generally produces more conservative PNEC estimates.

Consequently, the argument about whether to use one or the other approach for deriving PNECs turns on matters of principle. The use of the SSD approach is rejected by some (e.g. by the British Columbia Environment Ministry, Nagpal and Meays, 2009) and favoured by others (e.g. the pharmaceutical industry, Caldwell et al. 2008, 2012). Arguments against the SSD approach are (Nagpal and Meays, 2009):

- The results of an SSD are sensitive to the choice of NOECs.
- NOECs are literature values which are influenced by the experimental design (e.g. concentration levels and concentration spacings used in toxicity studies). A benchmark approach based on critical effect concentrations (e.g. EC10) would be more reliable.

- NOECs vary according to the end point employed in the toxicity test. A PNEC derived from NOECs with mixed end points is regarded by many scientists as less desirable than a PNEC obtained from NOECs with single end points (e.g. critical reproductive effects).
- SSDs do not necessarily protect the most sensitive species and life stage, and there is the issue of representativity of the selected species.

Conversely, the advantage of the SSD method is seen in its use of the entire sensitivity distributions of species in an ecosystem for the establishment of a PNEC instead of taking always the lowest long-term NOEC. Accordingly, if appropriate data are available, SSDs are favoured in the USA and the EU. In the EU, deterministic and SSD approaches can be used side-by-side.

*Use of non-endemic species for the derivation of HC05 values from SSDs*

The European Commission (2011 a, b) as well as the pharmaceutical industry (Caldwell et al. 2012) have included fish species not endemic in Europe for their SSDs. In contrast, in Canada, the USA and Australia only endemic wildlife species are used as test species to derive water quality values (Nagpal and Meays, 2009, USEPA 1985, Hose and Van den Brink 2004).

*The choice of assessment factors*

As can be seen from Table 1, the choice of assessment factors (AF) can have a considerable impact on the numerical value of PNECs for EE2 and E2. With the deterministic approach, AFs of between 2 and 20 were used, while with the SSD approach AFs between 1 and 5 were applied, in line with ECHA guidance.

Only the PNEC for EE2 proposed by the pharmaceutical industry was derived directly from an SSD, without application of an AF (Caldwell et al. 2012). The pharmaceutical industry opposes the application of an AF > 1 in this case, with the argument that a PNEC of 0.1ng/L is smaller than the NOECs for commonly tested fish species. As a result, so the argument goes, this PNEC will be protective for fish species commonly found in Europe.

In contrast, the European Commission (2011 a, b) and the European Commission Scientific Committee on Health and Environmental Risks (SCHER 2011 a,b) regard the application of an AF as essential, to compensate for the fact that most NOECs used in the various SSDs are not from multi-generation studies. According to SCHER, this is particularly relevant in view of evidence that the toxicity of EE2 and E2 increases from generation to generation. Furthermore, the endpoints included in the NOECs used for constructing the various SSDs are not always the most sensitive.

*Other issues*

Of all the PNECs derived for E2, the one suggested by the pharmaceutical industry (Caldwell et al. 2012) is the highest (2ng/L). The values used by all other authorities and authors are by a factor of 2 to 20 lower than the industry value. The lowest PNEC for E2 (0.1 ng/L) has been derived by the European Commission, using a deterministic approach based on a NOEC of 0.5 ng/L for semen quality and other reproductive endpoints from a 35 day study in the rainbow trout (Lahnsteiner et al. 2006). However, this value was not chosen as the basis for the draft EQS.

The HC05 of 0.8ng/L in the European Commission SSD approach was driven by inclusion of the Lahnsteiner study. The pharmaceutical industry opposed consideration of the Lahnsteiner study for deriving a PNEC for E2, however, the European Commission SCHER saw no flaw with this study (SCHER 2011 b).

Exposure assessments

Exposure assessments for EE2 and E2 in rivers have been conducted in numerous countries and economic areas, including the European Union, the USA, China, Canada, Japan and Australia. These were based on either Measured Environmental Concentrations (MECs) or on values derived through hydrological modelling, Predicted Environmental Concentrations (PEC).

*Measured Environmental Concentrations (MECs)*

There are significant technical problems with measuring EE2 and E2 levels in riverine waters, due to the detection limits often being higher than the biologically effective concentrations or the various proposed PNECs. Despite the fact that many studies returned non-detects, the EE2 and E2 concentrations measured in locations in the European Union, the USA, or China were in the range of several ng/L, depending on location (Laurenson et al. 2014, Wu et al. 2014, European Commission 2011 a,b).

*Predicted Environmental Concentrations (PECs)*

The use of models has helped to overcome the difficulties with measuring EE2 and E2 in surface waters. On the basis of assumptions about per capita EE2 usage and E2 excretion, per capita wastewater outputs, metabolic and wastewater treatment removal rates, and instream dilution and loss processes in water bodies, models can provide estimates of EE2 and E2 concentrations in surface waters. The models that have been used to generate PECs for EE2 and E2 include PhATE (Pharmaceutical Assessment and Transport Evaluation) in the USA, GREAT-ER (Geography Referenced Regional Exposure Assessment Tool for European Rivers) and GWAVA in the European Union. The models differ in their assumptions about per capita use of EE2, per capita water usage and available dilution in rivers.

By using PhATE, approximately 1% of river segments in the USA are estimated to exceed an EE2 concentration of 0.1 ng/L. According to GREAT-ER, 23% of river segments in Europe exceed that value (see the review in Laurenson et al. 2014). Johnson et al. (2013), using GWAVA, estimated that 12% by length of European rivers exceed the European Commission Draft EQS for EE2 of 0.035 ng/L, and 1% would reach concentrations greater than the Draft EQS of 0.4 ng/L for E2.

A major factor explaining these differences between the USA and Europe is the water flow available for dilution of STW discharges. This is generally larger in the USA than in Europe.

Humans are not the only source of emission of steroidal estrogens to surface waters, emissions from life stock and farming have also been recognised as relevant (Laurenson et al. 2014).

Risk assessments

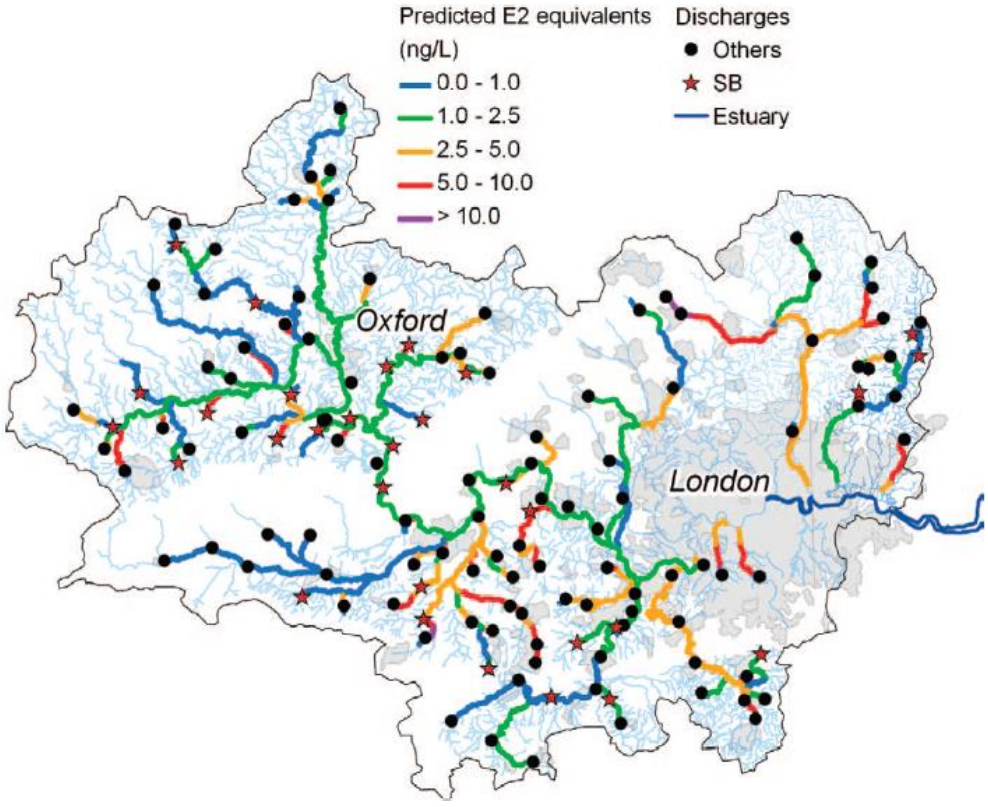
The possible risks to aquatic life of exposures to EE2 and E2 were assessed in several studies, by building risk quotients of MECs or PECs and various PNECs.

One such assessment, classed as a screening assessment, was conducted by CDER for EE2 in the USA (Laurenson et al. 2014), by comparing PECs obtained by modelling (PhATE) with the EE2 PNEC of 0.1 ng/L. The mean long-term PECs for EE2 were smaller than this PNEC in approximately 99% of US surface waters downstream of STWs, with a median PEC more than two orders of magnitude smaller than this PNEC. The approximately 1% of exceedances were in localized, effluent-dominated stream segments (Laurenson et al. 2014).

Due to the smaller water flow available for dilution of discharges into riverine waters, the situation in Europe, particularly the UK, is very different. In relation to the PNECs derived by the European Commission for EE2 and E2, 0.035 and 0.4ng/L, respectively, long river stretches were found to exceed these values (Johnson et al. 2013). Even compliance with the higher EE2 PNEC of 0.1 ng/L will be difficult in many areas of the European Union.

Because the focus on EE2 alone may underestimate the extent of exceedance of PNECs, attempts have been made to assess the risks from combined exposures to steroidal estrogens, including EE2, E2, estrone and estriol. PECs for these steroids were derived by modelling, converted into E2-equivalents, summed up and evaluated in relation to E2-equivalent PNECs of various magnitudes.

Williams et al. (2009) presented the first assessment of this kind for the United Kingdom. They evaluated the predicted E2-equivalents against an E2-equivalent PNEC of 1 ng/L (the value derived for E2 by the UK Environment Agency 2004) and found that only 39% of the modelled river segments were not at risk from combined endocrine disruption (E2-equivalent PEC < 1 ng/L). If the PNEC of 0.4 ng/L for E2 had been used in these assessments, an even higher proportion of river stretches would have been declared as "at risk". To illustrate this point, the figure below (Williams et al. 2009) shows the distribution of predicted E2-equivalents in the Thames catchment in the London metropolitan area. It is evident that only a few stretches would comply with an E2-equivalent PNEC of 0.4 ng/L, the Draft European Commission EQS for E2 (SB = secondary biological filter).




On the basis of short-term and long-term E2-equivalent PNECs of 5 and 2 ng/L, Anderson et al. (2012) conducted a similar analysis across 12 US watersheds. They found that only 0.8% of the segments of these river catchments would exceed 2 ng/L. The authors concluded that aquatic species in most US waters are not at risk from the release of steroidal estrogens by humans.

*The question of reproductive ability of fish populations*

While it is not under dispute that individual fish are likely to show signs of endocrine disruption such as intersex at EE2 and E2 levels found in many European surface waters, the debate has moved to the question whether this degree of endocrine disruption will affect fish populations to an extent that compromises their reproduction. Although there is evidence that cyprinid fish populations affected by mild intersex are able to sustain themselves (Hamilton et al. 2014, Harris et al. 2011), this question cannot currently be answered conclusively, especially not in relation to a concentration that will protect from population level effect. There is evidence that severe intersex, found particularly in older fish with long-term exposures, can compromise reproduction (Harris et al. 2011).

The pharmaceutical and water industries in the UK and the European Union are strongly opposed to implementing controls over the discharge of EE2 or E2 via STW effluents, unless clear evidence of significant ecological damage can be provided.

Risk management options

The UK Environment Agency has decided that there is a strong case for risk management measures (Gross-Sorokin et al. 2006). However, management options are limited: Reducing the levels of EE2 and E2 in the aquatic environment will require end-of-pipe solutions, with an upgrade of STW to remove steroidal estrogens from effluents. This is currently implemented in Switzerland.

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### *4.2 Case Study: Nonylphenols in the Aquatic Environment*

Summary

Nonylphenols were discovered as endocrine disruptors (estrogen mimics) not through systematic screening programmes, but by the accidental discovery of unusual estrogenic effects of cell culture media stored in laboratory plastic ware from which it leaked and contaminated the media. Since then, estrogenic effects of nonylphenols have been demonstrated in numerous assays and test systems. Water Quality Criteria (WQC) and Environmental Quality Standards (EQS) exist in the European Union, the USA and Canada. Chinese scientists have derived equivalent values for China. The various values range from 0.1 to 6.6 µg/L, with the USA WQC being the least restrictive. These variations are explained by the use of different sets of toxicity data, together with different methodologies for their derivation. In the USA, extrapolations from acute toxicity data to chronic toxicity were made by using acute-to-chronic ratios. In Canada, the European Union and China, WQC and PNECs were derived by using deterministic approaches, based on NOECs from critical toxicity studies, with application of an assessment factor of 10. The endocrine disrupting properties of nonylphenols were not decisive for deriving WQS or Predicted No-effect Concentrations (PNECs); other chronic toxicities were critical for setting these values. Nevertheless, European and USA authorities have assessed the degree of protection from endocrine disruption afforded by these values by comparison with data for endocrine disruptor tests in whole organisms and ascertained that such protection was achieved. Risk assessments were commonly performed by building risk quotient of measured nonylphenol concentrations and the respective WQC or PNECs applicable in the various legislations. These assessments have shown that nonylphenol WQC are exceeded in certain areas in Canada, the European Union and China.

Scope of this case study

This case study focuses on the effects of nonylphenol isomers on aquatic wildlife. In many risk assessment efforts this, rather than possible effects on human health, has been recognised as critical (ECHA 2014).

Discovery as an endocrine disruptor

The endocrine disrupting properties of nonylphenols were discovered accidentally when cell culture media stored in laboratory plastic ware was found to induce unusual cell-proliferative responses in estrogen-receptor positive human mammary gland cell lines (Soto et al. 1991). Estrogenic chemicals are capable of producing such effects in these cells. In painstaking investigations, nonylphenols were proven to leach from the plastics into the cell culture medium, and were shown to be responsible for the estrogenic effects. Since then, nonylphenols have been tested in numerous assays and test systems relevant for the detection of estrogen-like effects, including the pure steroid receptor, estrogen receptor-dependent gene reporter assays, cell lines and whole organisms including rats and fish.

Regulatory framework in selected jurisdictions

Nonylphenols are a group of different isomers, not a single chemical compound. This often complicates the interpretation of toxicological studies, because the precise composition and provenance of the material used was not specified. Of importance as endocrine disruptors are the para-substituted alkyl phenols, 4-nonylphenols. The alkyl side chain can be linear (CAS No 104-40-5) or branched (CAS No 84852-15-3). 4-nonylphenols with unspecified side chains are assigned CAS No 25154-52-3. Many regulatory values do not distinguish between these isomers and CAS No.

*European Union*

Nonylphenol (defined as CAS No 84852-15-3 and 25154-52-3) is a Priority Substance under the Water Framework Directive and is listed in the 2008/105/EC Environmental Quality Standards Directive (EQSD) and the 2013/39/EU Directive with the latest amendment of the list of Priority Substances (PS). All EU Member States are required to adhere to the Environmental Quality Standard (EQS) for freshwater of 0.3 µg/L. Pollution from Priority Substances should be progressively reduced.

*United States of America*

Section 304(a)(1) of the Clean Water Act of 1977 requires the US EPA to publish Water Quality Criteria (WQC) for pollutants. If these are adopted by States as Water Quality Standards (WQS), they become enforceable maximum acceptable pollutant concentrations in ambient waters in that State. The WQC for nonylphenol (final chronic value for freshwater) is 6.6 µg/L, not differentiating between the various isomers (US EPA 2005).

*Canada*

Canada's Water Quality Guidelines for the protection of aquatic life specify a concentration of nonylphenols of 1.0 µg/L in freshwater, and 0.7 µg/L in marine water (Canadian Council of Environment Ministers, CCEM 2002). The values apply to 4-nonylphenols with branched side chains (CAS No 84852-15-3).

*China*

There are currently no water quality criteria for nonylphenols in China, although several Chinese scientists have derived such criteria (Lei et al. 2012, Gao et al. 2014).

To our knowledge, water quality criteria for nonylphenols are not in place in Australia or Japan.

<u>Assessment and measurement endpoints for hazard characterisations</u>

A wide variety of assessment endpoints have been used for hazard characterisations of nonylphenols and these have formed the basis for the various regulatory values. Generally, whole organism endpoints were employed to derive water quality criteria. Endpoints relevant to endocrine disruption have played a relatively minor role in these efforts. Where they were used or where they proved critical for the derivation of WQC or EQS, measurement endpoints included reproduction, fecundity and biomarkers of estrogenicity such as induction of vitellogenin in fish. Biochemical and cell-based endpoints for estrogenicity were generally not employed.

Often, several toxicity endpoints were integrated, instead of selecting endpoints for specific toxicities, including endocrine disruption.

<u>Assessment values and their derivation</u>

WQC for nonylphenols and the associated Predicted No-effect Concentrations (PNECs) have been derived by a "deterministic" approach and by using Species Sensitivity Distributions (SSDs), often side-by-side. The "deterministic" approach focuses on a single experimental study considered to be "critical", which is then used to derive a No-Observed-Effect-Concentration (NOEC). Species Sensitivity Distributions (SSDs) are constructed on the basis of several NOECs for a variety of species with the aim of establishing a hazardous concentration (HC) that affects 5% of relevant taxa (HC05). NOECs and HC05 values are then combined with assessment factors (AF) to yield a PNEC.

Table 1 shows the various PNECs and freshwater Water Quality Criteria (WQC), together with values important for their derivation, arranged in descending order. These values ranged from 0.1 to 6.6 µg/L. Values based on endocrine disruption endpoints are shaded in grey. The PNECs that formed the basis for the European Union EQS are highlighted in bold.

**Table 1: Nonylphenol PNECs or WQC**

| PNEC or WQC (µg/L) | Approach | Basis (points of departure) | AF | Source and year |
|---|---|---|---|---|
| 6.6 | deterministic | Derived from a Final Acute value of 55.49 µg/L by using a Final Acute Chronic Ratio (FACR) of 8.412 | 8.412 (FACR) | US EPA (2005) |
| 1.59 | SSD | HC5: 1.59 µg/L, based on NOECs for chronic reproductive toxicity, specific for 4-NP with linear side chain CAS No 104-40-5 | 1 | Lei et al. (2012) |
| 1.3 | deterministic | Chronic NOEC: 10.3 µg/L, specific for 4-NP with unspecified side chains CAS No 25154-52-3 | 10 | Lei et al. (2012) |
| 1.34 | SSD | HC5: 1.34 µg/L, based on NOECs for chronic reproductive toxicity, specific for 4-NP with unspecified side chains CAS No 25154-52-3 | 1 | Lei et al. (2012) |
| 1 | deterministic | 91 day LOEC for growth reduction in rainbow trout: 10.3 µg/L | 10 | CCEM (2002) |
| 0.74 | deterministic | Chronic NOEC: 7.4 µg/L, specific for 4-NP with unspecified side chains CAS No 84852-15-3 | 10 | Lei et al. (2012) |
| 0.5 | deterministic | NOEC: 5 µg/L for chronic reproduction, specific for 4-NP with linear side chain CAS No 104-40-5 | 10 | Lei et al. (2012) |
| 0.5 | deterministic | NOEC: 5 µg/L for chronic reproduction, specific for 4-NP with unspecified side chains CAS No 25154-52-3 | 10 | Lei et al. (2012) |
| 0.48 | SSD | HC5: 1.43 µg/L, based on various endpoints which included NOECs and LOECs. Lowest value: LOEC = 0.3 µg/L for fecundity and biomarkers of estrogenicity in rainbow trout (Giesy et al. 2000) | 3 | Gao et al. (2014) |
| 0.42 | SSD | HC5: 2.12 µg/L based on combined marine and freshwater NOECs | 5 | ECHA (2014) |
| **0.3** | **deterministic** | **Long-term NOEC in freshwater algae *Scenedesmus subspicatus*: 3.3µg/L** | **10** | **EU EQS, EU (2005)** |
| 0.39 | deterministic | NOEC in marine mysid: 3.9 µg/L | 10 | ECHA (2014) |
| 0.16 | deterministic | Chronic NOEC: 1.65 µg/L, specific for 4-NP with linear side chain CAS No 104-40-5 | 10 | Lei et al. (2012) |
| 0.1 | deterministic | NOEC: 1 µg/L for chronic reproduction, specific for 4-NP with branched side chains CAS No 84852-15-3 | 10 | Lei et al. (2012) |

*Selection criteria for the choice of experimental studies*

Different experimental studies were chosen for the various PNECs and WQC listed in Table 1, but criteria for their selection were often not made explicit. Lei et al. (2012) based their selection on Klimisch scores of 1 as a minimum quality criterion, ECHA (2014) assigned Klimisch scores to some, but not all, studies and tolerated scores of 2.

US EPA (2005) and the Canadian CCEM (2002) only used results from test organisms resident in North America. US EPA (2005) excluded studies where the test organisms were inadequately described, where dosing was by injection, gavage or an artificial medium, and where the experimental model was not an intact organism.

Gao et al. (2014) used toxicity data from the US EPA ECOTOX database.

*Consideration of specific nonylphenol isomers*

The values listed in Table 1 were generally not derived for specific 4-nonylphenol isomers. US EPA did not specify isomers; most other values are for 4-nonylphenol with branched side chains and for unspecified side chains, i.e. CAS No 84852-15-3 and 25154-52-3 considered together. These distinctions are of importance because different isomers have different potencies, especially in relation to endocrine disruption. The only effort that derived different PNECs for the different CAS No is by Lei et al. (2012).

