ENDOCRINE-DISRUPTING CHEMICALS: A REVIEW OF THE STATE OF THE SCIENCE

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SUMMARY

In recent years, the possible effects of synthetic and naturally occurring chemicals with the potential to disrupt the endocrine system have been raised by scientists and environmental groups through the scientific literature, the Internet, books and television. These concerns were highlighted when research began to show that chemicals associated with adverse developmental effects in wildlife were also able to mimic the action of 17\(\beta\)-oestradiol, a female sex hormone.

The endocrine system is one of the signalling systems used to control the processes required for life. Other signalling systems include the nervous system and the immune system. These systems are integrated, which means that disruption of one can result in disturbances in the others.

The endocrine system uses hormones to carry messages from one part of a cell to another or from one part of the body to another. The hormones control processes such as reproduction, growth, development, energy use and maintenance of the internal environment (including blood pressure and heart rate). They interact with receptors located inside cells or on their surface – wherever activity is required. In the area of medical science, humans have benefited from taking advantage of our ability to disrupt the endocrine system – the contraceptive pill and providing insulin to diabetics are two well-known examples. It is becoming apparent that some synthetic chemicals can affect the health of organisms by either mimicking or blocking the action of these natural hormones or by interfering with the processes for making, excreting or delivering natural hormones to their site of action.

Synthetic chemicals that have been found to have this capacity include pesticides (e.g. the organochlorine insecticides, some herbicides and some fungicides), industrial chemicals (e.g. pentachlorophenol, polychlorinated biphenyls [PCBs], phthalate plasticisers, alkylphenol ethoxylates, bisphenol A) and pharmaceuticals (e.g. diethylstilboestrol [DES] and synthetic hormones in the contraceptive pill and in hormone replacement therapy). There are also naturally occurring chemicals in plants that have been found to have these effects (e.g. phytooestrogens). Naturally occurring hormones found in people and animals (including 17\(\beta\)-oestradiol and testosterone) can also interact with endocrine systems if they are released into the environment in an active form.

These chemicals can enter the environment by:

- direct, deliberate releases to land or water by chemical users;
- emissions to air from motor vehicles;
- emissions to air from various facilities;
- everyday use of chemicals and pharmaceuticals by householders and commercial users;
- accidental spills and releases;
- releases from plants into surrounding soils;
- indirect release to land or water from urban and rural run-off of stormwater;
- discharge from sewage treatment plants or pulp mills;
- disposal of animal wastes on land.

Once these chemicals are in the environment, they can be absorbed into the body directly from the air or the water or they can be taken in indirectly via ingestion of food or water. Chemicals that are not broken down during digestive processes can be absorbed into the blood and circulated throughout the organism which can then result in effects on the endocrine system.

The strongest supporting evidence for endocrine disruption involves high-level exposures to some of these chemicals of wildlife or people. Examples include:

- the effect of the drug diethylstilboestrol (DES) on the children of pregnant women who were given it to prevent miscarriage (the children were found to be significantly affected when exposed in utero – effects included cancer, malformations and sterility found only when they reached puberty or adulthood);
- severe infertility in sheep grazing on subterranean clover (containing phytooestrogens) in Western Australia since the 1950s.
Other impacts have occurred in wildlife populations exposed only to *seemingly low levels* of these chemicals. However, disruption of the endocrine system appears to be the most likely explanation for these effects. These include:

- the effect of tributyltin (TBT) anti-fouling paints on gastropods from rocky platforms (female snails developed penes, because TBT causes a build-up of testosterone);
- the effect of natural hormones, such as 17β-oestradiol, from sewage effluent discharged into rivers in the UK (fish have been found to have impaired reproduction).

A preliminary study in New South Wales, Australia, has provided limited evidence of endocrine disruption in aquatic animals downstream of a sewage treatment plant that discharges secondary treated effluent to a river. Studies at sewage treatment plants overseas indicate that even highly treated effluents are likely to have enough natural and/or synthetic hormones present to cause impacts in fish unless diluted significantly at discharge.

During many life stages, especially in mammals, disruption of the endocrine system might have little impact on the health of the individual, as feedback mechanisms control hormone signalling very sensitively. However, if an organism is exposed to low doses of these chemicals during a sensitive life stage (such as during foetal development) or is exposed to high doses during most life stages, serious health impacts can result.

It has been suggested that Australian marsupials could be susceptible to such effects during early development in the pouch, when they cannot access their mother’s protective detoxification systems. There is little available information so detailed research on the reproductive biology of these organisms and their sensitivities to these chemicals may be warranted.

There are two critical questions at the heart of this debate:

1) Are the current average exposures of people or wildlife high enough to be causing significant effects?
2) Are some of the reported adverse effects really related to disruption of the organism’s endocrine system or are the effects due to some other mechanism?

Information about what doses of these chemicals can cause impacts and what doses people and wildlife are being exposed to is currently being gathered through international collaboration and research. Strategies to direct research into areas where information is lacking are being pursued vigorously in the USA and Europe, especially in the area of potential effects in humans. The USA Government has provided $30-50 million to fund research. Chemical manufacturers are also investing significant amounts to gather the knowledge necessary to support decision-making.

Many of the chemicals thought to have the capacity to cause these effects – especially the organochlorine pesticides – were banned from use in many countries in the 1970s and 1980s, so exposures have been decreasing ever since. However, these chemicals are persistent, and small amounts are still present in the environment. Other chemicals discussed in this review are still in use. Owing to the uncertainty surrounding how much of a chemical is necessary to cause impacts, further research is required to allow determination of the best management approach. Many of these chemicals have a wide range of beneficial uses, and the risk of impacts will need to be weighed against the risk of losing those benefits.

**Key words:** endocrine disrupting chemicals, alkylphenol ethoxylates, natural and synthetic hormones.
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INTRODUCTION
During the 20th century, increasing numbers of synthetic chemicals were developed to meet a wide variety of medical, scientific, agricultural and industrial needs. While the wide use of these chemicals has had significant economic and social benefits (such as increased yields in agriculture and a dramatic increase in the variety of manufactured products available), it has also led to the release of many of the chemicals into the environment (Danzo 1997).

Public concern over the impacts of these chemicals on both human and environmental health has been growing, particularly during the last few decades. Recently, concern has focused on the possible effects on the endocrine system of a range of synthetic and natural chemicals that appear to disrupt normal hormonal function (Colborn et al. 1996). The chemicals of concern include natural hormones present in animals or plants, many of the chemicals that have been banned previously (such as the organochlorine pesticides), and a range of widely used chemicals that had not been investigated much including nonylphenol, octylphenol and bisphenol A. These synthetic chemicals have widely differing structures but can all interact with endocrine system functions. They have been labelled ‘endocrine-disrupting’ by a range of organisations including both environment groups and government agencies.

Although the international scientific community has not reached consensus on the linkages, if any, between synthetic chemicals and endocrine-related effects in human and wildlife populations, the potential implications of any linkage have stimulated significant coordinated research around the world, particularly in the USA and Europe.

This paper provides a review of the available scientific information to provide those interested with ready access to such information. A summary of current management responses by governments, including Australia, the USA, and the European Union (EU), is also provided.

HOW CHEMICALS CAN DISRUPT THE ENDOCRINE SYSTEM
The key fitting into the lock metaphor, used to describe the way hormone chemical messengers interact with their specific receptor to trigger an effect, implied that only the intended chemical messenger could fit into the lock. This implication then resulted in the assumption that the signalling system was protected against false messages. It has now been found that this is not the case.

Krimsky (2000) describes the disruption hypothesis “...the environmental endocrine hypothesis, asserts that a diverse group of industrial and agricultural chemicals in contact with humans and wildlife have the capacity to mimic or obstruct hormone function – not simply disrupting the endocrine system like foreign matter in a watchworks, but fooling it into accepting new instructions that distort the normal development of the organism...”. This is the major cause for concern: that these chemicals could possibly be causing subtle effects because they are tricking parts of the body’s complex communication systems into hearing slightly incorrect instructions.

These signalling systems are complex and there are numerous ways for disruption to occur. For example, there are now considered to be at least three different types of oestrogen receptors that have different roles and slightly different shapes, meaning that chemicals might interact with one receptor but not another. There is also a range of co-factors that must interact with the receptor for a signal to be sent properly. Some chemicals interact with the receptor, mimicking some or all of the behaviours of the natural chemical messenger or blocking the action of the natural chemical messenger. Other chemicals interfere with the production, storage or excretion of the natural chemical messenger or receptors. Others may interfere with the interaction of the hormone-receptor complex and the relevant co-factors.

Another possible example of a disruption mechanism was recently described by Li et al. (2003). They evaluated the links between the development of various cancers and exposure to DES. Based on their findings, they proposed an epigenetic mechanism which might make tissues more prone to cancer. The mechanism involves exposure to DES changing the methylation/demethylation patterns of DNA in oestrogen-responsive genes during early development, possibly by inducing the production of enzymes that methylate or demethylate DNA. In such situations the original DNA from the parents may be correctly formed, but the implementation of the information coded by the DNA can be flawed by the interaction with environmental factors such as exposure to hormonally active compounds like DES. Such flaws may not become obvious until the person reaches adulthood. Abnormal patterns of methylation have been found in many types of tumours, including breast and uterine, when these tumours develop during adult life.

Receptor-based Disruption
It has become clear over the last decade (and was known much earlier for some chemicals) that many naturally occurring and synthetic chemicals can interact with some hormone receptors and, as a result, turn on and off activities in the cell, disturbing its normal functioning (Stancel et al. 1995; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; Nikov et al. 2000). These chemicals may have a similar shape to the normal hormone and so can fit into the receptor. However, because they are not exactly the same shape as the normal hormone, they may trigger activities in a different order or with a different power or potency from the normal hormone, because the hormone-receptor complex ends up a slightly different shape. The differing shapes of the hormone-receptor complex and the different types of response elements (the place in the DNA where the complex attaches to trigger enzyme production) may open the way to the triggering of subtle effects by chemicals interacting with the hormone receptors, despite their lower potency. So even chemicals that have a much lower ability to fit into the receptor may still trigger some of the activities controlled by the normal hormone, or they may block the action of the receptor altogether.

Brzozowski et al. (1997) showed that the three-dimensional shape of the oestrogen receptor-ligand complex when
17β-oestradiol is bound to the receptor differed from when raloxifene, a classic receptor blocker, is bound to the receptor. Both chemicals bind to the same site in the protein but they bind differently resulting in different shapes.

There are also receptors, known as orphan receptors, that exist in cells but whose role in the normal functioning of the cell is not known (Tyler et al. 1998). Some chemicals, such as polychlorinated dibenzo-p-dioxins (PCDDs), have been found to interact with one of these receptors known as the Ah (aromatic hydrocarbon) receptor. The actions of this chemical-receptor complex are thought to disrupt the normal functioning of the oestrogen hormone-receptor complex. This is because when the PCDD-Ah receptor complex interacts with the part of the DNA that responds to it, the shape of that DNA region changes. This means that the oestrogen-receptor complex is unable to reach the part of the DNA it needs to interact with, and thus it cannot trigger any of the required functions. The possible effects of other orphan receptors have not been well studied. Ohtake et al. (2003) has found that, rather than interacting with relevant parts of the DNA, the Ah receptor-hormone complex may interact with the oestrogen receptor directly.

Plants also have a chemical signalling system. Plants are thought to produce chemicals such as the flavonoids as a deterrent to herbivores or as a protection from fungal or bacterial infections or to attract bacteria such as the nitrogen-fixing bacteria so important to legumes. The roots of some plants (such as alfalfa or soybean) release a range of chemicals which attract the nitrogen-fixing bacteria present in the soil. The Rhizobium bacteria infect the roots which then form nodules. The bacteria share carbon nutrients with the plant while taking atmospheric nitrogen and converting it into nitrogen species that plants can take up. Each plant produces a different set of attractant chemicals so they attract the specific bacteria they need. The process works via a receptor in the bacteria with which the chemicals the plant releases interact. The bacterial receptor is similar to the oestrogen receptor and so this symbiosis may also be disturbed by endocrine disrupting chemicals (McLachlan 2001).

**Non-receptor-based Disruption**

Many chemicals disrupt the endocrine system not by interacting with the receptors in the cell but by disturbing the signalling process before the hormone reaches the receptor. Several processes exist:

- **Turning off the production of the enzymes that produce the normal steroid hormones from cholesterol** – Quite a few steps are required to get from the absorption of cholesterol from the digestive tract to the final production of the hormone molecules. Many of the enzymes involved in the process are related to those involved in removing unusual chemicals from the body. The production of more of the enzymes that deal with the unwanted chemicals can limit the availability of enzymes necessary for normal hormone production (Quigley et al. 1995; Danzo 1997; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; van der Kraak et al. 1998a).

- **Turning on the production of enzymes that break down the normal hormones for excretion** – Some of the enzymes used to remove unwanted chemicals from the cell can also break down the hormones that are needed for normal cell functions. If an organism is exposed to a large amount of an unwanted chemical (or chemicals), it can increase the production of these enzymes, resulting in increased breakdown and excretion of the needed hormones as well as the unwanted chemicals (Quigley et al. 1995; Danzo 1997; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; van der Kraak et al. 1998a).

- **Interrupting the movement of hormones from the place of production to the site of action by changing the action of transport or binding proteins in the blood** – Chemicals that can bind to a particular receptor may also be able to bind to the binding protein that normally transports the relevant hormone around the body. If the binding protein is transporting the endocrine-disrupting chemical around rather than the hormone, less of the hormone is available to trigger the actions necessary for normal functioning. These binding proteins may also facilitate the movement of the hormone into the hormone-responsive cells. If this is the case, then the same binding protein may increase the amount of the endocrine-disrupting chemical reaching the site of action (Quigley et al. 1995; Danzo 1997; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; van der Kraak et al. 1998a).

- **Causing the production of more (or fewer) receptors** – The level of response of the signalling system depends on the number of receptors of the message that are present in the relevant tissue. If the number of receptors is increased or decreased, then the response may be different from that which is actually required for normal functioning at that time (Quigley et al. 1995; Danzo 1997; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; van der Kraak et al. 1998a).

- **Disrupting the storage or release of hormones** – The storage or release of hormones may be varied by the presence of an endocrine-disrupting chemical. This results in abnormal levels of the hormone being available for normal functioning (Quigley et al. 1995; Danzo 1997; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; van der Kraak et al. 1998a).

- **Interrupting the availability or action of secondary messengers (such as cyclic AMP) from cell surface receptors** – As described above, some hormones interact with receptors on the surface of the cell that trigger the release of a secondary messenger to carry the message into the cell. If the secondary messenger is not available in sufficient quantity, then the normal functioning of the signalling system can be inhibited (Quigley et al. 1995; Danzo 1997; Crisp et al. 1998; Barton and Anderson 1998; Tyler et al. 1998; van der Kraak et al. 1998a).

It is also possible that chemicals that can interact with the relevant receptor may also act via these other disruption mechanisms, potentially magnifying their overall impact.
In adults, hormone levels and activity are controlled by a complex system of feedback mechanisms (Crisp et al. 1998). These are required to maintain homeostasis (a steady state in the body). The body can usually deal with small disruptions in hormone activity. Adults must be exposed to relatively large concentrations of endocrine-disrupting chemicals for the feedback mechanisms to be disrupted, with resultant serious endocrine disturbance. In embryos, however, it is not known how well the complex feedback mechanisms can act. Mechanisms exist to protect the embryo from maternal 17\(^\text{-oestradiol}\) (one of the natural oestrogen hormones), but it is unclear whether these systems are capable of dealing with some of the synthetic chemicals shown to be capable of disrupting the endocrine signalling system. In the case of DES, which is discussed in greater detail below, these mechanisms do not protect the embryo from its potent oestrogenic effects.

The immune system may also be affected by endocrine-disrupting chemicals, because of the links between the immune and endocrine systems. This area has not been well studied, but significant effects on people’s health may be possible, and have already been shown to occur in sea mammals (Reijnders 1986; Tryphonas 1994; De Swart et al. 1995; Goldey et al. 1995; Lahvis et al. 1995; Crisp et al. 1998; Arcand-Hoy and Benson 1998; CSTEE 1999).

**Testing the endocrine-disrupting ability of chemicals**

In March 1998, the Intergovernmental Program on Chemical Safety (IPCS) Steering Group on Endocrine Disruptors agreed on the following definitions for endocrine-disrupting chemicals:

- **An endocrine disruptor** is an exogenous substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny or (sub)populations (MRC Institute of Environment and Health 1997; CSTEE 1999; Damstra et al. 2002).

- **A potential endocrine disruptor** is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism or its progeny or (sub)populations (MRC Institute of Environment and Health 1997; CSTEE 1999; Damstra et al. 2002).

This definition just indicates that the potential endocrine disrupting effects of a synthetic chemical detected in *in vitro* tests (i.e. tests conducted on cells in culture) must be confirmed by *in vivo* tests (i.e. tests conducted on intact organisms, such as fish or rats). This is a sensible requirement, considering the number of ways the endocrine system can be disrupted other than by direct interaction with the receptor (the basis for most *in vitro* tests).

At present there is no one definitive test to determine the capability and capacity of a chemical to disrupt the endocrine system, because of the many different ways by which these chemicals exert their effects (Arnold S et al. 1996; McLachlan 1997; OECD 1997; Ramamoorthy et al. 1997).

A number of tests are being developed, trialled and/or validated for use in evaluating whether a chemical could mimic the action of hormones (Jobling and Sumpter 1993; Harries et al. 1996; Klotz et al. 1996; Shelby et al. 1996; Arulwe et al. 1997; Tsutsui and Barrett 1997; Ankley et al. 1998; Thorpe et al. 2000; Legler et al. 2000; Lattier et al. 2001; Zillioux et al. 2001; Ackermann et al. 2002; Ottinger et al. 2002; Lattier et al. 2002; Panter et al. 2002; Tyler et al. 2002; MacLatchy et al. 2003; Seki et al. 2003a; Ankley et al. 2003; Segner et al. 2003a). A battery of tests that use both *in vivo* and *in vitro* techniques is considered to be the most appropriate way to screen for endocrine-disrupting potential (Kavlock et al. 1996; Ankley et al. 1997; EDSTAC 1998). Segner et al. (2003b) compared *in vitro* and *in vivo* evaluations of oestrogenic potencies of a number of chemicals. They found that all the assays showed that the chemicals were oestrogenic and that the same relative order of potency was demonstrated but the *in vitro* tests tended to overestimate the oestrogenic potency of the industrial chemicals evaluated, further confirming the need to use a battery of tests to evaluate chemicals.

Some researchers have investigated the mathematical relationships between a chemical’s structure and its ability to bind to the oestrogen receptor or the Ah receptor (Bradbury et al. 1996, 1998, Mekenyan et al. 1996). These quantitative structure-activity relationship (QSAR) techniques may provide a way of predicting the types of chemicals that trigger these receptors, which will help in prioritising chemicals for testing. It will be important, though, to confirm QSAR predictions in *in vivo* tests (MRC Institute of Environment and Health 1997).

The USA Government’s approach to evaluating the possible effects of endocrine-disrupting chemicals was to include requirements for the assessment of possible endocrine system effects in a number of pieces of legislation relating to a wide range of chemicals in 1996 (Federal Register 1998). The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was formed to assist in formulating the program of assessments and provided its recommendations to the USEPA in the second half of 1998.

At the end of 1998, the USEPA released its intended program, the Endocrine Disruptor Screening Program (EDSP), for meeting the requirements of the *Food Quality Protection Act* and the *Safe Drinking Water Act* 1996 (Federal Register 1998). The USEPA found the fact that EDSTAC was able to reach consensus on a range of recommendations to the Agency compelling, considering the diverse range of people who were members of EDSTAC. They also found the proposed program to be scientifically rigorous. The USEPA program, therefore, relies heavily on the recommendations made by EDSTAC (Federal Register 1998).

The EDSP is designed to consider effects on both humans and ecosystems. It has been designed to look at effects mediated via the oestrogen receptor, the androgen receptor and the thyroid. It also attempts to look for non-receptor-mediated effects that nonetheless affect the endocrine system. The program will concentrate on single chemicals, but mixtures will also be evaluated as techniques become available.
The program will involve a number of tiers that will use increasingly sophisticated tests. Tier 1 will screen chemicals for their potential to interact with the various receptors. Tier 2 will attempt to develop dose-response relationships for those chemicals that interact with the receptors in Tier 1, to provide the type of information required to evaluate the risk posed by these chemicals (Federal Register 1998).

