POISONING THE FUTURE

Impacts of Endocrine -Disrupting Chemicals on Wildlife and Human Health

GREENPEACE

september, 1997

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SUMMARY
Thousands of man-made chemicals have been released into the environment in vast quantities since the chemical industry began to boom in the 1950s. This has brought many, often unforeseeable, problems for the environment. Only very recently evidence of a potentially huge threat has been unfolding - that of endocrine disruption – the disruption of hormone systems in wildlife and humans by man-made chemicals.

In 1991, scientists hypothesised that endocrine-disruption could be the cause of declines in the populations of many wildlife species which have occurred over the past 50 years. Effects in wildlife which are suspected to result from exposure to endocrine-disrupting chemicals, include adverse effects on the development of young, reproductive problems and weakened immune systems. Human health could also be affected. The ability of these chemicals to interfere with hormone systems has potentially implicated them in decreasing sperm counts, increases in reproductive problems, reduced intellectual capacity and behavioural problems. Many of the effects appear to be caused by disturbing development of the embryo or foetus, either in the womb of mammals and humans or in the eggs of egg-laying animals. This can occur because the chemicals are passed on from a mother's body to the next generation.

It is imperative that precautionary action on endocrine-disrupting chemicals is taken now to safeguard the future. These chemicals could already pose a long-term threat to world biodiversity and to human society. Many scientists have expressed great concern about endocrine-disruption. At a meeting in Erice, Sicily, in 1995, scientists were certain that:

"A trivial amount of government resources is devoted to monitoring environmental chemicals and health effects. The public is unaware of this and believes that they are adequately protected. The message that endocrine disrupters are present in the environment and have the potential to affect many people over a lifespan has not effectively reached the general public, the scientific community, regulators or policy makers. Although this message is difficult to reduce to simple statements without over- or understating the problem, the potential risks to human health are so widespread and far-reaching that any policy based on continued ignorance of the facts would be unconscionable".

Endocrine-Disrupting Chemicals - A Global Problem

Over 50 chemicals, most of which are still in commercial use, have been identified as endocrine disrupters. However, those identified to date may only represent the tip of the iceberg. Of the thousands of synthetic chemicals which are currently produced, only a tiny fraction have been tested for their ability to disrupt hormones, since current regulation does not require such testing.

Chemicals which vary widely in their structure and which have numerous different uses have been identified as endocrine disrupters. They include chemicals in the following categories:
* **plastics:** Chemicals used in PVC and in the production of other plastics including certain phthalate plasticisers.
***organohalogen chemicals:** Particularly those containing chlorine (organochlorines), including PCBs, dioxins and many pesticides.
***pesticides:** a variety of different pesticides used in agriculture.

*** industrial chemicals:** various industrial chemicals, including alkylphenols which are derived from chemicals used in detergents and other products.

Many endocrine disrupting chemicals have become widespread contaminants across the globe. This is not only due to their vast usage on a worldwide scale, but also because some can be transported for thousands of kilometres on air currents. Many are persistent in the environment, taking years to degrade. Some persistent chemicals now pollute polar regions where they have never even been used.

Even low environmental levels of many endocrine-disrupting chemicals can lead to high levels in the body tissues of animals and humans. This is because many endocrine disrupters, most notably more persistent chemicals, become stored in fatty tissues. They build up to higher levels in fat as more of a chemical is taken in. For many, the levels in fat increase as one animal eats another, so that the highest levels are found in predator animals at the top of food webs.

Exposure to Endocrine Disrupters is Unavoidable

For the general population, the greatest exposure to endocrine-disrupting chemicals is from food intake. Since many are fat soluble, the highest levels are present in meat, fish and dairy products. Exposure may also come from pesticide residues remaining in fruit and vegetables, and from low levels in drinking water. Food packaging has been reported to contaminate food with endocrine-disrupters. Bisphenol-A is used in the lining of some food cans, and phthalates have been found in some food wrappers. Recent concern has also focused on PVC toys which could potentially expose children to certain hazardous chemicals including some endocrine-disrupters.

The developing young are directly exposed to endocrine-disrupting chemicals. Chemicals which are circulating in the maternal body may be passed directly to the eggs of egg laying animals, or through the placenta to the developing foetus in the womb in mammals, and through breastmilk to the nursing young. It is almost certain that every pregnant woman has endocrine-disrupting chemicals in her body that are transferred to the foetus in the womb, and are present in her breastmilk. It is thought that body fat is mobilised before pregancy and lactation, releasing persistent chemicals stored in the fat. This means that a proportion of the chemicals which have accumulated in a woman's body during her whole lifetime may be passed to her child.

Endocrine Disruption

There are profound interconnections between many body systems and the hormone (endocrine) system, including the nervous, reproductive and immune system. Hormones act like chemical messengers, providing communication between different parts of the body and regulating many body functions. For their messages to be interpreted, hormones bind to specific sites in cells called receptors, following which a particular biological effect will be triggered.

Endocrine-disrupting chemicals may upset the balance of hormones in the body and thereby disturb the regulation of body functions. Chemicals may interfere with the balance of hormones in a number of ways. They may bind to hormone receptors and consequently mimic or block the actions of hormones, alter the natural production and breakdown of hormones, or interfere with hormonal control by the brain.

It is particularly thyroid hormones and steroid hormones which are affected by endocrine disrupters. Steroid hormones include the male sex hormone testosterone, the female sex hormone estrogen, and other hormones which are produced by the brain and adrenal glands. To date, most research has focused on "estrogenic" chemicals which mimic the hormone estrogen. Less is known about effects on other hormones.

The developing young are the most vulnerable. Steroid and thyroid hormones play a major role in regulating the development of organs and tissues in the foetus and developing young. For instance, sex hormones are involved in controlling the development of the reproductive system, and thyroid hormones are important in regulating development of the nervous system. The foetus is exquisitely sensitive to changes in hormone levels. Even a single disturbance at a critical time when a body system is developing can cause irreversible changes, which in turn, can lead to permanent health effects in the young or later in life. Exposure to endocrine-disrupting chemicals during development may therefore lead to permanent effects on health. Since hormones which are affected by endocrine disrupters, and the processes of development which they regulate, are very similar in all vertebrates, these chemicals may cause effects in animals and humans alike.

The greatest effects of endocrine-disrupters can occur at the lowest doses. For many chemicals, increasing the dose will increase the toxic effect it causes. However, for endocrine-disrupters, lower rather than higher doses can have the most effect.

Health Effects of Endocrine Disrupters

Health effects which have been associated with exposure to endocrine-disrupting chemicals in the egg or in the womb, often represent diminished potential - a loss of health or competency. Such effects, including reduced fertility, reduced intellectual capacity or weakened immune systems, may only be subtle. They may not directly threaten the existence of an individual. However, considered at a population level, these sorts of effects could destabilise wildlife populations and change the whole character of human society.

Many different adverse effects in numerous wildlife species have been associated with exposure to endocrine-disrupting chemicals. The mechanism by which these effects have occurred is suspected to be endocrine-disruption, although this is not certain in most cases. The effects in wildlife have been recorded over the past 50 years, with many still becoming evident today. They include decreased hatching success of eggs in fish, birds and turtles, feminisation of male fish, embryo death and deformities in birds, reproductive problems in reptiles, birds, and mammals, and altered immune systems in marine mammals. In many cases these effects have lead to population declines.

Exposure to endocrine-disrupting chemicals may be linked to increases in reproductive disorders in humans. It is known that exposure in the womb to a man-made synthetic estrogen drug causes a range of reproductive problems in humans and in laboratory animals. Studies on animals also show that exposure to estrogenic chemicals and other endocrine disrupting chemicals cause very similar effects. From such evidence it has been suggested that exposure to endocrine-disrupting chemicals could be partly or wholly responsible for increases in the incidence of male reproductive disorders which have been recorded in many countries over the past 20-50 years. These effects include, reduced sperm count, an increased incidence of testicular maldescent, urethral abnormalities, testicular cancer and prostate cancer.

Exposure to estrogenic chemicals in the womb may also be causing earlier puberty in girls. Research indicates that exposure to some endocrine disrupters throughout life could be associated with increases in the incidence of breast cancer and endometriosis, and a shorter duration of lactation in women.

Studies on the general population suggest that exposure in the womb to PCBs and /or dioxins can reduce intellectual capacity and alter immune systems. Studies indicate that the levels of PCBs and dioxins present in body tissues in some women of the general population are sufficient to cause subtle effects on the nervous and immune systems of their children. Effects on the nervous system include slightly reduced IQ, attention deficits, poorer memory and slight adverse effects on psychomotor and neurological function. Whether such effects on the nervous and immune systems are caused by endocrine-disrupting mechanisms is uncertain.

Prospects for the Future

The fact that many of the effects of endocrine-disrupting chemicals impact on the next generation means that we may only see the consequences many years after exposure. It is not possible to predict what the consequences for our children and grandchildren will be if the production of endocrine-disrupting chemicals continues. Nevertheless, there is a potential for severe widespread impacts on wildlife, human health and human society. Global action of a precautionary nature is needed now to safeguard the future. These chemicals should be phased out and safer alternatives implemented where available. Industry must pursue clean production technologies with the aim of preventing further releases of these chemicals into the environment. This is not an extreme viewpoint or an impossible task. The goal to phase out toxic, persistent and bioaccumulative chemicals, some of which are endocrine disrupters, has already been embraced at several international government conventions. What is needed is implementation of these agreements and their extension to include endocrine disruption as a recognised hazard in its own right.

1. INTRODUCTION

Since the late 1950s to the present day, many dramatic declines in wildlife populations, caused by reproductive failure and problems with the development of young, have been associated with exposure to man-made chemicals. Yet it was not until 1991, that scientists realised that a common thread could link these problems in wildlife. Many of the observed effects were synonymous with what would be expected from disruption of the body's hormones. At that time, a meeting of expert scientists proposed a hypothesis that exposure to chemicals which disrupt hormones, endocrine-disrupting chemicals, could be to blame for the detrimental effects on health and declines of numerous wildlife species (Wingspread 1991).

The adverse effects in wildlife included decreased hatching success in fish, birds and turtles, reproductive abnormalities and decreased fertility in fish, birds, reptiles and mammals, behavioural abnormalities in birds, and compromised immune systems in birds and mammals. Such problems were not only recorded in species inhabiting heavily polluted areas, such as in the area of the US and Canadian Great Lakes, but were also evident in wildlife in many other regions of the world (Wingspread 1991, Colborn *et al.* 1993).

A high proportion of the adverse effects in wildlife, suspected to be caused by endocrine-disrupting chemicals, result not from their impact on adult animals but on the developing young. These chemicals are transferred from the mother's body to the egg, or to the developing foetus in the womb, and via mothers' milk to newborns. Research has shown that hormones are crucial for regulating the development of all animals which have backbones. By disturbing the delicate balance of hormones during development, endocrine-disrupting chemicals may permanently change the course of development, leading to a wide range of adverse effects. The reproductive, nervous and immune systems appear to be particularly vulnerable to these effects on the next generation.

In 1962, Rachel Carson warned of the dangers of man-made chemicals to the environment and to humans in her book, Silent Spring, concluding, "Our fate is connected with the animals". In 1993, after reviewing both human and wildlife data on endocrine-disrupters, scientists similarly proposed that wildlife could be acting like "sentinels", or mirrors to health effects which could also occur in humans (Wingspread 1993). Presently, it is only hypothesis rather than fact that endocrine-disrupting chemicals are affecting human health, but evidence is mounting. Research suggests that the chemicals may be implicated in the rise of several reproductive disorders in humans over the past few decades, including reduced sperm counts and increased breast cancer. They may also be associated with reduced intellectual capacity and behavioural problems. Like the effects in wildlife, many effects in humans appear to be on the next generation, although adults may be also be affected (Colborn *et al.* 1993, US EPA 1997).

There is already evidence which suggests that some endocrine-disrupting chemicals have reached levels in the environment where they could cause adverse effects on development in humans and wildlife. Effects on development are often not gross, but instead represent diminished potential - a loss of health and competency, such as reduced fertility, reduced intellectual capacity and weakened immune systems. These sorts of effects may not obviously threaten the existence of an individual but, considered at a population level, they could change the whole character of human society or destabilise wildlife populations. There is now great concern among many scientists that endocrine-disrupting chemicals could pose a long-term threat to world biodiversity and to human society (Alleva *et al.* 1995).

2. IDENTIFICATION OF ENDOCRINE-DISRUPTING CHEMICALS

2.1 The Chemicals

Chemicals that have very different structures and all kinds of different uses have been identified as being able to disrupt hormones. They include (1) chemicals used in PVC and other plastics production, including certain phthalate plasticisers (2) a variety of pesticides; (3) organohalogen chemicals, particularly those containing chlorine (organochlorines), such as PCBs, dioxins and many pesticides, and (4) various industrial chemicals including alkylphenols which are derived from chemicals used in detergents and other products, and bisphenol A for which the main uses are epoxy resins and polycarbonate plastics.

A list of known and suspected endocrine-disrupting chemicals is given in Table 1. Over 50 endocrine-disrupting chemicals have been identified but there could be many more. Conservative estimates suggest there are about 63,000 man-made chemicals in common use worldwide, and that 200 to 1000 new chemicals enter the market each year, (Shane 1994). Presently only a tiny fraction have been tested for their ability to disrupt hormones.

2.2 Identification

Endocrine-disrupting chemicals vary widely in their chemical structure. It is unlikely that the ability of a chemical to disrupt hormones will ever be predictable from structure alone (McLaclan 1993, Katzenellenbogen 1995). Scientific studies which have identified these chemicals have relied on using "in vitro" tests (cell culture) and "in vivo" (laboratory animals) tests. Current regulatory systems do not require chemicals to be screened for their ability to disrupt hormones, or tested for health effects resulting from endocrine disruption, although this is now under discussion (see section 15.1).

To date, most testing for endocrine disrupters has focused on "estrogenic" chemicals which mimic the female sex hormone estrogen. Many chemicals have been designated as being estrogenic based on laboratory tests which use animal or human cells grown in culture. Cell culture tests are now available which can identify whether a chemical is likely to be estrogenic in different kinds of animals. For example, a test has been developed which uses fish cells to find out whether chemicals which pollute the aquatic environment would be estrogenic to fish (Sumpter and Jobling 1995). A test using human cells has also been developed to predict whether a chemical would be likely to be estrogenic in humans (Soto *et al.* 1992, 1995). Over 30 chemicals have now been identified as being estrogenic using cell culture tests (eg. Soto *et al.* 1995). In addition, several chemicals have also been identified which interfere with male sex hormones known as anti-androgenic chemicals (Kelce *et al.* 1995, Danzo 1997). Some endocrine-disrupting chemicals, such as DDT, have also been identified by the sorts of adverse health effects they cause when tested in laboratory animals (Bustos *et al.* 1988).

Note that estrogenic and anti-androgenic effects represent only a subset of the potential effects of known or suspected endocrine disrupters, as sex hormones are only one component of the endocrine system. Testing for other effects has been extremely limited to date.

3. ENDOCRINE-DISRUPTING CHEMICALS IN THE ENVIRONMENT

Once discharged into the environment, endocrine-disrupting chemicals do not always remain close to where they are released, but instead may be transported over long distances in air currents or by water. Coupled with their immense usage on a worldwide scale, this means that, in many cases, endocrine-disrupting chemicals now present a global problem rather than just one of localised contamination. Furthermore, some of the endocrine-disrupting chemicals, in particular the organochlorine pesticides, PCBs and dioxins, are persistent in the environment, taking years or even decades to degrade.

3.1 Transport and Distribution of Endocrine-Disrupting Chemicals in the Environment

Chemicals can become airborne either by their direct emission to air, such as from factory or incinerator stacks, or by evaporation from the ground, water, or the leaves of sprayed crops. Once airborne, they may be transported for thousands of kilometres in the atmosphere before condensing and falling once more to the earth's surface.

Research has shown that many persistent organochlorines, appear to be carried on air currents from warmer regions of the globe towards polar regions where they are subsequently deposited. This "global fractionation" process is thought to have led to the high concentrations of these chemicals which are now found in Arctic and Antarctic regions, where they have never even been used (Iwata *et al.* 1993, Wania and Mackay 1996).

