

European Workshop on Endocrine Disrupters

18-20 June 2001,

Aronsborg (Bålsta), Sweden

Workshop Report

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Swedish National Chemicals
Inspectorate (KEMI)
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WHO
European Environment Agency**

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This report represents the output from a workshop held at Aronsborg (Bålsta), Sweden on 18-20 June 2001. The text was prepared by WRc-NSF on behalf of the European Commission, in consultation with the Chairpersons and Rapporteurs as well as with the members of the Organising/Technical Committee. It represents the opinions of the participants of the working sessions, taking into account additional comments made during the plenary sessions. It is not a consensus report and does not necessarily reflect the opinions of individual participants.

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Executive Summary

Background

In the past decade there has been growing concern about a range of substances which are suspected of interfering with the endocrine system - so-called "*endocrine disrupters*". In both human beings and wildlife endocrine disruption is a mechanism of effect related to the functioning of the endocrine system, (which influences development, growth, reproduction and behaviour). Endocrine disrupters may:

1. Directly damage an endocrine organ
2. Directly alter the function of an endocrine organ
3. Interact with receptors
4. Alter hormone metabolism either in an endocrine organ (for example inhibit steroidogenesis) or peripherally (for example increase in hepatic metabolism and clearance)

It should be recognised that substances may affect several mechanisms and that in order to consider the potential impact of endocrine disrupters it is necessary to understand the function of the hormones that they mimic or antagonise. As a consequence of the effects of these substances on the endocrine system adverse health effects such as cancer, behavioural changes and reproductive abnormalities may occur.

In December 1999, the European Commission adopted a Communication to the Council and to the European Parliament on a Community Strategy for Endocrine Disrupters: a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706). The Community strategy addresses the key requirements of further research; international co-operation; communication to the public; and appropriate policy action. A series of short-, medium- and long-term actions are recommended to meet these requirements.

Endocrine disruption is a broad topic which is constantly evolving in terms of science and policy and in terms of national and international activities. Therefore, it is essential to periodically bring together key personnel in order to review progress, to help co-ordinate and create synergies amongst personnel/activities and to make recommendations for future work/next steps. In June 2001 the Commission adopted a follow-up Communication to the Council and European Parliament on the implementation of the Community Strategy for Endocrine Disrupters (COM(2001)262). In this Communication the Commission announced its intention to hold a European Workshop on Endocrine Disrupters as a means to follow up on several elements of the Community Strategy.

Scope and objectives of the workshop

The workshop was organised in the context of the Community Strategy for Endocrine Disrupters (COM (1999) 706), in order to follow up on several elements of the strategy, as follows:

- information exchange and international co-ordination;
- research and development;

- development of test methods/testing strategy; and
- establishment of monitoring programmes.

The goal of the workshop was to convene representatives of EU Member State and Associated State governments, US and Japan governments, representatives of academia, industry and environmental NGOs as well as representatives of OECD, WHO, EEA and the European Commission in order to review progress, help coordinate and create synergies and make recommendations for future work/next steps on the workshop themes outlined above. Detailed objectives for each workshop theme are described in the individual chapters of this Report.

The workshop was structured into a series of plenary and parallel sessions each with a Chair and Rapporteur – see Workshop Programme in Appendix A. Feedback on the outcomes of each session was reported back to all participants throughout the workshop.

A total of 96 persons took part in the workshop, a list of whom is contained in Appendix B.

Conclusions and recommendations of the workshop

The workshop recognised that the area of endocrine disruption and potential effects of endocrine disrupting chemicals on human health and wildlife was one of increasing concern to all sectors of society. This concern was evident in the increasing level of research activity which is being funded both nationally and internationally by governments, regulatory bodies and industry. The importance of close liaison between the different bodies and sharing of information was highlighted to ensure that the best possible use is made of available resources. It was evident that endocrine disruption is a complex area to address and that there are still problems (particularly for human health) in establishing causal links between exposure to suspected endocrine disrupters and any effects measured. In recent years a considerable amount of data on endocrine disruption has been generated which has provided answers to a number of important questions. Work has also proceeded on the development of test methods under the auspices of bodies such as Organisation for Economic Cooperation and Development (OECD) and the United States Environmental Protection Agency (US EPA). However a significant number of questions still need to be answered and the challenge facing regulators, industry and academia is how these can be resolved in a rapid cost-effective manner whilst still providing scientifically robust outputs.

The following recommendations resulted from the workshop sessions:

Information exchange and international co-ordination (c.f Chapter 2)

- Easily accessible and regularly updated databases providing information on endocrine disrupting chemicals and on-going research projects need to be available to the scientific community.
- Literature information systems need to be established which provide information on the current situation regarding endocrine disruption at a range of levels (from

the simple to the technical) for different target groups (from the general public to researchers). In these systems links between key websites need to be established for easy access of information.

- The Global Assessment of Endocrine Disruption (GAED) state-of-the-science report needs to be periodically updated after its publication and resulting recommendations should be implemented.
- There is a need for better exchange of information and data between scientists involved in national research and their national representatives engaged in OECD activities.

Research and development (c.f. Chapter 3)

Endocrine disruption in humans

- Future studies should work towards a full appreciation of endocrine disruption effects on human health which includes all potential target sites (particularly those associated with adrenal function and steroidogenesis).
- Future studies need to focus on investigating the links between exposure to endocrine disrupters and identified effects on human health (decrease in semen quality, increase in hypospadias, testicular and prostate cancer in males and breast and ovarian cancers in females).
- More detailed assessments of exposure in humans (to all endocrine disrupting chemicals by all routes) are needed.
- There should be thorough investigations of aetiological factors in current human endocrine conditions.
- Further information on risk factors in epidemiology studies is needed (with environmental and socio-economic factors being considered).
- Research studies should include input from endocrinologists.

Endocrine disruption in wildlife

- Studies on natural variability of measures of endocrine disruption need to be carried out in accordance with the recommendations of the SETAC Endocrine Modulators in Wildlife: Assessment and Testing (EMWAT) workshop (Tattersfield *et al* 1997).
- The population consequences of endocrine disrupter effects in individuals need to be understood to provide a better understanding of the ecological relevance of the assessed effects.
- Further studies on the effects of endocrine disrupters on amphibians/ reptiles are needed since these important (and vulnerable) groups have endocrine systems

(e.g. thyroid) which are different to fish and they may show effects not evident in other taxa.

- Further studies should consider endocrine disrupter effects on birds, possibly through the development of a model *in ovo* system in which the effects on organism development are measured following injection of chemicals into the yolk of the egg.
- Test methods for invertebrates need to be developed taking into account the recommendations of the SETAC Endocrine Disruption in Invertebrates: Endocrinology, Testing and Assessment (EDIETA) workshop (DeFur *et al* 1999) However, this needs to be underpinned by a greater understanding of the endocrinology of this important taxonomic group.

Regional and global concerns

- The environmental reasons for regional differences in human health effects (such as differences in semen quality between Denmark and Finland) need to be identified. This may link chemical aetiology to health effects.
- For fish, a European comparison of endocrine disruption effects is needed, but this will require harmonisation of methodologies (for example the test species and test design).

Mechanisms of action of endocrine disrupters

- A better mechanistic understanding of the action of endocrine disrupters is needed, particularly where the responses are not receptor mediated.
- Future studies should not only focus on oestrogenic effects but consider other parts of the endocrine system: for example (anti-) androgenic, (anti-) thyroid and (anti-) adrenal effects.
- Future studies should address the effects of endocrine disrupters on immune systems and behavioural and neurological development.
- Greater use should be made of genomics (techniques to identify the DNA sequence of the genome), transcript profiling (techniques to identify the mRNA from actively transcribed genes), proteomics (techniques to identify the proteins in a biological sample) and metabolomics (techniques to identify the presence and concentrations of metabolites in a biological sample). These GTPM approaches require additional development and validation, but could provide a vast range of additional useful endpoints. They could provide powerful tools in the future which could be used to target testing, address low dose effects, mixtures and decrease the numbers of test animals used. Development of these tools should be focussed initially on endocrine disrupter related activities. Their careful application could assist research associated with safety assessment by enabling a more detailed appreciation of molecular mechanisms of action and facilitating more rapid screens for effects.

Measurement of exposure

There is a need to develop analytical procedures to assess exposure to endocrine disrupters, in particular this relates to:

- integrated analytical chemistry/Toxicity Identification Evaluation (TIE) procedures using validated *in vitro* tests;
- methods for the detection and quantification of endocrine disrupters in complex matrices such as sediments;
- information on the potential role of invertebrates in the transfer of endocrine disrupting chemicals from sediments to fish via the food chain.

Measurement of effects

Future studies should focus on:

- critical windows of exposure in humans and wildlife (especially for development);
- ways to enhance the predictive capability of endpoints;
- the issue of sensitive individuals in populations.

Furthermore future studies need to consider the issues of:

- low dose effects where there are unusual dose response curves that traditional regulatory toxicology protocols may miss. There is a need for a clear definition of what is meant by 'low dose effects' as well as clarification of exposure patterns and sensitive windows of exposure.
- the extent to which mixture effects occur in terms of:
 - antagonism, additivity or supra-additivity;
 - mixture effects involving more than one molecular target (in mammals what is the effect to the male foetus of exposure to a weak oestrogen and a weak anti-androgen?).
- the issue of adverse effects and what level of change should be regarded as significant. It is important that sufficient information is available on natural variability to allow potential changes due to chemicals to be identified.

Funding and policy issues

Mechanisms need to be established to facilitate long-term studies which address:

- Endocrine effects in long-lived species;

- Whether regulatory actions related to endocrine disrupters result in improvements in the environment.

Development of test methods and testing strategies (c.f Chapter 4)

- Existing OECD approaches for detecting oestrogenic, androgenic and thyroid effects in mammals, fish and birds represent a scientifically robust approach but there is a need:
 - for whole animal tests to cover all possible endocrine disrupting effects;
 - to focus on test methods assessing reproductive function and developmental effects;
 - to refine chronic mammalian tests test designs and enhance/improve the test endpoints to better cover sensitive periods of the test organisms life history and to better capture hormonal effects.
- OECD activities are not covering all of the issues and there is a need for further work:
 - to develop tests to address impacts on the adrenal system, pheromone signalling and the neuro-endocrine-immune system;
 - to develop tests using invertebrates (which are underpinned by a greater understanding of the endocrinology of the test species used);
 - to develop alternative techniques such as SARs and *in vitro* tests (beyond those currently being developed or evaluated) which could be used to target testing and limit *in vivo* testing. However, alternative tests need to be used responsibly and intelligently and *in vivo* testing is needed to validate SARs and *in vitro* systems;
- There is a need for a comparison of information on thyroid effects in different species so as to determine how to address the issue of species redundancy.
- The approach for determining whether testing of potential endocrine disrupters is required needs to be more sophisticated than being based on production volume data alone and should include data on the pattern of exposure of substances in the environment and their persistence, bioaccumulation and toxicity.
- There is a need for an intelligent approach to testing homologous series of chemicals and the use of bridging studies to assist in this regard. Further research is needed and OECD should be asked to co-ordinate standardisation and validation of partial lifecycle studies to be used for bridging.
- A simple framework to determine testing needs should be developed, with the approach used in the Biocides Directive being proposed as a possible example.
- Discussions should begin on developing an EU strategy for testing endocrine disrupters. The Annex V Committee to 67/548 should coordinate EU views and activities on test methods and strategies to feed into the OECD.

