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Endocrine disruption horizon scanning: current status of endocrine disruptor research and policy

Science Report – SC030276/SR4

SCHO0507BMQD-E-P

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Steve Killeen

Head of Science

This document is one of four reports produced under the *Endocrine disruption horizon scanning* project (SC030276), which is part of Environment Agency's R&D Project Initiation Document P6-020/U, *Development of methods for detection of endocrine disruption and application to environmental samples*.

The aim of the *Horizon scanning* project is to identify and review new and emerging aspects of endocrine disruption (ED). The full list of documents in the project is:

- *Endocrine disruption horizon scanning : Aquatic invertebrates review (SC030276/SR1)*
- *Endocrine disruption horizon scanning: Molecular and genomic contributions (SC030276/SR2)*
- *Endocrine disruption horizon scanning : Priority and new endocrine disrupting chemicals (SC030276/SR3)*
- *Endocrine disruption horizon scanning: Current status of endocrine disruptor research and policy (SC030276/SR4)*

Executive summary

The Environment Agency (England and Wales) has funded programmes of research into endocrine disruption (ED) and endocrine disrupting chemicals (EDCs) for many years. Its current portfolio of ED-related research projects is worth hundreds of thousands of pounds.

The aim of this report was to assess the current international status of ED, with regard to scientific research, regulatory and legislative issues, and chemical testing and screening strategies.

In 2003, the UK Institute of Environment and Health (IEH) conducted an extensive survey of governmental regulatory departments and non-government groups with an interest in ED issues, to provide similar information (IEH, 2003). The information collected in 2003 forms the basis of this report, supplemented with information from other sources, together with a more recent survey of contributors to IEH (2003).

IEH (2003) is a comprehensive review of the regulatory and scientific importance of ED in 2003, and given that research and regulation tend to progress quite slowly (typically over years), the review provides a good basis for this report. To obtain an up-to-date perspective on the ED issue for this study, a follow-up questionnaire was sent to representative participants of IEH 2003. In addition, a number of websites were examined for up-to-date information.

ED remains of medium to high political importance around the world, and is also viewed as important scientifically. However, competition from other environmental issues can overshadow ED. For example, climate change is currently very high on political and scientific agendas, and is seen as more of a priority. In terms of media and public awareness, ED varies from country to country, based mostly on local or national issues. There is a view that the initial impact of ED effects has now gone, and media and public interest has declined as a consequence. Again, interest has tended to shift to other environmental issues such as climate change. However, interested parties (such as the conservation groups) maintain that ED is very important.

There are clearly differences between the approaches used to research, regulate and screen for ED and EDCs around the world.

In Europe, member states generally look to the European Commission for guidance on legislation and prioritisation. For example, there is a large expectation that the EU's Registration, Evaluation and Authorisation of Chemicals scheme (REACH) will cover EDCs and help towards the regulation of these chemicals. In addition, member states tend not to have their own lists of priority EDCs, but instead adopt the prioritisation process that the EU has developed (the reports produced by BKH Consulting Engineers, The Netherlands). However, although none of the members states have their own ED research programmes *per se*, many do fund national ED research projects. For example, in Ireland the Environmental Protection Agency (EPA) has just completed a project investigating ED effects in Irish freshwaters, including EDC concentrations in sewage treatment work (STW) effluents and receiving waters, and ED effects on fish. The Danish EPA has completed similar studies, looking at STW effluents in rivers and run-off from fields treated with sewage sludge. EDCs are not regulated specifically. Most of the chemicals known to be ED active have other toxicological impacts. Thus, although there is no legislation specifically for ED, most EDCs are regulated under other legislative measures based on their overall toxicological profile

In Japan, government departments such as the Ministry of the Environment and the Ministry of Economy, Trade and Industry have developed a list of priority EDCs, which

will be tested and researched first. However, there is no legislation in Japan specifically for EDCs because, using the definition provided by the World Health Organisation (WHO), there is insufficient evidence to identify any chemical as an EDC. Regardless, there are a number of research programmes running in Japan on ED, with particular interest in the potential for humans (specifically children) to be exposed to EDCs through the consumption of food and beverages in plastic containers. Japan has also invested considerable effort to develop screening and testing methods.

In the US, legislation specifically covers EDCs. In particular, the Food Quality Protection Act 1996 requires pesticides to be tested for ED activity; the US EPA also has the power to test drinking water for EDCs under the Safe Drinking Water Act. The EPA has a large, long-term ED research programme on classes of chemicals, dose-response curves and modes of action identified as being important and having implications for future legislation. Other research includes: the integration of human and ecological data in risk assessments, sources of exposure, drinking water treatment, chemical mixtures, and impacts on the environment (including pulp mill and STW effluents). The US EPA has its own tiered screening strategy, driven by the 1996 legislation and the 1998 EDSTAC (Endocrine Disruptor Screening and Testing Advisory Committee) report. The three main initiatives are: (1) to develop and validate tests and screens; (2) to generate a list of chemicals for screening; and (3) to draft regulation on testing and screening processes, where industry will perform the tests. The emphasis is on assessing the ED activity of the chemical, regardless of the animal it is being tested on.

In terms of screening and testing, the Organisation for Economic Cooperation and Development (OECD) has a well-developed strategy that most countries support. Indeed, most of the countries actively involved in ED research have contributed to the OECD strategy to some extent, either in method development or validation exercises. Although some parts of the strategy are disputed (for example, which test organisms should be recommended), there is a general consensus that the OECD strategy will become a useful, internationally agreed strategy for screening purposes.

In summary, ED is still seen to be an important part of the scientific and regulatory agenda. Much effort continues to be spent on research of ED and EDCs, both on human health and environmental protection. There is a great deal of collaboration on the ED issue within Europe, and more specifically on the development of screening methods internationally.

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Contents

1	**Introduction**	**1**
2	**Methods**	**2**
2.1	Review of Institute of Environment and Health report (2003)	2
2.2	Follow-up questionnaire given to participants of IEH (2003) in 2006	2
2.3	Consultation with UK research councils	2
2.4	Web pages of regulatory and conservation agencies	3
3	**European Commission initiatives**	**4**
3.1	REACH	4
3.2	ED priority substances list	4
4	**IEH (2003)**	**6**
4.1	European Union countries	6
4.2	Non-European Union countries	17
4.3	Non-government organisations	25
5	**Research councils**	**34**
5.1	BBSRC	34
5.2	NERC	35
6	**Current Environment Agency endocrine disruptor projects**	**36**
6.1	ED Project Initiation Document (PID)	37
6.2	Fish population modelling (SC060002)	43
6.3	EDCAT 7 – Influence of endocrine disruption on reproduction in roach (SC050061)	44
6.4	Endocrine disruption demonstration programme (SC040058)	45
6.5	Endocrine disruption demonstration programme - Fish histopathology environmental monitoring (SC050020)	46
7	**Environment Agency perspective on human health aspects of ED**	**47**
8	**Summary and conclusions**	**49**
References		**51**
Websites		**52**
List of abbreviations		**53**
Appendix 1: Questionnaire used by IEH (2003)		**55**

Appendix 2: Update since 2003 questionnaire **60**

Appendix 3: People contacted with update questionnaire **62**

1 Introduction

Environmental impacts of endocrine disruption (ED) and endocrine disrupting chemicals (EDC) have received much attention over the last 20 years or so. In the UK in particular, significant findings of ED in the natural environment include imposex (the superimposition of non-functioning male genitalia in females) in the dogwhelk *Nucella lapillus* (e.g. Bryan *et al.*, 1987); and intersex (partial feminisation of males) in freshwater fish (see Gross-Sorokin *et al*., 2005). EDCs cover a wide range of chemical substances, including man-made chemicals, natural plant products and animal waste products (such as sewage). For example, imposex in dogwhelks was found to be caused by tributyl tin (TBT), which is the active substance of many anti-fouling paints. Intersex in fish has been traced to effluent from sewage treatment works (STWs), with the female hormones oestradiol (E2) and oestrone (E1) being identified as the specific EDCs (together with ethinyloestradiol (EE2), the active substance in female contraceptives) (see Gross-Sorokin *et al*., 2005). Because of the potential risks to wildlife, the Environment Agency (England and Wales) has a risk assessment strategy for EDCs. This report brings together the current views on ED and the current legislative status of EDCs from regulatory organisations and research funding bodies around the world.

In 2003, the UK Medical Research Council's Institute of Environment and Health (IEH) was contracted by the European Commission to perform a survey of international policy, regulation and scientific research on ED and EDCs and to collate this information for exchange and coordination in this area (IEH, 2003). Being only three years old and having a forward-looking focus, the survey's main findings are still relevant today, and have been drawn heavily upon for this report. Progress since IEH (2003) has been captured in a number of ways, including contacting original participants of IEH (2003), gathering information from regulatory/research organisation websites and reviewing published literature. A detailed explanation is provided in Chapter 2 (Methods).

This report provides an up-to-date summary of current international thinking, research and legislation on ED and EDCs. The report will guide the Environment Agency's ED strategy and risk assessments, ensuring that they are based on the most up-to-date information available on ED and EDCs.

2 Methods

Information for this review was collected from various sources, including: recent published reviews of ED policy and regulations; a questionnaire directed at regulatory authorities, research institutes and conservation agencies; consultation with research funding councils; and web pages from regulatory authorities.

2.1 Review of Institute of Environment and Health report (2003)

In 2003, the Institute of Environment and Health (UK) was commissioned by the European Commission (EC) to run an information exchange exercise on current ED research, policy and regulation. Representatives of regulatory and conservation organisations from a number of countries (mostly Europe, but also the United States and Japan) were interviewed using a standard questionnaire. This covered the current importance of ED, legislation of EDCs, priority substances, human health and wildlife concerns over EDCs, and EDC testing and screening strategies.

This report draws heavily on the IEH report (2003), a large project which collected much information. Although it is now three years old, this information is still largely relevant. This was confirmed by contacting everyone who participated in the interviews conducted by IEH, to ask for an update on their country's policy on EDCs.

The full questionnaire used in IEH (2003) is presented in Appendix 1.

2.2 Follow-up questionnaire given to participants of IEH (2003) in 2006

Representative participants from IEH (2003) were contacted again in 2006 to ask for an update on ED perspectives from their organisation and/or country. The same topics from 2003 were used and participants were asked only to provide information since 2003. Examples of new information included revised regulatory priorities, a revised policy for ED or EDCs, revised priority substances, revised environmental protection goals and so on.

The full list of questions asked in the questionnaire is presented in Appendix 2.

The full list of participants approached in 2006 is presented in Appendix 3.

2.3 Consultation with UK research councils

Research funded by the UK research councils often reflects current awareness, direction and priorities, and so the main councils that fund environmental research in the UK were consulted. Contacts at the Natural Environment Research Council (NERC) and the Biotechnology and Biological Sciences Research Council (BBSRC) were asked to provide information on the amount of funding spent on ED or EDC research, how budget spending was decided, and their thoughts on the future direction of ED research in the UK.

Each research council was asked the following questions:

- How much funding are/have you allocated to ED research?
- Where in your priorities does the area of ED research sit?
- What are your key ED research areas and needs for current and future funding?
- How do you decide which ED research to fund? For example, do you take leads from government, regulators, academics, or European initiatives?