Some endocrine disrupters are now very widespread across the globe. These include several persistent organochlorine pesticides, PCBs, dioxins and phthalates (Loganthan and Kannan 1994, Jobling *et al.* 1995). In addition, levels of lead, mercury and cadmium have become elevated, even in remote areas, as a result of man's activities (Pacyna 1997). Although many of the persistent organochlorine pesticides are now banned in Europe, their input into the environment continues in some developing countries where they remain in use, often in a widespread and poorly unregulated fashion (Iwata 1994, Wania and Mackay 1996). PCBs are no longer manufactured intentionally but, due to their persistent nature and continued input into the environment from wastedumps, there is not a global downward trend in their levels. It is estimated that about 31% of the PCBs which were manufactured have entered the environment, but more than double this amount still remain in use in older electrical equipment or is present in dumps and landfill sites or in storage (Tanabe 1988). This means that PCBs could continue to enter the environment in large quantities in the future if measures are not taken to prevent this. In addition, PCBs and dioxins continue to be generated as by-products of some chemical and combustion processes. Other known or suspected endocrine disrupters, including many modern pesticides, industrial chemicals and additives in consumer products, remain in widespread use globally.

3.2 Endocrine-Disrupting Chemicals in the Food Web

Most endocrine-disrupting chemicals which are persistent, such as the dioxins, PCBs, organochlorine pesticides and alkylphenols, are also soluble in fats. As a consequence of this, and because of the way that animals and humans deal with these toxic substances once they enter the body, they can become stored in fatty tissues. The levels of chemicals build up or "bioaccumulate", in the animal's fat as more of the chemical is taken in (Hall 1992, Warhurst 1995). For many of these chemicals, the levels in fat increase as one animal eats another, so that the highest levels are found in predator animals at the top of food webs, for example, humans, seals, dolphins and fish-eating birds.

Even low environmental levels of chemicals which bioaccumulate can lead to very high levels in the body tissues of animals. For example, in a food web in Lake Ontario, small aquatic organisms such as algae, accumulate PCBs at concentrations hundreds of times higher than levels in the surrounding water. Fish which eat the algae accumulate still higher levels, and predatory birds like herring gulls at the top of the food web which eat fish accrue the highest levels. The levels in their eggs can be 25 million times greater than the original level in the water (Colborn *et al.* 1990).

When persistent chemicals are stored in fatty tissue they are only very gradually released and excreted from the body. For example, it is estimated that the half life for residence of PCBs in the human body is 12 years, which means that after one single exposure to PCBs it would take 12 years for just half of it to be excreted from the body. However, as we are normally exposed to these chemicals at each meal , body levels inevitably build up (Hall 1992).

4. HUMAN EXPOSURE TO ENDOCRINE-DISRUPTING CHEMICALS

Exposure to man-made endocrine-disrupting chemicals is presently unavoidable because of their widespread presence in the environment. For the general population, by far the largest exposure comes from foods that are contaminated with these chemicals. As a result of the widespread occurrence of many endocrine-disrupting chemicals in the environment, most foodstuffs are contaminated by some of them. Since many of the chemicals are soluble in fat and/or bioaccumulate, the highest levels are found in meat, fish and dairy products. It has been estimated for the persistent organochlorine chemicals for example, that about 80% of our intake of these chemicals come from these foodstuffs (Hall 1992). Some individuals may also be exposed to endocrine disrupters as a result of handling chemicals at work.

4.1 Pesticide Residues in Food and Drinking Water

Aside from chemicals that have accumulated in foods, other dietary exposure may come from pesticide residues which remain on sprayed crops and contaminate drinking water. Spraying pesticides on crops means that a proportion of them may remain on the crops by the time they are harvested. A review of pesticides in the US reported that fruit and vegetable crops receive most of the pesticides applied to agricultural crops, and the contamination rate with pesticides is higher for fruits than for any other commodities (Culliney *et al.* 1992).

In The Netherlands, residues of several endocrine-disrupting pesticides were found to be present in foods that were tested, including carbaryl, dicofol, endosulfan, lindane and vinclozolin (Health Inspector for Public Health, The Netherlands, 1995). Another study revealed that 33.2% of fruit and vegetables produced in the Netherlands, and 59.4% of imported foods, contained detectable levels of pesticide residues (van Klaveren 1997).

Pesticides can leach from sprayed land into watercourses which are used for drinking water supplies. Monitoring in The Netherlands has shown that the most commonly detected pesticides in surface waters include several endocrine disrupters, namely organochlorine insecticides lindane, endosulfan and dieldrin, and the fungicide vinclozolin. In the Netherlands and most European countries, the safety limit for the total amount of pesticides in drinking water is sometimes exceeded (HMSO 1995).

4.2 Food Packaging and Processing

Some endocrine-disrupting chemicals are used in the production of food packaging materials. Several studies have shown that these chemicals can leach out of packaging into food they are in contact with.

Bisphenol-A, an estrogenic chemical, is present in lacquer coatings which are used to line the inside of some food cans. Tests on tins of peas, artichokes, green beans, mixed vegetables, corn and mushrooms found the liquid surrounding vegetables in food cans had estrogenic properties due to bisphenol-A. (Brotons *et al.* 1995). The amount of this chemical found in liquid from cans (highest level 80ug/kg) was well within the EC safety limit (3 mg/kg). However, this was set before it was known that bisphenol-A was estrogenic and, as such, cannot be regarded as protecting the health of the public. Bisphenol-A is also present in polycarbonate plastic which is widely used for packaging of food and drinks. Whether it leaches from this plastic into foodstuffs is yet to be tested (Feldman and Krishnan 1995).

Research indicates that some phthalates, including DBP and BBP which are are estrogenic, are present in foods from general environmental contamination (MAFF 1995, MAFF 1996). However, DBP has also been found in printing inks used in plastic food packaging and on paper and board packaging (Nerin 1993, MAFF 1995). Studies show that DBP can migrate from the packaging into a wide variety of foods it is in contact with (Nerin 1993, MAFF 1995).

4.3 PVC Toys

Some children's toys are made from soft PVC plastic which contain phthalate plasticisers (Meek and Chan 1994, Vinkelsoe *et al.* 1997). A recent study conducted by the Danish Environment Protection Agency, found that a number of phthalates could leach from three brands of teethers, which could potentially expose infants to these chemicals. The phthalates included BBP and DBP which are estrogenic. In addition, another estrogenic chemical, nonylphenol, was found to leach from the teethers (Vinkelsoe *et al.* 1997). As a result of the study, the manufacturer of the teethers withdrew these products from sale in Denmark, Spain, Greece and Italy.

Two major retailers in Denmark and Sweden reacted by withdrawing soft PVC toys from their shelves (ENDS 1997a). In The Netherlands, one of the main suppliers and importers of PVC toys with almost 50% of the market, has decided to stop selling PVC toys to children under 3, and has requested suppliers not to use PVC toys for children older than 3 years.

Recent research by Harris *et al.* (1997) has demonstrated that the isomeric phthalate DINP (diisononyl phthalate), commonly used at high concentrations in consumer products such as PVC toys, can also show weak estrogenic activity *in vitro* with human breast cancer cell lines. DEP (diethyl phthalate) also showed some activity, although this phthalate appears to be less widely used. In addition the authors note that there was little, if any, relationship between molecular structure and estrogenic activity, even within the phthalates as a chemical class.

5. EXPOSURE OF THE DEVELOPING YOUNG TO ENDOCRINE-DISRUPTING CHEMICALS

Prior to egg laying in fish, amphibians, reptiles and birds, and during pregnancy and lactation in mammals and humans, body fat is mobilised. This may release persistent chemicals present in the fat. Such chemicals, as well as others circulating in the maternal body, may be passed directly to the egg in egg-laying animals, or passed through the placenta to the developing foetus in the womb in mammals, and through breastmilk to the nursing young. The developing stages of life may, therefore, be directly exposed to endocrine-disrupting chemicals (Colborn *et al.* 1993).

Scientific experts at a meeting in Erice, Sicily (Alleva 1995), were certain that: "Gestational exposure to persistent man-made chemicals reflects the lifetime of exposure of females before they become pregnant. Hence, the transfer of contaminants to the developing embryo and foetus during pregnancy and to the newborn during lactation is not simply a function of recent maternal exposure".

5.1 Exposure in the Womb

The placenta, which connects the developing foetus in the womb to its mother, does not act like a barrier and will not protect the foetus from toxic substances circulating in the mother's body. (Hall 1992). For instance, it is common knowledge that smoking cigarettes during pregnancy can cause detrimental effects to the unborn child, such as retarding growth. Another example is the tragic effects which occurred on the development of limbs following the administration of the pharmaceutical drug thalidomide to pregnant women (Michal *et al.* 1993).

It is now known that many chemicals and pharmaceutical drugs can pass directly across the placenta to the developing foetus (Sullivan 1993). Endocrine-disrupting chemicals are no exception to the rule and many could be passed to the foetus in this way. Those known to cross the placenta include DDT, DDE, hexachlorobenzene, lead, methylmercury, PCBs and dioxins, and phthalates (Ando *et al.* 1986, Kanja *et al.* 1992, Koopman-Esseboom *et al.* 1994, Rice 1995, Howard *et al.* 1997 *in prep*).

Scientific experts at a meeting in Erice, Sicily (Alleva et. al. 1995), estimated with confidence that: *"Every pregnant woman in the world has endocrine disrupters in her body that are transferred to the foetus. She also has measurable concentrations of endocrine disrupters in her milk that are transferred to the infant".*

5.2 Exposure of the Nursing Young

Chemicals in a pregnant woman's body which circulate in her blood may contaminate her breast milk and pass to her nursing infant. Endocrine-disrupting chemicals which are often found in human breast milk (in parts per million or trillion levels) include PCBs, dioxins and the organochlorine pesticides DDT, DDE, dieldrin, hexachlorobenzene, hexachlorocyclohexane and chlordane (Galentin-Smith *et al.* 1990, Skaare and Polder 1990, Thomas and Colborn 1992, Stevens *et al.* 1993, Furst *et al.* 1994).

Persistent chemicals which have accumulated in a woman's body during her lifetime decrease in the mother's body as they pass to her breastmilk, and hence to her nursing infant. It has been estimated that if a child is breastfed for one year, it will accumulate 4-12% of the total amount of dioxins which it would be expected to accumulate during its whole lifetime in just its first year (US EPA 1994). Other estimates suggest that for dioxins and PCBs which are known to bioaccumulate, a woman reduces her body levels of these chemicals by over 50%,by breast feeding for 6 months. Most of this will pass to her baby via breastmilk (Lindstrom *et al.* 1994).

There has been concern among some scientists regarding the levels of certain persistent hormone-disrupting chemicals in breast milk, in particular PCBs and dioxins. However, breast-feeding is known to convey very important benefits to the overall health and development of infants. Consequently, despite the presence of these chemicals in breastmilk, health authorities still encourage and recommend breastfeeding (eg. WHO 1996, MAFF 1997).

6. LEVELS OF ENDOCRINE-DISRUPTING CHEMICALS IN THE GENERAL POPULATION

Persistent endocrine-disrupting chemicals which bioaccumulate in body fat are only excreted from the body at a very slow rate, and with continued exposure, their levels progressively build up over a lifetime. Mobilisation of stored contaminants from fat tissue only appears to take place during lactation and starvation, and as a result of disease (Thomas and Colborn 1992).

Some endocrine disrupters, such as phthalates, do not become stored in fat because they are more readily broken down and excreted from the body. However, people are continually exposed to these chemicals because they are ubiquitous in the environment. In addition, research by Dirven *et al.* (1993) indicated that, following occupational exposure, phthalates may persist in the human body for longer than previously assumed.

Levels of endocrine-disrupting chemicals in people's bodies do vary geographically. For instance, the general population of industrialised nations have higher body levels of industry-related chemicals, like PCBs and dioxins, than people in non-industrialised countries (Schecter 1996). Some populations have particularly high body levels of endocrine-disrupters due to a subsistence diet of fish and/or sea mammals. For example, in many parts of the Arctic, a reliance on marine food which contains high levels of PCBs, dioxins, methylmercury and organochlorine pesticides, has led to elevated exposure in indigenous communities (Kuhnlein *et al.* 1995, Gilman *et al.* 1997). For example, levels of organochlorines in women's breastmilk in Inuit communities of Arctic Quebec, Canada, were 3 to 7 times higher than in other parts of the province (Dewailly *et al.* 1994).

Localised chemical use can also be problematic. For instance DDT is still used in sanitation campaigns against malaria in Mexico. Levels of DDT in the general population and in workers are high, and it has been suggested that infants could be exposed to potentially deleterious levels through breastmilk. This research has generated much concern and calls for alternative measures to be taken (Lopez-Carrillio *et al.* 1996, Rivero-Rodriguez *et al.* 1997, Marien 1997).

Banning the use of persistent chemicals, such as DDT, has led to a gradual reduction in levels present in breastmilk in some European countries (Thomas and Colborn 1992). However, a study in Germany reported that following bans, DDE and PCBs levels had initially decreased but levels have since become more stable (Furst *et al.* 1994). With better technology and tighter restrictions on the release of dioxins into the environment, a recent European survey showed that levels in breastmilk were no longer increasing and had decreased in some countries (WHO 1996).

7. HOW ENDOCRINE-DISRUPTING CHEMICALS CAUSE HEALTH EFFECTS

7.1 Hormones in the Body - The Endocrine System

Hormones are natural chemicals which are produced by the body and are normally effective at very low concentrations. They act as chemical messengers, travelling in the blood and sending signals to cells to provide a means of communication between different parts of the body. The hormone system, is known as the endocrine system, because it is the endocrine glands which produce hormones.

Hormones are essential for maintaining the proper functioning of the body, being involved, for instance, in regulating reproductive functions, general body growth and metabolism, muscle and nervous system functions. During the early stages of life, in the embryo/foetus and developing young, hormones play a major role in the controlling the development of many tissues and organs, including the reproductive, immune and nervous systems.

There are several endocrine glands in the body which produce hormones. These include the gonads - testicles in men and ovaries in women, the thyroid gland in the throat, the pituitary gland in the brain, the pancreas in the abdomen and the adrenal glands. Each gland makes specific hormones and releases them into the blood. For example, the sex organs produce the female sex hormone estrogen and the male sex hormone testosterone, which control reproductive function, whilst the thyroid gland makes thyroid hormones, which regulates the use of food and body growth (Colborn *et al.* 1993, Colborn 1996, EPA 1997).

Hormones travel from their point of release in the bloodstream to particular tissues where they convey their messages. For the messages to be interpreted, hormones bind to special sites in cells called receptors. A hormone and receptor have a precise fit, like a lock and key, so that only a specific type of hormone can bind to a specific sort of receptor. For instance, estrogen can only bind to estrogen receptors to convey its messages. Once a certain number of connections between the hormones and their receptors have been formed, a particular biological effect in cells will be triggered (EPA 1997). Therefore, by binding to receptors, hormones cause biological effects which can lead to changes in the functioning of cells, tissues and organs. Although hormones are generally effective at low concentrations, they can affect major changes in organ or body function.

7.2 Hormones Which Are Affected by Endocrine Disrupters.

Steroid hormones include the male sex hormone testosterone, female sex hormones estrogen and progesterone, and various hormones produced by the pituitary and adrenal glands. These hormones, together with thyroid hormones, are not only found in humans but are produced by all animals which have a backbone - fish, amphibians, reptiles, birds and mammals. In all of these animals and in humans, the structure of these hormones is almost identical and they have very similar functions. It appears that, throughout evolution, these hormones have remained the same, being a successful way of regulating bodily processes in animals and humans alike. It is these steroid hormones and thyroid hormones in particular with which synthetic endocrine-disrupting chemicals are able to interfere (Colborn *et al.* 1993).

7.3 How Endocrine Disrupters Work

Endocrine-disrupting chemicals can interfere with the way in which steroid hormones and thyroid hormones normally work. As a consequence they may upset the delicate balance of hormones in the body, which, in turn, may lead to adverse effects on health.

A definition of an endocrine disrupter proposed by the US Environmental Protection Agency, is "an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour (US EPA 1997). Some of the mechanisms by which man-made endocrine-disrupters can interfere with hormones are outlined below:

* **Hormone Mimicry:** Some chemicals mimic hormones by binding to hormone receptors in cells, and thereby triggering the same biological effect as the hormone (McLachlan 1993). For example, many chemicals are now known which can mimic the female sex hormone estrogen by binding to estrogen receptors in cells. These are the so-called "estrogenic chemicals" because they act like the hormone estrogen (Soto *et al.* 1995).

***Blocking Hormone Receptors:** Some chemicals bind to hormone receptors and block them. This prevents hormones from binding to the receptors and exerting their normal biological effects (McLachlan 1993). A few chemicals are known which can block the male sex hormone, the "androgen", receptor. These include the pesticides vinclozolin and DDE, which is the breakdown product of DDT (Kelce *et al.* 1994 and 1995, Gray *et al.* 1994). These chemicals are known as "anti-androgenic chemicals".