Establishment of monitoring programmes (c.f Chapter 5)

- In humans large scale epidemiology studies investigating changes in reproductive function (for example sperm counts, semen quality, fertility rates) and malformations in fetuses, new born and young children, adult women (breast cancer and endometriosis) and adult males (testicular cancers) should be established.
- For human health monitoring further use should be made of registers of reproductive effects (sperm counts, semen quality, infertility levels) and malformations (male and female cancers) to identify spatial and temporal trends across Europe. However, it will be necessary to standardise methodologies so that the data can be interpreted both within and across countries.
- Extensive but cost-effective schemes should be established to monitor changes in parameters such as semen quality (linked with exposure studies) in appropriate target groups so that action can be taken before this is translated into decreases in fertility rates. Such programmes should work towards establishing the reasons for the differences in semen quality between countries such as Denmark and Finland.
- In the environment monitoring should initially focus on the assessment of “hot spots” using effects measures and attempt to identify the causative agents. An example of a cost-effective study which would build on existing programmes and provide a relevant and representative picture of the European situation would involve effects-based monitoring (primarily using standardised fish species) in rivers and estuaries of Germany, the Netherlands and the United Kingdom and in the Mediterranean Sea.
- Programmes need to be established which can provide information on background effects/concentrations (making greater use of tools such as environmental specimen banks). These can provide material that can be used to obtain data retrospectively to monitor temporal trends. However, it is important that there is harmonisation of approaches so that it is possible to compare results across European countries.
- Appropriate modelling approaches should be used with product registry data (production volumes and use patterns) to predict where “hot spots” of effects might be expected.
- Regulators and industry have to work together to ensure that current and realistic data on the production, patterns of use and releases of potential endocrine disrupters are available.

1. General Introduction

1.1. What is endocrine disruption?

In both human beings and wildlife endocrine disruption is a mechanism of effect related to the functioning of the endocrine system, (which influences development, growth, reproduction and behaviour) and in recent years there has been growing concern about a range of substances, which are suspected of interfering with the endocrine system - so-called "*endocrine disrupters*". Endocrine disrupters may:

1. Directly damage an endocrine organ
2. Directly alter the function of an endocrine organ
3. Interact with receptors
4. Alter hormone metabolism either in an endocrine organ (for example inhibit steroidogenesis) or peripherally (for example increase in hepatic metabolism and clearance)

It should be recognised that substances may affect several mechanisms and that in order to consider the potential impact of endocrine disrupters it is necessary to understand the function of the hormones that they mimic or antagonise. As a consequence of the effects of these substances on the endocrine system adverse health effects such as cancer, behavioural changes and reproductive abnormalities may result.

In December 1996 the first European workshop on the impacts of endocrine disrupters on human health and wildlife was organised in Weybridge, United Kingdom (EC 1996). At this workshop over 70 scientists and policy-makers from the European Union, the United States and Japan as well as from organisations such as Organisation for Economic Cooperation and Development (OECD), World Health Organisation (WHO) and non-governmental organisations such as the European Science Foundation (ESF), the Conseil European de l'Industrie Chimique (CEFIC) and WWF concluded *inter-alia* that:

- There is sufficient evidence that testicular cancer rates are increasing, and the apparent decline in sperm counts in some countries is likely to be genuine.
- Some cases exist in the EU where adverse endocrine effects, or reproductive toxicity, in birds and mammals coincide with high levels of substances, shown to have endocrine-disrupting properties in some test systems.
- The considerable uncertainties and data gaps should be reduced by research and monitoring into exposure and effects in wildlife and humans, but meanwhile, consideration should be given to measures to reduce exposure to endocrine disrupters in line with the "precautionary principle".

On 4 March 1999, the European Commission Scientific Committee for Toxicity, Ecotoxicity and the Environment (SCTEE) presented its Opinion "*Human and Wildlife Health Effects of Endocrine Disrupting Chemicals, with emphasis on Wildlife and on Ecotoxicology test methods*" (SCTEE 1999). It identified a "potential global problem" for wildlife and stated that "impaired reproduction and development causally linked to endocrine disrupting chemicals are well-documented in a number of wildlife species and have caused local and population changes. However, from the data presented

in the report it was evident that major areas of uncertainty remain (see Table 1.1) which need to be addressed.

Table 1.1 Areas of uncertainty in the effects of endocrine disrupters on human health and wildlife identified in the SCTEE report

Type of effects	Areas of uncertainty regarding endocrine disrupters identified in the SCTEE report
Human health	<ul style="list-style-type: none"> • No causative role for endocrine disrupters in the increased prevalence in cryptorchidism or hypospadias has been determined • The underlying reason(s) for the increased incidence in testicular cancer has not been identified • No causative role for endocrine disrupters in the increased prevalence of prostate cancer has been determined
Wildlife	<ul style="list-style-type: none"> • The inference of cause and effect relationships in wildlife studies is complex and difficult, since poor reproductive performance and subsequent population change is multi-factorially defined. In addition many other environmental and stock-related factors such as habitat destruction and fisheries impact may influence population structure and size. In this respect it is important to establish baseline data in non-exposed reference populations.

The SCTEE report stated that “the reported health effects which have been associated with endocrine disrupters should be further evaluated and the underlying causes identified. In this, special attention should be given to exceptionally high chemical exposures and to the health consequences of phytoestrogens in human food”.

With regard to effects on wildlife it recommended that research be carried out to:

- establish the long-term consequences at the population and community level of the observed effects in reproductive organ structure and function (such as vitellogenin induction, precocious female maturation, intersexuality, ovotestis formation, and altered behaviour), thyroid status and immune system;
- conduct further laboratory, semi-field and field studies to establish cause and effect relationships and assess the full environmental significance of endocrine disruption, including studies on effects in terrestrial systems and in amphibian and reptile populations in Europe.

1.2. Policy response

Following the first European workshop on endocrine disruption the phenomenon has attracted significant media attention and an increasing number of parliamentary questions has been addressed to the European Commission since 1997 concerning the use and regulation of a range of suspected endocrine disrupting substances. In addition from 1997 – 2000 a series of workshops have been organised to consider different aspects of endocrine disruption. The chronology of publication of policy documents and reports, workshops and meetings is summarised in Table 1.2.

In December 1999, the Commission adopted a Communication to the Council and to the European Parliament on a Community Strategy for Endocrine Disrupters: a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706). The Community strategy addresses the key

requirements of further research; international co-operation; communication to the public; and appropriate policy action. Recommendations are made for short-, medium- and long-term actions.

- Short-term actions represent a timeframe of 1-2 years and include:
 - the establishment of a list of substances for further evaluation of their role in endocrine disruption
 - the use of existing legislative instruments
 - the establishment of monitoring programmes
 - the identification of specific cases of consumer use for special action
 - information exchange and international co-operation
 - communication to the public
 - consultation of stakeholders
- Medium-term actions requiring a 2-4 year horizon include:
 - the development of agreed test methods (primarily in the framework of the OECD)
 - the gathering of research results from ongoing research projects
 - the launching of further research to address new research requirements
 - the identification of substitutes
 - voluntary initiatives

Finally, long-term actions requiring a timeframe of more than 4 years entail the adaptation/amendment of existing and proposed legislation in order to take specific account of endocrine disrupters.

At the Council of Ministers (Environment) meeting in Brussels on 30 March 2000 the Council held a policy debate in which it welcomed the Commission Communication on a Community Strategy for Endocrine Disrupters. During the debate, all delegations stressed the importance of this strategy which includes short, medium and long-term actions. Ministers noted that the strategy should be coordinated and be consistent with the general Community strategy on chemicals. The Council adopted Conclusions on the Commission Communication in which it stressed the precautionary principle, the need to develop quick and effective risk management strategies and the need for consistency with the overall chemicals policy. The Council invited the Commission to report back on the progress of the work at regular intervals, and for the first time in early 2001.

On 26 October 2000, the European Parliament adopted a Resolution on endocrine disrupters, emphasising the application of the precautionary principle and calling on the Commission to identify substances for immediate action.

In June 2001 the European Commission provided a Communication (COM(2001)262) to the Council and the European Parliament on the implementation of the Community Strategy on Endocrine Disrupters (COM(1999)706). In addition, on 31 May 2001, a dedicated call for research proposals on endocrine disrupters was published under the 5th Community Framework Programme on Research and Technological Development, with a deadline for submission of proposals of 14 September 2001.

1.3. Overall Chemicals Policy

On 13 February 2001, the Commission adopted a White Paper on a Strategy for a Future Chemicals Policy. One of the key elements of the proposed strategy is an authorisation procedure for substances of very high concern, namely, substances that are carcinogenic, mutagenic or toxic to reproduction and substances with POPs characteristics. This procedure would require authorities to give specific permission before such a substance could be used for a particular purpose, marketed as such or as a component of a product. Given that serious human health effects which have so far been associated with endocrine disrupting chemicals include testicular cancer, breast cancer, prostate cancer, decrease in sperm concentration and semen volume, cryptorchidism and hypospadias, it is likely that the majority of endocrine disrupting chemicals would fall under this authorisation procedure. Furthermore, adverse effects on the endocrine system of wildlife species have been causally linked to certain POPs, which would be subject to authorisation. In addition, the need for particular research efforts on endocrine disruption are highlighted in the White Paper. These include research into the development and validation of *in-vivo* and *in-vitro* test methods as well as modelling (SAR) and screening methods, and research into the effect of low doses, long-term exposure and exposure to mixtures of chemicals.

The White Paper emphasises that the protection of human health and the environment, including wildlife, should be balanced against protection of the welfare of laboratory animals. The Commission will therefore promote further development and validation of non-animal test methods, by maximising the use of non-animal test methods, encouraging development of new non-animal test methods and minimising test programmes.

Other issues pertinent to the issue of endocrine disruption, which are addressed in the context of the overall chemicals policy, include the rigorous testing for long-term effects of substances exceeding a production volume of 100 t and the obligation of manufacturers/importers and downstream users to carry out appropriate risk assessments.

1.4. Purpose of the Workshop

Endocrine disruption is a broad topic, which is constantly evolving in terms of science and policy and in terms of national and international activities. It involves stakeholders in governments, industry, academia and non-governmental organisations as well as the general public. Therefore, it is essential to periodically bring together key personnel in order to review progress, to help co-ordinate and create synergies amongst personnel/activities and to make recommendations for future work/next steps.

The current workshop was organised in the context of the Community Strategy for Endocrine Disruptors (COM (1999) 706), in order to follow up on several elements of the strategy, as follows:

- information exchange and international co-ordination
- research and development.

- development of test methods/testing strategy; and
- establishment of monitoring programmes

1.5 Description of the workshop

A Background Document was sent to all participants prior to the workshop, describing the objectives of each workshop session, the issues to be addressed during the session and providing background information on the workshop themes. It was recognised that there would be considerable overlap between the themes and that it would be difficult to define exact boundaries.

The workshop structure is shown in the Final Programme which is contained in Appendix A. A combination of plenary and parallel sessions was used. Each session had an identified Chair and Rapporteur and feedback on the outcomes of each session was reported to all participants throughout the workshop.

A List of Participants is given in Appendix B.

