Supporting the Implementation of the EU Community Strategy on Endocrine Disrupters

Susanne Bremer, Paolo Castello, Elise Grignard, Anne Milcamps, Sharon Munn, Clemens Wittwehr, and Maurice Whelan


1 Introduction

In 1998, the European Parliament responded to the increasing public concern about potential adverse effects of chemicals that interfere with the endocrine system in wildlife and in humans by calling on the European Commission to take specific action regarding “Endocrine Disrupters” (Tab. 1). The major objectives were to improve legislative frameworks and to reinforce research efforts and communication with the public.

In response to this request, in 1999 the European Commission (EC) published the EU Community Strategy on Endocrine Disrupters (European Commission, 1999), which presented a conceptual framework defining objectives and actions to address the identification and control of endocrine disrupters in Europe. An overview of these actions is presented in Table 2. Approximately every three years, the Commission has published a progress report describing the advancement of the implementation of the Strategy and initiatives taken (European Commission, 2001, 2004, 2007, 2011).

The EC’s Joint Research Centre (JRC) supports the implementation of the Strategy through a number of scientific/technical activities, such as the validation of new testing methods, the development of a web-based IT platform for endocrine active substances, and the development of an OECD Harmonised Template for reporting on intermediate toxicological effects. These policy support activities aim to provide tools and scientific knowhow that will reduce the number of animal experiments needed to assess the endocrine disrupting properties of chemicals while at the same time ensuring the maximum protection for human health.

Keywords: endocrine disrupters, European Community strategy for endocrine disrupters, OECD Harmonised Template, validation of transcriptional tests, EAS database

Tab.1: IPSC/WHO definition on Endocrine Disrupters

<table>
<thead>
<tr>
<th>Endocrine Disrupters</th>
<th>An Endocrine Disrupter is an exogenous substance or mixture that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential Endocrine Disrupters</td>
<td>A potential Endocrine Disrupter is an exogenous substance or mixture that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations.</td>
</tr>
</tbody>
</table>
### Tab. 2: Overview on the related actions needed to implement the Community Strategy

<table>
<thead>
<tr>
<th>Short term</th>
<th>Medium term</th>
<th>Long term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Establish a priority list of substances</td>
<td>Identification and assessment of EDs: including harmonization of the development and validation of new improved testing methods</td>
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</tr>
<tr>
<td>Establish monitoring programmes</td>
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<td></td>
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<tr>
<td>Information exchange and international co-ordination</td>
<td>Research and development on mechanisms, new risk assessment concepts, exposure assessment and environmental monitoring tools</td>
<td>Adapt and/or amend present EU legislative instruments including implementation of new legislative instruments</td>
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<tr>
<td>Collect, exchange, assess and provide information on EDs to the public</td>
<td></td>
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<tr>
<td>Consult with the stakeholders</td>
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### Tab. 3: Examples of EU legislation items in which EDs are of special relevance

<table>
<thead>
<tr>
<th>Legislation item</th>
<th>Specific relevance of Endocrine Disrupting Chemicals</th>
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</thead>
<tbody>
<tr>
<td>Reg. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)</td>
<td>For substance evaluation: information on endocrine disrupting (ED) properties might be asked by the competent Authority as additional information. For substance authorisation: REACH Art. 57 (f) states that substances having endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health or the environment and which give rise to a concern equivalent to carcinogenic, mutagenic, reproductive toxic substances, or persistent, or bio accumulative substances may be included in Annex XIV (List of substances subject to authorisation) Under Art 60(3), REACH also states that if it is not possible to determine a level of exposure to the substance above which humans should not be exposed, such substances may be only authorised if it is shown that socio-economic benefits outweigh the risk. By 1 June 2013 the Commission shall carry out a review to assess whether or not, taking into account latest developments in scientific knowledge, to extend the scope of Article 60(3) to substances identified under Article 57(f) as having endocrine disrupting properties. On the basis of that review the Commission may, if appropriate, present legislative proposals.</td>
</tr>
<tr>
<td>Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market (PPPR).</td>
<td>Under the PPPR provisions in relation to human health and ecotoxicology, an active substance shall only be approved if it is not considered to have endocrine disrupting properties that may cause adverse effects in humans/non target organisms, unless exposure is negligible. By the end of 2013, the European Commission must present a draft of the measures concerning specific criteria for the determination of endocrine disrupting properties.</td>
</tr>
<tr>
<td>Proposal for a Regulation concerning the placing on the market and use of biocidal products COM(2009)267</td>
<td>The proposed Regulation will repeal and replace the current Directive 98/8/EC. Under Article 5 of the proposal (exclusion criteria), the proposed regulation imposes specific conditions for the authorisation of active substances which have been classified carcinogen, mutagen or toxic for reproduction in accordance with Regulation (EC) No 1272/2008, or identified under REACH Article 57(f) as having endocrine disrupting properties.</td>
</tr>
<tr>
<td>Regulation (EC) 1223/2009 on cosmetic products</td>
<td>Endocrine disrupting substances are currently not restricted in the scope of Regulation (EC) 1223/2009 on cosmetic products. However, Article 15(4) calls upon the Commission to review this Regulation with regard to substances with endocrine-disrupting properties, when Community or internationally agreed criteria for identifying substances with endocrine-disrupting properties are available, or at the latest on 11 January 2015.</td>
</tr>
</tbody>
</table>
2 Legislative background