Robotic technology incorporating the in vitro assays listed under Tier 1 was being considered to enable a high-throughput screening program of 15000 chemicals to be undertaken to assist with priority-setting. According to the USEPA’s independent Science Advisory Board in its draft report on the EDSP, an initial trial in 1999 did not show the technology to be sufficiently robust (Federal Register 1998; SAB 1999). A call for additional research has now been issued providing up to $US 2 million to work through some of these issues (USEPA 2002). USEPA is now also researching the usefulness of a number of QSAR models to assist in priority-setting as they do not expect the high-throughput screening technology to be useful in the short-term. The USEPA is now proposing to choose 50 to 100 chemicals (pesticide actives, some pesticide inerts and high production volume industrial chemicals), based on the likelihood that people will be exposed to the chemicals, to evaluate the proposed Tier 1 tests (Federal Register 2002).

The Tier 1 evaluation involves the following screening assays:

In vitro
- oestrogen-receptor-binding/transcriptional activation assay;
- androgen-receptor-binding/transcriptional activation assay;
- steroidogenesis assay with minced testis.

In vivo
- rodent 3-day uterotrophic assay;
- rodent 20-day pubertal female assay with thyroid;
- rodent 5-7-day Hershberger assay;
- frog metamorphosis assay;
- fish gonadal recrudescence assay.

The Tier 2 testing phase involves the use of a range of existing assays for the evaluation of reproductive toxicity that have been modified to incorporate a much larger range of endocrine-related endpoints. The tests include the:

- 2-generation mammalian reproductive toxicity test;
- 2-generation avian reproduction test (using mallards or northern bobwhites);
- fish life-cycle test (using fathead minnows or sheepshead minnows);
- invertebrate reproduction test (using mysids or Daphnia).

One major difficulty with conducting this range of assays for a large number of chemicals is the number of laboratory animals that would be required (SAB 1999).

The Organisation for Economic Cooperation and Development (OECD) is coordinating developmental work internationally on methods for assessing chemicals for endocrine-disrupting potential (OECD 1997, 1999). The first phase of work to validate the rodent uterotrophic assay – a round robin trial of two forms of the test using a strong agonist and antagonist – has been completed. This test is fundamental to any testing program like that described above as it is considered the “gold standard” of tests to evaluate endocrine disruption potential (Kanno et al. 2001). The OECD is also convening a number of expert panels to assess the adequacy of tests proposed for assessing endocrine-disrupting chemicals in fish and birds.

**THE CHEMICALS**

Over the last ten years, numerous lists of chemicals that are potentially endocrine disrupting have been developed by numerous groups including governments, environmental and industry groups (Gray et al. 1994; Kelce et al. 1994; Toppari et al. 1995; Desbrow et al. 1998; Crisp et al. 1998; UK Environment Agency 1998; European Commission 1999; UK Environment Agency 1999).

The USA Government has taken the approach that most synthetic chemicals currently in use have not been fully evaluated for their potential to disrupt endocrine systems. As a result, they have recommended development of a program to go back and test synthetic chemicals for this potential. The process has not progressed as quickly as had been hoped due to difficulties in developing and validating the tests required (SAB 1999; Federal Register 1998, 2002; USEPA 2002).

The European Union decided to initially focus on the subset of natural and synthetic chemicals that have been shown or were thought to have this potential as noted by a range of governments and other groups. The subset of chemicals being evaluated consists of 553 synthetic chemicals and natural or synthetic hormones. Of these chemicals, 115 were found to already be regulated in Europe. Only nine of the chemicals with evidence of endocrine disrupting potential were found not to be regulated currently (European Commission 1999, 2001).

The natural and synthetic chemicals proposed as priorities for evaluation and possible action or by the EU are listed below.

**Synthetic Chemicals**

**Pesticides**

- DDT, dieldrin, aldrin, lindane, dichlorvos, endosulfan, trifluralin, demeton-S-methyl, dimethoate, permethrin, diazinon, chlorfenvinphos, endrin, toxaphene, chlordane, kepone, mirex, hexachlorobenzene, heptachlor, malathion, methyl parathion, ethyl parathion, amitrol, acetochlor, alachlor, nitrofen, prochloraz, dicofol, iprodione, carbendazim, triadimefon, propanil (fungicides).

- Atrazine, simazine, linuron, diuron, 2,4-D (herbicides).

- Vinclozolin, manebe, sodium metam, thiram, zineb, ziram (fungicides).
DDT, dieldrin, aldrin, lindane and endosulfan (i.e. organochlorine pesticides) have been used to control a variety of pests, including mosquitoes, since the 1940s (Crisp et al. 1998). The chemicals have been shown to exert effects on the endocrine system in a variety of ways. Some of the chemicals in this group are capable of interacting with the oestrogen receptor, while others – especially p,p’-DDE – cause effects by blocking the androgen receptor so that testosterone cannot interact with it (Kelce et al. 1995). The DDT-related chemicals are no longer used in most developed countries, but they are still used in the developing world to control the spread of malaria. These chemicals can also be distributed globally in the atmosphere. Atrazine and related herbicides are used to control weeds in crops (Short and Colborn 1999). These and other herbicides make up the most widely used class of pesticides. Many are suspected of having endocrine-disrupting potential. As they are used in large quantities, research is under way to further clarify their potential to act via this mechanism.

• Products containing tributyltin (TBT) have been widely used as paints on boat hulls to prevent fouling.

Industrial chemicals

• Alkylphenols are the breakdown products of alkylphenol polyethoxylates, which are non-ionic surfactants widely used in, for example:
  – industrial detergents for washing wool;
  – domestic detergents in some countries;
  – some pesticide formulations, where they help disperse the pesticide.

Alkylphenol polyethoxylates are non-ionic surfactants. In the USA this type of surfactant makes up 6% of the total surfactants produced. Nonylphenol ethoxylates are the most common of this group. These surfactants break down in sewage treatment plants to nonylphenol and nonylphenol mono-ethoxylate or diethoxylate, as well as a number of other products. These breakdown products are all quite stable and remain in the effluent and the sewage sludge. Almost all of the metabolites, particularly the nonylphenol-related compounds, have been found to have oestrogenic potential. They are presumed to exert this effect by binding to the oestrogen receptor, but they are less effective than natural hormones in binding to the receptor. The European Chemicals Bureau recommend that levels below 0.33 µg L\(^{-1}\) are unlikely to have effects on fish. On release into the environment these compounds adsorb onto particles and accumulate in the sediments where they can be broken down by microbiological processes, but this can take some time. They have been found in a wide variety of environmental samples. A study of 60 commercially available foodstuffs in Germany also found them to be ubiquitous in food (Brunner et al. 1988; Jobling and Sumpter 1993; Ahel et al. 1994a,b; Blackburn and Waldock 1995; Harries et al. 1996; Jobling et al. 1996; Nimrod and Benson 1996; Harries et al. 1998; Ferguson et al. 2001; European Chemicals Bureau 2002; Guenther et al. 2002).

• Bisphenol A is used in epoxy resins lining food cans and water pipes. It is also used in dental coatings and fillings and in PVC polymers.

• Phthalates, including diethylhexylphthalate, dihexylphthalate, butylbenzylphthalate, dipropylphthalate, di-n-butylphthalate, dicyclohexylphthalate, di-n-pentylphthalate and diethylphthalate, are used in the manufacture of plastics, including those used in toy manufacture and for food containers or wraps. In some cases they can leach out of the plastics while in use.

• Styrene.

• 3,4-dichloroaniline.

• Brominated flame retardants.

• Phenolics, including 4-chloro-2-methylphenol, 4-chloro-3-methylphenol, 4-t-butylphenol, 2,4-dichlorophenol and o-phenylphenol.

• Tetrachloroethene.

• 4-nitrotoluene.

• Resorcinol.

• Carbon disulfide.

• PCBs were used in a wide variety of electrical transformers and other equipment because of their very stable nature.

• PCDDs and polychlorinated p-dibenzofurans (PCDFs) are not used commercially but are formed as unwanted by-products in a range of chemical reactions, including:
  – incineration processes;
  – emissions from cars and steel foundries;
  – the manufacture of chlorinated compounds;
  – the bleaching of paper pulp.

PCBs, PCDDs and PCDFs enter the environment via emissions to the atmosphere or water from a variety of processes such as waste incineration, metal refining and chemical manufacturing. PCBs can also enter the environment via accidental spillage from transformers and other electrical equipment. These chemicals are thought to affect endocrine systems primarily through their interaction with the Ah receptor, which (among other things) triggers the induction of enzymes that are used to metabolise these chemicals. These enzymes are crucial to removing these unwanted chemicals from the body, but they can also metabolise natural hormones, possibly affecting normal functioning. The metabolites of some of these chemicals have more oestrogenic activity than the parent compounds. For example, hydroxylated PCBs are more oestrogenic than the original compounds. These chemicals are usually present as mixtures of similar compounds. It is thought that the more highly chlorinated PCDDs, furans and PCBs are antioestrogenic, while those that are less chlorinated have oestrogenic potential (Safe 1995; Chaloupka et al. 1992). Most studies with PCBs have found the mixtures to have little, if any, oestrogenic activity because of this mixing of compounds with antioestrogenic and oestrogenic activity. PCBs have been banned from production in many countries, including Australia (Safe 1995; McLachlan and Arnold 1996).
Pharmaceuticals
• 17α-ethinylestradiol is the synthetic oestrogen used in the contraceptive pill and other hormone treatments (see below, ‘Steroids’).
• 17β-trenbolone is a metabolite of trenbolone acetate – a synthetic steroid used widely to promote growth in beef cattle in the USA (see below, ‘Steroids’).
• Zeranol
• Melengestrol acetate

Natural Chemicals
Steroids
• 17β-oestradiol and oestrone – both naturally occurring hormones excreted throughout life by women and men.
• Progesterone
• Testosterone

The natural hormones 17β-oestradiol and oestrone and the synthetic hormone ethinylestradiol can be released into the environment from sewage treatment plants. These chemicals are excreted continuously by people. Previously it was thought that, once excreted, the chemicals retained no biological activity. Recent studies in the UK, however, have shown that they can be broken down in the sewage system to release the active chemical (Desbrow et al. 1998; Routledge et al. 1998a; Panter et al. 1999). They readily trigger hormone receptors, as would be expected, so only small amounts need to be present to trigger effects. Recent work has shown that oestrogenic effects in wild fish may be widespread in UK rivers. These effects have been shown to be associated with the amount of sewage effluent being discharged to a river (Jobling et al. 1998). The concentration of these natural hormones has also been shown to vary considerably with time in sewage effluent, making prediction of likely effects difficult (Rodgers-Gray et al. 2000). Natural and synthetic hormones, including anabolic steroids, such as 17β-trenbolone, that have androgenic effects, may also reach the aquatic environment in runoff from intensive agricultural activities (Renner 2002; Ankley et al. 2003).

Other Chemicals With Endocrine-disrupting Potential
Phytooestrogens
There has also been much discussion of the effects of natural plant chemicals that have oestrogenic potential. These chemicals are known as phytooestrogens. The most common are present in whole grains, fibres and soy products. These natural chemicals were listed by the UK Environment Agency as chemicals with oestrogenic potential but were not listed as a priority for the types of management actions listed in the body of the report (Barrett 1996; Zava et al. 1997; UK Environment Agency 1998).

People are often exposed to much greater quantities of phytooestrogens than to the synthetic chemicals previously discussed (Safe 1995; Crisp et al. 1998). The effects of such exposures are still unclear. It has been suggested that these chemicals may have preventive effects against some of the potentially hormone-related cancers discussed below, or against other effects of some of the synthetic oestrogen-like chemicals (Barrett 1996; Zava et al. 1997). For example, in Asia, people have much higher amounts of soy products in their diet than in the West, and they have much lower rates of breast and prostate cancer (Barrett 1996; Crisp et al. 1998). The reason for these differences is not yet known. Agreement has not yet been reached about the benefits of these chemicals versus the possible risks. One difference between these chemicals and many of the synthetic chemicals discussed above is the rapid metabolism of phytooestrogens compared with the long-term storage in fat tissue of compounds such as DDT. This could change the exposure regime and hence the possible impacts of the synthetic compounds (Safe 1995; Barrett 1996).

Research has also shown that there is a difference in the lipophilic nature of most of the synthetic chemicals thought to mimic oestrogen and the phytooestrogens. This could mean that the synthetic chemicals bind to the oestrogen receptor in a different way, triggering actions different from those triggered by the phytooestrogens. This hypothesis requires further scrutiny, but it could prove useful for explaining the differences among these chemicals and for classifying the potential impacts of synthetic chemicals (Cunningham et al. 1997).

Almstrup et al. (2002) developed an assay that evaluated the ability of a chemical to bind to the oestrogen receptor and the ability of the chemical to inhibit the aromatase enzyme. The aromatase enzyme converts testosterone to 17β-oestradiol. They tested a range of phytooestrogens and synthetic chemicals. They found that at low concentrations the phytooestrogens inhibited aromatase. At higher concentrations the phytooestrogens interacted with the receptor. The synthetic chemicals only interacted with the oestrogen receptor. As a result, this study found that the phytooestrogens had a U-shaped dose response curve because of their ability to exert these two different effects. The aromatase inhibition at low doses may also help explain the protective effect of phytooestrogens against breast cancer.

Newbold et al. (2001), however, have found that exposure to genistein in early life can result in the development of uterine cancer in mice when they reach adulthood. The pathway of exposure was via injection. The levels used in the experiment were slightly higher than the levels new-born children would be exposed to if they consumed soy based infant formula. Yellayi et al. (2002) also found adverse effects from exposure to genistein. These researchers looked at the effects of genistein on the thymus and a range of immunological indicators and found a dose-related decrease in the size of the thymus and impacts on the immunological indicators. These effects occurred at doses producing the same sorts of serum levels that a human infant would have if they consumed soy-based infant formula.
Mixtures of Chemicals

The effects of exposure to mixtures of chemicals with endocrine-disrupting potential have been of significant concern. It is rare for humans or other organisms to be exposed to these chemicals only one at a time. In reality, exposure usually involves a complex mixture of these chemicals. A number of studies, using in vitro and in vivo techniques, have looked at the effects of chemicals when organisms are exposed to the chemicals in a mixture as well as individually (Arnold S et al. 1996; McLachlan 1997; Ramamoorthy et al. 1997; Arcaro et al. 1998; Knudsen et al. 1998; Payne et al. 2000; Thorpe et al. 2001; Rajapakse et al. 2001; Charles et al. 2002; Rajapakse et al. 2002; Silva et al. 2002). Most studies have shown a lack of synergistic interaction when chemicals are studied in mixtures, but the studies have consistently shown that the effects are additive (i.e. the effect of the mixture is the sum of the effect for each chemical at the concentration at which it is present in the mixture), particularly if the chemicals are present at concentrations with similar levels of effect (i.e. small concentrations of natural hormones (highly potent) are present with much larger concentrations of the less potent synthetic chemicals can contribute equally to an effect).

Porter et al. (1999) studied the effects of mixtures of up to three chemicals on behaviour, the immune system and the endocrine system. Effects were most often seen in groups exposed to two or more of the chemicals. Mice exposed to just one of the chemicals rarely showed effects. The experiments were replicated a large number of times to provide confidence in the results. The doses used were about the same as the maximum acceptable concentrations in drinking water for the chemicals being studied. These levels occur in groundwater across the USA.

The issue of how to assess possible interactions between chemicals when exposure occurs in mixtures is not at all straightforward. It is to be tackled in the USEPA’s Endocrine Disruptors Screening Program as far as test procedures and the understanding of possible mechanisms allow by looking at a small number of commonly found mixtures (Federal Register 1998, 2002).

Silva et al. (2002) studied the interactions between eight oestrogenic chemicals to test what type of additive effect model might apply. They found that the concentration addition model best explained the results obtained when testing the mixtures using in vitro techniques. This would be expected if the chemicals all act via the same mechanism such as interaction with the oestrogen receptor. As a result of the additive effect in the mixture, a toxicity equivalency factor approach can be applied where the amount of each chemical present is multiplied by its potency to cause endocrine effects (usually ranked by comparison to some well studied and highly potent chemical that has these effects such as 17β-oestradiol). These results are added up to determine the total equivalent endocrine effect of a particular mixture. This type of approach has been successfully applied to assess the potential effects from dioxin-like chemicals. This group of researchers further investigated the additivity of these effects using 11 chemicals (Rajapakse et al. 2002).

Fenner et al. (2002) demonstrated the use of such an approach to evaluate the risks from nonylphenol ethoxylate breakdown products in Switzerland.

Exposure to these chemicals

Potentially endocrine-disrupting chemicals need to enter an organism before they can disrupt its endocrine system. As a result, all polymers with a molecular weight greater than 1000 have been excluded from the USEPA’s Endocrine Disruptors Screening Program, as they are unlikely to cross biological membranes and so cannot interact with the endocrine system (Federal Register 1998). An exception to this would be if a polymer was broken down in the environment into pieces that were small enough to cross membranes and these smaller molecules happened to have endocrine-disrupting potential. Breakdown might occur through the action of sunlight, changes in water or soil quality parameters, or exposure to enzymes exuded from a variety of organisms. Such processes may be quite slow, and the large number of synthetic and natural chemicals that are already small enough to cross biological membranes has meant that the USEPA’s Screening Program will focus on the smaller molecules. Polymers that are pesticide chemicals are not included in this exempt category. The various monomers or oligomers which join together to make up all polymers will also be screened, as they are small enough to cross biological membranes (Federal Register 1998).

Wildlife can be exposed to chemicals through a number of routes.

• Aquatic organisms are exposed via:
  • the water they live in;
  • the sediments they intentionally or accidentally eat;
  • the sediments they live in or on;
  • the food they eat.
• Terrestrial organisms are exposed via:
  • the air they breathe and the particles or vapours it contains;
  • the water they drink;
  • the food they eat;
  • the soil they eat while foraging for food;
  • in utero exposure from the mother’s body burden;
  • exposure from maternal deposition of chemicals into eggs (Crisp et al. 1998; CSTEE 1999).

Humans can be exposed in a variety of ways as well:

• the food we eat;
• the air we breathe and the particles or vapours it contains;
• the pharmaceuticals we ingest for medical reasons;
• the water we drink;
• the soil we accidentally or intentionally eat;
• in utero exposure from the mother’s body burden (Crisp et al. 1998; Federal Register 1998; CSTEE 1999).

For fat-soluble chemicals such as PCBs, for example, food is the major source for humans. Dairy products, meat and processed foods are all major contributors. Breast milk is also a contributor. These fat-soluble chemicals remain in the body for a long time, and their accumulation early in life contributes significantly (approximately 15%) to the adult exposure from the mother’s body burden.
body burden (Patandin et al. 1999). For natural hormones like 17β-oestradiol, the major exposure route for fish is probably absorption through the gills from the water in which they are living (Jobling et al. 1998; Harries et al. 1999).

**EFFECTS FOUND IN WILDLIFE**

The recently released Global Assessment of the State of the Science of Endocrine Disruptors concluded that “there is sufficient evidence to conclude that adverse endocrine-mediated effects have occurred in some wildlife species and laboratory studies support these conclusions” (Damstra et al. 2002). A review prepared for the EU by their Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) also noted that the main reason for concern about endocrine-disrupting chemicals is the potential effects they appeared to be having on wildlife (CSTEE 1999). The committee recommended that the EU give the issue high priority.

**Fish**

Two of the most studied examples of impacts on wildlife from endocrine-disrupting chemicals have been the effects of sewage and pulp mill effluents on fish. The Global Assessment found that these two examples provided some of the strongest evidence for adverse effects on wildlife that were being caused via an endocrine-mediated mechanism. There is now enough information available in relation to potential for effects from endocrine-disrupting chemicals in sewage effluents to undertake a type of screening level risk assessment.