* **Altering Hormone Metabolism:** Some chemicals do not directly interfere with hormones or their receptors, but upset the balance of hormones by interfering with their metabolism, ie., their synthesis or natural breakdown and elimination from the body. For instance, the organochlorine pesticides DDE, atrazine and kepone have been found to alter the metabolism of estrogen (Bradlow *et al.* 1995). Another example of altered hormone metabolism comes from a recent study of flounder fishes in Rotterdam harbour in the Netherlands. Female fish had raised estrogen levels, most likely due to the mixture of chemicals present in the harbour. The chemicals had the effect of impeding the normal processes in the liver which are responsible for the breakdown of estrogen (Janssen *et al.* 1997).

***Affects on Hormonal Control:** The brain and the endocrine system are profoundly interconnected with each other. While the brain regulates hormonal activity, hormones themselves influence brain function, including behaviour, in adults and affect brain development during early life.
The main control centre for the hormone system is the pituitary gland in the brain - which itself produces hormones that tell other endocrine glands whether to increase or decrease their production of hormones. The pituitary receives signals on how to act from another part of the brain, the hypothalamus, which constantly monitors the levels of hormones in the blood.
Some hormone-disrupting chemicals appear to affect the brain's control of the hormone system, either by their direct impact on steroid hormone levels, or indirectly by affecting the activities of the brain in other ways (Colborn *et al.* 1996, EPA 1997).

* **Ah Receptor:** The Ah receptor is not a hormone receptor but another sort of receptor in cells. Some chemicals, notably the dioxins and certain PCBs, can bind to these receptors and in so doing trigger many different biological effects, among which can be the disruption of hormones. It is thought that this is how these chemicals can cause anti-estrogenic effects and alter levels of thyroid hormones (Safe and Krishnan 1995). Besides the dioxins and PCBs, there are other chlorinated and non-chlorinated chemicals which are suspected of being able to bind to the Ah receptor (see table 2), (Giesy *et al.* 1994). These chemicals may therefore also be endocrine-disrupters. Indeed, there is experimental evidence for some, including certain PAHs, that they might be (Santodonato 1997).

Research has shown that while some chemicals may disrupt hormones by one of the above mechanisms, others may cause their effects in a multitude of different ways. Experiments with DDT for instance have clearly shown that it is estrogenic - it can bind to the estrogen receptor and causes adverse effects on health that would be expected for an estrogenic chemical. However, a recent study has shown that it can also bind to the androgen receptor, and so it is possible that it could cause adverse effects from this action too (Danzo 1997). Similarly, the organochlorine pesticides endosulfan and alachlor have been found to bind not only to the estrogen receptor but also to the progesterone receptor (Vonier *et al.* 1996).

Steroid hormones and their receptors are very similar in structure in all animals which have backbones (vertebrates), which would suggest that if a chemical was estrogenic in one species it could also be in another. It is not yet possible to generalise and say that this is the case, but most evidence supports the idea that if a chemical is estrogenic in one species it will be in others (Sumpter and Jobling 1995). Endocrine-disrupting chemicals could therefore have impacts on wildlife and humans alike on a global scale. Creatures without backbones (invertebrates), and plants have different hormones to vertebrates, but they too may be influenced by chemicals which disrupt hormones (US EPA 1997).

7.4 Is endocrine-disruption feasible at current environmental levels of chemicals?

It is accepted that endocrine-disrupting chemicals are generally very weak in comparison to real hormones. When estrogenic chemicals have been tested in cell culture for their ability to bind to hormone receptors, they are 1000 to 1,000,000 times less potent than the hormone estrogen (Soto *et al.* 1995, Harris *et al.* 1997). This implies that it would take 1000 to over 1,000,000 times more of the chemical than estrogen to achieve the same amount of binding to receptors.

Because of the apparent weak potency of the endocrine-disrupting chemicals, it is difficult to see how they could overcome the normal functioning of hormones in the body and cause adverse effects. There are however several reasons which may explain how endocrine-disrupting chemicals could have biological effects:

***Persistence and Bioaccumulation**
Many endocrine-disrupting chemicals build up (bioaccumulate) in the fatty tissues of animals and humans reaching levels which can be much higher than levels in the environment. Many are also persistent, taking a long time to degrade and remain in the body for years. As a result of these properties, some of the chemicals have now reached levels in the bodies of animals and humans which are millions of times higher than the levels of natural hormones in the body (Alleva *et al.* 1995).

*** Chemical Mixtures**
In the real world, humans and animals are exposed, not to individual endocrine-disrupting chemicals, but to complex mixtures of many different ones. Consequently, it is possible that the actions of these chemicals may add up together and cause cumulative effects. By mixing endocrine-disrupting chemicals together and testing them on human cells in culture, studies have revealed that the effects of the chemicals can indeed be additive (Soto *et al.* 1994, Soto *et al.* 1995). One study has found that mixtures of estrogenic chemicals caused effects that were even greater than additive, or ie. synergistic. In this study mixtures of estrogenic chemicals which are known to contaminate alligator eggs in Lake Apopka, Florida, were found to produce synergistic effects when tested on alligator estrogen receptors (Vonier *et al.* 1997).

***Binding Proteins**
Over 90% of natural estrogen circulating in the body becomes bound to special binding proteins in the blood. Only the remaining "free" estrogen is able to diffuse into cells and exert biological effects. However, many endocrine-disrupting chemicals do not bind significantly to the binding proteins, potentially leaving up to 100% of these chemicals circulating in blood free to enter cells and exert effects (vom Saal *et al.* 1995).

***Multiple Pathways of Action.**
Some endocrine-disrupting chemicals may not just exert their effects by one mechanism, such as mimicking estrogen, but may be capable of causing effects through two or more endocrine-disrupting mechanisms. This amplifies their potential for causing hormone-disrupting effects (Danzo 1997).

When the above points are considered, it becomes clear that, even though they are far less potent than normal hormones and are present at low concentrations in the environment, endocrine disrupters could pose a threat to the health of wildlife and humans.

8. HEALTH EFFECTS OF ENDOCRINE-DISRUPTING CHEMICALS

It has been hypothesised that endocrine-disrupting chemicals may be causing adverse effects to wildlife and humans alike. In some cases it is almost certain that detrimental effects in wildlife populations are caused through endocrine disruption. There is also growing evidence that endocrine disrupters could be implicated in human health effects, at levels currently found in the environment.

A critical piece of evidence which showed that the human body could mistake a man-made chemical for a hormone, arises from an unfortunate incident between 1945 and 1971, in which about 5 million women were given a synthetic drug during pregnancy. The drug, diethylstilbestrol (DES), is a man-made estrogen. It caused a range of reproductive and immune system abnormalities in sons and daughters born to these women (Blair 1992, Blair *et al.* 1992, Gray 1992, Toppari *et al.* 1995).

Further evidence has come from direct study of the effects of exposure to chemicals on human health. Most studies which are available on developmental effects of chemicals which have endocrine disrupting properties have examined effects of PCBs and dioxins. Scientific studies on laboratory animals have also provided information. In the context of endocrine-disruption, animal studies are useful as indicators of potential effects in humans because steroid hormones, and the developmental processes they regulate, are extremely similar in animals and humans.

Known and suspected effects of endocrine-disrupting chemicals on the reproductive, nervous and immune systems of wildlife and humans are discussed in the following sections. Many of the problems arise from effects during development in the egg or in the womb.

8.1 The Vulnerable Foetus and Young

Steroid hormones and thyroid hormones play a major role in regulating the development of tissues and organs in the foetus and young. Research has shown that the foetus is exquisitely sensitive to changes in hormone levels. A disturbance in hormone levels at a critical time when a body system is developing can lead to irreversible changes, which, in turn, can lead to permanent health effects in young or later in life (vom Saal *et al.* 1992, Colborn *et al.* 1993).

Since endocrine-disrupting chemicals are present in the eggs of animals, and can pass through the placenta to the developing foetus, or through breastmilk to the young in mammals and humans, they have the potential to disrupt hormones during development and cause permanent effects on health. As a consequence of the extreme sensitivity of a developing foetus, levels of endocrine-disrupting chemicals which cause little problem to the mother may be harmful to the foetus.

Not only is the foetus the most sensitive lifestage to the potential effects from endocrine-disrupting chemicals, it is also the most vulnerable. This is because mechanisms which provide some protection against toxic chemicals in the adult are not fully developed in the foetus.

9. MALE REPRODUCTION

9.1 Increasing Reproductive Disorders in Men

Over the last 50 years or so there has been a dramatic increase in the incidence of several reproductive disorders in men. Studies show that :

*Testicular cancer, a disease most common in men aged 20-45, has increased worldwide, rising by as much as 4-fold in some areas. It is now the most common form of cancer in men in some countries (Giwercman *et al.* 1993, Toppari *et al.* 1995).

*The incidence of testicular maldescent (undecended testicles) appears to have increased in several countries (Toppari *et al.* 1995).

*The incidence of boy's born with urethral abnomalities appears to have increased in many countries (Toppari *et al.* 1995).

*Prostate cancer is on the increase in a number of countries, particularly northern Europe and North America (Santti et. al. 1994).

* In 1992, a report suggested that sperm count had fallen by 50% in 50 years, following an analysis of results from 61 previous studies which were undertaken between 1938 and 1991 in several different countries (Carlsen *et al.* 1992). Subsequent studies carried out in Paris, Belgium and Scotland (UK) have also indicated that sperm counts have declined (Auger *et al.* 1995, Van Waeleghem *et al.* 1996, Irvine *et al.* 1996). These studies reported that sperm count has decreased over the past 20 years at a rate of 2% per year. The year of a man's birth was found to influence sperm count, such that a 20 year old man's sperm count today is lower than at 20 year olds sperm count would have been 10-20 years ago. Furthermore, the studies showed that it was not only sperm count, but other measures of sperm quality which have declined. The percentage of motile sperm in semen has decreased and the numbers of physically abnormal sperm has increased. Preliminary findings of a study in Bangalore, India, also suggest sperm count has decreased in the last 5 years (Mehta and Kumar 1997).

Sperm count does appear to vary geographically and may not be decreasing on a worldwide basis. Two studies in the US, and one in Toulouse, France, found no evidence that sperm count has fallen over the past 20-25 years (Fisch *et al.* 1996, Paulsen *et al.* 1996, Bujan *et al.* 1996).

9.2 Possible Causes of the Increasing Incidence of Male Reproductive Disorders

All of the above disorders of the male reproductive system have increased in many countries over a short time period. It is therefore most likely that they are due to changes in environmental influences or "life-style", rather than to genetic factors (Jensen *et al.* 1995). Based on the following evidence, this has led to the hypothesis that exposure to endocrine-disrupting chemicals in the womb, and possibly during childhood, could be partly or wholly to blame for the increased incidence of male reproductive disorders:

It is thought that all of the disorders probably have their origins during development in the womb and possibly also in childhood. A clue to what could have caused the increase in these disorders in recent years came from the exposure of pregnant mothers to DES. This synthetic estrogen drug caused boys who were born to the women to have an increased incidence of reproductive abnormalities similar to those listed above. There was a greater incidence of urethral abnormalities and testicular maldescent among the boys and, when they reached adulthood, many had reduced sperm counts and sperm quality. Experiments with laboratory rodents showed that comparable reproductive abnormalities occurred in the male offspring of animals treated with DES during pregnancy (Gill *et al.* 1981, Sharpe 1993, Toppari *et al.* 1995). A recent follow-up study on over 200 men who were exposed to DES in the womb found that, although there was no evidence of reduced fertility, the men were 3 times more likely to have malformations of the genitalia than unexposed men (Wilcox *et al.* 1995).

Results of the tragic experience with DES clearly showed that the human body could mistake a man-made estrogenic chemical for the real hormone estrogen. It also showed that that inappropriate exposure to additional estrogen during pregnancy can result in permanent effects in offspring, including an increase in male reproductive disorders. Following this line of thought, scientists have hypothesised that, if extra estrogen can cause these effects, exposure to environmental estrogenic chemicals in the womb may have the same effect (Sharpe and Skakkebaek 1993).
Furthermore, it is possible that other endocrine disrupters, including anti-androgenic compounds and chemicals that exert effects through the Ah receptor such as dioxin, could also cause similar effects. Considering this evidence, it seems very reasonable to suggest that the increase in male reproductive problems seen over the past few decades may be partly or wholly caused by increased exposure to endocrine-disrupting chemicals which have been introduced into the environment over the past 50 years (Jensen *et al.* 1995).

Presently, there is little direct evidence in humans of effects resulting from exposure to endocrine-disrupting pollutants. Nevertheless, the hypothesis is also supported by several laboratory animal studies described below. One study of women who had accidentally been exposed to high levels of PCB and dioxins whilst pregnant gave birth to boys who had slightly shorter penises at age 11-14 (Guo *et al.* 1993).

It has been argued that other factors can reduce sperm count in adult men, including drinking alcohol to excess, sexually transmitted diseases, some commonly prescribed drugs, smoking cigarettes and wearing tight underwear (Giwercman *et al.* 1993, Tiemessen *et al.* 1996, Vine 1996). Exposure to certain pollutants like lead or ionising radiation may also reduce sperm count. Such factors may have contributed to declining sperm counts in men reported over the past 20-50 years. However, these factors alone cannot account for increases in the other male reproductive problems which have occurred concurrently with sperm count decrease. Exposure to endocrine-disrupters during development maybe a more plausible explanation.

In addition, a recent study in Finland examined the reproductive tissues from two groups of middle-aged men after their death, in 1981 and in 1991. It found that the incidence of normal sperm production had deteriorated over the ten year period, and testicular weight had decreased by 5.8%. Sperm production was "normal" in 56% of the 1981 group, falling to only 27% in the 1991 group. The deterioration in sperm production could not be explained by alcohol drinking, smoking or drug taking. In fact, the results were more consistent with factors effecting the reproductive system during development and/or before puberty, such as endocrine disrupters. It was suggested that deteriorating sperm production may explain why sperm counts are declining (Pajarinen *et al.* 1997).

9.3 Laboratory Studies
Several studies have shown that when male animals are exposed to endocrine disrupters in the womb, they suffer from reproductive abnormalities. For instance, when pregnant mice or rats are given high doses an estrogenic chemical, such as DDT, methoxychlor, chlordecone, hexachlorocyclohexane, PCBs, or di-n-butylphthalate (DBP), or given the anti-androgenic chemicals DDE or vinclozolin, their male offspring have reduced testicular weight and/or a lower sperm count (Toppari *et al.* 1995, Gray *et al.* 1992, Wine *et al.* 1997, Kelce *et al.* 1995, Gray *et al.* 1994).

Only a few studies have investigated the effects of endocrine-disrupters on the male reproductive system at low doses which are more relevant to current environmental levels of these chemicals. The studies looked at the effect of exposing pregnant rodents to dioxin, an alkylphenol (octylphenol), and the phthalate BBP, at levels close to, or within an order of magnitude of, current exposure levels in humans. All 3 chemicals caused marked and significant decreases in sperm count and other reproductive abnormalities in the male offspring. For BBP, the level of exposure approached levels to which humans are exposed in everyday life (Mably *et al.* 1992, Gray *et al.* 1995a, Sharpe *et al.* 1995).

A recent study showed that exposing pregnant mice to synthetic estrogen, or to the estrogenic chemical bisphenol-A, caused their male offspring to have enlarged prostate glands. In humans, enlarged prostate glands may predispose individuals to prostate cancer in later life. The effect in mice occurred at doses close to those to which humans are exposed through eating canned vegetables, which can be contaminated with bisphenol-A from the lining of food cans (Nagel *et al.* 1997).

9.4 Wildlife Studies
Wildlife studies have revealed male reproductive problems in several species that have been associated with exposure to endocrine-disrupting chemicals:

***Fish**
Studies in the UK have found that sewage effluent may be disrupting reproduction in wild fish populations in rivers and estuaries. Research was initially prompted after fishermen found a high incidence of hermaphrodite fish (which had both testis and ova), near to sewage treatment works. Scientific investigations subsequently showed that when male fish were placed in cages in rivers near to sewage discharges, they became "feminised". There was an increased incidence of reduced testis size in the fish and they all produced high levels of a protein called vitellogenin. This protein is

normally only made by female fish because it forms yolk in fish eggs. It is only produced by male fish if they are exposed to estrogen. This indicated that something in the sewage effluent had estrogen-like activity (Purdom *et al.* 1994, Matthiessen 1996).