2.4 Web pages of regulatory and conservation agencies

Almost all regulatory and conservation agencies, government departments, research centres and universities have their own world-wide web (WWW) pages highlighting current policies, priorities and research. These pages provide a good source of information on current perspectives, especially if they are updated regularly, and so were reviewed for information for this study. WWW pages from the following organisations were reviewed:

- European Commission
- Japanese Ministry of the Environment
- National Institute of Environmental Health Sciences
- Organisation for Economic Cooperation and Development
- United States Environmental Protection Agency

3 European Commission initiatives

3.1 REACH

Perhaps the most significant development in legislation for hazardous substances is the proposed European regulatory framework known as REACH (Registration, Evaluation and Authorisation of Chemicals). The aim of REACH is "*to improve the protection of human health and the environment through the better and earlier identification of the properties of chemical substances. At the same time, innovative capability and competitiveness of the EU chemicals industry should be enhanced*." The Regulation also calls for the progressive substitution of the most dangerous chemicals when suitable alternatives have been identified. The REACH system will be used continuously and will allow more and more substances to be added.

The REACH framework was proposed in October 2003[1]. It has taken two years of negotiations on the initial proposal, and a first reading opinion from the European Parliament, for a common position to be agreed. REACH was formally adopted on 18 December 2006 following a second reading at the European Parliament on 13 December 2006. The Regulation was published on 30 December 2006, and will enter into force on 1 June 2007.

ED and EDCs received particular attention in the negotiations for REACH, because the initial proposal stated (Article 56(f)) that only EDCs "*for which there is scientific evidence of probable serious effects to human health or the environment*" would require authorisation through the REACH procedure. Conservation agencies and academic experts felt that a more precautionary approach should be applied for substances with known ED activities because, although they may not cause "serious effects" at present exposure levels, they can act in an additive nature.

More information on REACH can be found at the website:
http://ec.europa.eu/enterprise/reach/index_en.htm

3.2 ED priority substances list

In 2000 and 2002, BKH Consulting Engineers (Netherlands) were contracted by the EC to produce a list of chemical substances known to have ED properties (BKH 2000; BKH 2002). The compilation of the list was complemented by further work by WRc and BKH, to gain more information on some of the substances listed.

An initial list of 564 substances was compiled, of which 147 were identified as either persistent (remaining chemically active in the environment for many years), or produced in high volumes. Within those 147 persistent or high volume substances, 66 were known to have definite ED properties (identified as Category 1 substances), and 52 showed potential ED properties (Category 2 substances).

[1] This is after the consultation of experts for the IEH review. Consequently, although most representatives from EU member states were aware of the impending scheme, it was not being used at that time.

The list is a work in progress, and new ED substances can be added to it at anytime. It is used by all EU member states for legislation and regulation of EDCs. Further information on the list of priority EDCs can be found in BKH (2000; 2002) and WRC (2002), and also at the project website:
http://ec.europa.eu/environment/endocrine/index_en.htm

4 IEH (2003)

4.1 European Union countries

4.1.1 Denmark

Organisations consulted:

- Environmental Protection Agency (Danish EPA)

Relative importance of ED

ED is of high/very concern politically, but only medium concern scientifically. Public awareness and concern is medium to high, mostly focussed on the implications to human health – particularly pregnant women and infants.

Legislation – human health

Previous national legislation acts across all environmental media and considers ED and EDCs. The main legislation for EDCs is the Danish Chemical Substances Act and the Danish Food Act.

Internationally, Denmark works through the European Commission (EC), with EDCs expected to be covered within the REACH framework. Organisation for Economic Cooperation and Development (OECD) test methods will be used to implement REACH. Implementation of the EC's Water Framework Directive (WFD) will also impact on legislation of EDCs.

Statutory Orders exist for pesticides, and voluntary agreements exist for nonylphenol (NP) and nonylphenol ethoxylates (NPEs) in pesticides and detergents.

All 66 substances listed in the EC's priority ED substances, unless already banned or regulated via other legislation, are on the Danish List of Undesirable Substances.

Denmark is working hard to influence REACH negotiations for EDCS to be included in the authorisation procedure of REACH.

Legislation – environment and wildlife

Chemical legislation in Denmark includes humans and wildlife. As stated above, the WFD will be particularly significant for legislating against ED effects in wildlife.

Human health research

There has been no monitoring of EDCs and research is not focussed on any specific environmental compartment.

There has been research into changes in semen quality in young men, and development of test methods of fertility studies, via OECD.

Significant outcomes include a Danish and European database on semen quality and results shwoing declining reproductive health in men.

Environmental research

There is less funding available for environmental ED research, which currently only covers freshwater. Consequently, there is considerable collaboration with other Scandinavian countries.

Recent work includes studies of both natural and anthropgenic oestrogens in surface waters and waste, and their oestrogenic activities.

Significant outcomes include draft Scandinavian guidelines for a partial life-cycle test in zebra fish (*Danio rerio*), which has been fed into the international review for OECD.

Priority substances

All based on the Danish list of undesirable substances and EC priority list compiled by BKH (2000; 20002).

Testing/screening strategies and programmes

Denmark has taken an active role in the OECD Endocrine Disrupters Testing and Assessment (EDTA) task force, and the validation management group for mammalian and non-animal testing (quantitative structure-activity relationships (QSARs) and in *vivo*). Denmark has also contributed to the OECD development on the Herschberger and uterotrophic assays.

Update since 2003

Importance of ED

The status of ED in Denmark remains unchanged since 2003, in terms of its political and scientific importance. The Danish Environmental Protection Agency (EPA) still considers ED important, and has contributed actively to the REACH negotiations, including how to cover endocrine disrupters in the most suitable way.

Denmark has also been the lead on a Nordic project on how to use the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters as a basis for the regulation of substances with endocrine disrupting properties.

Research

The Danish EPA is still participating in the OECD work with uterotrophic and Hershberger assays; however, EPA-funded work to develop toxicological tests has now switched to the effects on thyroid function. In addition to experiments on rats, the Danish EPA is funding the development of an *in vitro* assay for measuring effects on the thyroid, and the subsequent testing of selected chemicals. The EPA will also be

involved in the OECD's upcoming work on the development of an enhanced one-generation test.

In terms of the environment, the Danish EPA has carried out a survey of oestrogens in the aquatic environment, including the effects of waste water effluents and run-off from farmland treated with sewage sludge.

The Danish EPA is still working towards the development of ecotoxicological tests in the OECD guideline programme; Denmark is the lead country for the development of the fish sexual development test and participates in validation exercises for the fish screening assay.

Priority substances

The Danish EPA has prepared a proposal for the EU Commission to ensure that the EU candidate/priority list of substances causing potential endocrine disrupting effects remains dynamic. In Denmark, parabens, some phthalates and siloxanes have been identified as important candidate EDCs since 2003.

Testing

Screening and testing strategies have not changed since 2003, nor have new ones been developed, because Denmark is following the work of the OECD and EU. However, the Danish EPA is heavily involved in the development of QSAR-methods, including methods for predicting endocrine disrupting effects.

Other

The Danish EPA has recently launched an information campaign directed towards pregnant and nursing mothers about chemicals in cosmetics, child care products and toys: www.babykemi.dk

4.1.2 Germany

Organisations consulted:

- Federal Institute for Risk Assessment (BfR)
- Federal Environmental Agency

Relative importance of ED

Political concern in EDs is generally medium, but sometimes high, and current awareness is maintained. Specific chemicals are usually of interest, and pharmaceuticals are receiving much attention. Scientific interest remains high, with a need for defined endpoints and modes of interest receiving particular attention; public interest varies, but there is rarely high concern.

Legislation – human health

Apart from the Plant Protection Act, which mentions but does not specifically control EDCs, Germany does not specifically legislate on EDCs. Germany will base its legislation on recommendations from the EU, potentially imposing stricter controls than those of the EU framework where necessary.

Legislation – environment and wildlife

As above, legislation will follow EU guidance. Voluntary agreements are made by about 80 per cent of companies. Water quality objectives are negotiated, although not legally binding.

Research – human health

The Federal Environment Ministry and the Federal Ministry of Education and Research have co-funded research into EDs in human and wildlife. Experts are also involved in a number of EU-funded research initiatives.

A database exists for human milk contaminants (20 years worth of data), including chemicals considered to be EDCs (PCBs, dioxins and so on). A national health and environment survey documenting chemical body burdens (including EDCs) is run by federal institutions.

Industry, academia and other stakeholders co-operate and consult with each other regarding such research initiatives.

Research – wildlife and environment

Wildlife and environmental research is conducted with human health research programmes; see above.

Priority substances

Reports from the University of Kiel were used in the BKH report on priority EDCs (BKH 2000; 2002). Other than this, EU guidance on priority EDCs will be followed.

Testing/screening strategies and programmes

Human health screening programmes are made within the OECD framework.

Wildlife screening and test methods (invertebrates) are feeding into the OECD framework, as are mammalian tests for the Herschberger and uterotrophic assays. Germany has been heavily involved with the development of the zebra fish assay, and a two-generation bird life-cycle assay (Japanese quail).

Work is focussing on oestrogens, but some anti-estrogen, androgen and anti-androgen activity is studied. Thyroid function (studied in short-clawed African toad *Xenopus sp.*) has also received attention.

REACH is expected to influence future screening and testing protocols.

Update since 2003

Unfortunately no reply was received to our request for an update since IEH (2003)

4.1.3 Ireland

Organisations consulted:

- Environmental Protection Agency (Irish EPA)
- Department of Agriculture and Food
- Health and Safety Authority

Relative importance of ED

Politically, ED is considered important in terms of environmental health (especially aquatic environments), although importance is low relative to other concerns. Ireland will follow guidance from the EU. A range of ED scientific research occurs under EU Framework programmes as well as national programmes – ED is considered of medium importance scientifically. Public awareness is low and this is reflected in ED being considered of low importance to the public.

Legislation – human health

None specifically for EDCs, although some chemicals known to be EDCs fall under other legislation (for example, pesticide and biocide testing and labelling). Integrated Pollution Control (IPC)/Integrated Pollution Prevention and Control (IPPC) licensing also controls the release of chemicals known to be EDCs.

Compliance with REACH is expected to cover EDCs.

Policy is formulated in consultation with stakeholders.

Legislation – environment and wildlife

As above. Nothing specific but will follow EU guidance.

Compliance with WFD will also consider chemicals known to be EDCs, but not EDCs specifically.

Research – human health

Nothing specific.

Research – wildlife and environment

Within the last few years, research programmes into EDC effects in fish have received funding worth several million pounds from the EU, UK[2] research councils and Irish academic institutes (such as University College Cork and Cork Institute of Technology).

[2] via collaboration with UK institutes

Research has included monitoring of EDCs in the aquatic environment (especially sewage effluent), effects in fish, chemical structure, *in vitro* toxicity, genotoxicity and ecotoxicity.

Priority substances

The Irish government is involved with the EU evaluation process of the BKH report, and will follow any recommendations. Any priority EDCs will also be re-examined for IPC/IPPC licensing, as well as monitoring under WFD.