**Sewage effluent – a case study**

**Problem Identification**

In the early 1990s anglers in the UK found hermaphroditic fish (also known as intersex fish) downstream of sewage treatment plants (STPs). This discovery has led researchers in the UK into a more than decade-long investigation of the possible causes of such effects in these effluents (Allen et al. 1999a and b; Desbrow et al. 1998; Harries et al. 1996, 1997, 1999; Jobling and Sumpter 1993; Jobling et al. 1996, 1998, 2002a,b; Lyé et al. 1997; Rodgers-Gray et al. 2000; Routledge et al. 1998a; Sheahan et al. 2002a,b; Thomas et al. 2002).

Initially, they investigated whether they could reproduce the type of effects (or related ones) being seen by the anglers. Male rainbow trout were caged downstream of STP discharge points and were shown to begin to produce significant quantities of vitellogenin. This is a protein produced in the liver of female fish as the first step in making the proteins that are incorporated into eggs. These proteins sustain the young until they hatch. Male fish can produce this protein but only do so if exposed to oestrogens or oestrogen-like substances.

The researchers initially expected that the effects noted in the caged fish could be due to the synthetic hormones used in the contraceptive pill and in hormone replacement therapy – like 17α-ethinyl-oestradiol – which are very resistant to degradation and keep their oestrogenic potential after excretion and might be present in sewage effluent (Blackburn and Waldock 1995; Jobling et al. 1996; Beer 1997; Harries et al. 1997; Crisp et al. 1998). However, none of the synthetic hormones could be found in sewage effluents at the time due to the lack of sufficiently sensitive analytical methods. Instead, another group of chemicals with oestrogenic potential was identified: the alkylphenol ethoxylates, such as nonylphenol ethoxylate (Jobling and Sumpter 1993). These chemicals are widely used surfactants or detergents. Exposure of male fish in the laboratory to these chemicals induced effects similar to those observed in field studies.

Studies continued as analytical procedures improved and it became clear that the alkylphenol ethoxylates did not explain the observations at all locations. Desbrow et al. (1998) used fractionation techniques coupled with an *in vitro* technique to determine that most of the oestrogenic effects were caused by the natural hormones 17β-oestradiol and oestrone as well as the synthetic hormone 17α-ethinyl-oestradiol in the effluent. The quantities in the effluents were extremely small but sufficient to have significant effects. The results of the fractionation study were confirmed by exposing male fish to these natural hormones at the concentrations found in the effluent; this induced the production of vitellogenin just as had been found in the fish caged in the effluents (Routledge et al. 1998a).

**Exposure Assessment**

The work of the UK researchers triggered studies to determine whether a range of chemicals including natural and synthetic hormones, alkylphenol ethoxylates and bisphenol A were present (and at what levels) in sewage effluents at various locations around the world.

A large number of studies has now been undertaken looking at the concentrations of these chemicals in sewage effluents and receiving waters (e.g. Aherne and Briggs 1989; Field and Reed 1996; Larsson et al. 1999; Ternes et al. 1999a,b; Belfroid et al. 1999; Williams et al. 1999; Rodgers-Gray et al. 2000; Komer et al. 2000; Sole et al. 2000; Fujita et al. 2000; Staples et al. 2000; Johnson et al. 2000; Baronti et al. 2000; García-Reyero et al. 2001; Huang and Seldak 2001; Tabata et al. 2001; Nasu et al. 2001; La Guardia et al. 2001; Tanaka et al. 2001; Snyder et al. 2001; Kuch and Ballaschmitter 2001; Williams et al. 2001; Witters et al. 2001; Lee et al. 2002; Kirk et al. 2002; Belfroid et al. 2002; Murk et al. 2002; Petrovic et al. 2002a,b; Sheahan et al. 2002a,b; Young et al. 2002; Kolpin et al. 2002; Atkinson et al. 2003; Williams et al. 2003; Schultz et al. 2003).

One of the steps needed to assess the likely risk posed by this issue is to clarify whether the chemicals are present in sewage effluent and/or receiving waters; which ones are present and how much is present. This constitutes the exposure assessment step of the risk assessment.

**Natural hormones**

The natural hormones, such as 17β-oestradiol, were not originally suspected of contributing to the effects seen in fish because when excreted by people the natural hormones are not biologically active. These molecules get attached (or conjugated) to sulfate or glucuronide (molecules that make them easier to get out of the cell and into the excretion
system). It is now clear that the inactivated (or conjugated) natural hormones are being reactivated in the sewerage system by being split from the conjugated group. Glucuronide conjugates are common amongst metabolites of organic compounds excreted by people and so it makes sense that there are microorganisms which gain energy by cleaving them present in sewage treatment systems (Ternes et al. 1999b; Panter et al. 1999; Renner 2002).

So when disposed of to the sewage system, the first step in the breakdown of these chemicals is one which reactivates them. Once deconjugated, 17β-oestradiol is then converted to oestrone, which is why the level of oestrone often increases across a sewage treatment plant rather than decreasing. Oestrone is about three to five times less potent than 17β-oestradiol but is more persistent than 17β-oestradiol in the treatment plant and the environment. The next step in the degradation process converts oestrone to oestriol which also has some oestrogenic activity (Young et al. 2002; Ternes et al. 1999b).

Numerous studies (listed above) have found that the levels of 17β-oestradiol and oestrone in sewage effluent range from <1 ng L\(^{-1}\) to 308 ng L\(^{-1}\). Studies have determined whether these chemicals are present in effluents in quite a number of countries including UK, Sweden, Germany, Japan, Canada, The Netherlands, Italy, and the USA.

Some of these studies have also looked at whether these chemicals are present in receiving waters. Concentrations up to 93 ng L\(^{-1}\) of 17β-oestradiol and 112 ng L\(^{-1}\) of oestrone have been found although most often the concentrations were in the range of 1-10 ng L\(^{-1}\).

Johnson et al. (2000) described a method for estimating likely levels of natural hormones in sewage effluents. To estimate steroid oestrogen input to a STP the amount of oestrogens excreted daily at differing lifestyles and by men and women is used, along with the amount of water used on average by each person using the sewage system. The population is assumed to be 50/50 males and females. Males excrete 1.6 µg d\(^{-1}\) 17β-oestradiol (E2), 3.9 µg d\(^{-1}\) oestrone (E1) and 1.5 µg d\(^{-1}\) oestriol (E3). Women between 15 and 59 years of age (60% of women) who are not pregnant excrete 3.5 µg d\(^{-1}\) E2, 8 µg d\(^{-1}\) E1 and 4.8 µg d\(^{-1}\) E3. Post-menopausal women (20% of women) excrete 2.3 µg d\(^{-1}\) E2, 4 µg d\(^{-1}\) E1 and 4 µg d\(^{-1}\) E3. Pregnant women (1 woman in 75 using UK estimate) excrete 259 µg d\(^{-1}\) E2, 600 µg d\(^{-1}\) E1 and 6000 µg d\(^{-1}\) E3. These values were all based on analysis of fairly small and old datasets so further work on clarifying the ranges of these excretion rates would be helpful. Using the above values and applying them to a plant that caters for a population of 100 000 (i.e. 20 ML d\(^{-1}\) discharge) influent is predicted to contain 0.016 µg L\(^{-1}\) E2, 0.038 µg L\(^{-1}\) E1 and 0.16 µg L\(^{-1}\) E3. For a treatment plant with full tertiary treatment up to 90% of these chemicals will be removed before discharge – i.e. leaving 2 ng L\(^{-1}\) E2, 4 ng L\(^{-1}\) E1 and 20 ng L\(^{-1}\) E3. These levels are about the same as those actually being measured in sewage effluents and lower than some of those measured. For those STPs that have only primary treatment as little as 10% of the steroid hormones may be removed leaving something like 14 ng L\(^{-1}\) E2, 34 ng L\(^{-1}\) E1 and 140 ng L\(^{-1}\) E3 in the effluent discharged into the environment.

Williams et al. (2003) determined the concentrations of 17β-oestradiol and oestrone in sewage effluent from three STPs every day for either 28 or 14 days. They compared the values found with the values predicted for each STP by the method of Johnson et al. (2000) described above. For oestrone the observed range at Great Billing STP was 0.8-11.2 ng L\(^{-1}\) and the predicted value was 8.4 ng L\(^{-1}\). For 17β-oestradiol the observed range at this STP was <1.0-2.0 ng L\(^{-1}\) compared to the predicted value of 1.7 ng L\(^{-1}\).

**Synthetic hormones**

17α-ethinylestradiol is the oestrogenic chemical used in the contraceptive pill and in hormone replacement therapy. As a result, it is excreted to the sewage system. 17α-ethinylestradiol was designed to be more persistent than the natural hormones to enable it survive the environment of the stomach long enough to be absorbed sufficiently. As a result, when present it persists through the STP. It may be adsorbed onto the solids also leading to its presence in biosolids (Ternes et al. 1999b).

One of the oldest studies noted above found levels of 17α-ethinylestradiol up to 15 ng L\(^{-1}\) in sewage effluent. More recent studies found 17α-ethinylestradiol concentrations to range from <1 ng L\(^{-1}\) to 10 ng L\(^{-1}\) in sewage effluent in Sweden, UK, Germany, Canada and The Netherlands. One study found up to 5 ng L\(^{-1}\) in surface waters in The Netherlands. A study undertaken by the USGS of levels of a range of pharmaceuticals, hormones and other chemicals in USA waterways found 17α-ethinylestradiol in 16% of samples with a median level of 73 ng L\(^{-1}\) but levels as high as 800 ng L\(^{-1}\) were found (Kolpin et al. 2002).

Johnson et al. (2000) also provided a method for estimating the concentration of 17α-ethinylestradiol in sewage effluents based on the number of women likely to be taking the pill or to be on hormone replacement therapy. They assumed 25% of women between 16 and 49 were taking the contraceptive pill, which contains an average of 35 µg d\(^{-1}\). They assumed that 3% of post-menopausal women were on hormone replacement therapy, the dose being on average 27.5 µg d\(^{-1}\). They also assumed that 26% of the dose received was excreted by the women. Using these assumptions and applying them to the same sized STP as discussed above, results in predicted concentrations of 17α-ethinylestradiol of the order of 3.5 ng L\(^{-1}\). Treatment may not reduce this concentration much and this concentration is within the actual range found at STPs.

**Nonylphenol ethoxylates**

Nonylphenol ethoxylates are commonly used as detergents as well as in paints and herbicides. When used in such products they have up to 18 ethoxy groups attached to them although the most common ones have 8-12 ethoxy groups attached. Upon discharge to the sewage system the ethoxy groups are cleaved one at a time until there are only one or two left. Degradation in the STP also results in cleavage of all the ethoxy groups producing nonylphenol. Another degradation route for these chemicals results in adding an acetate group onto the one or two ethoxy groups left. The parent chemical
with the large number of ethoxy groups is not oestrogenic but nonylphenol ethoxylates (with one or two ethoxy groups left), nonylphenol and the nonylphenol carboxylates are all oestrogenic to some extent (Fujita et al. 2000; Fenner et al. 2002).

Studies have evaluated the presence of nonylphenol ethoxylates in sewage effluents and receiving waters in Sweden, UK, Spain, Japan, USA and Germany. Nonylphenol has been found to be present in sewage effluents at concentrations ranging from undetected up to 289 µg L\(^{-1}\), nonylphenol ethoxylates have been found up to 60 µg L\(^{-1}\) and nonylphenol carboxylates have been found at concentrations up to 1000 µg L\(^{-1}\) (e.g. Aherne and Briggs 1989; Field and Reed 1996; Larsson et al. 1999; Ternes et al. 1999a,b; Belfroid et al. 1999; Williams et al. 1999; Rodgers-Gray et al. 2000; Korner et al. 2000; Sole et al. 2000; Fujita et al. 2000; Staples et al. 2000; Johnson et al. 2000; Baronti et al. 2000; Garcia-Reyero et al. 2001; Huang and Sedlak 2001; Tabata et al. 2001; Nasu et al. 2001; La Guardia et al. 2001; Tanaka et al. 2001; Snyder et al. 2001; Kuch and Ballschmitter 2001; Williams et al. 2001; Witters et al. 2001; Lee et al. 2002; Kirk et al. 2002; Belfroid et al. 2002; Murk et al. 2002; Petrovic et al. 2002a,b; Sheahan et al. 2002a,b; Young et al. 2002; Kolpin et al. 2002; Atkinson 2003; Williams et al. 2003; Schultz et al. 2003).

In surface waters nonylphenol has been found at concentrations up to 398 µg L\(^{-1}\) and nonylphenol ethoxylates at concentrations up to 100 µg L\(^{-1}\) (e.g. Aherne and Briggs 1989; Field and Reed 1996; Larsson et al. 1999; Ternes et al. 1999a,b; Belfroid et al. 1999; Williams et al. 1999; Rodgers-Gray et al. 2000; Korner et al. 2000; Sole et al. 2000; Fujita et al. 2000; Staples et al. 2000; Johnson et al. 2000; Baronti et al. 2000; Garcia-Reyero et al. 2001; Huang and Sedlak 2001; Tabata et al. 2001; Nasu et al. 2001; La Guardia et al. 2001; Tanaka et al. 2001; Snyder et al. 2001; Kuch and Ballschmitter 2001; Williams et al. 2001; Witters et al. 2001; Lee et al. 2002; Kirk et al. 2002; Belfroid et al. 2002; Murk et al. 2002; Petrovic et al. 2002a,b; Sheahan et al. 2002a,b; Young et al. 2002; Kolpin et al. 2002; Atkinson 2003; Williams et al. 2003; Schultz et al. 2003).

**Bisphenol A**

Bisphenol A is a plasticiser used in many products. The presence of bisphenol A in sewage effluents has been assessed at STPs in Sweden, Japan, Germany and The Netherlands. Concentrations found ranged from 0.001 to 1.4 µg L\(^{-1}\). Surface waters near manufacturers of bisphenol A were assessed; levels were undetectable at most sites and one site had levels of 2 to 8 µg L\(^{-1}\) (e.g. Aherne and Briggs 1989; Field and Reed 1996; Larsson et al. 1999; Ternes et al. 1999a,b; Belfroid et al. 1999; Williams et al. 1999; Rodgers-Gray et al. 2000; Korner et al. 2000; Sole et al. 2000; Fujita et al. 2000; Staples et al. 2000; Johnson et al. 2000; Baronti et al. 2000; Garcia-Reyero et al. 2001; Huang and Sedlak 2001; Tabata et al. 2001; Nasu et al. 2001; La Guardia et al. 2001; Tanaka et al. 2001; Snyder et al. 2001; Kuch and Ballschmitter 2001; Williams et al. 2001; Witters et al. 2001; Lee et al. 2002; Kirk et al. 2002; Belfroid et al. 2002; Murk et al. 2002; Petrovic et al. 2002a,b; Sheahan et al. 2002a,b; Young et al. 2002; Kolpin et al. 2002; Atkinson 2003; Williams et al. 2003; Schultz et al. 2003).

**Other chemicals**

Many other chemicals are present in sewage effluents but the ones listed above are the ones that have oestrogenic potential that are most commonly found.

**Effects Assessment**

**Vitellogenin induction and the development of intersex**

One of the first oestrogenic effects that the UK researchers demonstrated was linked to exposure to sewage effluents was the induction of vitellogenin in male fish. Male fish are capable of producing this egg yolk protein precursor but usually do not. If they receive a higher than normal dose of an oestrogenic chemical, the production of vitellogenin can be switched on in the liver of the fish. Exposure to a sufficiently high dose can cause the male fish to produce the same or higher levels of vitellogenin than spawning females.

The UK researchers began looking for this effect in wild-caught fish (Jobling et al. 1998; Harries et al. 1999; Lye et al. 1997; Allen et al. 1999a,b; Thomas et al. 2001; van Aarle et al. 2001). They found the effect in roach (**Rutilus rutilus**), gudgeons (**Gobio gobio**) and flounder (**Platichthys flesus**), and the effect was strongly related to the presence of sewage effluent, especially for the freshwater fish. Other researchers also found vitellogenin induction in wild-caught fish or in fish exposed in the laboratory to various oestrogenic chemicals (Folmar et al. 1996; Jones et al. 2000; Foran et al. 2002; Mills et al. 2003; Karels et al. 2003; Nichols et al. 1999; Hashimoto et al. 2000; Flammarion et al. 2000; Hecker et al. 2002; Fossi et al. 2001; Kramer et al. 1998; Panter et al. 1998; Panter et al. 2000; Roy et al. 2003). Fish study included Japanese medaka (**Oryzias latipes**), cunner (**Tautogolabrus adspersus**), carp (**Cyprinus carpio**), sheepshead minnow (**Cyprinodon variegatus**), fathead minnow (**Pimephales promelas**), flounder (**Pleuronectes yokohamae**), black seabream (**Abras brauma**), horsehead flounder (**Pleuronichthys variegatus**), English sole (**Pleuronectes vetulus**), bigmouth sole (**Hippoglossina stomata**) and swordfish (**Xiphas gradis**).

In evaluating the induction of vitellogenin in wild-caught fish, the original effect noted by the anglers — hermaphroditic fish — was also identified. The presence of eggs in the testis of the fish was termed intersex or testis-ova. The induction of vitellogenin and the development of intersex were found to be strongly associated in many studies, particularly for the more severe forms of intersex (Jobling et al. 1998; van Aarle et al. 2001; Metcalfe et al. 2001; Kramer et al. 1998; Panter et al. 1998). Induction of vitellogenin and/or development of intersex indicate that a fish has been exposed to oestrogenic chemicals but such effects had not been demonstrated to be necessarily adverse to the fish’s survival or its ability to reproduce until recently. Jobling et al. (2002a,b) investigated the link between intersex and reproductive success. They showed that fish with intersex produced reduced numbers and impaired sperm resulting in the production of fewer viable eggs clearly linking this effect to reproductive impairment.
A recently-released study investigating the effects of 17α-ethinylestradiol on maturing male rainbow trout found effects on reproductive success (reduced numbers of viable eggs when sperm were used to fertilise unexposed eggs) at concentrations that did not show histological changes like intersex – 10 ng L⁻¹ (Schultz et al. 2003). A full life cycle study of the effect of 17α-ethinylestradiol on the fathead minnow was conducted by Lange et al. (2001). They found no testicular tissue at all in any of the fish exposed to 4 ng L⁻¹ of 17α-ethinylestradiol. Plasma vitellogenin levels were induced in fish exposed to 16 ng L⁻¹. The no observed effect concentration from this study, which lasted almost a year, was 1 ng L⁻¹. The data from this study were reanalysed by Grist et al. (2003) to determine the impact of such effects on the rate of population growth. They found that the intrinsic population growth rate was significantly reduced at about 3 ng L⁻¹, an effect level similar to that found for changes in testicular tissue using more common toxicological procedures. Segner et al. (2003a) found effects on gonadal tissue in zebrafish when exposed to 3 ng L⁻¹ and vitellogenin was induced at 1.7 ng L⁻¹.

These studies found that these effects were occurring at levels actually found in the environment given that the studies were on wild-caught fish. Other studies listed above were conducted in the laboratory showing that these effects occurred at ng L⁻¹ levels of the natural and synthetic hormones.

Other studies have looked at the levels of nonylphenol ethoxylate-related chemicals needed to induce vitellogenin production or the development of intersex (Gimeno et al. 1997, 1998a, b; Gray and Metcalfe 1997; Christensen et al. 1999; Gronen et al. 1999; Dreze et al. 2000; Harries et al. 2000; Aehl et al. 2000; Seki et al. 2003). Effects were noted in fish exposed to levels around 1-10 µg L⁻¹. Seki et al. (2003b) also evaluated the persistence of the effects in medaka once they were removed from exposure to nonylphenol or octylphenol. Vitellogenin induction dissipated fairly rapidly, however, the intersex effects still remained after two months in clean water suggesting that such histopathological changes may be quite long lived.