Subsequent studies showed that the estrogenic activity of domestic sewage could be due to natural estrogens which are excreted by women and, to a much lesser extent, to synthetic estrogen from the contraceptive pill. However, this could not account for some of the effects being detected in fish, especially from industrial sewage (Desbrow *et al.* 1996).

Estrogenic chemicals including alkylphenols and phthalates have been detected in sewage (Jobling *et al.* 1995). Alkylphenols are now the prime suspects for causing the feminisation of fish in some areas. These chemicals are present in industrial sewage because of their formation as persistent breakdown products of alkylphenol ethoxylates, which are used in industrial detergents. The ethoxylates are also widely used as "inert" additives in pesticides, although the potential for contamination of soils and surface waters with alkylphenols from this source are not well studied.

Once discharged into the environment alkylphenols may bioaccumulate in fish tissue (Warhurst 1995, Schwaiger et. al. 1997). The most common ones are nonylphenol and octylphenol. Experiments found that these chemicals caused yolk protein to be produced by male fish, and reduced testes growth, at levels similar to those in some UK rivers (Jobling *et al.* 1996). Scientists have concluded that, at least for the river Aire in the UK where a wool scouring factory discharges these chemicals into the river, alkylphenols could be solely responsible for causing feminisation of male fish (Matthiessen 1996). Initial studies on wild fish in the Tyne estuary have found feminisation in male fish which is associated with nonylphenol levels, although it is not yet known whether other chemicals may be implicated (Lye and Frid 1997).

Effluent discharges from pulp and paper mills have also been reported to cause feminisation of male fish. These effects could relate to the presence of organochlorines in the effluent and possibly also to natural chemicals present in wood (Heuval *et al.* 1994, Landner *et al.* 1994).

*** Reptiles**

Following an extensive spill of pesticides, dicofol and DDT, in Lake Apopka, Florida, in 1980, the inhabiting population of alligators has continually declined. The decline appears to be caused by a decrease in hatching success of the alligator eggs, together with reproductive abnormalities in the young alligators. The young males have abnormally small penises, half to a quarter of the normal size, and abnormalities of the testes. Furthermore, they have altered levels of sex hormones. The research indicates that the gonads of the alligators are permanently changed during sexual development whilst in the egg, leading to the reproductive abnormalities. This is almost certainly because the eggs contain high amounts of estrogenic pesticides from the chemical spill and DDE, the breakdown product of DDT. DDE has two forms (isomers), one of which (o,p-DDE) is estrogenic and the other (p,p'-DDE) has anti-androgenic properties (Guillette *et al.* 1994 and 1995, Kelce *et al.* 1995, Vonier *et al.* 1996, Crain *et al.* 1997).

***Birds**

The Great Lakes, North America, were subjected to very high levels of organochlorine chemical contamination in the 1960s and 70s. Although levels have now substantially decreased, residues remain significant. In the 1970s, Herring Gull colonies around the Great Lakes regions suffered from sharp population declines. Studies showed that this was probably due to reduced numbers of male birds that were capable of breeding. The gonads of male chicks were found to be feminised, ie. they had female (ovarian) type structures. Subsequent experiments showed that the levels of chemicals such as DDT and DDE found in the gulls eggs were sufficient to cause these reproductive abnormalities in the male birds. Researchers suggested that estrogenic properties of such chemicals could be the cause of the problem (Fox *et al.* 1992, Fry 1995).

***Mammals**

The Florida Panther is an endangered species of wildcat with fewer than 50 remaining in their natural habitat. Many of the males suffer from health problems and reproductive problems, including a reduced sperm count, and an increased incidence of undescended testes in male cubs. The female panthers have very high levels chemicals in their bodies many of which are endocrine disrupters, including DDE, PCBs, mercury, methoxychlor, and transnonachlor. It is thought that the reproductive impairment of the males could be largely, if not entirely, a result of exposure to these chemicals from their mothers during development, and may be caused by endocrine-disruption (Facemire *et al.* 1995).

10. FEMALE REPRODUCTION

Human and animal studies indicate that some endocrine-disrupters can adversely affect the female reproductive system, both during development and during adulthood. However, it is not certain whether exposure to current environmental levels of these chemicals is having such effects.

10.1 Developmental Effects

The exposure of over 5 million women between 1945 and 1971 not only caused reproductive abnormalities in boys born to the women (see section 9.2), but also affected the girls. Many of the girls suffered from vaginal cancer in their teens, had reduced fertility and a high incidence of structural abnormalities of the reproductive system (Gray 1992).

When DES was given to pregnant laboratory rodents, it caused comparable reproductive problems in female offspring. This indicated that evidence from animal studies on endocrine disrupters and reproductive effects is very relevant to humans. Laboratory studies have also shown that when pregnant laboratory rodents are exposed to high levels of estrogenic pesticides, such as DDT, methoxychlor and chlordecone, their female offspring suffer from similar effects to those caused by DES. These include reduced fertility and various structural abnormalities of their reproductive systems (Gray 1992). Exposure to low doses of dioxin, that are within an order of magnitude of doses which may be expected from current environmental levels, also causes similar effects (Gray *et al.* 1995b).

From the above studies in animals, and from the unfortunate experience with DES in humans, it is plausible that exposure to estrogenic chemicals, and possibly to other endocrine disrupters, during development, could lead to reproductive abnormalities in female offspring. Presently, there is some evidence that exposure to endocrine disrupters in the environment, both during development and during adult life, may affect the female reproductive system:

Onset of Puberty
Animal studies have shown that exposure to increased levels of estrogens in the womb causes an earlier onset of puberty. Records show that women of industrialised countries now reach puberty at an earlier age. It has been suggested that this may be a result of exposure to estrogenic chemicals during development in the womb (Whitten 1992).

Increased Risk of Miscarriage and Occupational Chemical Exposure
Perchloroethylene (PERC) is the main solvent used in dry-cleaning. Consequently workers in the industry are exposed to this chemical (Aggazzoti *et al.* 1994). Studies have shown that women who work in the dry-cleaning establishments may have a greater risk of having miscarriages as a result of exposure to PERC (Olsen *et al.* 1990, Lindbolm *et al.* 1992, Kyyronen *et al.* 1989). PERC is suspected to be an endocrine disrupter, because it appears to affect pituitary function in the brain. It has been sugges-ted that endocrine disruption may be the mechanism accounting for the increased risk of miscarriage following exposure (Zielhuis *et al.* 1989, Ferroni *et al.* 1992).

10.2 Effects in Adult Women

Shortened Duration of Lactation
Declines in the duration of lactation have been reported throughout the world. This represents a serious public health concern because of the associated implications for increased infant illness and death, especially in developing countries.

The impact of DDE (breakdown product of DDT) on women's ability to lactate, has been investigated in North Carolina, US, and also in northern Mexico where DDE levels are very high due to continued use of DDT in the country. Both studies found that women with higher levels of DDE in their breastmilk lactated for shorter time periods than women with lower levels. The main reason why women lactated for shorter times was because they produced insufficient milk to continue breast feeding. Researchers think that DDE could be inhibiting lactation because of its estrogen-like effects. The study concluded that exposure to DDE, and possibly to other estrogenic pollutant chemicals, may therefore be contributing to lactation failure throughout the world (Gladen and Rogan 1995).

Breast Cancer.
The incidence of breast cancer has steadily risen worldwide since 1940. Known risk factors for the disease in women, such as family history of breast cancer, or age at puberty and menopause, at best can only account for a third of all cases, leaving the cause of the remaining cases uncertain (Harris *et al.* 1992).

Interestingly, there is good evidence in humans that elevated levels of the bodies own natural estrogen actually increases the risk of getting breast cancer (Toniolo *et al.* 1995). It is therefore plausible that exposure to estrogenic chemicals, which might amplify the effects of estrogen, could also increase the risk of breast cancer (Davis *et al.* 1993, Davis and Bradlow 1995).

Both animal and human studies indicate that estrogenic organochlorine chemicals could be involved in increasing the risk of breast cancer. Animal studies show that at high levels, PCBs and the pesticides DDT and atrazine cause an increased incidence of breast cancer (Stevens *et al.* 1994, Wetzel *et al.* 1994, see Davis *et al.* 1993). Not all research on women is consistent, but recent studies show that women from the general population who have higher body levels of DDE appear to have an increased risk of breast cancer (Wolff *et al.* 1993, Dewailly *et al.* 1993, Savitz 1994).

Further evidence that estrogenic organochlorine chemicals increase the risk of breast cancer, comes from looking at how they affect estrogen metabolism. Estrogen is normally broken down in the body to either of two forms - a "good" estrogen and a "bad" estrogen. The actions of the "bad" estrogen are thought to increase the risk of breast cancer, by causing breast cells to multiply. Studies in animals and women with breast cancer have found that the ratio of the two forms of estrogen is altered, so that there is too much "bad" estrogen. This implicates elevated levels of "bad" estrogen as a potential cause of breast cancer. When scientists tested the effect of several organochlorine pesticides on estrogen metabolism, they found that these chemicals also altered the ratio of the two forms of estrogen, such that levels of "bad" estrogen were significantly

elevated. This suggested that exposure to these pesticides, namely atrazine, DDE, DDT, and kepone, may influence estrogen metabolism in a way that could increase breast cancer risk (Bradlow *et al.* 1995).

As yet the hypothesis that estrogenic organochlorines may increase the risk of breast cancer is not proven. However, research does suggests that exposure to such chemicals could contribute to an increased risk and thereby account for many of the unexplained cases of this disease (Davis and Bradlow 1995).

Endometriosis
Endometriosis is a disease associated with infertility and chronic pain. The incidence of this disease is thought to have risen over the past few decades, and it is now estimated to affect 10% of all women of reproductive age in the US (Rier *et al.* 1995). Studies on monkeys have indicated that exposure to PCBs or dioxin can increase both the prevalence and severity of endometriosis (Rier *et al.* 1993). It is possible that dioxin may affect endometriosis because of its hormone-disrupting effects which could impact on the immune system (Rier *et al.* 1995).

It is of concern that exposure to dioxin was associated with an increased prevalence and severity of endometriosis in monkeys, at levels which are within an order of magnitude of current levels in people's bodies (US EPA 1994). This research suggests that PCBs and dioxins could also increase the threat of endometriosis in women, although direct human evidence is currently very limited. It is known that endometriosis is more prevalent in industrialised countries where the levels of these chemicals are the highest (Koninckx *et al.* 1995). One study on women found higher body levels of PCBs were linked with endometriosis, but another found no association with levels of PCBs or dioxins (see US EPA 1997). Further investigation is therefore needed to confirm whether or not PCBs and dioxins are linked to endometriosis in women. In the meantime, an attitude towards dioxin pollution in the environment which considers levels to be "too low to be harmful" could be over-optimistic (Koninckx *et al.* 1994).

10.3 Wildlife Studies

*** Reptiles**
Lake Apopka in Florida was heavily contaminated by a spill of dicofol and DDT in 1980, and young male alligators have altered sex hormone levels and suffer from reproductive abnormalities. The females too have been affected. They also have altered levels of sex hormones and abnormalities in the structure of their reproductive systems. Again, these abnormalities are most likely caused by permanent modification during development in the egg as a result of exposure to endocrine-disrupting chemicals (Guillette *et al.* 1994).

***Birds**
Pollutants which have been found in bird's eggs, including organochlorines, pesticides and heavy metals, have been reported to cause reduced hatchability of eggs, death and deformities in the embryo, and reduced survival of chicks hatched from eggs. Some of the effects may be due to the endocrine-disrupting properties of these chemicals (Fry 1995).

The most dramatic effect on the reproductive performance of wild birds was eggshell thinning caused by DDE, the breakdown product of DDT. From the 1950s to 70s, this resulted in the population decline of many predatory and fish-eating wild bird species (Fry 1995, Giesy *et al.* 1994).

Egg-shell thinning is now less common, although other breeding problems persist. Organochlorine pollution in the Great Lakes regions has decreased over the past two decades but still remains relatively high. Some bird populations such as double-crested cormorants and herring gulls have made dramatic recoveries since population crashes in the 1970s, but others, such as common and Fosters terns, continue to decline. Problems in these Great Lakes birds include death and deformities in embryos and chicks, (eg. crossed bill, lack of eyes, skeletal malformations), as well as other problems, including edema and behavioural changes. There is evidence that these effects, which result in reproductive failure of the birds, may result from contamination with dioxins, PCBs and possibly other chemicals which exert toxic effects through the Ah receptor (Giesy 1994).

Severely reduced breeding success in cormorants has been reported in the area of the Rhine and Meuse delta in the south-western part of The Netherlands. Egg shell-thinning and increased embryonic mortality is reported to be responsible for reduced hatching success of the birds. Research has shown that high levels of DDE are linked to the egg-shell thinning, whilst levels of PCBs are related to reduced hatching and breeding success of the birds (Dirksen *et al.* 1995).

***Marine Mammals**
Between 1950 and 1975, the population of common seals inhabiting the Wadden Sea, The Netherlands, collapsed from 3000 to less than 500 animals. In 1986, studies revealed that the female seals were experiencing reproductive failure due to problems relating to the process of implantation. This was found to be linked to high levels of PCBs in the seals. Female Baltic ringed seals and grey seals have also suffered from reproductive problems. Research suggests that this can been attributed to hormone-disrupting effects of PCBs and possibly other organochlorine pollutants in the seals (Reijnders 1986, Reijnders and Brasseur 1992).

The St. Lawrence estuary in Quebec, Canada, is heavily contaminated with organochlorines and other pollutants. The local population of Beluga whales was drastically reduced in the first half of the century by over-hunting. It has, however, failed to recover in the past 40 years, despite a reduction in hunting. Researchers think that this could be due to high levels organochlorine chemicals which contaminate the whales and a range of adverse health effects which could result from this contamination. The female whales suffer from reproductive problems. A very low proportion of the females are breeding. In addition, lesions have been detected in the mammary glands of 36% of the females which would seriously affect their ability to feed if any calves were produced. It is possible that the reproductive effects are caused by estrogenic activity of the organochlorine pollutants (DeGuise *et al.* 1995, US EPA 1997, see Johnston and McCrea 1992)

11. SEX RATIO

In humans and many other animals, sex ratio is determined in humans by the sperm, which carry either an X or a Y chromosome. All eggs have X chromosomes, so at fertilisation, either a female (XX) or a male (XY) is produced. Traditionally, the number of X and Y sperm produced was thought to be equal so that the number of males and females born should also be equal, giving a sex ratio of 0.5. In reality, there is a slight excess of males at birth, which may be accounted for by several different factors, including age of parents and time of insemination within the cycle (Moller 1996).

It has been hypothesised that the steroid hormone concentrations of both parents may influence sex determination, so that changes in levels may result in a skewed sex ratio. If this hypothesis is correct, with an increased exposure to man-made estrogenic chemicals in recent decades, it may be expected that the sex ratio would be altered, such that fewer males were born (Moller 1996).

There is some evidence to support this hypothesis. Following an accident at a chemical plant in Seveso, Italy, the local population was exposed to high levels of dioxin (TCDD). Research shows that exposure of both parents was associated with an significant increase in the proportion of girls born (Mocarelli *et al.* 1996). Another study showed that more females than males were fathered by men who were exposed to an endocrine-disrupting pesticide DBCP (see Moller 1996).

Recent studies have investigated trends in the sex ratio of the general population in The Netherlands and Denmark over the past few decades. It was found that the proportion of boys born over the past 50 years had slightly, but statistically significantly, decreased (Pal-de Bruin *et al.* 1997, Moller 1996). From 1950 to the mid 1990s the ratio declined from 0.515 to 0.513 in Denmark and from 0.516 to 0.513 in The Netherlands. If the hypothesis that hormone levels influence sex determination is correct, this may reflect the increased exposure to endocrine disrupting chemicals over the past 50 years. The Netherlands study concluded: *"our finding of a decreasing ratio of male to female newborn babies in The Netherlands can only add to concern about the potential hazards of environmental endocrine disrupters"* (Pal-de Bruin *et al.* 1997).

12. EFFECTS ON THE NERVOUS SYSTEM

There is evidence from human studies that exposure to PCBs and/or dioxins during development can lead to adverse effects on the nervous system of infants and children. These effects include reduced cognitive function, such as lower IQ, poorer short and long term memory, attention deficits and effects on psychomotor development. Although the effects which have been recorded are subtle in nature, they do represent diminished potential in children. Research suggests that some women of the general population currently have body levels of PCBs and dioxins which may cause such effects in their children.