Testing/screening strategies and programmes

There are no specific human health testing or screening programmes. Nor is there any specific wildlife testing or screening, although academic institutes actively research this area, and the EPA encourages the development of national testing facilities for EDCs.

The main areas of interest are oestrogenicity, vitellogenin (Vtg) induction, receptor binding, YES-assay, and the effects of oestrogen mimics in sheep.

Update since 2003

Although Ireland did not return a completed questionnaire supplying the specific information we requested, they did provide up to date information in the form of a report assessing EDCs in the Irish aquatic environment (Ireland EPA, 2005).

<u>Research</u>

Levels of oestrogenic chemicals in Irish freshwater systems, and of their effects on freshwater fish populations, were investigated between 2001 and 2005 for a project entitled *Estimation of Estrogenic Compounds in Irish Surface and Waste Waters* (Irish EPA, 2005). The project examined a number of different water bodies representing a variety of catchment usage and receiving varying amounts of waste water. Oestrogenic activity in the waterbodies was measured using *in vitro* bioassays, and effects on fish quantified *in vivo*. The report concluded:

1 Irish WWTP effluents are oestrogenic, although levels compare favourably with other European countries and the USA;

2 Irish rivers and lakes do not appear to be at general risk from significant concentrations of environmental oestrogens;

3 In general, wild fish populations do not appear to be at risk from oestrogenic chemicals; and

4 Judging from the limited number of sites examined in this study, Irish drinking water resources do not appear to be at significant risk from oestrogenic chemicals.

Recommended further work includes further characterisation of the River Liffey; a review of IPC licences to reduce the emission of EDCs to Irish waterbodies; detailed investigation of EDC levels in Irish coastal waters; use of geographic information system (GIS) modelling to predict EDC ‘hotspots’; and the investigation of the contribution of agricultural products (e.g. pesticides and vetinary pharmaceuticals) to oestrogenic activity in Irish waterbodies.

4.1.4 Italy

Organisations consulted:

- National Institute for Public Health

Relative importance of ED

ED is of low political importance and low to medium scientific importance, although the number of national ED-related projects is increasing. Public interest and awareness fluctuates but is generally low and focussed on human health issues.

Legislation – human health

There is no specific legislation for EDCs, although some chemicals known to be EDCs are covered via other means. As with other European countries, Italy will look to EU guidance, although as environmental protection moves to regional control individual regions might impose their own restrictions on EDCs.

Eight phthalates are banned for use in children's toys and food packaging.

Legislation – environment and wildlife

Nothing specific for EDCs, other than the ban on tributyl tin-based anti-fouling paints.

Research – human health

A number of human health research programmes that relate to EDCs have been funded through national government and the EU. These include using genetically modified models to study EDC mechanisms; effects of xenobiotics on male fertility; effects of persistent organohalide compounds on female reproduction; and effects of EDCs in food.

Biomonitoring of EDCs in humans and foods is a key research area, where the thyroid is seen as an important target for these chemicals.

Research – wildlife and environment

There are no national research programmes for EDCs and environmental protection, but there a number of individual projects relevant to ED.

Priority substances

The National Institute for Public Health has been involved with the BKH reports (2000; 2002).There is no specific prioritisation for EDCs, although some compounds have recently been examined under a reassessment of pesticides used in Italy.

Italy will follow EU guidance.

Testing/screening strategies and programmes

There are no specific national screening or testing programmes for human health, but Italian laboratories have contributed to the validation of the OECD uterotrophic assay.

The implementation of the WFD is expected to consider EDCs in the protection of water bodies.

A working group was established to examine environmental contaminants and human fertility, to promote the use of biomonitors as opposed to just chemical analysis.

Update since 2003

Our request for information updating IEH (2003) was passed on to the relevant specialists, however, no further response was received.

4.1.5 Netherlands

Organisations consulted:

- Ministry of Transport, Public Works and Water Management

Relative importance of ED

ED is of low/medium importance both politically and scientifically. ED is of low importance to the general public, although the more environmentally aware have concerns.

Legislation – human health

There is no specific national human health legislation for EDCs. Instead, EU legislation and guidance is used, for example REACH.

Legislation – environment and wildlife

Essentially, there is no specific national environmental legislation for EDCs either. Emissions to water bodies are regulated, but this is not specific for EDCs. For example, IPPC procedures cover discharges to water but this is not used to regulate EDCs specifically.

A national standard exists for tributyl tin, and the WFD is expected to cover EDCs. The Netherlands are collaborating with ED work in the EU and OSPAR (Oslo-Paris agreement). Also, the Environment Ministry has published a report on hormone disruption in ecosystems.

Research – human health

RIZA (Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling, or the Institute for Inland Water Management and Waste Water Treatment) is collaborating with the EDEN project (examining novel endpoints, low dose and mixtures in humans and wildlife), and RIKZ (Rijksinstituut voor Kust en Zee, or the National Institute for Coastal and Marine Management) is involved with the FIRE project (examining brominated flame retardants). Both of these projects are part of the CREDO project group and are due to finish in December 2007. Dutch national funding contributes to these programmes.

Other national research programmes include LOES (examining the occurrence and effects of oestrogenic chemicals in aquatic ecosystems); ENDIS-RISKS (examining the effects of EDCs in the Western Scheldt estuary); and field studies of imposex in marine gastropods and biomarkers in flounder for OSPAR and the EU BEQUALM programme.

Research – wildlife and environment

As above, the EDEN and FIRE projects also consider wildlife effects of EDCs.

Priority substances

There is no specific prioritisation process for EDCs, but the Netherlands is contributing to the development of the BKH list.

Testing/screening strategies and programmes

There are no screening or testing strategies specifically for EDCs and human health.

For wildlife, Vtg assays are used in bream and roach (as indicator species). RIVM (Rijksinstituut voor Volksgezondheid en Milieu, or the National Institute for Public Health and the Environment) have developed a test to examine the effects and ecological relevance of EDCs using zebra fish.

Tests focus on oestrogenicity and androgenicity (including anti-hormone responses). Thyroid hormone disruption is being investigated at several universities.

The Netherlands will look to EU and OECD developments for testing, and the outcomes of the FIRE and EDEN projects.

Update since 2003

Unfortunately no reply was received to our request for an update since IEH (2003).

4.2 Non-European Union countries

4.2.1 Japan

Organisations consulted:

- Ministry of Economy, Trade and Industry (METI)
- Ministry of the Environment (MOE)
- Ministry of Health, Labour and Welfare (MHLW)
- Chemicals Evaluation and Research Institute (CERI)

Perceived importance of ED

Politically, ED is perceived to be of medium to high interest. Interest peaked around 1998, but this has dropped due to lack of substances proven to cause adverse effects through ED specifically. Risk assessments are based on scientific knowledge; EDCS are considered, but are not given any more weight than other chemical substances.

There remains a lot of scientific interest in ED and EDCs (level perceived to be medium to high). Many issues remain of interest and are being researched.

Public interest in ED also peaked about 1998. The term 'environmental hormone issue' is commonly used by the media to describe ED. The media frequently report on this issue, although the accuracy of the reports may be questionable.

Legislation

According to the WHO and IPCS (International Programme on Chemical Safety) definitions of EDCs, there are no chemical substances proven to be EDCs and, therefore, the Japanese government does not legislate for them specifically. This applies to both human health and environmental protection.

Certain chemicals are considered under other legislation. For example, in terms of human health, a number of chemicals suspected of having ED properties are legislated for under drinking water and food safety legislation. However, this is due to their overall toxicity profile, and not specifically for their suspected ED activities

It is commonly accepted that more research is required, and Japan is collaborating with OECD, IUPAC/SCOPE[3], and other research initiatives. The OECD and WHO are considered the most appropriate organisations for developing strategies for the legislation of EDCs.

[3] Collaboration between IUPAC (International Union of Pure and Applied Chemistry) and SCOPE (Scientific Committee on Problems of the Environment)

Research

In 1998, the Japanese MOE established its *Strategic programmes on environmental endocrine disruptors* (SPEED '98). SPEED '98 had four main themes: (1) environmental monitoring and effects of EDCs on wildlife; (2) new technologies for dealing with EDCs; (3) risk assessment/management; and (4) international collaboration on ED research. SPEED '98 also defined a list of 65 chemical substances thought to be EDCs. Subsequent ED research has been based on SPEED '98.

Risk assessments for human health were conducted for all those chemical substances listed in SPEED '98 that were either produced or used by Japan; none was identified as hazardous due to ED effects. This work has now been extended to cover ED risks of these chemicals to the environment and wildlife. Chemicals on the EDC priority list are measured in water, aqueous sediments, air and wild animals by the MOE.

The MHLW funds a programme called the *Health and labour science research programme* which includes work on EDCs (for example, effects on offspring and development). In particular, the programme considers EDCs in drinking water (including monitoring) and low-dose effects as important.

The MOE monitors food and indoor air as part of a general monitoring programme for suspected substances, but this also includes EDCs. For human health, umbilical cord blood is sampled to measure foetal exposure, and epidemiology of suspected substances in children is also monitored. The MOE in particular considers the risk of EDC effects to be important, especially in children.

As stated, MOE monitors exposure of wild animals to EDCs; this is done annually. Effects are not attributable directly to ED, but all impacts on wildlife are considered important.

Priority substances

Japan works to the priority list identified by SPEED '98. This identified 67 substances considered to be EDCs, but this has since been reduced to 65 substances. However, the SPEED '98 list is under revision.

Testing and screening strategies

METI has a screening strategy that concentrates on human health; this includes *in vitro*, *in vivo* and definitive testing methods. *In vitro* methods include receptor binding and receptor gene assays for oestrogen receptor (ER) alpha, ER beta, androgen receptor (AR) and thyroid hormone receptor (TR); also, an aromatase assay using ovarian tumour cells and validation of a commercial reporter gene assay with an aryl hydrocarbon receptor (AhR) (CALUX^{TM}). Oestrogenicity and androgenicity are screened *in vivo* using the rodent uterotrophic and the rodent Hershberger assay, respectively; a repeated dose 28-day oral toxicity study is used to screen for overall activity *in vivo.* Reproductive toxicity is screened for using a two-generation reproductive toxicity test. These screens are continually progressing for different EDCs.

MOE has a screening strategy for mammals, fish, amphibians and invertebrates.

A one-generation test is used for mammals including thyroid and adrenal gland effects, although this is not specifically aimed at sex hormones.

In fish, sex steroids, vitellogenesis, and partial and full life-cyle tests are screened, also using ER reporter gene asays *in vitro*.

For birds (Japanese quail), a one-generation test using a Vtg kit is used for detecting oestrogenic effects.

A metamorphosis assay is used for ED screening in amphibians; also used is a thyroid assay, and a Vtg kit (for females).

A *Daphnia magna* life-cycle (one-generation) test is used for invertebrates.

One of the main problems with the interpretation of the screening tests is extrapolating the findings from one species to another. It is also difficult to assess effects of EDCs in the immune and neural/behaviour systems.

Update since 2003

Our request for information updating IEH (2003) was passed on to the relevant specialists, however, no further response was received.