Other effects

Batty and Lim (1999) investigated endocrine-disrupting effects in mosquito fish (Gambusia affinis holbrooki) exposed to sewage effluent in a river in New South Wales, Australia. Small numbers of fish were collected in 1995 and 1996 at a number of sites on South Creek, part of the Hawkesbury-Nepean River system. The body length and gonopodium length of each fish were measured. The gonopodium is a modified fin that male mosquitofish use to transfer sperm to the females. As such it is critical for reproduction. Fish collected downstream of the STP were shorter in 1996 and had shorter gonopodia in both years. The authors proposed two explanations for the reduction in gonopodium length. In the first, the water in the creek is more turbid and flows more quickly downstream of the STP, possibly giving a reproductive advantage to males with a shorter gonopodium. However, other studies, quoted by the authors, on mosquito fish and some related small species indicated that this was unlikely. The other explanation proposed was that testosterone levels in the male fish were affected in some way during the development of the gonopodium by exposure to a chemical or chemicals in the effluent. Doyle and Lim (2002) have recently reported similar effects when juvenile male mosquitofish were exposed to 17β-oestradiol (20, 100 and 500 ng L⁻¹) in the laboratory. They also found a significant decrease in sexual activity in the exposed fish.

Angus et al. (2002) also investigated the effects of living below a sewage treatment plant on male mosquito fish in a small river, this time in the USA. No effects were found on the length of the gonopodium nor did the male fish have increased levels of vitellogenin. The fish were found to have larger testes and livers than unexposed fish but they did not have any histological changes like the intersex condition found in fish in the UK. Efluent from this plant received significant dilution upon discharge – the annual mean percentage efluent levels in the river was 5.7%.

Bell (2001) evaluated the effect of 17α-ethinylestradiol on the behaviour of male three-spined sticklebacks (Gasterosteus aculeatus). The study showed that the exposed fish were less aggressive to competing males and exhibited less courting behaviours to female fish. Hormone levels were also affected. Such behaviourial effects can impact on the long-term viability of the population by reducing mating and, therefore, population growth rates.

Palace et al. (2001) studied the movement of lipid and changes in vitamin levels in juvenile lake sturgeon exposed to 17α-ethinylestradiol as these parameters were found to be affected in mammals exposed to this chemical. Under the conditions of the study vitellogenin induction was noted, as were significant changes in the distribution and levels of vitamin A and E in the exposed fish. It was also found that plasma levels of 17α-ethinylestradiol were higher than those in the water indicating bioconcentration. Changes in vitamin levels could be important as embryonic development is affected by such changes. Fish were only exposed for 25 days – effects may have been even more significant after longer exposures.

The UK researchers have also begun looking at the androgenic activity present in waterways and sewage effluents. Thomas et al. (2002) studied surface waters, sediment pore waters and particulate matter from a range of rivers as well as the sewage effluents being discharged to those waterways. About one quarter of the surface water samples contained detectable levels of androgenic activity. Only the effluent from primary treatment plants was found to contain detectable levels of androgenic activity. Fractionation of one of the effluents showed that a range of natural steroids and steroid metabolites were responsible for almost all the androgenic activity found.

Schoenfuss et al. (2002) exposed adult male goldfish to effluent from a large STP with advanced treatment for ten weeks and observed whether there were effects on reproductive behaviour and sperm production. They found that there were small but variable changes in the behaviour of the fish but no changes in sperm production. The levels of 17β-oestradiol found in the effluent were approximately 5 ng L⁻¹.
Porter and Janz (2003) assessed a range of effects in fish in a creek affected by sewage effluent and another close by with little surrounding land use. The Index of Biotic Integrity indicated that the community of fish in the affected creek was altered significantly. A habitat assessment of the two creeks indicated that they were similar and that both creeks contained good quality habitat so the changes in fish community structure were not due to habitat alteration. Fish from the affected creek also had elevated vitellogenin and testosterone levels; and altered condition factors.

**Predicted no effect concentrations**

The UK Environment Agency has proposed predicted no effect concentrations (PNEC) for the natural and synthetic hormones (Young et al. 2002). On considering evidence such as listed above they have determined that for the protection of freshwater life and marine life the concentration of 17α-oestradiol, oestrone and 17α-ethinyloestradiol in surface waters should not exceed 1, 3-5 and 0.1 ng L\(^{-1}\) respectively.

The European Union has proposed a predicted no effect concentration for nonylphenol and related chemicals (European Chemicals Bureau 2002). They calculated that a PNEC for nonylphenol of 0.33 µg L\(^{-1}\) should be protective of aquatic life, both for toxic effects and oestrogenic effects.

These PNECs are broadly equivalent to the Australian and New Zealand (2000) water quality guidelines (ANZECC and ARMCANZ 2000), and are used here as guidance levels for protecting aquatic life.

**Risk Characterisation**

Over the last decade a large number of studies have looked at the oestrogenic potential of sewage effluent. Damstra et al. (2002) in the WHO Global Assessment of the State of the Science of Endocrine Disruptors rated this issue as having some of the strongest evidence for adverse effects via endocrine-mediated mechanisms.

The many studies looking at the levels of various chemicals in sewage effluents have found that natural hormones (including 17β-oestradiol and oestrone) can be found at concentrations ranging from <1 to 308 ng L\(^{-1}\) (usually median values are 1-10 ng L\(^{-1}\)); synthetic hormones (17α-ethinyloestradiol) can be present at concentrations ranging from <1 to 10 ng L\(^{-1}\) and nonylphenol ethoxylate-related chemicals can be present at concentrations of the order of 1-100 µg L\(^{-1}\).

The UK Environment Agency and the European Chemicals Bureau have proposed predicted no effect concentrations (PNECs) for the protection of aquatic life for these chemicals. Natural hormones like 17β-oestradiol and oestrone need to be at levels below 1 and 3-5 ng L\(^{-1}\) respectively to minimise adverse effects on aquatic life. 17α-ethinyloestradiol, the synthetic hormone, needs to be below 0.1 ng L\(^{-1}\) to protect aquatic life. Nonylphenol needs to be kept at levels of the order of 0.33 µg L\(^{-1}\) to be protective of aquatic life.

It can be seen that these chemicals are often found to be present (and are predicted to be present) in sewage effluents at or above the concentrations recommended as protective of aquatic life indicating a potential risk to the environment depending on the amount of dilution available at discharge.

In the UK these findings have resulted in the UK Environment Agency deciding that sufficient evidence exists of risk of harm to fish to warrant the consideration of further risk management actions. The first step is a ranking of sewage treatment plants to determine which pose the highest risk to fish, which should be completed in late 2003. The second step is to consider the installation of additional treatment processes at the highest risk plants – approximately 100 plants based on a preliminary review. Additional treatment processes may include modern treatment processes not currently installed at older plants as well as novel new techniques specifically focused on the removal natural and synthetic hormones (UK Environment Agency 2002).

**Pulp Mills**

Evidence of masculinisation, feminisation and disruption of reproduction in fish downstream of pulp and paper mills has been documented for some time (Adams et al. 1992; Hodson et al. 1992; McMastor et al. 1992; Munkittrick et al. 1994; Gagnon et al. 1995; Kovacs et al. 1995; Brodeur et al. 1997; Hontela et al. 1997; Crisp et al. 1998; Mellanen et al. 1999; Jenkins et al. 2001; Karels et al. 2001; Larson et al. 2000; Larsson and Forlin 2002; van den Heuvel and Ellis 2002; Ellis et al. 2003; Sepulveda et al. 2003). It is now thought that these effects are due to disruption of the endocrine system by exposure to chemicals in the effluent from the pulping process. Various studies have found effects including increased activity of mixed-function oxidase in the liver, reduced plasma levels of sex steroids, decreased egg and gonad size, a decrease in the occurrence of secondary sex characteristics, an increased age to maturation, abnormal size distribution and age structure, physiological changes to gonadal tissue, and reduction in ability to make cortisol.

Because there are so many chemicals released in the pulping of wood, it is difficult to isolate the ones causing these effects. In early studies it was thought possible that dioxins formed in the bleaching process might have been the cause. The effects, however, remained after process changes that removed the production of dioxins. Recent studies have highlighted that naturally occurring phytosterols released from the wood during the pulping process could have endocrine-disrupting potential (MacLatchy et al. 1997; Crisp et al. 1998; Tremblay and van der Kraak 1998, 1999; van der Kraak et al. 1998b). Studies have focused on β-sitosterol, which is the major plant sterol found in pulp mill effluents. A series of in vitro and in vivo studies confirmed that β-sitosterol can interact with the oestrogen receptor and induce the production of vitellogen in male fish. This chemical was also found to reduce the plasma levels of a number of sex steroids and their intermediates, including cholesterol. Nakari and Erkoma (2003) conducted a multi-generation test on zebrafish using two phytosterol preparations – one from wood containing β-sitosterol and one from soy beans. The wood sterol mix caused vitellogen induction and significant changes in the sex ratios of the fish – increased male bias in the first generation and increased female bias in the second generation. The phytosterol mix from soy beans caused other effects. Kiparissis et al. (2001) identified genistein in bleached kraft mill effluent – another phytoestrogen found in plants.
Jenkins et al. (2001) investigated the possible causes of masculinisation of female fish downstream of a paper mill using a toxicity identification characterisation approach. They isolated an androgenic chemical – androstenedione – as a possible cause. It is possible that this chemical, a natural precursor for testosterone, is formed by bacteria modifying the phytoestrogens such as β-sitisterol and campesterol. It is also possible that it is present in small quantities in the wood. Durhan et al. (2002) further evaluated the possibility that androstenedione was the cause of masculinisation of female fish downstream of pulp mills. They found that androgenic activity was present in a fraction of the effluent that did not contain androstenedione so that this chemical was unlikely to be the cause of the masculinisation.

Dube and MacLatchy (2001) and Hewitt et al. (2002) investigated whether additional treatment of one of the sources of wastewater in a bleached kraft pulp mill reduced the potential of the final effluent to affect testosterone levels in the mummichog (Fundulus heteroclitus). They found reverse osmosis treatment of the effluent reduced the effects but could not identify specific chemicals that appeared to be causing the effects.

Larsson and Forlin (2002) provide further evidence that masculinising fish near pulp mills is due to some chemical in the mill effluent by showing the effects were not apparent after a short-term shutdown of a mill. In 1997 and 1998 the eelpout downstream of a large kraft pulp mill in Sweden showed a male-biased sex ratio. In 1999 a short shutdown occurred at the same time as the embryos’ gonads differentiated. As a result, no male bias showed up in the sex ratio of the fish. The male bias returned in 2000 as no shutdown occurred.

**Summary**

There is good evidence that fish are showing endocrine related effects downstream of pulp mills but it is still not clear which chemical or chemicals present in the pulp mill effluent are likely to be causing these effects.

**Endocrine effects of other chemicals and mixtures**

Salmon in the Great Lakes in the USA and Canada exhibit disruption of thyroid function and effects on fertility and on embryo survival and development (Moccia et al. 1981, 1986; Crisp et al. 1998). Disruption of the release of thyroid hormones can affect skeletal growth and behaviour, including migratory behaviour. It is thought that these effects are caused by chemicals that disrupt the endocrine system. Effects on thyroid pathology have been observed in all salmon analysed in the last 20 years, although similar effects in the thyroid gland have not been observed in other fish species in the lakes. As yet, no specific chemical has been linked to these effects. It does appear, however, that they are not caused solely by the low levels of iodine in some of the lakes, as the pattern of effects does not reflect the changes in iodine levels.

Nagler and Cyr (1997) looked at the effects of exposing male bottom-dwelling fish to contaminated sediments. The fish were exposed to sediments with varying degrees of contamination by PCBs and polyaromatic hydrocarbons (PAHs) for five months. Semen was then collected from each exposed male. The semen was used to fertilise eggs from an unexposed female, and hatching success was evaluated. Those fish exposed to the most contaminated sediments had a 48% lower hatching success rate than the fish exposed to the control sediments, a statistically significant difference. Sperm numbers did not vary between the treatments, but sperm quality seems to have varied, resulting in the differing hatching successes.

Fairchild et al. (1999) conducted a retrospective study linking catch size of Atlantic salmon with spraying of a particular pesticide in forests to control spruce budworm. One of the pesticides used in the budworm control contained nonylphenol. The study compared whether there was any pattern in the catch size that related to when the pesticide was sprayed. A significant proportion of the lowest salmon catches between 1973 and 1990 occurred when the nonylphenol-containing pesticide had been sprayed. Other pesticides used to control budworm but which did not contain nonylphenol did not show this relationship.

Bayley et al. (1999) exposed guppies to water containing either 17β-oestradiol or 4-tert-octylphenol. The adult males were exposed for four weeks and then mating behaviour was observed. Fish exposed to either chemical showed a dramatic decrease in sexual behaviours. As these behaviours are closely linked to reproductive success, chemicals that cause a decrease may result in a reduction in reproductive success. This technique may prove useful as a biomarker for oestrogenic effects.

Hemmer et al. (2001) exposed sheepshead minnow, an estuarine fish, to nonylphenol, methoxychlor or endosulfan to assess the impact of the chemicals on vitellogenin induction. They found that both nonylphenol and methoxychlor induced a dose-dependent response with a significant increase in vitellogenin at concentrations above 5.4 µg L⁻¹ for nonylphenol and above 2.5 µg L⁻¹ for methoxychlor. No vitellogenin induction was noted in the fish exposed to endosulfan.

Carlson and Williams (2001) studied the induction of vitellogenin in rainbow trout when exposed via their food to two hydroxylated PCBs and the two natural oestrogens – 17β-oestradiol and oestrone. All four chemicals induced the production of vitellogenin in a dose-dependent manner. 17β-oestradiol and oestrone induced vitellogenin at all concentrations tested but oestrone was about two to three times less potent. The di-ortho substituted PCB (PCB30-OH) was more potent than the mono-ortho substituted PCB (PCB61-OH).

Shimasaki et al. (2003) studied the effects of tributyltin in fish. They fed Japanese flounder (Paralichthys olivaceus) 0.1 or 1.0 µg g⁻¹ tributyltin from 35 days after hatching to 100 days after hatching. Sex-reversed males (i.e. females with male gonads) were noted in both treatment groups – 26% and 31% respectively. Significant inhibition of growth was also found.
Xu et al. (2002) collected silver carp (Hypophthalmichthys molitrix) from a lake in China in 1999. They found significant effects on thyroid hormones and retinol levels in the livers. The effects were negatively associated with levels of PCBs, dioxins and PAHs in the various ponds in which the fish lived – i.e. reduced levels of thyroid hormones and retinol levels with increased levels of pollutants.

Nagler et al. (2001) looked at a genetic marker in chinook salmon returning to spawn in the Columbia River. They found that 84% of the fish appearing to be females contained a genetic marker for the Y chromosome indicating that they were genetically male. This was not observed in female fish from a hatchery. It appeared that significant levels of sex reversal were occurring in the wild. This could lead to the development of YY male fish.

**Summary**

A wide range of endocrine related effects have been noted in fish both in the field and in the laboratory. Further work is needed to clarify the long-term implications.

**Invertebrates**

There is only limited understanding of the endocrine systems of invertebrates, making it difficult to assess whether effects seen when invertebrates are exposed to chemicals like 17β-oestradiol or 17β-ethynloestradiol are caused via an endocrine related mechanism. The biology of invertebrates is obviously different from vertebrates and they have a range of chemical messengers (e.g. ecdysteroids) that vertebrates do not have which may also be subject to disruption – the limited understanding about how such signalling systems work limits our ability to investigate such disruption (Segner et al. 2003a). A range of studies of the effects of various oestrogenic compounds have been conducted, but their relevance is as yet unclear in relation to endocrine disrupting chemicals, except those relating to TBT induced effects.

**Tributyltin (TBT)**

Matthiessen and Gibbs (1998) reviewed the state of knowledge of TBT-induced effects on molluscs. The development of male reproductive structures (including a penis and vas deferens) in female marine snails, a condition known as imposex, has been studied since the mid 1980s. Imposex has been found in many populations of marine snails around the world. The effect was linked to exposure to TBT antifouling paints used on boats. As a result, these paints have been banned in many countries on boats less than 25 m long. Reliable studies have now linked this effect to an accumulation of active testosterone in the female snails: Females exposed to testosterone alone developed imposex, but females exposed to testosterone plus an androgen receptor blocker (which stops the testosterone from acting) did not. It is not yet clear how TBT causes the increase in testosterone in these snails, but two theories are currently being investigated (Matthiessen and Gibbs 1998). One is that TBT blocks the enzyme aromatase, which converts testosterone into 17β-oestradiol. The alternative theory is that TBT blocks the excretion of testosterone. Regardless of which hypothesis is correct, this is clearly a case of disruption of the endocrine system in these organisms (Matthiessen and Gibbs 1998).

Recently, the World Health Organisation Global Assessment of the State of the Science of Endocrine Disruptors report determined that TBT-related effects were another strong example of endocrine disruption (Damstra et al. 2002).

More recently, Gooding et al. (2003) have conducted further studies on the mechanism of action of TBT in mud snails (Ilyanassa obsoleta). These authors consider it important to determine the mechanism of action of TBT to ensure that any replacement antifouling agents do not also have the same effect. They found that the two mechanisms listed above did not apply in this snail. Instead it appears that TBT affects the ability of the snail to attach testosterone to a fatty acid: a process used to store excess testosterone in fatty tissue until needed. As a result, more testosterone stays in the active form and so is available to affect the female snails.

TBT has also been found to affect shell development in oysters. Shell development appears to be controlled by oestrogen receptors; hence its disruption when 17β-oestradiol levels are reduced (Oehlmann et al. 1995; Crisp et al. 1998).

**Other organisms and chemicals**

Gross et al. (2001) have studied reproductive abnormalities in amphipods (Gammarus pulex) downstream of sewage treatment plants in the UK. Effects were not found in male amphipods but a large number of the females studied had abnormal eggs. Body size also appeared to be affected.

Moore and Stevenson (1991) reported the appearance of intersexuality in copepods near a sewage effluent discharge. Previously, they found individuals bearing both male and female structural characteristics to be a rare phenomenon. At this polluted site and other sites near STP discharges this effect was becoming more common.

Oehlmann et al. (2000) evaluated the effects of bisphenol A and octylphenol on the freshwater snail (Marisa cornuarietis) and the marine snail (Nucella lapillus). They found the female snails were significantly feminised with the development of ovarian and male reproductive structures and effect sizes were noted.

In other invertebrates, such as crustacea, the role of hormones is unclear. However, a number of studies have found effects in organisms such as water fleas when they are exposed to chemicals such as nonylphenol ethoxylates, nonylphenol, DES, bisphenol A, dieldrin and pentachlorophenol (Baldwin et al. 1995, 1997, 1998; Parks and LeBlanc 1996; Caspers 1998; Dodson et al. 1999). Characteristics affected include brood sizes, sex ratios and steroid metabolism.

Segner et al. (2003a) studied the effect of 17α-ethynloestradiol and bisphenol A on Hydra vulgaris, Gammarus pulex, Chironomus riparius, Hyalella azteca and Lymnaea stagnalis. They found effects on reproduction, differentiation, moultng, emergence times, population size, and sex ratios.
Summary

Some endocrine related effects have been shown in invertebrates particularly related to TBT exposure but due to the limited understanding of invertebrate endocrinology there may be a variety of other effects yet to be identified.

Reptiles and Amphibians

One of the best-known examples of the effects of endocrine-disrupting chemicals in wildlife was observed after a spill in 1980 of dicofol, a pesticide similar to DDT, which was contaminated with DDT metabolites. It affected the alligator population in Lake Apopka, Florida, USA (Guillette et al. 1994, 1995; Guillette 1995; Crisp et al. 1998). The alligator populations in Florida generally were thriving after protection under the Endangered Species Act, and so studies were begun to determine whether alligators could be harvested. The studies looked at how harvesting should take place: through egg collection, or by capturing of young alligators or adults. During this work it was found that the alligator population in Lake Apopka was, in fact, failing to maintain itself. Very few eggs, hatchlings or juveniles were found, and most of the eggs did not hatch. Of the few young that did hatch, most died within 10 days. Further work showed that the hatchlings that did survive had a variety of reproductive abnormalities. Juvenile alligators had abnormal plasma sex hormone levels, and males had a significantly reduced phallus size. Male hatchlings had ratios of 17β-oestradiol to testosterone of 2, compared with 0.5 in normal males from other locations. Females were super-feminised, having a ratio of 17β-oestradiol to testosterone of twice the normal ratio. Turtles from the lake showed similar problems. Heavy metals were present in the lake but did not appear to be causing the effects. It is thought that the effects are due to the antiandrogenic activity of DDE (a breakdown product of dicofol), but there is still some difficulty in establishing this link for all the observed effects.