*Consideration of endocrine disrupting effects*

Where toxicities other than endocrine disruption were decisive for the derivation of PNECs or WQC, several regulatory bodies also assessed the degree of protection from endocrine disrupting effects that results from the respective PNECs and WQCs by comparison with the outcome of critical endocrine disruption endpoints.

For example, US EPA (2005) stated that the USA WQC protect against endocrine disrupting effects because such responses had rarely been reported below the critical value of 6.6 µg/L.

ECHA (2014) noted that the most sensitive fully reliable LOEC in fish was 10 µg/L (endpoints: growth in rainbow trout and sex ratio changes in the zebrafish), but pointed out that first effects on semen quality may start around 0.75 µg/L. In amphibians, the LOEC is smaller than 10 µg/L. Both the EU EQS of 0.3 µg/L and the value of 0.39 used by ECHA for their risk assessment would protect against such endocrine disrupting effects.

*The choice between the deterministic and the SSD approach*

All the PNECs and WQC in Table 1 that are applied in jurisdictions (i.e. EU 2005, CCEM 2002 and US EPA 2005) were derived by deterministic approaches. However, ECHA (2014) also constructed SSDs and estimated HC5 values which were then compared with the deterministic values.

The authors of several Chinese efforts of deriving WQC for nonylphenols (Lei et al. 2012, Gao et al. 2014) expressed a preference for the SSD approach, with the argument that information from a multitude of studies is used, instead of placing a great deal of weight on just one critical study, as in the deterministic approach.

The differences in the various HC5 values derived using the SSD approach are due entirely to different sets of NOECS and LOECs employed for constructing the SSDs. Across the various efforts, no consistent selection criteria for the sets of toxicity values are discernible.

*Use of non-endemic species for the derivation of HC05 values from SSDs*

The EU values were derived by inclusion of species not resident in Europe, such as the fathead minnow or the zebrafish. The USA and Canada rely only on species resident in North America.

*The choice of assessment factors*

With the deterministic approach, an AF of 10 was typically used. With the SSD approach AFs between 1 and 5 were applied, in line with ECHA guidance.

Exposure assessments

Exposure assessments for NP in freshwaters and marine waters have been conducted in numerous countries and economic areas, including the European Union, the USA, China, Canada and Japan. Mostly, these were based on Measured Environmental Concentrations (MECs). Values derived through hydrological modelling, Predicted Environmental Concentrations (PEC), were not often used.

Risk assessments

The possible risks to aquatic life of exposures to nonylphenols were assessed in several studies, by building risk quotients of MECs and the PNECs or WQC applicable in the various jurisdictions.

*European Union*

By deriving risk quotients with the PNEC of 0.39 µg/L, ECHA (2014) concluded that there is no concern for most surface waters in the European Union, with the exception of some Member States where risk quotients exceeding 1 were found. If however, an additional factor of 10 was used to produce a value of 0.039 µg/L, with the aim of accounting for endocrine disrupting properties of nonylphenols, the measured concentrations in most European Union Member States would be of concern. If mixture effects that occur through joint action of NP with other estrogenic chemicals would be accounted for, even larger risk quotient would be the result (ECHA 2014).

*Canada*

Nonylphenol concentrations in Canadian freshwaters range between < 0.01 and 1.7 µg/L (CCEM 2002), thus sometimes exceeding the Canadian WQC of 1 µg/L. It is unclear what proportion of Canadian freshwaters exceed the WQC of 1 µg/L.

*China*

Gao et al. (2014) have presented calculations of risk quotients for a variety of Chinese rivers using their freshwater PNEC of 0.48 µg/L. The risk quotients in freshwater ranged from 0.04 to 69.7, with an average of 6.22. The highest RQ value of 69.7 was recorded at the urban riverine water of the Pearl River Delta in Southern China around the economically highly developed city of Guangzhou. The main sources of nonylphenol are from industrial wastewaters and domestic sewage.

Risk management options

In the European Union, nonylphenol is subject to risk management measures; it fulfils the criteria for being designated an endocrine disruptor laid down in the REACH legislation (ECHA 2014). This means that measures for substitution of nonylphenols should be implemented.

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### *4.3 Case Study: BPA, Bisphenol A, human health*

Summary

Bisphenol A (BPA) is an industrial chemical that is widely used as a monomer or additive for the manufacture of polycarbonate (PC) plastics and epoxy resins and other polymeric materials. It is also used in certain paper products, including thermal paper.

The endocrine disrupting properties of Bisphenol A (BPA) have been discovered through screening exercises back in 1930's.

BPA has been the subject of intense research and debate over the last decade, not least due to suspected low-dose effects (endocrine disrupting properties and developmental neurotoxicity) of the chemical and its presence in food contact materials resulting in exposure of vulnerable groups such as infants and young children.

What is unresolved is whether there are low dose effects and non-monotonic dose responses at the levels to which most people are exposed to BPA. Moreover the reference doses and the risk management of BPA are different internationally.

Scope of this case study

Many studies addressing BPA as endocrine disruptor for humans have focused on effects on male and female reproductive development (studies in rodents). Accordingly, this case study focuses on the effects of BPA on humans and the effect on aquatic wildlife will not be the focus in this report. However, also in the environment effects on BPA in several studies are seen[3]. In a recently published report from the EU commission it is stated (on BPA): "*With respect to vertebrate wildlife evaluation, a plausible link was established since in vitro and in vivo mechanistic data available (binding and agonistic activity to thyroid hormone receptor as well as transthyretin transactivation) were considered as likely to be responsible for the observed malformations in several frog species. Moreover, inhibition of sperm maturation in fish and skewed sex ratio in amphibians could be linked to increased vitellogenin synthesis in male fish suggesting estrogenic activity*".

Discovery as an endocrine disruptor

Chemists synthesized the chemical bisphenol A (BPA) in the laboratory in 1891. In the 1930's, scientists were searching for synthetic chemicals that could replace the expensive natural oestrogen in pharmacological applications. They identified BPA as a weak functional oestrogen. Its use as a pharmaceutical hormone was precluded by the invention of another synthetic chemical, DES, with even more potent estrogenic properties (Dodds and Lawson, 1938). DES was subsequently used as a pharmaceutical that showed severe side effects (Meyers, 1983). Since 1940's and 1950's BPA have been used to make polycarbonate plastics.

In 1993 a team of endocrinologists at Stanford University found an unknown oestrogenic substance that contaminated their assays. Finally, they identified BPA leaching from their polycarbonate cell culture dishes when they were autoclaved (Krishnan et al., 1993).

BPA has been the subject of intense research and debate over the last decade, not least due to suspected low-dose effects (endocrine disrupting properties and developmental neurotoxicity) of the chemical and its presence in food contact materials resulting in exposure of vulnerable groups such as infants and young children (DK-EPA, 2014).

[3] Reported in: EU RAR CAS: 80-05-7 EINECS No: 201-245-8, Environment Addendum of April 2008, 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A), Part 1 Environment.

Regulatory framework in selected jurisdictions

In recent years, the reproductive and developmental toxicity of BPA has been thoroughly evaluated at the national, European and international level. Moreover, during the last 10 years, several risk assessments on BPA have been performed by different regulatory bodies and expert groups in Europe, Canada, USA and Japan. The hazard assessments were mainly based on a comprehensive range of studies conducted in accordance with international testing guidelines and Good Laboratory Practices.

*European Union*

The EU risk assessments under the Existing Chemicals programme concluded that there was no consumer risk associated with the use of BPA.

However, in recognition of the uncertainty as to the effect associated with possible low-dose exposure of BPA and following pressure from several Member States, an EU ban prohibiting the use of BPA for the manufacture of polycarbonate infant feeding bottles was adopted in January 2011 and EU states outlawed the manufacture of polycarbonate feeding bottles containing the compound from March 2011, and banned their import and sale from June 2011[4].

## EFSA

In 2015 EFSA concludes that based on the t-TDI of 4 µg/kg bw/day, and using the EFSA estimates of the total exposure to BPA, there is no health concern.

## EU Member states

### Germany

In REACH a Substance Evaluation on BPA (with focus environment) was started in 2012 with Germany as the rapporteur Member State. One of the initial grounds for concern was: Potential endocrine disruptor. The status is that information is requested[5] on more testing[6].

### France

France suggested a harmonised classification and labelling for BPA (Reprotoxic Category 1B). The Committee for Risk Assessment (RAC) has adopted an opinion to strengthen the existing harmonised classification and labelling (CLH) of BPA from a category 2 reproductive toxicant to a category 1B reproductive toxicant regarding the adverse effects on sexual function and fertility in line with a proposal from the French competent authority[7]. The classification of bisphenol A (BPA) as a category 1B substance toxic for reproduction which will come into force on 1st March 2018[8].

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[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:026:0011:0014:EN:PDF

[5] https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e375d

[6] https://echa.europa.eu/documents/10162/84dbe057-2950-487a-8c72-aee0aacaf215

[7] https://echa.europa.eu/view-article/-/journal_content/title/rac-proposes-to-strengthen-the-classification-of-bisphenol-a

[8] http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.195.01.0011.01.ENG&toc=OJ:L:2016:195:TOC

Moreover, in May 2014, the French authorities submitted a proposal to restrict BPA because of health risks for pregnant workers and consumers exposed to it in thermal paper - for example when they handle cash register receipts. The population identified as being at risk is unborn children, who are exposed in the uterus. RAC agreed with the French proposal that BPA may have effects on the mammary glands, as well as on reproduction, metabolism and neuro-behaviour. In addition, and in line with the opinion of the European Food Safety Authority (EFSA), RAC also considered the effects on the immune system.

In September 2015, RAC concluded that the risk for the unborn children of female workers e.g. cashiers handling thermal paper, is not adequately controlled. However, the Committee did not identify a risk to consumers in handing receipts[9].

Moreover, France have ongoing work to identify BPA as SVHC57(f) for human health (to be submitted in the window of early 2017)[10].

### Denmark

In July 1, 2010 Denmark temporarily restricted BPA in baby bottles, sippy cups and packaging for baby food and "breast milk substitutes". The measure, labelled "temporary," is in effect until evidence proves BPA safe to developing nervous system and behaviour[11].

*United States of America*

FDA's current perspective, based on its most recent safety assessment, is that BPA is safe at the current levels occurring in foods. Based on FDA's ongoing safety review of scientific evidence, the available information continues to support the safety of BPA for the currently approved uses in food containers and packaging[12].

FDA's regulatory Centers and FDA's National Center for Toxicological Research continue to pursue a set of studies on the fate of BPA in the body from various routes of exposure and the safety of low doses of BPA, including assessing novel endpoints where questions have been raised.

Research studies pursued by FDA's National Center for Toxicological Research have recently completed a rodent subchronic study (Delclos et al. 2014) intended to provide information that would help in designing a long-term study that is now underway (CLARITY BPA). The subchronic study was designed to characterize potential effects of BPA in a wide range of endpoints, including prostate and mammary glands, metabolic changes, and cardiovascular endpoints. The study included an in utero phase, direct dosing to pups to mimic bottle feeding in neonates, and employed a dose range covering the low doses where effects have been previously reported in some animal studies, as well as higher doses where estrogenic effects have been measured in guideline oral studies. The results of this study showed no effects of BPA at any dose in the low-dose range (Delclos et al. 2014).

The FDA's National Center for Toxicological Research is now continuing with an additional study. Using the data and design from the rodent subchronic study, the National

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[9] https://echa.europa.eu/view-article/-/journal_content/56/10162/22052209

[10] Pers. Comm ANSES

[11] http://www.foodqualitynews.com/Regulation-and-safety/Denmark-bans-bisphenol-A-in-food-packaging-for-young-children

[12] http://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm064437.htm#summary

Toxicology Program/Food and Drug Administration (NTP/FDA) is conducting a long-term toxicity study of BPA in rodents to assess a variety of endpoints, including novel endpoints where questions have been raised. As an addition to this core study, FDA is providing extra animals and tissues to a consortium of grantees (Schug et al. 2013) selected and funded by the National Institute of Environmental Health Sciences to address other critical questions.

The 2014 hazard assessment by the FDA's BPA Joint Emerging Science Working Group reconfirms the previously identified NOAEL of 5 mg/kg bw/day for systemic toxicity from subchronic/multigenera-tional studies using rodents as the most appropriate NOAEL for a safety assessment of oral or dietary exposures. Available pharmacokinetic data and comparisons between ages and species further support use of this NOAEL as very conservative in extrapolating to humans. Compared to the 90th percentile exposures cited above for populations of 2 years old, the margins of safety exceed the uncertainty factor of 1000[13].

*Canada*

In its 2008 risk assessment, the Health Canada's Food Directorate did not revise the provisional TDI for BPA of 0.025 mg/kg bw per day set from the lowest NOEL of 25 mg/kg bw per day for general toxicity in a rat 90-day study (NTP, 1982), and concluded that the current dietary exposure to BPA through food packaging uses was not expected to pose a health risk to the general population, including new-borns and young children (Health Canada, 2008). Health Canada then estimated the probable daily exposure to BPA to vary from as low as 0.21 µg/kg bw for infants 8-12 months of age to as high as 1.35 µg/kg bw for 0-1 month old infants with the maximum formula intake and the maximum concentration of BPA migrating from epoxy lined infant formula cans (EFSA, 2015).

Over the years, Health Canada's Food Directorate has conducted periodic reviews of BPA as new information has become available relating to its toxicity and/or its potential exposure from food packaging applications. The purpose of these reviews was to determine whether dietary exposure to BPA could pose a health risk to consumers. However, due to the uncertainty raised in some animal studies relating to the potential effects of low levels of BPA, the Government of Canada is taking action to enhance the protection of infants and young children. It is therefore recommended that the general principle of ALARA (as low as reasonably achievable) be applied to continue efforts on limiting BPA exposure from food packaging applications to infants and new-borns, specifically from pre-packaged infant formula products as a sole source food, for this sensitive segment of the population[14].

Health Canada released an "updated assessment of BPA in 2012" and also concluded that there were no safety issue at the levels people are exposed to[15].

*Australia*

Food Standards Australia New Zealand (FSANZ) is an independent statutory agency established by the Food Standards Australia New Zealand Act 1991 (FSANZ Act). FSANZ is part of the Australian Government's Health portfolio. In a paper from 2010 FSANZ states that:

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[13] http://www.fda.gov/downloads/NewsEvents/PublicHealthFocus/UCM424266.pdf

[14] http://www.hc-sc.gc.ca/fn-an/securit/packag-emball/bpa/index-eng.php

[15] http://www.hc-sc.gc.ca/fn-an/securit/packag-emball/bpa/bpa_hra-ers-2012-09-eng.php

The weight of scientific evidence indicates that exposure to BPA in food does not present a significant human health and safety issue at current exposure levels. A recent FSANZ survey of BPA levels in food and beverages in Australia affirms the conclusion that consumers are exposed to very low levels of BPA through food and beverage consumption. Only a limited number of products were found with detected levels of BPA and no detectable levels of BPA were found in infant formula. These results provide additional assurance that BPA concentrations in Australian food do not pose a health risk to consumers[16].

*China*

In April 2011, China's Ministry of Health announced that the production and import of infant food containers containing bisphenol A (BPA), including baby feeding bottles, will be banned from 1 June, and that sales of such products will be banned from 1 September. The ministry says that while risk assessment of dietary exposure levels of BPA have demonstrated that the substance does not pose a health hazard, taking into account the potential of a low dose effect and the uncertainty of animal experiments, Canada, the EU and some US states have felt it necessary to introduce restrictions. China's production and imports ban will take effect on the same date as the ban on polycarbonate baby bottles in the EU[17].

Assessment and measurement endpoints for hazard characterisations

The scientific debate on the risks for public health of BPA focuses on its endocrine-active properties, which might adversely impact physical, neurological and behavioural development. In addition, other perturbations of physiology, both in animals and humans, have been brought in relationship to the endocrine active properties of BPA. Among these are e.g. obesity, modification of insulin-dependent regulation of plasma glucose levels, perturbation of fertility, proliferative changes in the mammary gland possibly related to the development of breast cancer, immunotoxicity and adverse effects on the cardiovascular system (EFSA, 2015).

In the recent evaluation made by EFSA, both kidney and liver effects and proliferative changes in mammary gland were considered likely and were used for hazard characterisation (EFSA, 2015).

Assessment values and their derivation

In 2006, EFSA set the TDI for BPA at 0.05 mg BPA/kg body weight (b.w.)/day. This is based on the No-Observed-Adverse-Effect-Level (NOAEL) of 5 mg/kg b.w./day that has been identified in two multi-generation reproductive toxicity studies in rodents, where the critical effects were changes in body and organ weights in adult and offspring rats and liver effects in adult mice, respectively (EFSA, 2006). The NOAEL for developmental toxicity was 50 mg BPA/kg bw/day and 600 mg BPA/kg bw/day for reproductive toxicity (Tyl et al., 2006). EFSA reviewed new scientific information on BPA in 2008, 2009, 2010 and 2011: EFSA's experts concluded on each occasion that they could not identify any new evidence which would lead them to revise the TDI for BPA of 0.05 mg/kg bw/day.

Based on new scientific findings and possibly triggered by several Member States aiming at further restrictions on the use of BPA in food contact materials, EFSA undertook a full re-evaluation of the human risks associated with exposure to BPA through the diet, also

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16 https://www.foodstandards.gov.au/science/surveillance/documents/BPA%20paper%20October%202010%20FINAL.pdf

17 http://en.nhfpc.gov.cn/

taking into consideration the contribution of non-dietary sources to the overall exposure to BPA. After public consultation EFSA published the new evaluation of bisphenol A (BPA) in January 21, 2015[18]. The EFSA evaluation of BPA consisted of a summary, Part 1: Exposure assessment and part 2: Toxicological assessment and risk characterisation. In this opinion EFSA establishes a new temporary TDI for BPA on 4 µg/kg bw/day. EFSA concludes that based on the t-TDI of 4 µg/kg bw/day, and using the EFSA estimates of the total exposure to BPA, there is no health concern.

The t-TDI vas based on a BMDL10 of 8960 µg/kg bw/day based on changes in relative kidney weights in the Tyl et al. (2008) study on mice and by using the HEDF of 0.068 based on the adult mouse. Multiplying the HEDF by the point of departure (i.e. a NOAEL or BMDL10) of a toxicity study yields a human-equivalent oral dose that can be used for risk assessment. To obtain the equivalent dose in humans, the HEDF of 0.068 is multiplied by the BMDL10 of 8960 µg/kg bw/day resulting in a human equivalent dose of 609 µg/kg bw /day. The overall uncertainty evaluation by EFSA (2015) included the effects on mammary gland as well as reproductive, metabolic, neuro-behavioural and immune systems (EFSA, 2015). The uncertainty evaluation approached "likely" in the (HED) dose range of 100-1000 µg/kg bw/day. EFSA (2015) therefore concluded that the uncertainty regarding the abovementioned effects at the HED of 100 µg/kg bw/day and higher should be taken into account when establishing a health-based guidance value by including an extra factor in establishing the t-TDI. Thus, as the reference point was 609 µg/kg bw/day based on the mean relative kidney weight and the lower end of the dose range for which the uncertainty evaluation for other endpoints approached "likely" is 100 µg/kg bw/day, a factor of 6 was applied. Applying the remaining assessment factor of 25 (remaining factor of 2.5 for interspecies differences, and factor 10 for intraspecies differences), the resulting t-TDI was 4 µg/kg bw/day (EFSA, 2015) (see table 1).

[18] http://www.efsa.europa.eu/en/efsajournal/pub/3978.htm

Table 1. TDI(Tolerable daily intake), RfD(Reference dose) , DNEL(derived no-effect level)

| TDI/RfD or DNEL internal<br><br>(µg/kg bw/day) | Basis (points of departure) | AF | Source and year |
|---|---|---|---|
| TDI<br><br>4 | Extrapolation from BMDL10 (8960) for kidney effects to cover also reproductive effects (e.g. mammary gland effects and ↓ male AGD). Human equivalent dose (HED) was 609 µg/kg bw/day based on the mean relative kidney weight. An extrapolation to cover uncertainty for other endpoints 100 µg/kg bw/day was used. | 25 (remaining factor of 2.5 for interspecies differences, and factor 10 for intraspecies differences, and 1 for toxicokinetic, reflecting that toxicokinetic intraspecies differences have been addressed when using the HED approach), the resulting t-TDI was 4 µg/kg bw/day (EFSA, 2015) | EFSA, 2015<br><br>(Based on Tyl et al. 2008) |
| TDI<br><br>**0.7** | Mammary hyperplasia in adult females.<br><br>NOAEL 25, LOAEL 80 | 25 (10 for intraspecies, 2.5 for toxicodynamics)<br><br>Conversion from rat to human using factor 0.72 (EFSA 2015) | DTU, 2015 Based on study by Delclos et al., 2014 and use of AF as in EFSA 2015. |
| RfD<br><br>50 | NOAEL of 5 mg/kg bw/day for systemic toxicity from subchronic/multigenerational studies | 100 (10 from interspecies differences, and factor 10 for intraspecies differences) | FDA, 2008<br><br>(Based on Tyl et al. 2008) |
| DNEL Internal<br><br>0.005, 0.01, 0.009 and 0.0025 µg/kg bw per day for neural-, female repro., metabolic- and mammary gland effects, respectively | Neurobehavioural development (NOAEL: 50 µg/kg bw per day from Xu et al., 2010), female reproductive system (NOAEL: 100 µg/kg bw per day from Rubin et al., 2001), metabolism and obesity (LOAEL: 260 µg/kg bw per day from Miyawaki et al., 2007) and mammary gland proliferation - changes in the structure that make it more susceptible to carcinogens (NOAEL: 25 µg/kg bw per day from Moral et al., 2008). | 300 (10 Interspecies x10 toxicokinetics/toxicodynamics x 3 uncertainty low dose non-monotonic dose-response relationships (NMDRs). | ANSES, 2013 (based on Xu et al., 2010; Rubin et al., 2001); Miyawaki et al., 2007; Moral et al., 2008). |

*The choice of critical experimental studies and selection criteria*

Different experimental studies were chosen for the various reference values listed in Table 1.