Table 1.2 Summary of the key events concerned with the assessment of the effects of endocrine disruptors

Date	Policy documents and reports	Workshops, OECD meetings and EU Stakeholder meetings
17-18 February 1997		A German Federal Environment Agency Workshop on the Effects of Endocrine Disruptors in the Environment on Neuronal Development and Behaviour, Berlin, Germany (UBA 1997)
March 1997		A workshop on screening methods for detecting potential (anti-) oestrogenic/androgenic chemicals in wildlife held in Kansas City, Missouri and sponsored by the United States Environmental Protection Agency, the Chemical Manufacturers Association and the World Wildlife Fund (Ankley <i>et al.</i> 1998).
10-13 April 1997		A Society for Environmental Toxicology and Chemistry (SETAC)/OECD/EC Expert Workshop on Endocrine Modulators and Wildlife: Assessment and Testing (EMWAT), Veldhoven, The Netherlands (SETAC 1997)
November 1997		Meeting organised by the Services of the Commission (DG XII) to identify the research being funded by the European Commission (under the Fourth Framework Programme) and by national bodies in 15 member and Associated States (EC1997)
10-11 March 1998		1 st Meeting of the Mammalian Effects Working Group of the OECD Task Force on Endocrine Disrupter Testing and Assessment (EDTA)
11-13 May 1998		A workshop on characterising the Effects on Human Health at Environmental Exposure Levels, Raleigh, North Carolina
October 1998	European Parliament adopted Resolution A4-0281/98 on endocrine-disrupting chemicals	
12-13 November 1998		2 nd Meeting of the Mammalian Effects Working Group of the OECD Task Force on Endocrine Disrupter Testing and Assessment
12-15 December 1998		A Society for Environmental Toxicology and Chemistry Workshop on Endocrine Disruption in Invertebrates: Endocrinology, Testing and Assessment (EDIETA), Noordwijkerhout, The Netherlands (SETAC 1999)
February 1999		1 st Meeting of the OECD Validation Management Group (VMG) for mammalian effects in Tokyo

Table 1.2 Continued

Date	Policy documents and reports	Workshops, OECD meetings and EU Stakeholder meetings
4 March 1999	The European Commission Scientific Committee for Toxicity, Ecotoxicity and the Environment (SCTEE) presented its Opinion <i>"Human and Wildlife Health Effects of Endocrine Disrupting Chemicals, with emphasis on Wildlife and on Ecotoxicology test methods"</i> (SCTEE 1999)	
April 1999		An Expert Panel meeting on Opportunities for Collaborative EU/US Research Programs as part of the EU/US Trans-Atlantic Co-operation in Human and Environmental Health in Ispra (EU/US 1999)
23-24 May 1999		OECD Expert Group on Test Guidelines for Avian Reproductive Toxicity Testing, Leipzig
27 May 1999		EU stakeholder meeting to discuss the establishment of a priority list of substances for further evaluation of their role in endocrine disruption.
May 1999		3 rd Meeting of the Mammalian Effects Working Group of the OECD Task Force on Endocrine Disrupter Testing and Assessment
28-29 October 1999		1 st OECD Expert Consultation on Endocrine Disrupter Testing in Fish (EDF1) in London
December 1999	The Commission adopted a Communication to the Council and to the European Parliament on a Community Strategy for Endocrine Disrupters: a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706)	
20-21 January 2000		2 nd Meeting of the OECD Validation Management Group (VMG) for Mammalian Effects in Paris
15-16 March 2000		2 nd OECD Expert Consultation on Endocrine Disrupter Testing in Fish (EDF2) in Tokyo
30 March 2000	Council of Ministers (Environment) adopt Council Conclusions on the Commission Communication COM(1999)706	
May 2000		4 th Meeting of the Mammalian Effects Working Group of the OECD Task Force on Endocrine Disrupter Testing and Assessment
10-11 May 2000		EU-US High level Consultation on the Environment agreed to share

		information on a regular basis on priority setting, screening and testing as well as research activities
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Table 1.2 Continued

Date	Policy documents and reports	Workshops, OECD meetings and EU Stakeholder meetings
27-30 May 2000		A workshop on "Hormones and Endocrine Disrupter in Food and Water: Possible Impact on Human Health" in Copenhagen
July 2000	Report (A5-0197) of the Committee on the Environment, Public Health and Consumer Policy of the European Parliament on the Commission Communication to the Council and to the European Parliament on a Community Strategy for Endocrine Disrupters (COM (1999) 706 final)	
26 October 2000	European Parliament adopted Resolution A5-0197/2000 on endocrine disrupters, emphasising the application of the precautionary principle and calling on the Commission to identify substances for immediate action	
8-9 November 2000		EU stakeholder meeting on the implementation of the Community Strategy for Endocrine Disrupters (COM(1999)706)
March 2001		1 st meeting of the OECD Validation Management Group (VMG) for Ecotoxicological Effects
May 2001		5 th Meeting of the Mammalian Effects Working Group of the OECD Task Force on Endocrine Disrupter Testing and Assessment
June 2001	The Commission adopted a Communication to the Council and to the European Parliament (COM(2001)262) on the implementation of the Community Strategy for Endocrine Disrupters: a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706)	

2. Information exchange and international co-ordination

2.1 Objectives of the session

- To exchange information and to stocktake on areas where international co-ordination can speed up and make efficient use of resources;
- To identify ways and means to facilitate international co-ordination on work on endocrine disruption.

Background information on this workshop theme is contained in Appendix C.

2.2 Issues to be addressed

1. How can a robust strategy be implemented to ensure current information on endocrine disruptors is available to all interested parties?
2. How can international links between regulators, industry and academia be further optimised with regard to co-ordination of research?
3. How can international and national websites providing information on endocrine disruption be integrated most effectively?
4. What types of coordinating activities or mechanisms are most feasible at an international level?

2.3 Report of the workshop discussions

This plenary session primarily consisted of a series of platform presentations describing the activities related to endocrine disruptors of the European Commission, the Organisation for Economic Cooperation and Development (OECD), the International Programme on Chemical Safety (IPCS), the United States Environmental Protection Agency (US EPA) and the Japanese regulatory agencies. The overheads from the presentations are given in Appendix G.

The discussions in the session following the presentations considered a number of issues:

- availability of information on project addressing endocrine disrupting effects in humans and wildlife and data on exposure to and effects of endocrine disrupting chemicals;
- liaison between national and international regulatory bodies on the nature and funding of work on endocrine disruption;
- communication of information to the public in an easily understood format.

2.4 Recommendations from the workshop

The following recommendations resulted from the workshop session:

- Easily accessible and regularly updated databases providing information on endocrine disrupting chemicals and on-going research projects (such as GEDRI and the MRC Institute for Environment and Health REDIPED database need to be available to the scientific community.
- Literature information systems need to be established which provide information on the current situation regarding endocrine disruption at a range of levels (from the simple to the technical) for different target groups (from the general public to researchers). In these system links between key websites need to be established for easy access of information.
- A committee for implementing and updating the Global Assessment of Endocrine Disruption (GAED) state-of-the-science report after its publication needs to be established and resulting recommendations should be implemented.
- There is a need for better exchange of information and data between scientists involved in national research and their national representatives engaged in OECD activities so that new findings and techniques are fed into test method development.

3. Research and Development

3.1 Objectives of the session

- To review recent findings on the range of effects associated with endocrine disruption.
- To share results/help create synergies and co-ordinate work amongst European researchers involved in national and Community research projects as well as in industry-funded research work.

Background information on this workshop theme is contained in Appendix D.

3.2 Issues to be addressed

1. What is the extent of the endocrine disruption problem?
 - *What is the latest evidence for effects in humans?*
 - *What is the latest evidence for effects in wildlife?*
 - *Are the problems of regional or global concern?*
2. What information is still missing? particularly concerning:
 - *mechanisms of action and the phenomenon of synergy (supra-additivity)*
 - *low dose effects*
 - *non-oestrogenic effects*
 - *concept of adverse effects and the issue of causality*
 - *effects on populations and ecosystems*
3. What other tools are needed to assess the effects of endocrine disrupters?
4. What research on endocrine disrupters is being carried out by national bodies?

3.3 Report of the workshop discussions

The session began with a presentation by the European Commission, Research Directorate which outlined the objectives of the forthcoming Dedicated Call for Proposals for Endocrine Disrupters under the 5th Community Framework Programme on Research and Development.

3.3.1 What is the extent of the endocrine disruption problem?

What is the latest evidence for effects in humans?

- There is no hard evidence for **causal** links between chemicals and endocrine disruption in humans. However, there are identified health problems that may result from exposure to endocrine disrupters.

- It was in general strongly doubted whether the proof of causality is possible at all, particularly in the case of cancers which are diseases with potentially long latency periods.
- Since the Weybridge workshop in December 1996 there is more knowledge available on exposure and more findings indicating an **association** of endocrine disrupter exposure and human endocrine effects even at very low doses. It is therefore considered more probable that endocrine disrupter effects are causally linked with negative influences on human health.
- Sperm quality is decreasing and testicular cancer is increasing but with strong regional variability.
- Trends in breast cancer and other effects in females are also of concern.
- A recent EU-funded study in the Netherlands, a study in Germany and studies in the United States have shown an association between environmentally relevant PCB concentrations and negative influences on neuronal development.
- Accidental poisoning of humans (e.g. the Yusho accidents) show that certain compounds are in principle capable not only of interfering with the endocrine system but also of inducing marked effects in the exposed population and their progeny at high and low doses.
- Exposure studies are in progress and are important in attempting to establish causal relationships. Use of biomarkers would add value to such studies.

What is the latest evidence for effects in wildlife?

- In fish endocrine mediated effects (VTG induction, intersex) were reported for other countries than the United Kingdom indicating a European or global dimension of the problem. Environmental androgens have been identified in fish exposed to pulp mill effluents (in Sweden and the United States), discharges from sewage treatment works (in Sweden) and in concentrated cattle feed operations (in the United States). In the United Kingdom and Denmark estuarine fish populations are affected.
- There is clear evidence of oestrogenic activity in United Kingdom estuarine sediments due to substances strongly adsorbed to particulates, though this is not as evident for freshwater sediments. Androgenic activity is lower than oestrogenic activity in United Kingdom estuaries and is mainly due to natural androgens.
- It is noteworthy that not only feminisation but also masculinisation occurs in eelpout from Sweden near pulp mills.
- Though individual effects are quite clear, in many cases the ecological relevance has not been addressed. Only a few studies link responses in individuals to population effects. One Canadian study on effects in salmon seems to demonstrate such a link.
- Studies with fish need to include assessments of reproductive success.
- For invertebrates, the endocrine signalling systems are highly variable so that a number of different invertebrates have to be considered. Only a few studies have been carried out with invertebrates but these have identified substances which result in oestrogenic, androgenic and anti-androgenic effects. The outcome of the SETAC EDIETA workshop has been almost totally ignored for the design of monitoring programmes and test development. This is surprising as TBT-induced imposex is often referred to

as one of the best documented examples for endocrine disruption in the animal kingdom.

- No data is available on most invertebrate groups that could play an important role in the transfer of endocrine disrupters from sediments to fish via the food chain.
- For amphibians there is a large body of work in the United States and Australia showing declines in populations, but less for Europe. Amphibians and reptiles are an important group with endocrine sub-systems (e.g. thyroids) which are different to fish. There is some indication that they are a vulnerable group.
- In birds there are known developmental problems (e.g. pesticide induced egg shell thinning) but contradictory population observations.
- Endocrine disrupters in soils may enter water systems via runoff and are not being adequately considered.
- Insufficient studies on natural variance have been carried out even though this was recommended by the SETAC EMWAT workshop.
- Despite the large number of national studies there has still been no coordinated European comparison of endocrine disruption effects in specific wildlife groups. This comparison could give important information on the environmental loads of endocrine disrupters.

Are the problems of regional or global concern?

- Although not recognised by all participants it was generally accepted that the problem of endocrine disruption is of global importance in terms of effects on human health and wildlife.
- In wildlife, VTG induction and intersex in fish populations, and imposex in snails are global problems.
- Immunotoxic effects in seals are at least of concern in the European scale with some evidence for a global problem.
- Endocrine disrupter problems need to be studied locally since the effects are variable and depend on local covariates.

