SCIENTIFIC REPORT OF EFSA

Scientific report of the Endocrine Active Substances Task Force[1]

European Food Safety Authority[2, 3]

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ABSTRACT

Discussions within the Scientific Committee and the Advisory Forum have called for the development of a common approach within EFSA towards endocrine active substances. The aim of this report by an internal EFSA task force is to clarify the state-of-play, and provide recommendations for scientific and communication issues. Both specific issues and new regulations make it necessary to follow up on recent developments with the EU bodies, Member States, and internationally, in order to avoid diverging assessment approaches and the duplication of work. The proposed actions for EFSA are to contribute to the work in progress under the auspices of DG Environment and to continue its participation in the ongoing OECD activities in the area of testing of chemicals. The development of a generally accepted risk assessment methodology is an additional challenge due to the complexity of the issues involved. Here, the task force recommends that EFSA continues its activities aimed at developing harmonised methodologies for risk assessment of combined exposures to endocrine active substances in food. EFSA should continue to build a dialogue to develop a common strategy with the EC, other EU bodies, Member States' Competent Authorities, international organisations and partners, as well as external experts and stakeholders on the before mentioned issues. In line with these recommendations, it is proposed that EFSA creates a working group of Panel experts and national experts to advise on prioritising the work on endocrine active substances. EFSA should also work with the experts in its Advisory Group on Risk Communications in conjunction with the communication experts from Member States, and continues to monitor and analyse media and stakeholder developments, in order to define a strategy for communications addressing both the collective group and specific endocrine active substances.

KEY WORDS

Endocrine active substances, endocrine disrupters, hazard identification, risk assessment, method harmonisation, common strategy, risk communication

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SUMMARY

The issue of endocrine active substances touches upon the activities of several of the EFSA Units and Panels, recent examples being the risk assessment of bisphenol A, the hazard assessment of isoflavones and the implementation of the new regulation on plant protection products. Previous examples include contaminants like organotins, dioxins, PCBs, and hormone residues.

Discussions within the Scientific Committee and the Advisory Forum have called for the development of a common approach within EFSA towards endocrine active substances. A first step in this process was to appoint a task force to identify trends and developments in the assessment of the health risks and risk communication issues EFSA may have to address. The aim of this report by the task force is to clarify the state-of-play, and provide recommendations for the scientific and communication issues.

Current activities and developments within EFSA, European Commission, other European Union bodies, and Member States are reviewed. Both specific issues and new regulations make it necessary to follow up on recent developments within the EU bodies and Member States, in order to avoid diverging assessment approaches and the duplication of work. One such issue that now needs to be addressed is the development of specific criteria for determining endocrine disrupting properties. The proposed action for EFSA is to contribute to the work in progress under the auspices of DG Environment.

International consensus on testing strategies to determine the endocrine activities of substances that may be of significance to human health is needed for both hazard identification as well as for risk assessment. The harmonisation with regard to the testing of chemicals has been an ongoing activity of the OECD for more than 30 years. A core activity on endocrine disrupters was initiated in 1997 and under this umbrella both specific tests and a tiered assessment framework have been developed. The task force recommends that EFSA continues its participation in the ongoing OECD activities in the area of the testing of chemicals and evaluates how the tiered testing approach might be applied within EFSA's work, not only to prioritise which substances in food and feed might require assessment for endocrine activity, but also to evaluate those substances which are prioritised.

The development of a generally accepted risk assessment methodology is an additional challenge due to the complexity of the issues involved. Multiple sources and routes of exposure exist for endocrine active substances, such as contaminants, residues or natural constituents of foods. From a toxicological point of view, the significance of the various adverse effects, gender and life stage must be assessed and different types of combined actions need to be considered. In addition, there is a need to have a better understanding of the significance of low-dose exposure, and an evaluation of the health risks and health benefits of certain naturally occurring substances, such as phytoestrogens.

The task force recommends that EFSA continues its activities aimed at developing harmonised methodologies for risk assessment of combined exposures to endocrine active substances in food. In order to ensure consistency between the approaches developed for risk assessment through other sources than food (non-dietary exposure), EFSA should continue to build a dialogue for developing a common strategy with the EC, other EU bodies, Member States' Competent Authorities, international organisations and partners, as well as external experts and stakeholders on the before mentioned issues. In line with these recommendations, it is proposed that EFSA creates a working group of Panel experts and national experts to advise in prioritising the work on endocrine active substances.

From a risk communications perspective, it appears that the concept of "endocrine active substances" is not well known and that the public debate has been largely shaped by the negative connotations associated with the term "endocrine disrupters." Further research on public perception is required in this area taking into account also perception regarding "natural" constituents of foods which have endocrine effects and which may be promoted for their health effects. It is recommended that EFSA

work with the experts in its Advisory Group on Risk Communications in conjunction with the communication experts from Member States, and continues to monitor and analyse media and stakeholder developments, in order to define a strategy for communications addressing both the collective group and specific endocrine active substances.

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BACKGROUND AS PROVIDED BY EFSA

The issue of endocrine active substances touches upon the activities of several of the EFSA Units and Panels, recent examples being the risk assessment of bisphenol A, the hazard assessment of isoflavones and the implementation of the new regulation on plant protection products. Previous examples include contaminants like organotins, dioxins, PCBs, and hormone residues.

The lack of coherence in terminology and the failure to recognise the distinction between hazard identification of substances with endocrine activity and the role of exposure in determining any risks can be a barrier for efficient communication within science, between scientists and risk managers, and with the public.

The scientific literature on the subject is growing steadily and new risk assessment approaches are emerging. There is a need to follow-up on these developments, to encourage harmonisation and to avoid diverging scientific opinions between the EU Member States and internationally.

Meanwhile the public interest in endocrine active substances is increasing and the media coverage is extensive. There is an obvious interplay between the scientific discourse and the public debate. These interactions also need to be taken into account to anticipate future needs in the risk communication.

Discussions within the Scientific Committee and the Advisory Forum have called for the development of a common approach within EFSA towards endocrine active substances. A first step in this process would be the development of a technical report.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The SCAF unit is requested to establish an internal task force, with the participation from the RA, SCA and COM directorates, to initiate the development of a common strategy towards endocrine active substances.

Specifically the assignments of the task force are to:

- Identify trends and developments in the assessment of the health risks of endocrine active substances;
- Identify risk communication and risk perception issues EFSA may have to address;

Develop a technical report, clarifying the state-of-play, and with recommendations for the scientific and communication issues.

1. Introduction

There are growing concerns about the potential adverse effects of chemicals on the endocrine system in humans and wildlife. These concerns are reflected in the fast growth of the scientific literature, the intense public debate, and different regulatory initiatives. Food and feed are primary routes of exposure for both man-made (synthetic) and natural endocrine active substances (EAS). The evaluation of effects from these substances is complex, but an important task in the safety assessment of food and feed.

The term *"endocrine disrupter*" (ED), was introduced in the early 1990s and later defined as (WHO, 2002): *"... an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."*. However, endocrine disruption is not a toxicological endpoint in itself, but rather a class of modes and mechanisms of action.

In this report, *"endocrine active substance (EAS)"* is used to describe any chemical that can interact directly or in-directly with the endocrine system, and subsequently result in an effect on the endocrine system, target organs and tissues. Whether the effect is adverse ("disruptive") or not will depend on the type of effect, the dose and the background physiological situation.

The risk assessment of EAS in food and feed calls for an integrated approach, where both cumulative and mixture exposure may need to be considered. Industrial chemicals, emitted directly or indirectly, may enter the food chain as contaminants, but in addition, a food risk assessment must also consider food additives, flavourings, substances migrating from food-contact materials, and naturally occurring EAS. The risk assessment approach for EAS needs to be consistent for all compound groups and routes of exposure, with regard to both hazard and exposure assessment, to characterise the risks. This full risk assessment approach is needed to support prioritisation and risk management decisions.

A first step in clarifying the state-of-play is to describe current activities and developments within the European Food Safety Authority (EFSA), European Commission, other European Union (EU) bodies, and Member States. The "Community Strategy for Endocrine Disrupters" was first published by the European Commission (EC) in December 1999, highlighting the occurrence of such compounds in the food chain (EC, 1999). This Communication was preceded by a 1996 report from the EU Scientific Committee on Food (SCF) about "Endocrine disruptors and food" (EC, 1997). The endocrine activity of chemicals in food and feed has subsequently been part of many European risk assessments. However, both specific issues and new regulations now make it necessary to follow up on recent developments within the EU bodies and Member States, in order to avoid diverging assessment approaches and the duplication of work.

Most of the work on development, validation and harmonisation of testing methods is done under the auspices of the Organisation for Economic Co-operation and Development (OECD). The Special Activity on Endocrine Disrupter Testing and Assessment was initiated in 1996[4]. Similarly, the World Health Organization (WHO) is addressing the issue of endocrine disruptors through the International Programme on Chemical Safety (IPCS)[5]. There is also a keen interest outside Europe in the issue of endocrine active substances in general and, in particular, bisphenol A (BPA), which needs follow up.

[4] http://www.oecd.org/document/62/0,2340,en_2649_34377_2348606_1_1_1_1,00.html
[5] http://www.who.int/ipcs/en/

It is a requirement in the General Food Law (EC/178/2002)[6] that measures relating to food safety should have a strong scientific basis. Science is developing very fast within the field of EAS and an assessment of the current status is therefore needed. Issues relating to combined and cumulative exposure, and possible low-dose effects and non-monotonic dose-response, are particularly challenging and difficult to evaluate. However, these issues must be addressed as science based risk assessments go beyond the hazard identification step.

A clarifying terminology and criteria could facilitate risk communication regarding EAS (Foster and Agzarian, 2008; Tyshenko *et al*., 2008). There is an obvious interplay between the scientific discourse and the public debate. These interactions need to be taken into account to anticipate future needs in risk communication. Furthermore, findings in risk communication research may provide guidance for new, more elaborated and targeted approaches to risk communication.

In this report we will address each of these four issues, with the purpose to provide a better description of the current situation regarding EAS, particularly in relation to the safety of food and feed. We will also try to provide an outlook and give some recommendations for the future.

[6] Commission Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L31/1, 1.2.2002, p.24 http://eur-lex.europa.eu/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

2. Current activities and developments within European Commission, other EU bodies, EFSA and Member States

2.1. European Commission and other EU bodies[7,8]

In March 1999, the Scientific Committee for Toxicity, Ecotoxicity and the Environment (SCTEE) issued a report on "Human and Wildlife Health Effects of Endocrine Disrupting Chemicals, with Emphasis on Wildlife and on Ecotoxicology Test Methods" (EC, 1999). The report identified a "potential global problem" for wildlife. It also stated that "impaired reproduction and development causally linked to endocrine disrupting substances are well-documented in a number of wildlife species and have caused local and population changes".

Against this background, a "Community Strategy for Endocrine Disrupters" was adopted by the EC in December 1999 (EC, 1999).

The objectives were to identify the problem of endocrine disruption, its causes and consequences and to identify appropriate policy action on the basis of the precautionary principle in order to respond quickly and effectively to the problem, thereby alleviating public concern. Four key elements were identified.

These were:

- the need for further research,
- the need for international co-ordination,
- the need for communication to the public,
- the need for policy action.

On this basis a set of appropriate actions was recommended:

Short-term actions:

- establishment of a priority list of substances for further evaluation of their role in endocrine disruption,
- use of legislative instruments,
- establishment of monitoring programmes to estimate exposure to and effects of the substances on the ED priority list,
- identification of specific cases of consumer use for special action,
- information exchange and international co-ordination,
- communication to the public,
- consultation of the stakeholders.

Medium-term actions:

- identification and assessment of endocrine disrupters,
- research and development,
- identification of substitutes and voluntary initiatives.

Long term actions:

- legislative actions.

[7] The text in section 2.1, to describe ongoing activities in the Commission, is largely based on comments and suggestions received from Patrick Murphy and Peter Korytar (DG Environment).

[8] Please note that for the introduction to chapter 2 (subchapter 2.1.) text has been taken from relevant EC webpages.

The text below does not discuss all actions of the Strategy but focuses on those that are most relevant.

2.1.1. Priority list of substances

Establishment of a priority list of substances for further evaluation of their role in endocrine disruption was one of the key short-term actions. Four contracts (one in 2000, two in 2002 and one in 2007) were mandated by the EC to gather scientific evidence on endocrine disruption of chemicals submitted by stakeholders. The reports thereof are available on the DG ENV website. A database covering 428 potentially endocrine active substances was developed and became available in 2007. It contains information on the results of *in vivo* and *in vitro* toxicological and ecotoxicological tests, as well as other data such as regulatory status, classification and labelling of these substances. The database can be downloaded freely from the Website of DG ENV[9]. DG ENV is currently considering revising this database with a goal to increase its scientific character and its level of interactivity, to enable uploading new information and making the information easily accessible to the scientific community, regulators, industry and civil society.

2.1.2. International cooperation

The EC participates in the OECD working group of the National Co-ordinators for the Test Guidelines Programme (WNT) and also in its subsidiary body the Endocrine Disruptors Testing and Assessment Advisory Group (EDTA AG). The aim of the WNT is to oversee the testing guidelines programme and of the EDTA AG to advise WNT on the issue of testing of endocrine disruptors. The EC has been involved in the organisation of the OECD Workshop on Endocrine Disruptors held in Copenhagen in 2009. The EDTA AG has considered the outcomes and recommendations from this workshop and decided to pursue work on preparation of a detailed review paper on new endocrine endpoints and on the preparation of guidance documents for assessment of endocrine disruptors.

Some ten years ago the EC provided financial contribution to the publication of a comprehensive report on *Global Assessment of the State-of-the-Science of Endocrine Disruptors*[10], which was published in 2002 by the IPCS. Recently, the United Nations Environment Programme (UNEP) and WHO have initiated a process to prepare a 10-year update of this report and the EC has been involved.

2.1.3. Research and development

On the medium-term scale, research is essential for understanding the phenomenon of endocrine disruption. Since the start of the 4th Framework Programme (FP4) in 1994, through FP5, FP6 and the ongoing FP7[11], the EC funds research on endocrine disruption. Up to now more than 80 projects were launched focusing on EDs' identification, risk assessment, education and information on chemicals as contaminants in the food chain[12].

2.1.4. Legislative actions

Long term actions included review and adaptation of existing legislation governing testing, assessment and use of chemicals and substances within the EU. Under this action, provisions on endocrine disruptors were included in Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (hereinafter referred to as the REACH Regulation) and the Regulation (EC) No 1107/2009 concerning the

[9] http://ec.europa.eu/environment/endocrine/strategy/short_en.htm
[10] http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/
[11] http://cordis.europa.eu/fp7/home_en.html
[12] http://ec.europa.eu/research/endocrine/index_en.html

placing of plant protection products on the market (hereinafter referred to as the PPP Regulation).

One of the key elements of REACH are the provisions regarding the authorisation of substances listed in Annex XIV of REACH and the initial procedure of identification of substances of very high concern (SVHC) and their inclusion on the so-called "Candidate List".

Substances which may be included in Annex XIV and the Candidate List are:

i) carcinogenic, mutagenic or toxic to reproduction (CMR cat. 1a and 1b),
ii) persistent, bio accumulative and toxic (PBT)
iii) very persistent and very bio accumulative (vPvB),
iv) substances for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to CMR, PBT or vPvB substances and which are identified on a case-by-case basis.

Pursuant to Article 57(f) of the REACH Regulation the latter (iv) covers substances having endocrine disrupting properties. Annex XIV inclusion of a substance is adopted by the EC through a Committee procedure on the basis of recommendations made by the European Chemical Agency (ECHA). Substances listed in Annex XIV can be placed on the market only if the authorisation is granted by the EC. Authorisation shall be granted only if the risks to human health or the environment are adequately controlled, but this does not apply to CMRs and endocrine disrupters for which it is not possible to determine a threshold, for PBTs and vPvBs. Authorisation may only be granted here if the socio-economic benefits outweigh the risk to human health or the environment and if there are no suitable alternative substances or technologies.