In EFSA, the changes in relative kidney weights in the Tyl et al. (2008) have been used for setting the TDI. Moreover, the overall uncertainty evaluation by EFSA (2015) was reported to also include the effects on the mammary gland as well as reproductive, metabolic, neurobehavioural and immune systems (EFSA, 2015).The same study (industry) was chosen for RfD setting in FDA. The DTU evaluation is based on a new statistical analysis of the data from Delclos et al. 2014. DTU evaluates that these data suggest that increased numbers of female animals with hyperplasia of the mammary tissue are found at 80 µg/kg bw/day BPA and higher doses. Such changes indicate an increased risk for breast cancer later in life. DTU finds that the study leads to a tentative LOAEL of 80 µg/kg bw/dag and a NOAEL of 25 µg/kg bw/day.

ANSES has chosen different studies on low dose exposure of BPA with neural-, female reproductive, metabolic- and mammary gland effects (ANSES, 2013) resulting in low internal DNELs.

*The choice of assessment factors*

### EFSA

In deriving a health-based guidance value, the CEF Panel (EFSA) used an uncertainty factor of 2.5 for inter-species differences (1 for toxicokinetics and 2.5 for toxicodynamics, reflecting the fact that toxicokinetic differences have been addressed by use of the HED approach) and an uncertainty factor of 10 for intra-species differences (see table 1). In addition, the CEF Panel considered that the extra factor of 6 should be included to take into account the uncertainty in the database, i.e. mammary gland, and reproductive, neurobehavioural, immune and metabolic systems. The CEF Panel applied, therefore, an overall uncertainty factor of 150 to the HED of 609 µg/kg bw per day and established a temporary Tolerable Daily Intake (t-TDI) for external oral exposure to BPA in humans of 4 µg/kg bw, based on the mean relative kidney weight effect in the mouse. The CEF Panel designated the TDI as temporary, pending the outcome of the long-term study in rats involving prenatal as well as postnatal exposure to BPA, currently being undertaken by NTP/FDA. This study will help resolve the uncertainties in the database (EFSA, 2015).

### French Agency for Food, Environmental and Occupational Health & Safety (ANSES)

In September 2011, ANSES published a report on BPA, including one part concerning its effects on human health and the other one on its uses (ANSES, 2011). In the hazard identification report "Effets sanitaires du bisphénol A" ANSES classified the effects of BPA on humans and animals as proven, suspected, controversial, or inconclusive (ANSES, 2011). Furthermore, it reached the conclusions that BPA exposure was associated with proven effects in animals and suspected effects in humans, also at levels of exposure below the current regulatory thresholds. These effects were the main focus of the subsequent risk assessment that was completed by ANSES in April 2013(ANSES, 2013).

Specifically, the characterisation of human health risks was conducted considering four toxicological endpoints for which the external threshold doses identified in oral studies in developing animals are reported: neurobehavioural development (NOAEL: 50 µg/kg bw per day from Xu et al., 2010), female reproductive system (NOAEL: 100 µg/kg bw per day from Rubin et al., 2001), metabolism and obesity (LOAEL: 260 µg/kg bw per day from Miyawaki et al., 2007) and mammary gland proliferation - changes in the structure that make it more susceptible to carcinogens (NOAEL: 25 µg/kg bw per day from Moral

et al., 2008). ANSES used a bioavailability factor of 3% to convert the external NOAEL/LOAEL from the experimental data into equivalent internal doses (internal NOAEL/LOAEL), taking into consideration the impact of first-pass metabolism on orally ingested BPA and assuming that only the unconjugated fraction of BPA was responsible for the effects observed. To obtain an internal Derived No Effect Level (DNEL) for each critical endpoint, ANSES applied an overall assessment factor of 300 to the internal NOAELs (or 900 if the starting critical dose was a LOAEL), consisting of a factor 100 to account for inter- and intra-species kinetic and dynamic differences, and an extra factor of 3 to account for uncertainties regarding possible low-dose effects of BPA and non-monotonic dose-response relationships (NMDRs). The resulting internal DNELs that were used in risk characterisation were 0.005, 0.01, 0.009 and 0.0025 µg/kg bw per day for neural-, female reproductive-, metabolic- and mammary gland effects, respectively (see table 1). The sources of BPA considered for BPA (probabilistic) exposure assessment were air, sedimented dust, food and beverages. Dietary (external) exposure (99th percentile) was estimated to be for children (3-17 yrs) 0.31 µg/kg bw per day, for adolescents (11-17 yrs): 0.12 µg/kg bw per day, and for pregnant women: 0.24 µg/kg bw per day (ANSES, 2013).

*Other issues*

No risk assessment has taken potential mixture effects due to exposure to other chemicals with similar types of effects as BPA into account. This means that the risk can be underestimated.

Moreover, the debate on non-monotonic dose-response relationships and low dose effects on BPA is ongoing. In a recent paper by Vandenberg and Prins (2016) it is stated: …, some effects are seen at low doses of BPA, but not at higher doses. These may be examples of nonmonotonic dose responses, which are common for hormones and EDCs, and can be explained by a number of endocrine-mediated mechanisms, including receptor down-regulation, receptor competition, and the overlap of competing monotonic curves.

ANSES provided a more lengthy discussion of non-monotonic relationships in their evaluation of BPA (ANSES, 2013). As mentioned above, ANSES do include an extra factor of 3 to account for uncertainties regarding possible low-dose effects of BPA and non-monotonic dose-response relationships (NMDRs) when deriving a DNEL (ANSES, 2013).

<u>Exposure assessments</u>

Highly exposed humans are according to EFSA's exposure assessment exposed to 1.01-1.06 µg/kg bw/day for men and women and 1.26-1.45 µg/kg bw/day for children (3-10 years) and teenagers. These exposures are around 3-4 times lower than the EFSA t-TDI of 4 µg/kg bw/day and EFSA concludes that the aggregated exposure to BPA indicates no health concern for BPA (EFSA, 2015).

However, DTU finds that the TDI for BPA should be 0.7 µg/kg bw/day (see table 1) to be sufficiently protective with regards to endocrine disrupting effects of BPA. Highly exposed humans including pregnant women and children are according to EFSA's exposure assessment exposed to around 1.4-2 times more than 0.7 µg/kg bw/day BPA. DTU concludes that this gives rise to health concern for highly exposed humans (DTU, Evaluation, 2015).

In 2012, a refined (probabilistic) exposure assessment of Canadians was conducted based on the collective results of a number of recent Canadian surveys, including results from a Total Diet Study. A mean exposure to BPA of 0.055 µg/kg bw per day was calculated for the general population, which is approximately 3 times lower than the exposure calculated in the risk assessment of 2008. This updated dietary exposure figure generally aligns with exposure estimates that are based on the results of population-based biomonitoring studies. Infants, as an age group, were exposed to the greatest

amount of BPA. The probable daily exposure to BPA varied from 0.083 µg/kg bw (0-1 month of age) to 0.164 µg/kg bw (4-7 months old infants). Collectively, also the BPA exposure estimates for infants were, on average, approximately 3-fold lower than those of 2008. Health Canada recommended the application of the general principle of ALARA (as low as reasonably achievable) to limit BPA exposure of new-borns and infants, due to uncertainties for low-dose neurodevelopmental and behavioural effects in rodents (EFSA, 2015).

In 2005, the Japanese AIST concluded that BPA was unlikely to pose unacceptable risks to human health at current exposure levels. Margins of exposure (MOEs) were calculated as 85,000-1,800,000 based on realistic exposure scenarios, and as >1,000 for adults and children based on worst-case scenarios. For these calculations, the NOAEL or the Benchmark Dose Lower Limit (BMDL) for three critical endpoints, namely lower body weight gain, liver and reproductive effects, were in the 5 to 50 mg/kg bw per day range. AIST updated the Hazard Assessment of BPA in 2011 (AIST, 2011). The oral NOAEL for BPA general toxicity was considered to be 3 mg/kg bw per day based on centrilobular hepatocyte hypertrophy in the two generation study in mice by Tyl et al. (2008). A total uncertainty factor of 25 was applied, consisting of 2.5 for inter-species differences (1 for toxicokinetics, and 2.5 for toxicodynamics), and of 10 for intra-species differences. According to the BPA exposure estimate in Japanese individuals, exposure was highest in 1 to 6 years old children with an estimated 95th percentile (in µg/kg bw per day) of 3.9 (males) - 4.1 (females). In adults, the 95th percentile of BPA intake (estimated from the amount of BPA excreted in 24-hour urine samples) was 0.037-0.064 µg/kg bw per day in men and 0.043-0.075 µg/kg bw per day in women. The relative margins of exposure (MoEs, i.e. ratio between the NOAEL and 95th percentile exposure data) were 730-770 for 1-6 yr old children and 40,000-81,000 for adults. These values were much larger than both the MoE (25) that was considered might possibly result in health effects in humans and the standard (conservative) MoE of 100, and thus the AIST concluded that the risk of BPA with regard to human health was very small (reported in EFSA, 2015).

Risk assessments

*EU*

In 2003, the European Chemical Bureau of the European Union published a comprehensive Risk Assessment Report (EU-RAR) for BPA in the context of Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances. The key health effects of BPA through different exposure routes were considered to be eye and respiratory tract irritation, skin sensitisation, repeated dose toxicity to the respiratory tract, effects on the liver and reproductive toxicity (effects on fertility and on development). With respect to human health risks, a need for further research was identified, to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on neurological and neurobehavioural development at low doses (EU-RAR, 2003).

In 2008, the EU-RAR (EU-RAR, 2008) was updated after evaluation of the two generation reproductive study in mice by Tyl et al. (2008) along with the new data on human exposure and effects of BPA that had become available since 2003. EU-RAR identified a NOAEL of 50 mg/kg/day from the multigenerational study and used it for risk characterisation purposes, in relation to effects on fertility. The Rapporteur came to the conclusion that there was no need for further information and/or testing and for risk reduction measures beyond those which were already being applied. However, Denmark, Sweden and Norway considered that the results of four neurodevelopmental studies

warranted further consideration and expressed a minority view[19] concerning this toxicological endpoint (EU-RAR, 2008).

## EFSA

EFSA established in 2015 a temporary Tolerable Daily Intake (t-TDI) of 4 µg/kg bw per day. Highly exposed humans are according to EFSA's exposure assessment exposed to 1.01-1.06 µg/kg bw/day for men and women and 1.26-1.45 µg/kg bw/day for children (3-10 years) and teenagers (EFSA, 2015). These exposures are around 3-4 times lower than the EFSA t-TDI of 4 µg/kg bw/day and EFSA concluded that there is no health concern for any age group from dietary exposure or from aggregated exposure (EFSA, 2015).

Risk management options

In recognition of the uncertainty as to the effect associated with possible low-dose exposure of BPA an EU ban prohibiting the use of BPA for the manufacture of polycarbonate infant feeding bottles was adopted in January 2011 and EU states outlawed the manufacture of polycarbonate feeding bottles containing the compound from March 2011, and banned their import and sale from June 2011.

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[19] Minority view from EU RAR (2008): "Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007."

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### *4.4 Case Study: DEHP, human health*

Summary

DEHP was discovered as a reproductive toxicant several decades ago and later when relevant endpoints for endocrine disruption became investigated, experimental exposures during development have demonstrated that DEHP can cause endocrine disrupting effects on male sexual development. The critical effect, assessment factor (AF) and the assessment values, e.g. DNEL or TDI, appear rather similar in Europe, the US and Canada and the values range from 34-60µg/kg bw/day. No additional AFs are included to address uncertainties related to endocrine disruption (e.g. threshold). Estimated exposures to DEHP also appear similar in Europe, the US and Canada and range from 1-30 µg/kg bw/day. Both Canada and the US have concluded that there is concern for human health, especially for children. In the EU it has been concluded that the combined exposure to DEHP, BBP, DBP and DIBP reaches levels that constitute a risk to children. The regulatory actions include a ban on DEHP in toys and childcare products at concentrations greater than 0.1% in EU and US as well as recommendations in the EU, US and Japan with regards to medical devices used for infants. In the EU, there is a proposal to ban also articles intended for use indoors in unsealed applications and articles that may come into direct contact with the skin or mucous membranes containing one or more of the 4 phthalates DEHP, DBB, BBP or DIBP in a concentration greater than 0.1 % by weight of any plasticised material.

Scope of this case study

This case study focuses on the endocrine disrupting effects of DEHP on male reproductive development in humans. Effects of DEHP on the environment are also relevant, but this is not the scope of this case study.

Discovery as an endocrine disruptor

The discovery of DEHP as an endocrine disruptor was not the result of systematic screening, nor was the endocrine disrupting property of DEHP predicted on the basis of the biological activity in animals or humans. DEHP was discovered as a reproductive toxicant several decades ago and gradually, as relevant endpoints for endocrine disruption became investigated, experimental exposures during development demonstrated that DEHP can affect male sexual development. The type of effects seen in male offspring, especially decreased anogenital distance, increased nipple retention and malformation of the external genitalia (hypospadias) clearly points to an anti-androgenic mode of action of DEHP. This is supported by mode of action studies showing decreased testosterone levels in male foetuses during sensitive periods of sexual development.

Regulatory framework in selected jurisdictions

*European Union*

DEHP is classified as toxic to reproduction in category 1B according to the CLP Regulation because it induces effects on reproductive organs and fertility in experimental animals exposed prenatally. The toxicological mode of action was later recognized to be the anti-androgenic properties of DEHP (ECHA/NA/14/56).

Under the REACH Regulation (EC) No. 1907/2006, the European Chemicals Agency (ECHA) has proposed adding DEHP to the list of substances for inclusion in Annex XIV for authorisation procedures. A restriction dossier was therefore submitted in 2011.

In 2014, Denmark proposed DEHP to be identified as a substance of very high concern (SVHCs) due to the endocrine disrupting properties for human health and the environment. The Member State Committee (MSC) unanimously agreed with the identification of DEHP as a substance giving rise to equivalent level of concern due to its endocrine disrupting properties to the environment. The MSC also unanimously

acknowledged that for DEHP there is scientific evidence of endocrine activity and of a link between this activity and adverse effects to human health. However, the MSC did not reach unanimous agreement on whether this constitutes an equivalent level of concern to CMRs (majority view), as a minority of members were of the view that the concern related to endocrine disruption is already covered by the existing identification as SVHC due to toxicity to reproduction. This MSC opinion with majority and minority views on the SVHC proposals will be sent to the European Commission for final decision.

DEHP is subject to a ban on use (0.1%) in toys and childcare articles, in cosmetics and in plastic materials and articles intended to come into contact with foodstuffs.

The risks posed by toys and childcare articles have to an extent been covered by the European Commission (Decision 1999/815/EC) temporary ban on DEHP in toys and childcare articles intended to be put into the mouth by children under three years of age. In September 2004, the EU Competitiveness Council replaced this temporary ban with permanent legislation within the framework of Directive on Restrictions on the Marketing and Use of Certain Substances and Preparations (76/769/EEC), which banned DEHP in toys and children's articles for all children because of the classification of DEHP as a reproductive toxicant.

Similar restrictions on DEHP, as one of many substances classified as Carcinogenic, Mutagenic and Reproductive Toxicants (CMR), have been adopted in the Cosmetics Directive 2003/15/EEC by European Parliament in February 2003. "The Scientific Committee on Cosmetics concluded that CMR substances pose a significant threat to the health of consumers when used in cosmetic products. Although the exposure routes are not the same, toys, food packaging materials and medical devices may be seen as parallel cases giving rise to direct exposure of (the) consumers."

In 2012, France became the first country in EU to ban the use of DEHP in paediatrics, neonatal and maternity wards in hospitals (http://healthierhospitals.org/media-center/news-updates/france-first-eu-country-ban-use-dehp-paediatrics-neonatology-and-maternity).

*United States of America*

### Federal Government (2012)

The federal government both regulates and continues to study phthalates. Federal entities involved in phthalate management and research include:

Consumer Products Safety Commission (CPSC) – Among other provisions, the Consumer Product Safety Improvement Act of 2008 (CPSIA) banned the use of DEHP in toys and child care articles at concentrations greater than 0.1 percent. The CPSIA tasks the CPSC with appointing a Chronic Hazard Advisory Panel and examining the cumulative health risks of phthalates and phthalate substitutes.

Food and Drug Administration (FDA) – The FDA regulates phthalates in food contact substances (such as plastic wrap), cosmetics, pharmaceuticals and medical devices. FDA announced in June 2008 that it is conducting a comprehensive inventory of regulated products that contain phthalate and is reviewing available use and toxicology information associated with phthalate exposure from FDA regulated products to better characterize any potential risk from these uses.

Environmental Protection Agency (EPA) – Existing EPA Actions affecting DEHP (and other phthalates) include:

- DEHP is regulated under the Safe Drinking Water Act. The highest concentration allowed, the maximum contaminant level (MCL), is 0.006 mg/L.
- DEHP is listed as hazardous air pollutant under the Clean Air Act.

- Under the Resource Conservation and Recovery Act (RCRA), phthalates are regulated as a hazardous waste if discarded as a commercial chemical product.
- DEHP is reportable to the Toxic Release Inventory (TRI) under section 313 of the Emergency Planning and Community Right-to-know Act (EPCRA).
- DEHP is included in the first group of 67 chemicals to be screened as part of the Endocrine Disruptor Screening Program (EDSP).
- Phthalates that are listed on the TSCA Inventory are subject to TSCA section 8(e) Inventory Update Reporting (IUR) requirements, including production and use information for sites having production volumes of at least 25,000lbs/yr. All eight Phthalates (10 CASRNs) included in this Action Plan are listed on the TSCA Chemical Substance Inventory.
- In 1989, EPA entered an Enforceable Consent Agreement under TSCA section 4 with six companies to perform certain chemical fate and environmental effects on certain Alkyl Phthalates (54 FR 618).
- In 2001, under the voluntary HPV Challenge Program, the Phthalate Esters Panel Testing Group of the American Chemical Council sponsored a phthalates ester category. The panel has submitted to EPA robust study summaries or other information for 26 phthalates, including DEHP.

### US State Governments

California, Vermont and Washington have established standards for the content of certain phthalates in children's articles. California prohibits the manufacture, sale, or distribution in commerce of any toy or child-care article that contains DEHP at greater than 0.1%.

Vermont prohibits the manufacture, sale, or distribution in commerce of any toy or child-care article intended for use by a child younger than three years old that contains DEHP, DBP, or BBP in concentrations greater than 0.1% (CRS, 2008).

As part of a statute concerning chemicals in children's products generally, Washington prohibits a manufacturer, wholesaler, or retailer from manufacturing, knowingly selling, offering for sale, or distributing for sale or for use in the state a children's product or product component containing phthalates (DEHP, DBP, BBP, DINP, DIDP, DnOP) individually or in combination, at a concentration exceeding 0.1% by weight (CRS, 2008).

Other States such as Hawaii have introduced legislation to prohibit the manufacture, sale, or distribution of certain toys and child care articles containing certain types of phthalates (Hawaii House of Representatives, 2009; CRS, 2008).

Proposition 65 California Proposition 65: The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency added DEHP to the list of more than 750 chemicals known to the state to cause reproductive toxicity for the developmental and male reproductive endpoints.

*Japan*

In 2002, the Japanese Ministry of Health, Labour and Welfare recommended that healthcare professionals do not use medical devices made of PVC in which the plasticiser DEHP is used; alternative devices should be used instead.

*Australia*

In 2006, the Australian Government declared the phthalates DEHP, DIDP, DMP, DINP, DBP, BBP, DnOP, DEP and bis(2-methylethyl) phthalate as Priority Existing Chemicals and initiated public risk assessments for these phthalates. Phase 1 "the development of the public health hazard assessments" was concluded in 2008 (Australian Government,

2008a-h). Phase 2 "the development of the risk assessments" is currently in progress (Australian Government, 2009).

Assessment and measurement endpoints for hazard characterisations

Developmental toxicity manifested as effects on male sexual development is used as critical effect. The endpoints considered include among others AGD, NR and testicular toxicity in rat offspring and these effects are generally recognized to be due to endocrine disruption.

Assessment values and their derivation

In the EU, the Tolerable Daily Intake (TDI) value of DEHP was previously established (RAR 2008 and ECB 2008) at 48 µg per kg bw per day, based on a NOAEL of 4.8 mg/kg/d for reproductive toxicity in rats and applying an assessment factor of 100. Based on the same studies, EFSA rounded the TDI to 50 µg/kg bw/d (EFSA 2005). The current derived No Effect Level (DNELs) adopted by ECHA for DEHP is 35 µg/kg bw/day (ECHA 2013).

In 2015, SCENIHR supported the TDI value of 50 µg/kg bw/d, considering that the new studies are in line or not sufficiently robust to justify the derivation of a new TDI (SCENIHR, 2015). The assessment values, i.e. DNEL, TDI, are shown in table 1. They appear rather similar, i.e. range from 0.03-0.06 mg/kg/d, and generally an AF of 100 for intra- and interspecies differences is used. Although the effects of DEHP are generally recognized to be due to endocrine disruption, no additional AFs are included to address uncertainties related to endocrine disruption (e.g. threshold, non-monotonic dose-response).