3.3.2 What information is still missing?

Mechanisms of action and the phenomenon of synergy (supra-additivity)

Information is needed on:

- genomic- and proteomic-based approaches related to endocrine disrupter exposure and effects;
- SARs especially those describing processes other than receptor binding;
- the effects of endocrine disrupters on transcription mechanisms;
- effects on gene expression and on sexual development
- behavioural effects including brain development;
- genotoxic mechanisms leading to effects in male germ cell lines;
- ways to enhance the predictive capability of endpoints;
- the identification of risk factors in epidemiology studies;
- anti-androgenic effects;
- the problem of sensitive individuals in populations;

- the extent to which synergism exists;
- effects of mixtures and metabolites;
- critical windows of exposure.

A German *in vitro* study shows moderate over-additive effects of endocrine disrupters but the conclusion was not clear because of methodological shortcomings. Antagonistic or supra-additive effects need to be shown to be reproducible before they can be considered to constitute mechanisms of actions.

Low dose effects

- There is some evidence that a threshold concentration or dose for endocrine disrupters might not exist. Low dose endocrine disrupting effects have been shown not only for bisphenol A but also for other suspected compounds.
- Definitive data is needed on whether threshold levels of endocrine disrupters are found for all endpoints. Full consensus was not reached on this issue.
- Differences between laboratories might be due to genetic or general biological variation in the animal models. A step-wise approach is recommended for the investigation of inter-laboratory differences.
- Low dose toxicology is an important research topic and can benefit from lessons learned in immunotoxicology.
- There is a need to address long-term low dose effects.
- Food chain exposure assessment should be studied.
- Additional information is needed on the existence of irregular dose-response relationships.
- The results of studies need to be linked to mechanisms of action.
- Low dose issues complicate evaluation of effects and the use of data for risk assessment. Similar assessment strategies as for carcinogens (acceptable risk) should be considered to address this issue.

Non-oestrogenic effects

- There is only a historical (or traditional) reason for focusing on oestrogenic effects. Other parts of the entire endocrine system such as androgens, the thyroid, and the adrenal system have to be considered.
- A number of different endpoints are of interest, especially for research.
- However, it must be recognised that only a few chemicals are well documented.
- It is important to consider the effects of endocrine disrupters on a wide range of taxonomic groups which may have different endocrine systems (for example invertebrates). These may result in different response patterns and sensitivities.
- The main emphasis to date has been on receptor mediated effects while *in vivo* studies can additionally address potential indirect effects of test compounds.
- Techniques such as genomics and proteomics may provide the technological answer but validation work has still to be completed. Issues surrounding the

link to regulation and how should results be interpreted also need to be resolved.

Concept of adverse effects and the issue of causality

No consensus was reached on the concept of adverse effects. Two different views were put forward from different parallel groups:

1. All measured physiological effects should not necessarily be considered to be adverse. In human and ecological studies the evidence for causality may only be circumstantial and based on weight of evidence.
2. Any change from the norm is believed to be adverse and should be considered for regulatory purposes. This requires historical controls or good baseline knowledge.

On the issue of causality, research is urgently needed (see Section 3.3.1).

Effects on populations, ecosystems and communities

- Information on population effects is important but has not yet been clearly established in the field. Identification of causal relationships between endocrine disrupter exposure and population effects remains problematic.
- It should be remembered that although the ultimate objective of ecotoxicology is the protection of wildlife populations, data on the effects on individuals can be used for hazard assessment and decision-making,
- Some full life cycle tests may allow estimations to be made of possible population effects using modelling approaches.
- Effects on sensitive groups need to be addressed.
- There is a need for dialogue between laboratory-based and field-based workers.
- There are different problems for humans and ecosystems.
- Ecosystem effects are hard to define and this is a developing scientific discipline which is important for the risk assessment of endocrine disrupters.

3.3.3. What other tools are needed to assess the effects of endocrine disrupters?

- Further epidemiological studies particularly if the data could be more than just indicative of effects.
- Advanced analytical techniques to determine endocrine disrupter doses at the target sites; analytical chemistry of endocrine disrupters in conjunction with TIE studies;
- General genomic methods, particularly those for key species with well sequenced and widely accessible genomes.
- Approaches for assessing different responses among groups in the same population.

- Approaches whereby the same (wild) organisms can be studied in the laboratory and the field, especially where these organisms can be used to measure a range of endpoints (for example sticklebacks for evaluating both oestrogenic and androgenic effects).
- Tools to address exposure to endocrine disrupters, effects on immune systems and behavioural and neurological development; testing of taxa such as invertebrates.
- Literature information systems and databases on endocrine disrupting chemicals and on-going research projects (as infrastructure support for research purposes) should be established. One of the working groups encouraged the Commission to take more pronounced responsibility in this field.
- Mechanisms to periodically update the GAED state-of-the-science report after its publication and to implement its recommendations.
- Mechanisms to provide updates on the development of new tools.
- Mechanisms to implement recommendations from the SETAC EMWAT and EDIETA workshop publications.

3.3.4. What research on endocrine disrupters is being carried out by national bodies?

- The use of three spined sticklebacks (*Gasterosteus aculeatus*) to identify endocrine disrupters. Swedish EPA Research Grant 2001-2002 to Bengtsson and Hahlbeck.
- Crustacean full life cycle tests to assess effects of chemicals on reproduction and development. Swedish Mistra Research Grant 1999-2002 (Part of National Research Programme A New Strategy for the Risk Management of Chemicals) to Bengtsson and Breitholtz.
- In June 2001 the Austrian Research Co-operation on Endocrine Modulators (ARCEM) launched a 3 year research programme on endocrine disruption (<http://www.arcem.at>). The study will assess concentrations of relevant endocrine disrupting substances in ground- and surface water via chemical analysis methods as well as new *in vitro* screening methods (yeast assay and MCF7 assay). The effect of these chemicals on fish species like brown trout and chub will be assessed in the laboratory and field by methods like the vitellogenin assay, histopathological analysis of fish gonads and morphometric studies. Taking into account all available results, the risk to fish and humans (drinking water) will be quantified. A further main focus will be the identification of effective techniques for eliminating relevant endocrine disrupting substances in the processes of drinking water purification and waste water treatment.

3.4 Recommendations from the workshop

A plenary session reviewed the feedback from each of the breakout groups and considered the views expressed. It was evident that there was agreement between the groups on key areas which needed to be addressed in forthcoming research and development activities. The following overall recommendations resulted from the workshop session:

3.4.1 Endocrine disruption in humans

- Future studies should work towards a full appreciation of endocrine disruption effects on human health which includes all potential target sites (particularly those associated with adrenal function and steroidogenesis).
- Future studies need to translate associations between exposure to endocrine disrupters and identified effects on human health (decrease in semen quality, increase in hypospadias, testicular and prostate cancer in males and breast and ovarian cancers in females) into causative links. It should be recognised that since cancers are multi-factorial diseases with a potentially long latency period it may be extremely difficult to establish causality between exposure and the suspected effect.
- More detailed assessments of exposure in humans (to all endocrine disrupting chemicals by all routes) are needed.
- There should be thorough investigations of aetiological factors in current human endocrine conditions (for example thelarche in Puerto Rican baby girls).
- Further information on risk factors in epidemiology studies is needed (with environmental and socio-economic factors being considered).
- Research studies should include input from endocrinologists.

3.4.2 Endocrine disruption in wildlife

- Studies on natural variability in measures of endocrine disruption need to be carried out in accordance with the recommendations of the SETAC EMWAT workshop.
- The population consequences of endocrine disrupter effects in individuals need to be established to provide a better understanding of the ecological relevance of the assessed effects.
- Further studies on the effects of endocrine disrupters on amphibians/ reptiles are needed since this important (and vulnerable) group have endocrine systems (e.g. thyroid) which are different to fish and they may show effects not evident in other taxa.
- Further studies should consider endocrine disrupter effects on birds, possibly through the development of a model system in which the effects on organism

development are measured following injection of chemicals into the yolk of the egg.

- Test methods for invertebrates need to be developed (taking into account the recommendations of the EDIETA workshop) but this needs to be underpinned by a greater understanding of the endocrinology of this important taxonomic group.

3.4.3 Regional and global concerns

- The environmental reasons for regional differences in human health effects (such as differences in semen quality between Denmark and Finland) need to be identified. This may link chemical aetiology to health effects.
- For fish, a European comparison of endocrine disrupter effects is needed, but this will require harmonisation of methodologies (for example the test species and test design).

3.4.4 Mechanisms of action of endocrine disrupters

- A better mechanistic understanding of the action of endocrine disrupters is needed, particularly where the responses are not receptor mediated.
- Future studies should not focus on oestrogenic effects but consider other parts of the endocrine system: for example (anti-) androgenic, (anti-) thyroid and (anti-) adrenal effects.
- Future studies should address the effects of endocrine disrupters on immune systems and behavioural and neurological development.
- Greater use should be made of genomics (techniques to identify the DNA sequence of the genome), transcript profiling (techniques to identify the mRNA from actively transcribed genes), proteomics (techniques to identify the proteins in a biological sample) and metabonomics (techniques to identify the presence and concentrations of metabolites in a biological sample). These GTPM approaches require additional development and validation, but could provide a vast range of additional useful endpoints. Their careful application could assist research associated with safety assessment by enabling a more detailed appreciation of molecular mechanisms of action and facilitating more rapid screens for effects.

3.4.5 Measurement of exposure

There is a need to develop analytical procedures to assess exposure to endocrine disrupters, in particular this relates to:

- integrated analytical chemistry/TIE procedures;
- methods for the detection and quantification of endocrine disrupters in complex matrices such as sediments;

- information on the potential role of invertebrates in the transfer of endocrine disrupting chemicals from sediments to fish via the food chain.

3.4.6 Measurement of effects

Future studies should focus on:

- critical windows of exposure in humans and wildlife (especially for development);
- ways to enhance the predictive capability of endpoints;
- the issue of sensitive individuals in populations.

Furthermore future studies need to consider the issues of:

- low dose effects where there are unusual dose response curves that traditional regulatory toxicology protocols may miss;
- the extent to which mixture effects occurs in terms of:
 - antagonism, additivity or supraadditivity;
 - mixture effects involving more than one molecular target (in mammals what is the effect to the male foetus of exposure to a weak oestrogen and a weak anti-androgen?).
- the issue of adverse effects and what level of change should be regarded as significant. It is important that sufficient information is available on natural variability to allow potential changes due to chemicals to be identified.

3.4.7 Funding and policy issues

Mechanisms need to be established to facilitate long-term studies which address:

- Endocrine effects in long-lived species;
- Whether regulatory actions related to endocrine disrupters result in improvements in the environment.

4. Development of test methods and testing strategies

4.1 Objectives of the session

- To review progress on test method development in the framework of the OECD;
- To discuss with EU Member and Associated States an appropriate testing strategy in the light of existing EU legislation and the recent White paper on a future chemicals policy.
- To identify research requirements to underpin the development of test methods at OECD.

Background information on this workshop theme is contained in Appendix E.

4.2 Issues to be addressed

1. Have appropriate test species and test endpoints been identified for detection of potential endocrine disrupters; what are the key criteria for making such an assessment?
2. Will the test procedures being proposed provide robust repeatable data for the purposes of hazard/risk assessments?
3. Will the data generated by the proposed test procedures be sufficient and consistent with the overall approach to hazard identification and risk assessment in existing Community legislation?
4. Are there specific issues related to test methods and testing strategies for endocrine disrupters which need to be further considered in the future chemicals policy?
5. Do the proposed test endpoints consider the potential effects of all types of endocrine disrupters, since concern to date has focussed on oestrogen and androgen agonists or antagonists and thyroid effects but not adrenal effects?
6. Could further use be made of current or modified *in vitro* tests as an appropriate means of screening potential endocrine disrupting chemicals provided that the responses measured in these tests are representative of those recorded in *in vivo* tests?
7. Are additional mechanisms needed to ensure effective communication of (i) results from new relevant research to test method development and (ii) results from validation work on test method development, including new research needs, back to research and development?