Pursuant to Article 138(7) of the REACH Regulation, the EC shall carry out a review by 1 June 2013 to assess whether or not, taking into account the latest developments in scientific knowledge, to extend the scope of Article 60(3) of the Regulation as regards endocrine disruptors, or in other words whether all endocrine disruptors could be authorised only via the socio-economic route.

Regulation (EC) No 1107/2009[13] concerning the placing of plant protection products (PPPs) on the market provides that an active substance shall only be approved if it is not considered to have endocrine disruptive properties that may cause adverse effects in humans or on non-target organisms, unless the exposure of humans is negligible. By 14 December 2013, the EC shall present to the Standing Committee on the Food Chain and Animal Health (SCFCAH) a draft of the measures concerning specific criteria for the determination of endocrine disrupting properties.

In order to fulfil the obligations from the two Regulations (REACH and PPPs), the EC has commissioned a study on the "State-of-the-art of the assessment of endocrine disrupters". This study will be completed by September 2011. The contractor will review the information provided by all completed or on-going EU projects, relevant scientific opinions from EU Scientific Committees and Agencies as well as relevant WHO activities. Inter alia, the final report will give an overview of all scientific results of regulatory relevance and describe the relevant scientific state of the art.

[13] Commission Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal L 309, 24.11.2009, p. 1-50.

The report will also deal with:

- criteria for identification/determination of endocrine disrupting properties and ways to set such criteria,
- threshold values in hazard assessments of endocrine disrupters and ways to define it,
- suitability and availability of tests to identify substances with endocrine disrupting properties and to cover various mechanisms of endocrine disruption,
- suitability of testing schemes to cover all mechanisms and all kinds of substances (e.g. pesticides, biocides, industrial chemicals, pharmaceuticals, natural and synthetic hormones).

The EC intends to formally consult EFSA on the final report in view of the definition of the criteria, before any proposal can be submitted by the EC in the framework of Regulation (EC) No 1107/2009. This consultation should take place in October 2011.

In addition, the EC also has an obligation to review the Regulation (EC) No 1223/2009[14] on cosmetic products in regard to endocrine disrupters when criteria for identifying endocrine disruptors are available, or at the latest on 11 January 2015.

2.1.5. Communication to the public

With the aim of making information available and accessible to the public and stakeholders in an appropriate form, the Commission has set up two websites on endocrine disruptors. One is hosted by DG ENV[15] and provides overview of the issue of endocrine disruptors and overview of the activities performed by the Commission under the Community Strategy, including a series of reports documenting the progress achieved by implementing actions. The second website is hosted by DG RTD[16] and contains an overview of all research projects funded via Framework Programmes including published summaries of the final reports.

2.1.6. Mixture toxicity

In December 2009 the Council of the European Union (hereinafter referred to as the Council) adopted conclusions on combination effects of chemicals (Council, 2009). In particular, the Council invited the EC, drawing on existing and future research and paying appropriate attention to the costs and benefits:

- to make recommendations as to how exposure to multiple EDs should be further addressed within the relevant existing Community legislation, inter alia in the context of its forthcoming report on the implementation of the Community strategy on endocrine disrupters to be completed by 2010,
- to assess how and whether relevant existing Community legislation adequately addresses risks from exposure to multiple chemicals from different sources and pathways, and on this basis to consider appropriate modifications, guidelines and assessment methods, report back to the Council by early 2012 at the latest,
- to pay appropriate attention to the precautionary principle and the potential risks of chemical combination effects when drawing up further proposals, inter alia by assessing the need for risk management measures to protect the environment and human health.

[14] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Official Journal L 342, 22.12.2009, p. 59-209

[15] http://ec.europa.eu/environment/endocrine/documents/index_en.htm

[16] http://ec.europa.eu/research/endocrine/index_en.html

These conclusions were partly based on the results from a study commissioned by the Danish Environmental Protection Agency, (hereinafter referred to as the Danish EPA), assessing health risks from exposure to multiple chemical substances in consumer products (Danish Ministry of the Environment, 2009; ULSOP 2009). One of the conclusions from this study was that: *"The contributions that 2-year-olds absorb especially from the phthalate DBP (mostly from foods, if we discount the rubber clogs) and dioxin and dioxin-like PCBs (mostly from foods and partly from the indoor climate) constitute a risk for antiandrogenic disruptions to the endocrine system."* The EC has, very recently, organised a workshop on Mixture Toxicity at which endocrine disruptors have also been duly considered. A summary report of this workshop is available on-line (EC, 2010).

In this context, a request for an opinion on the toxicity and assessment of mixtures of chemicals has been sent to the non-food scientific committees, the Scientific Committee on Health and Environment (SCHER), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and the Scientific Committee on Consumer Safety (SCCS).

The committees are asked to advise the EC on:

- the scientific evidence of combined toxicity of substances,
- the capacity of current regulatory approaches to provide sufficient levels of protection,
- the advantages and disadvantages of different models assessing the effects of mixtures of chemicals,
- the most effective ways to target resources,
- the identification of major knowledge gaps.

In developing its opinion, the SCHER/SCENIHR/SCCS are requested to take account of the latest scientific information and to consult with prominent experts and with relevant agencies such as the EFSA, the European Medicines Agency (EMA), the European Environment Agency (EEA), the European Chemicals Agency (EChA) as well as experts and organisations outside the EU.

2.2. EFSA

EFSA's Scientific Panel on Plant Protection Products and their Residues (PPR) has recently published opinions on cumulative and synergistic risks from pesticides (EFSA, 2008b; 2009). The PPR Panel is currently working on the development of probabilistic methodologies to assess the cumulative exposure and risk to pesticide residues in food, and, will also identify by the end of 2011 "cumulative assessment groups" of pesticides. These are substances that can be grouped together for risk assessment based on the identification of similar mode/mechanism of toxicological action. Although to date no formally agreed testing strategies for the identification and characterisation of EAS are available, substances having or as suspected to have such features are already particularly considered in the on-going work on the establishment on cumulative assessment groups. The PPR Panel also follows, very closely, the on-going activities with regard to the development of testing strategies and decision criteria for EAS both in the EC, OECD and EU Member States.

Risk assessment of PPPs under Directive 91/414/EEC[17] is organised and coordinated by EFSA's Pesticide Risk Assessment Peer Review Unit (PRAPeR). A significant number of PPPs have or are suspected to have endocrine active properties (McKinlay *et al*., 2008). Until agreed specific scientific criteria for the identification of EAS are established and mandatory regulatory measures for these are in force (see "Legislative action" above), endocrine effects of pesticides

[17] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230, p. 1-32.

are evaluated on a case by case basis within the already existing provisions for pesticide risk assessment.

EFSA's Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has already published two opinions on bisphenol A in which the available information on the endocrine active properties of the substance are extensively presented and discussed (EFSA, 2006a; 2008c). Publication of another opinion on bisphenol A is expected in 2010 in which low dose and neurodevelopmental effects of the substance, in particular, are critically evaluated. The guidelines issued by the SCF for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation are still in use (EC, 2001a).

EFSA´s Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) will consider endocrine effects for the preparation of guidance on submissions for food additive evaluations that will replace the current available guidelines on submissions for food additive evaluations by the SCF (EC, 2001b).

EFSA´s Scientific Panel for Contaminants in the Food Chain (CONTAM) has already issued a number of scientific opinions on substances/substance groups that are present as contaminants in food and have endocrine active properties. For instance those which have already been evaluated, organotins (EFSA, 2004a), polychlorinated biphenyls (PCBs) (EFSA, 2005a), hexachlorobenzene (EFSA, 2005b), brominated flame retardants (EFSA, 2006b), dichlorodiphenyltrichloroethane (DDT) (EFSA, 2006c), hormone residues (EFSA, 2007a), chlordane (EFSA, 2007b), glucosinolates (EFSA, 2007c) and perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts (EFSA, 2008a).

Within the frame of an internal mandate EFSA's Assessment Methodology Unit (AMU) has established an EFSA Working Group on isoflavones (EFSA-Q-2009-00457, M-2009-0062). Isoflavones are secondary plant constituents that have endocrine active features and are present in certain foodstuffs and food supplements. The working group will report on the identification and characterisation of potential health hazards/health benefits associated with isoflavone consumption in December 2010. An isoflavones database has been created by an external contractor in collaboration with the WG and AMU to assist in this work.

2.3. EU Member States

Denmark has been very active, authorities as well as stakeholders, in the field of the assessment of EAS. The Council conclusions (see "Legislative action" above), directly referenced a report carried out by the Danish EPA: "Survey and Health Assessment of the Exposure of 2 year-olds to Chemical Substances in Consumer Products (Danish Ministry of the Environment, 2009), focussing on substances with known endocrine disrupting effects in animal studies. A centre for endocrine disrupters was established in 2008 which functions as a network of scientists and relevant institutions for knowledge building and preventive work.

Denmark is also actively involved in the Nordic Group for the Development of Test methods (*Nord-Utte*) in which representatives from Sweden, Finland, Norway and Denmark work on the development of methods for the detection of endocrine disrupting substances.

The German Federal Institute for Risk Assessment (BfR) has in response to the provisions laid down in the new EU PPP Regulation[18] initiated work to develop assessment and decision

[18] Commission Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal L 309, 24.11.2009, p. 1-50.

criteria for EAS. An expert workshop was hosted in November 2009 and recommendations and proposals drawn from it by BfR have been published in form of a report (BfR, 2010a) which was also presented to the EC, EFSA and OECD.

One of the outcomes of a Consumer Forum on Endocrine Disruptors that was held in Berlin early 2010, as a joint event between BfR and the French Agency for Food, Environmental and Occupational Health Safety (ANSES), was the publication of a draft concept paper for the stepwise assessment of endocrine disruptors under the new PPP Regulation (BfR, 2010b).

The Italian National Committee for Biosafety, Biotechnology and Life Sciences formed recently a working group to identify priorities and objectives for an "environment and health" project platform that would focus on the assessment and management of hazards associated with endocrine disrupters (EDs) and that could be adopted as a model for other emerging contaminants[19]. The working group considered particularly methods for the surveillance of contamination and exposure, the assessment of risk to the environment and to human health as well as hazard prevention, management and reduction and identified critical issues and future objectives as a means to strengthening the link between increased knowledge and prevention.

The Chemicals Regulation Directorate (CRD) of the UK has very recently issued a draft report on the "Regulatory Definition of an Endocrine Disrupter in Relation to Potential Threat to Human Health" in which a strategies to address regulatory challenges in regard to endocrine disrupters imposed by the new EU PPP Regulation and REACH are presented.

[19] http://www.iss.it/binary/inte/cont/IE_Environment_and_Health.pdf

3. International activities and developments, especially with regard to testing and assessment methodology

3.1. Background

International harmonisation activities with regard to testing and assessment of chemicals have been ongoing for more than 30 years under the auspices of the OECD. These harmonisation activities have been different whether concerning an industrial chemical or a pesticide.

In 1981 the OECD Council agreed by consensus a Decision concerning the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals, which is built on the OECD Test Guidelines (TGs) and Good Laboratory Practice (GLP) Principles. The significance of this decision is that Council Act requires all OECD governments to accept chemical test data developed for regulatory purposes in another country if these data were developed in accordance with the OECD TGs and GLP Principles.

The OECD Guidelines for the testing of chemicals are agreed by OECD Member countries' consensus. They consist of a basic set of tools based on validated experimental protocols, and are primarily used in regulatory safety testing and subsequent chemical product notification and registration. They can also be used for a variety of other purposes including the selection/ranking of candidate chemicals during Research and Development of new chemicals and products, as well as in toxicology research. The TGs are periodically updated in order to keep pace with progress in science and regulatory needs. In addition, new TGs are developed and agreed upon, based on specific needs identified by OECD Member countries.

Historically toxicity data packages have been specified in the data requirements for pesticides (which, unlike the TGs are a prerogative of national authorities/the European Community) and are the basis to detect adverse effects on living organisms exposed to certain active substances (hazard assessment). Specific data requirements for pesticide and more recently biocide active substances include toxicokinetics, metabolism pathways, genotoxicity, carcinogenicity, toxicity for the reproduction and developmental toxicity studies within others. The mechanism and mode of action behind observed adverse effects is not always clearly identified; in the EU it has been assumed that adverse effects resulting from an endocrine activity would be detected within the extensive data required according to Council Directive 91/414/EEC[20] for PPPs or Directive 98/8/EC[21] of the European Parliament and of the Council for biocides products and taken into consideration in the toxicological risk assessment. Once an adverse effect has been noted, that is sufficient for toxicological risk assessment purposes, it is not necessary to request further testing to address the mechanism. For industrial chemicals such rigorous data requirements were not requested on a legislative basis by Regulatory Authorities. To streamline and improve the former EU legislative framework, the REACH Regulation entered into force on 1 July 2007.

3.2. International regulatory approaches to the testing of endocrine active substances

3.2.1. Organisation for Economic Cooperation and Development

Following growing public concern, in 1997, the OECD Environment Directorate was requested by member countries and the international industry to initiate a core activity on Endocrine Disrupters Testing and Assessment. The objectives were to provide specific endocrine testing and assessment strategies for regulatory use in addition to the standard test guidelines.

[20] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230 , 19.08.1991, p. 1 - 2

[21] Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of the placing of biocidal products on the market. Official Journal L 123, 24.04.1998, p. 1-63

To achieve these objectives, a Task Force, the Endocrine Disrupters Testing and Assessment Task Force (EDTA-TF) was created. In 2002, the Task Force agreed and adopted a Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupting Chemicals (see Annex 1). The CF is a toolbox including screening and testing methods that were considered useful for the assessment of oestrogen-, androgen- and thyroid-mediated modes of action. It is composed of five levels ranking different types of *in silico*, *in vitro* and *in vivo* assays, to be used according to countries regulatory needs. Three technical expertise Validation Management Groups, on mammalian tests (VMG-mammalian), on ecotoxicity tests (VMG-eco) and on non-animal tests (VMG-NA) were tasked with the development and validation of suitable test methods for the assessment of endocrine active substances (see Annex 2) and reported to the EDTA and WNT. In 2009, the work of the VMG-mammalian was completed, as for example with the uterotrophic (TG 440), Hershberger (TG 441) and the 28-day repeated dose toxicity study in Rodents (TG 407) TGs. The first *in vitro* TG to come from the VMG-non animal is the TG 455 in 2009, a steroidogenesis test guideline is forthcoming and a large number of further *in vitro* tests are still in pre-validation. For the VMG-eco there are three new test guidelines as the 21-Day Fish Assay, A Short-term Screening for Oestrogenic and Androgenic Activity, and Aromatase inhibition (TG 230), Fish Short Term Reproduction Assay (TG 229) and the Amphibian Metamorphosis Assay (TG 231). In the EU, TG 230 is proposed to be used and not TG 229. In addition a retrospective validation and preliminary Draft Test Guideline of the Androgenised Female Stickleback Screening Assay is currently in review. The purpose of the test is mainly to detect anti-androgen activity[22].

At the request of the WNT, the EDTA TF has been replaced with the EDTA Advisory Group (EDTA AG) to address cross-cutting issues and the review of the Conceptual Framework. The first step of the EDTA AG was to gather information from countries on their approaches for assessing endocrine disrupters. A workshop was held in September 2009 and the report of the Workshop was utilised in the refocusing of the work of the EDTA AG (OECD, 2009a). Ongoing work includes:

1. the development of a guidance document on the assessment of endocrine active substances, focusing on oestrogen, androgen, thyroid and steroidogenesis modalities if the aim is to increase evidence that a substance is not an EDC. This guidance document will not present a testing strategy; it will only recommend the most appropriate assay that could be performed. At this stage, the guidance document will only assist in increasing the evidence,

2. the development of a Detailed Review Paper (DRP) on new endocrine endpoints building on a previous DRP on the use of metabolising systems for *in vitro* testing of EDs. The DRP will utilise the EU paper on "State-of–the-art assessment of endocrine disrupters" including criteria for classification as a starting point (to be available by September 2011) (refer to chapter 2). The scope of the DRP is the evaluation of the potential endocrine or neuroendocrine perturbations in vertebrates that may lead to, but not limited to, neurodevelopmental effects, developmental susceptibility to cardiovascular disease, obesity, lipid metabolism or metabolic disorders, and is under the lead of the US EPA and the EC.