Table 1 Assessment values and their derivation

| Organisation | Critical effect (animal species) | Critical dose (NOAEL/LOAEL/BMD) | AF | DNEL/TDI/reference dose (mg/kg/d) |
|---|---|---|---|---|
| ECHA (2013) | testicular toxicity in rat offspring | NOAEL = 4.8 mg/kg/d; NOAEL (corrected, absorption) = 3.36 mg/kg/d | 100 | 0.034 |
| EFSA (2005) | testicular toxicity in rat offspring | NOAEL = 5 mg/kg/d | 100 | 0.050 |
| ATSDR (2002) | Aspermatogenesis (F344 rats) | NOAEL = 5.8 mg/kg/d | 100 | 0.060 |
| Canada (1994) | Developmental toxicity (mice) | NOAEL = 44 mg/kg/d | 1000 | 0.044 |

ECHA has rejected claims by a group of NGOs that there are "procedural and substantive flaws" in the draft opinions, adopted by its Committees on Risk Assessment (RAC) and Socio-Economic Analysis (SEAC), which concluded that authorisations for the use of the phthalate DEHP should be granted. NGOs have argued that it is wrong to set a DNEL for DEHP, because REACH does not allow them to be set for endocrine disruptors, and DEHP's classification as a reprotoxicant "is mediated by an endocrine mode of action" (https://chemicalwatch.com/22717/echa-defends-its-views-on-dehp-authorisation, 28 January 2015). ECHA's responses to Chemical Watch points out that REACH allows DNELs

to be set for reproductive toxicants. The RAC acknowledges that the reproductive toxicity of DEHP is mediated by an endocrine mode of action but finds that it is appropriate to establish the reference DNEL because the substance has been identified according to Article 57(c) and not (f).

Exposure assessments

In the EU RAR from 2008, the mean DEHP intake in one year-old children estimated using the dietary concentration in 2001 was 5.7-6.1 µg/kg/day (5 to 95 percentiles: 0.8 to 17.5 µg/kg/day). The mean DEHP intake in all age groups was 1.8-1.9 µg/kg/day (5 to 95 percentiles: 0.4 to 5.4 µg/kg/day).

NTP in the US has estimated that the general population of the United States is exposed to DEHP levels ranging from 1 to 30 µg/kg bw/day (NTP 2006).

Estimated daily intake of DEHP for the general population in Canada is 9-19 µg/kg bw/day (Canada 1994).

Based on Danish biomonitoring data on urinary phthalate metabolite excretion in pregnant women and collected in 2011-12, the estimated mean daily intake was 1.54 µg/kg/day (25 to 95 percentiles: 0.7 to 3.4 µg/kg/day). This is very similar to the levels in the DEMOCOPHES study (Frederiksen et al. 2013). Here, the intake levels in pregnant women were estimated to be 2.6 µg/kg/day (25 to 95 percentiles: 1.1 to 5.1 µg/kg/day). The intake levels in children were estimated to be higher, i.e. 4.1 µg/kg/day (25 to 95 percentiles: 1.6 to 10.3 µg/kg/day).

Risk assessments

*EU*

EU Risk Assessments have been made for DEHP individually.

The ANNEX XV RESTRICTION REPORT (Danish EPA 2011) concludes that the combined exposure to DEHP, BBP, DBP and DIBP from food, dust and indoor air combined with normal handling and use of a few selected articles containing one or several of these phthalates reaches levels that constitute a risk to children. A comparison with biomonitoring data of urine metabolites further confirmed that risk.

For DEHP, DBP, BBP and DIBP a ban is proposed on the placing on the market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes containing one or more of these phthalates in a concentration greater than 0.1 % by weight of any plasticised material. The proposal is to ban the placing on the market of articles intended for use indoors in unsealed applications and articles that may come into direct contact with the skin or mucous membranes containing one or more of the 4 phthalates DEHP, DBB, BBP or DIBP in a concentration greater than 0.1 % by weight of any plasticised material.

*NTP US*

The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) has reached the following conclusions on the possible effects of exposure to DEHP on human development and reproduction (NTP 2006). (Note that the possible levels of concern, from lowest to highest, are negligible concern, minimal concern, some concern, concern, and serious concern):

- There is serious concern that certain intensive medical treatments of male infants may result in DEHP exposure levels that adversely affect development of the male reproductive tract. DEHP exposure from medical procedures in infants was estimated to be as high as 6000 µg/kg bw/day.

- There is concern for adverse effects on development of the reproductive tract in male offspring of pregnant and breastfeeding women undergoing certain medical procedures that may result in exposure to high levels of DEHP.
- There is concern for effects of DEHP exposure on development of the male reproductive tract for infants less than one year old. Diet, mouthing of DEHP-containing objects, and certain medical treatments may lead to DEHP exposures that are higher than those experienced by the general population.
- There is some concern for effects of DEHP exposure on development of the reproductive tract of male children older than one year. As in infants, exposures of children to DEHP may be higher than in the general population.
- There is some concern for adverse effects of DEHP exposure on development of the male reproductive tract in male offspring of pregnant women not medically exposed to DEHP. Although DEHP exposures are assumed to be the same as for the general population, the developing male reproductive tract is sensitive to the adverse effects of DEHP.
- There is minimal concern for reproductive toxicity in adults exposed to DEHP at 1–30 µg/kg bw/day. This level of concern is not altered for adults medically exposed to DEHP.
- U.S. Environmental Protection Agency's (EPA's) current management plan (2012) includes DEHP

*Canada (British Columbia)*

Based on limited available data on concentrations of bis(2-ethylhexyl) phthalate in food, indoor air, ambient air, drinking water, soil, and children's products, the total average daily intakes of bis(2-ethylhexyl) phthalate have been estimated for various age groups in the general population (Canada 1994) . The estimated average daily intakes of bis(2-ethylhexyl) phthalate for some age groups of the general population in Canada may slightly exceed the tolerable daily intake developed on the basis of studies in laboratory animals. Based on these considerations, there is insufficient information to conclude whether bis(2-ethylhexyl) phthalate is entering or may enter the environment in a quantity or concentration or under conditions that are having a harmful effect on the environment. It has been concluded, however, that bis(2-ethylhexyl) phthalate is not entering the environment in a quantity or concentration or under conditions that constitute a danger to the environment on which human life depends. It has also been concluded that bis(2-ethylhexyl) phthalate may enter the environment in a quantity or concentration or under conditions that may constitute a danger in Canada to human health.

References

Canada (1994) Priority substances list assessment report http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl1-lsp1/bis_2/bis_2_ethylhexyl_phthalate-eng.pdf

ECHA(http://echa.europa.eu/view-article/-/journal_content/title/the-member-state-committee-unanimously-agreed-to-identify-the-phthalate-dehp-as-an-svhc-because-of-its-endocrine-disrupting-properties-in-the-environm )

NTP 2006. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di(2-Ethylhexyl) Phthalate (DEHP), https://ntp.niehs.nih.gov/ntp/ohat/phthalates/dehp/dehp-monograph.pdf

SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks) 2015, Scientific

### *4.5 Case Study: Mancozeb*

Summary

Mancozeb and its metabolite ethylene thiourea (ETU) were known thyroid toxicants before the endocrine disruption hypothesis emerged. Plant protection products undergo broadly comparable registration process in all jurisdictions and the substance-specific monographs produced by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR) play an important role in the harmonisation of acceptable daily intakes (ADI = 0.05 mg/kg bw/day) for human health risk assessment. Full reports or summaries of the evaluation of mancozeb were made publicly available (in English) by Canada (2013), the United States (2005) and the European Union (2005, revised in 2009). As the latest JMPR evaluation of mancozeb is now 23 years old, the more recent reregistration proposal by Health Canada (2013) has derived a lower ADI of 0.008 mg/kg bw/day. This did not however influence the results of risk assessments which find no concern related to the thyroid toxicity of mancozeb or ETU via food or drinks. Another shift probably more likely to be related to the passage of time and expansion of knowledge than fundamental differences in regulatory processes is that the ecotoxicological effects of mancozeb are more readily attributed to endocrine disruption (Health Canada 2013). Evidence for endocrine-disrupting properties of mancozeb has recently been reviewed as part of a screening exercise related to the impact assessment of various options for science-based criteria required by European legislation. Mancozeb was found to be an endocrine disrupter under all options but the interim criteria currently in place.

Scope of this case study

This case study focuses on the consideration of the thyroid disrupting effects of mancozeb in humans and wildlife during authorisation of its uses as a pesticide or biocide. In many risk assessment efforts, thyroid toxicity has been recognised as the critical endpoint. Media-oriented legislation that may have addressed the potential human or ecological effects of mancozeb or its degradation product was considered beyond the scope of this case study.

Discovery as an endocrine disruptor

Mancozeb is a broad-spectrum ethylene(bis)dithiocarbamate (EBDC) fungicide launched in 1961 by Rohm and Haas (Klittich, 2008). It is a complex of two dithiocarbamates, zineb and maneb, that controls many fungal diseases in a wide range of field crops, fruits, nuts, vegetables, and ornamentals grasses. It is also used as a slimicide in water-cooling systems, in sugar, pulp, and paper manufacturing, as antioxidant in rubber, and as a scavenger in waste-water treatment. It plays an important role in the management of pest resistance and is still the largest selling fungicide in the world.

Discovery of thyroid toxicity of ethylene(bis)dithiocarbamates (EBDCs) predates the launch of mancozeb as a pesticide. Smith et al. (1953) reported thyroid changes in rats fed mancozeb throughout their lives in 1953. Most of this toxicity is thought to be associated with one of the principal metabolites of EBDCs, ethylenethiourea (ETU). ETU is readily formed in soil and water, during storage, processing and cooking of produce, and as a catabolite in mammals. Both mancozeb and ETU have been found to inhibit thyroid peroxidase (Hurley et al. 1998). Although this mechanism of thyroid disruption is not species-specific, marked differences between rodents and primates in inhibition of thyroid peroxidase in vitro underlie to the assumption that rodents are much more sensitive than humans (IARC Monograph 1987).

As a recognised thyroid toxicant, mancozeb was included in some early priority lists of chemicals to be screened for endocrine disrupting properties. The Japanese SPEED’ 98, and the European BKH database are examples.

Regulatory framework in selected jurisdictions

Residues of mancozeb are currently analysed following non-specific acid digestion of whole samples, converting all dithiocarbamates to carbon disulphide (CS2), which is then quantified by spectrophotometry or gas chromatography. As a result, limit values at the national or international level are commonly set for any dithiocarbamate, determined as CS2. Several problems arise with this quantification method. Firstly, the samples themselves may contain sulphides leading to an overestimation of dithiocarbamate residues or false-positive results. Secondly, this method does not allow attribution of any contravention to a specific dithiocarbamate.

As a result, a group Acceptable Daily Intake (ADI) and Maximum Residue Limit (MRLs) are generally set for all or selected dithiocarbamates, and separately for their common metabolite ETU.

*Joint FAO/WHO Meeting on Pesticide Residues (JMPR)*

Before considering specific jurisdictions, it is worth noting that two United Nations institutions, the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), jointly develop their own scientific risk assessments as part of the JMPR. The purpose of this ad hoc expert meeting is to provide advice on the acceptable levels of pesticide residues in food moving in international trade. The focus of these activities is therefore on the protection of human health.

The WHO Core Assessment Group is responsible for reviewing pesticide toxicological data and estimating Acceptable Daily Intakes (ADI), acute reference doses (ARfDs) and characterizes other toxicological criteria.

The FAO Panel is responsible for reviewing pesticide data residue and for estimating maximum residue levels, supervised trials median residue values (STMRs) and highest residues (HRs) in food and feed. Maximum residue levels (MRLs) are recommended to the Codex Committee on Pesticide Residues for consideration to be adopted by the Codex Alimentarius Commission. MRLs take account both of good agricultural practice and toxicological data and should be understood as the minimum quantity required to achieve effective protection against plant pathogen infections, and still be acceptable for consumer intake.

Mancozeb was repeatedly evaluated by the JMPR in 1967, 1970, 1974, 1977, 1980 and 1993. A temporary ADI of 0.025 mg/kg bw/day set 1967 was reduced to 0.005 mg/kg in 1974. An ADI of 0.05 mg/kg bw/day was finally established at the 1980 Meeting for mancozeb or the sum of maneb, mancozeb and zineb, of which not more than 0.002 mg/kg bw may be present as ETU. This was then reduced to 0.03 mg/kg at the 1993 Meeting (JMPR, 1993).

*European Union*

Use of mancozeb as a plant protection product is currently authorised in the European Union until 31/01/2018 according to the Plant Protection Product Regulation (EC) No 1107/2009 (a two years extension was granted in 2013 to allow applicants to complete the renewal procedure). Currently, the ADI is set at 0.05 mg/kg bw, the ArfD at 0.6 mg/kg bw and the acceptable occupation exposure level (AOEL) at 0.035 mg/kg bw/day. MRLs set for individual food items are publicly available via the EU pesticide database.

*United States of America*

The US Environmental Protection Agency published its Reregistration Eligibility Decision on mancozeb in 2005. The chronic Population Adjusted Dose (cPAD) used its risk assessment of dietary exposure of the general population, a dose metric equivalent to the ADI, was determined as 0.05 mg/kg bw/day and its acute PAD (aPAD), a dose metric

equivalent to the ArfD was determined as 1.3 mg/kg bw/day. Maximum residue limits are referred to as 'tolerances'. The United States and Canada, along with other OECD member countries, use the OECD MRL Calculator to calculate pesticide tolerances or MRLs. The OECD MRL Calculator replaces and supersedes the (North American Free Trade Agreement (NAFTA) MRL Calculator.

*Australia*

The Australian Pesticides and Veterinary Medicines Authority is a government statutory authority established in 1993 to centralise the registration of all agricultural and veterinary chemical products into the Australian marketplace. Previously each State and Territory government had its own system of registration. The APVMA is responsible regulating 'agvet' chemicals up to—and including—the point of retail sale. Beyond this point the state and territory governments are responsible, including controlling the use of these chemicals. Dithiocarbamates are listed under Schedule 20 of the Australia New Zealand Food Standards Code – Standard 1.4.2 – Agvet chemicals that commenced on 1 March 2016. As such MRLs have been set for dithiocarbamates measured as CS2 (https://www.legislation.gov.au/Details/F2016C00812).

Summaries of registration applications are made publicly available for individual products but no public record of decisions of evaluation could be located by our online searches.

*Canada*

Mancozeb is currently under re-evaluation by Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the Pest Control Products Act. A consultation document proposing continued registration of most mancozeb uses in Canada and phase-out of certain uses with risk concerns was made publicly available. The online consultation is now closed and the report considering comments received is being finalised. (http://www.hc-sc.gc.ca/cps-spc/pest/part/consultations/_prvd2013-01/prvd2013-01-eng.php#a4).

Canadian MRLs are also calculated using the OECD MRL Calculator. Under NAFTA, Canada, the United States and Mexico are committed to resolving MRL discrepancies to the broadest extent possible. Canadian MRLs are specified in the proposed re-evaluation report of mancozeb (Health Canada 2013) and differences with US tolerances are presented in Annex IX of the same document. MRLs may vary from one country to another for a number of reasons, including differences in agricultural practices and the locations of the field crop trials used to generate residue data.

*Japan*

Under the Law to Partially Revise the Food Sanitation Law (Law No. 55, 2003), the Ministry of Health, Labour and Welfare (MHLW) introduced the positive list system for agricultural chemicals remaining in foods. This system prohibits the distribution of foods that contain agricultural chemicals above a certain level if MRLs have not been established. MRLs for sum of residues of dithiocarbamates as CS2 are established (http://www.m5.ws001.squarestart.ne.jp/foundation/agrdtl.php?a_inq=28700 ).

*China*

China operates a pesticide registration system under the Regulations on Pesticide Administration 2001. No substance specific publicly available information in English could be retrieved during our online searches.

<u>Assessment and measurement endpoints for hazard characterisations</u>

Mancozeb and its metabolite ETU are recognised thyroid toxicants and in line with the JMPR evaluation, thyroid toxicity is considered the critical endpoint for mammalian

toxicity and thereby human health. Mancozeb is classified as toxic for the (aquatic) environment. The extent to which effects seen in fish, birds and other taxa are considered to be related to the endocrine system appears to be itself correlated with the date of the evaluation.

Whilst some effects on fish, birds and other taxa have been noted in earlier available evaluations and risk assessments (DG SANCO 2005, USEPA 2005), these could not be clearly related to the endocrine system. No mention of amphibians was found in either document despite the fact that ETU is known to alter metamorphic development and thyroid gland histology in the amphibian metamorphosis assay. The focus of the following sections will therefore be limited to human health.

Assessment values and their derivation

*Human health: acceptable daily intakes (ADI)*

The review report by the European DG SANCO and the USEPA's Reregistration Eligibility Decision for mancozeb were both published in 2005 and were consistent with the last JMPR evaluation in 1993. The ADI (or cPAD) in all three evaluations were derived from a two-year dietary study in rats. The No Observable Adverse Effect Level (LOAEL) was 125 ppm, equal to 4.8 mg/kg bw/day, based on decreased body-weight gain, decreased T3, T4 values, increased TSH values, increased absolute and relative thyroid weight, thyroid follicular cell hypertrophy, hyperplasia, and nodular hyperplasia, in both sexes at 750 ppm.

Under the Food Quality Protection Act, USEPA did consider the application of an additional tenfold (10X) safety factor, to account for potential pre-and postnatal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. As the dataset did include acceptable reproductive and developmental toxicity studies that did not show any indication of increased susceptibility to fetuses or offspring, this special safety factor was reduced to 1. No additional uncertainty factors were deemed necessary to account for uncertainties in the toxicology database. As a result, all three evaluations applied a safety factor of 100 and supported an ADI of 0.05 mg/kg bw/day.

The only difference was that the JMPR decided to establish a lower group ADI of 0-0.03 mg/kg bw for mancozeb, alone or in combination with maneb, metiram, and/or zineb, because their parent residues cannot be differentiated using presently-available analytical procedures.

The more recent Proposed Re-evaluation Decision by Health Canada departs from these evaluations. The ADI was derived from a one-year dog toxicity study with a NOAEL of 2.3 mg/kg bw/day based on thyroid hormone effects. In addition to the standard uncertainty factors of 100-fold for interspecies extrapolation and intraspecies variability, an additional 3-fold factor for database uncertainty (lack of ETU DNT and mancozeb immunotoxicity studies) was applied. The additional Pest Control Product Act factor for the protection of infants and children was reduced to one-fold as the selected endpoint was deemed to provide adequate margins to the reproductive and developmental endpoints of concern yielding a composite assessment factor is 300. The ADI derived by Health Canada by this method is equal to 0.008 mg/kg bw/day.

*Environment: consideration of endocrine disrupting effects*

Mancozeb is classified as dangerous to the environment. While there are notable differences between the USEPA Reregistration Eligibility Decision and the European Review Report (USEPA 2005, DG SANCO 2005) such as the presence or absence of effects in birds, both reports concurred that there was little evidence that any observed effect could be related to endocrine toxicity. Neither report made any mention of

amphibians, despite the fact that the ETU is known to alter metamorphic development and thyroid gland histology in the amphibian metamorphosis screening assay.

By contrast, the more recent Canadian Proposed Re-evaluation Decision (Health Canada 2013) interpreted effects in birds, mammals, amphibians, freshwater fish and invertebrates as 'indicative of hormonal disruption and would tend to support the concern that mancozeb (as parent and/or complex form) and ETU may be potential endocrine disrupting compounds'.

Exposure assessments

Exposure assessment for the general population, sensitive subgroups, workers, bystanders and various environmental receptors are expected to vary with national or local circumstances. Typically estimated environmental exposures are derived from the results mandatory fate, transport and transformation tests combined with data on pesticide use, whilst dietary intakes are derived from MRLs or monitoring data (from field trials or market basket surveys) when available and food intake surveys.

Risk assessments

*European Union*

The Theoretical Maximum Daily Intake (TMDI; excluding water and products of animal origin), based on MRLs and the FAO/WHO European Diet (August 1994), was 39 % of the ADI for a 60 kg adult and DG SANCO concluded that mancozeb fulfilled safety requirements (DG SANCO 2005).

*United States*

Aggregated risks from chronic exposure to both mancozeb and ETU via food and drinking water intake for the general population and sensitive subgroups were all found to be less 100% of the cPAD and therefore were not considered of concern (USEPA 2005).

*Canada*

Aggregated risks from chronic exposure to both mancozeb and ETU via food and drinking water intake for the general population and sensitive subgroups were all found to be less 100% of the ADI and therefore were not considered of concern. However, a lifetime aggregate cancer risk for ETU was 8 × 10-6 which was found to be of concern (Health Canada 2013).

Health Canada (2013) considered that some of the toxicity observed in environmental receptors may be related to endocrine disruption and also subsequently found that mancozeb may pose a risk to beneficial arthropods used in Integrated Pest Management programs, birds, small wild mammals, and to aquatic organisms. ETU may also pose a risk to small wild mammals.

The risk quotient calculated for chronic effects for amphibians on the thyroid histology did exceed the level of concern. However, this was deemed a highly conservative endpoint because it is unknown whether the observed histological changes to the thyroid will result in decreased survival. An endpoint for developmental effects in the forelegs of frogs following exposure to ETU is considered to be more severe and could result in the decreased survival of amphibians. When this endpoint is used to calculate the risk quotient the level of concern is not exceeded and Health Canada therefore concluded that amphibians are not at risk (Health Canada 2013).