4.2.2 United States of America

Organisations consulted:

- Environmental Protection Agency (US EPA)
- National Institute of Environmental Health Sciences (NIEHS)

Perceived importance of ED

Within the US EPA, ED is considered to be very important, and is one of six main research initiatives. In the wider political arena, however, it is probably only of medium importance, generally driven by public pressure.

Scientific interest in ED is high, and there is a recognised need for further research. ED is particularly important for research groups investigating reproductive and developmental aspects of human biology.

Media interest and/or concern in ED is low, due to competition from other topics. However, occasionally ED will be raised in the news as research findings become public. For example, when the effects of atrazine on frogs were reported it raised public interest to a very high level. Otherwise public interest is generally low, apart from those involved in interest groups, such as the World Wildlife Fund (WWF).

Legislation

The only legislation that applies specifically to EDCs is that of the Food Quality Protection Act 1996, which requires pesticides to be tested for ED activity. Consequently, the US EPA's entire ED screening and testing programme is based on this legislation. The Safe Drinking Water Act 1996 does not legally require the testing of chemicals in source water for ED activity, although it does give the US EPA the authority to do so.

The Food Quality Protection Act requires that risks from chemical residues in food are assessed on a cumulative basis, and are considered on a 'real world' basis. Additional sources must also be considered so that the total exposure is evaluated.

There is no additional legislation to protect the environment or wildlife from EDCs, other than that already in place for other toxic chemicals; there is nothing specific for EDCs. The WWF has recently pushed for specific ED legislation to protect wildlife.

Research

In 1998, the US EPA established a research plan (EDSTAC '98) for ED and EDCs that was designed to last for 10 years, but now extends to 2012. The programme covers both human health and wildlife protection. Areas of particular importance are the identification of classes of chemicals, elucidation of dose-response curves and modes of action, all of which have implications for future legislation.

A number of other research projects complement the main ED research programme, including routes of exposure to humans and wildlife, human studies (for example, EDC

levels in children at nursery), the integration of human and ecological data in risk assessments, sources of exposure, drinking water treatment, chemical mixtures, and impacts on the environment (which consider pulp mill and STW effluents).

This work is funded by a combination of US EPA money and grants. Internal research and development is the main driver for such research, but mandates are also important. The US EPA consults with external groups, including academia, industry and NGOs when formulating research plans, which are all subject to external peer review.

The National Health and Environmental Effects Research Laboratory (NHEERL) of the US EPA is the largest individual laboratory researching ED in the United States. For human health specifically, ongoing research by the NHEERL is investigating the effects of atrazine in causing the early formation of tumours in mammals; the developmental toxicity (in terms of postnatal losses) of the surfactant perfluoro-octane sulphonate (PFOS), used in Scotchguard among other products; the mode of action of conazole fungicides in causing thyroid tumours; and the mode of action of chlorinated and brominated by-products of disinfection processes, which cause reproductive or developmental problems. In addition, research is underway to identify the effects of multiple-chemical exposure on reproductive development, and whether the effects of multiple chemicals with different modes of action act additively or synergistically.

In terms of specific wildlife protection, high density animal feed facilities are being investigated by the NHEERL because effluents from these sites have been shown to be androgenic, although the causal substance has not yet been identified. Another research area is investigating the effects of trembolone on wild fish. Several projects are examining ED in sheepshead minnow.

The US EPA Office of Research and Development (ORD) is progressing the use of computational technology, where advances in molecular biology, molecular chemistry and computation are combined; it is hoped that computational technology can be applied to ED.

NIEHS also has an active ED research programme, and as with the US EPA, human and environmental effects are generally not segregated (on the assumption that if the environment is affected there is a risk to humans). NIEHS funds various wide-ranging research projects. In particular, ED effects on female and male human reproductive development (*in vivo*) are considered important. ED signalling is another key area, particularly dose-response relationships. Important current research is using multigenerational studies on oestrogenically-active substances (such as ethinyloestradiol, EE2), so that their effects can be compared across males and females at different levels of exposure. This will help to determine whether our understanding of the effects and modes of action of these chemicals is correct. Another important area is the development of guidance on the production of authoritative monographs on human reproduction and development effects, which will provide weight-of-evidence information and a scale for the adverse effects of substances.

All research is subject to peer review and consultation with advisory boards including academia, industry and NGOs; decisions are announced publicly and subject to comment.

Priority substances

The US has a completely different approach to priority substances compared with that of the EU. There is no list of substances for priority regulatory consideration or control, nor are there plans to establish one.

In the US, the chemical registration system only applies to pesticides. For substances already registered, the burden lies with the US EPA to prove there is an ED problem. The US EPA must be advised of new substances, but there is no set requirement for datasets to be supplied. There has been pressure to develop a list of known EDCs, but nothing is planned.

However, a priority list has been established for screening and testing purposes, based on exposure criteria using monitoring data. The initial list had 50-100 chemicals.

Testing and screening strategies

The US EPA has a tiered screening strategy driven by the 1996 legislation and the 1998 EDSTAC report. The three main initiatives are: (1) to develop and validate tests and screens; (2) to generate a list of chemicals for screening; and (3) to draft regulation on testing and screening, where industry will perform the tests. The emphasis is on assessing the ED activity of the chemical, regardless of the animal it is being tested on. Screens at each tier will include both *in vitro* and *in vivo* tests.

Tier 1 screens will be mammalian-based, although fish and amphibians will also be considered. Mammalian tests will include human/rat ER/AR assays, uterotrophic and Hershberger assays, and a rat pre-pubertal assay. A reproductive test will be used for fish, and a thyroid-related test will be used in frogs. Furthermore, details of ER and AR binding are being studied, in an attempt to incorporate the information into quantitative structure-activity relationships (QSARs).

Tier 2 screens will be multigenerational for rats, fish, birds, amphibians and mysid shrimp. Endpoints measured will include oestrogenicity, androgenicity, thyroid function, steroidogenesis, aromatase activity, mammary gland development, prostate development and general health endpoints.

To date, the frog tail re-absorption assay and fish gonadal recrudescence assays have had problems in development, resulting in the further development of a pre-metamorphosis assay for amphibian thyroid function, and a true reproductive screen being developed for fish.

The NIEHS has initiated several studies for screening purposes. Multigenerational studies (up to five generations) have been used to gain baseline data for the development of shorter term assays. Also, much work has gone into the development of genomic techniques, including transfection and microarray assays. One key area of work has been the pharmacology of EDCs, with the aim of identifying and separating true hormonal responses and non-hormonal responses to EDCs. The NIEHS has also considered transgenic models and whether these offer a useful approach.

NIEHS is government-funded and specifically looks at the effects of EDCs on human health, rather than general ED activity. Its interest is in whether ED can result in human disease.

The NIEHS is also actively involved in the development and assessment of high throughput screens. The pharmaceutical industry leads in this aspect of screening, but the pesticide industry could make more use of these screens if motivated to do so. Regulatory agencies such as the US EPA are further behind, but the NIEHS wants to be involved at the forefront of this science. Again, genomics (and to a much lesser extent, proteomics) is one of the main areas being assessed and developed for this purpose.

Update since 2003

Importance of ED

The ED issue remains important politically and scientifically, but the media is more focussed on climate change and so public awareness has decreased. However, public concern was raised following the detection of feminized fish in the Potomac River and in rivers in Colorado.

Research

Within the EPA there has been some shift of resources away from endocrine disruptors. This reflects the view that the development of screens and tests has been completed and these assays are now in the pre-validation or validation stages. Within the Office of Science Co-ordination and Policy (OSCP), priorities have not changed.

The detection of feminized fish led to a set of Congressional hearings on endocrine disruptors, which should ensure that more agencies and programs work together on this issue. The monitoring of rivers and other bodies of water falls chiefly under the US Geological Survey (USGS). USGS noted the feminized fish program and is stepping up its monitoring and follow-up accordingly, which is promoting greater cooperation between USGS and EPA's Office of Water.

The primary mission of the OSCP with respect to endocrine disruptors is to ensure the development and validation of screens and tests for endocrine disruptors. These screens and tests will then be implemented by the Office of Pesticide Programs which will require industry to test the chemicals that it produces and registers as pesticides.

In terms of human health, the US EPA's research program has been quite broad. It has featured basic research on mechanisms of action and identification of effects of endocrine disruptors in model species of relevance to human health and the environment. It has also resulted in the development of test methods for effects and for characterization of chemicals suspected of being EDCs. In addition, the US EPA has investigated the efficiency of wastewater treatment facilities in removing various types of EDCs.

NIEHS has funded research in human health aspects of ED, but not wildlife or environmental aspects.

Priority substances

The U.S. has taken the position that existing hazard information is not adequate for prioritizing chemicals for screening or testing for endocrine disrupting potential. The U.S. experimented with the development of high throughput screens and QSARs in the late 1990's.

The US EPA is still developing its priority list of EDCs for screening, as identified from the *Endocrine disruptor screening programme* (EDSP). The OSCP plans to implement the screening of priority chemicals in early 2008. Chemicals testing positive in the Tier 1 screen would be tested in Tier 2 to characterize the effects on human health and the environment. A general review of the success of this limited screening would be conducted by the EPA and a peer review panel before continuing to screen additional compounds.

The original list of priority substances included between 50 and 100 chemicals, selected on the basis of their relatively high potential for human exposure, rather than a combination of exposure and effects factors. Following several projects this list was

reduced, firstly to 37 and then to 28 chemicals. The list remains to be finalised but is expected to be published in 2007.

Further information can be found at:

http://www.epa.gov/scipoly/oscpendo/pubs/prioritysetting/approach.htm

The screening/testing strategy has not changed since 2003. The US EPA plans to update the screens and tests as the science evolves. For instance, tests using animal tissue (such as the sliced testes for steroidogenesis) are being replaced by the assay using the H295R adrenocarcinoma cell line, and binding assays using rat uterine or prostate tissue for ER and AR binding, respectively, are being replaced by recombinant receptors.

4.3 Non-government organisations

4.3.1 Organisation for Economic Cooperation and Development (OECD)

Perceived importance of ED

Within the OECD, ED is considered to be of high importance. ED has a high political profile within the member states of the OECD, and consequently receives considerable funding for test guideline development. This work requires a substantial scientific understanding and this is reflected in the scientific effort ED receives: of all the work relating to ED carried out by the OECD, approximately 80 per cent is spent on science, with only 20 per cent devoted to policy.

Media and public interest varies, but is generally medium to low, and led by animal welfare and environmental groups. The OECD ensures that such groups are included in, but do not dictate, the ED issue.

Legislation

Legislation varies from member state to member state, and consequently the protection afforded also varies. There are also differences between existing and new chemicals. Existing chemicals are difficult to assess with current information. In contrast, testing requirements for new chemicals (particularly pharmaceuticals, pesticides and food additives) are generally adequate, certainly enough to identify potential risk. This is not the case for new industrial chemicals, which are not subject to such detailed testing.

EU legislation appears to match the OECD approach, and member states appear happy to follow the testing guidelines recommended. However, there are discrepancies with the US, which is generally more rigid in collecting chemical activity information and prefers its own testing methods. For example, the mysid life-cycle assay is highly developed in the US, but is not seen to be ecologically relevant in other countries. The OECD has addressed this issue by documenting the mysid test and other potential arthropod tests to encourage discussion of a preferred test species. Selection of the preferred fish species for testing is at a similar stage.