4.3. Report of the workshop discussions

This report provides a summary of the discussions of the working groups with regard to the issues identified in Section 4.2.

4.3.1. Have appropriate test species and test endpoints been identified for detection of potential endocrine disrupters; what are the key criteria for making such an assessment?

There was agreement on the existing OECD approaches for detecting oestrogenic, androgenic and thyroid effects in mammals, fish and birds, but it was recognised there was a need:

- for whole animal tests to cover all possible endocrine disrupting effects (including adrenal function and steroidogenesis);
- to focus on test methods assessing reproductive function and developmental effects.

For chronic mammalian tests there is a need for the refinement of test designs and enhancement/improvement of test endpoints to better cover sensitive periods of the test organisms life history and to better capture hormonal effects.

It was recognised that the number of tests in the overall battery need to be limited but they need to be able to obtain information about differences between species and strains.

It was recognised that currently the OECD activities are not covering all of the issues and there was a need for further work on the development of:

- tests using invertebrates (which is underpinned by a greater understanding of the endocrinology of the test species used and builds on the recommendations of the SETAC EDIETA workshop, DeFur *et al* 1999);
- alternative techniques such as Structure Activity Relationships (SARs) and *in vitro* tests (beyond those currently under development) which could be used as a complementary tool (but not a substitute) for *in vivo* testing as part of a strategy to target testing and limit the use of animals. It was recognised that within the United States Endocrine Disrupter Screening Programme, SAR models (based on oestrogen and androgen receptor binding) and *in vitro* tests (including oestrogen and androgen receptor binding reporter gene assays and a steroidogenesis assay) are being evaluated as part of the overall testing programme (see Appendix G). In Japan an inter-ministry collaboration exercise on endocrine disruption has also been developing SARs. Attention should be focused on models involving processes other than receptor binding.
- Genomics, transcription profiling, proteomics and metabonomics (GTPM) which could provide powerful tools in the future and which could be used to target testing, address low dose effects, mixtures and decrease numbers of animals used in testing. It was suggested that the development of these tools which has been started should be focussed initially on endocrine disrupter related activities.

ECETOC have recently published an introductory document on this subject (ECETOC 2000).

It was also recognised that there is a need for better exchange of information and data between scientists involved in national research and those engaged in OECD activities.

The workshop session noted that OECD had developed criteria for test guidelines which would be applicable to tests for endocrine disrupters. It was proposed that these would be discussed at the next competent authorities meeting.

4.3.2. Will the test procedures being proposed provide robust repeatable data for the purposes of hazard/risk assessments?

Clearly the provision of robust and repeatable data is an essential element of the tests method development programme but at present it was not possible to answer the question for most of the proposed OECD tests.

The workshop session noted that the overall view of the work carried out to date was positive but also noted that any assessment of a test procedure had to bear in mind the role of the procedure, thereby ensuring it was 'fit for purpose'.

The data on the work carried out on the OECD Uterotrophic Test Guideline had been independently reviewed and the data along with a summary of the review was available for inspection. This process would be carried out for the other methods under development by the OECD.

It was proposed that the results of the enhanced TG407 could be compared with the results of the pubertal assays when these were completed.

For all the tests developed it was recognised that there was a need to assess false positive and negative rates so that it would be possible to ascribe a level of confidence to the predictive capacity of a given test.

4.3.3. Will the data generated by the proposed test procedures be sufficient and consistent with the overall approach to hazard identification and risk assessment in existing Community legislation?

The workshop session made the following observations regarding the development of testing strategy for endocrine disrupters by OECD:

- the OECD programme has to date focussed on development of test methodologies rather than strategies;
- that no testing strategies can guarantee complete safety;
- that any test strategy should be considered as a whole and will have to take account of pragmatic considerations such as costs and animal welfare issues.

It was noted that there may be limitations of the current testing requirements for both:

- new chemicals which principally focus on acute toxicity and take limited account of potential endocrine disruption effects;
- pesticides which though more rigorously tested may also need to be assessed to take account of endocrine disruption endpoints particularly in relation to effects on invertebrates which may not be covered by existing procedures.

The workshop session raised concerns that endocrine disruption should be taken into account for all chemicals assessment and management including food additives, cosmetics, human and veterinary medicines. There is also a need for integrated risk assessment so that findings from ecotoxicity testing are considered in mammalian risk assessment and vice versa.

It was noted that:

- there is an international activity (IPCS/WHO) on integrated risk assessment of endocrine disrupters using information in the published literature.
- risk assessments had been carried out in different countries for the same chemicals and also that some of these chemicals had been used as reference chemicals in the OECD work. The results of these could be compared and any lessons learned.
- A NATO Advanced Research Workshop on “Endocrine Disrupters and Cancerogenic Risk Assessment” was held in Poland in May 2001 and the proceedings will be published by the end of May 2002.

In terms of potential data gaps those identified in the discussion of Issue 1 were reiterated, namely

- that the OECD focus effort on test method development for invertebrates (particularly molluscs);
- that *in vitro* screening tests are needed, with information being available about the predictive capability of the tests relative to *in vivo* tests.

It was noted that as more information becomes available there would be a need to revisit these issues.

The workshop also identified a need for a comparison of information on thyroid effects in different species so as to determine how to address the issue of species redundancy.

In terms of test design testing strategies should be designed to pick up apical endpoints relevant to all endocrine disrupting mechanisms with the determination of an endocrine disrupting mechanism being of secondary importance. However, knowing that a chemical has endocrine disrupting potential will influence the dosing regime in the test.

4.3.4. Are there specific issues related to test methods and testing strategies for endocrine disrupters which need to be further considered in the future chemicals policy?

Animal welfare issues

In terms of the issue of animal welfare and the need to minimise animal testing the workshop session had the following conclusions:

- there was support for the use of SARs and *in vitro* test systems as a supplement to *in vivo* tests but a recognition that these need to be used responsibly and intelligently;
- there was support for a more flexible approach to testing guided by SARs and *in vitro* testing, for example some Tier 1 screening may be unnecessary;
- it was noted that *in vivo* testing was needed to validate SARs and *in vitro* systems
- *In vivo* testing would continue to be needed but there was a recognition of the 3 Rs – replacement, refinement and reduction (as defined by Russell and Birch 1959) ;
- the most effective possible use of whole animals studies should be made to include all potential target sites (for example the adrenals) and ensure that the test species used are well-characterised (this being particularly important in the case of invertebrates and fish;
- its was noted that an increase animal testing may be needed now to improve understanding so that fewer animals could be used in future;
- in *in vivo* tests better use should be made of statistical techniques which take account of all of the concentration (or dose) response data and do not focus only on the NOEL (No Observed Effect Level) or NOEC (No Observed Effect Concentration). This will be the most efficient way to use test organisms and the data derived from them.

Drivers for testing

The workshop session considered the drivers for conducting tests on potential endocrine disrupters and had the following conclusions:

- the approach for determining whether testing of potential endocrine disrupters is required needs to be more sophisticated than being based on production volume data alone and should include data on the pattern of exposure of substances in the environment and their persistence, bioaccumulation and toxicity. For example the use of only *in vitro* testing for chemicals produced in 1 – 10t quantities was questioned but no consensus was reached;
- there were requirements on primary producers of chemicals and downstream users where chemicals were used for purposes other than those originally specified;

- there was a need for an intelligent approach to testing homologous series of chemicals and the use of bridging studies to assist in this regard. Further research was needed and OECD should be asked to co-ordinate standardisation and validation of partial lifecycle studies to be used for bridging;
- it was suggested that a simple framework to determine testing needs should be developed, with the approach used in the Biocides Directive being proposed as a possible example;
- it was recommended that discussions should begin on developing an EU strategy for testing endocrine disrupters. It was noted that it may be too early to do this but scientific and political discussions were needed on issues raised by chemicals currently going through ESR process and the application of interim arrangements. It was recommended that Annex V Committee to 67/548 should coordinate EU views and activities on test methods and strategies to feed into the OECD;
- there are potential limitations on the current capacity for testing chemicals in Europe and the associated costs (financial and number of animals) could be high.

Low dose effects

The discussions on the workshop session on low dose effects resulted in the following conclusions:

- there is a need for a clear definition of what is meant by 'low dose effects';
- further research is needed to resolve the issue and this should include clarification of exposure patterns and sensitive windows of exposure;
- there are plausible mechanisms for low dose effects but questions remain about the significance of the effects;
- there are examples of low dose effects in immunotoxicology;
- if low dose effects are confirmed, the implications for testing, risk assessment and risk management will need to be assessed.

Mixtures

The discussions in the workshop session on the effects of mixtures of endocrine disrupters resulted in the following conclusions:

- the issue of mixtures is one which applies generally and not just to endocrine disrupters. It is an extremely complicated issue, which cannot be easily solved at the risk assessment stage;

- with endocrine disrupters acting through the same mechanism additivity but not synergy has been observed. It should be possible to address the issue of mixtures through the activities of industry consortia and regulatory assessment of product groups instead of individual chemicals;
- work carried out on assessing the effects complex emissions and whole effluents using a Direct Toxicity Assessment approach and assessing receiving waters using biological effects measures was noted, and particularly the ability of such approaches to detect unexpected effects.
- Suggested approaches to dealing with mixture issues include restrictions on use, possibly related to potency, or a safety factor approach.

4.3.5. Do the proposed test endpoints consider the potential effects of all types of endocrine disrupters, since concern to date has focussed on oestrogen and androgen agonists or antagonists and thyroid effects but not adrenal effects?

The workshop session considered that not all potential effects of endocrine disrupters were currently being considered, gaps including impacts on the adrenal system, pheromone signalling and the neuro-endocrine-immune axis.

In wildlife studies it was considered that other potential effects of endocrine disrupters could be assessed by making greater use of histopathology.

4.3.6. Could further use be made of current or modified *in vitro* tests as an appropriate means of screening potential endocrine disrupters provided that the responses measured in these tests are representative of those recorded in *in vivo* tests?

The workshop session considered that further use could be made of *in vitro* tests for pre- screening (and possibly screening), but there was a need for

- the validation of the techniques based on the principles of the OECD Workshop on Harmonisation of Validation and Acceptance Criteria for Alternative Toxicological Test Methods held in Solna in January 1996 (OECD 1996);
- an understanding of the rates of false positives and negatives;
- identification of the limitations of the approach (for example the absence of consideration of metabolism effects).

There was no discussion of how *in vitro* tests might be used in practice but there were suggestions that the European Union could learn from the experiences from the United States and Japan. The information on *in vitro* tests was continually increasing and needed to be constantly re-assessed.

It was again noted that there was a need to carry out *in vitro* and *in vivo* tests in parallel to enable comparisons to be made, but also that this had significant financial implications.

4.3.7. Are additional mechanisms needed to ensure effective communication of (i) results from new relevant research to test method development and (ii) from validation work on test method development, including new research needs back to research and development?

In terms of effective communication the workshop session had mixed experiences, with some members of group feeling that communication was good while others felt they were not aware of what was happening.

It was noted that information on the OECD programme was available on their website but this was not widely known. It was stated that OECD was doing more to publicise the website. It was also noted that there was an OECD reference set of chemicals which could be publicised.