3. additionally the EDTA AG will continue the coordination on a policy basis of the work of the VMGs.

The endocrine disrupters/endocrine active substances test methods currently in (pre)validation are summarised in Annex 3.

[22] http://www.oecd.org/document/62/0,3343,en_2649_34377_2348606_1_1_1_1,00.html

3.3. Country specific approaches

3.3.1. The United States

Legislation in the United States of America (US) requires the testing of chemicals for endocrine activity (the Food Quality Protection Act of 1996, Public Law 104-170, and Safe Drinking Water Act Amendments of 1996, Public Law 104-182. The description given below is mainly based on a previous review (Jacobs *et al.*, 2008).

In the US, the Environmental Protection Agency (hereinafter referred to as the US EPA) was charged to develop an Endocrine Disruptor Screening Program[23] (EDSP) using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may affect human health by mimicking oestrogen. For this purpose, the US EPA established in October 1996 an Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC[24]) to advise on establishing a program and considering possible testing strategies. EDSTAC recommended broadening the screening programme to include the androgen and thyroid hormone systems and to include effects on wildlife. The same Committee concluded that there is a need to include both *in vitro* and *in vivo* assays in a tiered approach (see Annex 4). The proposed Tier 1 screening phase comprises both *in vitro* and *in vivo* assays, aimed primarily at detecting chemicals with endocrine activity and thus potential human health effects. Among the recommended *in vitro* assays included in the battery are cell-free receptor binding; transcriptional activation; steroidogenesis in testis and Leydig cell cultures. The Tier 2 test battery consists solely of *in vivo* assays, and includes two generation reproductive mammalian tests, as well as tests for environmental effects on target wild-life species. It is intended that the Tier 2 battery will provide the definitive data on EAS suitable for risk assessment purposes. As the tests that have shown greater promise for development for screening and testing batteries have been developed, they have generally been included in the rolling work plan of the OECD TG development.

The EDSTAC Tier 1 testing scheme requires that both *in vitro* and *in vivo* screens should be developed, and it is essential that the former test methods are developed and validated so that they can be used first in the test battery for prioritising chemicals for further testing, and secondly to then facilitate the interpretation of data from animal tests. As such, it is crucial that false negative results are minimised. A certain level of false positive results is acceptable, although should be low, given that a false positive result is likely to lead to animal testing with the resultant financial and animal costs.

Priority Setting is one of three major components of the EDSP of the US EPA (the other two are assay development and validation, and development of program policies and procedures to require testing). The Priority Setting is based on four categories:

> i) chemicals unlikely to interact with hormone systems (e.g. certain polymers, strong mineral acids/bases);
> ii) chemicals without sufficient existing data to determine if Tier 2 testing is required;
> iii) chemicals with sufficient existing data to determine if Tier 2 testing is required; and
> iv) chemicals with sufficient data to support a hazard assessment.

The selection of the first chemicals to be screened is based on the potential human exposure. It includes pesticide active substances found in food and water, for residential use and occupational contact, and High Production Volume (HPV) inert ingredients detected in human and environmental monitoring data. A list was issued on the 15th April 2009 including 58

[23] http://www.epa.gov/scipoly/oscpendo/index.htm
[24] http:// www.epa.gov/scipoly/oscpendo/pubs/edspoverview/edstac.htm

pesticide active substances and 9 HVP/pesticide inert ingredients for prioritisation for endocrine activity testing. As defined, it does not correspond to a list of "known" or "likely" EAS, is simply a high priority list for testing on the basis of high volume production. The program started in Autumn 2009 with the publication of the final notice of Tier 1 tests, Screening Battery, posting of final Test Method Protocols and issuance of Test Orders for 67 chemicals (to the manufacturers and importers) requiring all assays in the Tier 1 battery.

In addition, *in silico* tools are also been developed for prioritisation of testing. For example, an expert system for oestrogenic activity of pesticide inerts and biocides has been developed and reviewed based on data obtained from fish liver oestrogen receptor (ER) experiments (see US EPA FIFRA SAP August 25-27, 2009: The Use of Structure Activity Relationships of Estrogen Binding Affinity to Support Prioritization of Pesticide Inert Ingredients and Antimicrobial Pesticides for Screening and Testing at: http://www.epa.gov/scipoly/sap/meetings/2009/082509meeting.html).

A comparison of the US EPA EDSP approach with the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals is presented in Annex 5.

Regarding naturally occurring endocrine active substances, the US National Institute of Environmental Health Sciences, National Toxicology Program (NIEHS/NTP) have recently conducted a safety evaluation of soy infant formulae. NTP-CERHR[25] Expert Panel Report on the Reproductive and Developmental Toxicity of Soy Formula 2006, revisited in 2009 and published in 2010.

3.3.2. Canada

Legislation in Canada requires the testing of chemicals, with the Canadian Environmental Protection Act of 1988 (CEPA, 1988) and the Canadian Environmental Protection Act of 1999 (CEPA, 1999). These Acts require that existing and new substances are assessed to determine their potential risks to human health and/or the environment, and the ways in which humans or the environment can be exposed to the substances, and are administered by both Health Canada and Environment Canada.

Canada also collaborates closely with other countries and the OECD, for example, through Health Canada's Pesticide Management Regulatory Agency (PMRA) there is a close collaboration with the US and with Australia for new chemicals. Canada is involved in a number of OECD validation and policy activities. The VMG-mammalian group was chaired by Canada and Canada currently co-chairs the EDTA AG. Health Canada and Environment Canada have been involved in developing recommendations on isoflavones, and regulations for bisphenol A and other EAS as part of the existing and new substances legislation, and Hazardous Products Act.

3.3.3. Japan

The following descriptions of activities in Japan come from the contributions to a recent OECD workshop (OECD, 2009a):

> *"The Ministry of Health, Labour and Welfare (MHLW) established a Committee on Health Effects of Endocrine Disrupters, which addresses the evaluation of risk of endocrine disrupters on human health, the necessity to prompt action to protect human health, and the risk communication to the general public. The Committee has developed*

[25] Center for the Evaluation of Risks to Human Reproduction

a framework for the testing of possible endocrine disrupter chemicals, which consists of two tiers: 1) screening assays (including in silico, in vitro and in vivo assays) and 2) definitive tests. The MHLW has carried out screening tests (i.e. including the Hershberger Bioassay and the rodent Uterotrophic Bioassay) on a number of chemicals and a priority list for future definitive testing was established based on the results from these screening assays.

The Ministry of Economy Trade and Industry (METI) established an advisory body - the Endocrine Disruptive Effect Subcommittee. So far, METI funded studies on hazard assessment of 15 chemicals of potential concern as endocrine disrupters (no significant risk to human health was identified). Moreover, METI has been involved in the OECD Test Guideline Programme and conducted non-animal testing (receptor binding assay, reporter gene assay, steroidogenesis assay, QSAR) and animal testing (Uterotrophic assay, Hershberger assay, enhanced 407 Test Guideline, in utero and lactational exposure study, two-generation reproductive toxicity study) of a number of substances.

The Ministry of Environment (MOE) established the "Strategic Program on Environmental Endocrine Disrupters 98" (SPEED 98). In addition the ExTEND 2005 programme was established by MOE in 2005 and it involves basic research on the mechanisms of endocrine disruption, environmental monitoring (observation of wildlife and measurement environmental concentrations and exposure levels), development of test methods, hazard and risk assessment, risk management, promotion of information sharing and risk communication, organization of annual international symposia." (OECD, 2009a)

Japan also conducted a risk assessment of isoflavones.

3.3.4. Republic of Korea

A description of activities in the Republic of Korea was presented at the same OECD workshop (OECD, 2009a):

"The relevant ministries of the Government of Korea have established a Mid-Long Term Research Plan for Endocrine Disrupters. The resulting research projects, conducted in the years 1999-2005, dealt mainly with environmental monitoring of endocrine disrupters and the assessment of ecological effects. They included monitoring of terrestrial and marine environment, livestock, food, agricultural products, farmland and drugs. The ministries have recently revised and produced and new five-years research plan (2007-2011). It is focused on reviewing the results of the previous research projects and on preparing appropriate plans for safety management of endocrine disrupters in each ministry."

Recently, South Korea has been participating in the validation of the HeLa oestrogen receptor (ER) antagonist *in vitro* assay (OECD lead by Japan).

3.4. Industry initiatives

3.4.1. Business and Industry Advisory Committee to OECD (BIAC)

The BIAC views on EAS assessment were also reported at the OECD workshop in 2009 (OECD, 2009a):

"BIAC supports the use of a tiered hierarchical scientific framework (comprising of 3 stages) in which validated screening assays are used to identify substances with endocrine activity and prioritize substances for further, more definite testing that

provides data on adverse effects and dose response which are necessary for hazard and risk characterization. Definitive testing using validated harmonized protocols are necessary to identify adverse effects caused by alterations to endocrine system functions.

Using such a tiered approach the results from definite tests must outweigh or supersede results from screening assays in guiding policy and management in both the public and private sectors.

The interpretation of data coming from testing using the tiered approach should be based on a weight-of-evidence evaluation. For hazard characterization the chemical industry supports the development of a weight-of-evidence evaluation process that consists of a comprehensive, objective, transparent and balanced interpretation of the totality of scientific evidence regarding hormonal activity and adverse effects that might result in endocrine mechanisms."

The proposed 3-stage testing approach consists of (OECD, 2009a):

1. **Initial Assessment -** evaluation of all available data (including production volume and patterns of use, exposure information, predicted environmental properties, toxicological data from existing studies, Quantitative Structure-Activity Relationship (QSAR) and molecular screening results; this stage corresponds to level 1 of the OECD CF.

2. **Screening -** This step allows to efficiently and effectively develop information as to whether a substance has the potential to interact with one or more components of the endocrine system. It includes *in vitro* assays and *in vivo* assays providing mechanistic information/data on single mechanisms and *in vivo* assays providing information on multiple endocrine mechanisms. As the results from assays comprising the Stage 2 provide only mechanistic information and not evidence for adverse effects, these screening results do not indicate that a compound is an "endocrine disrupter". This stage largely corresponds to levels 2, 3 and 4 of the OECD CF.

3. **Definitive Testing -** This step aims at evaluation of apical endpoints, adverse effects and dose response to accurately and effectively identify and characterize the hazard(s) from chemicals. Overall, hazard characterization for hormonally active chemicals requires an objective evaluation of whether the effects produced are adverse and whether adverse effects are due to a hormonal activity of the chemical. It is important to stress that all stages should make use of standardized, validated and internationally harmonized test methods. In case that new and novel methods, and studies with non-standard species which provide important scientific information, are used, BIAC suggests doing a thorough review of the study report, if possible replicating the study in another laboratory. This stage corresponds to level 5 of the OECD CF.

Industry (BIAC) and NGOs (environmental and animal welfare - ICAPO) participate in OECD advisory groups. Technical input can be provided in the VMGs and EDTA, and in the WNT they are observers without any voting rights.

3.4.2. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) funded by the Chemical Industry

ECETOC held a workshop to explore guidance on interpreting Endocrine Disrupting Effects in June 2009. To quote in summary (ECETOC, 2009a):

> *"Fifty-five invited experts (from academia, regulatory bodies and industry) discussed an approach developed by an ECETOC Task Force and distributed to participants in the form of a Task Force report. This report outlined an approach developed by the Task Force providing guidance in the form of flowcharts that could be used as a decision tree for the identification of endocrine disrupting effects in human health (toxicology) and environmental assessments (ecotoxicology). The aim of the workshop was to assess the suitability of such an approach. It was also intended as an open forum for critical analysis and an opportunity to propose improvements to the scheme. The workshop consisted of a series of invited presentations. The first outlined the regulatory background to the issue. The second reported on German national initiatives to develop toxicology criteria for endocrine disrupters. This was followed by presentations from the ECETOC Task Force introducing the ECETOC approach, including detailed explanations (with case studies) for its application in the toxicology and ecotoxicology fields."*

Following the workshop, ECETOC published a revised guidance on identifying endocrine disrupting effects (ECETOC, 2009b).

3.4.3. The soy industry: Council for Responsible Nutrition

There are a number of European and international industry umbrella groups relating to soy production and marketing of nutritional supplements, infant formula. The Council for Responsible Nutrition (industry) coordinated on behalf of the soy and supplement industry a symposium to advocate beneficial health effects of isoflavones in May 2009.

3.5. Further approaches under development

3.5.1. Computer-Based Approaches

A number of computer-based expert systems for predicting metabolism and metabolism-dependent toxicity are undergoing development, and have been reviewed (Jacobs *et al*, 2008); and a more updated review is under preparation. The OECD validation criteria for QSAR development are available in Guidance Document No. 69 (OECD, 2007a). There are many reasonable oestrogen receptor (ER) QSARs available, androgen receptor (AR) and thyroid receptor (TR) much less so.

Strategically, molecular modelling and QSAR techniques have value in the first Tiers of integrated testing strategies for processing chemicals, in levels 1 and 2 of the OECD Conceptual Framework. However the practical limitations (such as applicability domains) of these *in silico* tools with respect to regulation of chemicals needs to be acknowledged, to provide realistic expectations of such methods.

3.5.2. TTC approach

The EC Joint Research Centre (JRC) held a workshop on threshold of toxicological concern (TTC) approaches in 2008 "Thresholds of Toxicological Concern for Endocrine Active Substances in the Aquatic Environment" with the participation of the OECD, industry, EC and academia, there was a consensus on the approaches to be taken particularly on oestrogenic substances (Gross *et al.,* 2009). The paper proposes further steps in investigation to develop the applicability of this approach.

4. Challenges to risk assessment of food and feed

It is not the intention of this chapter to review the state of the art in research on EAS, since that task has been commissioned from a contractor by the EC, with a planned delivery date in 2011. Rather it is to indicate some of the issues that are challenging in the risk assessment of EAS and to highlight some areas that are particularly pertinent to risk assessment of food and feed.

4.1. Multiple modes of action

As mentioned in the Introduction, endocrine activity is not a toxicological endpoint in itself. It describes a varied group of actions, involving many organs and tissues in the body, on which substances may act either directly or indirectly to affect the endocrine system. These actions may be reversible (as occurs during natural hormonal cycles) or may lead to persistent, irreversible changes (for example, during development). It is this considerable variety of potential outcomes of such actions that pose one of the challenges to risk assessment of EAS in food and feed.

When the issue of environmental endocrine disrupters was first raised in 1991 at the Wingspread Conference (Colborn and Clement, 1992), the focus was on oestrogenic activity and interaction with nuclear steroid receptors. Since then, concern about other potential actions of environmental chemicals with endocrine activity has broadened to include, androgenic and anti-androgenic activities, anti-thyroid activity, interaction with the hypothalamic-pituitary-adrenal axis, inhibition of steroid hormone synthesis, up regulation of hepatic enzyme systems that alter the metabolism or protein binding of endogenous hormones, and receptor interactions with membrane-bound steroid receptors, retinoid receptors, aryl hydrocarbon receptors, glucocorticoid receptors and immune cell receptors. From these varied actions, a wide variety of effects may result, including carcinogenesis, reproductive toxicity, developmental abnormality, central nervous system and immune changes. More recently, the potential role of EAS in the aetiology of human conditions or diseases, such as obesity, diabetes, cardiovascular disease, and neurobehavioural changes has been raised. Although many of the mechanisms by which EAS may cause such effects have not yet been precisely defined, it is known that there are several potential receptors and cross-talk mechanisms between receptors that could be involved (e.g. between oestrogen receptors (ER) and aryl hydrocarbon receptors (AhR); constitutive androstane receptors (CAR), pregnane X receptors (PXR), peroxisome proliferator-activated receptors (PPAR) and retinoid X receptors (RXR)) (IPCS, 2002; Jacobs *et al*., 2002; Jacobs, 2005; Hotchkiss *et al*., 2008; Swedenborg *et al.*, 2009), and these diseases, mechanisms and modes of action will be explored further in the new ED DRP being developed at the initiative of the OECD EDTA-AG (as indicated in chapters 2 and 3).