The preferred approach for legislation is that chemicals should be assessed on their complete chemical and toxicological profiles, and not just whether they are EDCs (that is, there should not be legislation specifically for EDCs).

Research

The OECD has no research programmes specifically on ED effects on human health or wildlife. All research effort is directed to testing and method development.

Priority substances

The OECD has collated all published lists of priority substances, and after examining the criteria used, has formulated a list of the most frequently cited substances for testing and regulation. Many of these are already subject to regulatory control, based on other aspects of their toxicological profiles.

Testing and screening strategies

The OECD has a five-level conceptual framework for screening and testing, which covers human health and environmental protection:

Level 1 – Sorting and prioritising using existing chemical information, such as physico-chemical properties, exposure data and hazard/risk data.

Level 2 – *In vitro* testing to demonstrate mechanisms, such as: ER/AR, TR receptor binding affinity; QSARs; thyroid function; fish hematocyte Vtg assay; transcriptional activation; steroidogenesis.

Level 3 – *In vivo* testing of single ED mechanisms and effects, using: uterotrophic assay; Hershberger assay; non-receptor mediated hormone function; fish Vtg assay.

Level 4 – *In vivo* testing of multiple ED mechanisms and effects, using: male and female pubertal assays; intact adult male assay; fish gonadal histopathology; frog metamorphosis assay.

Level 5 – *In vivo* testing of ED effects and other mechanisms, using: one- and two-generation assays; reproductive screening test; combined 28-day reproductive screening test; partial and full life-cycle tests for birds, fish, amphibians and invertebrates.

The main endpoints for measurement are oestrogen and androgen agonism, oestrogen and androgen antagonism, and thyroid hormone interference.

With wildlife screening, there is an emphasis on determining whether reproductive effects found in arthropods also affect vertebrate or mammalian reproduction. This applies to other, non-reproductive effects.

Update since 2003

The OECD continues to coordinate the validation of screening assays to detect ED for both human health and the environment. In 2005, the OECD published a guidance document on how to conduct validation studies in OECD countries. This will ensure that all OECD validation studies follow the same principles. Some of the assays for ED screening are now at the stage of peer review and will be developed as OECD test guidelines.

For human health, the uterotrophic and Hershberger bioassays are the most advanced (peer review completed and peer review underway, respectively). For the environment, the 21-day fish screening assay and the 21-day frog metamorphosis assay are currently being validated. The experimental work is being undertaken by laboratories in OECD member countries; the OECD does not provide funding for these experiments and participating laboratories have to financially support their work by themselves.

Recently, the OECD contributed to a special issue of the journal Ecotoxicology documenting developments in the use of invertebrates in ED testing (Gourmelon and Ahtiainen, 2007). Issues that the OECD are currently addressing are:

- what is the importance of mechanistic information in regulating chemicals?, and;
- what is the best way to address the issue of possible endocrine disruption in invertebrates while integrating these tests in a regulatory scheme? (Gourmelon and Ahtiainen, 2007).

4.3.2 European Chemical Industry Council (CEFIC)

Perceived importance of ED

ED has attracted a lot of attention, for example via the EC White Paper on chemical regulation, and it has been identified as an area of toxicology that requires further attention. CEFIC recognises that ED is important politically and scientifically, and devotes a considerable amount of its science and research resources to this area. Public interest varies, but is thought to be of medium importance (there is an elevated awareness).

Legislation

CEFIC is actively involved in the development of a scientific knowledge base for ED, with a view to filling gaps in our understanding and improving safety. EDCs do not warrant special attention, but the overall risk of a chemical is important. With reference to the EC White Paper, this is particularly the case for substances that have cancer, mutagenicity or reproductive toxicities.

Changes in legislation should be made at EU level, and CEFIC strongly supports the OECD with regard to ED and EDCs. CEFIC takes an active role in the development and validation of OECD activities.

Research

Roughly 33 per cent of CEFIC's long-range research initiative budget is spent on ED, focussing on male reproductive health. CEFIC has funded human health-related ED research at a number of universities attempting to identify causal factors of health effects (including occupational). Reliable baseline information on cryptorchidism (undescended testes) and hypospadias is also being collected. Exposure of soils and drinking water to chemicals is being investigated (for example, from point sources in estuaries), though this is in terms of the general toxicity profile of the chemicals.

CEFIC has funded a number of environmental ED studies, including the EDMAR[4] project (providing roughly 20 per cent of the total budget), some of Brunel University's work on sex ratios of fish population, and Dutch research on thyroid function.

CEFIC has also collaborated on OECD research programmes.

Priority substances

CEFIC has been actively involved with the EU's attempts to compile a priority list of chemical substances, and will follow EU initiatives in this area.

[4] Endocrine Disruption in the Marine Environment (see Allen et al 1999a; 199b)

Testing and screening strategies

CEFIC works closely with the OECD on the development of testing and screening programmes and endorses the approach to identify, and gain international agreement on, common screening and testing methods. This applies to both humans and wildlife.

Update since 2003

Unfortunately no reply was received to our request for an update since IEH (2003).

4.3.3 Okazaki National Research Institutes (Japan)

Perceived importance of ED

Political importance of ED is difficult to quantify, although ED has made an impact and led to new chemical regulations. Greater interest in improved regulation has developed alongside knowledge gained on ED. Scientific interest in ED is high, particularly to identify and understand the mechanisms involved.

Although the media interest in ED has declined, public interest remains high, especially in certain human-health related issues (for example, the possibility of BisA leaching from the lining of drinks cans and from polycarbonate food bowls).

Legislation

At the moment there are not enough data to legislate specifically for EDCs, and it takes a great deal of time to establish testing strategies. An EDC testing framework has been set up, but regulation remains several years in the future.

Research

The institute does not research human health issues, but is actively involved in several aspects of ED research with regard to environmental protection and health of wildlife. At the institute, research is focussed on sex differentiation and gonadal development in different animals. The institute also collaborates on a number of ED projects in laboratories around the world, with some of the leading academic ED researchers.

Priority substances

The institute is not involved with this.

Testing and screening strategies

Human health is not studied at the institute, but it is involved in the development of testing strategies for oestrogenicity and androgenicity in a number of animals, including medaka, alligators, daphnia, mosquito fish, zebra fish and roach. This work is collaborative with other international laboratories, and has focussed on the development of reporter gene assays and ER and AR receptor binding.

Although the institute does not formally collaborate with the OECD, the results form institute research programmes are shared with the OECD.

Update since 2003

<u>Importance of ED</u>

Funding of ED research is a problem. The Japanese government usually funds science topics for only five years, and because large research initiatives into ED and EDCs began in Japan in 1997, funding has been greatly reduced.

In 2005, Japan's Ministry of Environment (MOE) changed its strategy for EDC issues from that presented in SPEED '98 to the recommendations from another project, ExTEND 2005. The new strategy has no list of priority substances to be studied, presumably due to pressure from industry; instead, all chemicals should be assessed. However, the Society for EDC is still actively researching the topic.

Public awareness of ED issues has probably reduced because the media rarely report new findings.

Research

The biology institute has been funded by the Ministry of Health Labour and Welfare (MHLW) to research some basic biology of ED in humans/mammals. It has been working to identify the mechanism by which EDCs affect developing rodents. In addition the institute has identified the functional mechanism of diethylstilbestrol (DES), which induces the persistent phosphorylation of ER; this causes the production of EGF-related growth factors, which in turn stimulate the phosphorylation of ER again. The institute has also clustered oestrogen responsive genes in mouse uterus and vagina.

Professor Iguchi has worked to establish a law banning the use of DEHP and DINP (phthalates) in materials that come into direct contact with food, or are used in chewing toys.

In terms of wildlife, the MOE has funded research to establish microarrays for *Daphnia magna*, medaka and algae. For *Daphnia* specifically, the institute has worked on the clustering of *Daphnia* expressed sequence tags (ESTs) and preliminary microarray. In addition, the Institute has demonstrated that juvenile hormone agonists (pesticides) induced 100 per cent male offspring in *Daphnia*, which usually produce only females by parthenogenesis. Oestrogen receptors from several animal species have been cloned and reporter gene assays produced. Professor Iguchi has supported the OECD validation and management group by reviewing *in vitro* assays using fish cells and genes.

Priority substances

Although not involved in prioritisation, the institute has contributed to fish testing which has further demonstrated the ED activities of nonylphenol, octylphenol, bisphenol A and o,p-DDT in medaka.

Other significant developments since 2003

The MHLW and METI are currently working to establish docking mode (in silico three day QSAR) and have found that 2,000 out of 200,000 chemicals can potentially bind human ER alpha. The MHLW is also working on low dose effects and establishing a life-long testing method.

4.3.4 World Wildlife Fund (WWF)

Perceived importance of ED

From the WWF perspective, ED is very important both politically and scientifically, and the WWF is actively trying to link these two arenas, so that effects demonstrated by science can be used effectively in policy making.

Media awareness is good, but requires new perspectives on the issue to remain newsworthy. Public awareness varies, and tends to be focussed on specific topics, such as sperm quality or other human health disorders.

As an indication of how important the WWF views ED, the US branch (WWF-US) employs two full-time scientists and one support person specifically to work on ED, and a number of other staff members allocate significant time to the subject.

Legislation

Current legislation and regulation with regard to EDCs is insufficient, both in Europe and the US. Many substances have not been tested for ED activity, and the lack of effort in addressing mixtures is particularly concerning.

Legislative areas that the WWF is particularly concerned with are: the lack of information on complex environmental mixtures with regard to exposure and human health (for example, hormone-related cancers); differences in species sensitivity and the lack of testing on the most sensitive animal groups, particularly among invertebrates; and improved testing during regulatory assessments, complemented with improved environmental monitoring of those chemicals, including their breakdown products, that pass regulatory controls.

The WWF would like to see a more coordinated approach to regulation of EDCs in Europe, and believes that this can only be achieved at EU level. In the US, the WWF has intervened in the US EPA's regulatory process on pesticides during review stages. In particular, WWF-US is concerned with the tendency of the US EPA to use weight-of-evidence, whereby the supply of extensive datasets funded by industry can lead to biased assessments.

Research

The WWF has funded some fundamental research into human health and wildlife ED issues, working in association with independent academics. The WWF also assesses and interprets the scientific information available on ED and EDCs, for the purposes of scientific review articles, briefing papers and so on.

Priority substances

Although not directly involved with the prioritisation of EDCs, this is an area that the WWF is particularly interested in and one where it would like to see a lot more international agreement and collaboration.

In Europe, the EU list and the data provided by BKH have been very useful, but this initiative needs to be progressed in a coordinated way for future regulatory use. In the US, the WWF has intervened where necessary with the US EPA's prioritisation work, which it feels does not receive enough funding.

Testing and screening strategies

The WWF does not do any chemical testing, but does scrutinise and comment on such testing. For example, the WWF has observer status in the OECD test method development.

Regarding current testing and screening strategies, tests should look at endpoints beyond oestrogenicity and androgenicity, and should include investigations into thyroid function and signalling. Furthermore, assessments of ED should include not just hormones, but also associated enzymes, neurotransmitters, growth factors and proteins.