Ultimately, the European Community Strategy on Endocrine Disrupters is intended to provide the knowledge needed to adapt and/or where necessary to amend present EU legislation concerning the risk management of chemicals in various sectors to take specific account of potential endocrine disrupting effects. Several pieces of EU legislation already refer to endocrine disruption; some of the most important examples are summarized in Table 3. However, none of these Regulations and/or Directives currently goes so far as to define the standard information requirements that would identify a chemical as an endocrine disrupter. The European Commission therefore is committed to take action on this matter by proposing specific science-based criteria for the evaluation of substances with potential endocrine disrupting properties. The obligation for the Commission to propose these criteria by 2013 is explicitly laid down only in the Regulation (EC)1107/2009 on Plant Protection Products (PPPR). For the sake of simplicity and consistency, however, it would be advantageous if the same criteria could be applied across all the relevant pieces of legislation.

Various position papers and proposals for regulatory definitions and/or assessment criteria have been published recently, anticipating the 2013 deadline. These include the joint UK-Germany proposal for “A regulatory definition of an endocrine disrupter in relation to human health” (Deutschland and UK, 2011) and the Danish proposal on “The establishment of Criteria for endocrine disrupters and options for regulation” (Danish Ministry of the Environment, 2011). In order to harmonize different point of views and approaches, a working group with experts from different Member States will be established to host science-focused debates under the chairmanship of the JRC. In this context it will be important to define the role of newly developed, and possibly validated, *in vitro* and *in silico* methods that can be used to assess the endocrine disrupting properties of chemicals and to provide the necessary understanding on the mode of action of the investigated substance.

3 Development of a new OECD Harmonised Template for intermediate effects

The OECD Harmonised Templates (OHTs, http://www.oecd.org/ehs/templates) are standard data formats for reporting data on the effects of chemicals on human health and the environment. They are usually associated with OECD guidelines and allow an easy exchange of data among the OECD Member States to facilitate the mutual acceptance of test data. The European Chemicals Agency (ECHA)’s International Uniform Chemical Information Database (IUCLID5), for example, is based on this standard. However, currently existing OHTs do not allow reporting observations at the molecular, cellular or tissue level, unless they are connected to a specific “apical endpoint.” Consequently, it is often difficult if not impossible to use the OHT framework to associate data derived from an *in vitro* test, a read-across algorithm, or a computational prediction model that could prove invaluable in understanding the complexity of endocrine pathways associated with numerous possible adverse health outcomes. The JRC recently has been mandated by the OECD to develop a new Harmonised Template (OHT 201) for “intermediate effects,” which would allow the reporting of events observed at different biological levels in order to establish a mode of action of a hazardous substance. The process is being undertaken in consultation with various experts, and a first draft proposal is envisaged as the basis for a 2012 discussion with the relevant stakeholders.

4 EU IT platform for scientific information on Endocrine Active Substances

Collecting information on the endocrine activity of substances is currently not a standard information requirement under EU legislation. However, such data often are generated as part of internal screening processes and priority setting during product development, research activities, or as mechanistic support data for the explanation of observed adverse effects. In order to make full use of these data for the implementation of the existing legislative provisions, the JRC started to develop an interactive web-based IT system on Endocrine Active Substances (EAS Web Portal). Initially populated with scientific data collected by the EC Directorate General Environment (http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm), the system also will be open to contribution from third parties. After reviewing the needs and requirements of such a database (Berggren et al., 2011), a vision for a future EAS Database and Web Portal has been elaborated (Fig. 1). The first version of the system should be available on-line in 2012.

5 Development and validation of new test methods

More than 80 projects with a total budget of more than 150 k€ have been funded via the Community Framework Program for R&D, with the goal of understanding the mechanisms of endocrine disrupters and their consequences for human health and the environment (http://ec.europa.eu/research/endocrine/index_en.html). The development of monitoring tools and the establishment of new predictive toxicological methods were the subject of research activities.