Consideration of mixture effects

The potential for mixture effects is not addressed consistently by the various authorities. For the purpose of its reregistration eligibility decision, the USEPA had considered the possibility that mancozeb may act in concert with other dithiocarbamates. After a thorough review of relevant mechanistic data, the Agency reached the conclusion that mancozeb did not share a common mechanism with other substances and it was therefore not necessary to consider mixture effects. It nonetheless recognised that dithiocarbamates share a common metabolite, ETU, and its effects were considered (USEPA 2005).

The European report made no mention of mixture effects at the time of publication (DG SANCO 2005). However, the European Food Standard Agency (EFSA) has since investigated the grouping of pesticides for the purpose of deriving MRLs. A methodology was developed resting on the assumption that pesticides causing the same specific phenomenological effects, can produce joint, cumulative toxicity – even in the absence of a similar mode of action. The application of the approach was carried out with thyroid disrupters and resulted in a much larger assessment group of around 100 active substances, including mancozeb (EFSA 2013).

Health Canada refers to the possibility of mixture effect considering the only two ethylene bisdithiocarbamate fungicide registered for food use in Canada, mancozeb and metiram, nabam being registered for industrial uses only. Exposure to ETU in the environment or in occupational settings from non-pesticidal sources were not considered as they are regulated separately (Canadian Environmental Protection Act, 1999). Further, as the aggregate exposure from food and water to ETU derived from mancozeb was found to be of concern on its own due to carcinogenic risk, a cumulative risk assessment was considered redundant.

Risk management options

In the United States, the Reregistration Eligibility Decision details a number of restricted uses and protection measures related to residential or occupational exposure. The potential for endocrine disruption from the available human health and ecological effects data was considered. For human health risk assessment, thyroid effects are considered in the human health risk assessment. For possible hormonal effects in birds and mammals, it was suggested that mancozeb may be subject to additional testing and screening when the appropriate screening and/or testing protocols being considered under the Endocrine Disrupter Screening Program have been developed. Mancozeb was not included in list 1 or list 2 of the EDSP.

Health Canada has also proposed a number of risk management measures in relation to the risk to beneficial predatory arthropods, birds, small mammals and aquatic organisms ranging from labelling requirements to the implementation of spray buffer zones (Health Canada 2013).

In the European Union, DG SANCO in its review report also recommended that Member States request additional studies for birds and mammals and developmental toxicity and that they consider risk mitigation measures for the protection of birds, mammals, aquatic organisms and non-target arthropods. Recent EU legislation has introduced endocrine disrupting properties as a hazard-based ‘‘cut-off’’ criterion for the approval of active substances as pesticides and biocides (Reg. (EC) No 1107/2009). Marx-Stölting et al. (2014) compared two options for the science-based criteria to decide whether a substance should be considered an endocrine disrupter with the interim criteria in place until a decision has been reached by the Commission. Mancozeb was one of the substances evaluated as part of this exercise and for which the evaluators’ opinion differed when additional elements such as potency were included. More recently, evidence for endocrine-disrupting properties of mancozeb were reviewed as part of a screening exercise related to the impact assessment of various options for science-based criteria required by European legislation. Mancozeb was found to be an endocrine disrupter under all options but the interim criteria currently in place (DG SANTE 2016).

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### *4.6 Case Study: Prochloraz, human health*

Summary

Prochloraz is a broad-spectrum imidazole fungicide. It was first synthesized in mid and late 1970's (PPDB, 2016) while The Boots Company presented and produced it commercially from 1980 (INCHEM, 2001).

Prochloraz is widely used in gardening and agriculture. It is used on wheat, barley, mushrooms, cherries, turf on golf courses, and in flower production, for instance, in Ecuador, where roses are treated with prochloraz prior to export to the USA (Vinggaard et al. 2006).

Its fungicidal activity is due to inhibition of 14 alpha- demethylase (CYP 51), an enzyme required for the synthesis of fungal cell walls. Prochloraz is recognized as an endocrine disrupter causing adverse effect on male reproductive development by multiple mechanisms of action in non-target species including inhibition of enzymes of steroidogenesis (CYP 19, CYP 17 and 5 α-reductase) and AR antagonism. Other possible mechanisms of action are ER antagonism and AhR agonism (OECD, 2012). Prochloraz was one of the case studies used for the OECD GD 150 developed to evaluate whether the conclusions and next steps recommended in the guidance document for identification of ED was sensible and helpful when assessed in light of comprehensive datasets (OECD, 2012). Prochloraz is on the European Union (EU) Prioritization List as Cat. 2 (potential).

Prochloraz has earlier on been authorized for use as a pesticide in many parts of the world, and in the EU (apart from DK and Malta) the approval expires 31st December 2021. The ADI for prochloraz on 0.01 mg/kg is based on the effects on a 2 year study in dogs. Plant protection products undergo broadly comparable registration process in all jurisdictions and the substance-specific monographs produced by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR) play an important role in the harmonization of acceptable daily intakes (ADI = 0.01 mg/kg bw/day) for human health risk assessment. The JMPR report mentions that the intake range is 7-10% (% of maximum ADI).

The potential for mixture effects of prochloraz and other anti-androgens has been shown in studies of developing rats. However, the cumulative risk does not appear to have been addressed by the various authorities.

Scope of this case study

This case study focuses on the endocrine disrupting effects of Prochloraz on male reproductive development in humans. Effects of Prochloraz on the environment are also relevant, but are considered to be outside the scope in this document.

Discovery as an endocrine disruptor

Prochloraz is an imidazole fungicide, and its regulatory toxicological data package has been primarily generated in the 1980s-1990s and in these studies the endocrine activity or the endocrine disrupting effects of prochloraz have not been in focus. Prochloraz has been tested in a full set of regulatory toxicological studies including two multi-generation reproductive toxicity studies, which was performed according or comparable to the US EPA OPPTS 870-3380, OECD TG 416 (1983) (EFSA conclusion, 2011). These guidelines, however, precede both OPPTS and OECD harmonization and lack specific parameters to identify anti-androgenicity (e.g., sperm parameter, onset of puberty) so the discovery of prochloraz as an ED was not part of regulatory testing. Besides these regulatory studies, prochloraz has been extensively studied in mode of action studies during the last 15 years (Melching-Kollmuss et al. 2016). Screening studies have shown that prochloraz elicits multiple mechanisms of action in vitro, as it antagonises the androgen and the oestrogen receptor, agonises the Ah receptor and inhibits aromatase activity (Vinggaard et al. 2006).

Regulatory framework in selected jurisdictions

*European Union*

In 2008 the EU Commission decided not to include prochloraz (among a number of other active substances) in Annex I to Council Directive 91/414/EEC (EU, 2008). However, prochloraz was approved on 1 January 2012 by Commission Implementing Regulation (EU) No 1143/2011 as an active substance in accordance with Regulation (EC) No 1107/2009, and is thus authorised for use as an active substance in pesticide products. It was a specific provision of the approval that the applicant was required to submit to the European Commission further studies as regards comparison and verification of the test material used in the mammalian toxicity and ecotoxicity dossiers against the specification of the technical material and further studies regarding the environmental risk assessment for the metal complexes of prochloraz by 31 December 2013.

This EU approval expires 31st December 2021. At the EU Member State level it has been approved in 25 EU countries (except Malta and Denmark) (EU, 2016).

*United States of America*

The chemicals listed as U.S. EPA registered are those that can currently be legally used in the U.S., except in states where state laws are stricter than federal laws and prohibit such use. It is unclear whether any regulatory actions have been taken on prochloraz and whether it can be used legally in the USA (EPA, 2016).

*Japan*

The Food Safety Commission of Japan (FSCJ) has not conducted a risk assessment of prochloraz. Under the Law to Partially Revise the Food Sanitation Law (Law No. 55, 2003), the Ministry of Health, Labour and Welfare (MHLW) introduced the positive list system for agricultural chemicals remaining in foods. This system prohibits the distribution of foods that contain agricultural chemicals above a certain level if MRLs have not been established. MRLs for prochloraz are established for the sum of residues of prochloraz prochloraz and each of N-folumyl-N-1-propyl-N-[2-(2,4,6-trichlorophenoxy) ethyl] urea and N-propyl-N-[2-(2,4,6-trichloro phenoxy) ethyl] urea, and 2,4,6-trichlorophenol, which are individually calculated as prochloraz and is in the range of 0.05-10 (JFCRF, 2015).

*China*

China operates a pesticide registration system under the Regulations on Pesticide Administration 2001. No substance-specific publicly available information in English could be retrieved during our online searches.

*Australia*

In Australia, 10 products with prochloraz as active ingredient are listed by the Australian Pesticides and Veterinary Medicines Authority (APVMA)(APVMA, 2016a). For most of the products an expiry date of 30/6 2017 is included.

APVMA (Australian Pesticides and veterinary Medicines Authority) have nominated the triazoles fungicides and prioritised them for reconsideration but prochloraz is not included in this prioritisation (APVMA, 2016b).

Assessment and measurement endpoints for hazard characterisations

Prochloraz is a fungicide belonging to the imidazole group and acts as an inhibitor of ergosterol biosynthesis in fungi. Imidazole fungicides inhibit the activity of lanosterol 14α-demethylase, which is a fungus-specific cytochrome P450 enzyme. Prochloraz has

been shown to react through several endocrine disrupting mechanisms, such as AR antagonist and can interfere with testosterone synthesis by inhibiting the CYP450 17a-hydroxylase / 17,20-lyase as shown in vitro studies (Vinggaard et al., 2005, 2006). Moreover in vivo it can affect the development of several androgen-dependent tissues (Vinggaard et al., 2002; Vinggaard et al. 2005, Laier et al. 2006, Taxvig et al. 2008). Common features for the azole fungicides are that they increase gestational length and affect steroid hormone levels in foetuses and/or dams. In addition, studies indicate that prochloraz may also affect thyroid hormone levels and cause effects on the sexually dimorphic development of the brain (Vinggaard et al. 2005). In the majority of studies, male offspring, exposed in utero to prochloraz often showed no statistically significant changes in anogenital distance (AGD) with doses from 25-150 mg/kg but find significant nipple retention (NR) (Vinggaard et al. 2005; Christiansen et al. 2009; Noriega et al. 2005 and Melching-Kollmuss et al. 2016). One study has found both a decrease in male AGD at 50 and 150 mg/kg and also increased NR (Laier et al. 2006).

Assessment endpoints used to derive for points of departure (NOAELs) for prochloraz can be grouped into two broad categories, long-term studies on adult laboratory animals, and two-generation studies in rodents.

In long-term studies, prochloraz induced liver weight increases, and this measurement endpoint was used to estimate NOAELs for the derivation of ADI values.

Reproductive and developmental toxicity of prochloraz was also assessed in two-generation toxicity studies. In these studies, reproductive outcomes were measured in terms of extended gestation lengths, dystocia and reduced live birth and viability indices.

Assessment values and their derivation

The lowest NOAELs from long-term studies were 0.9 mg/kg body weight/day (mg/kg bw/day) in dogs, 5.1 mg/kg bw/day in rats and 7.5 mg/kg bw/day in mice all increased liver weight and histopathology (EFSA conclusion 2011). The ADI (acceptable daily intake) of prochloraz is 0.01 mg/ kg bw which is based on the lowest NOAEL of 0.9 mg/kg bw, derived in dogs based on liver weight increases (EFSA, 2011).

Prochloraz was evaluated in two key two-generation toxicity studies from 1993 and 1982 where overall reproductive performance was impaired following prochloraz administration to rats. Effects on reduction in body weight and body weight gain, increased liver weight and deaths were associated with dystocia and extended gestation length. Developmental toxicity was observed as reduced mean litter size, increased total litter loss, reduced live birth index, impaired growth and adverse effects on organ weights. In the 1993 study the agreed parental and reproductive NOAEL is 50 ppm (2.26 mg/kg bw/d), and the offspring NOAEL is 150 ppm (6.58 mg/kg bw/d). In the study from 1983 the agreed parental NOAEL is 150 ppm (13 mg/kg bw/d), the reproductive NOAEL is 37.5 ppm (3.1 mg/kg bw/d), and the offspring NOAEL is 150 ppm (13 mg/kg bw/d. (EFSA, 2011).

In the developmental toxicity studies, there was no evidence of teratogenicity, and the relevant maternal and developmental NOAELs are 25 mg/kg bw/d for the rat and 40 mg/kg bw/d for the rabbit. Public literature reports effects of prochloraz on reduced anogenital distance (Vinggaard et al, 2005 in EFSA, 2011) and increased nipple retention (Christiansen et al. 2009 in EFSA, 2011) in rats, with the NOAEL for these effects being 30 mg/kg bw/d and 5 mg/kg bw/d, respectively (EFSA, 2011). The higher NOAELs in reproductive toxicity studies of prochloraz, reproductive toxicity is not the critical toxicity in the derivation of ADIs. Critical for the derivation of ADIs is toxicity to the liver in long-term studies. Assessment values and the basis for their derivation are shown in the table below. The ADI for prochloraz has been based on the long term effects on dogs.

Table 1. ADI(Acceptable daily intake), ARfD(Acute Reference dose) , AOEL (Acceptable Operator Exposure Level)

| Organisation | Critical effect (animal species) | Critical dose (NOAEL/LOAEL/BMD) | AF | DNEL/ADI/reference dose (mg/kg/d) |
|---|---|---|---|---|
| EC (DAR 2007) | Increased liver weights | 0.9 (found in the 2-year dog study) | 100 | 0.01 (ADI) |
| EC (DAR 2007) | Increased liver weights | NOAEL of 2.5 mg/kg bw/d (considering the effects observed<br><br>in the 90-day dog, multigeneration rat and 14-day dog studies) | 100 | 0.025 (ARfD) |
| EFSA (2011) | Increased liver weights | NOAEL of 2.5 mg/kg bw/d (found in the 90-d dog study) with 70% correction for oral absorption AOEL = 2.5/100 x 0.7= 0.0175 | 100 | AOEL 0.02 (AOEL) |
| FAO and JMPR (2001) and JMPR (2004) | Increased liver weights | Prochloraz was evaluated by the FAO/WHO JMPR several times from 1983- 2001. The 2001 JMPR confirmed the ADI of 0.01 mg/kg bw/d and set an acute reference dose of 0.1 mg/kg bw (JMPR 2001). | 100 | ADI 0.01<br><br>ARfD 0.1 |

Exposure assessments

In 2008, the average exposure to prochloraz in the general Danish population was estimated as 0.004 µg/kg bw/day (FVST, 2008), with a 95th percentile of 0.011 µg/kg bw/day (Jensen et al. 2013).

In a research study, estimates for human intake of anti-androgenic chemicals (including prochloraz) were made and a TMDI (Theoretical Maximum Daily Intake) for France was set at 14 µg / kg d (Christiansen et al. 2012 referred to Menard et al., 2008).

Risk assessments

*EU*

The overall conclusion from the evaluation made by DG SANCO is that it may be expected that plant protection products containing prochloraz will fulfil the safety requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC (EU, 2016). Estimates of acute dietary exposure of adults and children revealed that the Acute Reference Dose (ARfD) would not be exceeded (EU, 2016).

The EFSA Draft Assessment Report (EFSA DAR, 2007) writes that the risk assessment carried out indicates that the estimated risk to the bystander will not exceed the AOEL (0.0175 mg/kg bw) under practical conditions of use.

*Japan*

The Food Safety Commission of Japan (FSCJ) has not conducted a risk assessment of prochloraz[20].

Mixture effects

The potential for mixture effects of prochloraz and other anti-androgens has been shown in studies of developing rats. For example, a mixture of the pesticides epoxiconazole, mancozeb, prochloraz, tebuconazole and procymidone caused severe effects on gestation length, nipple retention and genital malformations at dose levels where the individual pesticides caused no or smaller effects when given alone (Hass et al. 2012). Generally, the mixture effect predictions based on dose-additivity were in good agreement with the observed effects.

The potential for mixture effects is not directly addressed by the various authorities. However, the European Food Standard Agency (EFSA) has investigated the grouping of pesticides for the purpose of deriving MRLs. A methodology was developed resting on the assumption that pesticides causing the same specific phenomenological effects, can produce joint, cumulative toxicity – even in the absence of a similar mode of action. The application of the approach was carried out with anti-androgens in a scientific report submitted to EFSA and resulted in an assessment group of around 25 active substances (Nielsen et al. 2012. The assessment group does not include prochloraz as it was not included in Annex I of Council Directive 91/414/EEC (up to 31st of May 2009).

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### *4.7 Case Study: Procymidone, human health*

Summary

Procymidone is a dicarboximide fungicide. Procymidone is recognized as an endocrine disrupter causing adverse effect on male reproductive development due to AR antagonism. Earlier on, procymidone has been authorized for use as a pesticide in many parts of the world, but is no longer approved in the EU and the USA. The ADI for procymidone has been based on the effects on reproduction, such as infertility and abnormalities of the male sexual organs in adults and in pups. In the last decade, testing during sensitive windows of development in relation to endpoints reflective of anti-androgenic effects (e.g. anogenital distance) and negative impacts on male reproductive development has begun. This has resulted in lower points of departure (NOAELs) with correspondingly lower ADIs in EU, Australia and Japan. In EU, the recognition of the severity of such effects has also lead to the lowest ADI due to the use of an additional assessment actor of 3. To reflect the decrease in the ADI, the Maximum Residue Limits (MRL) in food had to be lowered in EU. The various ADIs exist next to each other and the variation from the lowest to the highest is around 30 fold. Risk assessments of consumer intake in the EU and Australia have not signalled concerns. The potential for mixture effects of procymidone and other anti-androgens has been shown in studies of developing rats. However, the cumulative risk does not appear to have been addressed by the various authorities.

Scope of this case study

This case study focuses on the endocrine disrupting effects of procymidone on male reproductive development in humans. Effects of procymidone on the environment are also relevant, but this is outside the scope in this document.

Discovery as an endocrine disruptor

Procymidone is a dicarboximide fungicide. Procymidone was discovered as a reproductive toxicant during regulatory testing and the type of effects seen in male offspring, especially decreased anogenital distance and malformation of the external genitalia (hypospadias) clearly points to an anti-androgenic mode of action. This was supported by in vitro studies showing AR antagonism. The characteristics of procymidone were similar in assays for binding to androgen receptors in rats and humans and the concentrations required to inhibit activity by 50% (IC50) values for procymidone were similar to those of the anti-androgen prostate-cancer drug flutamide (JMPR 2005).

Regulatory framework in selected jurisdictions

*European Union*

Procymidone had been authorized for use as a pesticide in EU, but the inclusion on the list of approved pesticides expired in 30 June 2008 and the authorization was withdrawn 1 July 2008 (EU database 2016).

Procymidone is classified as a toxic to reproduction in category 1B (May cause harm to the unborn child) and as a carcinogen in category 2 (Limited evidence of a carcinogenic effect).

*United States of America*

The chemicals listed as U.S. EPA registered are those that can currently be legally used in the U.S., except in states where state laws are stricter than federal laws and prohibit such use. Procymidone is not on this list and consequently cannot be used legally in the USA.

*Canada (British Columbia)*

Health Canada sets maximum residue limits (MRLs) in food and MRLs for procymidone were established for wine, grapes and raisins in 2008. The MRLs are also calculated using the OECD MRL Calculator. Under NAFTA, Canada, the United States and Mexico are committed to resolving MRL discrepancies to the broadest extent possible.

*Japan*

Under the Law to Partially Revise the Food Sanitation Law (Law No. 55, 2003), the Ministry of Health, Labour and Welfare (MHLW) introduced the positive list system for agricultural chemicals remaining in foods. This system prohibits the distribution of foods that contain agricultural chemicals above a certain level if MRLs have not been established. MRLs for procymidone are established (MHLW 2016).

*China*

China operates a pesticide registration system under the Regulations on Pesticide Administration 2001. No substance specific publicly available information in English could be retrieved during our online searches.

*Australia*

In Australia, 18 products with procymidone as active ingredient are listed by the Australian Pesticides and Veterinary Medicines Authority (APVMA 2016). For most of the products an expiry date of 30/6 2017 is included, but for others the status is 'approved' and there is no information on expiry dates.

Assessment and measurement endpoints for hazard characterisations

The assessment endpoints used for hazard characterisations of procymidone were for carcinogenicity (testicular interstitial cell tumours) and for endpoints characteristic of reproductive toxicity, including infertility and abnormalites of the male sexual organs in adults and pups. Changes in anogenital distance, hypospadias, testicular atrophy and undescended testes, increased weight of the testes and decreased weight of the prostate, epididymis and seminal vesicles in pups were used in later studies of effects mediated by endocrine mechanisms.

Assessment values and their derivation

Procymidone was evaluated by the FAO/WHO JMPR in 1981, 1989, 1990, 1993, 1998 and 2001. No ADIs were established in 1981 and 1982. In 1989, an ADI of 0–0.1 mg/kg bw was established based on the NOAEL of 12.5 mg/kg bw per day identified in studies of reproductive toxicity in rats. In 2001, the JMPR evaluation was (FAO 2001): *"In a long-term feeding study reported in rats, testicular interstitial cell and ovarian stromal hyperplasia, and an increased incidence of testicular interstitial cell tumours, were observed at 1000 and 2000 ppm. In a 2-generation study in rats, infertility and abnormalites of the male sexual organs were observed in adults and in pups at the highest dose level of 750 ppm. The JMPR evaluated that the effects on reproduction and the induction of testicular tumours in the long term rat study can be explained by the effects of procymidone on the endrocrine system. The JMPR allocated an ADI of 0 to 0.1 mg/kg body weight for procymidone, based on sub-chronic effects in rats, mice and dogs and on chronic effects in mice and rats*".