Crain et al. (1998) studied levels of sex steroids and thyroid hormones in alligators from three lakes in Florida. The first was Lake Apopka. The second was a reference lake – Lake Woodruff – located in a National Wildlife Refuge. The third was Lake Okeechobee, which is affected by agricultural and municipal run-off and is eutrophic. Levels of sex and thyroid hormones were significantly different in the alligators from Lakes Okeechobee and Apopka from those in Lake Woodruff. Testosterone concentrations in male alligators from the two affected lakes were significantly lower than in the males from Lake Woodruff. Thyroid hormone levels were also quite different. The differences were most pronounced in Lake Apopka. This study may indicate that the ongoing impacts of urban and agricultural activities are affecting some of the lakes in Florida, rather than that these effects occurred just as a result of the dicofol spill in Lake Apopka.

Sex is determined in many reptiles by the temperature at which the eggs are incubated, rather than by sex chromosomes. This factor was used in laboratory studies, where alligator eggs from a clean lake were painted with DDE, 17β-oestradiol or nothing and then incubated at a temperature that should have resulted in mostly males (Crisp et al. 1998). 17β-oestradiol produced 80% female hatchlings, and DDE produced 20% female, 40% intersex and 40% male hatchlings. The study also found that both DDE and 17β-oestradiol produced hatchlings with abnormal sex hormone ratios, similar to those seen in the hatchlings from Lake Apopka.

Podreka et al. (1998) studied the effects of DDE on the sexual differentiation of the marine turtle Chelonia mydas. Eggs were collected on Heron Island in Queensland, Australia, and painted with DDE just before the period when incubation temperature determines the sex of the organisms. The dose when corrected for background was up to 543 ng g⁻¹ DDE. Some of the eggs were incubated at the temperature that should have produced mostly males, and others at the temperature that should have produced mostly females. The sex ratios for each group remained as would normally be expected based on the incubation temperature. This species does not appear to be susceptible to endocrine-disruption when exposed to these levels of DDE.

Palmer et al. (1998) investigated the induction of vitellogenin in frogs as a marker of exposure to oestrogen-like compounds. Male African clawed frogs (Xenopus laevis) were exposed to DES by being immersed in water containing the chemical to mimic the main environmental exposure pathway. After an 11-day exposure period, plasma vitellogenin levels were significantly increased. Other chemicals were then studied with this method, including chlordane, dieldrin, endosulfan and toxaphene. At the exposure concentration used, endosulfan was toxic to the frogs. The frogs exposed to dieldrin and toxaphene showed significant levels of vitellogenin. Those exposed to chlordane had vitellogenin levels no different from those of the unexposed frogs. This species does appear to be sensitive to the endocrine-disrupting potential of most of these chemicals.

Hayes et al. (2002a) examined the effects of atrazine, a commonly used herbicide, on the sexual development of the African clawed frog (Xenopus laevis). At 25 µg L⁻¹ serum testosterone levels were reduced 10-fold and at levels as low as 0.1 µg L⁻¹ gonadal abnormalities (such as multiple gonads in the one animal) were noted. They hypothesize that atrazine induces increased activity of the aromatase enzyme which converts testosterone to 17β-oestradiol – the opposite effect to that hypothesized for tributyltin in molluscs.

Hayes et al. (2002b, 2003) added to the study discussed above using another species of frog – the leopard frog (Rana pipiens) – and studying the effects of atrazine in both the laboratory and the field. At exposure to levels of atrazine of 0.1 ppb the laboratory study found retarded gonad development and testicular oogenesis (i.e. intersex – the presence of oocytes in testicular tissue). Exposures of 25 ppb did not show these effects to the same extent. Field surveys showed these effects were widespread (up to 92% males affected at some sites) as was atrazine contamination – only one site showed levels <0.2 ppb. It was also noted that the peak time of the year for atrazine use is also the time when larvae are developing and may therefore be most sensitive.
Mayer et al. (2003) evaluated the impact of exposure to 4-tert-octylphenol on sexual differentiation in bullfrog tadpoles. They found that sexual differentiation commenced early in the exposed tadpoles. The exposed tadpoles also exhibited changes in the normal male and female patterns of expression of steroidogenic factor 1 (SF-1) which is involved in regulating the metabolism of hormones. Such effects may have impacts on reproductive success.

Bevan et al. (2003) exposed embryos of the commonly used frog, *Xenopus laevis*, to a range of endocrine-disrupting chemicals including 17β-oestradiol, nonylphenol, octylphenol, methoxychlor, p,p'-DDE and 17α-methyltestosterone. Short-term exposure (less than two days) to nonylphenol was found to cause significant effects (including mortality, changes in body shape, changes in eye development etc.) on the developing embryos at concentrations similar to those found in primary treated sewage effluent. The other chemicals were also found to cause a range of effects in the embryos.

Brasfield et al. (2002) studied the induction of vitellogenin production in male Western fence lizards (*Sceloporus occidentalis*). 17α-ethinylestradiol was injected into the lizards and even at the lowest dose tested (0.0003 mg kg⁻¹) induction of vitellogenin was found.

**Summary**

Vitellogenin induction, changes in sexual differentiation, changes in hormone levels, and developmental difficulties have all been found to occur in reptiles and amphibians when exposed to potentially endocrine disrupting chemicals.

**Birds**

Endocrine-disrupting chemicals are thought to be responsible for a decline in the hatching success of birds in a variety of habitats in the USA and Europe (Guillette 1995; Feyk and Giesy 1998; CSTEE 1999; Fox 2001). Eggshell thinning and other effects in raptors highlighted the accumulation of persistent chemicals through the food chain. It has been suggested that eggshell thinning was due to disruption of the bird’s endocrine system by persistent chemicals like DDT. In fact, since the reduction in the use of these chemicals, there has been a widespread reduction in eggshell thinning among fish-eating birds. Bignert et al. (1995) studied museum-collected eggs in Sweden and showed that the thickness of eggshells of the Baltic guillemot has increased since the 1970s, when DDT was banned, but it is still not back to pre-1946 levels. The research undertaken since this effect was noticed has not yet clarified the mechanism of eggshell thinning. Some recent studies have shown that eggshell thinning may have been caused by DDE inhibiting the synthesis of prostaglandin, resulting in a reduction in calcium transport to the eggshell, but numerous other theories have been suggested as well, some endocrine-related, others not. It is still unclear whether this is an endocrine-related effect.

These chemicals, however, could still be acting as disruptors of the endocrine systems of birds. For example, exposure to chemicals thought to have endocrine-disrupting potential has been related to a variety of symptoms in these birds, including skewed sex ratios, abnormal reproductive tract development, abnormal plasma levels of sex hormones, abnormal nesting and mating behaviour and poor egg quality. Another example is the observation around Lake Ontario of complete or partial reproductive failure from the 1970s onward in bald eagles, double-crested cormorants, common terns and herring gulls. Even by the mid 1990s bald eagles were still not nesting normally (Guillette 1995; Feyk and Giesy 1998; CSTEE 1999; Fox 2001). Laboratory studies where eggs were exposed to environmental concentrations of such chemicals have produced the same kinds of abnormalities in reproductive tract development as seen in the wild. Current studies show that the concentrations of these chemicals in the worst-affected areas in the USA have decreased to the point where the young survive to hatch and grow, but that the chemicals may be modifying the reproductive behaviour of these birds and affecting future fertility.

Gulls in California and around the Great Lakes have been shown to be less susceptible to eggshell thinning, but more susceptible to feminisation caused by some of these chemicals (Crisp et al. 1998; Feyk and Giesy 1998). This has resulted in skewed sex ratios relative to populations from unpolluted areas, unusual behaviour and population declines. Around Puget Sound, Washington, USA, these effects have also been seen in gull populations and could be caused by exposure to PCBs and PAHs.

Abnormal thyroid structure has been noted in herring gulls living around the Great Lakes. Although iodine, essential for normal thyroid functioning, is naturally low in the area, the observed effects could not be fully explained by the iodine levels in the lakes. Moccia et al. (1986) found that the more severe effects corresponded to areas more contaminated with PCBs and PAHs. There has also been a decrease in the incidence and severity of these effects with the decline in contaminant levels in the lakes.

Fox (2001) reported that birds living and breeding near pulp and paper mills discharging to the Strait of Georgia in Canada showed significant reproductive defects including lack of survival of young, changes in hormone levels and deformities in the young. Behaviour effects were also noted.

17β-oestradiol has been found to affect the song nuclei in the central nervous system of zebra finches and, therefore, their singing behaviour. Investigations have found that these processes are also highly responsive to other oestrogenic/antioestrogenic chemicals like dicofol, methoxychlor and octylphenol in a way that is similar to 17β-oestradiol (Laessig et al. 1999).

**Summary**

A variety of reproductive and endocrine-related effects have been seen in birds across the USA and Europe (Fry 1995; Feyk and Giesy 1998; CSTEE 1999). These effects have been associated with contamination by a range of chemicals, including PCBs, DDT, DDE, methylmercury, methoxychlor, PCDDs, PCDFs and PAHs, all of which have been shown to be potentially endocrine-disrupting. The range of birds that have shown such effects include the white-tailed sea eagle, peregrine, osprey, hawk, Forster’s tern, western gull, herring gull, cormorant, duck, common tern, crowned night heron and...
After the first calf has been born the mother’s PCBs levels are transferred to the yolk of the egg, because once the egg is laid the chick is fairly well protected from external or maternal influences. This limits the sorts of chemicals that can affect birds in this way to those that are persistent.

Mammals - Wildlife

Endocrine-disrupting chemicals have been linked to effects in some mammals.

Disruption of immune systems, rendering marine mammals vulnerable to infection by common bacteria and viruses, is thought to have resulted from contamination of these mammals with chemicals such as PCBs. The evidence seems to be growing. This is thought to be the cause of massive die-offs of seals from canine distemper virus in the North Sea and dolphins in the Mediterranean Sea and off the east coast of the USA (Kajiwara et al. 2002; Kennedy et al. 2000; O’Shea et al. 1999; Schumacher et al. 1993). Researchers found a link between increasing levels of pollutants (especially DDT analogues and some PCBs) and the degree of immune system suppression in animals, but this was based on a very small sample size. When the immune system is suppressed it is difficult to fight off infections and so the organisms become susceptible to diseases that they normally fight off. Other researchers question this hypothesis due to the lethality and virulence of the morbillivirus (O’Shea 2000). Hypotheses unrelated to endocrine-disrupting chemicals have also been proposed, but evidence is also weak for these (Lahvis et al. 1995; Crisp et al. 1998).

Impairment of reproductive success in seals and mink has been linked to the concentrations of chemicals with endocrine-disrupting potential in the fish they eat. The seal population in the Wadden Sea in the Netherlands has decreased dramatically over the last 25 years. The drop has been associated with PCB contamination of the fish the seals eat, as the fish are exposed to pollutants carried by the Rhine River to the ocean. An experiment where seals were fed fish from polluted and unpolluted areas was conducted to test the observation (Reijnders 1986). Additional laboratory experiments on small groups of seals have provided further evidence for the involvement of PCB contamination (De Swart et al. 1995). These studies have shown reduced reproductive success in seals fed fish from the Wadden Sea, and impaired immune systems. The fish were analysed for PCBs, PCDDs, PCDFs, dieldrin, β-hexachlorocyclohexane and DDT and the effects found were most clearly related to the PCB concentrations found in the fish. Other effects noted in these studies included abnormal growth of the cortex of the adrenal gland and effects on the action of the thyroid gland (Crisp et al. 1998; CSTEE 1999).

Schwacke et al. (2002) have evaluated the risk of reproductive effects in bottlenose dolphins, with regard to the levels of PCBs found in their blubber. They found that at two of the locations studied there was a high risk that the first calf born to each mother would not survive. This is because PCBs in the mother move into the calf during gestation and lactation. After the first calf has been born the mothers’ PCBs levels are significantly lower and therefore the exposure of further calves is expected to be below the levels that might cause impacts.

Chiba et al. (2001) investigated whether there was a relationship between the chlorinated hydrocarbon levels in the blubber of two types of seals and the seals’ thyroid hormone levels. They analysed seal blubber for PCBs, DDTs, chlordanes and lindane. A negative correlation was found between PCBs and total triiodothyronine (T3) levels in plasma. None of the other thyroid hormones were significantly related to the levels of the various chlorinated hydrocarbons found.

The population of beluga whales in the St Lawrence estuary in Canada is thought to have impaired reproductive success, as it has not recovered noticeably from overfishing in the first half of the 20th century (De Guise et al. 1995; Crisp et al. 1998). This could be due to exposure to contaminants in the fish they consume. The whales live in a highly polluted area, and they do not seem to be reproducing at a normal rate. Abnormalities have been seen in individuals. For example, one whale was found to be hermaphroditic, with two ovaries, two testes, a complete male genital tract and a partial female genital tract. This population is also known to have a high prevalence of cancers and frequent infections by common bacteria, which suggests immunosuppression. All of these effects might be related to the whales’ exposure to contaminants such as PCBs, dieldrin and PCDD, but the evidence is very limited at this stage.

Florida panthers have a very high rate of undescended testes (cryptorchidism). These animals also have a high rate of sperm abnormalities. The cause of this was thought to be inbreeding, but work by Facemire et al. (1995) indicates that endocrine disruption could also be the cause. Genetic diversity was found to be of a similar level to that in other cat populations, and cryptorchidism is not common in other cats regardless of the degree of inbreeding. Further study of serum sex hormone levels showed abnormally high levels of 17β-oestradiol in both apparently normal males and affected males. These levels were similar to those found in the females. Testosterone levels were higher in these males, but their high 17β-oestradiol levels resulted in abnormal testosterone to 17β-oestradiol ratios. Analysis of several dead panthers found high levels of mercury. The panthers eat raccoons, which could be the source of the mercury. Further work is needed to clarify what causes these effects in the panthers (Crisp et al. 1998).

Summary

Chemicals suspected of having endocrine disrupting effects have been shown to be present when reproductive effects, effects on the immune system and some birth defects were found in various marine mammals. A similar range of effects has been documented in other mammals, including bears, mink and otters. These effects include reproductive impairment, immune suppression and masculinisation, and have been associated with exposure to PCBs, DDT-like compounds, PCDDs, mercury and dieldrin (CSTEE 1999).
Severe infertility was reported in Western Australia in sheep in the early 1940s. It took only a few years of study to determine that phytooestrogens in subterranean clover were the likely cause (Adams 1998). However, a good understanding of how these chemicals exerted their effects, which of the phytooestrogens was responsible, and the extreme sensitivity of sheep to these effects took about ten years. Part of the delay was due to the reliance on normal laboratory assays on rats, mice or guinea pigs. Sheep, unlike most other mammals, continue to develop in response to oestrogen throughout their lives. As a result, exposure to the phytooestrogens caused continued differentiation of cells in the cervix throughout life, resulting in changes that made it difficult for sperm to be transported through the cervix and into the reproductive tract. As recently as the 1980s, fertility rates on one farm reduced to less than 40% when a field reverted to a clover monoculture after grasses failed to seed for two seasons because of climatic conditions.

**EFFECTS FOUND IN PEOPLE**

The recently-released Global Assessment of the State of the Science of Endocrine Disruptors (Damstra et al. 2002) concluded that “studies examining EDC-induced effects in humans have yielded inconsistent and inconclusive results, which is responsible for the overall data being classified as ‘weak’. This classification is not meant to downplay the potential effects of EDCs; rather, it highlights the need for more rigorous studies” and “The only evidence showing that humans are susceptible to EDCs is currently provided by studies of high exposure levels. Our understanding of the effects of chronic, low levels of EDCs are much more obscure. In particular, the relationship between early-life exposures to EDCs in humans and functioning in adult life is poorly understood. This is a concern because laboratory animal studies have indicated that early life stages may be especially sensitive to the effects of EDCs. Only recently have human epidemiological studies been conducted with the necessary rigour to sufficiently address potential cause-

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**Figure 1. How the hormone system signalling works in a cell.** At any step indicated here it is possible that a variety of chemicals may interfere. Chemicals may also interfere with the production of the natural hormone or with the metabolism and excretion of the natural hormone (adapted from SPEED 1998).
and-effect relationships in regards to EDC exposures.” The report contains a summary of the evidence – both that obtained in the laboratory using animal models and from epidemiological investigations. The report then discusses a series of case studies looking at specific situations thought to represent endocrine disruption. Those studies relating to effects in people showed that based on current evidence, there is weak to moderate evidence for the hypothesis that particular effects were due to exposure to particular chemicals (such as the relationship between the incidence of breast cancer in women and exposure to PCBs, DDT, DDE). They also concluded that there was weak to moderate evidence that the mechanism by which these chemicals caused these effects related to disruption of some aspect of the endocrine system (Damstra et al. 2002). So further work is needed to clarify both whether or not there is a relationship between exposure to particular chemicals and a particular effect, as well as to determine the likely mechanism by which the chemical causes an effect. Some of the effects that have been proposed as potentially being due to endocrine disruption in people are described below.

Effects in Children

Sex Ratios

Owing to the effects noted in a number of groups exposed to chemicals with endocrine-disrupting potential, the proportion of boys to girls at birth is being studied as a potential indicator of endocrine-related effects in the general population.

Studies of men exposed to a range of chemicals including dibromochloropropane, dioxin related chemicals, PCBs and pesticides have shown significant decreases in the proportion of sons born. (Potashnik et al. 1984; Williams et al. 1992; Garry et al. 1996; James 1997; Davis et al. 1998; Rogan et al. 1999; Ryan et al. 2002; del Rio Gomez et al. 2002). In some cases the men were shown to father less than 20% male children. In some of the studies the sex ratio in exposed women was also evaluated. The studies of women exposed to PCBs and dioxin related chemicals showed no changes in the sex ratio of their children.

Results like those described above have triggered the evaluation of sex ratios in the general population in a number of countries. In Canada the proportion of boys at birth decreased from 51.5% to 51.3% from 1970 to 1990 (Allan et al. 1997). During the same period the proportion in the USA decreased from 51.3% to 51.2%. In Scandinavia the proportion decreased from 51.4% to 51.3%; and in the UK the proportion decreased from 51.45% to 51.1%. Given the numbers of children born in each of these countries each year these seemingly small changes in the proportion of male babies translates to a large numerical difference in the numbers of boys and girls being born (Dickinson and Parker 1996; Davis et al. 1998; Moller 1998). On the other hand, there has been an increase in the sex ratio reported in Italy, Greece and the Netherlands (Damstra et al. 2002).

It is not clear why these changes are occurring. There was a significant increase in the ratio of boys to girls earlier this century, so it is a parameter that can change over time. A range of factors have previously been shown to affect the sex ratio including older fathers and mothers, in vitro fertilisation, ovulation induction (using drugs), and a range of diseases (Damstra et al. 2002). Although one explanation of the current trend may be related to exposure to chemicals that have oestrogenic potential, there may also be other mechanisms (Williams et al. 1992; Davis et al. 1998; Moller 1998; Damstra et al. 2002). Another hypothesis may be that the ratio is re-equilibrating in response to improvements in medical care during the 20th century (Damstra et al. 2002).

It has also been noted that the changes described above in the general population are still quite small and that future studies should focus on particular subpopulations that have been exposed to endocrine-disrupting chemicals to get a better understanding (James 1998a, b).

Development and function of the reproductive organs

Chemicals with endocrine-disrupting potential can affect adult reproductive systems, but they may have a greater impact if exposure occurs before or soon after birth as they may impact on the normal development of reproductive organs or secondary sexual characteristics (Crisp et al. 1998; Damstra et al. 2002). In humans, as in all mammals, embryos develop into females unless the presence of a Y chromosome triggers the production of a number of hormones that cause the formation of male reproductive organs. Sexual differentiation begins as early as week 6 of gestation, but the development of the reproductive tract continues throughout gestation. The production of particular hormones in appropriate quantities is essential for the development of a normal male. The extent and timing of any interference in the release of these hormones determine the extent of effects. Much of the understanding of the potential effects of such disruption has developed from studies in organisms which showed the absence or overexpression of one of these hormones due to some defect (Damstra et al. 2002).

The most commonly evaluated effects in boys are hypospadias (where the urethra opens onto the underside of the penis) and undescended testes or cryptorchidism.