An individual substance may also produce endocrine effects via more than one mechanism of action. For example, steroids may combine with receptors to produce a full agonist effect or partial agonist effect. Substances which act as partial agonists but occupy most of the receptors will also demonstrate antagonistic activity in the presence of a stronger agonist. The extent to which agonistic or antagonistic activity is expressed will depend on the tissue, the presence of other endogenous or exogenous substances that are also ligands for the same receptor, and their relative concentrations at the receptor (Granner, 2001). This phenomenon is well known and exploited for therapeutic benefit in endocrine pharmacology. A substance may also have more than one mechanism of action via different pathways but give rise to the same phenotypic effect. For example, the pesticides prochloraz and linuron are both androgen receptor antagonists but also inhibit biosynthesis of testosterone, with both mechanisms contributing to an anti-androgenic mode of action (Hotchkiss *et al*., 2008).

4.2. Physiological context

A big challenge in the risk assessment of EAS is the fact that many different organs, tissues and hormone levels must be considered and many of the endocrine pathways and end effects in the body naturally undergo large changes, depending on life stage, gender, ovarian cycle, pregnancy, diurnal variation, etc. Thus, judgement as to whether a change in a particular parameter is within the normal range of variation, and whether or not it should be considered adverse, can be more complex than, say, a judgement as to whether a particular set of changes in liver enzymes and histopathology indicate an adverse effect on the liver.

Endocrine pathways are also regulated by positive and negative feedback loops, which can enable the body to maintain normal homeostasis (Darlington and Dallman, 2001), despite exposure to exogenous EAS in the environment and in the diet. Thus, for example, identification that a substance has a potential to bind to endocrine receptors *in vitro* does not, by itself, demonstrate that a substance will have endocrine activity *in vivo*, unless some measure of downstream effect is also observed. Without such information, there will be an insufficient basis for risk assessment of actual human exposures *in vivo*.

It is also recognised that there are considerable inter-species differences in endocrine biology, for example in the timing of critical windows of vulnerability to EAS during development, or in the hormones required to maintain pregnancy, and similar inter-species differences in endocrine-mediated pathogenesis. Given this complexity, there is a need among risk assessors for in depth expertise in both the endocrine system and toxicology. Otherwise, test results can be poorly understood, resulting in inappropriate extrapolation of risk from data on laboratory animals and wildlife to humans.

4.3. Adequacy of current test protocols

Testing approaches and test protocols have been discussed in detail in Chapter 3. In the explosion of concern about the possible risks from exposure to EAS, it is often forgotten that many of the previous and current standard test protocols for *in vivo* toxicity studies assess numerous endpoints that are relevant to detection of perturbations to the endocrine system. These include general subchronic and chronic toxicity studies, carcinogenicity bioassays, reproductive and developmental studies, including developmental neurotoxicity. Some OECD test guidelines, such as the repeated-dose 28-day study (TG 407) and the two-generation reproduction study (TG 416) have been updated to include additional (but optional in the case of TG 407) endpoints specific to detection of EAS (OECD, 2001, 2006). New OECD test guidelines for additional *in vivo* studies have also been adopted in recent years, (e.g. TG 440, the uterotrophic assay and TG 441, the Hershberger assay) (OECD, 2007b, 2009b). *In vivo* studies are usually regarded as the "gold standard" for risk assessment of endocrine activity. In many of the guidelines for toxicity testing prior to authorisation of substances deliberately added or otherwise present in food and feed, such studies will have been routinely conducted. Thus, in sectors such as pesticides, food and feed additives, there is already a considerable body of evidence about whether or not an authorised substance has endocrine activity.

However, it is also evident that existing standard protocols may not cover all potential effects that could be induced by EAS. For example, effects on gastro-intestinal hormones, or reproductive senescence are not covered. Similarly, while existing protocols may allow for the measurement of a number of endogenous hormones, such measurements are not always undertaken, or the results may be confounded by stress or the inconsistency of diurnal variations. Test protocols may also allow for relevant tissues to be examined, but not in a way that might reveal subtle changes, such as effects on the weight of the thyroid (as for the updated TG 407), and also for example for the weight of (subdivisions of) the prostate gland, or histological effects on mammary gland development that are only visible at the microscopic level, which have been reported following treatment with bisphenol A and dibutyl phthalate

respectively (Welshons *et al.*, 1999; Vom Saal and Myers, 2010; Lee *et al*., 2004). It is because of the inconsistency in some of these measurements, revealed by inter-laboratory validation studies, that they are not obligatory in standard test protocols.

Test protocols for *in vitro* approaches to hazard identification of EAS are also being developed and validated under the auspices of OECD, though to date, only one (an assay for oestrogen-agonist activity at the level of the human oestrogen-receptor-alpha) has been adopted. Such assays will be useful for screening substances suspected of having endocrine activity, or structurally related to known EAS, and for providing mechanistic information, but at present are not required routinely for substances deliberately added to food or feed.

4.4. Low-dose issue

The issue of possible effects at low doses, below what was previously considered to be a no-effect level, has been central to the controversy about the risks of human exposure to EAS for over a decade. It was examined in depth in 2000 by a panel of over 40 academic, government and industry scientists, with relevant expertise, in the low-dose peer-review on endocrine disruptors that was convened by the US Toxicology Program/National Institute of Environmental Health Sciences at the request of the US Environmental Protection Agency (Melnick, 2002). That panel concluded (*inter alia)* as follows:

> *"Low-dose effects, as defined for this review, were demonstrated in laboratory animals exposed to certain endocrine-active agents. The effects are dependent on the compound studied and the end point measured. In some cases where low-dose effects have been reported, the findings have not been replicated. The toxicologic significance of many of these effects has not been determined."*

Since then there have been many more publications on low-dose effects, but the difficulties outlined above with respect to replication and toxicological significance of low-dose effects largely remain. Regarding the shape of the dose-response curve at low exposures, the panel commented that:

> *"The shape of the dose–response curves for effects of estrogenic compounds varies with the end point and the dosing regimen. Theoretical models based on mechanisms of receptor-mediated processes, as well as empirical models of endocrine-related effects, produced dose–response shapes that were either low-dose linear, or threshold appearing, or non-monotonic (e.g., U-shaped or inverted U-shaped)."*

It is considered by some scientists that because exposure to exogenous EAS will be superimposed on underlying and varying physiological hormonal states, and because endogenous hormones act at very low blood concentrations, then low-dose effects (e.g. by dose addition) might be anticipated. Further, because of the well-known agonist/antagonist profiles of hormones and other EAS, there is biological plausibility for the possibility of low-dose effects, for the occurrence of non-monotonic dose-responses, and for qualitatively differing effects on the endocrine system at low and high doses of the same substance.

On the other hand, the theoretical background for non-monotonic dose-response curves for xenobiotics has also been questioned (Kitchin and Drane, 2005; Mushak, 2007; Mayo and Spanos, 2008). In the NTP-CERHR (2008) review on bisphenol A, for example, it was stated:

> *"Every chemical that produces low dose cellular and molecular alterations of endocrine function also produces a cascade of effects increasing in severity resulting in clearly adverse alterations at higher doses, albeit the effects can be different from those seen at low doses. With these endocrine disrupters, but not BPA, the low dose effects are often*

causally linked to the high-dose adverse effects of the chemical. This is true for androgens like testosterone and trenbolone, estrogens like DES, 17β-estradiol and ethinyl-estradiol, xenoestrogens like methoxychlor and genistein, and antiandrogens like vinclozolin, for example. Hence, the failure of BPA to produce reproducible adverse effects via a relevant route of exposure, coupled with the lack of robustness of many of the low dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the inability to reproduce many of these effects .... strains the credibility of some of these study results. They need to be replicated using appropriate routes of exposures, adequate experimental designs and statistical analyses and linked to higher dose adverse effects if they are to elevate our concerns about the effects of BPA on human health".

This lack of scientific consensus on whether low-dose effects have biological plausibility or have been adequately demonstrated empirically is a further challenge for risk assessment of EAS at the present time.

The frequency with which EAS possessing oestrogenic activity have been implicated in low-dose effects may have an explanation in the evolutionary biology of oestrogen receptors. ER is known to be the earliest ancestral receptor of the entire steroid superfamily of nuclear receptors (Thornton *et al*., 2003) and this long evolutionary history may explain its apparent undiscriminating behaviour with respect to ligand binding. There is also widespread occurrence of oestrogen receptors (ER-α and ER-β) in both vertebrates and some invertebrates, though not in plants or insects. In plants, phytoestrogens initiate activity when they bind to a membrane tyrosine kinase receptor (Levin, 2009a). Even some bacteria contain transcriptional activator protein receptors called nodulation D protein (NodD), which share genetic homology with human ER and respond to similar ligands; NodD bind phytoestrogens released by plants to bring about symbiotic nitrogen fixation (Fox, 2004).

Nuclear oestrogen receptors, ER-α and ER-β, in addition to binding physiological oestrogenic hormones, also bind numerous other ligands, including natural phytoestrogens and xenobiotic oestrogens. However, the majority of these other ligands are weak oestrogens in terms of their binding affinities and initiation of downstream events, with relative potencies *in vitro* of 10^{-3} or less, compared with the most potent physiological oestrogen, 17β-oestradiol.

It is partly this weakness of the nuclear receptor binding that has generated scepticism about the likelihood of effects of environmental oestrogens *in vivo*. However, it has also been known for many years that some effects of oestrogens are much more rapid in onset, occurring within minutes, than can be accounted for by the pathway of transport of ligand into cells, binding to nuclear receptors, followed by ligand-activated transcription and subsequent modulation of gene expression, which takes some hours. Rapid-onset actions have also now been demonstrated for xenobiotic oestrogens and are known to be non-genomic and mediated via membrane-localised oestrogen receptors. Binding at the membrane receptor then triggers intracellular signalling pathways via second messengers such as calcium, cGMP and cAMP (Levin, 2009b). Membrane oestrogen receptors have been identified as ER-α and ER-β which has been trafficked from inside the cell to the cell membrane. More recently, a novel receptor, termed G-protein coupled ER1 (GPER), belonging to an entirely different family from ER-α and ER-β, has also been identified as another membrane-based oestrogen receptor (Maggiolini and Picard, 2010). When activated, membrane-based oestrogen receptors switch on protein kinase pathways, alter membrane electrical properties and cause rapid influx of calcium ions. The downstream consequences of activation of membrane-based oestrogen receptors include such diverse events as prevention of vascular injury and cardiac hypertrophy, regulation of sexual behaviour and pain perception in the central nervous system, survival of osteoblasts, fluid resorption in the colon, and development of interneuron connectivity and dendritic spine density in the brain (Levin, 2009b; Leranth *et al*., 2008). Bisphenol A, for example, given subcutaneously, has been

shown to antagonise spine synapse formation induced by oestrogens and testosterone in the limbic area of the brain of gonadectomised female and male rats. Similarly, it abolished the synaptogenic response to subcutaneous oestradiol in the hippocampus and prefrontal cortex of ovariectomised monkeys, the magnitude of the effect being modulated by the presence or absence of soy in the diet (Leranth *et al*., 2008). Such effects, if mediated via membrane oestrogen receptors, open up the possibility that an EAS, given orally, even if rapidly inactivated by metabolism in the liver, might nevertheless be capable of inducing rapid-onset, low-dose effects from the small amounts of free substance in the plasma.

4.5. Implications of low-dose effects for testing

It is evident from the published literature that there is not yet international consensus on how to test for low-dose endocrine effects, nor, more importantly, how to interpret the results from low-dose studies.

In the EFSA evaluations of bisphenol A, for example (EFSA, 2006a, 2008c,d), while all available studies have been equally scrutinised, irrespective of the sponsor or authors, the studies reporting low-dose effects have been found to be generally less robust (though not "ignored" as some media reports have stated), compared with large studies conducted to standard protocols and conforming with GLP. The problems identified included the use of small numbers of animals, single doses, or too widely spaced dose intervals to demonstrate a U-shaped dose-response, and where several doses had been used, lack of dose-response. The biological and toxicological significance of some of the reported observations were also unclear, particularly where contrary results had been obtained in different studies (EFSA, 2006a).

For those who consider that the possibility of non-monotonic dose-response curves must be investigated for EAS, this implies that studies conducted to standard protocols in which the doses tested are usually in the milligram/kg body weight per day range, and in which the lowest dose tested does not produce any effect, may fail to identify low-dose effects in the microgram/kg body weight per day range. However, this is a different issue from whether the low-dose studies published to date have been of adequate design, appropriate route of administration, and quality of execution to produce robust and replicable results for risk assessment of oral exposures in humans. It is this key issue which has largely determined the differences in view between different groups of risk assessors looking at the outcomes of the same low-dose studies on bisphenol A. Because such effects are unexpected or unusual, some risk assessors consider that, as a minimum, low-dose effects should be replicated in well-designed studies before they can be used in risk assessment.

Apart from the study design issues already identified above, there is lack of scientific consensus on the appropriate selection of species and strain of laboratory animal for the study of EAS (Vom Saal and Hughes, 2005a), the influence of background diet and housing conditions (Vom Saal *et al*., 2005b; Verwer *et al*., 2007), the use of appropriate positive controls (Vom Saal *et al*., 2010; Gray *et al*., 2010), and appropriate statistical evaluation of results (Haseman *et al*., 2001; Scholze and Kortenkamp, 2007). These issues will take some time to resolve, but in the meantime, risk assessors are faced with the challenge of evaluating and interpreting the results of existing low-dose studies.

4.6. Hazard identification versus risk assessment

As already discussed in Chapter 3, a number of organisations (e.g. OECD, US EDSTAC) have been charged with the task of developing schemes for the screening of substances for endocrine activity. Such schemes usually involve a tiered approach to testing, starting with *in vitro* studies and progressing to *in vivo* studies of increasing complexity. While the validity and reliability of the individual tests included in such screens have mostly been evaluated in inter-laboratory

trials, the reliability of the screens as a whole to detect novel EAS has yet to be shown. Such screens are costly to apply to large numbers of substances and will likely reveal a substantial number of substances showing effects at the *in vitro* level, which then require *in vivo* follow-up.

It is for this reason that it will be important for risk assessors to reach international consensus on the ability of currently used apical 'gold standard' *in vivo* tests, which are often required elements in applications for authorisation of substances in food and feed, to cover the full range of possible adverse effects on the endocrine system.

In tiered screening approaches, the initial tests undertaken will provide information on hazard identification, but not hazard characterisation or risk characterisation. Some Regulatory decisions in areas of chemical regulation in the EU to which the Classification, Labelling and Packaging (CPL) Regulation applies (EC, 2008), are currently based on hazard identification (e.g. consumer use of products, including plant protection products, containing chemicals that have been classified as carcinogenic, mutagenic or toxic to reproduction (CMR). While in other areas, including some within EFSA's food remit such as food additives and food contact materials to which the CPL Regulation does not apply, regulatory decisions are based on risk assessment and not just hazard identification. This allows an acceptable daily intake to be set for substances that, for example, are toxic to reproduction, provided a clear no-effect level has been identified in relevant *in vivo* testing.

Both types of regulatory approach, utilising hazard identification for some decisions and full risk assessment for others, impact on substances on which EFSA advises. An approach to EAS that relies on hazard identification and does not allow the possibility of risk assessment could lead to restrictions in the use of products which might otherwise be considered safe under normal conditions of use. The divergence in approach may become further highlighted as the Community approach to the assessment of plant protection products with endocrine activity is elaborated over the next 3 years; for instance, in accordance with the new marketing of plant protection products regulation, there will need to be agreed scientific criteria for concluding that a component of a plant protection product has "endocrine disrupting properties that may cause adverse effects in humans" (EC, 2009). It is not yet clear whether the criteria will allow risk assessment or be limited to hazard identification for determination of endocrine activity. Thus EFSA may be required to advise on some EAS utilising only hazard identification, whereas for others it may still utilise full risk assessment. Should such a divergent approach emerge, it would pose challenges, particularly for risk communication.