With regard to wildlife , some behavioural changes in birds have been associated with exposure to man-made chemicals. In the Great Lakes region in the 1970s, impaired incubation and chick rearing behaviours in gulls has been associated with exposure to organochlorines. Also, female-female pairs of birds have been found tending abnormally large clutches of eggs. This phenomenon has been recorded between the late 1960s and 1980s in Western gulls in Southern California, and in populations of Herring gulls and Caspian Terns from the Great Lakes regions. It has been suggested that the altered behaviour of these gulls could be due to a reduction in the number of males in the colonies which are capable of breeding. This could be due to exposure to organochlorine chemicals which may cause feminisation of male embryos or a reduction in male chick survival (see also section 9.4), (Fox 1992, Fry 1995).

12.1 Development of the Nervous System

Thyroid hormones and gonadal sex hormones play a role in controlling development of the nervous system both in the womb and during childhood (Porterfield 1994, Hines 1992). A disturbance in the levels of these hormones at critical times when the nervous system is developing can cause changes which may, in extreme cases, even result in permanent brain damage. For instance, in a condition known as congenital hypothyroidism, a deficiency in thyroid hormones during late foetal life and early infancy, causes permanent mental retardation. Treatment with hormones after birth can prevent mental retardation, but children may still suffer from various psychomotor problems, memory problems and slightly reduced IQ (Porterfield 1994).

It is known that PCBs and dioxins cause damage to the developing nervous system, but how they do this is not well understood. It is possible that they cause this damage by disrupting thyroid hormones and sex hormones during development.

Thyroid hormones

Animal studies show that PCBs and dioxins can alter the levels of thyroid hormones during development. Certain PCBs can also alter the levels of natural chemicals which are essential for the transmission of nerve signals (neurotransmitters). Both of these effects may explain why these chemicals cause adverse effects on the developing nervous system (Morse *et al.* 1992, Seo *et al.* 1995, Seegal and Shain 1992).

In humans, exposure to PCBs and dioxins in the womb has been associated with changes in thyroid hormone levels. In The Netherlands, a study was undertaken on healthy women and their babies from the general population who lived in the Zaandam region, near Amsterdam. The study calculated a baby's exposure to PCBs and dioxins in the womb by measuring the level of these chemicals in the mothers. It found that babies who had a higher exposure to PCBs and dioxins in the womb had slightly altered thyroid hormone levels (Pluim *et al.* 1992, Brouwer *et al.* 1995).

Sex hormones
In mammals and humans there are some differences between males and females in the structure and functioning of the brain. This accounts for a number of differences in behaviour and learning between the sexes. The sex hormones, estrogen and androgens, partly control the development of such differences in the brain, although exactly how they do this is not fully understood.

It is known that disturbances in the delicate balance of estrogen and androgens during development can lead to permanent effects on behaviour in laboratory rodents (Dohler and Jarzab 1992). Some endocrine-disrupting chemicals have also been shown to alter behaviour in animals. For example, one study showed that male mice which were exposed in the womb to the synthetic estrogen DES, had altered urine-marking behaviour. This is a behaviour which plays a major part in determining reproductive success in male mice. Exposure to the estrogenic chemicals DDT and methoxychlor also caused the same effects. There was evidence that the effects could have been caused by the binding of these chemicals to estrogen receptors in the developing brain (vom Saal *et al.* 1995). Other research on behaviour in rodents found that exposure of females in the womb to estrogenic pesticides, including DDT, methoxychlor and chlordecone, causes them to have more masculine-like sex behaviour (Gray 1992).

12.2 Effects of PCBs and Dioxins on the Human Nervous System

There are several studies involving groups of children whose development has been followed from birth, to investigate whether exposure to PCBs and dioxins in the womb, and through breastfeeding, has an impact on health. All but one of these studies was carried out on women and their children from the general population. The other study focused on pregnant women who were exposed to high levels of PCBs/dioxins following an accident in Taiwan.

The studies estimated a baby's exposure to PCBs/dioxins in the womb and through breastfeeeding by measuring levels of the chemicals in their mothers bodies and breastmilk. In general, they found that exposure to higher levels of PCBs/dioxins in the womb is associated with a number of subtle adverse effects on neurological and intellectual function:

YuCheng "Oil Disease" Incident, Taiwan.
In 1979, 2000 people in Taiwan were accidentally exposed to high levels of PCBs and dioxins after eating contaminated rice oil. Women who were pregnant at the time of the incident gave birth to children who, on average, have slightly reduced cognitive function. The effects did not improve by age 7 in the children, indicating they were probably permanent. Their IQ was about 5 points lower than unexposed children and they suffered from mildly disordered behaviour. Furthermore, due to the persistence of PCBs and dioxins in the body, children born up to 12 years after the accident

suffered from the same problems. (Chen *et al.* 1992, Guo *et al.* 1994 and 1995, Yu *et al.* 1994).

Lake Michigan Study, US.
Studies were undertaken on women from the Lake Michigan area who had eaten moderate amounts of fish from the lake over several years before becoming pregnant. Lake Michigan fish are known to contain relatively high amounts of PCBs and, consequently, the women had body levels of PCBs which were slightly above those of the general population. The studies compared development of their children with that of children whose mothers had not eaten fish and who therefore had slightly lower body levels of PCBs.

It was found that infants and children who had been exposed to higher levels of PCBs in the womb showed small, but significant, deficits in cognitive function (Jacobson *et al.* 1985, Jacobson and Jacobson 1993). The effects are long-term, still being apparent in the children at age 11. Problems include deficits in general intellectual ability, especially with respect to reading skills, poorer short and long-term memory, and deficits in attention. The most highly exposed children had a 6.2 point decrease in IQ. Even though scores on these tests for the children fell within the normal range, they were at the lower end of the normal range, which means the children would be expected to perform less well at school (Jacobson and Jacobson 1996).

North Carolina Study, US
This study investigated women and their children from the general population. Children who were exposed to higher levels of PCBs in the womb had deficits in their psychomotor development up to the age of 2. No effects on cognitive development were found (Rogan and Gladen 1992).

The Netherlands Study
The Netherlands study investigated healthy women and their babies from the general population who lived in the Rotterdam or Groningen area. There was a slight adverse effect on psychomotor development in infants who were exposed to higher levels of PCBs and dioxins in the womb and during breastfeeding (Koopman-Esseboom *et al.* 1995). There was also a slight adverse effect on neurological development in the children detected at 18 months of age. Tests on neurological development looked at movement coordination, (eg. sitting, crawling, standing and walking), and are a way of measuring the quality and integrity of brain function (Huisman *et al.* 1995). At the age of 2 years and 7 months, tests on a subgroup of infants found slight changes in some measures of neurological development in the more highly exposed individuals. These changes were regarded as "unwanted" by researchers, and it was proposed that they could be due to the action of dioxins on thyroid hormones during development (Ilsen *et al.* 1996).

12.3 Possible Consequences of Effects on the Nervous System

Although effects which have been found on the nervous system are relatively subtle in nature and may not greatly affect an individual, they do, nevertheless represent diminished potential of individuals. When considered at a population level, such effects could be far reaching. For example, a 5 point drop in IQ across a population would, in effect, halve the number of people with high powered minds who have the ability to become the most gifted doctors, scientists, writers etc., and would greatly increase the number of slow learners who require special remedial education. This could fundamentally change the character of society (Colborn 1996).

Experts at a meeting in Erice, Sicily (Alleva et. al. 1995), expressed concern about neurological effects resulting from exposure to endocrine disrupters. They were certain that: "Endocrine-disrupting chemicals can undermine neurological and behavioural development and subsequent potential of individuals exposed in the womb or, in fish, amphibians, reptiles and birds, the egg. This loss of potential in humans and wildlife is expressed as behavioural and physical abnormalities. It may be expressed as reduced intellectual capacity and social adaptability, as impaired responsiveness to environmental demands, or in a variety of other functional guises. Widespread loss of this in nature can change the character of human societies or destabilise wildlife populations. Because profound economic and social consequences emerge from small shifts in functional potential at the population level, it is imperative to monitor levels of contaminants in humans, animals, and the environment that are associated with disruption of nervous and endocrine systems and reduce their production and release".

13. IMMUNE SYSTEM

The immune system consists of a network of various types of specialised cells which circulate in the body and serve to prevent infection and disease. Some toxic chemicals can alter the levels or functioning of immune system cells. This can lead to a decrease in resistance to infection and tumours, or an increase in auto-immune diseases (Thomas 1990).

Research has shown that the immune system and the endocrine system are closely inter-connected. For instance, steroid hormones, including estrogen, testosterone, and hormones produced by the pituitary and adrenal glands, are all involved in the regulation of the immune system (Grossman 1984, Berczi 1989).

13.1 Effect of Endocrine-Disrupters on Immune System Development

Presently, little is known about the potential impact that endocrine-disrupting chemicals could have on the immune system during development. There is evidence that exposure to the synthetic estrogen drug DES during development in the womb, leads to permanent changes in the immune system of both laboratory rodents and humans. For example, women who were exposed to DES in the womb, had changes in some immune system cells, and had an increased susceptibility to auto-immune and other diseases associated with defects in immune system regulation (Blair *et al.* 1992, Blair 1992).

Evidence from studies on DES raises the possibility that exposure to estrogenic or other endocrine-disrupting chemicals during development, could alter development of immunity and lead to permanent changes in immune system function in later life. Animal studies do show that exposure to some estrogenic chemicals during development, including DDT and chlordane, cause suppression of the immune system (Rehana and Rao 1992, see Holladay and Luster 1996). However, it is not known whether immune suppression in these studies was caused by disruption of hormones or by other mechanisms.

13.2 Human Studies

Several studies have shown that some women of the general population have body levels of chemicals with the ability to disrupt hormones, ie. PCBs and/or dioxins, which are within the range at which changes in the immune systems of their babies may occur. However, such effects may be caused by mechanisms other than endocrine disruption.

Increased Infections

Inuit women inhabiting Arctic Quebec have relatively high body levels of persistent organochlorines because of their seafood-rich diet. Research has shown that higher levels of PCBs and dioxins in their breastmilk were linked with an increased incidence of ear infections in their babies (Dewailly *et al.* 1993). Other studies on women who ate fish from the Great Lakes before becoming pregnant, reported that higher body levels of PCBs in the women were associated with an increased incidence of bacterial infections in their babies (Bernier *et al.* 1995, see Tryphonas 1995).

Changes in Immune System Cells
In the 1970's, residents of Times Beach in Missouri, USA, were accidentally exposed to high levels of dioxins because waste oil which was sprayed on roads for dust control was contaminated. Children born to women who were pregnant during the incident, were found to have changes in the number of some immune system cells at the ages of 9-14. This indicated that effects on their immune systems were long-term (Smoger *et al.* 1993).
A recent study in The Netherlands focused on healthy women and their babies from the general population. Some of the women had higher levels of PCBs and dioxins in their bodies and breastmilk than others. It was found that infants who were exposed to higher levels of these chemicals in the womb, and via breastfeeding, had changes in the number of certain immune system cells. It is not known what effect such changes could have on their health (Weisglas-Kuperus *et al.* 1995).

13. 3 Wildlife Studies

Mass Mortalities in Marine Mammals
Several marine mammal populations around the world have suffered from sudden dramatic losses in numbers since the mid-1980s, in what have become known as "mass mortalities". In both dolphins and seals, there is evidence which suggests that high body levels of persistent, bioaccumulative chemicals like PCBs and organochlorine pesticides may be resulting in weakening of the immune system, and leaving the animals more susceptible to infections. In turn, the infections may lead to their death. It is not known whether the mechanisms leading to suppression of the immune system in these cases are connected with disruption of hormones.

In 1988, an estimated 20,000 harbour seals died in seas around Germany, Sweden, The Netherlands and the UK. The cause was a virus (phocine distemper virus), similar to that which causes distemper in dogs. Since then, experiments with captive seals have shown that feeding fish which contains high levels of organochlorines to seals, causes suppression of their immune system, weakening their ability to cope with disease (Ross *et al.* 1995, de Swart *et al.* 1996). It is thought that the mass mortality of seals could be due to suppression of their immune systems by these chemicals. Further evidence is provided by the fact that seals which died had higher body levels of organochlorines than surviving seals, and those living in more highly polluted waters had higher death rates than those in less polluted regions (Simmonds *et al.* 1993).

Mass mortalities of dolphins have been occurring since the late 1980s. Huge numbers of striped dolphins died between 1990 and 1992 around the whole of the Mediterranean, possibly due to a virus named dolphin morbillivirus. The dead animals were found to have higher body levels of PCBs than healthy animals (Aguilar and Borrell 1994). Similarly, in the late 80s and early 90s, many bottlenose dolphins died along the eastern coast of the United States. They were also contaminated with high levels of PCBs and other organochlorines. In both mass mortalities, is possible that these contaminants could have contributed to suppression of the immune systems, rendering the dolphins more susceptible to infections which subsequently led to their death. This is further supported by a study on bottlenose dolphins resident in Florida. Blood samples taken

from the animals found that suppression of the immune system increased with increasing levels of pollutants in the dolphins especially DDT, DDE and PCBs. More research is necessary to confirm these findings (Lahvis *et al.* 1995).

Increased infections in Marine Mammals
A small isolated population of beluga whales in the St. Lawrence estuary has very high body levels of organochlorine pollutants. Studies show that they suffer from a high incidence of infections from mildly pathogenic bacteria. The data strongly suggest that immunosuppression in the animals could be related to the high levels of contaminants in their body tissues. In addition, immune system suppression leading to decreased resistance to tumours may be a contributing factor in the high prevalence of tumours found in the belugas over the past 15 years. (de Guise *et al.* 1995).

14. PHYTOESTROGENS: NATURALLY OCCURRING CHEMICALS IN PLANTS WHICH AFFECT HORMONE SYSTEMS

About 300 different plants are known to produce and contain a number of natural chemicals called phytoestrogens. These are thought to serve a variety of functions, acting as natural fungicides, regulating plant hormones, deterring herbivores from eating them and as a protection against ultraviolet radiation from the sun. Plants which contain phytoestrogens include many in our diet, such as whole cereal grains, seeds, soy, cabbage, beet, broccoli and peas (Barrett 1996).

When some phytoestrogens are eaten, they are be broken down in the gut to form estrogen-like compounds, which are able to bind to estrogen receptors. Phytoestrogens spend relatively little time in the body before being excreted. However, during this time they may affect sex hormones and their binding proteins. Studies show they can result in lowering the biological activity of sex hormones in the body. Some also have antioxidant properties.

Research suggests that phytoestrogens in the diet have many beneficial effects, but harmful effects have also been found. For example, in Australia, female sheep which grazed for prolonged periods on a species of clover containing phytoestrogens suffered sharp declines in fertility. Phytoestrogens may act as a defence mechanism, to deter herbivores from heavy predation on a single plant species (Adlercreutz 1995, Barrett 1996).

If phytoestrogens can affect the fertility of sheep, they may be also be able to affect human fertility. This is indeed the case for a few plants which have been known to herbal medicine for centuries and used as contraceptives (Colborn *et al.* 1996). However, eating a variety of plants in a normal diet does not seem to affect human fertility. For example, Asians consume very high levels of phytoestrogens in their diet without affecting their fertility. The most likely explanation is that, as humans and animals have evolved alongside plants, any harmful effects on fertility that a normal diet may cause, may have been selectively bred out of the populations long ago (Hughes 1988, Barrett 1996).

It has been suggested that phytoestrogens in soy-based infant milk formulas may have adverse effects on sexual development. Presently there is no human evidence to substantiate this, and the levels in soy infant milk are at least 100 times lower than doses reported to affect sexual development in rodents (Kardinaal 1997).

Beneficial health effects have been associated with eating a diet which is rich in grains and vegetables, containing high quantities of phytoestrogens. Studies on populations who consume suggest that such a diet suggest it has a protective effect against several hormone-related diseases. These include estrogen-related cancers, such as breast cancer and prostate cancer, colon cancer, and possibly osteoporosis and cardiovascular diseases. Whether the protective effect against such diseases is a direct result of the phytoestrogens themselves, or due to other factors, is uncertain. Preventative effects of phytoestrogens on such diseases have been demonstrated in some laboratory studies (Adlercreutz 1995, Barrett 1996).