The FP-6 project “ReProTect” (http://www.reprotect.eu/) aimed to develop new methods reflecting relevant toxicological targets of reproductive toxicants. In all, 33 participating European research institutions established a set of *in vitro* methods that can be combined in a battery approach for assessing reproductive toxicants. This test battery was challenged in a blind study (Schenk et al., 2010) and was demonstrated to possess an impressive level of predictivity. The battery also included a number of tests capable of detecting substances with estrogenic or androgenic properties (Tab. 4). Several of these tests were then submitted to the European Centre for the Validation of Alternative Methods (ECVAM) at the JRC to be considered for formal validation.
The “MELN” test, the first test of the ReProTect test battery, is currently under (pre-)validation. This test is based on MCF-7 cells that have been stably transfected with the estrogen-responsive gene (ERE-βGlo-Luc/SVNeo) (Balaguer et al., 1999). Since two datasets of 16 chemicals produced during the ReProTect project already demonstrated a reliable level of transferability and reproducibility (Witters et al., 2010), ECVAM currently undertakes finalization of the prevalidation through the generation of a third dataset. To facilitate an international consensus on the outcome, the study is managed in collaboration with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Japanese Center for the Validation of Alternative Methods (JACVAM), and its progress is regularly reported at the OECD Validation Management Group “non animal.” One aim is to assess whether the MELN test can meet the standards currently under development for the OECD Performance-Based Test Guideline (PBTG) for transcriptional activation in vitro assays to detect chemicals with estrogenic activity. These standards will be based on successfully validated assays such as the transcriptional assay for detection of estrogenic agonist-activity of chemicals using HeLa-9903 cells (OECD TG455) and the transcriptional assay for identifying estrogenic agonists and antagonists using BG1Luc4E2 cells (BG1Luc ER TA). The concept of PBTG will allow similar tests demonstrating their reliability and relevance through the performance standards to be annexed to the OECD guideline.

Nevertheless, the increasing family of transcriptional assays proposed as methods to detect endocrine disrupting properties requires an in-depth scientific review so that only the most reliable and informative tests will be considered for formal validation. Even if the experimental design of the various tests is similar, the intrinsic properties of the cellular models might differ, including the stability of the genetically engineered cell lines. In order to obtain more objective information on the sensitivity and specificity of the various cellular models, the JRC has started to automate a number of transcriptional assays so that they can be run on a High Throughput Screening (HTS) platform available in its laboratories. The validated BG1Luc ER TA assay developed by XenobioticDetection Systems Inc. (XDS, USA) is the first among a series of tests that will be assessed (NICEATM, 2011).
Tab. 4: Assays targeting endocrine disrupters developed under the EU ReProTect project

<table>
<thead>
<tr>
<th>Name</th>
<th>Type of assay</th>
<th>Cells</th>
<th>Genetic modifications</th>
<th>Validation status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat recombinant Androgen Receptor Binding Assay</td>
<td>receptor binding</td>
<td>n. a.</td>
<td>Rat hinge region and an LBD, thioredoxin-tagged</td>
<td>optimization, submitted to ECVAM</td>
<td>(Freyberger et al., 2010a)</td>
</tr>
</tbody>
</table>
| PALM                               | transcriptional activation | PC3 (human prostate cancer cell line) | – full length human AR  
– luciferase gene under the control of the androgen-dependent Mouse Mammary Tumor Virus (MMTV) promoter | optimization, submitted to ECVAM       | (Freyberger et al., 2010c)     |
| AR-CALUX                           | transcriptional activation | U2-OS (human osteosarcoma cell line) | – full-length human androgen receptor  
– firefly luciferase reporter gene under control of 3 androgen response elements placed upstream of the minimal human E1B TATA promoter | optimization, submitted to ECVAM       | (van der Burg et al., 2010a) |
| human recombinant Estrogen Receptor-α Binding Assay | receptor binding  | n. a.                              | full length human estrogen receptor-alpha                                                                   | validated                              | (Freyberger et al., 2010b)     |
| ERα-CALUX                          | transcriptional activation | U2-OS (human osteosarcoma cell line) | – full length human ERα  
– firefly luciferase reporter gene under control of 3 estrogen response elements placed upstream of the minimal human E1B TATA promoter | optimization, submitted to ECVAM       | (van der Burg et al., 2010b) |
| MELN                               | transcriptional activation | MCF-7 (human breast adenocarcinoma cells) | firefly luciferase reporter gene under control of 1 estrogen response element placed upstream of the minimal rabbit β-globin promoter | validation ongoing                     | (Witters et al., 2010)        |

n.a.: not applicable

6 Conclusion

Stimulated by the European Community Strategy for Endocrine Disrupters, considerable scientific information and knowledge has been gained regarding the mechanisms of action of endocrine active compounds, their effects on human health and the environment, and the methods by which substances of concern can be detected and assessed. Accordingly, action is being considered to adapt and/or amend legislation covering the risk management of chemicals, as appropriate. Translating the latest scientific knowledge and methodology related to the identification and characterization of endocrine disrupting hazards into
mainstream regulatory toxicology remains a challenge. However, the numerous collaborative efforts under way at the international level are helping to exploit the latest scientific findings in this field, as well as to expedite the development of appropriate regulatory measures, mitigate the risks that may be posed by endocrine disruptors, and allay the concerns of the citizen.

References

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