In 2007 in the EU, the toxicological profile of procymidone was investigated by the rapporteur Member State France in the framework of the peer review under Directive 91/414/EEC and again in 2007 in view of the extension of the Annex I inclusion. An ADI of 0.025 mg/kg bw/day was previously assigned to procymidone (EC, 2007). France proposed to set a lower ADI value of 0.0028 mg/kg bw/d than previously derived in the

first peer review (France 2007). Member States and the European Commission confirmed that this toxicological reference value should be used for the risk assessment of MRLs although there was no formal adoption of these values by the Standing Committee on Food Chain and Animal Health (EFSA 2011).

In the EFSA peer review, an ADI of 0.025 mg/kg bw/day was assigned, the value being based on the NOAEL for the rat multi-generation study, 2.5 mg/kg bw/day (50 ppm), and a safety factor of 100 (EFSA 2009). The effects noted at 250 ppm (12.5 mg/kg bw/day) in pups were: reduced anogenital distance, hypospadias, testicular atrophy and undescended testes. Considering the additional information submitted in the framework of the renewal of the Annex I inclusion, the RMS concluded that increased weight of the testes and decreased weight of the prostate, epididymis and seminal vesicles were seen even at 50 ppm. Thus, the following assessment factors were proposed: a 3-fold factor (LOAEL → NOEL), a 10-fold factor for interspecies variability, a 10-fold factor for intraspecies variability and a 3-fold factor for the severity of the effects giving a safety factor of 900 and an ADI of = 0.0028 mg/kg bw/day. If the 50 ppm level was regarded as a LOAEL, the use of a safety factor of 1000 would lead to a similar ADI of 0.0025 mg/kg bw/day.

In 2009, EFSA self-tasked to revise the previously performed risk assessment of MRLs established for procymidone because Member States and the European Commission agreed on lower toxicological reference values (EFSA 2009). EFSA proposed to lower the MRLs for 24 different food commodities in order to reduce the acute and/or consumer exposure to a level where no negative consumer health effects are expected. Thus, these MRLS are currently used in EU.

In 2004, the Australian Government, Department of Health derived an ADI of 0.03 mg/kg bw/day for procymidone and this value still apply (Australian Government, Department of Health, 2016).

In 2014, the Food Safety Commission of Japan (FSCJ) in 2014 derived an ADI for procymidone based on summary reports made by applicants and documents of the EU, JMPR and others (Food Safety Commission of Japan, 2014). The lowest no observed adverse effect level (NOAEL) was 3.5 mg/kg bw/day, obtained in a developmental toxicity study in rats. FSCJ specified an acceptable daily intake (ADI) of 0.035 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

Assessment values and the basis for their derivation are shown in the table below. Earlier on, the ADI of 0.1 mg/kg bw/day for procymidone has been based on the effects on reproduction, such as infertility and abnormalities of the male sexual organs in adults and in pups (FAO (2001) and JMPR (1989-2005)). Many of the conventional studies of toxicity with procymidone were relatively old, were performed before the widespread use of GLP and some contained relatively limited information. Within the last decade, the ADI has decreased around 3-30 fold reflecting testing during sensitive windows of development combined with assessment of sensitive endpoints for anti-androgenic effects on male reproductive development (e.g. anogenital distance) as well as recognition in EU of the severity of such effects. The various assessment values and ADI exist next to each other.

Table 1: Assessment values for procymidone and their derivation

| Organisation | Critical effect (animal species) | Critical dose (NOAEL/LOAEL/BMD) | AF | DNEL/ADI/reference dose (mg/kg/d) |
|---|---|---|---|---|
| | | | | |
| EC (EFSA 2011) | Reduced anogenital distance, hypospadia, testicular atrophy, undescended testes | LOAEL = 50 ppm (2.5 mg/kg bw/d) | 900 incl.an additional AF of 10 for severity | 0.0028 |
| EC (DAR 2006 and 2007) | Reduced anogenital distance, hypospadia, testicular atrophy, undescended testes | NOAEL = 50 ppm (2.5 mg/kg bw/d) | 100 | 0.025 |
| Food Safety Commission of Japan (FSCF 2014) | Reduced anogenital distance, hypospadia | NOAEL 3.5 mg/kg bw | 100 | 0.035 |
| Australian Government, Department of Health (2016), from 2004-2016 | Increased (parental) testes weights and decreased epididymides and prostate weights at 250 ppm (12.3 mg/kg bw/d) | NOAEL = 50 ppm (2.5 mg/kg bw/d) | 100 | 0.03 |
| FAO (2001) and JMPR (1989-2005) | Infertility and abnormalities of the male sexual organs in adults and in pups; testicular tumours in adults | NOAEL = 12.5 mg/kg bw/d; Testicular tumours at higher doses | 100 | 0.1 |

Exposure assessments

The average exposure to procymidone in the general Danish population was in 2007 calculated to 0.012 µg/kg bw/day (Danish Ministry of Food, 2007). Based on probabilistic methods the 95 percentile was calculated to 0.041 µg/kg bw/day (Jensen et al. 2013).

Risk assessments

A consumer risk assessment for procymidone was published by EFSA in 2011 (EFSA 2011). No chronic intake concerns were identified for any of the European diets and the

total intake values accounted for a maximum of 93% of the ADI in the subgroup of French toddlers. Also, no acute intake concerns were identified.

Due to specific occupational health and safety concerns for women of child-bearing age, Food Standards Australia New Zealand (FSANZ) undertook a National Estimated Short Term Intake (NESTI) and a National Estimated Dietary Intake (NEDI) calculation to ascertain whether any public health and safety concerns existed from residues of procymidone for females aged 16 to 44 years (FSANZ 2007).

The Australian Pesticides & Veterinary Medicines Authority (APVMA) has deleted the uses and MRLs for some specific commodities and withdrew the permits for others. The NEDI of residues (based on the MRL) in food was 40% of the acceptable daily intake (ADI) of 0.03 mg/kg bw/day. Further, in later Australian Total Diet Surveys (ATDS) the estimated dietary exposure to Procymidone was less than 1% of the ADI for adult females 25-34 years of age. On the basis of results from the NEDI and the results from the ATDSs, FSANZ considers that chronic dietary exposure to the potential residues associated with MRLs for Procymidone would not present a risk to the health and safety of women of child-bearing age.

Mixture effects

The potential for mixture effects of procymidone and other anti-androgens has been shown in studies of developing rats. For example, a mixture of the pesticides epoxiconazole, mancozeb, prochloraz, tebuconazole and procymidone caused severe effects on gestation length, nipple retention and genital malformations at dose levels where the individual pesticides caused no or smaller effects when given alone (Hass et al 2012). Generally, the mixture effect predictions based on dose-additivity were in good agreement with the observed effects.

The potential for mixture effects is not directly addressed by the various authorities. However, the European Food Standard Agency (EFSA) has investigated the grouping of pesticides for the purpose of deriving MRLs. A methodology was developed resting on the assumption that pesticides causing the same specific phenomenological effects, can produce joint, cumulative toxicity – even in the absence of a similar mode of action. The application of the approach was carried out with anti-androgens in a scientific report submitted to EFSA and resulted in an assessment group of around 25 active substances Nielsen et al 2012). The assessment group does not include procymidone as the focus was only on pesticides approved for use in EU, i.e. those included in Annex I of Council Directive 91/414/EEC (up to 31st of May 2009).

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### *4.8 Case Study: Benzophenone-3*

Summary

Benzophenone-3, also commonly referred to as oxybenzone, is a light absorber widely used in sunscreens, cosmetics, as well as plastics intended to come in contact with food. The present case study focused on its use in sunscreen lotions. The possibility that benzophenone-3 may affect the hormonal system was first raised when the National Toxicology Program in the United States published a report reviewing in vivo experimental evidence of the effects of benzophenone-3. The consistency of an effect on sperm density, estrous cycles and other parameters was thrown into doubt following the publication the following year of an industry sponsored study that did not find any effect. The ability of benzophenone-3 to bind with the estrogen receptor in vitro when several UV screens were tested in the MCF-7 proliferation assay and the uterotrophic assay. The Danish Centre for Endocrine Disrupters recently reviewed all publicly available epidemiological and (eco)toxicological data and concluded that benzophenone-3 would be considered a suspected endocrine disrupter (category 2a) if the criteria proposed by the Danish Government were applied.

A major difference in the manner sunscreen ingredients are regulated in different jurisdictions is whether they are considered drugs/therapeutic goods (US, Canada, Japan, Australia) or cosmetics (EU, China). Nonetheless, all countries apply restriction for use in terms of the maximum concentration of benzophenone-3 allowed in the formulation of the product, although these vary from 5-15% by weight. The processes by which such restrictions are decided are rather opaque and apart from in Europe, our searches did not uncover any documentation of these decisions online. The opinion of the European Scientific Committee on Consumer Products did consider reproductive toxicity but not the estrogenic activity in Benzophenone as it had concluded in an earlier opinion that it did not endanger human health under current conditions of use. The point of departure to estimate the margin of exposure was derived from a teratogenicity study that only showed effects at the highest dose concomitant with signs of maternal toxicity. The margin of exposure was calculated as 112 which is greater than 100 and the use of benzophenone-3 at concentrations up to 6% by weight was considered safe for use. A large biomonitoring program in the United State showed that benzophenone-3 is bioavailable and exposure is widespread (97% of the sampled population). Benzophenone-3 is known to enhance the dermal absorption of other substances and there are some restrictions on formulations containing both benzophenone-3 and insect repellents. Investigation of the dermal absorption of a mixture of sunscreen ingredients including benzophenone-3 over a week in human volunteers observed no effect on endogenous reproductive hormone homeostasis. Beside stated restrictions with regards to benzophenone-3 concentration in product formulation, its use is generally considered safe and no further risk management options are recommended to protect human health.

Scope of this case study

Benzophenone-3, also commonly known as oxybenzone, is an ultraviolet (UV) light absorber. It is used to help prevent potential damage from sunlight exposure, often in combination with other benzophenones, in many consumer products including sunscreens but also other cosmetic products such as hairspray or nail varnish, in paints and inks, as well as in plastic intended to come in contact with food. The focus of this case study is on its use as an over-the counter sunscreen.

As it is incompletely absorbed by skin, it pollutes surface and coastal waters directly or via sewage effluent discharges. Water pollution, bathing water quality, secondary poisoning through fish consumption and effects on coral reefs were considered beyond the scope of this case study.

Discovery as an endocrine disruptor

Benzophenone-3 occurs naturally in flower pigments. It has been synthesised and used commercially as a UV light absorber and stabiliser since the 1970's. In 1992, the National Toxicology Program published a technical report on 2- and 13-week toxicity studies of 2-hydroxy-4-methoxybenzophenone, also known as benzophenone-3 or oxybenzone (French, 1992). The report concluded that benzophenone-3 was a moderate reproductive toxicant at high dietary doses on the basis of observed increases in estrous cycle length and decreases in epididymal sperm density and suggested that such toxicity at least in females may operate via hormonal mechanisms. The summary of comments from peer reviewers reveals that the fact that the lack of a NOAEL for decreased epididymal sperm density in the 13-week dermal study in mice was subject to some debate. Mention was made of a subsequent study of the effect of topically applied benzophenone-3 on sperm production in another strain of mice, sponsored by the Cosmetic Toiletry and Fragrance Association, that failed to show statistically significant decreases in epididymal sperm density or any other effects on the reproductive system (Daston et al. 1993). It was deemed too difficult to compare the results of these toxicity studies with human exposure under conditions of used deemed safe at the time and this report did not lead to any revision of safe condition of use.

The hormonal activity of benzophenone-3 was not investigated further until Schlumpf et al (2001), following reports of high concentrations reported in German fish, decided that their potential for bioaccumulation warranted that six frequently used UV filters be screened in vitro and in vivo for estrogenic activity. Benzophenone-3 was found to be an estrogen receptor agonist in vitro in MCF-7 breast cancer cells but showed only weak in vivo uterotrophic activity in the immature rat assay. This study prompted a request to the then European Scientific Committee on Cosmetic products and Non-Food Products intended for consumers (SCCNFP) to evaluate the possible estrogenic effects of organic UV filters (SCCNFP, 2001). It concluded that at least as far as benzophenone-3 is concerned, the results of Schlumpf et al were in line with those of another study carried out according to Good Laboratory Practice (GLP) standards commissioned by the cosmetic industry. Further, the Committee proposed that the weak estrogenic activity of benzophenone-3 in vivo was related to the fact that about 1% of the dose is metabolised to p-hydroxy-benzophenone in rats, itself a compound which might exhibit an estrogenic effect. Since then, benzophenone-3 has been screened in a large number of in vitro assays, most of which show estrogenic activity (there are however also studies that found no in vitro estrogenicity) but also other modes of action for this compound such as androgen and progesterone receptors antagonism and binding to the thyroid hormone receptor (Danish Centre on Endocrine Disrupters, 2012). Benzophenone-3 was included by ChemSec on the SINList 2.0[21] and the Danish Centre on Endocrine Disrupters categorised it as a suspected endocrine disrupter (category 2a) on the basis of an evaluation of results from human health, in vitro/vivo studies and studies in the environment and the Danish proposal for criteria (Danish Centre on Endocrine Disrupters, 2012).

Regulatory framework in selected jurisdictions

*International Cooperation on Cosmetics Regulation (ICCR)*

The ICCR is a voluntary international group of cosmetics regulatory authorities from Brazil, Canada, the European Union, Japan and the United States established in 2007. This group of regulatory authorities meet on an annual basis to discuss common issues on cosmetics safety and regulation. No information on sunscreen could be found on their website.

[21] www.sinlist.chemsec.org

*European Union*

Sunscreen products are cosmetics according to Regulation (EC) No 1223/2009. The safety of cosmetic products is in the EU based on the safety of the ingredients, the latter being evaluated by toxicological testing. Until recently, this was done by using experimental animals. This regulation introduced a ban on animal testing, making the use of validated alternative methods in toxicological testing compulsory. Only replacement methods are allowed. Via the combination of a testing and marketing ban, in vivo testing outside the EU was allowed for repeated dose toxicity (including skin sensitisation testing), developmental toxicity and toxicokinetics until 11 March 2013.

For sunscreen products, there are also specific recommendations on efficacy claims[22]. Annex VI of the Cosmetics Regulation list UV filters allowed in cosmetic products. Benzophenone is authorised in concentrations up to 10% by weight.

*United States of America*

Sunscreen ingredients are regulated under the Federal Food, Drug, and Cosmetic Act (FD&C Act)[23]. Because the Food and Drug Agency (FDA) regulates sunscreen ingredients as drugs, each active ingredient must be approved before it can be allowed on the market under a Monograph process. The monograph process allows companies to avoid other more rigorous regulatory pathways. These monographs specify conditions whereby over-the-counter (OTC) drug ingredients are generally recognized as safe and effective. Historically, the human safety of sunscreen active ingredients contained in sunscreen products has been based on decades-long human experience, as well as preclinical and clinical safety testing.

Benzophenone was first approved for use in OTC sunscreen in the Advanced Notice of Proposed Rulemaking in 1978 in concentrations up to 6% by weight.

The Sunscreen Innovation Act (SIA) signed in November 2014 amends the FD & C Act to establish a process for the review and approval of OTC sunscreen active ingredients. The FDA published a draft guidance on sunscreens which describes the safety and efficacy requirements that each sunscreen ingredient will need to meet in order to be included in the OTC sunscreen monograph (FDA 2015). The FDA will publish the final guidance in late 2016.

*Australia*

UV filters can be regulated either as therapeutic goods or cosmetic sunscreens depending the stated (primary or secondary) purpose of the product and its efficacy, i.e. its sun protection factor (SPF). Primary sunscreens, products used primarily for protection from UV radiation, with a SPF of 4 or more and moisturisers containing sunscreen with SPF greater than 15 are regulated as therapeutic goods by the Therapeutic Good Administration (TGA). Therapeutic sunscreens are required to be included in the Australian Register of Therapeutic Goods (ARTG) before they can legally be marketed in Australia. Most secondary sunscreens (those with a primary purpose other than sunscreening but that also contain sunscreening agents) are regulated as cosmetics. The regulators are the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Australian Competition and Consumer Commission (ACCC)[24].

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[22] Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto (notified under document number C(2006) 4089) (Text with EEA relevance)

[23] http://www.fda.gov/regulatoryinformation/legislation/federalfooddrugandcosmeticactfdcact/

[24] https://www.tga.gov.au/about-sunscreen-regulation

The Australian regulatory guidelines for sunscreens (ARGS) have been developed to provide guidance to sponsors and manufacturers, and to assist in the understanding of the regulatory requirements for sunscreens in Australia (TGA, 2016). Benzophenone is listed in this guidance document as a permitted active ingredient for therapeutic sunscreens with a maximum concentration of 10% by weight.

*Canada*

Sunscreens can be classified either as natural health products or drugs depending on their medicinal ingredient (Health Canada, 2013). Accordingly, sunscreens will be subject to either the Food and Drug Regulations administered by the Therapeutic Products Directorate or the Natural Health Products Regulations administered by the Natural Health Products Directorate. Benzophenone-3 is listed as a drug medicinal ingredient and is authorised in concentrations up to 6% by weight.

Under the auspices of the Canada-United States Regulatory Cooperation Council, Health Canada and U.S. Food and Drug Administration will coordinate and adjust their respective Over-the-Counter (OTC) monographs development processes for OTC drugs to reduce the regulatory burden on stakeholders. Health Canada is currently updating its approach to sunscreens, while the US FDA is due to issue its final guidance document on sunscreens at the end of 2016[25].

*Japan*

In Japan, cosmetics are regulated by the Ministry of Health, Labour and Welfare (MHLW) under the Pharmaceutical Affairs Law (PAL). For legal purposes, beauty products are divided into quasi-drugs and cosmetics. Sunscreens are classified as quasi-drugs, therefore, they require approval of their formulations, ingredients, use levels and functionalities, in addition to stability testing and a certificate showing no animal-derived materials were used (EU-Japan Centre for Industrial Co-operation, 2015).

MHLW notification allows benzophenone-3 for cosmetic use in concentrations of up to 5% by weight.

*China*

In China, sunscreens are considered special use cosmetics and require a Hygiene license from China Food and Drug Administration (CFDA). Cosmetics need to be tested in CFDA-accredited labs in China during the registration process even if they have been tested abroad or assessed. Hygiene safety tests include physio-chemical, microbiological and toxicological studies, which are mandatory for non-special use cosmetics. For special use cosmetics, such as sunscreen, human safety tests are also required[26].

The CFDA has published a consolidated Inventory of Existing Cosmetic Ingredients in China (2014) for public consultations[27]. Benzophenone-3 figures on this list and its maximum level of use already approved is 15% by weight.

<u>Assessment and measurement endpoints for hazard characterisations</u>

[25] http://www.hc-sc.gc.ca/ahc-asc/legislation/acts-reg-lois/rcc-ccmr/wp-counter-pt-comptoir-eng.php

[26] http://www.cirs-reach.com/news-and-articles/guidance-on-regulations-compliance-of-cosmetic-products-in-china-2016.html

[27] http://www.cirs-reach.com/Cosmetic_Inventory/China_IECIC_Inventory_of_Existing_Cosmetic_Ingredients_in_China.html

The publicly available documentation on standard information requirements for sunscreens focus on the evaluation of their efficacy rather than their safety. Maximum allowed concentration of the active ingredients are specified and as illustrated in the above section vary between 5-15% by weight. It is however unclear how and on the basis of which data these concentrations were derived. As benzophenone-3 has been used as a sunscreen ingredient for decades, it is possible that such values were mainly based on human experience and reports of allergic skin reactions. No safe level for benzophenone-3 in the body have been established.

The only notable exception is the European Union where the Scientific Committee on Consumer Products (SCCP) reviewed the Submission I dossier on the UV-filter Benzophenone-3 first submitted by COLIPA, the European Cosmetics Toiletry and Perfumery Association, in December 2005, applying for a maximum allowed concentration up to 6%. In December 2006, SCCP adopted an opinion concluding that the data presented were insufficient to calculate the margin of safety of Benzophenone-3 under the proposed conditions of use and requesting a dermal absorption study.

The applicants resubmitted a dossier with the additional required information in December 2007 and the SCCP published a second opinion in December 2008 (SCCP, 2008). The point of departure selected for its risk assessment was selected from a teratogenicity study in rats showing that benzophenone-3 caused some skeletal aberrations only at the highest dosage level (400 mg/kg bw/day), which also caused maternal toxicity. The NOAEL-value for maternal and developmental toxicity was 200 mg/kg bw/day. The opinion of the European Scientific Committee on Consumer Products did consider reproductive toxicity but not the estrogenic activity in Benzophenone as it had concluded in an earlier opinion that it did not endanger human health under current conditions of use (SCCP, 2006).

Exposure assessments

*United States of America*

Benzophenone-3 can be absorbed through human skin and excreted in the urine, mostly as a glucuronidated conjugate. The U.S. Center for Disease Control (CDC) National Health and Nutrition Examination Survey (NHANES) has been monitoring urinary benzophenone-3 levels in the general population over 6 years old since 2003 and found that it could be detected in 97% of samples (Calafat et al. 2008). The analysis showed that female participants had slightly higher urinary levels than males. The geometric mean and 95th percentile concentrations were 22.9 µg/L (22.2 µg/g creatinine) and 1,040 µg/L (1,070 µg/g creatinine), respectively.