4.7. Exposure to naturally occurring EAS

One feature unique to risk assessment in food and feed is the presence of naturally occurring EAS, which have been present in (or on) food and fodder for centuries. Red clover disease in sheep, for example, in which infertility from the oestrogen content is an early manifestation, has been recognised since the mid 20th century (Adams, 1995). Similarly, it has been known for many decades that there are anti-thyroid agents, notably nitrate, glucosinolates and isoflavones in leguminous food plants such as soy, lentils and peanuts, also the *Brassica* family (such as broccoli and cabbage), linseed/flaxseed, millet, all of which can cause goitre in humans and animals, and that these may co-exist with anti-goitrogenic substances in the same plants (Marine *et al*., 1933; McCarrison, 1933; Astwood, 1949; Care, 1954; Ekpechi *et al*, 1966; Gaitan, 1990).

Oestrogenic substances are particularly widespread in plants. They are collectively termed phytoestrogens, comprising isoflavones, coumestans and lignans, of which isoflavones in soybeans and coumestans in *Medicago* species such as alfalfa, are well-known (Livingston, 1978; Fletcher, 2003; Dixon, 2004). The oestrogenic substance zearalenone is also found in *Fusarium* species of fungi, which are common contaminants of cereals (Zinedine *et al*., 2007;

Mantovani *et al*., 2009). This raises the possibility not only of adverse effects from natural substances present in the diet, but equally, the possibility that humans and animals have evolved efficient mechanisms for dealing with such substances (e.g. by activation of receptors that induce detoxifying enzymes (Jacobs *et al.*, 2005). Natural anti-androgenic substances are far less widespread than natural oestrogenic substances, but are known to occur, for example in American saw palmetto (Tacklind *et al*., 2009).

Information on human and animal exposure to naturally occurring EAS in individual foods and crops used in feed is available (see, for example Potter and Steinmetz, 1996; Peeters, 2007). There is also much interest to exploit the properties of phytoestrogens as dietary supplements or pharmaceuticals, although safe and efficacious levels have yet to be established (Scott *et al*., 2009). A hazard assessment of the possible effects of exposures to the naturally occurring EAS isoflavones is currently underway at EFSA.

Another consequence of the presence of naturally occurring EAS in animal feed, including laboratory animal feed in which soy is a common ingredient, is that experiments that have undertaken on other EAS in the past may have been confounded by components in the background diet, particularly if subtle effects were sought. More recently, this has been recognised and the OECD Test Guideline for the Uterotrophic Bioassay in Rodents (OECD, 2007b), for example, discusses the possible need to modify the background diet. However, the need to measure EAS in background diets and to eliminate such substances is not always done.

It should also be noted that EAS other than phytoestrogens may be unavoidable contaminants in food, such as arsenic (Davey *et al*., 2007, 2008) and dioxins (EFSA, 2004b).

4.8. Exposure to EAS in combination

Currently, the majority of substances deliberately added or present as contaminants in food and feed are risk assessed individually. In response to scientific and public concerns, extensive efforts are now in train to apply existing methods and to develop new methods for the assessment of chemicals present in mixtures, or chemicals to which there may be co-exposure, either by the same route or by different routes (see also section 2.1.5 in this report). Such assessments are most advanced with respect to groups such as dioxins/dioxin-like PCBs or pesticides, which act by the same mechanism of action (EFSA, 2007d). However, more systematic approaches are now being considered for other types of chemical. In 2008, for example, the US National Research Council recommended that the US EPA undertake cumulative risk assessment of combined exposures to phthalates, several of which target the male reproductive system (NAS, 2008), and this is now being undertaken by the US National Toxicology Program. The IPCS/WHO has recently published a draft framework for the risk assessment of combined exposures to multiple chemicals (IPCS, 2010). It does not specifically address EAS but its tiered approach would be applicable to any combination of substances including EAS. WHO, OECD and the ILSI Health and Environmental Sciences Institute (HESI) are convening an International Workshop in February 2011 to discuss the outcome of case studies utilizing the proposed WHO/IPCS framework. It is envisaged that the presentations and discussions will enable the Workshop to derive general conclusions and make recommendations for further work.

The possibility of additive or synergistic effects from combined exposures to mixtures of chemicals with similar modes of action has been a common topic of discussion and research (see for example Cassee *et al*., 1998; McCarty and Borgert, 2006; Lambert and Lipscomb, 2007; Teuschler, 2007). There is also a rich literature on the experimental outcomes of combining EAS in *in vitro*, laboratory animal and environmental models, with some effects conforming the rules of dose addition while others appear to act synergistically (see for example Kortenkamp, 2007, 2008; Christiansen *et al*., 2008; Flippin *et al*., 2009).

There will no doubt be a need to develop risk assessment capacities to assess combined exposures to EAS, as there have been to assess combined exposures to certain pesticides.

4.9. Improving risk assessment of EAS

Risk assessment of EAS requires extensive knowledge and skilful judgement. However, many of the challenges in risk assessment of EAS cannot yet be met due to gaps in knowledge and lack of consensus on the significance and interpretation of the results of low-dose exposures. Currently, risk assessors are required to advise on EAS in the context of considerable uncertainty, and for some substances the considerable volume of evidence has not necessarily reduced that uncertainty (e.g. bisphenol A).

The complexity of the biology, toxicology and risk assessment of EAS can appear daunting, involving understanding of multiple pathways and receptor cross-talk, consideration of both natural and man-made chemicals, some with both agonist and antagonist actions, against a background of normal and varying, physiological hormone actions. Nevertheless, the explosion of research in EAS is providing a huge amount of information and a better understanding of the relevance (or sometimes irrelevance) of animal models for human risk assessment.

There are however, many useful and validated tests for detection of EAS, and test methodology, while still requiring development in some areas, is not a major stumbling block (see section 3 of this report). One of the tasks to be undertaken by the OECD EDTA AG in 2011 is to develop a Detailed Review paper on promising assays or endpoints which are not yet addressed in current test guidelines. These assays or endpoints would evaluate potential endocrine or neuroendocrine perturbations in vertebrates that may lead to, but not limited to, neurodevelopmental effects, developmental susceptibility to cardiovascular disease, obesity, lipid metabolism or metabolic disorders.

There are a number of issues requiring continued, long-term research that are unlikely to be resolved in the near future. One area being actively developed is that of *in silico* tools, including computer models for expert systems and statistical models such as quantitative structure-activity relationships (QSARs) and SARs. Most of these need considerable development before they will be useful as a tool for reproductive and developmental screening and risk assessment. To date, only QSARs and expert systems for oestrogen receptor-related mechanisms have received much use. There are also androgen receptor, pregnane X receptor, and thyroid receptor models available that are commonly used in the pharmaceutical industry. The multiple pathways and endpoints that are potentially involved in the actions of EAS indicate that the development of useful predictive QSARs, will require their use in a battery type of approach if adequate sensitivity and predictive capability is to be achieved.

Human research on EAS poses additional difficulties, especially with respect to efforts to relate early exposures to later emerging disease. Human epidemiological studies are often deficient in characterising chronic exposures, or in characterising exposure at relevant life stages. For example, there is concern about the possible role of exposure to EAS in the development of breast cancer, but the most vulnerable period for exposure may be in the teenage and young adult years, a time far removed from the appearance of the disease. Similarly, the investigation of whether exposure of young male infants to soy-based infant formula may cause reproductive disorders in adult life, or protective effects (as reported for the prostate) requires long-term studies. Assessment of exposure at the critical period may require prospective studies stretching over many years.

In the context of food and feed, knowledge of exposure ranges can be variable, from comprehensive (e.g. bisphenol A) to poor (e.g. for newly identified contaminants). There is a

need to gather better exposure data on EAS that are naturally present, are created during the manufacturing process or are unavoidable contaminants in food and feed.

A number of publications have advocated a weight of evidence approach to the risk assessment of EAS (see for example IPCS, 2002, which suggests the use of modified Bradford Hill criteria). The integration of *in silico, in vitro* and *in vivo* evidence from humans, laboratory animals and wildlife, and the goal of establishing agreed scientific criteria for concluding from such evidence whether a substance is an EAS with adverse effects in humans and animals, or which can reasonably be predicted to adversely affect humans or animals, will reduce the uncertainties and result in more robust risk assessment. Until that point is reached, risk assessors can only offer a provisional opinion on the likelihood of risk to humans from EAS, particularly at low levels of exposure.

5. Endocrine Active Substances – Communications aspects

5.1. Introduction

EFSA's Communications Directorate has produced a number of communications outputs linked to EFSA's risk assessments on individual substances which are considered to be endocrine-active (notably bisphenol A (BPA), but also PFOA/PFOS and dioxins), rather than communicating on the overall issue or concept of "endocrine active substances". Recent developments on bisphenol A and the associated media coverage, have raised the level of interest in EAS generally. Many of the articles refer to emotive topics affecting vulnerable groups such as BPA in baby bottles and the potential link between EAS and the early onset of menarche. More specifically, scientific methodologies are being brought into question; can the risks that EAS pose be effectively assessed given the uncertainties about which biological effects, noted in animals, may be relevant for human health?

To date, in line with the prevailing trends in terms of legislation and risk assessment, the majority of this risk communications work has focused on individual substances or topics (such as pesticides) rather than EAS as a horizontal grouping cutting across several different areas of EFSA's mandate.

Experience gained to date, as well as insights from the limited academic literature focussing on risk communications and EAS, as well as media analysis conducted by the Communications Directorate, suggests that communicating on this subject poses a number of specific challenges, not least the terminology used and the widespread and growing interest among the media and general public.

Careful consideration therefore, needs to be given to the extent to which EFSA should communicate on EAS as a whole, and the form that any communication activities should take. The results of the 2010 Food Risk Eurobarometer survey, as well as discussions with the EFSA Advisory Forum Working Group on Communications and EFSA's Advisory Group on Risk Communication, will provide important contributions to shaping the future approach.

5.2. Specific challenges when communicating on endocrine active substances

"Hormone mimics", "Hormonally active agents/chemicals/substances", "Endocrine active chemicals", "Endocrine active substances", "Endocrine disrupters", "Endocrine disruptors", "Endocrine modulators". In the absence of consensus on scientific terminology, the mainstream media has opted to translate these emotive but technical terms into headlines, coining terms such as "Killer chemicals" and "Gender benders". As a result, communicating on EAS presents a number of particular challenges; not least terminology and the resulting public perception that all such substances are dangerous. The level of interest in and concern about, the endocrine-related properties of these substances can often be disproportionate to the risks they actually pose.

Furthermore, there is a tendency in the media to portray the question of endocrine activity in black and white terms. Substances which are considered to be "endocrine disrupters" are generally viewed as being artificial and dangerous, not always with due consideration given to the absolute or relative levels of risk that they pose. However, naturally occurring phytoestrogens found in foods (such as those in soy-based products) can sometimes be presented as having beneficial effects, whilst there may also be possible associated risks. It

would be important for EFSA to consider these issues in any future communication activities related to EAS as a whole.

Enhanced communication activities on the way in which EFSA currently carries out its work, explaining the four main principles of scientific risk assessment could help to explain that endocrine activity is only one of a number of different toxicological endpoints, which EFSA regularly takes into consideration in all aspects of its work. This would also help to dispel the impression given by certain media articles that this is somehow a new area of science, which has not previously been considered by risk assessors, who are still using outdated methods.

The way that endocrine active substances are regulated can also have implications for risk communications. For instance the new regulation on plant protection products introduces a mainly hazard-based approach for regulating use of these substances by indicating that an active substance used in pesticides "*..shall only be approved if…it is not considered to have endocrine disrupting properties that may cause adverse effects in humans, unless the exposure of humans to that active substance .. is negligible.*" A perceived lack of distinction between hazard and risk can in turn fuel the debate that risk assessors may not have the correct methodologies to identify the potential negative impact that EAS may have on human health, particularly at low dose levels.

A limited amount of academic research has been carried out on communications-related aspects specifically related to EAS, notably two articles published in the Journal of Toxicology and Environmental Health in 2008 and The Ethical Significance of Language in the Environmental Sciences: Case Studies from Pollution Research, published in Ethics, Place & Environment (2009).

On the issue of terminology, Foster and Agzarian (2008) note that there continues to be controversy – in both the scientific and lay press – surrounding how best to define "endocrine disrupters", resulting in ambiguous use of terminology and confusion in the literature. The authors reasonably argue that effective communication on this issue needs to be based on clear definitions of exactly which substances we mean when referring to "endocrine disrupters" or "endocrine active substances", where the latter is certainly more neutral and science-based. Work on such definitions is on-going within the international scientific community, and any EFSA communications work in this area should be fully in line with the outcome of these discussions.

Tyshenko *et al.* (2008) argue that the public and scientific experts tend to hold different views on the risks posed by EAS as each group defines risk differently and has different values and interpretations of concepts such as risk and uncertainty. In the case of EAS in particular, the authors claim that the divide between expert knowledge and non-expert information is so profound that different language terms are used. This assertion is at least partially borne out by a brief analysis of media coverage, which indicates that negative and scary terms such as "gender benders" are often used in media reports in this area.

With regards to the negatively biased term "disrupter", Tyshenko *et al.* (2008) argue that the different terminology used in scientific and lay circles adds to the public uncertainty and the inability to effectively assess scientific information in order to form judgments on the relative risk posed by EAS. Scheufele and Lewenstein (2005) support this by outlining how news stories are interpreted differently based upon the framing of associated terminology, whilst Elliott (2009) argues for the merits of ethical scrutiny for a balanced language to be used in environmental sciences. Use of the term "endocrine active substances", although scientifically more accurate/comprehensive, should therefore be considered carefully within this broader context, as it is not widely used or understood and could possibly add to the overall confusion and suspicion surrounding this issue.

5.2.1. Media monitoring

Along with genetically modified organisms (GMOs) and health claims, bisphenol A is one of the key subjects in the media coverage related to EFSA over the last few years. There is a steady stream of articles on BPA from many different EU Member States (most notably France, but also Germany, the UK, Denmark, Sweden and Austria) and also internationally. Bisphenol A is the subject of a significant number of media requests received by the EFSA press office, as well as requests for media interviews.

Using EFSA's internal media monitoring tools, keyword (Boolean) searches were conducted for the geographical region of Europe in the areas of Health OR Living/Lifestyle OR Risk News OR Science/Technology in English, French and German languages between 1 January 2008 and 30 August 2010 (this timeframe includes the Irish dioxin in pork crisis). Table 1 shows the volume of media articles generated. EAS as a group, and the individual substances involved, have been more widely covered in the English language media than the Francophone or German language media (although these figures are likely to be significantly biased by the number of sources available in the different languages).

From a risk communications perspective, it is significant to note from the table below, how one endocrine active substances is perceived to have these traits (bisphenol A) whilst despite generating the highest number of articles, during this period, there were no articles in any of the three languages that linked dioxins with endocrine disrupters.

Table 1
Volume of media articles generated following a search with EFSA's media monitoring tools using (a combination of) various keywords related to endocrine active substances for the period January 2008-September 2010

endocrine disrupter	41
endocrine disruptor	119
bisphenol A	694
endocrine disrupter AND bisphenol A	12
endocrine disruptor AND bisphenol A	45
dioxin	929
endocrine disrupter AND dioxin	0
endocrine disruptor AND dioxin	0
perturbateur endocrinien	49
bisphénol A	213
perturbateur endocrinien AND bisphénol A	17
dioxine	289
perturbateur endocrinien AND dioxine	0
Endokrine Disruptoren	4
Bisphenol A	115
Endokrine Disruptoren AND Bisphenol A	1
Dioxin	320
Endokrine Disruptoren AND Dioxin	0

EFSA has commissioned its external media monitoring providers to produce a detailed analysis of how EAS have been covered in the European and international media since 2008. This report will be available in autumn 2010 and will provide a comprehensive overview of the evolution in EAS-related media coverage.