As with other aspects of ED research and regulation, there is a need for a more coordinated approach to testing and screening, including better integration of the various methodologies into an overall process. In addition, the process is taking too long, perceived to be due to a lack of funding which is particularly apparent in the lack of academic specialists participating in method development.

Update since 2003

Because of commitments to working towards influencing the implementation of REACH, the WWF were unable to provide an update to their statements made in 2003.

However, a brief communiqué stated that the WWF felt that the ED issue is more critical than it was 10 years ago. Important points identified were the potential for epigenetic effects, and the links between hormone disruption and adverse effects on brain development, as well as obesity and diabetes.

Most importantly, the WWF has tried very hard to get EDCs included in REACH. During the "*Impacts of Endocrine Disrupters*" conference held in Helsinki, 2006 (a 10th anniversary follow-up meeting of a similar meeting held in Weybridge, UK), the WWF orchestrated a statement on ED and Article 56(f) of REACH, which was signed by 38 of Europe's leading hormone disruption scientists. The statement expressed concerns over the phrasing of the proposed regulation, and recommended that:

"*where scientific evidence shows a substance to have endocrine disrupting properties, there should not be a need to show that serious effects are probable, before this substance is subject to the REACH authorisation procedure*".

The statement provided alternative wording and said that if alternative, non-ED active chemicals were available to those with ED properties, they should be used instead.

5 Research councils

The two main research councils in the United Kingdom responsible for funding biological, environmental and ecological research are the Natural Environment Research Council (NERC) and the Biotechnology and Biological Sciences Research Council (BBSRC).

5.1 BBSRC

BBSRC is a non-departmental public body (NDPB) and is supported through the Science Budget by the Department of Trade and Industry via the Office of Science and Innovation.

The BBSRC is the UK's main funder of basic and strategic biological research. It supports research and training in universities and research centres throughout the UK, and promotes knowledge transfer from research to applications in business, industry and policy, and public engagement in the biosciences.

BBSRC is investing an estimated £380 million in UK research for 2006/2007.

5.1.1 Funding

There is currently one studentship and three projects being supported by the BBSRC for ED- and EDC-related research, worth a total of £830,000. Spending for the year 2005/2006 was:

Studentship (1):	£ 14,000
Grants (3):	£163,000
TOTAL	£177,000

5.1.2 Strategy for ED

The BBSRC does not currently have any specific priority or policy on ED or EDC research.

The BBSRC accepts responsive mode applications in all areas of its remit. The remit of the BBSRC has the potential to include ED applications, and applications are received in this area, although ED applications form only a very small percentage of the total applications received. The main sections of the BBSRC that receive ED-related applications are Animal Sciences, Agri-Food, and Genes, and Developmental Biology. Any EDC applications submitted to BBSRC are assessed equally alongside all other applications in that grants round.

5.2 NERC

The Natural Environment Research Council (NERC) is also an NDPB, funded mainly by government through the Department of Trade and Industry's Office of Science and Innovation. Although NERC recieves public money, it remains independent of government.

The NERC is the UK's main agency for funding and managing research, training and knowledge transfer in the environmental sciences. NERC supports research in universities and in its own research centres.

The NERC invested an estimated £375 million in UK research for 2006/2007.

5.2.1 Funding

The NERC currently supports ED research grants/fellowships to the value of £3 million.

Past spending on ED research is more difficult to quantify, although completed grants on electronic record total £820,000.

In additions, the NERC has supported at least 10 PhD studentships relevant to ED (with funds of approx £50,000 each).

The NERC also invests in research via NERC Research Centre Programmes, for example at the Centre for Ecology and Hydrology (CEH), but it has not been possible to quantify how much money has been spent on ED research in such centres.

The NERC has no specific allocation of funding towards ED research. All projects are awarded competitively via responsive research calls or directed programmes in competition with other research areas.

5.2.2 Strategy for ED

ED is not indicated as a priority by the NERC. In terms of impacts on natural ecosystems or the *Environment and human health programme*, ED remains within the general remit of the NERC, and academics are free to bid for funding from the responsive research schemes to support work in this area, but all applications are assessed equally in competition with other subjects. Funding decisions are based on scientific excellence.

The NERC has not identified any key ED research areas/needs for current or future funding. The NERC remains independent and all funding decisions on research applications (including ED) are led by academics (such as the NERC Peer Review College) with some additional inputs from government and regulators if appropriate (such as the NERC Affiliate College).

6 Current Environment Agency endocrine disruptor projects

As the leading public body for protecting and improving the environment in England and Wales, the Environment Agency has committed a significant amount of its science and research budget towards ED and EDC research. Many projects have been completed and published, and are available on the Environment Agency website: www.environment-agency.gov.uk. In addition, the Environment Agency is currently running a number of ED-related research projects.

This chapter summarises current Environment Agency-led ED projects. The projects outlined in this chapter have received a total funding of just over £1 million from the Environment Agency, the Department for Environment, Food and Rural Affairs (Defra), UK research councils and industry:

Environment Agency	*c.* £700,000
Defra	*c.* £48,000
BBSRC	*c.* £173,000
Industry	*c.* £118,000

6.1 ED Project Initiation Document (PID)

6.1.1 Horizon scanning (SC030276)

Aims:

A comprehensive review of the literature and active research groups to provide an update on current knowledge on endocrine disrupting chemicals and their effects in the following areas:

- invertebrate endocrine disruption;
- genomic techniques;
- priority and new EDCs;
- current status and future direction of research.

Timescale:

September 2004 to April 2007.

Outputs:

- internal report updating the Environment Agency (1999) report on invertebrate endocrine disruption (Science Report SC0300276/SR1);
- internal report reviewing genomic techniques available for ED (Science Report SC0300276/SR2);
- internal report listing priority and new endocrine disrupting chemicals (Science Report SC0300276/SR3);
- internal report on current status and future direction of ED research (Science Report SC0300276/SR4; i.e. this report).

Collaborative information:

None.

Contribution to Environment Agency ED strategy:

The reviews will enable the Environment Agency to make decisions on the regulation of EDCs with the most relevant and up-to-date information.

6.1.2 Ontogeny of sex determination and development (SC030299)

Aims:

- to document normal sexual differentiation and development in juvenile roach (atlas of ontogeny) in order to better understand the effects of endocrine disrupting chemicals on the sexual development of wild fish in UK rivers;
- to determine the effects of long-term exposure to environmental concentrations of exogenous steroid oestrogen (ethinyloestradiol) on sex determination, sexual differentiation, sex cell development and early life in the roach
- using the molecular tools developed as part of NERC-funded research, to investigate the potential for simple molecular probes to be used for signalling and diagnosis of endocrine disruption/intersex in roach.

Timescale:

December 2004 to November 2007.

Present outputs:

Draft atlas of ontogeny documenting normal sexual differentiation and development.

Expected outputs:

- science report on the effects of long-term exposure of roach to ethinyloestradiol;
- science report appraising genomic tools as a non-lethal survey technique and a roach microarray.

Collaborative information:

None.

Contribution to Environment Agency ED strategy:

This work package will enable the Environment Agency to better predict the potential impacts of long-term oestrogen exposure to a native fish species. It will clarify the mechanistic (genomic) links between exposure and effect and identify sensitivities of key life stages to guide hazard assessment and protection goals.

6.1.3 Fathead minnow bioassay (SC030278)

Aims:

- to adapt an existing laboratory bioassay, which has already been validated against individual EDCs, to assess ED effects of whole effluents and environmental samples;
- to produce standard operating procedures (SOP) and quality control criteria for the fathead minnow screening and pair breeding assays.

Timescale:

February 2005 to December 2007.

Expected outputs:

This project will produce validated *in vivo* tools (and associated SOPs and quality control criteria) which are fit for testing sewage effluents within the *Endocrine disruption demonstration programme.*

Collaborative information:

This is a collaborative project with financial input from AstraZeneca.

Contribution to Environment Agency ED strategy:

The bioassay and methods developed in this project will be used within the *Endocrine disruption demonstration programme* to assess the holistic ED effects of sewage treatment work effluents.

6.1.4 The characterisation and functional role of novel oestrogen receptors (ER) in the prosobranch mollusc *Marisa cornuarietis* (SC030187)

Aims:

- to understand the mode of action of EDCs in gastropods, in order to be able to state with confidence that reproductive effects observed in this species are caused by endocrine disruption;
- to characterise the oestrogen receptor (ER) *in vitro;*
- to characterise the ER *in vivo* – this will involve determining the baseline expression of the ER in unexposed molluscs through one annual cycle and how this differs on exposure to EDCs.

Timescale:

October 2004 to July 2007.

Expected outputs:

Science report describing the findings of this project.

Collaborative information:

This project is mainly funded by a BBSRC grant, with contributions from the Environment Agency and AstraZeneca.

Contribution to Environment Agency ED strategy:

This work package will provide information on the mode of action of EDCs in steroids. This will feed into both the European risk assessment of bisphenol A and the development of a mollusc ED biomarker (see project below) to protect an invertebrate group and reduce vertebrate testing.

6.1.5 Molluscs as an indicator species of endocrine disruption (SC030279)

Aims:

- to determine whether selected UK species are sensitive/responsive to known EDCs in mesocosm exposures;
- to establish controlled cultures of sensitive species in the laboratory;
- to expose snails to EDCs in controlled conditions and measure reproductive and developmental effects and biomarkers.

Timescale:

August 2004 to October 2007.

Expected outputs:

Development of a bioassay that can be used to assess the effects of sewage effluents and EDCs in a non-vertebrate species.

Collaborative information:

None.

Contribution to Environment Agency ED strategy:

This project will give a clear indication of the sensitivity of gastropods to EDCs and a comparison of sensitivity with fish may be possible. The results will indicate the level of protection afforded to an invertebrate trophic layer by current control measures. The project will also help to explain the decreases in gastropod diversity downstream of STWs reported by Environment Agency monitoring staff.

6.1.6 Catchment-based risk assessment of steroid oestrogens from sewage treatment works (SC030275/3)

Aims:

To carry out a national catchment-based risk assessment of steroid discharges from domestic effluents, which will help to prioritise river reaches at risk of exceeding biological thresholds of effect.

Timescale:

January 2006 to August 2007.

Expected outputs:

- science report on the methodology and results of the risk assessment model;
- risk maps of steroid discharges.

Collaborative information:

None.

Contribution to Environment Agency ED strategy:

The Environment Agency committed to carrying out a national catchment-based risk assessment of steroid discharges to run alongside the *Endocrine disruption demonstration programme*. The assessment will allow STWs that may require improvement to be identified in time for future water industry investment rounds (PR09 and beyond).

6.2 Fish population modelling (SC060002)

Aims:

To use both mathematical and statistical models to predict the potential effects of endocrine disruption on the long-term stability of roach populations subjected to a range of exposure scenarios.

Timescale:

March 2006 to May 2009.

Expected outputs:

A science report describing the model, inputs, assumptions and results of the predictive modelling. The mathematical modelling will also predict an effect concentration (such as predicted no effect concentration, or lowest effect concentration) for population stability.

Collaborative information:

The mathematical modelling is a collaborative project with financial input from Defra and contribution from AstraZeneca. The statistical modelling project is being conducted as an internal piece of work.