*European Union*

In their 2008 opinion, the SCCP considered the newly provided in vitro dermal absorption (then Draft OECD TG 428: Percutaneous Absorption: in vitro Method (2000)) study scientifically acceptable. It showed a mean dermal absorption level of 19.3 µg/cm² or 3.1% of the applied dose for a sunscreen containing the maximum requested concentration of 6%. The mean value plus 2 standard deviations (9.9% [mean (3.1%) + 2 SD (2*3.4%)]) was be used for the calculation of the systemic exposure dose for an adult weighing 60kg (SCCP, 2008) equal to 1.78 mg/kg bw/day.

Risk assessments

*European Union*

On the basis of the hazard characterisation and exposure assessment described in the previous sections, the SCCP (2008) derived a margin of safety of 112. As this is above 100, it concluded that the use of benzophenone-3 as a UV-filter up to 6% in cosmetic

sunscreen products does not pose a risk to the health of the consumer, apart from its contact allergenic and photoallergenic potential.

*China*

Although no risk assessment was publicly available for benzophenone-3, the CFDA website states a safety risk assessment report is compulsory for the registration of domestic special cosmetics and imported cosmetics. There is however as yet no official guidance for the safety evaluation of cosmetic products in China and draft Guidelines on Safety Risk Assessment of Cosmetic Products have been compiled based on the Guidance for Safety Evaluation of Cosmetic Products in Europe.

Consideration of mixture effects

There is no routine requirement to consider the possibility of mixture effects. Nonetheless monographs generally mention 'synergistic percutaneous permeation', or the fact that benzophenone-3 can enhance the penetration of other chemicals such as insect repellents. A recent study investigating the dermal absorption of 10% of Benzophenone-3 in a sunscreen formulation and 10% of other UV filters reported no effect on endogenous reproductive hormone levels in humans after topical application (Janjua et al. 2004). It is cited in the SCCP opinion (2008) as evidence that benzophenone-3 does have any effects on hormone homeostasis.

Risk management options

The use of benzophenone-3 as a sunscreen ingredient below the stated maximum concentrations is generally considered safe for human use and no further risk management options have been proposed.

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TGA (Therapeutic Goods Administration) (2016) Australian regulatory guidelines for sunscreens. Department of Health, Australian Government. 43 pp.

### *4.9 Analysis of commonalities and differences in the regulatory status and in assessment valu*

The eight case studies presented in this chapter show that endocrine disrupting chemicals are treated in a variety of ways in the different legalities that we examined. In this section, a summary and a comparison of the case study findings is given, with the aim of identifying the degree of commonalities and differences, and whether any differences are the result of scientific disputes or simply a reflection of the features of the respective regulatory systems in the various legalities.

#### 4.9.1 Discovery as endocrine disruptors

The endocrine disrupting properties of five of the chemicals we considered were discovered in the context of scientific research activities; only three – two pesticides, prochloraz, procymidone and a cosmetic ingredient, benzophenone-3 – were identified through regulatory testing efforts. This may be due to the fact that pesticides and cosmetic ingredients are subject to rather extensive and relatively uniform regulation and testing across legislations, and also suggests that the framework of established regulatory testing is ill-equipped for identifying chemicals with endocrine disrupting properties.

#### 4.9.2 Regulatory status

All the pesticides analysed in our case studies are subject to regulations and restrictions in all the legalities considered in this project, although there are important differences in detail: Procymidone is not authorised for use in the EU and the USA, but is used in Japan and Canada (we were unable to establish its regulatory status in China and Australia). Similar considerations apply to benzophenone which is either treated as a cosmetic ingredient, with the restrictions that implies, or as a drug (e.g. USA). The precise commonalities and differences are manifestations of the details of the respective regulatory systems which e.g. in the EU facilitate withdrawal of authorisation for placement on the market.

The picture is much more varied for an industrial chemical such as bisphenol A. Restrictions apply in some legalities (e.g. EU and China), while in others, bisphenol A is essentially unregulated (USA, Canada, Japan, Australia). This is a reflection of differences in the regulatory regimes and of differences in the level of concern with which bisphenol A is treated (higher in the EU than anywhere else).

The use of DEHP is restricted in the EU and the USA, but not in Canada, Japan or China.

The regulatory status of nonylphenol varies considerably across the legalities we analysed, with water quality criteria implemented in the EU, the USA and Canada, but not in China, Japan and Australia.

In contrast, the status of ethinylestradiol is rather uniform. With the exception of Canada, which has established water quality criteria, there are no environmental standards implemented in any of the other legislations.

#### 4.9.3 Assessment values

The assessment values (acceptable daily intakes) that are applied for the pesticides considered in the case studies are rather uniform across all legislations apart from procymidone where the values vary by a factor of approximately 30. This appears to be a result of the internationally harmonised procedures of hazard characterisation that have evolved over the years in the area of pesticides. It is of note that the assessment values derived for prochloraz are based on toxicities unrelated to endocrine disruption.

A similar, rather uniform picture emerges for DEHP where the assessment values utilised in the different countries and legislations do not differ much, with the exception of the EU where a range of values is applied.

Greater differences became obvious for the environmental standards used for ethinylestradiol and nonylphenol, and for bisphenol A.

The water quality criteria (or equivalent) that are in use for risk assessments for ethinylestradiol vary by a factor of approximately 15. These differences are explained by the use of different experimental studies for the derivation of the values, and by the application of differing assessment factors.

In the case of nonylphenol, the greater differences between the various assessment factors (factor of approximately 60) are due to the fact that their derivation was based on distinct chemical entities (linear or branched side chain), with quite different toxic properties.

The greatest variations are apparent with bisphenol A where the assessment values in use internationally differ by no less than 10,000-fold. This is driven by the use of a variety of assessment endpoints, not all of which relate to endocrine disruption, and the application of widely differing assessment factors, reflecting differences in the evaluation of adversity, and a lack of scientific agreement about the basis for hazard characterisations.

The Table below gives a summary of the case study findings.

Table 1: Compilation of assessment values for case study substances

| Substance | EU | USA | Canada | Japan | China | Australia |
|---|---|---|---|---|---|---|
| **Ethinylestradiol** | | | | | | |
| Discovery | Endocrine disrupting effects in fish discovered accidentally | | | | | |
| Regulatory Status | Not regulated | Not regulated | Water quality standard | Not regulated | Not regulated | Not regulated |
| Assessment value (ng/L) | 0.035 | 0.1 | 0.5 | - | - | - |
| **Nonylphenol** | | | | | | |
| Discovery | Accidentally | | | | | |
| Regulatory Status | Water quality standard | Water quality standard | Water quality standard | Not regulated | Not regulated | Not regulated |
| Assessment value (µg/L) | 0.5 | 6.6 | 1 | - | 0.1-0.7 | - |

| Substance | EU | USA | Canada | Japan | China | Australia |
|---|---|---|---|---|---|---|
| **Bisphenol A** | | | | | | |
| Discovery | scientific screening | | | | | |
| Regulatory Status | Restrictions | Not regulated | Not regulated | Not regulated | restrictions | Not regulated |
| Assessment value (µg/kg/d) | 0.7 – 4 | 50 | - | - | - | |
| **DEHP** | | | | | | |
| Discovery | Accidentally | | | | | |
| Regulatory Status | Restrictions | Restrictions | Not regulated | Not regulated | | Not regulated, risk assessment |
| Assessment value (mg/kg/d) | 0.034 – 0.05 | 0.06 | 0.044 | - | - | - |
| **Mancozeb** | | | | | | |
| Discovery | scientific studies | | | | | |
| Regulatory Status | Restrictions | Restrictions | Restrictions | Restrictions | Restrictions | Restrictions |
| Assessment value (mg/kg/d) | | 0.05 | 0.008 | | | |
| **Prochloraz** | | | | | | |
| Discovery | regulatory testing | | | | | |
| Regulatory Status | Restrictions | Unclear | Restrictions | Restrictions | Unclear | Restrictions |
| Assessment value (mg/kg/d) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| **Substance** | **EU** | **USA** | **Canada** | **Japan** | **China** | **Australia** |
| **Procymidone** | | | | | | |
| Discovery | regulatory testing | | | | | |
| Regulatory Status | ban | ban | Restrictions | Restrictions | Unclear | Unclear |
| Assessment value (mg/kg/d) | 0.0028 – 0.025 | - | - | 0.035 | - | - |
| **Benzophenone-3** | | | | | | |
| Discovery | regulatory testing | | | | | |
| Regulatory Status | Restrictions | Restrictions | Restrictions | Restrictions | Restrictions | Restrictions |
| Assessment value | - | | | | | |

In summary, the differences and commonalities in the different legalities in dealing with endocrine disruptors are mainly an expression of the different features of the respective legal systems. In some cases, differences in scientific approaches are also playing a role. The impact these differences have on the final outcome of the derivation of regulatory values (e.g. water quality standards, acceptable daily intakes and similar) varies from compound to compound, but is occasionally considerable.

The differences in the respective assessment values could diminish if more consistent methods of hazard assessment were applied across the various legalities, with uniform, transparent and agreed criteria as to the selection of studies for hazard characterisations, and more transparency in the choice of assessment factors.

## 5. THE INTERNATIONAL WORKSHOP ON COMMONALITIES AND DIFFERENCES IN APPROACHE

### *5.1 Introduction*

This document is the report of a Workshop held on 19-20 September 2016 in Hotel Bloom in Brussels, in connection with the project Mapping commonalities and differences in approaches for testing and assessment of endocrine disruptors within the EU and among relevant international trading partners.

This report describes the workshop objectives and agenda, and gives an account of workshop presentations, working group discussions and recommendations.

The objective of the project Mapping commonalities and differences (…) itself is to compare approaches for the regulatory screening, testing and assessments of substances for the endocrine disrupting properties within the European Union (EU) and among relevant international trading partners, as well as the results of said approaches, in order to establish commonalities and differences and assess the drivers for these differences, with the following specific objectives:

1. To gain an overview of regulatory screening, priority setting, testing and assessment approaches applied to identify and assess endocrine disruptors in EU Member State, at EU level and by relevant international trading partners (US, Canada, Australia, Japan, China) focusing in particular on case studies of application;

2. To map out commonalities and differences in the screening, priority setting, testing and assessments approaches addressing the used methodologies, type of data considered, technical assessments of specific cases and interpretation of results of specific cases;

3. To ascertain the extent to which differences are due to variations in scientific approaches or in different legislative frameworks and regulatory culture;

4. To identify opportunities to foster international cooperation on scientific issues related to promoting chemical safety in regards to potential for endocrine disruption.

#### 5.1.1 Workshop objectives

The following objectives were formulated for this workshop:

1. To present to participants an overview of the field of endocrine disruption,

2. To inform participants about project achievements thus far,

3. To produce an overview of differences and commonalities in screening and testing in different legalities outside the EU,

4. To investigate the scope for data sharing at the international level,

5. To investigate commonalities and differences in setting priorities for screening and testing for endocrine disrupting properties,

6. To consider international cooperation for research and horizon scanning for endocrine disruptors,

7. To investigate the scope for harmonisation of hazard and risk assessment for endocrine disruptors at the international level.

Objectives 1 – 3 were to be addressed by presentations from the contractor and by workshop participants.

Objectives 4 – 7 were the topic of four working groups with the following thematic orientations, which were set up during the workshop:

**Working Group 1**: The scope for data sharing on ED hazards and exposures at the international level

**Working Group 2**: Setting priorities for screening and testing for ED properties – commonalities and differences and scope for common principles

**Working Group 3**: Research needs and horizon scanning in the ED arena – prospects for international cooperation?

**Working Group 4**: Harmonisation of hazard and risk assessment for endocrine disruptors at the international level – opportunities and limitations

5.1.2 Workshop participants

Invited workshop participants were from EU Member State competent authorities, competent authorities from the USA, Japan, China (unfortunately unable to attend) and Australia, and international experts.

A list of participants can be found in Annex 1 of this report.

5.1.3 Workshop agenda

The workshop agenda can be found in Annex 2 of this report.

5.1.4 Workshop materials

To enable preparation for the workshop, participants received workshop materials in advance, prepared by the contractor. These materials consisted of:

- An overview summary of legislative frameworks and approaches in different legalities, relevant to endocrine disruptors
- Five case studies of hazard and risk assessments for specific endocrine disrupting chemicals in different legalities
- Seven theses on commonalities and differences in approaches for testing and screening of endocrine disruptors, intended to trigger discussions at the workshop

### *5.2 Workshop report: Summary of formal presentations*

In this section, a brief summary of the formal workshop presentations is given. The presentations are available in Annex 3 to this report.

**Prof Andreas Kortenkamp** (Brunel University London): *Milestones, discoveries and set-backs in endocrine disruptor research – a potted history*

Andreas Kortenkamp began by sketching out the beginnings of endocrine disruptor research in the 1980s, with research into DES and the importance of timing of exposure. The 1990s saw by chance discoveries of important endocrine disrupting chemicals, including nonylphenol, tributyl tin, and steroidal estrogens as causing feminisation in fish. Research into the causes of deterioration of male reproductive health gave rise to the testicular dysgenesis hypothesis. By the mid 1990s, the importance of endocrine disruptors with estrogenic, anti-androgenic and thyroid disrupting modalities was established.

Further underlining the importance of timing and windows of heightened susceptibility, the 2000s saw the establishment of the male programming window during pregnancy as the key period of susceptibility to anti-androgenic endocrine disruptors. This coincided with the discovery of phthalates as suppressors of androgen synthesis. Similar

developments unfolded in the area of thyroid research, as substances eliciting thyroid insufficiency in neonatal life were identified as risk factors for compromised brain development. Perchlorate, various UV filter substances and soy isoflavones were found to be thyroid disruptors.

Endocrine disruptor research also uncovered issues of a more generic nature, applicable to many chemicals. Prominent are the issues of non-monotonic dose-response relationships, low dose effects and of combined exposures.

More recent developments include the discovery of a number of chemicals inhibiting steroid modifying enzymes and the consequences of such inhibition, the importance of disruption of prostaglandin signalling as a new modality in endocrine disruption, which brought the issue of analgesics to the fore, the role of epigenetics, and the topic of differentiation of adipose tissue precursors and obesogens.

Curiously neglected are the topics of progesterone signalling and female reproductive health. Regulatory testing and the development of test methods have not kept up with these developments. The focus is still on testing for estrogens, anti-androgens and thyroid disruptors.

**Prof Ulla Hass** (Technical University of Denmark): *Commonalities and differences - a summary of project outcomes thus far*

Ulla Hass summarised the work conducted for this project by the contractor thus far. On the basis of an overview of the current status of EDC regulation in the EU, USA, Japan, Canada, China and Australia, several commonalities and differences became apparent:

A concern for endocrine disruptors is common to all jurisdictions considered, with a recognition of the need for testing and screening. Important differences concern the finer points of testing and screening, and of priority setting in the different jurisdictions: In some jurisdictions there is a strong focus on screening for endocrine activity without necessarily testing for adverse effects or vice versa. There are also differences concerning priority setting for testing and screening. The legal framework in the EU is based on production volume as a strong marker for priority setting, whereas in other jurisdictions (e.g. USA) endocrine activity or exposures are a strong stimulus. There are also differences concerning the issue of who should conduct testing – the manufacturer or governmental institutions?

Common to many jurisdictions is the fact that the toxicity produced by endocrine disruptors is the subject of general chemical legislation, as well as specific regulatory domains (pesticides, food additives, pharmaceuticals etc.). Differences in approaches relate to the question as to whether additional regulatory approaches are needed to deal with endocrine disruptors, or whether they are adequately addressed by existing laws. There are also differences in relation to regulatory approaches, which range from soft voluntary agreements or recommendations to consumers to actual restrictions in the use of chemicals.

In conclusion, there is common concern about endocrine disruptors. The differences that have become apparent in screening and testing approaches can be become significant strengths, if there is international cooperation. It is necessary to expand the focus of regulation beyond estrogens, antiandrogens and thyroid disruptors, and to move beyond high production volume chemicals to the large numbers of substances that have not been tested at all.

**Dr Patience Browne** (USEPA and OECD): *Prioritization and Screening Chemicals for Endocrine Bioactivity in the US*

Patience Browne traced the evolution of the USA EDSP from the first list (issued in 2009) with pesticide chemicals which were selected on the basis of potential routes of

exposures (67 substances), to the second list (revised in 2013) which was based on registration review schedule for pesticides and nationally regulated drinking water contaminants or unregulated chemicals. By exclusion of naturally occurring and untestable chemicals, the second list was revised to contain 109 chemicals.

Computational tools and models are used in EDSP to rapidly prioritise chemicals for endocrine activity and for further testing, to contribute to the weight of evidence evaluation of a potential biological activity and to provide alternative data for specific screening assays. In connection with the ToxCast programme, these approaches were first developed and evaluated for estrogenic chemicals, but are to be rolled out for (anti)-androgens and other endocrine modalities as well.

The inclusion of computational approaches in screening activities relies on the successful identification of reference chemicals with proven activity. Reference chemicals are also needed for the evaluation of model performance. EDSP will develop additional predictive models for estrogens, (anti)androgens and thyroid disruptors by integrating more assays that are representative of key events in adverse outcome pathways. Similar approaches are developed for substances that interfere with steroidogenesis.

To improve in vitro-in vivo extrapolations, current efforts are also focused on evaluating the impact of metabolism and bioactivation of endocrine active chemicals.

**Dr Jun Kanno** (JBRC, NIHS, Japan): *The Concept of "Signal Toxicity" for the Planning of Research and Testing of Endocrine Disrupting Chemicals - beyond EATS*

Jun Kanno expanded ideas of hormonal signalling and endocrine disruption into the concept of signal toxicity and demonstrated with original, unpublished data sets how these ideas can be harnessed for a more inclusive, comprehensive approach for endocrine disruptor testing beyond estrogens, (anti)androgens and thyroid disruptors.

**Dr Sharon Munn** (DG Joint Research Centre): *The EASIS data base (Endocrine Active Substances Information System) – an update*

Sharon Munn presented the history, structure, content and the evolution of the Endocrine Active Substances Information System (EASIS) data base at the Joint Research Centre.

EASIS's pre-history began in 1999 with the adoption of the EU Community Strategy for Endocrine Disruptors. One short-term action of the Strategy was to establish a priority list of substances for further evaluation of their role in endocrine disruption. This list (here termed the "DG ENV list") was established between 2000 and 2006 and contains over 500 substances. It uses a categorisation system, with category 1 assigned to substances with in vivo evidence of endocrine disruption in at least one species, category 2 for substances with in vitro evidence, and category 3 for cases with no evidence or no data.

In 2010, a follow-up process began, with DG ENV requesting JRC to develop a new system, called EASIS. EASIS was to adhere to international standardised data models and allow the hosting of non-guideline test data (from in vitro, in silico and in vivo methods). Unlike the DG ENV list, EASIS does not categorise substances according to evidence of endocrine disruption. Because EASIS contains both positive and negative data, the mere presence of a substance in EASIS does not allow any conclusions as to its endocrine disrupting properties. EASIS is compatible with the OECD Harmonised Templates (OHT).

EASIS's content is currently evolving from a read-only data base for everyone, where JRC adds new data, to a system where third parties can be granted write access for the upload of data which are then curated by JRC.

In summary, EASIS is a source of data relevant to the research community and to assessors of toxicity/ecotoxicity data with respect to the identification of endocrine disruptors. EASIS captures endocrine disruptor mode of action data, together with adverse effect data, in a structured knowledge base that follows the OHT.

**Dr Olwenn Martin** (Brunel University London): *Weighing and integrating evidence in hazard and risk assessment of endocrine disruptors*

Olwenn Martin presented preliminary findings from the contractor's work and summarised recent developments in the field of evidence assessment and integration, especially for endocrine disruptors.

On the basis of the case studies conducted by the contractor for specific endocrine disruptors in which commonalities and differences in testing and evaluation were analysed, it is possible to define some important issues for weighing and integrating evidence.

It was notable that the endocrine disrupting properties of some chemicals were not detected through routine guideline testing. The effects were discovered accidentally.

Common, uniform, and agreed criteria for the selection of studies relevant for the derivation of regulatory quality standards for endocrine disruptors (ADI, EQS, water quality standards etc.) are missing. There is a lack of transparency in the selection process.

There is no consensus about what is to be considered an adverse effect relevant for endocrine disruption.

To improve this state of affairs, it would be desirable to make the selection of studies for deriving regulatory values transparent. Clarity about value judgements inevitably involved in the definition of adversity is also needed. The incorporation of peer reviewed literature is important, as is the need to consider all the evidence accessible in the literature.

These demands and requirements necessitate the adoption of systematic review protocols and methods for evidence integration and for judging data quality and reliability of studies that go beyond the familiar Klimisch scores. Olwenn Martin gave a summary of recent developments in these areas (the IARC system, GRADE, SYRINA and other decision tools such as from the OECD and EDSP). Endocrine disrupting chemicals present a difficult challenge to existing methodologies, as an assessment of adversity together with an endocrine mode of action is required.

### *5.3 Workshop report: Working group deliberations*

The formal workshop presentations were followed by working group discussions. This section of the report gives a summary of these discussions and the recommendations made in each group.

Each working group elected a rapporteur and a member of the contractor's project team was assigned to assist with record keeping, as follows:

**Working group 1**: rapporteur - Dr Patience Browne, project team - Dr Olwenn Martin

**Working group 2**: rapporteur - Dr Sharon Munn, project team - Prof Ulla Hass

**Working group 3**: rapporteur - Dr Sander van der Linden, project team - Dr Sofie Christiansen

**Working group 4**: rapporteur - Dr Henrik Holbech, project team - Prof Andreas Kortenkamp

The presentations and summary slides from the working groups are available in Annex 4 of this report.

5.3.1 Working group 1: The scope for data sharing on ED hazards and exposures at the international level

The discussions in this group focused on three topics relevant to the sharing of data at the international level: Copyright and proprietary data, reporting and data requirements, and biomonitoring data. For each of these topic areas, the group discussed commonalities, obstacles and solutions or opportunities for overcoming the obstacles.