5.2.2. Forthcoming Eurobarometer/AFWGC/AGRC discussions

EFSA is currently working on a Eurobarometer survey, which will provide detailed information on consumer perceptions of a range of different food-related risks in all EU member states. Although in the survey the endocrine disrupters' concept as such was not explored, reference was made to substances found in plastic or other materials in contact with food. This was done in the form of a table where respondents were asked to state whether they are worried or not about a range of specific substances found in foods(eg additives, pesticides, GMOs etc). This, together with planned discussions on this issue with both the EFSA Advisory Forum's Working Group on Communications and EFSA's Advisory Group on Risk Communication, may further help to shape our future communications approach.

5.3. Considerations for future work

EFSA employs a range of different tools (press releases, web, events, newsletters, other publications) and approaches when communicating with its various target audiences. Taking into consideration the points raised above, further reflection will be required on how previous learning can best be employed, as well as the extent to which it is desirable for EFSA to proactively communicate on this issue as a whole (and when).

As with all other aspects of EFSA's communication work, cooperation and coherence with national, European and international partners – including the European Commission (SANCO, ENV and RTD), WHO, OECD, ECHA and Member States – will be key to effectively transmitting appropriate messages on EAS in general and EFSA's work in this area in particular.

A final point for consideration is that communications outputs on EAS will largely be shaped by political, scientific and regulatory developments in this area over the coming months and years. Constant monitoring of media coverage on the subject, as well as regular contact with all concerned parties and stakeholders will be essential to ensure that the most up-to-date information is rapidly incorporated into EFSA's communication strategy.

6 OUTLOOK AND RECOMMENDATIONS

The Task Force is coming to the following conclusions and recommendations:

a) Although agreement exists on the general definition of an EAS, *specific criteria for the determination of endocrine disrupting properties of significance to human health* remain to be adopted for the purpose of implementing certain European Union legislation (e.g. Regulation (EC) No 1107/2009 concerning placing plant protection products on the market).

b) International consensus on testing strategies for determining endocrine disrupting properties of significance to human health is needed for both *hazard identification* as well as for *risk assessment*. The Task Force considers it important to start *building consensus on these testing schemes*.

   The Task Force notes in this regard that the *harmonisation with regard to testing of chemicals* has been an ongoing activity of the OECD for more than 30 years. A core activity on endocrine disrupters was initiated in 1997 and under this umbrella both specific tests and a tiered assessment framework have been developed.

   Recommendations:

   The proposed action for EFSA is to contribute to the work in progress under the auspices of DG Environment regarding the development of criteria for determining endocrine disrupting properties.

   In addition, the Task Force recommends that EFSA continues its participation in the ongoing OECD activities in the area of testing of chemicals and evaluates how the tiered testing approach might be applied within EFSA's work, not only to prioritise which substances in food and feed might require assessment for endocrine activity, but also to evaluate those substances which are prioritised.

c) Regarding risk assessment, the *development of a methodology which is generally accepted* is an additional challenge due to the complexity of the issues involved. Multiple sources and routes of exposure exist for many individual EAS. Besides, there is exposure to several different EAS such as contaminants, residues or natural constituents of foods that could act in combination. From a toxicological point of view, the significance of the various adverse effects, gender and life stage must be assessed and different types of combined actions need also to be considered. In addition, there is a need to have a better understanding of the significance of *low-dose exposure to EAS,* and an evaluation of the health risks and health benefits of certain naturally occurring EAS, such as phytoestrogens.

   Recommendations:

   The Task Force recommends that EFSA continues its activities aimed at developing harmonised methodologies for risk assessment of combined exposures to endocrine active substances in food.

   In order to ensure consistency between the approaches developed for risk assessment through other sources than food (non-dietary exposure), EFSA should continue to build a dialogue for developing a common strategy with the EC, other EU bodies, Member States' Competent Authorities, international organisations

and partners, as well as external experts and stakeholders on the before mentioned issues.

In line with these recommendations, it is proposed that EFSA creates a working group of Panel experts and national experts to advise in prioritising the work on EAS.

d) From a risk communications perspective, it appears that the concept of "endocrine active substances" is not well known and that the public debate has been largely shaped by the negative connotations associated with the term "endocrine disrupters."

e) Grouping these substances together can be potentially misleading as EAS are heterogeneous substances that the risk manager may need to address individually, taking specific measures for specific substances.

f) Further research on public perception is required in this area taking into account also perception regarding "natural" constituents of foods which have endocrine effects and which may be promoted for their health effects.

Recommendation:

Working with the experts in its Advisory Group on Risk Communications in conjunction with the communication experts from Member States, EFSA will examine the outcomes from the 2010 Food Risk Eurobarometer and will continue to monitor and analyse media and stakeholder developments in this area in order to define a strategy for the Authority on EAS communications addressing the collective group and specific substances.

REFERENCES

Adams NR, 1995. Detection of the effects of phytoestrogens on sheep and cattle. Journal of Animal Science 73,1509-1515.

Astwood EB, 1949. The natural occurrence of antithyroid compounds as a cause of simple goiter. Annals of Internal Medicine 30, 1087-11103.

BfR (Bundesinstitut für Risikobewertung), 2010a. Establishment of assessment and decision criteria in human health risk assessment for substances with endocrine disrupting properties under the EU plant protection product regulation. http://www.bfr.bund.de/cm/218/establishment_of_assessment_and_decision_criteria_in_human_health_risk_assessment_for_substances_with_endocrine_disrupting_properties.pdf

BfR (Bundesinstitut für Risikobewertung) 2010b. Draft Concept Paper Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg. (EC) No 1107/2009). http://www.bfr.bund.de/cm/218/development_of_a_stepwise_procedure_for_the_assessment_of_substances_with_endocrine_disrupting_properties.56746.pdf

Care AD, 1954. Goitrogenic properties of linseed. Nature 173, 172-173.

Cassee FR, Groten JP, van Bladeren PJ and Feron VJ, 1998. Toxicological evaluation and risk assessment of chemical mixtures. Critical Reviews in Toxicology 28, 73-101.

Christiansen S, Scholze M, Axelstad M, Boberg J, Kortenkamp A and Hass U, 2008. Combined exposure to anti-androgens causes markedly increased frequencies of hypospadias in the rat. International Journal of Andrology 31, 241-248.

Colborn T and Clement C, 1992. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Princeton, NJ.

Council of the European Union, 2009. Combination effects of chemicals http://register.consilium.europa.eu/pdf/en/09/st17/st17820.en09.pdf

Danish Ministry of the Environment, 2009. Survey and Health Assessment of the exposure of 2 year-olds to chemical substances in Consumer Products. http://www2.mst.dk/udgiv/publications/2009/978-87-92548-81-8/pdf/978-87-92548-82-5.pdf

Darlington DN and Dallman MF, 2001. Feedback Control in Endocrine Systems. In: Becker KL Ed. Principles and Practice of Endocrinology and Metabolism. Third Edition. Lippincott Williams & Wilkins, Philadelphia, pp. 50-56.

Davey JC, Bodwell JE, Gosse JA and Hamilton JW, 2007. Arsenic as an endocrine disruptor: effects of arsenic on estrogen receptor-mediated gene expression in vivo and in cell culture. Toxicological Sciences 98, 75-86.

Davey JC, Nomikos AP, Wungjiranirun M, Sherman JR, Ingram L, Batki C, Lariviere JP and Hamilton JW, 2008. Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor-and thyroid hormone receptor-mediated gene regulation and thyroid hormone-mediated amphibian tail metamorphosis. Environmental Health Perspectives 116, 165-172.

Dixon RA, 2004. Phytoestrogens. Annual Review of Plant Biology 55, 225-261.

EC (European Commission), 1997. Reports of the Scientific Committee for food on Endocrine disruptors and food. http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_40.pdf

EC (European Commission), 1999. Communication from the Commission to the Council and the European Parliament. Community Strategy for Endocrine Disrupters. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:1999:0706:FIN:EN:PDF

EC (European Commission), 2001a. Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation. http://ec.europa.eu/food/fs/sc/scf/out82_en.pdf

EC (European Commission), 2001b. Guidance on submissions for food additive evaluations by the Scientific Committee on Food. Opinion expressed on 11 July 2001. Reference SCF/CS/ADD/GEN/26 Final. 12 July 2001. http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

EC (European Commission), 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Union, L353/1-1355, 31.12.2008.

EC (European Commission), 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union, L309/1-50, 24.11.2009.

EC (European Commission), 2010. Workshop on the State of the Art Report on Mixture Toxicity, Summary overview of the different sessions and the chair's summary of the main messages. http://ec.europa.eu/environment/chemicals/pdf/Summary_Combination%20Effects.pdf

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) 2009a. Workshop: Guidance on Interpreting Endocrine Disrupting Effects 29-30 June 2009, Barcelona. Workshop Report No. 16 and addendum. Brussels, 2009.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2009b. Guidance on Identifying Endocrine Disrupting Effects. Technical Report No. 106. Brussels, 2009.

EFSA (European Food Safety Authority)), 2004a. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] to assess the health risks to consumers associated with exposure to organotins in foodstuffs. (Question No. EFSA-Q-2003-110). Adopted on 22 September 2001. EFSA Journal (2004) 102, 1-119. http://www.efsa.europa.eu/en/scdocs/scdoc/102.htm

EFSA (European Food Safety Authority), 2004b. Dioxins: Methodologies and principles for setting tolerable intake levels for dioxins, furans and dioxin-like PCBs. EFSA Scientific Colloquium Summary Report. European Food Safety Authority, Parma, December 2004.

EFSA (European Food Safety Authority), 2005a. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to the presence of non dioxin-like polychlorinated biphenyls (PCB) in feed and food. (Question No. EFSA-Q-2003-114). Adopted on 8 November 2005. EFSA Journal (2005) 284, 1-137.http://www.efsa.europa.eu/en/scdocs/scdoc/284.htm

EFSA (European Food Safety Authority), 2005b. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to Hexachlorobenzene as undesirable substance in animal feed. (Question No. EFSA-Q-2005-185) Adopted on 13 September 2006. EFSA Journal (2006) 402, 1-49. http://www.efsa.europa.eu/en/scdocs/scdoc/402.htm

EFSA (European Food Safety Authority), 2006a. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A). Question number EFSA-Q-2005-100. Adopted on 29 November 2006. EFSA Journal (2006) 428, 1-75. http://www.efsa.europa.eu/en/scdocs/doc/428.pdf

EFSA (European Food Safety Authority), 2006b. Advice of the Scientific Panel CONTAM related to relevant chemical compounds in the group of brominated flame retardants for

monitoring in feed and food. (Question No. EFSA-Q-2005-244). Adopted on 24 February 2006. EFSA Journal (2006) 328, 1-4. http://www.efsa.europa.eu/en/scdocs/scdoc/328.htm

EFSA (European Food Safety Authority) 2006c. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to DDT as an undesirable substance in animal feed. (Question No. EFSA-Q-2005-182). Adopted on 22 November 2006. EFSA Journal (2006) 433, 1-69. http://www.efsa.europa.eu/en/scdocs/scdoc/433.htm

EFSA (European Food Safety Authority), 2007a. Opinion of the Scientific Panel on contaminants in the food chain (CONTAM) related to hormone residues in bovine meat and meat products. (Question No. EFSA-Q-2005-048). Adopted on 12 June 2007. EFSA Journal (2007) 510, 1-62. http://www.efsa.europa.eu/en/scdocs/scdoc/510.htm

EFSA (European Food Safety Authority), 2007b. Chlordane as undesirable substance in animal feed[1] - Scientific Opinion of the Panel on Contaminants in the Food Chain. (Question No. EFSA-Q-2005-181). Adopted on 7 November 2007. EFSA Journal (2007) 582, 1-53. http://www.efsa.europa.eu/en/scdocs/scdoc/582.htm

EFSA (European Food Safety Authority), 2007c. Glucosinolates as undesirable substances in animal feed - Scientific Opinion of the Panel on Contaminants in the Food Chain. (Question No. EFSA-Q-2003-061) Adopted on 27 November 2007. EFSA Journal (2008) 590, 1-76. http://www.efsa.europa.eu/en/scdocs/scdoc/590.htm

EFSA (European Food Safety Authority) 2007d. Cumulative Risk Assessment of Pesticides to Human Health: The Way Forward. EFSA Scientific Colloquium Summary Report. European Food Safety Authority, Parma, October 2007.

EFSA (European Food Safety Authority) 2008a. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain. (Question number: EFSA-Q-2004-163). Adopted on 21 February 2008. EFSA Journal (2008) 653, 1-131. http://www.efsa.europa.eu/en/scdocs/scdoc/653.htm

EFSA (European Food Safety Authority), 2008b. Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. (Question No. EFSA-Q-2006-160). Adopted on 15 April 2008. EFSA Journal (2008) 704, 1-84. http://www.efsa.europa.eu/en/scdocs/scdoc/705.htm

EFSA (European Food Safety Authority) 2008c. Toxicokinetics of Bisphenol A. Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC). (Question No EFSA-Q-2008-382). Adopted on 9 July 2008. EFSA Journal (2008) 759, 1-10. http://www.efsa.europa.eu/en/scdocs/doc/759.pdf

EFSA (European Food Safety Authority), 2008d. Statement of EFSA on a study associating bisphenol A with medical disorders. Prepared by the Unit on food contact materials, enzymes, flavourings and processing aids (CEF) and the Unit on Assessment Methodology (AMU). (Question No EFSA-Q-2008-702). Issued on 22 October 2008. EFSA Journal (2008) 838, 1-23.

EFSA (European Food Safety Authority), 2009. Scientific Opinion on Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health. (Question No. EFSA-Q-2007-183). Adopted on 19 June 2000. EFSA Journal 2009; 7 (9); 1167. [187 pp.]. Available at: http://www.efsa.europa.eu/en/scdocs/scdoc/1167.htm

Ekpechi OL, Dimitriadou A and Fraser R, 1966. Goitrogenic activity of cassava (a staple Nigerian food). Nature 210, 1137-1138.

Elliott KC, 2009. The Ethical Significance of Language in the Environmental Sciences: Case Studies from Pollution Research. Ethics, Place & Environment 12, 157-173.

Fletcher RJ, 2003. Food sources of phyto-oestrogens and their precursors in Europe. British Journal of Nutrition 89, Suppl 1, S39-S43.

Flippin JL, Hedge JM, DeVito MJ, Leblanc GA and Crofton KM, 2009. Predictive modeling of a mixture of thyroid hormone disrupting chemicals that affect production and clearance of thyroxine. International Journal of Toxicology 28, 368-81.

Foster, WG and J Agzarian, 2008. Toward less confusing terminology in endocrine disruptor research. Journal of Toxicology and Environmental Health Part B: Critical Reviews 11, 152-161

Fox JE, 2004. Chemical communication threatened by endocrine-disrupting chemicals. Environmental Health Perspectives 112, 648-653.

Gaitan E, 1990. Goitrogens in Food and Water. Annual Review of Nutrition 10, 21-37.

Granner DK, 2001. Hormonal Action. IN: Becker KL Ed. Principles and Practice of Endocrinology and Metabolism. Third Edition. Lippincott Williams & Wilkins, Philadelphia, pp 36-49.

Gray LE Jr, Ryan B, Hotchkiss AK and Crofton KM, 2010. Rebuttal of "Flawed experimental design reveals the need for guidelines requiring appropriate positive controls in endocrine disruption research" by vom Saal. Toxicological Sciences 115, 614-620.

Gross *et al.,* 2009. Thresholds of toxicological concern for endocrine active substances in the aquatic environment. Integrated Environmental Assessment and Management 6, 2-11

Haseman JK, Bailer AJ, Kodell RL, Morris R and Portier K, 2001. Statistical issues in the analysis of low-dose endocrine disruptor data. Toxicological Sciences 61, 201-210.

Hotchkiss AD, Rider CV, Blystone CR, Wilson, VS, Hartig PC, Ankley GT, Foster PM, Gray CL and Gray LE, 2008. Fifteen years after "Wingspread" – environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicological Sciences 105, 235-259.