Toppari et al. (1995) reported that the rates of genital defects such as hypospadias and cryptorchidism were increasing in a number of countries. Paulozzi (1999) assessed international trends in hypospadias and cryptorchidism in data from an international clearing house for birth defects managed by the World Health Organization. Data from 29 registries (by both countries and regions) were evaluated. Data collection began mostly in the early to mid 1970s. The registries report birth defect rates among 4 million births a year. The rates of hypospadias and cryptorchidism varied considerably between countries. The general trends in the data showed no generalised increasing rate for these defects. In the 1970s and early 1980s, the USA, Japan and Scandinavia reported increasing rates of hypospadias. By 1985, rates in most countries had levelled off. Cryptorchidism rates seem to have been declining since the mid 1980s. There is difficulty in getting consistent methodologies used for diagnosis of these conditions, which also adds to the variability in rates (Damstra et al. 2002; Toledano et al. 2003).
Endocrine Disrupting Chemicals Review

Damstra et al. (2002) note that if there were a relationship between exposure to persistent endocrine disrupting chemicals via breast feeding and hypospadias then the rate should be highest in first-born males, the prevalence should decrease as the number of children a woman has increases and maternal age at first pregnancy should also be related to increased prevalence. These relationships have been found in some studies. The high variability in the rates of these conditions, however, makes it difficult to assign such relationships with confidence.

These effects have also been noted in a range of studies where children have been exposed to various chemicals during development. Exposure to chemicals including DES and various pesticides has shown an association with increasing incidence of hypospadias and/or cryptorchidism (Gill et al. 1979; McLachlan 1993; Wilcox et al. 1995; McLachlan and Arnold 1996; Garcia-Rodriguez et al. 1996; Sloth Weidner et al. 1998; Crisp et al. 1998; Damstra et al. 2002).

Damstra et al. (2002) conclude that, given the mechanism by which the reproductive tract is formed in males, it is plausible that chemicals that increase testosterone turnover, decrease the synthesis of testosterone and other androgens, or antagonise androgen interactions with the androgen receptor, could result in an increase in the prevalence of undescended testes.

DES has also been shown to have effects in female children. DES was given to pregnant women in the 1940s, 1950s and 1960s to prevent miscarriage and was used widely in the livestock industry to promote rapid growth. It was used in these areas because it was known to have marked oestrogenic activity. In the early 1970s it became clear that DES caused a rare form of cancer in the daughters of women who had taken the drug. It also caused reproductive dysfunction and a range of malformations in the reproductive organs of these daughters (Crisp et al. 1998). Studies of the effects of DES on people who were exposed and on laboratory animals have shown the many effects that synthetic oestrogens can have in the developing foetus (Degan 1995; McLachlan and Arnold 1996; Crisp et al. 1998). DES interacts with the oestrogen receptor almost as well as 17β-oestradiol, the most common natural oestrogen hormone. Its concentration in blood cannot be controlled as tightly as that of 17β-oestradiol, because DES does not bind to the transport proteins in the blood. This means that any DES absorbed by the body is freely available to trigger oestrogenic effects.

Colon et al. (2000) investigated premature breast development in girls in Puerto Rico. The rate of premature breast development in Puerto Rico is the highest in the world. Serum samples were analysed for a range of pesticides and phthalate esters. No relationship was found between the pesticides and cases of premature breast development. However, significantly higher levels of phthalate esters were found in 68% of girls who showed this effect. The paper noted that analysis of phthalate esters is subject to numerous interferences which the analytical procedures attempted to take into account.

Other effects in children

A number of studies have evaluated the association between exposure to chemicals such as PCBs and intellectual development. Yung-Cheng et al. (1992) studied children exposed prenatally to high levels of PCBs in Taiwan. The use of PCB-contaminated cooking oil was the reason for the exposure. Exposed children were compared with unexposed children and siblings of exposed children born before the contamination. It was found that their cognitive development was impaired in comparison with the controls and unexposed siblings.

Jacobson and colleagues in a number of studies have looked at children born to mothers who had consumed varying amounts of fish from Lake Michigan, USA, during and immediately before pregnancy. The lake is known to be contaminated with PCBs and other chemicals (Fein et al. 1984; Jacobson et al. 1990a, b; Jacobson and Jacobson 1996). At birth these children were smaller than paired controls. At four years of age the children were smaller and less active and had poorer short-term memory. At eleven their intelligence was measured using IQ scores. A significant decrease was found in those children whose mothers had consumed the most fish from this lake, after controlling for confounding factors such as socioeconomic status.

Vreugdenhil et al. (2002) have been studying a group of Dutch children since birth. They have looked at a range of effects in the children in relation to prenatal/perinatal exposure to PCBs and dioxins, determined by analysis of cord and maternal plasma and breast milk where relevant. In this part of the study the relationship between play behaviours at age seven and early exposures to these chemicals was determined. They found that higher prenatal exposure to PCBs in boys led to less masculine play behaviours while for girls it led to more masculine play behaviours. For dioxin exposure the relationship was found to be with the more feminine play behaviours. In boys and girls the higher the dioxins exposure, the more feminine the play behaviours. Childhood play shows definite differences based on the sex of the child and so it is likely that sex hormones have a role in the development of these behaviours. No sex hormone levels were measured in the children at birth.

Lonky et al. (1996) studied children born between 1991 and 1994 to mothers who had consumed fish from Lake Ontario. Babies were assessed after 24 and 48 hours. Those born to mothers that had consumed large quantities of fish were found to score more poorly in most of the tests. No difference was found between the two groups of children for birth weight or head circumference.

Effects on development such as those found in Taiwan and the USA may be due to interference with thyroid hormone action – another part of the endocrine system (Tilson and Kodavanti 1997; Brouwer et al. 1998; Sher et al. 1998; MacLusky et al. 1998; de Vito et al. 1999; Oliver Cheek et al. 1999). PCBs are hydroxylated during metabolism in the body, and the hydroxylated PCBs then compete with one of the thyroid hormones, thyroxine, for binding to the transport protein transthyretin. The foetus then receives hydroxylated PCBs...
as well as thyroxine, and consequently may not get enough thyroxine. Sufficient thyroid hormone levels are essential for normal brain development. Alternative non-endocrine mechanisms may also cause the same effects.

The potential effects of other chemicals on thyroid hormones have also been studied. Porter et al. (1993) studied the effects of a mixture of methomyl, aldicarb and metribuzin (pesticides) on hormone levels in rats during 2-week and 6-week exposures. They found that the animals exposed to metribuzin alone or to all three pesticides together had significant increases in thyroxine levels. Thyroxine stimulates oxygen consumption, regulates lipid and carbohydrate metabolism, controls cell differentiation in numerous tissues, and is essential for normal growth and development in the reproductive and nervous systems. High levels of this hormone can indicate hyperactivity, hypermetabolic rates and hyperirritability. Such effects can make learning difficult. The study also looked at levels of growth hormone somatotropin but found few effects.

Longnecker et al. (2001) investigated the relationship between the serum concentrations of DDE, a DDT metabolite, in mothers and whether the babies were born pre-term or were small for their gestational age. They used serum samples that had been stored between 1959 and 1966 so looked at DDE levels much higher than current levels. They found that the risk of pre-term birth increased steadily with increasing DDE concentrations. The relationship between DDE levels and the size of the child at birth was less consistent but also showed an increasing risk with increasing concentrations. Pre-term birth is a contributor to infant mortality and so the authors were concerned that this potential effect of continued use of DDT for control of malaria vectors (i.e. mosquitoes) be thoroughly evaluated. This effect may be caused by DDE interfering with the action of hormones like testosterone (i.e. an endocrine disruption mechanism) or it could be due to DDE being toxic to cells in the placenta which would impact on the size of the placenta and, therefore, the size of the baby and/or when the baby is born (i.e. not an endocrine disruption mechanism).

Birnbaum and Fenton (2003) reviewed the evidence relating to the susceptibility of developing organisms to cancer if they are exposed to environmental chemicals during this stage of rapid growth and differentiation. The authors suggest that the implication of the growing evidence is that the causes of endocrine-related cancers or susceptibility to cancer more generally may be related to exposures during development rather than any exposure at or near the time of detection of the cancer. Childhood cancers are rare so any epidemiological study looking at links between exposures of children or parents to chemicals (or other environmental factors) and a particular type of cancer will be extremely difficult making this hypothesis difficult to assess even if biologically plausible.

### Effects in Women

**Endometriosis**

Endometriosis is a reproductive disorder that has been related to malfunctions in the immune system and the endocrine system. In this condition, cells like those lining the uterus are located in abnormal positions throughout the abdomen. In the USA it may affect five to six million women between the ages of 15 and 45 (Rier et al. 1995; Crisp et al. 1998). It can cause infertility, painful menstruation and extreme pelvic pain. Very little is known about its causes, and treatments are limited. Damstra et al. (2002) found that the condition can be aggravated by additional exposure to oestrogens. An imbalance between oestrogens and progesterone may be involved in the development of the condition. Combined oral contraceptives can assist in treatment and this is thought to be due to the progesterone. At menopause the disease is eliminated.

A number of studies have looked at the link between endometriosis and organochlorine exposure, especially exposure to PCBs and PCDDs. One study in rhesus monkeys found an association between the level of PCDD exposure and the severity of endometriosis (Rier et al. 1993). A more recent study found that 2,3,7,8-tetrachlorodibenzo-p-dioxin promoted the development of endometriosis in rats and mice, more so in mice (Arnold D et al. 1996). Other studies, including one epidemiological study, showed no relationship between serum levels of these chemicals and endometriosis (Boyd et al. 1995). There is evidence that women exposed to DES in utero have an increased frequency of endometriosis (Crisp et al. 1998). Another study found that PCBs and PCDDs that were able to interact with the Ah receptor significantly enhanced the growth of these cells (Johnson et al. 1997). This work may provide some information about a possible mechanism. A study looking at the women exposed to dioxin in Seveso, Italy, where an accident in a chemical plant released a cloud of chemicals containing dioxin that impacted on more than 30 000 people, has found a doubled risk of endometriosis among women with serum levels of TCDD above 100 ng L⁻¹. However, the study may have underestimated the risk, given difficulties in diagnosis and under-representation in the youngest women who were likely to be the most heavily exposed (Eskenazi et al. 2002). The available evidence neither confirms nor refutes the possible role of these chemicals in the development of endometriosis. Further studies might provide insights into the mechanism that causes this debilitating disease (CSTEE 1999; Damstra et al. 2002). Another paper by Rier and Foster (2002) reviews the relationship between dioxins and endometriosis. They suggest there is now compelling evidence from animal experiments supporting the relationship but that there is still no clear understanding of the potential mechanism by which exposure to dioxins might cause endometriosis.

Sinaii et al. (2002) surveyed women with surgically diagnosed endometriosis and found significantly higher rates of a number of immunological and endocrine disorders in the women.
Breast cancer

Breast cancer rates vary between countries, but it is one of the most common cancers in women (CSTEE 1999). The highest rates are in the USA and Western Europe. About one in eight women in the USA will get breast cancer during their lifetime (Davis et al. 1993; Crisp et al. 1998). This rate has been increasing by about 1% a year over the last 20 years. This is thought to be due in part to improved detection techniques (Feuer and Wun 1992). In Europe there is a two-fold difference between the countries with the highest and lowest rates (Switzerland and Spain) (CSTEE 1999).

A variety of factors are known to be related to the likelihood of contracting breast cancer, including age, race, family history, and total lifetime oestrogen exposure, which varies with the following factors: unopposed oestrogen therapy, late first pregnancy, late menopause and age at which menstruation starts (Davis et al. 1993; Crisp et al. 1998; CSTEE 1999). The development of the disease is complex, and research has found it difficult to isolate causes. There is some evidence that long-term contraceptive use is a risk factor. There is also a weak association between exposure to DES and later development of breast cancer. Therefore, it does appear that there is a link between exposure to strongly oestrogenic chemicals such as oestradiol and DES and development of breast cancer.

There have now been quite a large number of studies looking at the link between levels of various persistent organochlorine compounds and the incidence of breast cancer (Damstra et al. 2002). Some studies have suggested that women exposed to other oestrogenic chemicals (such as DDT, PCBs and PAHs) have an increased risk of breast cancer (Wolff et al. 1993; Davis and Bradlow 1995; Hoyer et al. 1998; Li and Li 1998; Warner et al. 2002; Calle et al. 2002; Damstra et al. 2002). A number of other studies have shown no association (Key and Reeves 1994; Krieger et al. 1994; Hunter et al. 1997; Safe 1997; van’t Veer et al. 1997; Gammon et al. 2002; Calle et al. 2002; Damstra et al. 2002).

Warner et al. (2002) used data from the Seveso Women’s Health Study to look at breast cancer rates in the women exposed to dioxin in the Seveso explosion. They found that there was a significant association between serum dioxin concentrations in samples taken in 1976 shortly after the explosion and current breast cancer incidence in the women. Further follow-up is planned.

The most up to date evidence does not support a link between exposure to DES and later development of breast cancer. Therefore, it does appear that there is a link between exposure to strongly oestrogenic chemicals such as oestradiol and DES and development of breast cancer.

Other effects

Cohn et al. (2003) found an association between levels of DDT and DDE in blood in women at the time they gave birth to daughters and the length of time it took for those daughters to get pregnant when they were 28-31 years old. It took longer to get pregnant for those daughters whose mothers had higher DDT levels in their blood. For those women that had higher levels of DDE, their daughters took less time to get pregnant when controlled for DDT blood levels.

Exposure to DES in utero caused a number of effects in women. These included cancer of the vagina at puberty, deformities of the reproductive organs, infertility, endometriosis and breast cancer. Rates for such effects, apart from breast cancer and endometriosis, have not been found to be increasing in the general population so far. Laboratory studies of a variety of chemicals have shown a range of effects on the ovaries or reproductive tract, although these effects are not yet showing up in the general population (McLachlan 1993; McLachlan and Arnold 1996; Crisp et al. 1998; Palmer et al. 2002; Damstra et al. 2002).

Effects in Men

Sperm count

The most commonly-discussed effect that endocrine-disrupting chemicals are supposed to have caused is a marked decrease in average sperm counts.

In 1992, Carlsen et al. published the results of a meta-analysis of 61 previously-reported studies of sperm counts in men from 1938 to 1991. They found that the average sperm count had decreased by 50% (from 113 million mL⁻¹ to 66 million mL⁻¹) over the 50 years studied. These authors and others postulated that this drop was due to widespread exposure to oestrogenic chemicals (Sharpe 1993; Sharpe and Skaakebaek 1993). There has been, however, much discussion of this study and the validity of the conclusion of a decrease in sperm counts given the timing of the studies, the size of the studies and some of the confounding factors (Bromwich et al. 1994; Farrow 1994; Olsen et al. 1995; Sherins 1995; de Kretser 1996; Crisp et al. 1998; CSTEE 1999).

Other studies of the available data on sperm counts at specific clinics or in specific regions have been conducted with a mix of results (MacLeod and Wang 1979; Ginsburg et al. 1994; Auger et al. 1995; Toppari et al. 1995; Fisch et al. 1996; Irvine et al. 1996; Paulsen et al. 1996; Swan et al. 1997; Selevan et al. 2000).

The appropriateness of determining trends in sperm counts for the total male population from single samples from individuals is subject to debate, especially where those individuals are evaluated for a range of reasons not as a random sample of the population. The variability in the sperm counts in individuals is large, making single samples problematic (Neubert and Chahoud 1996; Damstra et al. 2002).

More recent re-evaluations of the available data (Swan et al. 1997; Heinize 1998; Oreguela et al. 1998; Becker and Berhane 1998; Swan et al. 1998a, b; Swan et al. 2000) show that there was a decline in sperm densities in Europe (1971-1990) and in the USA (1938-1988), but there was no clear trend in non-Western countries, due probably to the few studies available. These results and the results at individual clinics in single locations (listed above) indicate that there may well be significant regional differences in actual sperm counts as well as in trends in the sperm counts.
The trends in sperm counts in males exposed to DES in utero are conflicting. Some studies found reductions in sperm counts while others did not (Damstra et al. 2002).

Effects of endocrine-disrupting chemicals, including PCB congeners, p,p'-DDE and phthalates, on sperm counts are plausible. The existing evidence is inconclusive. Studies are underway which have been designed to overcome some of the difficulties highlighted by the numerous evaluations of existing data (Damstra et al. 2002; Hauser et al. 2003; Duty et al. 2003a,b).

Jouannet et al. (2001) and Swan et al. (2003a) report on some of these new studies which have attempted to remove some of the difficulties discussed above. Jouannet et al. (2001) evaluated sperm counts in a number of cities in Europe – Copenhagen, Paris, Edinburgh and Turku. Using strict controlled protocols and adjusting for all the factors known to affect mean sperm counts, they found a significant difference in mean sperm count in the various towns. Turku in Finland had the highest mean sperm count while Copenhagen in Denmark had the lowest. Swan et al. (2003a) found similar differences across a range of cities including New York, Columbia (Missouri), Minneapolis and Los Angeles in the USA. Columbia (a semi rural/agricultural area) had a significantly lower mean sperm count than the other three cities. These new studies appear to strongly support the observation that sperm counts vary geographically.

Swan et al. (2003b) investigated whether there was a link between pesticide exposure and the decreased sperm quality found in the men from Missouri discussed above. They found an association between exposure to alachlor, atrazine and diazinon (as demonstrated by their presence in urine sampled at the same time as the sperm samples were taken) and decreased sperm quality. The association was quite strong even though the sample size was small and the study focused on men who had demonstrated fertility because their wives were pregnant at the time of recruitment for the study.

Testicular cancer
The rates of cancers in male reproductive glands are increasing. The incidence of testicular cancer in men under the age of 50 has increased by between 2% and 4% per annum since the 1960s in UK, the Nordic and Baltic countries, Australia, New Zealand and the USA. Denmark has the highest rate, where the lifetime risk is 0.25% (i.e. 1 in 400 men) (Toppari et al. 1995; Crisp et al. 1998; CSTEE 1999). There are marked differences in rates among countries and among races that are difficult to explain. For example, Finland has an incidence rate 80% lower than the rate in Denmark. Also, white men appear to be more susceptible than black men.

The cause of the increased incidence of testicular cancer is unknown, but one hypothesis is that it is due to damage sustained during development in utero as a result of disruption of the endocrine system (Toppari et al. 1995; Crisp et al. 1998; CSTEE 1999). No single study of DES sons showed an increase in testicular cancer but a meta-analysis of the available studies did show an increase in these men (Gill et al. 1979; Wilcox et al. 1995). Another study in Sweden of patients with testicular cancer found an association with exposure to PVC plastics during their working life (Hardell et al. 1997). It was difficult to confirm what these men might have been exposed to because the study was a retrospective survey of patients with cancer along with a matched set of control people who didn’t have cancer - it depended heavily on their memories of exposure (Damstra et al. 2002).

The available epidemiological evidence supports the idea that testicular cancer is a disease that begins early in life (Moller 1998). It is associated with low birth weight, neonatal jaundice, high maternal age, and malformations of the genital organs, including cryptorchidism. The causal factors seem to be related to foetal life, but whether oestrogenic chemicals are involved at all in development of the disease is not yet clear.

Moller (1998) studied male fertility and offspring sex ratios in men with testicular cancer and compared the rates with randomly selected controls. Fertility in the affected men (measured by the number of children) was lower than in the controls. The offspring sex ratio was 47% boys for the affected men, much lower than in the control group (52%) and in the normal population of Denmark (51.4%). The author proposed that these three effects were linked, but noted that the sex ratio in the general population did not predict the overall testicular cancer rate in the population of Denmark, and that the differences in testicular cancer rates in Scandinavian countries could not be related to differences in sex ratios. This indicates that there are likely to be other factors involved in the development of this disease. Other studies noted by the author have not found a lowering in the sex ratio in offspring of men who develop testicular cancer.

Hardell et al. (2003) evaluated the levels of organochlorine pesticides in blood samples taken from men recently diagnosed with testicular cancer and their mothers along with appropriately matched controls. They found no difference between the organochlorine pesticide concentrations in the two groups of men. There was, however, a significant difference found in the mothers. Mothers with higher levels of PCBs, hexachlorobenzene and nonachlordane were three to four times more likely to have a son with testicular cancer and the odds ratios were significant. The mechanism by which this cancer was caused is still to be determined, but this study highlights the difficulty in linking exposure to chemicals to particular diseases given that the timing of exposure can be so long before diagnosis.