*Copyright and proprietary data*

Common to all jurisdictions is that specific data may be requested for a substance if it presents concerns regarding endocrine disrupting properties. Summary hazard data can be shared at the international level, as can substance evaluations. This is particularly important for countries and jurisdictions that have not implemented data and information requirements and thus have to conduct regulation on the basis of data already available (as is the case e.g. in Canada). Exposure data are generally publicly available and can be readily shared.

The group identified several obstacles to data sharing at the international level: The biggest problem is with sharing proprietary data that emanate from specific regulatory domains which place data and information requirements on registrants, as is the case with several EU regulations such as PPPR, BPR and REACH. These data can be shared within the EU, but not beyond. The group noted that several countries do not have data and information requirements implemented, and therefore depend on already available data (see above).

The group saw significant opportunities in developing common guidance for hazard assessments of endocrine disruptors, which should include data from new methods and non-guideline studies. At a minimum, this guidance should be developed within the EU (EFSA, ECHA, to improve consistency between different regulatory domains), but also internationally, beyond the EU.

It is also necessary to arrive at a common definition for endocrine disruptors. Although the WHO definition is widely accepted in the EU, the definitions used by e.g. US EPA have

been criticised as lacking consistency[28]. There is large scope for international harmonisation.

*Reporting and data requirements*

Obstacles to better international sharing of data are language barriers, and the fact that many data are in non-standard templates, making them difficult to integrate into databases.

The group noted that there are challenges and difficulties in the correct reporting of data, and the validation of existing data. There is little point in sharing data that are not validated. An issue raised was whether the existing OECD Harmonised Templates, which as such seem to be ideal for international data sharing, include the appropriate endpoints needed for the reporting of endocrine disrupting effects.

To overcome these difficulties, agencies should be encouraged to use standard reporting formats for data on endocrine disrupting effects. Ideally, this requirement should extend to the realm of primary research, with funders requiring reporting in standardised formats. The same should apply also to scientific publishers who could make publication of data dependent on adherence to standardised data format. This would also help making data from non-guideline studies more useful for hazard- and risk assessment.

*Biomonitoring*

The group also discussed the possibilities of data sharing for exposure data, and focused on biomonitoring data. There are challenges in estimating correctly the external exposures that correspond to the various levels of endocrine disruptors measured in tissues and body fluids, and validation procedures are required before data can be shared internationally.

The opportunity in this area is an international harmonisation of how biomonitoring data are gathered and interpreted.

### 5.3.2 Working group 2: Setting priorities for screening and testing for ED properties – commonalities and differences and scope for common principles

The group began their deliberations by discussing how priorities for screening and testing are developed in different countries and identified various commonalities:

Common in Japan and the EU is a focus on using surrogates for exposures as an element in prioritisations. These are usually in examining production volumes and usage patterns of chemicals. Common to Japan and the EU is also that the literature or (in the EU) the submissions of registrants are reviewed for alerts for endocrine disrupting effects or endocrine disruptor modes of action. Screening is based on existing data, no new data are generated. In the EU, internationally validated screening assays for mode of action, such as those in the OECD Conceptual Framework, Level 2 or 3 are requested in some legislation (e.g. on plant protection products[29]), but only if there is evidence in mandatory in vivo studies long term toxicity and carcinogenicity, as well as reproductive toxicity[30]) or in the public literature[31] that the active substance may have endocrine

[28] See https://chemicalwatch.com/14867/epa-urged-to-define-endocrine-disruptors-consistently

[29] Regulation (EU) No 283/2013 points 5.8.3 and 8.2.3 and the associated Communication 2013/C 95/01 points 5.8.3 and 8.2.3

[30] Regulation (EU) No 283/2013 points 5.5 and 5.6

disrupting properties. Thus, these assays are not used for the screening of all active substances, but only for those substances where some alerts are highlighted in other in vivo mandatory studies. In themselves, they are not mandatory data requirements. In Japan, government-funded testing programmes are aligned to the OECD Conceptual Framework, with estrogenicity, (anti)androgenicity, thyroid disruption and steroidogenesis the focus of funding for testing.

In the USA, the situation regarding prioritisation and screening efforts is slightly different. With the EDSP and ToxCast/Tox 21, there are government-funded high through-put schemes for the screening of substances, in addition to specific data requirements for registrants in the pesticide area. The USA also plans mixture testing using high through-put methods, for substances that affect the same pathways via similar modes of action, and also for substances that produce common adverse outcomes, but via different modes of action.

In summary, the group realised that there were more commonalities than differences in the prioritisation for screening and testing for endocrine disruptors. They noted that the reporting of mode of action data, such as those from in vitro mode of action assays, are not core data requirements in any legislation, but may be triggered in some EU legislations if concern about endocrine disrupting properties arises from other toxicity testing (see above).

The group elaborated several suggestions for a way forward at the international level: Essential is the sharing of data and assessments, and steps should be taken to facilitate this at the international level. Cooperation in terms of research funding was also seen as essential to avoid duplication of efforts.

Looking to the future, the group saw the necessity of addressing mode of action data and adverse effects at the same time. The vision would be to use data from mode of action screens for the prediction of adverse effects. This will necessitate integration within an adverse outcome pathway framework, with the aim of establishing the degree of change required in an upstream key event to elicit adverse effects.

The group made several recommendations, as follows:

- Implement OECD Conceptual Framework Level 2 and 3 assays in data and information requirement directives
- Provide guidance as to how the data generated are to be used for hazard and risk assessment (as is currently being done in the process of updating OECD guidance document 150)
- Identify gaps in validated assays and prioritise, which should be filled (as is currently done at the OECD with guidance documents 97, 178 and others)
- Translate assays developed in a research context into validated assays (aspects of this activity are ongoing at the OECD)
- Explore the use of additional endpoints reflective of endocrine disruption in existing, validated assays
- Generate priority lists of substances to be subjected to screening and testing

### 5.3.3 Working group 3: Research needs and horizon scanning in the ED arena – prospects for international cooperation?

[31] Regulation (EC) No 1107/2009 Article 8.5

This group started the discussion with some research that has been reviewed by the OECD, and relevant projects for EDs, as outlined by the OECD, which concern:

- Metabolism (not just for endocrine disruptors)
- Thyroid disruption (with the development of adverse outcome pathways and prioritising assays for validation, as e.g. in the OECD thyroid scoping document)
- Non-genotoxic carcinogens (validation and integration of test methods, to lead to an integrated approach to testing and assessment (IATA)[32]
- Systematic uncertainty analysis of standard animal testing and assessment approaches to identify appropriate reference data and information for the validation of defined in vitro and in silico approaches and to identify benchmarks for the performance of the latter (e.g. initiated in OECD now for non-genotoxic carcinogenicity including ED and the derivation for a Point of Departure for risk assessment, but this is also needed for other areas).

Specifically for endocrine disruptors, the need for a defined testing strategy was recognised, using in vitro and in silico methods, with the aim of helping the risk assessors worldwide with the interpretation of the data. Research that can underpin the development of such a testing strategy needs to be funded.

Several aspects were discussed that could make the tools that would make up a testing strategy more useful. Risk assessors' needs in terms of data and test requirements should be better communicated to scientists and test developers. This could be achieved by involving the agencies more in research programmes, especially in relation to the validation of test methods, as now described by several OECD documents, but also by research funding bodies supporting such grant applications. The group saw this as essential for international mutual acceptance.

Generally, the group felt that research funding should be directed more towards a focus on regulatory science, as this would stimulate a better integration between science and regulation. An issue for debate was whether research funding should be directed towards the screening of many compounds for a limited number of endocrine-relevant pathways, or whether resources would be better allocated to the detailed and extensive characterisation of a few compounds in terms of their endocrine disrupting properties. One way of resolving this could be in building up the EU equivalent of the National Toxicology Programme in the USA.

The group reached a consensus that the development and translation of adverse outcome pathways to defined approaches to testing and assessment for endocrine disruption should be a priority area in future research funding. Not only would this help in terms of providing an organising principle that would help the regulator understand several different sets of data, but it could also function as a central depository which would reflect the state of the science for a certain pathway or adverse outcome, and be reflective of the maturity of the adverse outcome pathway.

The group also discussed the known publication bias towards positive findings. Publication of negative findings is important not only to prevent the funding of research programmes that turn out to be unsuccessful and fruitless, but also to put the positive data into perspective in weight of evidence assessments and systematic reviews. This dilemma could be addressed by obliging funded projects to make their data available, including negative results. Alternatively, or additionally, there could be funding for publishing the negative data in journals, e.g. a Journal of Confirmation Sciences, or a Journal of Negative Findings.

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[32] See: Jacobs et al. (2016) ALTEX, http://www.altex.ch/resources/altex_2016_4_359_392_Jacobs11.pdf; and Paparella et al. (2016) ALTEX, http://www.altex.ch/resources/epub_Paparella_of_1610241.pdf

Several participants supported that more resources should be put towards researching novel pathways in endocrine disruption. On the other hand it was also raised that funding should focus on the OECD identified priority projects and to translating available knowledge for EATS pathways to defined in vitro and in silico approaches including kinetic modelling (QIVIVE) for regulatory application: At present testing and assessment for endocrine disruption relies largely on animal testing and this is prohibitive for the assessment of a larger number of substances and mixtures and for retesting chemicals according to progress in the development of scientific and toxicological understanding.

The group identified another research need in the area of mixtures of endocrine disruptors.

Finally, the research and assessment efforts spent on substances before they are put on the market should be complemented by some post-marketing research to confirm the initial exposure assessments, e.g. to confirm the hypothesis that chemicals do not appear in e.g. breast milk. Similar considerations apply to the post-market monitoring of endocrine-related effects in workers or environmental organisms exposed to agrochemicals. These efforts should be funded by the producers of chemicals.

The group ended the discussions by considering whether the regulatory sector would be ready to utilise new research data.

#### 5.3.4 Working group 4: Harmonisation of hazard and risk assessment for endocrine disruptors at the international level – opportunities and limitations

The group embraced the need for harmonisation of hazard and risk assessment for endocrine disruptors internationally, but stressed that even in the EU essential guidance for conducting such assessments is missing. There is great scope for developing such guidance jointly between ECHA and EFSA.

The group discussed the possibility of harmonising hazard assessment approaches internationally, beyond the EU. To avoid "reinventing wheels", this should borrow from principles of hazard identification and characterisation developed for carcinogens by IARC. However, the aspect of mode of action evaluation will have to be elaborated, considering that hazard assessment for endocrine disruptors requires consideration of adverse effects and endocrine related modes of action at the same time.

As part of these efforts of harmonising hazard assessments, criteria for the selection of studies that can meaningfully be used in this process must be developed. These criteria should take into consideration the choice of endocrine related adverse endpoints, and also quality criteria. The harmonisation of hazard identification at the international level could find expression in the inclusion of an endocrine disruptor class in GHS.

The group identified data availability as a bottleneck for harmonisation and noted that the EU does not use in vitro mode of action assays for the screening and testing, e.g. to establish endocrine relevant modes of action. The suggestion was made to ensure that relevant in vitro assays from the OECD Conceptual Framework Level 2 and further assays from level 3 are implemented in EU regulations for data and information requirements, e.g. in the context of pesticides, biocidal products and REACH. Such efforts should be based on careful consideration of which assays should be selected and should be embedded in developments of testing strategies, including decision trees for waiving further testing, should certain outcomes be negative, or to trigger earlier further testing in case of positive outcomes.

Finally, institutional aspects of harmonisation were discussed. Various options were considered, ranging from the creation of the equivalent of IARC for endocrine disruptors

to the setting up of a body under the umbrella of UNEP or the use of OECD (EDTA AG and TFHA) as the platform for international institutionalisation.

### *5.4 A summary of working group recommendations: The way forward*

In summary, all working groups saw a need for international cooperation and harmonisation in the area of endocrine disruptors. To achieve this goal, quite a few recommendations were made, often common to several working groups.

In this section, a summary of these recommendations is drawn out from the account of the working group deliberations. The first two of these recommendations concern the "what?" of harmonisation, the remainder the "how?".

**Recommendation 1**: Develop international guidance for harmonised hazard identification and assessment of endocrine disruptors (articulated by working groups 1 and 4)

Elements of this guidance should be:

- A common definition of endocrine disruptors,
- The development of approaches that can assess adversity and endocrine-related modes of action at the same time,
- The description of sets of tests essential for hazard assessment,
- The elaboration of criteria for the selection of studies for hazard assessments,
- The development of a standardised reporting format for data describing hazards,
- The elaboration of quality criteria for studies and data sets,
- The development of suitable weight of evidence approaches.

There is also scope for developing hazard assessment guidance into one for risk assessment.

**Recommendation 2**: Develop a strategy for the testing of endocrine disruptors (articulated by working group 2, and in parts by groups 3 and 4)

This should entail:

- The identification of gaps in internationally validated tests for endocrine disruption, with respect to endocrine disruptor effects not yet covered,
- Consideration of additional endpoints of endocrine disruption that could be incorporated in existing tests,
- The development of a staged testing strategy with a decision tree that would allow discontinuation of further testing in case of negative results (after thorough evaluation of all available information about the initial concern, data on similar substances and metabolic differences between species) and further testing in case of positive results,
- An assessment of already existing scientific tests (as currently conducted at the OECD working group of National Coordinators), with a view of taking them forward into the validation process.

The development of such a strategy could also be supported by the adverse outcome pathway conceptual framework and it will have to be underpinned by further research

funding, especially for progressing OECD agreed priority areas towards regulatory applicability and the assessment of novel pathways and novel effects.

**Recommendation 3**: Implement assays described in the OECD Conceptual Framework, Level 2 and 3 in legislations for data and information requirements (articulated by working groups 2 and 4)

This was seen as essential to create the data necessary for judging whether chemicals induce adverse effects through endocrine-mediated modes of actions. However, it was also acknowledged that prior to this, testing strategies need to be established and harmonised that allow optimal use of the in vitro and in silico approaches (see working group 3).

**Recommendation 4**: Create an institutional platform for international harmonisation of hazard and risk assessment for endocrine disruptors, and for the exchange of data and assessments (articulated by working groups 3 and 4)

For international cooperation and harmonisation to become a reality, an appropriate institutional structure was seen as essential. Suggestions for realising this idea included:

- The creation of an agency that is the IARC equivalent for endocrine disruptors, under the auspices of WHO,
- As above, but under the umbrella of UNEP,
- To continue to support the relevant platforms at the OECD

## 6. POSSIBILITIES FOR FOSTERING INTERNATIONAL COOPERATION ON SCIENTIFIC ISSUES RELATED TO PROMOTING CHEMICAL SAFETY IN REGARDS TO THE POTENTIAL FOR ENDOCRINE DISRUPTION

There is widespread recognition of the need for international cooperation on promoting chemical safety in relation to chemicals with endocrine disrupting properties. Furthermore, as has become apparent during the international workshop organised in connection with this project, there is considerable willingness among risk assessment practitioners in competent authorities to move towards an international harmonisation of approaches. This has found expression in the four recommendations from the workshop (see section 5.4).

These recommendations concern the

- development of international guidance for harmonised hazard assessment of endocrine disruptors,
- development of a strategy for the testing of endocrine disruptors
- implementation of existing tests and assays for the identification of endocrine disruptors, as described in the OECD Conceptual Framework, Level 2 and 3, and
- creation of an institutional platform for international harmonisation of hazard and risk assessment for endocrine disruptors, and for the exchange of data and assessments

The implementation of the last of these recommendations would appear to be essential to initiate the process of international harmonisation in the assessment of endocrine disruptors. However, it is beyond the scope of this project to elaborate the finer organisational and institutional details of this process.

## 7. CONCLUSIONS

The inventory of completed and on-going activities in screening, priority setting, testing and assessment of chemicals for their endocrine disrupting properties in the EU (including within the Member States) and relevant international trading partners (US, Canada, Australia, Japan, China), as well as the expert consultation about ongoing activities showed that there is a common concern about the harmful effects of endocrine disrupting properties across all legalities.

Despite this common concern, that there are significant differences relating to the question whether endocrine disrupting chemicals require dedicated regulatory systems and structures, or whether the adverse effects produced by endocrine disrupting chemicals can be dealt with adequately by regulating toxic effects within the existing regulatory structures.

The eight case studies of ethinylestradiol and estradiol, nonylphenol, bisphenol A, di-ethyl-hexyl phthalate (DEHP), mancozeb, prochloraz, procymidone and benzophenone-3 support the conclusion that the framework of established regulatory testing is ill-equipped for identifying chemicals with endocrine disrupting properties.

It can also be concluded that there are benefits in the international harmonisation of the ways in which chemicals are dealt with. This is substantiated by the commonalties in which all the pesticides analysed in the case studies (mancozeb, prochloraz, and procymidone) are treated across all legalities.

In contrast, there are considerable disparities in the ways in which industrial chemicals such as bisphenol A, nonylphenol and DEHP are handled in the different legalities. These are a reflection of differences in the regulatory regimes and of differences in the level of concern with which these chemicals are regarded.

In conclusion, the differences and commonalities in dealing with endocrine disruptors in the various legalities are mainly an expression of the features of the respective legal systems and less so of differences in data interpretation. However, in some cases, including ethinylestradiol, nonylphenol and bisphenol A, diverging scientific approaches also play a role. The impact of these differences on the derivation of regulatory values (e.g. water quality standards, acceptable daily intakes and similar) can be considerable.

It has also become apparent that the differences in the assessment values for specific chemicals could diminish if more consistent methods of hazard assessment were applied across the various legalities. This concerns the application of uniform, transparent and agreed criteria for the selection of studies for hazard characterisations, as well as for the choice of assessment factors.

During the workshop which was organised in connection with this project, these conclusions were echoed among workshop participants. The need for international cooperation in promoting the chemical safety of chemicals with endocrine disrupting properties was recognised. There was also a willingness to move towards an international harmonisation of approaches, including internationally harmonised guidance for hazard and risk assessment of endocrine disruptors.

# 8. ANNEXES

## *Workshop participants*

This list encompasses the actual participants of the workshop and the people who express an interest in being kept informed.

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***Workshop Agenda***

# International workshop on commonalities and differences in approaches for testing and assessment of endocrine disruptors within the EU and among relevant international trading partners

19-20 September 2016, Hotel Bloom, Rue Royale 250, 1210 Bruxelles, Belgium

*Note on modus operandi during the workshop:*

*Workshop discussions and deliberations will be conducted on the basis of detailed material and background documents which will be made available in advance (early Sept 2016)*

*Registration and coffee breaks will take place on floor 1 in the meeting lounge, all plenary sessions take place in Meeting Room II, and working groups will take place in meeting rooms II and III.*

*Free wifi is available (password: LOVE)*

**Monday, 19 September 2016**

9:00 Arrival and registration

9:30 **Dr Bjorn Hansen** (DG ENVI): Welcome from the European Commission

9:45 **Prof Andreas Kortenkamp** (Brunel University London): Milestones, discoveries and set-backs in endocrine disrupter research – a potted history

10:00 Discussion

10:15 **Prof Ulla Hass** (Technical University of Denmark): Commonalities and differences - a summary of project outcomes thus far

10:40 Discussion

10:45 **Dr Patience Browne** (USEPA): Prioritization and Screening Chemicals for Endocrine Bioactivity in the US

11:15 Discussion

11:30 COFFEE BREAK

12:00 **Dr Jun Kanno** (JBRC, NIHS, Japan): The Concept of "Signal Toxicity" for the Planning of Research and Testing of Endocrine Disrupting Chemicals - beyond EATS.

12:30 Discussion

12:45 LUNCH (Restaurant)

14:00 **Dr Sharon Munn** (DG Joint Research Centre): The EASIS data base (Endocrine Active Substances Information System) – an update

14:30 Discussion

14:35 **Dr Olwenn Martin** (Brunel University London): Weighing and integrating evidence in hazard and risk assessment of endocrine disruptors

15:00 Discussion

15:05 **Commonalities and differences in screening, testing, interpreting endocrine disruptors – General discussion: what is the problem and where is a way ahead?**

Moderation: Prof Andreas Kortenkamp

15:35 COFFEE

16:00 **Prof Andreas Kortenkamp** (Brunel University London): Introduction to Working Groups: The Scope for International Collaboration

Assembly of 4 parallel running working groups on identifying the scope for international collaboration

**Working Group 1: The scope for data sharing on ED hazards and exposures at the international level**

**Working Group 2: Setting priorities for screening and testing for ED properties – commonalities and differences and scope for common principles**

**Working Group 3: Research needs and horizon scanning in the ED arena – prospects for international cooperation?**

**Working Group 4: Harmonisation of hazard and risk assessment for endocrine disruptors at the international level – opportunities and limitations**

17:00 Working Group Rapporteurs: First round of discussion highlights

17:30 Closure

17:45 Drinks

**Tuesday, 20 September 2016**

9:00 **Dr Bjorn Hansen** (DG ENVI): Introduction to Working groups: recap from day 1

9:15 Working Groups resume

10:30 COFFEE

11:00 Presentation of results: from commonalities and differences to opportunities for collaboration

Moderation: **Dr Bjorn Hansen** (DG ENVI)

11:00 Working Group 1

11:30 Working Group 2

12:00 Working Group 3

12:30 LUNCH (Meeting Lounge)

13:30 Working Group 4

14:00 General discussion

Moderation: **Dr Bjorn Hansen** (DG ENVI)

15:00 COFFEE

15:30 Conclusions and wrap-up

Moderation: **Dr Bjorn Hansen** (DG ENVI)

16:00 Close

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