IPCS (International Programme on Chemical Safety), 2002. Global Assessment of the State-of-the Science of Endocrine Disruptors. Eds Damstra T, Barlow S, Bergman A, Kavlock R, Van der Kraak G. International Program on Chemical Safety, World Health Organization, WHO/PCS/EDC/02.2.

IPCS (International Programme on Chemical Safety/ World Health Organisation), 2009. Draft WHO/IPCS Framework for Risk Assessment of Combined Exposures to Multiple Chemicals. http://www.who.int/ipcs/methods/harmonization/areas/combined_exposure/en/index.html

Jacobs MN, 2005. Nuclear receptors and dietary ligands. In: Zemplini J and Dakshinamurti K (Eds.), 'Nutrients and Cell Signaling': 'Oxidative Stress and Disease' series. Marcel Dekker Inc., New York.

Jacobs MN, Janssens W, Bernauer U, Brandon E, Coecke S, Combes R, Edwards P, Freidig A, Freyberger A, Kolanczyk R, Mc Ardle C, Mekenyan O, Schmieder P, Schrader T, Takeyoshi M and van der Burg B, 2008. The use of metabolising systems for in vitro testing of endocrine disruptors, Current Drug Metabolism 9, 796-826.

Jacobs MN and Lewis DFV, 2002. Steroid hormone receptors and dietary ligands – A selected review. Proceedings of the Nutrition Society 61, 105-122.

Jacobs MN, Nolan GT and Hood SR, 2005. Lignans, bacteriocides and organochlorine compounds activate the human pregnane X receptor (PXR). Toxicology and Applied Pharmacology 209, 123-133.

Kitchin KT and Drane JW, 2005. A critique of the use of hormesis in risk assessment. Human and Experimental Toxicology 24, 249-253.

Kortenkamp A, 2007. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. Environmental Health Perspectives 115 Suppl 1, 98-105.

Kortenkamp A, 2008. Low dose mixture effects of endocrine disrupters: implications for risk assessment and epidemiology. International Journal of Andrology 31, 233-240.

Kortenkamp A and Faust M, 2010. Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. International Journal of Andrology 33, 463-474.

Lambert JC and Lipscomb JC, 2007. Mode of action as a determining factor in additivity models for chemical mixture risk assessment. Regulatory Toxicology and Pharmacology 49, 183-194.

Lee KY, Shibutani M, Takagi H, Kato N, Takigami S, Uneyama C and Hirose M, 2004. Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology 203, 221-238.

Leranth C, Hajszan T, Szigeti-Buck K, Bober J and MacLusky NJ, 2008. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. Proceedings of the National Academy of Sciences USA 105, 14187-14191.

Levin ER, 2009a. Membrane oestrogen receptor alpha signalling to cell functions. Journal of Physiology 587(Pt 21), 5019-5023.

Levin ER, 2009b. Plasma membrane estrogen receptors. Trends in Endocrinology and Metabolism 20, 477-482.

Livingston AL, 1978. Forage plant estrogens. Journal of Toxicology and Environmental Health 4, 301-324.

Maggiolini M and Picard D, 2010. The unfolding stories of GPR30, a new membrane-bound estrogen receptor. Journal of Endocrinology 204, 105-114.

Mantovani A, Frazzoli C and La Rocca C, 2009. Risk assessment of endocrine-active compounds in feeds. Veterinary Journal 182, 392-3401.

Marine D, Baumann EJ, Webster B and Cipra A, 1933. The occurrence of antigoitrous substances in plants**.** Journal of Experimental Medicine 57, 121-137.

Mayo DG and Spanos A, 2008. Risks to health and risks to science: the need for a responsible "bioevidential" scrutiny. Human and Experimental Toxicology 27, 621-625.

McCarrison R, 1933. The goitrogenic action of soybeans and groundnut. Indian Journal of Medical Research 21, 179.

McCarty LS and Borgert CJ, 2006. Review of the toxicity of chemical mixtures: Theory, policy, and regulatory practice. Regulatory Toxicology and Pharmacology 45, 119-143.

McKinlay RJA, Plant, JNB Bell and N Voulvoulisl, 2008. Endocrine disrupting pesticides : Implications for risk assessment. Environment International 34, 168-183.

Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T, Moore J, Leakey J, Haseman J and Kohn M, 2002. Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. Environmental Health Perspectives 110, 427-431.

Mushak P, 2007. Hormesis and its place in nonmonotonic dose–response relationships: some scientific reality checks. Environmental Health Perspectives 115, 500-506.

NRC (National Research Council), 2008. Phthalates and Cumulative Risk Assessment: The Task Ahead. Committee on the Health Risks of Phthalates, National Research Council. The National Academies Press, Washington DC.

NTP-CERHR (National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction), 2008. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. National Toxicological Program-Center for the Evaluation of Risks to Human Reproduction, September 2008. NIH Publication No. 08-5994. Available from http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.pdf

OECD (Organisation for Economic Co-operation and Development), 2001. OECD Guideline for the Testing of Chemicals, 416. Two-Generation reproduction Toxicity Study. Adopted 22 January 2001. Available at: http://lysander.sourceoecd.org/vl=1277159/cl=11/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s16/p1.idx

OECD (Organisation for Economic Co-operation and Development), 2006. Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats. Series on Testing and Assessment No 59, ENV/JM/MONO(2006)26.).

OECD (Organisation for Economic Co-operation and Development), 2007a. OECD Series on Testing and Assessment No. 69. Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models. Available at: http://www.oecd.org/document/30/0,3343,en_2649_34377_1916638_1_1_1_1,00.html

OECD (Organisation for Economic Co-operation and Development), 2007b. OECD Guideline for the Testing of Chemicals, 440. Uterotrophic Bioassay in Rodents: A Short-Term Screening Test for Oestrogenic Properties. Adopted 16 October 2007. Available at: http://lysander.sourceoecd.org/vl=873813/cl=17/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s34/p1.idx

OECD (Organisation for Economic Co-operation and Development), 2009a. Report from the OECD EDTA Workshop 2009 ENV/JM/MONO(2010)2 Series on Testing and assessment no. 118.

OECD (Organisation for Economic Co-operation and Development), 2009b. OECD Guideline for the Testing of Chemicals, 441. Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties. Adopted 7 September 2009. Available at: http://puck.sourceoecd.org/vl=10988707/cl=12/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s56/p1.idx

Peeters PHM, Slimani N, van der Schouw YT, Grace PB, Navarro C *et al*., 2007. Variations in Plasma Phytoestrogen Concentrations in European Adults. Journal of Nutrition 137, 1294-1300.

Phillips KP and Foster WG, 2008. Endocrine Toxicants with Emphasis on Human Health Risks. Journal of Toxicology and Environmental Health, Part B 11, 149-151.

Phillips KP, Foster WG, Leiss W, Sahni V, Karyakina N, Turner MC, Kacew S and Krewski D, 2008. Assessing and Managing Risks Arising from Exposure to Endocrine-Active Chemicals. Journal of Toxicology and Environmental Health, Part B, 11, 351-372.

Potter JD and Steinmetz K, 1996. Vegetables, fruit and phytoestrogens as preventive agents. IARC Scientific Publications 139, 61-90.

Scheufele D and Lewenstein B, 2005. The public and nanotechnology: how citizens make sense of emerging technologies. Journal of Nanoparticle Research, 7, 659-667.

Scholze M and Kortenkamp A, 2007. Statistical power considerations show the endocrine disruptor low-dose issue in a new light. Environ Health Perspectives 115 Suppl 1, 84-90.

Scott EN, Gescher AJ, Steward WP and Brown K, 2009. Development of dietary phytochemical chemopreventive agents: biomarkers and choice of dose for early clinical trials. Cancer Prevention Research (Phila Pa). 2, 525-530.

Swedenborg E, Rüegg J, Mäkelä S and Pongratz I, 2009. Endocrine disruptive chemical: mechanisms of action and involvement in metabolic disorders. Journal of Molecular Endocrinology 43, 1-10.

Tacklind J, MacDonald R, Rutks I and Wilt TJ, 2009. *Serenoa repens* for benign prostatic hyperplasia. Cochrane Database Systematic Reviews 15 (2):CD001423. The Cochrane Library 2010, Issue 4.

Thornton JW, Need E and Crews D, 2003. Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling. Science 301, 1714-1717.

Teuschler LK, 2007. Deciding which chemical mixtures risk assessment methods work best for what mixtures. Toxicology and Applied Pharmacology 223, 139-47.

Tyshenko MG, Philips KP, Mehta M, Poirier R and Leiss W, 2008. Risk communication of endocrine-disrupting chemicals: Improving knowledge translation and transfer. Journal of Toxicology and Environmental Health Part B: Critical Reviews. 11, 345-350.

ULSOP (The School of Pharmacy, University of London), 2009. State of the Art Report on Mixture Toxicity – Final Report, Executive Summary. http://ec.europa.eu/environment/chemicals/pdf/report_Mixture%20toxicity.pdf

Verwer CM, van der Ven LT, van den Bos R and Hendriksen CF, 2007. Effects of housing condition on experimental outcome in a reproduction toxicity study. Regulatory Toxicology and Pharmacology 48, 184-193.

Vom Saal FS and Myers JP, 2010. Good Laboratory Practices are not synonymous with good scientific practices, accurate reporting, or valid data. Environmental Health Perspectives 118, A60.

Vom Saal FS and Hughes C, 2005a. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environmental Health Perspectives 113, 926-933.

Vom Saal FS, Richter CA, Ruhlen RR, Nagel SC, Timms BG and Welshons WV, 2005b. The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A. Birth Defects Res A Clinical and Molecular Teratology 73, 140-145.

Vom Saal FS, Akingbemi BT, Belcher SM, Crain DA, Crews D, Guidice LC, Hunt PA, Leranth C, Myers JP, Nadal A, Olea N, Padmanabhan V, Rosenfeld CS, Schneyer A, Schoenfelder G, Sonnenschein C, Soto AM, Stahlhut RW, Swan SH, Vandenberg LN, Wang HS, Watson CS, Welshons WV and Zoeller RT, 2010. Flawed experimental design reveals the need for guidelines requiring appropriate positive controls in endocrine disruption research. Toxicological Sciences 115, 612-613.

Welshons WV, Nagel SC, Thayer KA, Judy BM and Vom Saal FS, 1999. Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicology and Industrial Health 15, 12-25

Zinedine A, Soriano JM, Moltó JC and Mañes J, 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food and Chemical Toxicology 45, 1-18.

ANNEX 1

Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

Level 1 Sorting & prioritization based upon existing information	- physical & chemical properties, e.g., MW, reactivity, volatility, biodegradability, - human & environmental exposure, e.g., production volume, release, use patterns - hazard, e.g., available toxicological data	
Level 2 *In vitro* assays providing mechanistic data	- ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis *in vitro* - Aryl hydrocarbon receptor recognition/binding - QSARs	-High Through Put Prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
Level 3 *In vivo* assays providing data about single endocrine Mechanisms and effects	- Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non-receptor mediated hormone function - Others (e.g thyroid)	- Fish VTG (vitellogenin) assay (estrogenic related)
Level 4 *In vivo* assays providing data about multiple endocrine Mechanisms and effects	- enhanced OECD 407 (endpoints based on endocrine mechanisms) - male and female pubertal assays - adult intact male assay	- Fish gonadal histopathology assay - Frog metamorphosis assay
Level 5 *In vivo* assays providing data on effects from endocrine & other mechanisms	- 1-generation assay (TG41 5 enhanced) [1] - 2-generation assay (TG41 6 enhanced) [1] - reproductive screening test (TG421 enhanced)[1] - combined 28 day/reproduction screening test (TG 422 enhanced)[1] 1 Potential enhancements will be considered by VMG mamm	- Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)

Notes to the Framework

Note 1: Entering at all levels and exiting at all levels is possible and depends upon the nature of existing information needs for hazard and risk assessment purposes

Note 2: In level 5, ecotoxicology should include endpoints that indicate mechanisms of adverse effects, and potential population damage

Note 3: When a multimodal model covers several of the single endpoint assays, that model would replace the use of those single endpoint assays

Note 4: The assessment of each chemical should be based on a case by case basis, taking into account all available information, bearing in mind the function of the framework levels.

Note 5: The framework should not be considered as all inclusive at the present time. At levels 3,4 and 5 it includes assays that are either available or for which validation is under way. With respect to the latter, these are provisionally included. Once developed and validated, they will be formally added to the framework.

Note 6: Level 5 should not be considered as including definitive tests only. Tests included at that level are considered to contribute to general hazard and risk assessment.

ANNEX 2

Test Guidelines and other documents related to endocrine disrupter testing and assessment (adopted or under development) [26]

Adopted Test Guidelines
Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals (TG 455), 2009
Uterotrophic Bioassay in Rodents: a Short-term Screening Test for Oestrogenic Properties (TG 440), 2007
Hershberger Bioassay in Rats: a Short-Term Screening Assay for (Anti)Androgenic Properties (TG 441), 2009
Fish Short Term Reproduction Assay (TG 229), 2009
21-Days Fish Assay: A Short-term Screening for Oestrogenic and Androgenic Activity, and Aromatase inhibition (TG 230), 2009
28-days repeated dose toxicity study in Rodents (TG 407), last update in 2008
One-Generation Reproduction Toxicity Study (TG 415), 1983
Two-Generation Reproduction Toxicity (TG 416), last update in 2001
Amphibian Metamorphosis Assay (TG 231), 2009
Daphnia magna Reproduction Test (TG 211), last update in 2008
Reproduction/Developmental Toxicity Screening Test (TG 421), 1995
Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (TG 422)1996

Guidance Documents published in the OECD Series on Testing and Assessment
No 106: Guidance Document for Histologic Evaluation of Endocrine and Reproductive Tests in Rodents
No 82: Guidance Document on Amphibian Thyroid Histology, 2007
No 71: Guidance Document on the Uterotrophic Bioassay - Procedure to Test for Antioestrogenicity, 2007

[26] Report from the OECD EDTA Workshop 2009 ENV/JM/MONO(2010)3 Series on Testing and assessment no. 118 Part 2

Detailed Review Papers, Background Review Documents, Validation Reports, and Peer Review Reports published in the OECD Series on Testing and Assessment
No 111: Report of the Expert Consultation to Evaluate an Estrogen Receptor Binding Affinity Model for Hazard Identification
No 110: Report of the Validation Peer Review for the Hershberger Bioassay (Weanling Model)
No 109: Literature review on the 21-Day Fish Screening Assay and Fish Short-term Reproduction Assay
No 108: Report of the Validation of the Hershberger Bioassay (Weanling Model)
No 97: Detailed Review Paper on the Use of Metabolising Systems for In Vitro Testing of Endocrine Disruptors, 2008
No 95: Detailed Review Paper on Fish Life-Cycle Tests, 2008
No 94: Report of the Validation Peer Review for the 21-Day Fish Endocrine Screening Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No 93: Report of the Validation of an Enhancement of OECD TG 211: Daphnia Magna Reproduction Test
No 92: Report of the Validation Peer Review for the Amphibian Metamorphosis Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No 91: Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3)
No 90: Background Review Document on the Rodent Hershberger Bioassay
No 86: Report of the OECD Validation of the Rodent Hershberger Bioassay: Phase 2: Testing of Androgen Agonists, Androgen Antagonists and a 5 α-Reductase Inhibitor in Dose Response Studies by Multiple Laboratories
No 85: Report of the Validation Peer Review for the Hershberger Bioassay, and the Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No 83: Summary Report of the Peer Review Panel on the Stably Transfected Transcriptional Activation Assay for Detecting Estrogenic Activity of Chemicals, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-Up of this Report
No.81: Summary Report of the Validation Peer Review for the Updated Test Guideline 407, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No. 78: Final report of the Validation of the 21-day Fish Screening Assay for the Detection of Endocrine Active Substances. Phase 2: Testing Negative Substances
No. 77: Final Report of the Validation of the Amphibian Metamorphosis Assay: Phase 2 - Multi-chemical Interlaboratory Study
No. 76: Final Report of the Validation of the Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances: Phase 1 - Optimisation of the Test Protocol
No. 73: Report of the Validation of the Rat Hershberger Assay: Phase 3: Coded Testing of Androgen Agonists, Androgen Antagonists and Negative Reference Chemicals by Multiple Laboratories. Surgical Castrate Model Protocol