The workshop suggested that scientists could be encouraged to post endocrine disrupter data on the worldwide web but there would be question marks about the quality assurance/control applied to the generation of the data. However, there were mixed views about the value of quality assurance/control systems such as Good Laboratory Practice (GLP). It was noted that the Endocrine Disrupter Testing Assessment (EDTA) Validation Management Group for Mammalian Effects (VMGMammalian) had agreed that its work would follow GLP standards but there would be no auditing. It was recognised that GLP does not guarantee quality studies.

4.4 Recommendations from the workshop

The following recommendations resulted from the workshop session:

4.4.1 Test methods and test design

Existing OECD approaches for detecting oestrogenic, androgenic and thyroid effects in mammals, fish and birds represent a scientifically robust approach but there is a need:

- for whole animal tests to cover all possible endocrine disrupting effects (particularly adrenal function and steroidogenesis);
- to focus on test methods assessing reproductive function and developmental effects;
- for the refinement of chronic mammalian test designs and an enhancement/improvement of the test endpoints to better cover sensitive periods of the test organisms life history and to better capture hormonal effects.

OECD activities are not covering all of the issues and there is a need for further work on the development of:

- to develop tests to address impacts on the adrenal system, pheromone signalling and the neuro-endocrine-immune system;

- tests using invertebrates (which are underpinned by a greater understanding of the endocrinology of the test species used);
- alternative techniques such as SARs and *in vitro* tests (beyond those currently being developed or evaluated) which could be used to target testing and limit *in vivo* testing. However, alternative tests need to be used responsibly and intelligently *and in vivo* testing is needed to validate SARs and *in vitro* systems.
- Genomics, transcription profiling, proteomics and metabonomics (GTPM) approaches which could provide powerful tools in the future and which could be used to target testing, address low dose effects, mixtures and decrease numbers of animals used in testing. It was suggested that the development of these tools which has been started should be focussed initially on endocrine disrupter related activities.
- Comparison information on thyroid effects in different species so as to determine how to address the issue of species redundancy.
- A clear definition of what is meant by 'low dose effects' and further research is needed to resolve the issue which should include clarification of exposure patterns and sensitive windows of exposure.

4.4.2 Drivers for testing

- The approach for determining whether testing of potential endocrine disrupters is required needs to be more sophisticated than being based on production volume data alone and should include data on the pattern of exposure of substances in the environment and their persistence, bioaccumulation and toxicity.
- There is a need for an intelligent approach to testing homologous series of chemicals and the use of bridging studies to assist in this regard. Further research is needed and OECD should be asked to co-ordinate standardisation and validation of partial lifecycle studies to be used for bridging.
- A simple framework to determine testing needs should be developed, with the approach used in the Biocides Directive being proposed as a possible example.
- Discussions should begin on developing an EU strategy for testing endocrine disrupters. The Annex V Committee to 67/548 should coordinate EU views and activities on test methods and strategies to feed into the OECD.

5. Establishment of monitoring programmes

5.1 Objectives of the session

- To define monitoring objectives, information needs and design requirements for monitoring programmes;
- To review available information from Member States/organisations/inventories;
- To review availability and identify development/validation for appropriate environmental tools and models for estimation of exposure.

Background information on this workshop theme is contained in Appendix F.

5.2 Issues to be addressed

1. What information needs to be generated from an integrated monitoring programme for endocrine disrupters?
2. How can a cost-effective monitoring programme be designed?
3. Under what circumstances should chemical-specific measurement and biological effects assessment be used for monitoring of endocrine disruption and what criteria need to be considered when making these decisions?
4. What biological effects assessment techniques are available to monitor endocrine disruption in humans and wildlife?
5. In the environment how can locations most at risk from inputs of endocrine disrupting chemicals be identified?
6. What monitoring information is currently available at national levels for potential endocrine disrupters in environmental compartments (water, soil, air and biota) as well as human exposure routes (water, food and consumer products)?
7. What gaps are there in existing monitoring programmes which need to be addressed with specific regard to endocrine disruption?
8. What is the current status of exposure models with regard to being used as an integral part of monitoring programmes?
9. What further work or information is necessary for the development, validation and implementation of exposure models?

5.3 Report on the workshop discussions

5.3.1. What information needs to be generated from an integrated monitoring programme for endocrine disrupters?

In considering the establishment of a monitoring programme for endocrine disrupters there are a number of policy issues that need to be addressed, namely that:

- the information generated needs to be 'fit for purpose' (particularly in relation to providing information for answering policy questions);
- it includes measurements of exposure and effects (on all potential endocrine target sites) in an integrated chemical and biological (ecotoxicological and, where appropriate ecological¹) approach;
- it provides data which can be used to protect sensitive environmental compartments and also sensitive target groups.

For the protection of the human health or the environment it is also important that monitoring programmes are able to answer a number of key questions, namely:

- what are the nature of the endocrine disrupter effects in the target group?
- what concentrations of causative agents are required to cause endocrine disrupter effects?
- at which locations are endocrine disrupting effects causing problems?
- are regulatory measures resulting in improvements in the environment with regard to endocrine disrupter effects?

What are not in place, yet needed, are programmes to determine if there are general effects caused by yet unknown chemicals on human health and wildlife (at a variety of ecological levels).

Furthermore for any biological-based monitoring programme of endocrine disrupters in wildlife it is also important to understand baseline levels of effects and the natural variability in effects under non-impacted conditions in order that actual effects can be distinguished from normal fluctuations. However, a key question is "what baseline should be used", which is often confounded by a problem with a lack of previous baseline data. Where the data is available it is important to examine historical 'baseline' monitoring data to detect possible long-term trends in populations. However, the wide range of endpoints that may be affected by endocrine disrupters means that there is generally a need to supplement these historical records.

Wider use needs to be made of environmental specimen banks that at present are only used in Germany and Nordic countries. These can provide material that can be used to obtain data retrospectively to monitor temporal trends. However, it is

¹ It has to be remembered that TBT-induced imposex in molluscs was initially identified as a result of the data generated by populations surveys of organisms.

important that there is harmonisation of approaches so that it is possible to compare results across European countries. It is also becoming increasingly important to be able to subsequently measure effects in stored samples.

5.3.2. How can a cost-effective monitoring programme be designed?

The workshop recognised that it is a significant challenge to prepare a cost-effective monitoring programme for endocrine disrupters that delivers key information on exposure and effects. A large amount of monitoring for endocrine disrupter effects in humans and wildlife has been carried out by different European countries on a national basis, but fewer studies have involved pan-European monitoring programmes. However, these studies need to be conducted using harmonised approaches so that the effects across Europe as well as within countries can be evaluated. The costs for such programmes and the time taken to initiate them may be excessive if all European countries are involved. Therefore, consideration should be given to initially investigating a sub-set of all countries which are likely to reflect the extent of the endocrine disrupter related human health and wildlife problems across all countries. These programmes should be used to investigate how cost-effective monitoring programmes can be established on a pan-European basis. In the first instance attention should also be paid to integrating new programmes with those that are already on-going such as:

- The **COMmunity Programme of Research on Environmental Hormones and ENdocrine Disrupters (COMPREHEND)** partnership consists of 13 research laboratories from 7 European countries. The partnership originated from the EurAqua network of freshwater research organisations and is funded by the European Commission (DG 12) under Framework IV (Contract No. ENV4-CT98-0798). The objectives of the programme are to identify the principal active oestrogenic compounds in complex sewage treatment works and industrial effluents, evaluate the natural partitioning of oestrogenic substances, evaluate long-term fisheries data for evidence of impacts that may be related to ED effects, develop new detection systems and apply existing screens to complex effluents.
- the **Endocrine Disruption in the MARine Environment (EDMAR)** programme in the United Kingdom. The EDMAR Programme is a joint initiative between the Department of the Environment, Transport and the Regions (DETR)², the Ministry of Agriculture Fisheries and Food (MAFF), the Environment Agency, the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER) and the European Chemical Industry Council. The programme involves 5 major laboratories and is investigating whether there is evidence of changes in the reproductive health of marine life and, if so, is seeking to identify possible causes and potential impacts on populations. The results from the programme will help to ensure that any actions necessary to protect the marine environment in the United Kingdom are targeted appropriately.
- The Dutch National Investigation on Estrogenic Compounds in the Aquatic Environment (LOES) which is coordinated and financed by the Dutch

² The Environment element of DETR and MAFF and now combined in the Department for Environment, Food and Rural Affairs (DEFRA)

government. The LOES project involves 13 different governmental bodies and universities, and it is linked to the EU funded COMPREHEND programme. The programme has the objectives of investigating the occurrence of known or suspected (xeno)-estrogens in different compartments of the Dutch aquatic environment (including waste water, surface water, drinking water and rain water), identifying sources of estrogenic activity in environmental samples and detecting possible impacts on natural fish populations. An additional objective of the programme is to recommend the compounds and biological effect techniques that would be most appropriate for continued monitoring.

It was recognised that the conclusions from the workshop session need to be considered in the light of recommendations made at the at the SETAC-Europe/OECD/EC Expert Workshop on Endocrine Modulators in Wildlife: Assessment and Testing (EMWAT)(Tattersfield *et al* 1997). The EMWAT workshop stated that *"The establishment of a limited number of co-ordinated programmes that cross international boundaries will maximise the possibility of finding evidence of endocrine modulation in wildlife including those areas which are not necessarily heavily contaminated. These programmes should be composed of scientists from different scientific disciplines that are the most competent for the task, regardless of nationality or affiliations. These programmes would examine a wide variety of endpoints in a few species at predetermined sites over at least three years. At this time, the results would be evaluated and the programme size adjusted accordingly. Another objective of such a programme would be to improve awareness and technical competence of citizens and scientists of OECD-member countries concerning the issue of endocrine modulation."*

The workshop session also identified the issue of the extent to which industry should contribute to the costs of monitoring and whether the 'polluter pays' principle was being applied appropriately within the EC in terms of recovering costs for environmental assessments.

Monitoring strategies

The workshop considered that monitoring programme for endocrine disrupters should consist of two elements:

1. ***hypothesis driven effects-based monitoring*** at hotspots based on modelling using production and use pattern data followed by the identification of causative agents using a combination of analytical chemistry/toxicity identification evaluation (TIE) exercises. The effects-based monitoring needs to be able to assess oestrogenic, (anti)-androgenic, thyroid and adrenal effects;
2. ***investigative monitoring*** at locations where there is little understanding of potential problems and a need to address specific issues.

It needs to be recognised that although the philosophy for human health and environmental monitoring may be the same the practicalities may require different mechanisms.

Examples of proposed monitoring schemes

Humans

For human health further use could be made of registers of reproductive effects (sperm counts, semen quality, infertility levels) and malformations (male and female cancers) to identify spatial and temporal trends across Europe. However, it will be necessary to standardise methodologies so that the data can be interpreted both within and across countries. It is also necessary to establish extensive but cost-effective schemes to monitor changes in parameters such as semen quality (linked with exposure studies) in appropriate target groups so that action can be taken before this is translated into decreases in fertility rates. Such programmes would work towards establishing the reasons for the differences in semen quality between countries such as Denmark and Finland.

Wildlife

The workshop session considered it important to revisit the recommendations from two previous workshops whose outputs were still considered relevant, namely the:

- SETAC-Europe/OECD/EC Expert Workshop on Endocrine Modulators in Wildlife: Assessment and Testing (EMWAT)(Tattersfield *et al* 1997);
- The SETAC Workshop on Endocrine Disruption in Invertebrates: Endocrinology, Testing and Assessment (EDIETA) (DeFur *et al* 1999).