The link between testicular cancer and exposure to endocrine-disrupting chemicals during development is, therefore, not strong at this stage and needs to be studied further.

Prostate cancer
Prostate cancer is the second leading cause of cancer deaths in American men (Garnick 1994; CSTEE 1999). There has been much improvement in its detection. However, death rates from it continue to increase despite improved diagnosis and treatment. Different races have different susceptibilities to it. It is very rare in Asian people, much higher in white people and even higher in black people. Also, not all men who are found to have prostate cancer die from it. Some forms seem to remain latent (Garnick 1994).
This cancer is difficult to study in laboratory animals, as the incidence is much lower in rodents than it is in people (Crisp et al. 1998). Work is continuing on developing appropriate animal models. Two epidemiological studies have been conducted, one on farmers exposed to herbicides and one on coke oven workers (Crisp et al. 1998). Both studies found a small but significant association between exposure to the chemicals and prostate cancer rates. The weight of evidence at this stage is weak, and further work is needed to establish whether there is a link and to determine other causes of the increasing incidence rate (Garnick 1994; Crisp et al. 1998; CSTEE 1999).

**Summary**

A wide range of health effects in adults and children have been linked to exposure to a range of chemicals that have endocrine disrupting potential. The major factors making it difficult to conclude with confidence that these links exist are the lack of actual exposure data showing how much of these chemicals children or adults have been exposed to and the small sizes of the groups studied to date which are, in some cases, so small that, even if there were an effect, it would be difficult to detect. Another aspect that is important is the timing of exposure - it may be that exposures early in life predispose individuals to health effects making the gathering of strong evidence difficult. As a result, the current evidence is considered inadequate to support the proposed links even though the biological plausibility of the links is considered strong and further work is, therefore, necessary (Moline et al. 2000; Damstra et al. 2002).

**COMPLEXITIES OF LOW-DOSE RESEARCH AND NATURAL VARIABILITY**

There has been much discussion about the repeatability of many of the studies showing that a range of chemicals has endocrine-disrupting potential, especially with regard to effects at very low doses. As a result, there are widely differing views among researchers on whether many of the chemicals can actually cause impacts at the doses being proposed. Some examples of the difficulties are discussed below.

**Natural Variability in Test Populations and Results**

Sharpe et al. (1995) showed that exposure to octylphenol (a breakdown product of surfactants) or butylbenzylphthalate (a plasticiser) caused small but significant (and repeatable) decreases in testicular weight in adult rats that were exposed during development (i.e. in utero). Another study by other researchers was not able to repeat these experiments despite using an almost identical study design (Ashby et al. 1997). Sharpe et al. (1998) discuss this difficult issue. They discuss the variability not only in the findings for the environmental chemicals, but also for DES, the positive control used in animal models. Two epidemiological studies have been conducted. This paper makes some detailed recommendations about how to conduct experiments to deal with this issue so as to clarify the relevance of these low dose effects.

**Difficulty in Selecting Appropriate Doses and in Evaluating Subtle Effects**

In 1998, vom Saal et al. released a study of the possible effects of bisphenol A, a chemical used in plastics production. The study was published in the peer-reviewed literature. They found that, if mice are exposed while pregnant to bisphenol A or octylphenol at very low concentrations, quite a number of significant effects occurred in the young. The concentrations used in the experiment were derived by using in vitro assays with the oestrogen receptor to determine the potency of a chemical to interact with this receptor compared with 17β-oestradiol. The change in 17β-oestradiol concentration required to cause effects in the whole animal and the potency of the chemical to interact with the receptor were then used to determine the experimental concentrations
of the chemicals being tested. Changes in 17β-oestradiol concentrations of as little as parts per trillion (ng L\(^{-1}\)) cause impacts in the foetus. (Further information on determining appropriate doses for these studies is described by Welshons et al. [1999]). Therefore, if a chemical is 1/1000th as potent, concentrations as low as parts per billion (µg L\(^{-1}\)) may cause equivalent effects. Such concentrations may be present in the environment, depending on the chemical. Mice were exposed to 2 or 20 µg kg\(^{-1}\) of bisphenol A or octylphenol. Effects such as decreased sperm count and increased prostate size were noted, as were effects on other glands and organs. Some effects were seen in the lower-dose animals but not in the higher-dose animals. This phenomenon is to be investigated, but it highlights the difficulty in choosing doses in these experiments.

In October 1998, an industry-funded study was released that attempted to repeat the vom Saal et al. (1998) study described above. This study used more animals and four different doses but was unable to find any health effects even in mice exposed to DES – the positive control (a chemical that is known to cause the effects being investigated and is used to check that the animals are responding normally). This study has been published in the peer-reviewed literature (Cagen et al. 1999). However, before its publication it was described as ‘fundamentally flawed’, as it did not find any effects in the positive controls (i.e. the DES-exposed animals) (ENDS 1998a; Macilwain 1998). This lack of effects may be because the dose of the chemicals used was too low to cause effects, despite the findings of the vom Saal et al. (1998) study. It could also possibly be due to some problem in the experimental design. Further work is needed to clarify the effects of bisphenol A at very low doses.

Tinwell et al. (2002) provides another example of these difficulties. This study attempted to repeat another study where bisphenol A had been shown to have effects at doses as low as 20 µg kg\(^{-1}\). Again the repeat study did not show the same effects as those seen in the original study. The authors note that two things may be influencing these types of studies – some subtle, unspecified differences in the bioassay (like housing, diet, degree of handling, noise, strain of animal, etc) or the multiplicity of biological endpoints and the intrinsic variability in them for individual animals resulting in statistical analysis issues.

**Difficulty in Ensuring Laboratory Animals are Exposed Only to the Chemical Under Investigation**

Thigpen et al. (1999) reported the high content of phytooestrogens in a range of diets fed to laboratory animals. Most of the commonly used diets contained soybean meal, resulting in the presence of genistein and daidzein, two potent phytooestrogens. Evaluations of the oestrogenicity of various chemicals using animals with already higher than normal oestrogenic responses may be compromised. This is a factor that needs to be considered when researchers interpret data from such experiments.

More recently Hunt et al. (2003) and Howdeshell et al. (2003) have shown that exposure to bisphenol A can come from unexpected sources. Normal practice in handling laboratory animals involves the use of appropriate cages and water bottles. Often these are made from various plastics. Both groups have found that bisphenol A can leach in significant quantities from cages and water bottles, compromising experiments attempting to evaluate endocrine-disrupting effects. Hunt et al. (2003), when investigating effects unrelated to endocrine disruption, found that their control animals were producing baby mice which had such significant genetic defects that most died. They tracked the unexpected exposure down and found that damage to the cages during washing had meant that bisphenol A could easily leach out so exposing the mice. Howdeshell et al. (2003) filled different types of cages with water for one week at room temperature to determine how much bisphenol A leached from the cages under these conditions. Used polycarbonate cages leached enough bisphenol A to cause effects in the MCF-7 *in vitro* test. New cages leached much smaller amounts. The effects were further demonstrated *in vivo*.

**Difficulty in Estimating Exposure to Endocrine-disrupting Chemicals**

Exposure to bisphenol A from its use in dentistry or in the coating of cans used for food has been evaluated in a number of studies (Brotos et al. 1995; Olea et al. 1996; Hoyle and Budway 1997; Welshons et al. 1997; Imai 1999; Olea 1999). These studies have found that people may be exposed to levels similar to those that caused effects in vom Saal et al.’s study discussed above. However, there has been much discussion about the validity of the exposure estimates used, especially those estimated for the dentistry uses. The usual practice in the management of chemicals is to use them in such a way as to ensure that exposures are much lower than doses likely to cause effects. This is difficult to achieve when there is such disagreement about what dose is likely to cause effects.

There has also been much discussion about the dose of particular phthalates received by infants when they chew on plastic toys. Laboratory tests simulating the release of these chemicals during chewing of the toys were shown to underestimate the amount of phthalate released when compared with the release rates when adult volunteers chewed on the toys (US CPSC 1998). Until this finding was reported, these laboratory simulations were used to estimate children’s exposure and therefore risk. Now there is little agreement on what techniques are suitable. It is also not known with much certainty how long children spend chewing these toys. One study by Dutch researchers attempted to quantify this by observing children at play, but the study has been criticised due to the difficulty in confidently reproducing children’s behaviours and the lack of inclusion of guidance from an expert committee advising the European Commission in the development of the test procedure (ENDS 1998b; Konemann 1998). These two types of information are essential for proper estimation of the dose to which infants are exposed. Without such information, reliable estimates of dose cannot really be developed, which makes the assessment of possible risks difficult.
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Non-monotonic Dose-response Curves
There has been much discussion in the media and in scientific circles about the shape of the dose-response curve for endocrine disrupting chemicals. Some studies have found that effects can be seen at very low doses while slightly higher doses can show no effects and then at high doses different types of effects may be found. The usual dose-response curve in toxicology shows increasing effects with increasing dose – the dose makes the poison. It has been suggested by some that the differences seen with endocrine disrupting chemicals may be because at low doses the chemicals are disrupting the normal messages while at high doses the chemicals are having other effects including overwhelming the cell’s defences. If chemicals do act via a non-monotonic dose response curve then standard toxicology tests may overestimate the dose that starts to cause effects so providing less protection than desired (NTP 2001; National Research Council 1999; Krimsky 2000; Cavieres et al. 2002; Oehlmann et al. 2000; Wetherill et al. 2002).

Cavieres et al. (2002), for example, evaluated the effects of a commercially available herbicide mixture on embryo implantation and litter size in mice. The herbicide mixture contained 2,4-D, mecoprop, dicamba and some non-active ingredients. The largest effect on litter size was found in the lowest dose used which was similar to those that might exist in the environment when the herbicide is used. There was seasonal variation in the effects caused by each dosage level.

Summary
These examples highlight the difficulties in assessing subtle effects. They also highlight why some researchers think that action is urgently required to control exposure to chemicals that may have endocrine disrupting potential while others do not. The proposed screening and testing program required in the USA and in Europe will have to meet such challenges if chemicals are to be classified appropriately. There will also be a need for adequate risk-risk comparisons (i.e. risk of continued use of a particular chemical versus risk of that type of product being unavailable – e.g. use of phthalate plasticisers in medical plastic goods such as IV equipment) to ensure effective chemicals management.

KNOWLEDGE GAPS
From the previously-described studies it appears that there are many situations in which endocrine-disrupting chemicals could be responsible for the effects seen, but in most of these cases causal evidence is not strong. It is likely that linking specific effects to specific exposures will always be difficult, owing to the complexities of environmental exposures to chemicals, the time lag before effects might show, and the often subtle nature of the effects. Some significant unanswered questions prevent us from conclusively determining the impacts of these chemicals (USEPA 1998). These questions include:

- What concentrations of chemicals can cause these effects?
- What concentrations of chemicals are people or wildlife exposed to (and when)?
- What are the effects of exposure to mixtures of these chemicals?
- What is the role of phytooestrogens?
- How do these chemicals affect Australia’s unique wildlife?
- Are natural and synthetic hormones that are potentially present in sewage effluents having effects on aquatic organisms in NSW?

What Concentrations of Chemicals Can Cause These Effects?
There is much controversy over how to determine what concentrations of particular chemicals in food or water might cause endocrine effects (USEPA 1998; Federal Register 1998, 2002). Over the last few years, in vitro systems have been developed to help rapidly screen chemicals for these effects. These systems usually involve a receptor, such as the oestrogen receptor, in a situation where only one response is possible if it is triggered. Such screening has found that the synthetic chemicals thought to have the potential to interact with the receptor must be present at concentrations many times higher than the natural hormone 17β-oestradiol to cause the same response. The numerous mechanisms by which chemicals may affect the endocrine system make such screening only partly useful (see Figure 1). The USEPA’s EDSP proposes to use a range of in vitro and in vivo tests to screen and test chemicals to determine what doses might cause impacts. Work has yet to commence, however, due to the difficulty in developing and validating the tests to be used. As a result, for many chemicals it is still not clear what concentrations can cause impacts, especially whether or not possible effects at very low doses need to be considered. With the recent research indicating that test animals may be exposed to endocrine disrupting chemicals in their normal environments in laboratory colonies it may be some time before all the bugs are worked out of these types of tests. The magnitudes of concentrations, however, of natural and synthetic hormones that affect fish are already becoming much clearer.

What Concentrations of Chemicals Are People or Wildlife Exposed to (and When)?
Knowledge of both what concentrations people and wildlife are exposed to and when they are exposed is crucial to understanding how these chemicals might affect human health and the environment (USEPA 1998; CSTEE 1999; Damstra et al. 2002). An organism may be exposed to a chemical all day every day, but may only be affected if exposed when just born or during the reproductive phase. Exposure must be estimated during these critical times to determine whether effects are likely. For a chemical that bioaccumulates, estimates of exposure all year round are necessary to determine whether effects are likely at some time in the future. Designing studies to provide these different types of information about exposures is very difficult, especially when, for many
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organisms, it is not known which life stages might be critical or when they occur.

It is clear that, for some wildlife, the concentrations of particular chemicals already appear to be high enough to cause effects. For example, TBT concentrations in marinas were high enough to cause effects in marine snails; and the levels of 1β-oestradiol and oestrone being discharged from STPs into UK rivers seem to be high enough to cause intersex in fish. For most potential endocrine-disrupting chemicals, however, the situation is less clear. More information about body burdens and better modelling tools to help estimate environmental levels are needed to answer this question.

What are the Effects of Exposure to Mixtures of These Chemicals?
People and the environment are usually exposed to a mixture of synthetic chemicals rather than to individual chemicals. It is difficult to evaluate such exposures. At present, regulatory test methods do not usually deal with mixtures, and research into this area has proven difficult. One group of researchers found greatly increased effects when evaluating a mixture of chemicals using an in vitro test (Arnold et al. 1996). These results were withdrawn, however, as no one was able to reproduce these findings – not even the original laboratory. There is, however, still some evidence for increased effects during exposure to mixtures of chemicals (Porter et al. 1999).

Other studies have evaluated whether there are additive effects amongst chemicals that interact with the endocrine receptor. It has now been fairly consistently demonstrated that mixtures of chemicals interact in an additive way - i.e. the effect of the mixture is the sum of the effect each chemical exerts at the concentration at which it is present in the mixture (Arcaro et al. 1998; Knudsen et al. 1998; Thorpe et al. 2001; Rajapakse et al. 2002; Silva et al. 2002). The USEPA’s EDSP intends to evaluate a number of mixtures of chemicals but have deferred such testing until the procedures are confirmed for individual chemicals (McLachlan 1997; Federal Register 1998, 2002).

What is the Role of Phytooestrogens?
Exposure of people to phytooestrogens in their diet is widespread and has been occurring for as long as people have been eating plants. These natural chemicals are present at much higher levels in people’s diets than any of the synthetic chemicals thought to have endocrine-disrupting potential previously discussed in this review (Safe 1995; Crisp et al. 1998). As yet, there is little understanding of the possible effects of such exposure and whether it overpowers exposure to the synthetic chemicals that have this potential. There is also evidence that exposure to phytooestrogens can have beneficial effects. The effects that these natural chemicals have on ecosystems are even less well understood. Further research to investigate the effects of these natural chemicals in people and wildlife is necessary to clarify their risk and to assist in the assessment of the risk posed by synthetic chemicals (Crisp et al. 1998; Zava et al. 1997).

How do These Chemicals Affect Australia’s Unique Wildlife?
Australia has a variety of wildlife not found elsewhere in the world. There is some concern that marsupials could be particularly vulnerable to endocrine disruption effects. If they are exposed during the development that takes place in the pouch, they are without the protection of the mother’s detoxification system, unlike placental mammals (Bolton and Ahokas 1995). So any chemicals present in the mother’s milk may be able to cause effects until the normal detoxification systems develop, but little is known at present to determine whether this is the case. Also, for a few marsupial species, their development to weaned infant may take much longer than for placental mammals, which could prolong such effects.

Are Natural and Synthetic Hormones That Are Potentially Present in Sewage Effluents Having Effects on Aquatic Organisms in NSW?
The presence of natural and synthetic hormones at levels capable of affecting the endocrine systems of aquatic organisms has been demonstrated in the UK (Jobling et al. 1998; Harries et al. 1999). In the USA, where STPs use a higher level of treatment and have greater dilution at discharge, neither natural nor synthetic hormones were detected (Nichols et al. 1999). In NSW, Australia, the level of treatment at many STPs discharging to rivers is quite high, and the number of people being served by each individual plant is likely to be much smaller than in the UK. The sorts of dilution possible in such locations, however, are frequently low, so it is not known whether the effects in fish that are being seen in UK rivers are likely to be found in NSW rivers. One study has shown possible endocrine-disruption effects in mosquitofish in a small creek downstream of an STP in NSW (Batty and Lim 1999; Doyle and Lim 2002).

INTERNATIONAL RESPONSES TO THE CONCERNS

United States of America
The USEPA Science Policy Council’s interim position on endocrine-disrupting chemicals was presented in 1997 (Crisp et al. 1998). The USEPA expressed concern about the possibility of adverse effects on human health and the environment associated with exposure to endocrine-disrupting chemicals. The current state of the science, however, does not yet allow widespread agreement about the extent of the problem. Identifying environmental agents likely to cause such effects and gaining a greater understanding of how these agents might exert their effects (particularly on children and vulnerable ecosystems) are high priorities. Where possible the USEPA is doing further research and testing to fill these knowledge gaps. This is necessary before the risks from such chemicals can be adequately assessed. The USEPA has undertaken a range of activities to meet some of these needs:
The USEPA was a cosponsor (with the Centers for Disease Control and Prevention and the Department of the Interior) of the detailed review and interpretation of the existing literature by the National Academy of Sciences' National Research Council, released in August 1999.

The Food Quality Protection Act (passed in 1996) and the Safe Drinking Water Act (amended in 1996) both require the development of a screening and testing strategy for evaluating chemicals for their potential to cause effects via endocrine system disruption, to start within four years.

A long-term research strategy is being implemented.

The USEPA is coordinating research in this area across the USA Federal Government and is pursuing coordination of research internationally.

The Food Quality Protection Act (passed in 1996) and the Safe Drinking Water Act (amended in 1996) both require the evaluation of chemicals for their potential to cause effects via endocrine system disruption, as discussed previously. To develop the strategy for screening and testing chemicals to enable such evaluation, a committee was formed – the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC). The Committee met during 1997 and 1998. It agreed to look at procedures that investigated the possible human and ecological effects of chemicals that could interact with the oestrogen, androgen or thyroid receptors. A report outlining the findings of the Committee was finalised in October 1998 (EDSTAC 1998).

The USEPA outlined how they proposed to implement the EDSTAC recommendations in December 1998 (Federal Register 1998). The EDSTAC report was also reviewed by the USEPA's Science Advisory Board (SAB 1999). The process for implementing the EDSTAC recommendations has not progressed smoothly since then. Developing and validating appropriate tests for assessing endocrine disrupting potential has taken longer than anticipated. The in vitro tests that were to be used to quickly screen a large number of chemicals have been found to not be as robust as hoped. The current proposal is to test 50-100 chemicals using the tests available. These chemicals are ones that have been found to be in the environment and that studies have not yet shown whether or not they have endocrine disrupting potential (Federal Register 2002).

The National Academy of Sciences' National Research Council completed an extensive review of the literature in 1999. The conclusions of the report are listed above (under ‘Conclusions’) (National Research Council 1999).

In December 1998 the USA Consumer Product Safety Commission released its findings on the safety of phthalates in plastic toys for children. These chemicals have been implicated as endocrine disruptors. They are used to soften the plastic used in toys. The study concluded that few, if any, children are at risk from ingestion of diisononyl phthalate via this route. However there are still some areas of uncertainty which need further work. Regardless of the results, 90% of manufacturers have indicated that they will be removing these chemicals from this type of product. Dummies and teats for bottles are usually made from rubber or silicone, but those made with plastic softened with phthalates have been removed from the market (US CPSC 1998).