No. 68: Summary Report of the Uterotrophic Bioassay Peer Review Panel, including Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No. 67: Report of the Uterotrophic Bioassay: Additional Data Supporting the Test Guideline on the Uterotrophic Bioassay in Rodents
No. 66: OECD Report of the Validation of the Rodent Uterotrophic Bioassay: Phase 2 - Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories
No. 65: OECD Report of the Initial Work Towards the Validation of the Rodent Uterotrophic Assay - Phase 1
No. 62: Final OECD Report of the Initial Work Towards the Validation of the Rat Hershberger Assay: Phase-1, Androgenic Response to Testosterone Propionate, and Anti-Androgenic Effects of Flutamide
No. 61: Report of the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1B)
No. 60: Report of the Initial Work Towards the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine active Substances (Phase 1A)
No.59: Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats
No. 57: Detailed Review Paper on Thyroid Hormone Disruption Assays
No. 55: Detailed Review Paper on Aquatic Arthropods in Life Cycle Toxicity Tests with an Emphasis on Developmental, Reproductive and Endocrine Disruptive Effects
No. 47: Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances
No. 46: Detailed Review Paper on Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances
No. 38: Detailed Background Review of the Uterotrophic Bioassay
No. 21: Detailed Review Paper: Appraisal of Test Methods for Sex Hormone Disrupting Chemicals

Projects included in the work plan of the Test Guidelines Programme
Test Guideline on Copepod Reproduction and Development
Test Guideline for Mysid Life Cycle Toxicity Test
Test Guideline for Fish Sexual Development Test
21-Day Female Stickleback Endocrine Screening Assay
Test Guideline for a Chironomid Life-Cycle Toxicity Test
Test Guideline for the Medaka Life-Cycle (MLC)/ Multi generation Test (MMT)
Test Guideline on Amphibian Growth, Development and Reproductive Assay
Test Guideline for Stably Transfected Transcriptional Activation (STTA) Assay for the Detection of Estrogen Receptors Agonists and Antagonists (LUMI-CELL® ER Assay)
Test Guideline for Human Recombinant Estrogen Receptor Alpha Binding Assays (hrERA, 2 protocols)
Test Guideline for H295R Cell-Based Steroidogenesis Assay

Test Guideline for Stably Transfected Transcriptional Activation (STTA) Assay for the detection of androgenic and anti-androgenic activity of chemicals
Stably Transfected Transcriptional Activation (STTA) Assay for the detection of anti-estrogenic activity of chemicals
Guidance Document on the Weanling Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties
Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads

ANNEX 3

The ED/EAS test methods currently in (Pre)Validation*, under the auspices of the OECD[27]

Receptor Binding Assays				
hERα	The Freyburger Wilson Assay (FWA) assay protocol utilises the Pan Vera (now Invitrogen) hERα full length ER, and the CERI protocol utilises the CERI-ERα, which contains the ligand binding domain of hrERα	binding	Validation started in June 2007 in 5 laboratories	US lead (US EPA) international collaboration study (EC ECVAM; CERI, Japan)
hAR	Human recombinant AR assay. Ligand binding domain expressed in E. coli.	binding	Under development. Approximately 900 chemicals have been tested	METI, Japan
rAR	Rat recombinant AR assay. (ligand binding domain is identical to human LBD)	binding	Some limited prevalidation work has been conducted in the EU funded project ReProTect. Options are currently being explored	Lead and international collaboration work under discussion
hTR	Human recombinant TR assay. Full-length expressed in *E. coli.* TRs α1 and β1 binding assays	binding	Under development. Approximately 60 chemicals have been tested using both receptors	METI, Japan
Transactivation Assays				
hERα	HeLa-9903 cells with plasmids containing hERα cDNA driven by SV40 promotor and luciferase reporter plasmid	Stable ag/antag	The agonist assay is validated (TG455). International validation of the antagonist assay started in 2008	CERI/MHLW, Japan Japan lead (JaCVAM), international collaboration study (EC ECVAM, CERI, Japan, South Korea)
	HeLa-9903 cells: hERα/pcDNA3.1 receptor expressing plasmid and ERE-AUG-Luc+ reporter plasmid	Transient, ag	Prevalidated and validated in Japan using same test chemicals as hERa-HeLa cell line. Should be considered for	CERI/MHLW, Japan

[27] Updated from Jacobs *et al*, 2008 "The Use of Metabolising Systems for *In Vitro* Testing of Endocrine Disruptors", Current Drug Metabolism, 2008, 9, 796-826

			(preliminary) peer review	
	MELN. MCF-7 cells with endogenous ERα + luciferase stably transfected	ag/antag	Prevalidation in 2007. Report awaited	EC/ECVAM
	ER-CALUX. T47 D (human breast cancer) cells with endogenous ERα + luciferase stably transfected	ag/antag	Going through optimisation (probably no further development)	EC/ECVAM
	LUMI cell, BG1 cells with endogenous ERα + luciferase stably transfected (XDS Inc)	ag/antag	Validation initiated in late 2007, not yet completed	US lead (ICCVAM) international collaboration study with ECVAM and JaCVAM
ERβ	HeLa, hERß/pcDNA3.1, ERE-AUG-Luc+	Transient ag	Completed data collection for 250 compounds	CERI/MHLW, Japan
AR	CV-1 cells hAR/pcDNA3.1 receptor expressing plasmid and ARE-AUG-Luc + reporter plasmid	Transient ag/antag	Prevalidated and validated in Japan in 4 laboratories, with 5 chemicals. Should be considered for (preliminary) peer review	CERI/MHLW, Japan
	AR-Ecoscreen™ stable CHO clone	Stable ag/antag	Prevalidated and validated in Japan in 4 laboratories, with 5 chemicals. Peer reviewed early 2007	CERI/MHLW, Japan
	PALM. PC-3 (prostate adenocarcinoma) cells stably transfected with hAR and luciferase reporter gene	ag/antag	Prevalidation completed in 2008, report not yet available	EC/ECVAM
	CALUX. U2-OS (bone cell) cells stably transfected with hAR and luciferase reporter construct	ag/antag	Prevalidation report not yet available	EC/ECVAM
TRβ	RXR co-transfected CHO cells are used	Transient ag/antag	Under development, 150 chemicals tested so far	MHLW, Japan

Aromatase & Steroidogenesis Assays				
	Microsomal aromatase assay, KGN cells		Prevalidated. Peer review report not yet available	
	Steroidogenesis, H295R cell based assay		Validation and peer review by December 2008. SPSF submitted. Draft TG ongoing	US lead international collaboration study

* Prevalidation: The initial phase(s) of a validation study. A small-scale study intended to obtain preliminary information on the relevance and reliability of a test method. Based on the outcome of those studies, the test method protocol may be modified or optimised to increase intra- and/or inter-laboratory reproducibility and accuracy in subsequent validation studies[28].

[28] OECD (2005). OECD Series on Testing and Assessment No. 34. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Available at: [http://www.oecd.org/document/30/0,3343,en_2649_34377_1916638_1_1_1_1,00.html]

ANNEX 4

US EPA EDSP screening and testing battery[29]

US EPA EDSP Tier 1 creening Battery:

- In vitro
    - Estrogen receptor (ER) binding – rat uterine cytosol [US], and human ER [US lead, EC and Japan]
    - Estrogen receptor α (hERα) transcriptional activation – Human cell line (HeLa-9903) [OECD Test Guideline 455, Japan]
    - Androgen receptor (AR) binding – rat prostate cytosol
    - Steroidogenesis – Human cell line (H295R) [US lead, draft test Guideline should be finalised by early 2011 in OECD program]
    - Aromatase – Human recombinant microsomes
- In vivo
    - Uterotrophic (rat) [OECD TG 440]
    - Hershberger (rat) [OECD TG441TG 441]
    - Pubertal female (rat) (no OECD TG)
    - Pubertal male (rat) (no OECD TG)
    - Amphibian metamorphosis (frog) [OECD TG 231]
    - Fish short-term reproduction [OECD TG 229]

US EPA EDSP Tier 2

- Mammalian two-generation rat (may be replaced by extended F1-generation)
- Avian two-generation (Japanese quail) [US lead, OECD validation program]
- Amphibian growth/reproduction (*S. tropicalis*) [US/Japan lead, OECD validation program]
- Fish multigeneration (medaka) [US/Japan lead, OECD validation program]
- Mysid multigeneration [US lead, OECD validation program]

[29] Source: Jacobs *et al*, 2008 "The Use of Metabolising Systems for *In Vitro* Testing of Endocrine Disruptors", Current Drug Metabolism, 2008, 9, 796-826

ANNEX 5

Comparison of the US EPA EDSP approach with the OECD CF for the Testing and Assessment of ED Chemicals (source: report from the OECD EDTA Workshop 2009 ENV/JM/MONO(2010)3 Series on Testing and assessment no. 118 Part 2)

OECD Conceptual Framework			**Approach in the U.S. EPA EDSP**			
Level	**Assays for mammalian toxicity**	**Assays for ecotoxicity**	**Stage**	**Description**	**Assays for mammalian toxicity**	**Assays for ecotoxicity**
Level 1: Sorting and prioritization based upon existing information	- Physical & chemical properties, e.g. MW, reactivity, volatility, biodegradability - Human & environmental exposure, e.g. production volume, release, use patterns - Hazard, e.g. available toxicological data		**Not described**			
Level 2: *In vitro* assays providing mechanistic data	- ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis *in vitro* - Aryl hydrocarbon receptor recognition/binding - QSAR - High-throughput pre-screens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)		**Tier 1** **Screening Assays (mode of action)**	*In vitro* assays providing mechanistic information / data	- Estrogen receptor (ER) binding (rat uterus or recombinant) - Estrogen receptor α (hERα) transcriptional activation (human cell line (HeLa-9903)) - Androgen receptor (AR) binding (rat prostate) - Steroidogenesis (human cell line (H295R)) - Aromatase (human recombinant)	
Level 3: *In vivo* assays providing data about single mechanisms and effects	– Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non-receptor binding mediated hormone function - Others (e.g. thyroid)	- Fish vitellogenin (VTG) assay (estrogenic related)		*In vivo* assays providing mechanistic information / data on single or multiple endocrine mechanisms	- Uterotrophic (rat) - Hershberger (rat) - Pubertal female (rat) (no OECD TGs for this assay) - Pubertal male (rat) (no OECD TGs for this assay) - Amphibian metamorphosis (frog)	- Fish short-term reproduction - Amphibian metamorphosis (frog)
Level 4: *In vivo* assays providing data about multiple mechanisms and effects	- Enhanced OECD TG 407 (endpoint based endocrine effects) - Male and female pubertal assays - Adult intact male assay	- Fish gonadal histopathology assay - Frog metamorphosis assay				
Level 5: *In vivo* assays providing data on effects on endocrine & other mechanisms	- One-generation assay (TG 415 enhanced) - Two-generation assay (TG 416 enhanced) - Reproductive screening test (TG 421 enhanced) - Combined 28-day/reproduction screening test (TG 422 enhanced)	Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)	**Tier 2** **Definitive (dose-response) testing**		- Two generation mammalian assay (TG 416 enhanced) or - Extended one-generation assay (*currently being drafted*)	- Avian two-generation reproductive effects* - Fish multigeneration reproductive effects* - Amphibian growth and reproduction test* - Invertebrate multigen (daphnia or copepod)* ** currently being validated*

GLOSSARY AND ABBREVIATIONS

AhR:	Aryl hydrocarbon Receptor
AMU Unit:	EFSA's Assessment Methodology Unit
ANS Panel:	EFSA's Scientific Panel on Food Additives and Nutrient Sources added to Food
ANSES:	French Agency for Food, Environmental and Occupational Health Safety
AR:	Androgen receptor
BIAC:	Business and Industry Advisory Committee to OECD
BfR:	German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
BPA:	Bisphenol A
CEF Panel:	EFSA's Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEPA	Canadian Environmental Protection Act
CERI:	Chemicals Evaluation Research Institute, Japan
CERHR:	Center for the Evaluation of Risks to Human Reproduction
CF:	The OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals
CONTAM Panel:	EFSA´s Scientific Panel for Contaminants in the Food Chain
CRD:	The UK Chemicals Regulation Directorate
CYP:	Cytochrome P450
DATEX:	Data Collection and Exposure
DG ENV	EC Directorate-General for the Environment
DNA:	Deoxyribonucleic acid
DRP:	Detailed Review Paper
EA:	Endocrine Activity
EAS:	Endocrine Active Substance
EC:	European Commission
ECETOC:	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA:	European Chemicals Agency

ED:	Endocrine Disrupter (or Disruptor)
EC/ECVAM:	The European Commission European Centre for the Validation of Alternative Methods
EDSP:	The US Endocrine Disruptor Screening Program
EDSTAC:	The US Endocrine Disruptors Screening and Testing Advisory Committee
EDTA TF:	The OECD Endocrine Disrupters Testing and Assessment Task Force
EDTA AG:	The OECD Endocrine Disrupters Testing and Assessment Advisory Group
EEA:	European Environment Agency
EFSA:	European Food Safety Authority
EPA:	Environmental Protection Agency
EMA:	European Medicines Agency
ER:	Estrogen receptor
EU:	European Union
GLP:	Good Laboratory Practice
GMO	Genetically modified organism
HeLa	Cell type
hERα:	Estrogen receptor α
HPV:	High Production Volume
ICCVAM:	The US Interagency Coordinating Committee on the Validation of Alternative Methods
IHCP:	The EC JRC, Institute for Health and Consumer Protection
IPCS:	International Programme on Chemical Safety
JaCVAM:	Japanese Centre for the Validation of Alternative Methods
JRC:	The EC Joint Research Centre
MAD:	Mutual Acceptance of Data
METI:	Ministry of Economy, Trade and Industry, Japan
MHLW:	Ministry of Health, Labour and Welfare, Japan
MOE:	Ministry of Environment, Japan

MSs:	Member States
NIEHS:	US National Institute of Environmental Health Sciences
OECD:	Organisation for Economic Cooperation and Development
NTP:	National Toxicology Program
PBT	Persistent, bioaccumulative and toxic
PCBs:	Polychlorinated biphenyls
PFOA:	Perfluorooctanoic acid
PFOS:	Perfluorooctyl sulfonate
PPPs	Plant protection products
PPR Panel:	EFSA's Scientific Panel on Plant Protection Products and their Residues
PMRA	Health Canada's Pesticide Management Regulatory Agency
PRAPeR Unit:	EFSA's Pesticide Risk Assessment Peer Review Unit
QSAR:	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCS:	Scientific Committee on Consumer Safety
SCFCAH:	Standing Committee on the Food Chain and Animal Health
SCF:	Scientific Committee on Food
SCTEE:	Scientific Committee for Toxicity, Ecotoxicity and the Environment
SCHER:	Scientific Committee on Health and Environment
SCENIHR:	Scientific Committee on Emerging and Newly Identified Health Risks
SPEED 98:	Strategic Program on Environmental Endocrine Disrupters 98, Japan
SVHC:	Substances of very high concern
TG:	Test Guideline
TGP:	Test Guidelines Programme
TR:	Thyroid receptor
TTC:	Threshold of Toxicological Concern
US:	The United States of America

US EPA:	The United States Environmental Protection Agency
VMG-eco:	The OECD Validation Management Group for ecotoxicity tests
VMG-mammalian:	The OECD Validation Management Group for mammalian toxicity tests
VMG-NA:	The OECD Validation Management Group Non-Animal tests
vPvB	Very persistent and very bioaccumulative
WHO:	World Health Organization
WNT:	The OECD Working Group of National Co-ordinators of the Test Guidelines Programme