The SETAC EMWAT workshop developed a generalised scheme for monitoring the effects of xenobiotics, including endocrine disrupters, on wildlife (see Table 5.1). The methodology recognised that *“there are several situations that the investigative programme can address: general screening for potential adverse effects of endocrine disrupters where there is little understanding of potential problems, or increasingly well characterised situations where a putative endocrine disrupting compound(s) is known to occur in the environment. If there is a little more detail on the system, for example evidence that endocrine disrupters are present, then monitoring may progress at a more detailed level on individuals. The species examined should be selected for characteristics which would maximise their exposure or risk but it must be acknowledged that factors associated with risk may not always be evident (e.g. TBT). If there are detailed data available that there are individual changes and suspect compounds, then monitoring may progress at a still more detailed level, concentrating on specific indicator measurements to provide information on degree of contamination, geographical scale, impacts of remediation”*.

Subsequently the SETAC EDIETA workshop recommended that *“the general strategy used to design monitoring programmes for endocrine disruption in invertebrates can be divided into techniques for use in:*

- *General monitoring when impacts are unknown;*
- *Targeted monitoring to follow up on existing knowledge about possible ED effects;*

- *Active monitoring with, for example caged sentinel species; and*
- *Bioassay-led fractionation procedures and other rapid screening techniques designed to throw light on causality”*

Table 5.1 Proposed generalised scheme for monitoring for effects of xenobiotics, including endocrine modulators (after Tattersfield *et al* 1997)

Aim	Knowledge	Techniques to be applied or information gathered
1) To establish if there are any sorts of effects on wildlife populations in a locale	None	<p><i>Generalised screening surveys:</i> A wide range of endpoints should be measured. Possible measurement endpoints could be selected from below: (a) <i>Community measures</i> e.g. Abundance, Number of taxa, Number of individuals (b) <i>Population effects</i> e.g. Age structure, Sex ratios (c) <i>Individual effects</i> e.g. Reproductive performance (e.g. Fecundity, Fertility), Gonad size, Secondary Sex characteristics, Growth/Development, Age/Growth relationships, Developmental Deformities (Lesions, Histology), Clinical measures (Hormones, Substrates, Enzymes), Immune function, Behaviour (Migratory/Dispersive, Reproductive).</p>
2) Identify causality	Population-level effects have been observed	<p><i>Identification of causality via combination of analytical determinations and verification of effects:</i> Use information gained in generalised screening survey or any background information available. Analyse cause of population level effects (i.e. what are the symptoms in an individual) Consider possible natural causes Determine possible sources of xenobiotics Selective/stratified sampling across effects gradient or between affected and non-affected environments (e.g. affected and non-affected nearby lakes) Perform TIE, Bioassay-directed fractionation.</p>
3) Determine magnitude and extent of effects and evaluate endocrine modulation risks relative to other stressors	Endocrine modulation effects observed or expected	<p><i>Surveying directed at specifically looking for endocrine-mediated effects:</i> Determine mechanisms of action Choose target species carefully (i.e. species selection should be exposure driven; e.g. if the xenobiotic is in the sediment, choose a bottom-dwelling species) Screening at the individual level (sex ratio, histology etc): Select and apply suitable biomarkers (if general type of effect is known) Analyse environmental samples chemically to identify and quantify xenobiotics Assess effects on translocated sentinel species.</p>

Table 5.1 continued

Aim	Knowledge	Techniques to be applied or information gathered
4) Validation of field observations	Confirmation/Verification	<i>In situ bioassays or validation in the laboratory to confirm that organisms respond in the same way as observed in the field to environmentally relevant concentrations of individual chemicals or mixtures:</i> Expose test organisms to; (a) known, environmentally relevant concentrations of suspect chemicals (b) chemical fractionation's of environmental samples © environmentally relevant mixtures

Note, there are various ways of conducting a survey: if not looking for anything specific other than general well-being of the population then the scheme is entered at Phase 1; if that survey indicates effects on reproduction then it might be that these effects are due to endocrine modulation and therefore you would move to Phase 3. However, if the original survey is aimed at looking specifically for evidence of endocrine modulation, then the initial survey would start at Phase 3. The phases are not sequential and one might move up or down the scheme depending upon the aims of the study.

5.3.3. Under what circumstances should chemical-specific measurement and biological effects assessment be used for monitoring of endocrine disruption and what criteria need to be considered when making these decisions?

The workshop session stated that an integrated approach using chemical analysis and biological effects assessment (using diagnostic biomarkers and bioassays) was needed to monitor endocrine disruption in humans and wildlife. However, it was recognised that with the large number of potential endocrine disrupting chemicals in use chemical-specific targeted monitoring programmes should be carried out when strong (ideally) causal links have been established between the presence of given substances in the environment and endocrine disruption effects in humans and wildlife.

Since it has not been established which of the myriad of substances present in the environment constitute an endocrine disruption problem it is more appropriate initially to use biological effects measures since they provide an overall measure of effects which also take into account interactions between substances. However, if biological effects-based measures are used it is also important to attempt to identify the causative agents, therefore, there is also a need for effective and integrated analytical chemistry/toxicity identification evaluation (TIE) exercises.

The current absence of causal links between concentrations of substances in humans and resulting effects means that an effects-based approach is recommended. For wildlife links between exposure and effects are becoming clearer for certain types of effects in particular target groups, such as the role of natural and synthetic steroids in causing oestrogenic effects (such as VTG induction and intersex) in fish. However, there is not yet sufficient understanding for regulators to establish environmental target levels for these substances against which chemical monitoring can be conducted (see also Chapter 3).

5.3.4. What biological effects assessment techniques are available to monitor endocrine disruption in humans and wildlife?

In considering biological effects assessment techniques which are available to monitor endocrine disruption in humans and wildlife it should be remembered that this is a continually evolving area and that it is important to link the outputs from research and development with the needs for monitoring.

Humans

The following techniques should be used to assess endocrine disruption in humans:

- Large scale epidemiology studies investigating changes in reproductive function (for example sperm counts, semen quality, fertility rates) and malformations in foetuses, new born and young children, adult women (breast cancer and endometriosis) and adult males (testicular cancers);
- Mammalian toxicity tests being developed by the OECD (and other bodies such as the US EPA) which model endocrine disruption effects on reproductive function and development in humans.

Wildlife

A general monitoring programme to establish the general status of wildlife populations would require a broad range of indicators to determine the magnitude and extent of population or community-level responses (see Table 5.1). The techniques currently available (see also report on the workshop session on development of test methods and a testing strategy) include:

- *In vitro* tests, such as the Estrogen Receptor mediated Chemical Activated Luciferase Gene Assay (ER-CALUX) and the Yeast Screen (YES) Assay to assess the potency of individual endocrine disrupters and environmental samples;
- Laboratory-based whole organism tests (principally with fish) which measure the effects of individual endocrine disrupters and environmental samples on biomarkers (for example vitellogenin induction in males), cell/tissue structure and function and reproductive function (for example fertilisation and hatching success and sex ratios of offspring);
- *In situ* deployments of sentinel species (principally fish) which measure the type of parameters used in laboratory-based tests;
- Measurement of effects in field populations which involve the parameters used in laboratory-based tests.

It is important that the ecological relevance of *in vitro* and *in vivo* tests is evaluated in terms of their capability to predict populations effects.

5.3.5. In the environment how can locations most at risk from inputs of endocrine disrupters be identified?

Xenobiotic-induced effects on the endocrine system are most likely to be found near sites of heavy human activity. These include areas used for agricultural, industrial and/or recreational purposes. Point sources such as effluent outfalls (from sewage treatment works and industrial plants), incinerators and treated fields should receive a high priority for monitoring based on the results of existing monitoring exercises (for example COMPREHEND, EDMAR and LOES). Therefore, it is recommended that a number of monitoring programs should be implemented at selected sites throughout OECD member countries. One of the main objectives of these programmes will be to develop a common methodology through a series of 'demonstration programmes'. Multi-disciplinary approaches on carefully targeted sites can focus effort for what will often be expensive studies.

Appropriate modelling approaches can be used with product registry data (production volumes and use patterns) to predict where "hot spots" of effects might be expected. It is important that regulators and industry work together to ensure that current and realistic data on the production, patterns of use and releases of potential endocrine disrupters are available. The BKH report on the development of a

mechanism for identifying priority substances has generated a large body of current information on substances considered in the priority setting exercise.

It is also important that consideration is given to which environmental compartment (water column, sediment, soil, air) may be most at risk from endocrine disruptors depending on the physico-chemical characteristics of potential endocrine disruptors and their pattern of release into the environment.

5.3.6. What is the current status of exposure models with regard to being used as an integral part of monitoring programmes?

It was clear that the targeting of monitoring programmes could be improved by having good information on the potential sources of endocrine disrupting substances. The workshop recognised that currently available models (see Table 5.2) can highlight the areas where monitoring are needed, but that these need to be used and developed based on their limitations. The current models may not solve all the problems associated with identifying areas of risk, but they can provide a mechanism for improving understanding.

Table 5.2 Examples of exposure models which could be used as part of monitoring programmes

Model	Description of the model
EUSES	The E uropean U niform S ystem for the E valuation of S ubstances model is a distribution type model using substance inherent properties and pre-defined European environmental scenarios to establish the distribution of a substance within the environment and the likely concentrations. The scenarios are differentiated according to a local, regional and continental scale but are not allocated to certain regions in Europe.
GREAT-ER	G eography referenced R egional E xposure A ssessment T ool for E uropean R ivers calculates the realistic Predicted Environmental Concentration (PEC) distribution of 'down the drain' chemicals in receiving riverine water due to regular use by modelling the waste water path. Emissions originate from point sources, which are linked to river stretches using Geographic Information Systems (GIS). Variability in the input data (for example variable water flows and flow velocities, as well as uncertainties concerning the input parameters are reflected by combining deterministic and stochastic model parts through the use of Monte Carlo simulations. GREAT-ER was designed in the context of post-screening risk assessments for substances, that is its aim was to assess the concentrations of pollutants in surface waters based on actual production figures. The validity of GREAT-ER has been validated by means of monitoring in different catchments in a number of European countries

It is important to know the uncertainties associated with a model, and what answers they can and cannot provided with a certain degree of confidence. This is often dependent on the level and quality of the data which is used to parameterise the model. There will be greater uncertainties associated with the outputs of a model where the proportion of default rather than measured values is used.

Overall the ideal situation for risk assessment purposes is one where there is a combination of predictive modelling and measured data.

There is increasing activity in the development of models for assessing exposure with improvements to both EUSES and GREAT-ER either on-going or planned and

both regulatory and industrial bodies (such as CEFIC³) funding work into the development of exposure models

5.4. Recommendations from the workshop

The following recommendations resulted from the workshop session:

- In humans large scale epidemiology studies investigating changes in reproductive function (for example sperm counts, semen quality, fertility rates) and malformations in fetuses, new born and young children, adult women (breast cancer and endometriosis) and adult males (testicular cancers) should be established.
- For human health monitoring further use should be made of registers of reproductive effects (sperm counts, semen quality, infertility levels) and malformations (male and female cancers) to identify spatial and temporal trends across Europe. However, it will be necessary to harmonise methodologies so that the data can be interpreted both within and across countries.
- Extensive but cost-effective schemes should be established to monitor changes in parameters such as semen quality (linked with exposure studies) in appropriate target groups so that action can be taken before this is translated into decreases in fertility rates. Such programmes should work towards establishing the reasons for the differences in semen quality between countries such as Denmark and Finland.
- In the environment monitoring should initially focus on the assessment of “hot spots” using effects measures and attempt to identify the causative agents. An example of a cost-effective which would build on existing programmes and provide a relevant and representative picture of the European situation would involve effects-based monitoring (primarily using standardised fish species) in rivers and estuaries of Germany, the Netherlands and the United Kingdom and in the Mediterranean Sea. Combined analytical chemistry and Toxicity Identification Evaluation (TIE) exercises would be used to identify the causes of the observed effects.
- Programmes need to be established which can provide information on background effects/ concentrations (making greater use of tools such as environmental specimen banks). These can provide material that can be used to obtain data retrospectively to monitor temporal trends. However, it is important that there is harmonisation of approaches so that it is possible to compare results across European countries.
- Appropriate modelling approaches should be used with product registry data (production volumes and use patterns) to predict where “hot spots” of effects might be expected.
- Regulators and industry have to work together to ensure that current and realistic data on the production, patterns of use and releases of potential endocrine disruptors are available.