There are major differences between phytoestrogens and man-made endocrine-disrupting chemicals. Phytoestrogens are readily broken down and excreted from the body. Consequently they spend very little time inside the body. The situation with man-made chemicals is different. Humans and animals have not evolved alongside vast quantities of man-made endocrine-disrupting chemicals in the environment. Unlike phytoestrogens, many are persistent and cannot be broken down or detoxified. These may bioaccumulate in body fat, remaining in the body for long periods of time (Barrett 1996).

15. PROSPECTS FOR THE FUTURE

15.1 Regulation

Presently, governments employ two basic strategies which attempt to protect the environment and human health from harmful effects of chemicals. These are prevention and regulation.

Prevention

To prevent harmful effects of chemicals on the environment necessitates government authorities to take measures to ban the production and use of chemicals in a country. For example, several persistent organochlorine chemicals have been banned in the US and most European countries, including PCBs, DDT and toxaphene.

Nevertheless, exposure to such chemicals continues because of their persistence, recirculation in the environment, and passage from one generation to the next. Furthermore, most bans are not implemented globally. For example, DDT and other persistent organochlorine pesticides continue to be produced and used in bulk in Asia, Africa and Latin America. Once released into the environment, these chemicals add to the global burden of contamination. A further problem is the export of chemicals (Bason and Colborn 1992), or the technology to make chemicals, from a country where they are banned to a country where bans are not in place.

Current Regulatory Strategies

Present regulations do not consider the potentially harmful effects of endocrine disrupters to the environment or to human health. Consequently no chemical has been banned or restricted soley because it is an endocrine disrupter. Chemicals with a known ability to interfere with hormone systems, including some industrial detergents, pesticides, mixing agents, softeners in plastics, flame retardants and preservatives, are still being manufactured and used in bulk quantities in Europe and the US. Others, such as dioxins, are produced as unwanted by-products of existing industrial and waste management processes. Exposure to these chemicals therefore continues.

The current regulatory system aims to set quantities or rates at which chemicals can legally be released into the environment. In Europe, limits are generally based on the process of hazard assessment. In recent years, the process of risk assessment, has also been increasingly implemented.

Hazard assessment involves the use of toxicity testing to identify the potential impact of a chemical on the environment. Chemicals are tested either vitro and/or in laboratory aninmals. Information is generated on the relationship between the dose of a chemical and the size of the particular biological effect it causes - known as the dose-response relationship. This information is interpreted in order to set an exposure level for the environment and for humans which is thought to be "safe" (Johnston *et al.* 1996 *in press*). This generally includes a safety factor which is largely arbitrary in nature.

The process of risk assessment also uses data from toxicity testing. In addition, it attempts to estimate exposure to that chemical from emissions, and finally the probability of health effects from the exposure. It determines what are deemed to be 'acceptable risks' from the release of chemicals into the environment. For instance, in the US, a risk of less than one in a million individuals contracting cancer from exposure to a chemical in the environment is generally considered to be an acceptable risk on which to base legal limits for the release of many chemicals. Risk assessment therefore attempts to provide a more accurate assessment of the dangers presented by chemicals by linking known hazards with estimates of exposure and by replacing safety factors with numerical estimates of risk. In practice, the uncertainties which prevail in estimates of both hazard and exposure often lead to risk assessments which are incomplete or yield information which is of little greater utility than a hazard assessment.

In hazard and risk assessment, the toxicity tests are based upon the ability of a chemical to produce an adverse effect on health such as causing cancer. Testing of some chemicals also takes into account their effects on development. For instance, after exposing animals to a chemical in the womb, the effects on reproduction would be investigated by checking whether the animals were fertile when they reached adulthood. However, with regard to endocrine-disrupting chemicals, such endpoints would often not be sensitive enough to detect adverse health effects caused by these chemicals. For example, animals may still be fertile after being exposed to a chemical in the womb, but their sperm count may have been substantially reduced (Gray 1992). Therefore, the dose of an endocrine-disrupting chemicals that are calculated by present methods to be "safe" may actually cause harmful effects.

In summary, toxicity tests for the purposes of hazard and risk assessment are not currently designed to look specifically for endocrine-disrupting effects. Current endpoints which are used in toxicity tests would potentially leave many health effects caused by endocrine-disrupting chemicals undetected. Consequently, based on the current system, the legal limits which have been set for the permitted release of endocrine-disrupting chemicals into the environment, or for allowable levels in products, are unlikely to be protective of human health.

Yet a further problem which questions current "safe" limits set for endocrine-disrupters and other chemicals is the process of risk assessment itself. This is because the calculations in the process of risk assessment often depend on highly uncertain and subjective assumptions. This frequently leads to a high degree of uncertainty and in risk assessment for legislative purposes. Consequently, it is questionable whether this process can be protective of public health. Furthermore, risk assessment generally only considers the effects of single chemicals, whereas, in the real world, humans are exposed to mixtures of numerous chemicals. Research has shown that endocrine disrupters may have cumulative effects when mixed together. This could further confound attempts to set safety limits for such chemicals (Johnston *et al.* 1996a, Johnston *et al. in press*).

Proposed Future Screening and Testing Programs for Endocrine Disrupters
To date, the identification of endocrine-disrupting chemicals has been largely based on specific scientific studies. No routine screening to identify endocrine disrupters been undertaken by government authorities. However, future programs to screen chemicals for their ability to interfere with hormone systems are currently under discussion in the US.

In the US, the Environmental Protection Agency (US EPA), has been given the task of developing a strategy to screen and test common chemicals for endocrine disrupting effects within the next two years. With over 63,000 chemicals in commercial use, this prospect is daunting. The EPA have already started to whittle down the vast numbers of chemicals in commercial use to about 17,000 common chemicals which are presently produced in bulk quantities. It is likely that this number will be further reduced by focusing on chemicals found in drinking water, food, ambient air and household products. Current legislation requires that all pesticides will have to be screened and endocrine-disrupting chemicals in drinking water identified (Patlak 1997).

There are many problems which face the development of tests to screen large numbers of chemicals for endocrine-disrupting effects. To minimise costs and time, the use of *in vitro* testing using cell culture is the method of choice. Presently, *in vitro* tests are available which can detect estrogenic chemicals. However, there are problems with using such tests. The effects of an estrogenic chemical can vary depending on dose, timing, tissue type, organism, and interaction with other hormones and metabolism. This means that a chemical which is estrogenic in one *in vitro* test system, may be innocuous in a different test system. It is therefore likely that the US EPA will develop a screening plan that uses several different *in vitro* tests.

The use of *in vitro* tests rather than testing in laboratory animals means that effects of a chemical on the developing foetus cannot be detected. Also, some chemicals may not estrogenic in an *in vitro* test, but exert estrogenic effects in an animal. This is because the chemical itself is not estrogenic, but when it is broken down in the body, the resulting product is estrogenic. It is therefore argued by some that a testing program should also include testing chemicals in laboratory animals. Again, this is not without its problems, including ethical considerations. There is one test which can detect whether a chemical is estrogenic to the developing reproductive system in animals. However, this test can take up to 18 months to complete. This would be too expensive financially and too time consuming as a test for screening vast numbers of chemicals. According to toxicologist Paul Foster, of the Chemical Industry Institute of Toxicology in Research Triangle Park, US, "there would be enough work to keep labs going for the next three to four hundred years" (Patlak 1997).

Given the problems of developing a screening strategy to detect endocrine disrupters, it is probable that a group of *in vitro* tests will be used. It is possible that following an initial screening to identify estrogenic chemicals, there may be further testing for developmental effects in laboratory animals (Patlak 1997).

Further Problems Facing Future Screening and Testing Programs for Endocrine Disrupters.
Endocrine disruption is a much wider problem than estrogenicity alone:
Since most research to date has focused on estrogenic chemicals, future plans for screening chemicals for risk assessment purposes are concentrating on estrogenic effects. However, while attracting the greatest concern to date, estrogenic effects represent only one aspect of a complex range of potential interferences with the hormone system. Effects of chemicals on male sex hormones, androgens, on thyroid hormones, on hormones from the adrenal glands and those produced by the brain are not being considered. Thus, in the rush to develop methods to screen chemicals for estrogenicity, other hormones affected by endocrine-disrupters and the resulting impacts on health, could be increasingly overlooked.

Classical Risk Assessment Ceases to be of Any Use for Endocrine Disrupters:
In risk assessment, toxicity tests to determine the effects of endocrine disrupters in laboratory animals, would not only be time consuming and expensive, but are also plagued by several other problems. The effects of endocrine disrupters can vary depending on which tissues are tested, and the time during development when the chemical is given. The effects are often subtle in nature and may not be detectable until adulthood. This makes it both time consuming and very difficult to predict with certainty the effects of endocrine disrupters (Howard 1997).

Furthermore, the greatest effects of endocrine disrupters often occur at the lowest doses. In contrast, in classical toxicology, the dose-response curve of many chemicals is usually linear, with small effects occurring at low doses and more drastic effects occurring at the highest doses. However, increasing the dose of endocrine disrupters may have less effect because of receptor "down regulation", in which the body reacts to hormonal over-stimulation by reducing the number of hormone receptors it produces. This results in an upside down U-shaped dose-response curve. The shape of the dose-response curve for endocrine-disrupters makes it very difficult to predict what effects would be at different doses. It would be extremely difficult, if indeed possible, to determine a minimum dose which caused adverse effects (Patlak 1997, ENDS 1997b). Classical risk assessment may, therefore, be of little use in assessing risks of endocrine disrupters.

This presents a massive dilemma for industry because of the reliance on risk assessment for regulation of chemicals. Presently, the chemical industry play down the concerns, arguing that as there is insufficient information to assess risks at present, it is too early to take action. However, in reality, risk assessment will never provide the protection required. As noted by Professor vom Saal (University of Missouri), (ENDS 1997b),

"There are no safe doses of endocrine disrupters, just as there are no safe doses of carcinogens"

Defining Endocrine disruption:
An endocrine disrupter has been defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour (US EPA 1997). However, the effects of interference with hormones in themselves are not considered to be "adverse" effects by regulators. An adverse effect would only be considered as an effect which manifested in a physiological outcome in an animal. For instance, the US EPA (1997) state that:

"Based on current science, the Agency does not consider endocrine disruption to be an adverse effect *per se*, but rather to be a mode or mechanism of action potentially leading to other outcomes, for example, carcinogenic, reproductive of developmental effects, routinely considered in reaching regulatory decisions".

However, as previously discussed, there are many problems with testing for the effects of endocrine disrupters in animals and to wait for scientific proof from such studies before taking any action could take decades. Therefore, once a chemical is identified as being able to interfere with hormones by *in vitro* test systems alone, or by *in vivo* tests, this should be sufficient information to warrant action. Such chemicals should be targeted to be phased out of production and use.

15.2 The Way Forward
It is clear that more research is needed to understand fully the mechanisms, the effects and the consequences of endocrine disruption. However, scientific certainties about the effects and the risks posed by endocrine-disrupting chemicals could be decades away. To wait for conclusive scientific proof that these chemicals are adversely affecting human and wildlife populations before taking action could have devastating consequences for future generations. Action at a global level is needed now to deal with what has become a global problem.

From the discussion above, it is evident that current methods of regulating chemicals based on risk assessment and hazard assessment are wholly unsuitable for assessing risks and effects of endocrine disrupters. There is only one clear way forward which can avoid all these problems and safeguard future generations - the adoption of the precautionary principle and the implementation of zero discharge strategies. This means prevention of pollution at source, and implementation of clean production in industry and agriculture.

Adoption of the Precautionary Principle
The precautionary principle acknowledges that, if further environmental degradation is to be minimised and reversed, precaution and prevention must be the over-riding principles of policy. It requires that the burden of proof should not be laid upon the protectors of the environment to demonstrate conclusive harm, but rather on the prospective polluter to demonstrate no likelihood of harm. The precautionary principle is now gaining acceptance internationally as a foundation for strategies to protect the environment and human health (Stairs and Johnston 1991).

It is already known that over 50 man-made chemicals, most of which are still in use, can interfere with steroid or thyroid hormones. This should be sufficient evidence to justify the phase out these chemicals under the precautionary principle. For some endocrine disrupters, including many persistent, bioaccumaltive chemicals, safer alternatives are already available which could be used. For instance, phthalate plasticisers are used in the production of PVC, and in manufacturing this plastic, dioxins are produced as unwanted by-products. However, there are already alternative materials which can be used for many applications of PVC (Greenpeace 1996).

Phasing out chemicals requires careful planning to take into account the many factors involved. For example, a proposed plan to phase out organochlorine chemcials was made to the governments of Canada and the US by the International Joint Commission for the Great Lakes. In essence, it requires orderly timetables to be set for the elimination of chemicals, together with implementing safer alternatives and looking after the interests of workers. For example, it is necessary to:

"Consult with industry and other interests to develop timetables to sunset the use of chlorine and chlorine containing compounds as industrial feedstocks, and examine the means of reducing and eliminating other uses, recognising that socioeconomic considerations must be taken into account in developing the strategies and timetables".

and, essential that:

"Governments, industry and labour begin devising plans to cope with economic and social dislocation that may occur as a result of sunsetting persistent toxic substances" (IJC 1994).

Adoption of Zero Discharge
The aim of "zero discharge" is to halt environmental emissions of all hazardous substances into the environment. Although it is sometimes discussed as being simplistic or even impossible, it is a goal whereby regulation can be seen as resting places on the way to achieving it (Sprague 1991).

Zero discharge necessitates the adoption of clean production techniques both in industry and agriculture. It is essential that the change to clean production and material use should be fully supported by fiscal incentives and enforceable legislation.

The principle of clean production has already been endorsed by the Governing Council of the UNEP and has received growing recognition at a wide range of international fora. For example, the Fourth Ministerial Conference on the Protection of the North Sea committed signatory states to the cessation of all discharges, emissions and losses of hazardous substances within 25 years (MINDEC 1995). This essentially represents the adoption of a zero discharge strategy (Johnston 1996b). The North Sea States agreed at the conference:

"to pursue the development and use of clean technology for production processes",

and,

"to give priority to the development of environmentally sound products, taking into account the whole life cycle of substances or products".

The UK government was the only country at the North Sea Conference to object to the target of cessation within 25 years, but recently, the new UK government has agreed on a new approach to policy, including a commitment to address discharges of hazardous substances.

Industry Must Take Responsibility
In the long-term, industry must adopt the principles of clean production. In the interim and thereafter, the responsibility must rest with those who manufacture and market chemicals to assure product safety beyond reasonable doubt. In the judgement of experts taking part at the Erice meeting on endocrine disrupters in 1995, (Alleva *et al.* 1995),

"Manufacturers should be required to release the names of all chemicals used in their products with the appropriate evidence that the products pose no developmental health hazard".

Presently, emphasis must be placed on the identification of safer substitute processes and products, many of which already exist. Industry must urgently prepare for this change in markets.

Politicians Must Take Responsibilty
At a political level, several important statements have already been made, and decisions taken on chemicals which are persistent organic pollutants. These chemicals include some endocrine-disrupting chemicals:

* Paris Commission (PARCOM 1992) - Concerns over the toxicity and persistence of nonylphenol, a breakdown product of nonylphenol ethoxylate (NPE) detergents, led to a recommendation by the Paris Commission to phase out their household use by 1995 and industrial use by the year 2000.

* Oslo and Paris Commissions (1992) – 13 nations on the North East Atlantic and the Commision of European Communities agreed to eliminate discharges of persistent, bioaccumulative toxic substances, particularly organohalogens.

While decisions taken at these conferences are extremely important, ultimately to solve problems posed by persistent toxic chemicals, and other endocrine disrupters, decisions need to be taken at a global level. For successful implementation, these decisions need to be legally binding. This was recognised by experts at a conference in Tromso, Norway, in June 1997, in relation to problems of persistent chemicals in the Arctic. The Arctic Monitoring and Assessment Programme (AMAP 1997) reached the conclusion that,

"The long-term reduction of exposure to persistent organic pollutants can only be accomplished through international conventions on bans and restrictions in production and use of these substances".

Increasingly the issue of endocrine-disruption is also being recognised by political bodies in its own right:

* In a statement issued on 11th April 1997, the Health Council of the Netherlands warned that the entire population may be exposed to endocrine-disrupting chemicals and that some effects on human development and reproduction may be predicted (Health Council of the Netherlands 1997).

* A draft strategy to address hazardous substances, including endocrine disrupters, will be considered by the joint meeting of the Oslo and Paris Commissions (OSPAR) in September 1997. Recently, the UK government noted that they would work with other states to address the emerging issues such as endocrine disrupters which could pose serious problems.

Although these are important steps forward, there is still a long way to go. It is essential to ensure that decisions taken at political level will lead to effective action. Endocrine-disrupting chemicals are a global problem and require global responsibility.