In December 1998, the USA National Institute of Environmental Health Sciences and the National Toxicology Program formed a new Center for the Evaluation of Risks to Human Reproduction to provide a ‘readily and publicly available, scientifically authoritative mechanism for the evaluation of human and experimental evidence for adverse effects on reproduction’ (Anon 1999). Since its establishment the centre has evaluated phthalates, ethylene glycol, propylene glycol, methanol, 1-bromopropane and 2-bromopropane for their ability to disturb reproduction or development. These chemicals were chosen either because they were high production volume chemicals that had a high exposure risk to people or because they had already been shown to have some effect on reproduction or development. The centre also conducted an extensive review into the confusing topic of effects at low doses, which included statistical reassessment of the raw data from a number of studies. (Federal Register 2000; CERHR 2003).

Europe

Various European governments have reviewed the literature on chemicals with endocrine-disrupting potential to determine what action, if any, is required. The UK, Denmark and Germany are among these (Institute for Environment and Health 1995; Toppari et al. 1995; Umweltbundesamt 1996). The Working Group on Endocrine Disruptors of the CSTEE of Directorate 24 (Consumer Policy and Consumer Health Protection) also prepared a review of the literature for consideration by the European Commission (CSTEE 1999).

In 1998 the European Environment Agency (EEA) and the United Nations Environment Program published a report entitled Chemicals in the European Environment: Low Doses, High Stakes? (Gee 1999). The report noted that, although there was currently not much direct scientific evidence of widespread impacts on human health or the environment from manufactured chemicals (apart from a few notable exceptions), the evidence was growing for effects on people and the environment from chemicals acting as neurotoxins, endocrine disruptors, or allergens. As some of the hazards were serious and irreversible and may take some time to appear, it may be necessary to take action to reduce exposure even in the absence of definite proof of harm. As a result, the report encouraged the reduction of chemical loads to the environment, particularly for those chemicals that are persistent and bioaccumulate (UNEP 1998).

In January 1998, a discussion paper was released by the UK Environment Agency. The paper acknowledges that much is unknown about the potential effects of endocrine-disrupting chemicals, but that some actions can and should be considered now. These actions include encouraging industries to reduce or eliminate their use of compounds found to have the potential to disrupt endocrine systems, developing environmental quality standards for priority substances to
assist in setting discharge limits and managing catchments, and carrying out targeted monitoring programs to improve the information base on the occurrence of these chemicals in the environment. Comments on the paper closed in April 1998; the final strategy was released in 2000 (UK Environment Agency 1998, 1999, 2000).

Phthalates, which are used as softeners in the PVC used for making teething toys for children, have been implicated as endocrine-disrupting. In September 1998, studies in Europe were released showing that in most situations the migration rates of di-isononyl phthalate from the toys was not enough to exceed safety limits. However, there is much disagreement about the methodology used to estimate migration rates, including how long children are likely to have these toys in their mouths. Austria, Sweden and Denmark have all moved to ban the use of phthalates in PVC toys for children under the age of three. Norway, Italy, Greece, Finland and Germany look set to impose such a ban as well. As a result, toy manufacturers such as Mattel are removing it from their products (ENDS 1998b, 1999a; Konemann 1998).

The UK Environment Agency has been developing regulatory standards for a number of endocrine disrupting chemicals including alklyphenol ethoxylates and the natural and synthetic oestrogen hormones. The European Chemical Bureau has also developed regulatory standards for alklyphenol ethoxylates (UK Environment Agency 1998; ENDS 1999b, Young et al. 2002; Williams et al. 2001; European Chemicals Bureau 2002).

In December 1999 the European Commission released its strategy for action relating to endocrine-disrupting chemicals (European Commission 1999). In the short term, a priority list of substances for further evaluation will be established. Once this list is established, member states will be encouraged to take any appropriate legislative action to fully evaluate these chemicals as quickly as possible and to establish monitoring programs to estimate exposure to the nominated chemicals. Also, any special cases where more sensitive groups of consumers might be the most exposed will be identified, and the need for any action will be considered. International cooperation, information exchange and better communication with the public will also be pursued. In the medium term, research will be funded to look at the mechanisms involved and to develop tests to better assess the potential of chemicals to have these effects. In the long term, legislative actions will be considered both within Europe and in the development of treaties by international organisations. In 2001 the European Commission released a paper detailing progress on implementation of the strategy (European Commission 2001). The list of chemicals to be assessed for endocrine disrupting potential has been identified to guide future research; workshops have been held to further discuss the issue and funding for research has been provided to foster knowledge generation. Newly proposed European legislation to more effectively manage the use of chemicals has included consideration of effects such as endocrine disruption.

Basler and Lebsanft (1999) present the German government’s position. They stated that current findings did not provide evidence of a risk to human health from exposure to oestrogenic substances except in situations where high concentrations of relevant chemicals may be present. Continued monitoring of developments in the field will be undertaken to inform further decisions regarding research priorities and policy initiatives. In Europe actions are being considered for a range of substances including bisphenol A, benzylbutylphthalate, nonylphenol and organotin compounds.

Japan

Japan’s Environment Agency released a report in September 1998 stating its position on endocrine-disrupting chemicals (SPEED 1998). Its view of the issue can be summarised in the following:

‘We can tell from scientific knowledge that it is a serious problem that has the potential to cause major harm to the reproductive processes of humans and animal species.’

The overall approach proposed in the report was to undertake a comprehensive assessment of risks followed by adopting appropriate actions where necessary. Initially, the following areas are targeted for action:

• promotion of field investigations into the present state of environmental pollution and adverse effects on wildlife and humans of endocrine-disrupting chemicals;
• promotion of research, and development of screening and testing methods;
• promotion of environmental risk assessment, risk management and information dissemination, including the implementation of a Pollutant Release and Transfer Register (similar to the Australian National Pollutant Inventory);
• strengthening of international networks (SPEED 1998).

Since 1998 the Japanese Ministry of the Environment have undertaken a range of in vivo, in vitro and ecosystem studies to better understand the potential risks relating to the endocrine disrupting potential of prioritised chemicals. As of November 2002 they have completed most of the proposed tests for 12 priority chemicals and have studies on another 16 chemicals in the pipeline. The first 12 priority chemicals were tributyltin, octylphenol, nonylphenol, di-n-butylphthalate, octachlorostyrene, benzophenone, dicyclocetylphthalate, di-(2-ethylhexyl)phthalate, triphenyltin, benzylbutylphthalate, diethylphthalate and di-(2-ethylhexyl)adipate. The chemicals currently being investigated include pentachlorophenol, amitrole, bisphenol A, 2,4-dichlorophenol, 4-nitrotoluene, dipentylphthalate, dihexylphthalate, dipropylphthalate, hexachlorobenzene, hexachlorocyclohexane, chlordane, oxychlordane, trans-nonachlor, DDT, DDE and DDD (Ministry of the Environment 2002a).

The Ministry of the Environment has also surveyed waters, wildlife, food, indoor air and the atmosphere to determine levels of potential endocrine disrupting chemicals in the various compartments of the environment. In waters a survey of 23 different chemicals was undertaken at more than 170 sites, which included rivers, lakes, marine and groundwaters.
The 23 chemicals were also surveyed in sediments at almost 50 sites. These chemicals were also surveyed in birds and frogs. These chemicals have also been assessed in atmospheric samples at about 20 locations (Ministry of the Environment 2002b,c,d,e,f).

The Ministry of the Environment has also sponsored a yearly conference on the issue since 2000 (Ministry of the Environment 2003).

International Governmental Organisations

The issue of endocrine-disrupting chemicals was raised at the second Intergovernmental Forum on Chemical Safety (IFCS) in 1997. The Forum agreed that ‘a rapidly growing body of scientific research indicates that a number of substances have the potential to interfere with the normal functions of the body governed by the endocrine system’, and that as a result, effective coordination of activities is essential to allow adequate assessment of the issue (IFCS 1997).

The Forum requested that organisations participating in the Inter-Organisation Program for the Sound Management of Chemicals (IOMC) undertake the following:

1) Compile and harmonise the definitions and terms appropriate to endocrine-disruption.
2) Promote coordinated research strategies and processes, and identify research priorities and gaps for all relevant research disciplines.
3) Delineate testing methods, harmonise guidelines, and identify testing priorities and gaps.
4) Adopt and maintain an inventory of research activities and other relevant and related information.
5) Facilitate information exchange on:
   • existing and new evaluations of the scientific issues related to endocrine disruption;
   • research and testing results;
   • surveys and survey results;
   • meetings, workshops and conferences;
   • actions and options to manage risks and hazards.

The Forum also requested that the participating organisations keep the IFCS Forum and Inter-Sessional Group informed of progress (IFCS 1997).

In December 1998, at a meeting of the Inter-Sessional Group of the IFCS, a progress report was provided on activities in this area. The IOMC participating organisations – the IPCS and the OECD – have, as a result, initiated a range of activities in compliance with the Forum directive. The OECD is coordinating global and regional activities such as workshops and is working on appropriate OECD testing guidelines (activities 1, 3 and 5). The IPCS is developing an international inventory of research activities in the area and is coordinating the preparation of an international assessment of the science (activities 2 and 4). The international assessment was completed in 2002 and is available at the IPCS site. The global inventory of research is now available on the Internet at the IPCS site (IFCS 1999).

Australia

In April 1998 the Australian Academy of Science held a one-day workshop to discuss Australia’s role in this issue. A short summary of the proceedings was published (AAS 1998). The main outcomes of the workshop were that:

- Australian research should focus on our unique flora and fauna.
- Communication between industry, government and the community needs to be improved – the Academy agreed to look into the feasibility of becoming a clearing house for relevant information (AAS 1998).

The National Industrial Chemical Notification and Assessment Scheme published an information paper in 1998, and updated it in 2003, to provide advice on the position of the various Commonwealth departments responsible for chemical management. These departments support the position of the IFCS discussed above and are keeping a watching brief on international developments (NICNAS 1998; NICNAS 2003).

In April 2003, the Swedish Chemicals Agency started a concerted effort to reduce the use of endocrine disrupting chemicals in the country. This effort is called ‘Chemical Safety in Sweden 2005’ and the Ministry of the Environment is responsible for coordinating the effort.

A workshop in 2004 provided a roundup of research in Australia on endocrine disrupting chemicals. There are currently about 25 projects underway in Australia looking at the potential impacts of endocrine disrupting chemicals with about half of these projects being undertaken by postgraduate students. Many of these projects are just commencing so presentations covered experimental design rather than results. Most of the projects involve looking for particular chemicals in the environment with smaller numbers looking at the effects caused by particular chemicals, tools to assist in investigating these chemicals and their effects or modelling the fate of these chemicals once released to the environment. Monitoring projects cover a wide range of possible sources of these chemicals (sewage effluent and pesticides) as well as the environments in which they may be found including urban areas, various rural locations and remote tropical locations as well. The use of in vitro techniques is being developed by a number of researchers to assist in monitoring efforts. Effects on wild fish and laboratory-raised fish and frogs are also being investigated. Treatment technologies are also being investigated (CSIRO 2004).

CONCLUSIONS

The possible effects in people and wildlife of exposure to a wide range of chemicals acting via disruption of the endocrine system have been described as ‘very serious’ and ‘widespread’ by scientists and environmental groups in both the scientific literature and the print and electronic media during the last ten years. However, there is still much discussion about the strength of the evidence that exists, especially with regard to current exposures.

Many organisations throughout the world, including those responsible for environmental protection or public health in the UK, USA, Denmark, Germany and the European Union, have, in a relatively short period, gathered a large amount of information on the potential for endocrine disruption by chemicals. They have used this information to assist in determining the need for changes in chemical management.

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The available information on the impacts of endocrine-disrupting chemicals provides support for the following conclusions:

- A variety of wildlife populations living in contaminated areas seem to have impaired reproduction. At least some of these effects are likely to be due to endocrine disruption. The best-known example is the effects of TBT products on marine snails. There appears to be little doubt that this is a case of disruption of the endocrine system.
- Exposure of wildlife to natural hormones released into the environment from sources like STPs has affected fish in rivers in the UK. There can be little argument that these effects are mediated via the endocrine system, as they involve exposure to the same chemicals used in the body for signalling by the endocrine system.
- Disruption of the endocrine system caused by various medical conditions or due to particular drugs has been shown to be able to cause significant effects in people.
- Embryos are likely to be the most sensitive human life stages, as their endocrine systems are in the process of developing. Adults are less sensitive to these effects, as their endocrine systems are fully developed and include various protection mechanisms, such as feedback loops.
- Current exposures of the general human or most wildlife populations to many of the chemicals being shown to have endocrine-disrupting potential do not seem to be high enough to be causing widespread endocrine system disruption, but work is continuing to confirm this observation.
- Owing to the complex nature of the endocrine system and our limited understanding of some aspects of it, controversy continues as to whether chemicals are likely to have endocrine-disrupting effects at the concentrations present in the environment. Recent studies show that some of these chemicals may be able to cause effects at environmentally relevant doses.
- Specific widespread effects in humans that might be from environmental exposure to chemicals with endocrine-disrupting potential have not been demonstrated unequivocally.

The Committee on Environment and Natural Resources (CENR) of the USA Government’s National Science and Technology Council made the following comments, which support the conclusions reached above, in a report on research articles, conference proceedings and information available from reputable sources on the Internet have provided the basis for this review.

‘While exposure to high concentrations of chemicals such as DDT, polychlorinated biphenyls, diethylstilbestrol and PCDDs can clearly induce adverse effects, whether similar effects occur in response to low level ambient exposures in the general human population or among wildlife species is unknown. At present, scientific knowledge is inadequate to fully inform public policy and a [US] government-wide coordinated research effort that addresses the key scientific uncertainties related to the adverse effects of endocrine-disrupting chemicals is needed.’

‘Because of the endocrine system’s critical roles in normal growth and development and in reproduction, even small disturbances in endocrine function have the potential to exert profound and lasting effects’ (CENR 1998).

Strategies to direct research into areas where information is lacking are being pursued vigorously in the USA and Europe, especially in the area of effects in humans. The USA Government has provided $US30-50 million to fund research. The CENR report noted above stated that to properly fill the current knowledge gaps to enable appropriate risk assessment would take even greater funding, totalling some $US50-75 million over the next three to five years (CENR 1998). Chemical manufacturers are also investing significant amounts to gather the knowledge necessary to support decision-making. The general international consensus is that there is still much uncertainty about whether widespread effects are occurring but that it is possible to cause effects by disrupting the endocrine system.

A number of extensive reviews of the available literature have now been completed. The USA National Academy of Science sponsored one that was completed in 1999 after numerous delays. It also supports the views listed above. The panel of experts brought together to undertake this review found it difficult to reach conclusions about much of the evidence. The differences in opinion resulted in part from differing views on the value of the various types of evidence available. This highlights how the controversy about this issue has arisen. The review agreed on the following:

- Disruption of the endocrine system can occur through numerous mechanisms, not just interaction with receptors, and more research is required into these indirect or secondary effects.
- Exposure to a range of chemicals with endocrine-disrupting potential has resulted in adverse reproductive, developmental, neurological and immunological effects in people and wildlife and in laboratory studies. Whether this is due to the chemicals disrupting the endocrine system or because of other effects is still to be determined in most cases.
- The evidence to date does not strongly support the carcinogenic effects of these chemicals, especially with relation to endocrine-related tissues or glands, but future studies need to be carefully designed because of the long latency before disease develops, and because exposures usually occur to mixtures of these chemicals.
- Determining exposures to these chemicals is difficult, as they are not usually routinely monitored, and potential exposures can be complicated by natural hormones (steroids and phytooestrogens) and hormonal drugs. This adds to the difficulty in determining whether effects might be occurring (National Research Council 1999).
The WHO Global Assessment of the State of the Science of Endocrine Disrupters was released in 2002. Again, the conclusion reached by the numerous authors was that:

- This state-of-the-science assessment has revealed that our current understanding of the effects posed by EDCs to wildlife and humans is incomplete.
- The evidence that high-level exposure may impact both humans and wildlife indicates that this potential mechanism of toxicity warrants our attention.
- Uncertainty over the possible effects of chronic, low-level exposures to a number of chemicals with endocrine disrupting potential and the fundamental roles played by the endocrine system in maintaining homeostasis make understanding the potential effects posed by exposure to these chemicals an obvious international priority.
- There is a need to identify life stages and species that are more vulnerable to the effects of EDCs and to understand how this mechanism of toxicity may affect individual populations and communities (Damstra et al. 2002).

Some countries have already decided to take a precautionary approach and limit particular uses of some of the chemicals still in use to minimise impacts on sensitive populations. Some European countries are encouraging industries to consider the use of alternative chemicals wherever this is possible. A number of European countries have now banned the use of phthalates in toys that infants chew. These actions are being required even though there is still much uncertainty as to whether there is any risk to children. In the UK, the wool scouring industry has voluntarily replaced alkylphenol ethoxylate surfactants with surfactants that do not appear to have endocrine-disrupting potential.

In NSW, Australia, many of the chemicals that are classified as pesticides have already been withdrawn from the market; had their use restricted or were never registered for use in Australia. Pesticides including aldrin, dieldrin, heptachlor, dichlorvos, trifluralin, demeton-S-methyl, permethrin, chlordane, chlorfenvinphos, endrin, toxaphene, kepone, mirex, hexachlorobenzene, acetochlor, nitrofen, DDT, alachlor, maneb, vinclozolin and sodium metam fall into these categories. TBT compounds have also had their use restricted and can now, for example, only be used in NSW on vessels over 25 metres in length. PCBs can no longer be imported and their use is being phased out.

Based on this review, however, there are a number of areas where scientific information is limited that are particularly relevant for Australia. These areas include:

- the potential sensitivity of marsupials and monotremes to these effects
- natural hormone levels in sewage effluents discharged to rivers in Australia, particularly where dilution is minimal.

Other knowledge gaps described earlier in this review are likely to be filled by the research programs under way worldwide.

A wide range of chemicals may have the potential to disrupt the endocrine system. Many of them have already been banned from use for other reasons. Others are still in use. Owing to the uncertainty still surrounding how much of a chemical is necessary to cause effects, further research is required to allow determination of the best management approach. Many of these chemicals have a wide range of beneficial uses, and the risk of effects will need to be weighed against the risk of losing the benefits.

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SOME USEFUL WEB SITES

WHO State of the Science Assessment on Endocrine Disruptors
http://www.who.int/pcs/emerg_site/edc/global_edc_TOC.htm

US EPA EDC Sites
http://www.epa.gov/scipolicy/oscpendo/
http://www.epa.gov/edocrine
http://www.epa.gov/ORD/NRMRL/EDC/

International Program on Chemical Safety (WHO) EDC Site
http://www.who.int/pcs/emerg_main.html

EU EDC Site
http://europa.eu.int/comm/environment/edocrine/index_en.htm

OECD EDC Sites
http://www.oecd.org/document/62/0,2340,en_2649_34377_2348606_1_1_1_1,00.html
http://www.oecd.org/document/63/0,2340,en_2649_34377_2350207_1_1_1_1,00.html

UK Environment Agency EDC Sites
http://www.environment-agency.gov.uk/yourenv/issues/main/?lang=en&region=andprojectstatus=andtheme=and
subject=andsearchfor=endocrineandtopic=andareaa=andmonth=
http://www.environment-agency.gov.uk/commndata/105385/139909

Ministry of the Environment Japan EDC Site

Australian Government EDC Sites

USA Geological Survey EDC Site
http://www.cerc.usgs.gov/Other_Webs/endocrine/summary.htm

Global EDC Research Inventory
http://oaopub.epa.gov/edocrine/pack_edri.all_page

NIEHS Centre for the Evaluation of Risks to Human Reproduction
http://cerhr.niehs.nih.gov/

NTP EDC Low Dose Peer Review Report

Our Stolen Future EDC Site
http://www.ourstolenfuture.org/index.htm

European Chemical Industry Council EDC Site
http://www.cefic.org/lri/Templates/shwStory.asp?NID=34andHID=411andPHID=408

Tulane University – an educational service about endocrine disruptors
http://www.tmc.tulane.edu/cbr/ECME/EEHome/default.html

WWF EDC Site
http://www.worldwildlife.org/toxics/progareas/ed/index.htm

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