³ Further information is available at the CEFIC website, <http://www.cefic.org>

6. Conclusions and Recommendations

The workshop recognised that the area of endocrine disruption and potential effects of endocrine disrupting chemicals on human health and wildlife was one of increasing concern to all sectors of society. This concern was evident in the increasing level of research activity which is being funded both nationally and internationally by governments, regulatory bodies and industry. The importance of close liaison between the different bodies and sharing of information was highlighted to ensure that the best possible use is made of available resources. It was evident that endocrine disruption is a complex area to address and that there are still problems (particularly for human health) in establishing causal links between exposure to suspected endocrine disrupters and any effects measured. In recent years a considerable amount of data on endocrine disruption has been generated which has provided answers to a number of important questions. Work has also proceeded on the development of test methods under the auspices of bodies such as Organisation for Economic Cooperation and Development (OECD) and the United States Environmental Protection Agency (US EPA). However a significant number of questions still need to be answered and the challenge facing regulators, industry and academia is how these can be resolved in a rapid cost-effectively manner whilst still providing scientifically robust outputs.

The recommendations resulting from the workshop sessions are summarised in Table 6.1.

Table 6.1 Summary of the recommendations of the different workshop sessions

Workshop session	Recommendations
Information exchange and international coordination	<ul style="list-style-type: none"> • Easily accessible and regularly updated databases providing information on endocrine disrupting chemicals and on-going research projects (such as GEDRI and the MRC REDIPED database need to be available to the scientific community; • Literature information systems need to be established which provide information on the current situation regarding endocrine disruption at a range of levels (from the simple to the technical) for different target groups (from the general public to researchers). In these systems links between key websites need to be established for easy access of information. • The Global Assessment of Endocrine Disruption (GAED) state-of-the-science report needs to be periodically updated after its publication and resulting recommendations should be implemented. • There is a need for better exchange of information and data between scientists involved in national research and their national representatives engaged in OECD activities.

Workshop session	Recommendations
Research and development	<p>Endocrine disruption in humans</p> <ul style="list-style-type: none"> • Future studies should work towards a full appreciation of endocrine disruption effects on human health which includes all potential target sites (particularly those associated with adrenal function and steroidogenesis) • Future studies need to focus on investigating the links between exposure to endocrine disrupters and identified effects on human health (decrease in semen quality, increase in hypospadias, testicular and prostate cancer in males and breast and ovarian cancers in females). It should be recognised that since cancers are multifactorial diseases with a potentially long latency period it may be extremely difficult to establish causality between exposure and the suspected effect. • More detailed assessments of exposure in humans (to all endocrine disrupting chemicals by all routes) are needed • There should be thorough investigations of aetiological factors in current human endocrine conditions (for example thelarche in Puerto Rican baby girls) • Further information on risk factors in epidemiology studies is needed (with environmental and socio-economic factors being considered) • Research studies should include input from endocrinologists <p>Endocrine disruption in wildlife</p> <ul style="list-style-type: none"> • Studies on natural variability in measures of endocrine disruption need to be carried out in accordance with the recommendations of the SETAC EMWAT workshop • The population consequences of endocrine disrupter effects in individuals need to be understood to provide a better understanding of the ecological relevance of the assessed effects. • Further studies on the effects of endocrine disrupters on amphibians/ reptiles are needed since this important (and vulnerable) group have endocrine systems (e.g. thyroid) which are different to fish and they may show effects not evident in other taxa • Further studies should consider endocrine disrupter effects on birds, possibly through the development of a model <i>in ovo</i> system in which the effects on organism development are measured following injection of chemicals into the yolk of the egg • Test methods for invertebrates need to be developed (taking into account the recommendations of the SETAC EDIETA workshop) but this needs to be underpinned by a greater understanding of the endocrinology of this important taxonomic group <p>Regional and global concerns</p> <ul style="list-style-type: none"> • The environmental reasons for regional differences in human health effects (such as differences in semen quality between DK and SF) need to be identified. This may link chemical aetiology to health effects • For fish, a European comparison of endocrine disrupter effects is needed, but this will require harmonisation of

	methodologies (for example the test species and test design)
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Workshop session	Recommendations
Research and development cont'd	<p><i>Mechanisms of action of endocrine disruptors</i></p> <ul style="list-style-type: none"> • A better mechanistic understanding of the action of endocrine disruptors is needed, particularly where the responses are not receptor mediated • Future studies should not only focus on oestrogenic effects but consider other parts of the endocrine system: for example (anti-) androgenic, (anti-) thyroid and (anti-) adrenal effects • Future studies should address the effects of endocrine disruptors on immune systems and behavioural and neurological development • Greater use should be made of genomics (techniques to identify the DNA sequence of the genome), transcript profiling (techniques to identify the mRNA from actively transcribed genes), proteomics (techniques to identify the proteins in a biological sample) and metabonomics (techniques to identify the presence and concentrations of metabolites in a biological sample). These GTPM approaches require additional development and validation, but could provide a vast range of additional useful endpoints. They could be used to target testing, address low dose effects, mixtures and decrease numbers of animals used in testing. Development of these tools should be focussed initially on ED-related activities. Their careful application could assist research associated with safety assessment by enabling a more detailed appreciation of molecular mechanisms of action and facilitating more rapid screens for effects. <p><i>Measurement of exposure</i></p> <p>There is a need to develop analytical procedures to assess exposure to endocrine disruptors, in particular this relates to:</p> <ul style="list-style-type: none"> • integrated analytical chemistry/TIE procedures using validated <i>in vitro</i> systems • methods for the detection and quantification of EDs in complex matrices such as sediments • information on the potential role of invertebrates in the transfer of endocrine disrupting chemicals from sediments to fish via the food chain <p><i>Measurement of effects</i></p> <p>Future studies should focus on:</p> <ul style="list-style-type: none"> • critical windows of exposure in humans and wildlife (especially for development) • ways to enhance the predictive capability of endpoints • the issue of sensitive individuals in populations <p>Furthermore future studies need to consider the issues of:</p> <ul style="list-style-type: none"> • low dose effects where there are unusual dose response curves that traditional regulatory toxicology protocols may miss. There is a need for a clear definition of what is meant by 'low dose effects' together with clarification of exposure patterns and sensitive windows of exposure • the extent to which mixture effects occurs in terms of: <ul style="list-style-type: none"> - antagonism, additivity or supra-additivity

	<ul style="list-style-type: none"> - mixture effects involving more than one molecular target (in mammals what is the effect to the male foetus of exposure to a weak oestrogen and a weak anti-androgen?).
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Workshop session	Recommendations
Research and development cont'd	<p>Measurement of effects cont'd</p> <ul style="list-style-type: none"> • the issue of adverse effects and what level of change should be regarded as significant. It is important that sufficient information is available on natural variability to allow potential changes due to chemicals to be identified <p>Funding and policy issues</p> <ul style="list-style-type: none"> • Mechanisms need to be established to facilitate long-term studies which address: • Endocrine effects in long-lived species <p>Whether regulatory actions related to endocrine disrupters result in improvements in the environment</p>
Development of test methods and testing strategies	<ul style="list-style-type: none"> • Existing OECD approaches for detecting oestrogenic, androgenic, thyroid effects in mammals, fish, birds represent a scientifically robust approach but there is a need: <ul style="list-style-type: none"> - for whole animal tests to cover all possible endocrine disrupting effects (particularly adrenal function and steroidogenesis); - to focus on test methods assessing reproductive function and developmental effects; - to refine chronic mammalian tests test designs and enhance/improve the test endpoints to better cover sensitive periods of the test organisms life history and to better capture hormonal effects. • OECD activities are not covering all of the issues and there is a need for further work on the development of: <ul style="list-style-type: none"> - to develop tests to address impacts on the adrenal system, pheromone signalling and the neuro-endocrine-immune system; - tests using invertebrates (which are underpinned by a greater understanding of the endocrinology of the test species used); - alternative techniques such as SARs/<i>in vitro</i> tests (beyond those currently being developed or evaluated) which could be used to target testing and limit <i>in vivo</i> testing. However, alternative tests need to be used responsibly and intelligently and <i>in vivo</i> testing is needed to validate SARs/<i>in vitro</i> systems. • There is a need for a comparison of information on thyroid effects in different species so as to determine how to address the issue of species redundancy. • The approach for determining whether testing of potential endocrine disrupters is required needs to be more sophisticated than being based on production volume data alone and should include data on the pattern of exposure of substances in the environment and their persistence, bioaccumulation and toxicity. • There is a need for an intelligent approach to testing homologous series of chemicals and the use of bridging studies to assist in this regard. Further research is needed and OECD should be asked to co-ordinate

	<p>standardisation and validation of partial lifecycle studies to be used for bridging;</p> <ul style="list-style-type: none"> • A simple framework to determine testing needs should be developed, with the approach used in the Biocides Directive being proposed as a possible example;
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Workshop session	Recommendations
Development of test methods and testing strategies cont'd	<ul style="list-style-type: none"> • Discussions should begin on developing an EU strategy for testing endocrine disrupters. The Annex V Committee to 67/548 should coordinate EU views and activities on test methods and strategies to feed into the OECD.
Establishment of monitoring programmes	<ul style="list-style-type: none"> • In humans large scale epidemiology studies investigating changes in reproductive function (for example sperm counts, semen quality, fertility rates) and malformations in foetuses, new born and young children, adult women (breast cancer and endometritis) and adult males (testicular cancers) should be established. • For human health monitoring further use should be made of registers of reproductive effects (sperm counts, semen quality, infertility levels) and malformations (male and female cancers) to identify spatial and temporal trends across Europe. However, it will be necessary to standardise methodologies so that the data can be interpreted both within and across countries. • Extensive but cost-effective schemes should be established to monitor changes in parameters such as semen quality (linked with exposure studies) in appropriate target groups so that action can be taken before this is translated into decreases in fertility rates. Such programmes should work towards establishing the reasons for the differences in semen quality between countries such as Denmark and Finland. • In the environment monitoring should initially focus on the assessment of "hot spots" using effects measures and attempt to identify the causative agents. An example of a cost-effective study which would build on existing programmes and provide a relevant and representative picture of the European situation would involve effects based monitoring (primarily using standardised fish species) in rivers and estuaries of Germany, the Netherlands and the United Kingdom and in the Mediterranean Sea. Combined analytical chemistry and Toxicity Identification Evaluation (TIE) exercises would be used to identify the causes of the observed effects. • Programmes need to be established which can provide information on background effects/ concentrations (making greater use of tools such as environmental specimen banks). These can provide material that can be used to obtain data retrospectively to monitor temporal trends. However, it is important that there is harmonisation of approaches so that it is possible to compare results across European countries. • Appropriate modelling approaches should be used with product registry data (production volumes and use patterns) to predict where "hot spots" of effects might be expected.

	<ul style="list-style-type: none">• Regulators and industry have to work together to ensure that current and realistic data on the production, patterns of use and releases of potential endocrine disrupters are available.
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