Contribution to Environment Agency ED strategy:

The outputs of the model will provide further evidence that ED is a real issue, and that the investment needed to improve certain STWs is justified.

6.3 EDCAT 7 – Influence of endocrine disruption on reproduction in roach (SC050061)

Aims:

- to develop a suite of DNA microsatellite markers that can be used to assign parentage to roach;
- to determine to what degree intersex male roach contribute to the next generation when they spawn with unaffected male fish;
- to assess how the ratio of intersex male fish and unaffected male fish affects the reproductive performance of the intersex fish;
- to assess how the degree of competition for females affects the reproductive performance of intersex male fish.

Timescale:

January 2006 to March 2009.

Expected outputs:

Collaborative information:

None.

Contribution to Environment Agency ED strategy:

The results of this project provide a first step in answering whether the occurrence of intersex fish in British rivers presents a threat to long-term population sustainability. The results will also feed into the fish population modelling project SC060002 (described above).

6.4 Endocrine disruption demonstration programme (SC040058)

Aims:

The Environment Agency has made a commitment to provide resources, jointly with the water companies and Defra, for project management of the demonstration programme. In particular this is to:

- manage data exchange between the Environment Agency and the programme;
- coordinate sampling and analysis activities on the programme's sites with programme and operational staff;
- analyse data to feed into existing STW risk assessments;
- support the implementation of Environment Agency science project outcomes within the demonstration programme (especially the fathead minnow test and *in vitro* screening of effluents).

Timescale:

January 2005 to March 2010.

Expected outputs:

The project will produce six-monthly progress reports jointly produced by the project manager and the contractor, a science report at the end of the ecological appraisal stage, and another at the end of the programme.

Collaborative information:

The programme costs are borne by the water industry. Management of the programme is collaboratively funded by the water industry, Defra and the Environment Agency.

Contribution to Environment Agency ED strategy:

The Office of Water Services (Ofwat), Defra and the Environment Agency have designed the programme collaboratively. The programme will investigate options available to the water industry for removing steroid oestrogens from STW effluents and is being undertaken as part of the current price review of water companies (PR 09).

6.5 Endocrine disruption demonstration programme - Fish histopathology environmental monitoring (SC050020)

Aims:

To evaluate the success of the *Endocrine disruption demonstration programme* by demonstrating reversal of intersex pathology in the wild fish (roach) population.

Timescale:

May 2005 to April 2011,

Expected outputs:

Collaborative information:

None.

Contribution to Environment Agency ED strategy:

The demonstration programme is limited to evaluating the reduction achieved by different technologies on effluent hazard. It does not extend to environmental monitoring of fish to demonstrate that the technologies introduced have reduced the effect (intersex occurrence and severity) in the field, which this project covers.

7 Environment Agency perspective on human health aspects of ED

The Environment Agency human health science team has considered human health aspects of ED and EDCs, and recommends that although there is no clear justification for additional regulatory measures on the basis of human health concerns, the possibility of harm to human health from environmental exposure to endocrine disrupting substances should be taken seriously. A clear strategy remains to be finalised. Compared with our knowledge of ED effects in fish, very little is known with regard to human health.

The following text is taken from a briefing note that was drafted for inclusion in the revised Environment Agency ED Strategy Document. The revised strategy is not yet published.

> "*There is considerable evidence in Europe and elsewhere of adverse trends in the incidence of several conditions relating to male reproductive health, most notably testicular cancer, hypospadias, cryptorchidism and reduced semen quality. Although environmental causes - including environmental chemical exposures - have been suggested, the evidence for this is at present inconclusive. This may be because there is no such link, or because there is a multitude of factors influencing human reproductive health (of which chemical exposure is but one), which makes it difficult or impossible to separate out any one of those factors alone as being 'causative'.*
>
> *Either way, it can be concluded that chemical exposure is not a major cause of these observed adverse trends. Indeed, the IPCS Global Assessment on endocrine disrupters concluded that analysis of the human data, while generating concerns, has so far failed to provide firm evidence of direct causal associations between low level (general population) exposure to endocrine disrupting chemicals and adverse health outcomes. If, as discussed above, exposure to chemicals was a major factor, it is perhaps more likely that those exposures would be occurring as a result of the ingestion of pharmaceuticals, or contaminated food, or consumer/personal product use rather than directly through environmental exposures*." (Paul Harrison, 2005)

At a Committee on Toxicity (COT) meeting in February, a workshop was held on *The development and function in adulthood of the male reproductive system - Potential chemical-induced effects*. This was an open seminar, with invited expert speakers. Whilst the incidence of some effects - testicular cancer and, possibly, cryptorchidism (undescended testes) - was agreed to be on the increase, the evidence for other trends in male reproductive health, such as decreasing sperm count and sperm quality, was less clear cut. Of particular note was the view that changes in hormonal milieu in utero caused by weak environmental oestrogens are unlikely to be sufficient to cause the postulated effects in humans. Causal effects of anti-androgens are likely to be more plausible. Other factors which may adversely affect male reproductive health were also discussed; these include lifestyle, birthweight and genetic factors as well as, possibly, non-endocrine disrupting chemicals.

The COT meeting reiterated the uncertain nature of some of the data on trends in male reproductive health. Nonetheless, an increase in certain adverse effects (notably testicular cancer) is clear. The once-prevailing view that weak environmental

oestrogens might be a causal factor appears to be losing ground, with other EDCs highlighted as one of several plausible alternatives.

Another meeting, the Committee on Carcinogenicity (COC) reported the following information regarding testicular cancer:

"A number of factors have brought testicular cancer to the attention of the COC: more than 90 per cent of testicular cancer occurs in men under 45 years old; it is the commonest cancer in men of this age; and incidence rates in Great Britain have more than doubled since the mid-1970s. The COC has reviewed an overview of risk factors for the disease and will consider whether a more detailed review of the possible involvement of chemical causes of testicular cancer is warranted. Cryptorchidism (undescended testes) is a well-established risk factor and it is possible that the in utero environment during a critical developmental window is important. Several reports have suggested that men engaged in some occupations may be at increased risk, but there are conflicting views in the literature."

Because it occurs in younger men, and its incidence in the UK is increasing, testicular cancer is of particular current interest and the COCs deliberations are likely to receive considerable attention. Further review by the Committee is likely to include consideration as part of the postulated testicular dysgenesis syndrome as well as examining any evidence for the possible involvement of particular chemicals.

8 Summary and conclusions

This report outlines the current awareness, legislation and research on ED and EDCs. Much of the information was taken from a detailed survey of international regulatory agencies and laboratories specialising in ED, which the Institute of Environment and Health performed in 2003 (IEH, 2003). Given that research initiatives and regulatory changes tend to move quite slowly (over years), the survey provided a good basis for this review. To obtain an up-to-date perspective on the ED issue, a follow-up questionnaire was sent to all participants of IEH 2003. Not all those approached replied to this call for information, and the detail of information supplied by those who did varied. However, the information is still a good representation of the current status of ED legislation and science.

Other sources of information for this report included websites of regulatory, research and conservation agencies; UK research councils; published initiatives on ED legislation and science such as the EU's derivation of a priority list of EDCs (BKH, 2000; 2002); and colleagues within the Environment Agency.

In Europe, the European Commission has a dedicated science and risk strategy for EDCs and, therefore, all member states follow EU guidance with regard to ED and EDCs. For example, member states of the EU have not developed their own lists of priority ED substances, but use the EC's priority list developed by BKH (2000; 2002). The majority of European countries also anticipate that the EC's Registration, Evaluation and Authorisation of Chemicals (REACH) system will provide a mechanism by which EDCs can be regulated. However, although European countries take a general lead from the EC with regards to ED, they may have their own research and/or regulatory agendas. For example:

UK	The demonstration programme and associated fish modelling work, and ED effects in molluscs; also effects on male reproduction.
Ireland	ED effects in freshwater ecosystems.
Denmark	ED effects in freshwater ecosystems; also fertility research.
Germany	ED effects in humans and wildlife.
Italy	Fertility research, water contamination.

With regard to testing and screening of EDCs, European member states support the strategy being developed by the OECD. However, there is some concern over the suitability of some of the recommended testing species with regard to their relevance to European ecosystems. For example, the US has developed a two-generation life-cycle test using a sub-tropical species of mysid shrimp (*Americamysis bahia*) and this test had been recommended in the OECD strategy. However, this species is absent in European waters; the OECD has accepted this and is looking towards finding alternative species for invertebrate testing. In spite of some problems, however, European countries seem content to use OECD guidance for testing and screening.

Japan and America are also heavily involved with ED and EDCs, and there are similarities and differences in their approach to ED regulation, research and screening.

Japan has no legislation for EDCs because, according to the definition provided by the WHO, there are no EDCs in Japan. However, increasing importance is being placed on chemical risk assessments and Japan now has a priority list for EDCs, which has 65 chemicals of concern. This will form the basis of their chemical risk assessment. ED research is focussed on both human health and wildlife impacts, such as environmental monitoring, breast cancer and chemical exposure, and QSAR. Japan collaborates

closely with the OECD for testing and screening research, including the validation exercise.

The US carries out most of the ED research being conducted around the world. US legislation is very different from European legislation, because in the US there is specific legislation for the testing of chemicals in food and drinking water that have ED activity. Consequently, a comprehensive testing strategy has been developed, including the derivation of a list of priority substances. However, the legislation is somewhat contradictory since inclusion on the priority list only requires that the chemical be tested, and not necessarily regulated. The US has also spent much effort on developing testing and screening methods. A number of research projects may feed into ED regulation.

At present, no country has legislation in place specifically to control or regulate EDCs (with the exception of tributyl tin on small sailing vessels). What tends to happen is that chemicals suspected of having ED activities also have other toxicological properties and are therefore regulated in accordance with their overall toxicological profile. In spite of the lack of specific legislation, a number of countries have lists of priority EDCs and there is concern that there is no agreed or formulated objective of such lists.

In general, all of the agencies and laboratories working on ED issues are keen for closer collaboration to prevent duplication of effort and allow more targeted and coordinated research efforts. Where such arrangements exist, such as the ED strategy of the EU or the OECD's screening and testing strategy, there is a general agreement to follow the guidelines produced.