The Public Needs Information
Existing information regarding the potential impacts of endocrine-disrupting chemicals is not being communicated effectively to the public. There is an urgent need to give the public information about chemicals used in the products they buy, to enable people to make informed decisions.

15.3 Conclusions
There is increasing evidence of adverse changes in health and development in humans and wildlife at a population level. In many cases these effects are suspected to be caused by interferences with hormone systems, possibly as a result of exposure to endocrine-disrupting chemicals. We may never have scientific proof that a particular chemical or group of chemicals is responsible. However, the potential impacts that endocrine-disrupting chemicals may be having now, and on future generations, are so far reaching that to not take action now is unthinkable.

Presently there is no consensus to take action to phase out all known endocrine-disrupting chemicals and exposure to these chemicals therefore continues. We must learn rapidly from the mistakes of the past. History has shown us that we have been very bad at predicting risks of exposure to chemicals. It is essential that products and processes are no longer assumed to be safe until proven otherwise. If not, we will continue to experiment with our lives and the lives of our children.

The contention from industry that there is still insufficient information on which to act is untrue. Although the problem is complex, involving a wide range of chemicals and effects, there is already enough evidence regarding some chemicals for them to be banned. The fact that a chemical is able to interfere with hormones in *in vitro* or *in vivo* tests should be enough for it to be targeted for elimination.

The only way forward is to take action to phase out known endocrine-disrupting chemicals, implement safer alternatives, and rapidly adopt clean production technologies. Such action needs to be enforced by legally binding international agreements.

Table 1. KNOWN AND SUSPECTED ENDOCRINE DISRUPTERS AND THEIR USES

CHEMICAL	USES/COMMENTS
ORGANOHALOGENS	
Dioxins	Unintentionally produced by-products of processes in which chlorine and chlorine derived chemicals are produced, used and disposed of. Combustion processes such as waste incineration are a major source.
Several Polychlorinated biphenyls (PCBs)	Now banned worldwide, but still found in old electrical equipment. Due to persistence they still ubiquitous in the environment. Inputs into the environment continue from waste dump leakage.
Perchloroethylene (PERC)	The main solvent used in dry-cleaning. Water-based alternatives to dry-cleaning are now available.
Halogenated phenols: pentachlorophenol (PCP),	Used as a wood preservative and in textiles. Now banned in some European countries.
Polybrominated bisphenol-A,	Widely used as a flame retardant in plastics.
4-Cl-3-methylphenol,	Used in cosmetic products.
4-Cl-2-methylphenol	Used as an additive in pesticides.

PESTICIDES	
amitrole, benomyl, carbaryl, carbofuran, several conazole fungicides (eg. propiconazole), diazinon, linuron, mancozeb, maneb, metiram, metribuzin, oxydemeton-methyl, parathion, phenylphenol, procymidone, certain synthetic pyrethroids (eg. permethrin, phenothrin), thiram, tributyl tin (TBT), vinclozolin, zineb, ziram	Used in agriculture and aquaculture. Other applications include, eg. the use of TBT in anti-fouling paints on ships.
Organochlorine Pesticides: alachlor, atrazine, chlordane, chlordecone (kepone), DDT, DDE, DBCP, dicofol, dieldrin, endosulfan, hexachlorobenzene, beta-HCH, gamma-HCH (lindane), methoxychlor, mirex, toxaphene, transnonachlor	
PLASTICS	
Phthalate plasticisers: eg. benzylbutylphthalate (BBP), di-n-butylphthalate (DBP)	Used in PVC, polyvinyl acetate, polyurethane and some polystyrene plastics. About 90% used in the manufacture of PVC to make numerous products eg. flooring, water pipes, cables, furniture, children's toys, pharmaceutical packaging including blood bags. Phthalates are also used in non-plastic applications eg. Paints, pesticides, inks, hairspray and insect repellents.

INDUSTRIAL CHEMICALS

ALKYLPHENOLIC CHEMICALS: several including 4-nonylphenol, 4-tert-octylphenol	Breakdown products of industrial alkylphenol ethoxylate detergents and pesticide additives. Also used in paint, textiles, metal finishing, certain plastics and lubricating oils.
Bisphenol-A	Main uses in polycarbonate plastics and epoxy resins. Also for coating thermosensitive paper, stabiliser for PVC softeners, tyre production.
t-Butylhydroxyanisole (BHA)	Used as an antioxidant, especially in foods
Lead	Main use is in batteries. Also used in the production of chemicals, petrol additives, various metal products and ammunition.
Methylmercury (organic form of mercury)	Mercury uses include electrical equipment, batteries, production of chlorine gas. It is a by-product of gold mining. Several uses eg. in fungicides have declined in past 20 years.
Cadmium	Main uses for batteries and metal plating, also for pigments, plastics and synthetics.
Styrenes	Used to produce polystyrene; also released from some polystyrene applications.
Certain polyaromatic hydrocarbons	Formed in combustion processes eg. burning fossil fuels. Ubiquitous in the environment and food.
Dimethyl formamide (DMFA)	Common industrial and laboratory solvent. Used in the production of synthetic leather products.
Ethylene glycol	Common industrial and laboratory solvent. Used in the production of polymers for fabrics, and even in food products.

Source: Adapted from Colborn *et al.*1993, Jobling and Sumpter 1993, White et al. 1994, Bradlow *et al.* 1995, Jobling *et al.*1995 and 1996, Kime 1995, Soto *et al.* 1995, Toppari *et al.* 1995, Lyons 1996, Ren *et al.* 1996, Korner *et al.* 1997 and personal communication, Moore and Waring (1997), Santodonato 1997.

Table 2: Compounds that may have the potential to cause adverse effects through the Ah receptor mediated mechanism of action (depending on experimental evidence or structure).

Polycyclic aromatic hydrocarbons (PAHs)	Polychlorinated fluorenes
Polychlorinated biphenyls (PCBs)	Polychlorinated dihydroanthracenes
Polychlorinated dibenzo-p-dioxins	Polychlorinated diphenylmethanes
Polychlorinated dibenzofurans	Polychlorinated phenylxylylethanes
Polychlorinated napthalenes	Polychlorinated dibenzothiophenes
Polychlorinated diphenyltoluenes	Polychlorinated quaterphenyls
Polychlorinated diphenyl ethers	Polychlorinated quaterphenyl ethers
Polychlorinated anisole	Polychlorinated biphenylenes
Polychlorinated phenoxy anisoles	Polychlorinated thioanthrenes
Polychlorinated xanthenes	Polybrominated diphenyl ethers
Polychlorinated xanthones	Polychlorinated azoanthracenes
Polychlorinated anthracenes	

Source: Giesy *et al.* (1994).

REFERENCES

Adlercreutz H. (1995). Phytoestrogens: epidemiology and possible role in cancer protection. Environmental Health Perspectives 103 (Suppl. 7): 103-112.

Aggazzotti G., Fantuzzi G, Predieri G., Gobba F.M., Calvalleri A. (1994). Occupational and environmental exposure to perchloroethylene (PCE) in dry cleaners and their families. Archives of Environmental Health 49 (6): 487-493.

Aguilar A. and Borrell A. (1994). Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990-1992 Mediterranean epizootic. The Science of the Total Environment 154: 237-247.

Alleva E., Brock J., Brouwe A., Colborn T., Fossi C., Gray E., Guillette L., Hauser P., Leatherland J., MacLusky N., Mutti A., Palanza P., Porterfield S., Santti R., Stein S.A., vom Saal F. and Weiss B. (1995). Consensus Statement from the work session on environmental endocrine disrupting chemicals: neural , endocrine and behavioural effects, held at Erice, Sicily, November 5-10, 1995.

AMAP (1997). Arctic Pollution Issues. A State of the Arctic Environmental Report. AMAP, Arctic Monitoring ans Assessment Programme, Oslo. ISBN 82-7655-060-6.

Ando M., Saito H. and Wakisaka I. (1986). Gas chromatographic and mass spectrometric analysis of polychlorinated biphenyls in human placenta and cord blood. Environmental Research. 41: 14-22.

Auger J., Kuntsmann J.M., Czyglik F. and Jouannet P. (1995). Decline in semen quality among fertile men in Paris during the past 20 years. The New England Journal of Medicine. 332 (5): 281-285.

Barrett J. (1996). Phytoestrogens: friends or foes? Environmental Health Perpectives 104 (5): 478-482.

Bason C.W. and Colborn T. (1992). US application and distribution of pesticides and industrial chemicals capable of disrupting endocrine and immune systems. In: Colborn T. and Clement C., (Eds.), Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Princeton, New Jersey: 335-344.

Berczi I. (1989). Immunoregulation by neuroendocrine factors. Dev. Comp. Immunol. 13: 329-341.

Bernier J., Brousseau P., Krystyniak K., Tryphonas H. and Fournier M. (1995). Immunotoxicity of heavy metals in relation to Great Lakes. Environmental Health Perspectives 103 (Suppl. 9): 23-34.

Blair P.B., Noller K.L., Turiel J., Forghani B. and Hagens S. (1992). Disease patterns and antibody responses to viral antigens in women exposed in *utero* to diethylstilbestrol. In: Colborn T. and Clement C., eds. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Princeton, New Jersey:283-288.

Blair P.B. (1992). Immunologic studies of women exposed in utero to diethylstilbestrol. In: Bradlow H.L., Davis D.L., Lin G., Sepkovic D. and Tiwari R. (1995). Effects of pesticides on the ratio of 16a/2-hydroxyestrone: a biologic marker of breast cancer risk. Environmental Health Perspectives 103 (Suppl. 7): 147-150.

Brotons J.A., Olea-Serrano M.F., Villalobos M., Pedraza V. and Olea N. (1996). Xenoestrogens released from laquer coatings in food cans. Environmental Health Perspectives 103 (6): 608-612.

Brouwer A., Ahlborg U.G., van den Berg M., Birnbaum L.S., Rund-Boersma E., Bosveld B., Denison M.S., Gray L.E., Hagmar L., Holene E., Huisman M., Jacobson S.W., Jacobson J.L., Koopman-Esseboom C., Koppe J.G., Kulig B.M., Morse D.C., Muckle G., Peterson R.E., Sauer j. P.J.J., Seegal R.F., Smits-van Prooije A.E., Touwen B.C.L., Weisglas-Kupertis N. and Winneke. (1995). Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. (Outcome of a meeting held from May 9-11, 1994 in Wageningen, Netherlands under auspices of the EERO Foundation Training and Assessment, Wageningen, Netherlands. Eur. J. Pharmacol. *Environ. Toxicol &* Pharmacol. Section 293: 1-40.

Bujan L., Mansat A., Pontonnier F., Mieusset R. Time series analysis of sperm concentration in fertile men in Toulouse, France between 1977 and 1992. BMJ 312: 471-472

Bustos S., Denegri J.C., Diaz F. and Tchernitchin A.N. (1988). p,p'-DDT is an estrogenic compound. Bull. Environ. Contam. Toxicol 41: 496-501.

Carlsen E., Giwercman A., Keiding N. and Skakkebaek N.E. (1992). Evidence for decreasing quality of semen during the past 50 years. British Medical Journal 305: 609-613.

Chen J., Guo Y.L., Hsu C.C. and Rogan W.J. (1992). Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat degraded PCBs. JAMA 268 (22): 3213-3218.

Colborn T., Davidson A., Green S.N., Hodge R.A., Jackson C.I. and Liroff, R.A. (1990). "Great Lakes Great Legacy?" Washington, D.C.: The Conservation Foundation.

Colborn T., vom Saal F.S. and Soto A.M. (1993). Developmental Effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives. 101 (5): 378-384.

Colborn T., Dumanoski D. and Myers J.P (1996). In: Our stolen future. A Dutton Book, published by Penguin Books, USA.

Crain D.A., Guillette L.J., Rooney A.A. and Pickford D.B. (1997). Alterations in steriodogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants. Environmental Health Perspectives 105 (5): 528-533.

Culliney T.W., Pimentel D. and Pimentel M.H. (1992). Pesticides and natural toxicants in foods. Agriculture, Ecosystems and Environment 41: 297-320.

Danzo B.J. (1997). Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. Environmental Health Perspectives 105 (3): 294-301.

Davis D.L., Bradlow H.L., Wolff M., Woodruff T., Hoel D.G. and Anton-Culver H. (1993). Medical Hypothesis: Xenoestrogens as preventable causes of breast cancer. Environmental Health Perspectives 101 (5):372-377.

Davis D.L. and Bradlow H.L. (1995). Can environmental estrogens cause breast cancer? Scientific American, Oct. 166-172.

Desbrow C., Routledge E., Sheehan D., Waldock M. and Sumpter J. (1996). The identification and assessment of oestrogenic substances in sewage treatment works effluents. Published by: Environment Agency, P2-i490/7.

Dewailly E., Dodin S., Verreault R., Ayotte P., Sauve L. and Morin J. (1993). High organochlorine body burden in breast cancer women with estrogen receptors. In: Dioxin'93, 13th International Symposium on Chlorinated Dioxins and Related Compounds
Vienna, September 1993. Organohalogen Compounds. 13: 385-388.

Dewailly E., Ryan J.J., Laliberte C., Bruneau S., Weber J-P., Gringras S. and Carrier G. (1994). Exposure of remote maritime populations to coplanar PCBs. Environmental Health Perspectives Supplements. 102 suppl. 1: 205-209.

Dirksen S., Boudewijn J., Slager L.K., Mes R.G., van Schaick M.J.M. and de Voogt P. (1995). Reduced breeding success of cormorants *(Phalacrocorax carbo sinensis)* in relation to persistent organochlorine pollution of aquatic habitats in The Netherlands. Environmental Pollution 88: 119-132.

Dirven H.A.A.M., van den Broek P.H.H. and Jongeneelen F.J. (1993). Determination of four metabolites of the plasticizer di(2-ethylhexyl)phthalate in human urine samples. Int. Arch Occup Environ Health 64: 555-560.

Dohler K.D. and Jarzab B. (1992). The influence of hormones and hormone anatonists on sexual differentiation of the brain. In: Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection, T. Colborn and C. Clement (eds.). Princeton Scientific Publishing Co., Inc., Princeton, New Jersey: 231-260.

ENDS (1997a). Reassurances on phthalates fail to convince. The ENDS Report 270, (July): 33-34. Published by Environmental Data Services Ltd.

ENDS (1997b). Industry and scientists in cross-fire on endocrine-disrupting chemicals. The ENDS Report 268, (May): 26-29. Published by Environmental Data Services Ltd.

Facemire C.F., Gross T.S. and Guillette J. (1995). Reproductive impairment in the Florida panther: Nature or nurture? Environmental Health Perspectives 103 (Suppl. 4): 79-85.

Feldman D. and Krishnan A. Estrogens in unexpected places: possible implications for researchers and consumers. Environmental Health Perspectives 103 (Suppl. 7): 129-133.

Ferroni C., Selis L., Mutti A., Folli D., Bergamaschi E. and Franchini I. (1992). Neurobehavioural and neuroendocrine effects of occupational exposure to perchloroethylene. Neurotoxicology 13: 243-248.

Fisch H. and Goluboff E.T. (1996). Geographical variations in sperm counts: a potential cause of bias in studies of semen quality. Fertility and Sterility 65: 1044-1046.

Fox G.A. (1992). Epidemiological and pathobiological evidence of contaminant-induced alterations in sexual development in free-living wildlife. In: Colborn T., and Clement C., eds. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc, Princton, New Jersey: 147-158.

Fry D.M. (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. Environmental Health Perspectives 103 (Suppl. 7): 165-171.

Furst P., Furst C. and Wilmers K. (1994). Human milk as a bioindicator for body burden of PCDDs, PCDFs, organochlorine pesticides, and PCBs. Environmental Health Perspectives Supplements. 102 suppl. 1: 187-193.

Galetin-Smith R., Pavkov S. and Roncevic N. (1990). DDT and PCBs in human milk: Implication for breast feeding infants. Bull. Environ. Contam. Toxicol. 45: 811-818.

Giesy J.P. Ludwig J.P. and Tillitt D.E. (1994). Deformities in birds of the Great Lakes region: asssigning causality. Environmental Science and Technology 28 (3): 128-135.

Gill W.B., Schumacher G.F.B., Hubby M.M. and Blough R.R. (1981). Male genital tract cheanges in humans following intrauterine exposure to diethylstilbestrol. In: A.L. Herbst and H.A. Bern, eds. Developmental Effects of Diethylstilbestrol (DES) in Pregnancy. Thieme-Stratton Inc. New York: 103-119.