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CREDO	http://www.credocluster.info/intro.html
Defra	http://www.defra.gov.uk/
EDEN	http://www.credocluster.info/eden.html
EDSTAC	http://www.epa.gov/scipoly/oscpendo/edspoverview/edstac.htm
Environment Agency	http://www.environment-agency.gov.uk
EC	http://ec.europa.eu/environment/endocrine/index_en.htm
EURISKED	http://www.credocluster.info/eurisked.html
FIRE	http://www.credocluster.info/fire.html
IUPAC	http://www.iupac.org/index_to.html
IUPAC/SCOPE	http://www-cger.nies.go.jp/ipcc/HP-SCOPE/scopeiupac.html
METI	http://www.meti.go.jp/english/
MHLW	http://www.mhlw.go.jp/english/index.html
MOE	http://www.env.go.jp/en/chemi/ed.html
NIEHS	http://iccvam.niehs.nih.gov/methods/endocrine.htm
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SCOPE	http://www.icsu-scope.org/
USEPA	http://www.epa.gov/scipoly/oscpendo/edspoverview/index.htm
WHO	http://www.who.int/ipcs/publications/endocrine_disruptors/endocrine_disruptors/en/index.html
WWF	http://www.pmac.net/theos.htm

List of abbreviations

AhR	Aryl hydrocarbon receptor
AR	Androgen receptor
BBSRC	Biotechnology and Biological Sciences Research Council
CEFIC	European Chemical Industry Council
CEH	Centre for Ecology and Hydrology
CERI	Chemicals Evaluation and Research Institute
COC	Committee on Carcinogenicity
CREDO	Cluster of research into endocrine disruption in Europe
Defra	Department for Environment, Food and Rural Affairs
DES	Diethylstilbestrol
E1	Oestrone
E2	Oestradiol
EC	European Commission
ED	Endocrine disruption
EDC	Endocrine disrupting chemical
EDCAT	Endocrine disruption in catchments
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDTA	Endocrine Disrupters Testing and Assessment
EE2	Ethinyloestradiol
EPA	Environmental Protection Agency
ER	Estrogen receptor
EST	Expressed sequence tag
EU	European Union
ExTEND	Perspectives on endocrine disrupting effects of substances
IEH	Institute of Environment and Health
IPCS	International Programme on Chemical Safety
IPPC	Integrated Pollution Prevention and Control
IUPAC	International Union of Pure and Applied Chemistry
METI	Ministry of Economy, Trade and Industry
MHLW	Ministry of Health Labour and Welfare (Japan)
MOE	Ministry of the Environment
NERC	Natural Environment Research Council

NGO	Non-government organisation
NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute of Environmental Health Sciences
NP	Nonylphenol
NPE	Nonylphenol ethoxylate
OECD	Organisation for Economic Cooperation and Development
Ofwat	Office of Water Services
ORD	Office of Research and Development (US)
OSPAR	Oslo-Paris Agreement
PCB	Polychlorinated biphenol
PR09	Price review 2009
QSAR	Quantitative structure-activity relationship
REACH	Registration, Evaluation and Authorisation of Chemicals
RIVM	National Institute for Public Health and the Environment
SCOPE	Scientific Committee on Problems of the Environment
SOP	Standard operating procedures
SPEED	Strategic programmes on environmental endocrine disruptors
STW	Sewage treatment work
TBT	Tributyl tin
TR	Thyroid hormone receptor
US EPA	United States Environmental Protection Agency
Vtg	Vitellogenin
WFD	Water Framework Directive
WHO	World Health Organisation
WWF	World Wildlife Fund
YAS	Yeast androgen screen
YES	Yeast oestrogen screen

Appendix 1: Questionnaire used by IEH (2003)

GENERAL

1. On what basis are you providing this information? (e.g. as a representative of the whole organisation or of a particular department or division)
2. General role and responsibilities of your organisation?
3. Which *other* national bodies have an interest, or role, in the endocrine disrupters issue?
4. What is perceived to be the *relative importance* of endocrine disruption in your country/organisation? (Low/Medium/High)
    - Politically?
    - Scientifically?
    - How important would you say this issue was to the media/public?

LEGISLATIVE ISSUES

1. Could you describe the *national legislative base* in relation to endocrine disruption and possible effects in HUMANS (media to consider – soil, ground water, drinking water, waste water, food)

    Please consider:

    - *Current* national legislation
    - Any *proposed* national actions
    - *Progress* to date/intended time-scales and perceived *obstacles* to progress nationally
2. Does the process of developing your policy/regulatory position on endocrine disrupters involve *consultation* with NGOs, industry or other stakeholders?
3. What *international, multinational or bilateral activities* are in progress with regard to policy development or legislative matters?
4. In addition to the programmes discussed above in relation to human health, are there national policy/legislative activities in progress *specifically* focused on protection of the ENVIRONMENT and WILDLIFE from endocrine disruption (media to consider – soil, groundwater, drinking water, waste water, food)?

    Please consider:

    - *Current* national legislation
    - Any *proposed* national actions
    - *Progress* to date/intended time-scales and perceived *obstacles* to progress nationally

5. Does the process of developing your national policy/regulatory position on endocrine disrupters involve *consultation* with NGOs, industry or other stakeholders?
6. Does it involve *interactions* with the OECD or other international body?
7. What *international, multinational or bilateral activities* are in progress with regard to policy development or legislative matters?
8. Are there any current or proposed legislative requirements *specifically* relating to the screening or testing of chemicals for endocrine disrupting activity, or would general chemical regulations be used? If the latter, what legislative instruments would apply?

HUMAN HEALTH RESEARCH PROGRAMMES

1. What relevant *national research programmes* are you aware of that relate to the effects of endocrine disruption on HUMAN HEALTH? (exclude testing)

   For each programme consider:

   - Is it *national* or *international*?
   - What is the *scope* of the research?
   - Does it *specifically* or *purposely* cover the following media - soil, groundwater,
   - drinking water, wastewater?
   - Does it involve *monitoring* for endocrine disrupting chemicals?
   - Which *institutions* are involved and how is the work *funded*?
   - What *areas* or *issues* are of particular concern?
   - What have been the *achievements* to date?
   - What are the *time-scales*?
   - Does this involve *co-operation* with, or *involvement* of, NGOs, industry or the OECD?
2. Could you advise on any *key documents/web sites*?

ENVIRONMENT/WILDLIFE RESEARCH

1. *In addition* to research of relevance to human health, are you aware of any additional *national research programmes* on the effects of endocrine disruption on the ENVIRONMENT or WILDLIFE? (exclude testing)

   For each programme consider:

   - Is it *national* or *international*?
   - What is the *scope* of the research?
   - Does it *specifically* or *purposely* cover the following media - terrestrial, freshwater, ground water, marine/estuarine?

- Does it involve *monitoring* for endocrine disrupting chemicals?
- Which *institutions* are involved and how is the work *funded*?
- What *areas* or *issues* are of particular concern?
- What have been the *achievements* to date?
- What are the *time-scales*?
- Does this involve co-*operation* with, or *involvement* of, NGOs, industry, or the OECD?

2. Could you advise on any *key documents/web sites*?

PRIORITY SUBSTANCES

1. What activities are you *funding* or *involved in* that are intended to assist in the identification or *prioritisation* of endocrine disrupting chemicals?
2. What is the overall *approach/objective*?
3. Are you *funding* specific aspects?
4. Which *institutions* are involved in or undertaking the activities?
5. Is it a *national*, *international* or *bilateral* effort?
6. Does this work involve *interactions/consultation/collaboration* with NGOs or industry?
7. Does it involve the OECD?
8. What is the *current* status of activities?
9. What *results* have been produced to date?
10. What, if any, *obstacles* exist?
11. Are there plans for *national* regulatory action following prioritisation?
12. What plans are for there for *further activities*?
13. Can you suggest any relevant *key documents/web sites*?

TESTING STRATEGIES & SCREENING/TESTING PROGRAMMES

1. What *approach* are you following with regard to developing a testing strategy and screening/testing methods relating to HUMAN HEALTH concerns?
2. What *additional approaches* are being followed in relation to a testing strategy or screening/testing methods for the protection of the ENVIRONMENT or WILDLIFE, and what groups of animals and/or sentinel species are being considered?
3. What *endocrine disrupting activities* (e.g. oestrogenicity, androgenicity) are being explored in the screening and testing strategies for humans or wildlife (if different)?

4. What *progress* has been made to date in developing a national strategy and/or methods for screening/testing? (Please consider humans and wildlife/environment separately)
5. What *problems* have been encountered so far during development of a strategy or screening/test methods?
6. How is work on strategy and method development being *funded*?
7. Are you undertaking the work solely on a *national* basis or as part of an *international* collaborative programme?
8. What aspects (if any) are being undertaken in conjunction with the OECD? Does it involve interactions or collaborations with NGOs or industry?
9. How have the results of screening or testing development work conducted to date been *used*?
10. Has any *formal regulatory screening/testing* of chemicals been undertaken (i.e. actual testing, not method development/validation)? If so by whom and on what?
11. What *use* has been made of any results of formal regulatory screening or testing work conducted to date?
12. What *difficulties* have been encountered with regard to interpreting any formal screening/testing data?
13. How is the screening/testing of chemicals for endocrine disrupting potential for regulatory purposes, being *funded*? (or how will it be funded?)
14. What are the *time-scales* for planned outstanding activities?
15. Can you advise on any *key documents or web sites* relating to your organisation's activities with regard to the screening or testing of chemicals for endocrine disrupting activity?
16. With regard to *high-throughput screens*, have you any particular comments with regard to their use in your organisation's screening or testing strategies?

    In particular, please consider:

    - Approach (e.g. inclusion in national research programmes)
    - Position in any screening and testing strategy
    - Models in development/validation
    - Results/current status
    - Obstacles/problems encountered
17. Does this work involve interactions/consultation/collaboration with NGOs or industry?
18. Is the OECD involved?

OTHER POLICY/RESEARCH INITIATIVES

1. Is your country/organisation involved in any other *related* policy or research initiatives/programmes (either on a national or international basis) that may *impinge* or *have bearing* on the endocrine disruption issue?

   If so, for each could you please describe their:

   - Scope
   - Time-scales
   - Research funding arrangements
   - Achievements
   - Particular areas/issues of concern

2. Also, do they involve interactions/consultation/collaboration with NGOs or Industry?

3. Do they involve the OECD?

SPECIFIC RECOMMENDATIONS/SUGGESTIONS

1. Are there any other specific comments, recommendations or suggestions that you wish to make? (open discussion)

Appendix 2: Update since 2003 questionnaire

Current perspectives on endocrine disruption. Review update from 2003 – 2006.

[These question are taken from the IEH report, specifically to gain an update on that report]

General

Has the relative importance of endocrine disruption changed within your organisation, both in terms of science and regulation, since 2003?

Has public awareness/concern of endocrine disruption changed since 2003?

Has your organisation influenced monitoring programmes, influencing policy development or legislation of endocrine disruption since 2003?

Human Health

Has your organisation funded or become involved in any new research programmes for endocrine disruption and human health, other than those described in 2003?

- If the answer is yes, what is the scope, media under investigation, and time scale for delivery? Have there been any outputs yet?

Has your organisation been involved with the development of testing or screening programmes relating to endocrine disruptors and human health?

Wildlife / Environment

Has your organisation funded or become involved in any new research programmes for endocrine disruption and wildlife or the environment, other than those described in 2003?

- If the answer is yes, what is the scope, media under investigation, and time scale for delivery? Have there been any outputs yet?

Has your organisation been involved with the development of testing or screening programmes relating to endocrine disruptors and wildlife / environment health?

Priority Substances

Has your organisation funded or become involved in any work to assist in the identification of priority endocrine disrupting chemicals, other than those described in 2003?

Have any chemicals been identified as priorities since 2003?

Testing

Have existing screening / testing strategies changed, or new ones been developed, since 2003?

- If so how, or what are they? Have any outputs been put into use since 2003?

Other

Is there any other significant information regarding endocrine disruption, or endocrine disrupting chemicals that has emerged in your organisation / country since 2003?

Appendix 3: People contacted with update questionnaire

Contact		Organization	email
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