Gilman A.P., Van Oostdam J.C., Hansen J.C., Odland J.O. Tchachtchine V., Walker J., Lagerkvist B.J., Olafsdottir K., Weber J.P. Bjerregaard P. and Klopov V. (1997). The AMAP International Symposium on Environmental Pollution in the Arctic. (Extended Abstracts volume1: 57-58). Tromso, Norway, June 1-5, 1997.

Giwercman A., Carlsen E., Keiding N. and Skakkebaek N.E. (1993). Evidence for increasing incidence of abnormalities of the human testis: A review. Environmental

Health Perspectives Supplements. 101 (Suppl. 2): 65-71.

Gladen B.C. and Rogan W.J. (1995). DDE and shortened duration of lactation in a northern Mexican town. American Journal of Public Health 85 (4): 504-508.

Gray L.E. (1992). Chemical-induced alterations of sexual differentiation: a review of effects in humans and rodents. In: Colborn T. and Clement C., (Eds.), Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Princeton, New Jersey: 203-230.

Gray L.E., Ostby J.S., and Kelce W. (1994). Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. Toxicology and Applied Pharmacology 129: 46-52.

Gray L.E., Kelce W.R., Monosson E., Ostby J.S. and Birnbaum L.S. (1995a). Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: Reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. Toxicology and Applied Pharmacology 131: 108-118.

Gray L.E. and Ostby J.S. (1995b). In utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. Toxicology and Applied Pharmacology 133: 285-294.

Greenpeace (1996). Building for the Future. A guide to building without PVC. Published by Greenpeace UK, Canonbury Villas, London, N1 2PN.

Grossman C.J. (1984). Regulation of the immune system by sex steroids. Endocrine Reviews 5 (3): 435-455.

Guillette L.J., Gross T.S., Masson G.R., Matter J.M., Percival H.F. and Woodward A.R. (1994). Developmental abnormalites of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environmental Health Perspectives 102 (8): 680-688.

Guillette L.J., Crain A.D., Rooney A.A. and Pickford D.B. (1995). Organization versus activation: The role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environmental Health Perspectives 103 (Suppl 7): 157-164.

de Guise S., Martineau D., Beland P. and Fournier M. (1995). Possible mechanisms of action of environmental contaminants on St. Lawrence Beluga Whales (*Delphinapterus leucas*). Environmental Health Perspectives 103 (Suppl 4): 73-77.

Guo Y.L., Lai T.J., Ju S.H., Chen Y.C. and Hsu C.C. (1993). Sexual development and biological findings in Yucheng Children. In:Dioxin '93. 13th International Symposium on Chlorinated Dioxins and Related Compounds, Vienna September 1993. Organohalogen Compounds, 14: 235-238.

Guo Y.L., Chen Y.C., Yu M.L. and Chen C.H. (1994). Early development of Yu-Cheng children born seven to twelve years after the Taiwan PCB outbreak. Chemosphere 29 (9-11): 2395-2404.

Guo Y.L., Lambert G.H. and Hsu C-C. (1995). Growth abnormalities in the population exposed in utero and early postnatally to polychlorinated biphenyls and dibenzofurans. Environmental Health Perspectives 103 (Suppl.6): 117-122.

Hall R.H. (1992). A new threat to public health: Organochlorines and food. Nutrition and Health. 8: 33-43.

Harris C.A., Henttu P., Parker M.G., Sumpter J.P. (1997). The estrogenic activity of phthalate esters *in vitro*. Environmental Health Perspectives 105 (8): 802-811.

Harris J.R., Lippman M.E., Veronesi U. and Willett W. (1992). Breast Cancer (First of three parts). The New England Journal of Medicine. 327 (5):319-328.

Health Council of the Netherlands (1997). Committee on Endocrine disrupters in humans. Hormone disrupters in humans. Rijswijk: Health Council of the Netherlands, 1997; publication no. 1997/08. ISBN 90-5549-156-X.

Health Inspector of Public Health, The Netherlands, (1995). (Hoofdinspectie Gezondheidsbescherming). Report of Monitoring Results, Concerning Directive 90/642/EEC.

Heuvel van den, M.R., Munkittrick K.R., van der Kraak G.J., McMaster M.E., Porrt C.B., Servos M.R. and Dixon D.G. (1994). Survey of receiving-water environmental impacts associated with discharges from pulp mills. 4. Bioassay derived 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalent concentration in the white sucker (*Catostomus commersoni*) in relation to biochemical indicators of impact. Environmental Toxicology and Chemistry 13 (7): 1117-1126.

Hines M. (1992). Surrounded by estrogens? Considerations for neurobehavioural development in human beings. In: Colborn T. and Clement C., eds. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Princeton, New Jersey: 261-281

HMSO (1995). Pesticides in surface waters. ISBN 92 871 2776 X. (Cited in The ENDS Report 248, Oct. 1995: 10-11. Published by Environmental Data Services Ltd.)

Holladay S.D. and Luster M.I. (1996). Alterations in fetal thymic and liver hematopoietic cells as indicators of exposure to developmental immunotoxicants. Environmental Health Perspectives 104 (Suppl 4): 809-813.

Howard C.V. Chemical mixtures and synergism... the end of traditional toxicology? Presented at: Effects of Environmental Pollutants on Reproduction. Halle (Saale), Germany, 11-13th April.

Howard C.V. (1997 *in prep*). Phthalates in cord blood.

Hughes C.L. (1988). Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. Environmental Health Perspectives 78: 171-175.

Huisman M., Koopman-Esseboom C., Lanting C.I., Van der Paauw C.G., Tuinstra L.G.M., Fidler V., Weiglas-Kuperus N., Sauer P.J.J., Boersma E.R. and Touwen B.C.L.(1995). Neurological condition in 18-month old children perinatally exposed to PCBs and dioxins. Early human development 43: 165-176.

Ilsen A., Briet J.M., Koppe J.G., Pluim H.J. and Oosting J. (1996). Signs of enhanced neuromotor maturation in children due to perinatal load with background levels of dioxins. Chemosphere 33 (7): 1317-1326.

IJC (1994). International Joint Commission on the Great Lakes. Seventh Biennial Report. Windsor: International Joint Commission.

Irvine S., Cawood E., Richardson D., MacDonald E. and Aitken J. (1996). Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. British Medical Journal 312: 467-471.

Iwata H., Tanabe S., Sakai N. and Tatsukawa R. (1993). Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ. Sci. Technol. 27 (6): 1080-1098.

Iwata H., Tanabe S., Sakai N., Nishimura A. and Tatsukawa R. (1994). Geographical distribution of persistent organochlorines in air, water and sediments from Asia and Oceania, and their implications for global redistribution from lower latitudes. Environmental pollution 85: 15-33.

Jacobson S.W., Fein G.G., Jacobson J.L., Schwartz P.M. and Dowler J.K. (1985). The effect of interuterine PCB exposure on visual recognition memory. Child Development 56: 853-860.

Jacobson, J.L. & Jacobson, S.W. (1993). A 4-year followup study of children born to consumers of Lake Michigan fish. J. Great Lakes Res. 19, 4: 776-783

Jacobson J.L. and Jacobson S.W. (1996). Intellectual impairment in children exposed to polychlorinated biphenyls in utero. New England Journal of Medicine 335 (11): 783-789.

Janssen P.A.H., Dalessi D.L.W.M., Lambert J.G.D., Vethaak A.D., van Wezel A.P. and Goos H.J.Th. (1997). Oestrogenic effectsin the flounder, *Platichthys Flesus,* after exposure to polluted harbour sediment. (Abstract). Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands, 6-10 April.

Jensen T.K., Toppari J., Keiding N. and Skakkebaek N. (1995). Do environmental estrogens contribute to the decline in male reproductive health? Clinical Chemistry 41 (12): 1896-1901.

Jobling S. and Sumpter J.P. (1993). Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquatic toxicology 27: 361-372.

Jobling S., Reynolds T., White R., Parker M.G. and Sumpter J.P. (1995). A variety of environmentally persistent chemicals, including some phthalte plasticizers, are weakly esttrogenic. Environmental Health Perspectives 103 (6): 582-587.

Jobling S., Sheahan D., Osbourne J.A., Matthissen P. and Sumpter J.P. (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology and Chemistry 15 (2): 194-202.

Johnston P.J and McCrea I. (1992). Death in Small Doses. Published by Greenpeace International, ISBN 1871532 61 2.

Johnston P.A., Santillo D. and Stringer R. (1996a). Risk assessment and reality: recognising the limitations. In: Environmental Impact of Chemicals: Assessment and Control,: Quint M.D , Taylor D. and Purchase R. (eds.) Published by The Royal Society of Chemistry, special publication no. 176, ISBN 0-85404-795-6: Chapter 16, 223-239.

Johnston P.A., Stringer R.L. and Santillo D. (1996b). Effluent complexity and ecotoxicology: regulating the variable within varied systems. TEN 3 (4): 115-120.

Johnston P.A., Stringer R., Santillo D. and Howard V. (1996, *in press*). Hazard, exposure and ecological risk assessment.

Kanja L.W. Skaare J.U., Ojwang S.B.O. and Maitai C.K. (1992). A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood, and human milk from mother/infant pairs.Arch. Environ. Contam. Toxicol. 22: 21-24.

Kardinaal A.F.M. (1997). Phytoestrogens - health benefits and safety. (Abstract). Endocrine Active Compounds in Food and Environment - setting the research agenda - in cooperation with VLAG, and WAS. April 11th, Conference Centre "The Reehorst", Ede, The Netherlands.

Katzenellenbogen J.A. (1997). The structural pervasiveness of estrogenic activity. Environmental Health Perspectives 103 (Suppl 7): 99-101.

Kelce W.R., Monosson E., Gamcsik M.P., Laws S.C. and Gray L.E. (1994). Environmental hormone disrupters: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. Toxicology and Applied pharmacology 126 (2): 276-285.

Kelce W.R., Stone C.R., Laws S.C., Gray L.E., Kemppainen J.A. and Wilson E.M. (1995). Persitent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature 375: 581-585.

van Klaveren J.D. (1997). Resultaten residubewaking in Nederland (Results of residue monitoring in The Netherlands). RIKILT-DLO. Part of: KAP, Kwaliteitsprogramma agrarische producten). January 1997.

Kime D.E. (1995). The effects of pollution on reproduction in fish. Reviews in Fish Biology and Fisheries 5: 52-96.

Koninckx P.R., Braet P., Kennedy S.H. and Barlow D.H. (1994). Dioxin pollution in and endometriosis in Belgium. Human Reproduction 9 (6): 1001-1002.

Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Van der Paauw C.G., Tuinstra L.G.M.Th., Boersma E.R. and Sauer P.J.J. (1994). PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 28 (9): 1721-1732.

Koopman-Esseboom C., Weisglas-Kuperus N., de Riddler M.A.J., Van der Paauw C.G., Tuinstra L.G.M.T. and Sauer P.J.J. (1995). Effects of PCBs/dioxin exposure and feeding type on the infants mental and psychomotor development. In: Effects of perinatal exposure to PCBs and dioxins on early human development by C. Koopman-Esseboom, ISBN 90-75340-03-6, (Chapter 8).

Korner W., Hanf V., Schuller W., Zwirner M. and Hagenmaier H. (1997). Assessment of estrogenic potency of halogenated phenols and cresols. (Abstract). Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands, 6-10 April.

Kuhnlein H.V., Receveur O., Muir D.C.G., Chan H.M. and Soueida R. (1995). Arctic indigenous women consume greater than acceptable limits of organochlorines. Journal of Nutrition 125 (10): 2501-10.

Kyyronen P., Taskinen H., Lindbohm M.L., Hemminki K. and Heinonen O.P. (1989). Spontaneous abortions and congenital malformations in women exposed to tetrachloroethylene in dry-cleaning. J. Epidemiol. Community Health 43: 346-351. (Cited in Aggazzotti et al. 1994).

Lahvis G.P., Wells R.S., Kuehl D.W., Stewart J.L., Rhinehart H.L. Via C.S. (1995). Decreased lymphocyte responses in free-ranging bottlenose dolphins (Tursiops truncatus) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environmental Health Perspectives 103 (Suppl 4): 67-72. (Cited in US EPA 1997).

Landner L., Grahn O., Hardig J., Lehtinen K-J., Monfelt C. and Tana J. (1994). A field study of environmental impacts at a bleached kraft pulp mill site on the Baltic Sea coast. Ecotoxicoloty and Environmental Safety 27: 128-157.

Lindbohm M.L., Taskinen E., Kyyronen P., Sallmen P., Anttila A. and Hemminki K. (1992). Effects of parental occupational exposure to solvents and lead on spontaneous abortion. Scand. J. Work Environ. Health 18 (Suppl 2: 37-39. (Cited in Aggazzotti et al. 1994).

Lindstrom G., Dahl P. and Rappe C. (1994). Postnatal exposure of polychlorinated biphenyls (PCBs) from breast milk by three Swedish infants, two respectively three months post partum. 14th International Symposium on Chlorinated Dioxins, PCBs and Related Compounds, Kyoto, Japan. Organohalogen compounds 21: 255-258.

Longanathan, B., and Kannan, K. (1994). Global organochlorine contamination trends: an overview. AMBIO 23 (3):187-189.

Lopez-Carrillo L., Torres-Arrela L., Torres-Sanchez L., Espinosa-Torres F., Jimenez C., Cebrian M., Waliszewski S. and Saldate O. (1996). Is DDT use a public health problem in Mexico? Environmental Health Perspectives 104 (6): 584-588.

Lye C. and Frid C.L.J. (1997). Impacts of oestrogenic compounds in estuarine waters. (Abstract). Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands,
6-10 April.

Lyons G. (1996). Pesticides posing hazards to reproduction. A report for WWF on pesticides which disrupt hormones and reproduction. Published by WWF-UK.

Mably T.A., Bjerke D.L., Moore R.W., Gendron-Fitzpatrick A. and Peterson R.E. (1992). In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductive capability. Toxicology and Applied Pharmacology 114: 118-126.

MAFF (1995). Ministry of Agriculture, Fisheries and Food (UK), Food Safety Directorate. Phthalates in paper and board packaging. Food surveillance information sheet number 60, May 1995.

MAFF (1996). Ministry of Agriculture, Fisheries and Food (UK), Food Safety Directorate. Phthalates in food. Food surveillance information sheet number 82, March 1996.

MAFF (1997). Dioxins and Polychlorinated biphenyls in foods and human milk. Food surveillance information sheet 105, June 1997.

Marien K. (1997). DDT/DDE infant exposure. Environmental Health Perspectives 105 (1): 14-15.

Matthiessen P. (1996). Evidence for contamination of surface waters with chemicals oestrogenic to fish. Presented at: Oestrogenic Chemicals in the Environment 9-10 May, 1996 - One Whitehall Place, London. Organised by IBC UK Conferences Ltd, Gilmoora House, 57/61 Mortimer Street, London, W1N 8JX.

McKinney J.D., and Waller C.L. (1994). Polychlorinated biphenyls as hormonally active structural analogues. Environmental Health Perspectives 102 (3): 290-297

McLachlan J.A. (1993). Functional toxicology: a new approach to detect biologically active xenobiotics. Environmental Health Persepectives 101 (5): 386-387.

Meek M.E. and Chan P.K.L. (1994). Bis(2-ethylhexyl)phthalate: evaluation of risks to health from environmental exposure in Canada. Environ. Carcino. & Ecotox. Revs. C12 (2): 179-194.

Mehta R.H. and Kumar T.C.A. (1997). Declining semen quality in Bangaloreans: a preliminary report. Current Science 72 (9): 621-622.

Mennear J.H. and Lee C-C. (1994). Polybrominated dibenzo-p-dioxins and dibenzofurans: Literature review and health assessment. Environmental Health Perspectives. 102 Suppl. 1: 265-274.

Michal F., Grigor K.M., Negro-Vilar A. and Skakkebaek N.E. (1993). Impact of the environment on reproductive health: Executive summary. Environmental Health Perspectives Supplements 101 (Suppl. 2): 159-167.

MINDEC (1995). Ministerial Declaration of the Fourth International Conference on the Protection of the North Sea, Esbjerg, Denmark.

Moore A. and Waring C. Sublethal effects of pesticides on pheromonal mediated endocrine function in mature male atlantic salmon. (Abstract). Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands, 6-10 April.

Mocarelli P., Brambilla P., Gerthoux P.M., Patterson D.G. and Needham L.L. (1996). Change in sex ratio with exposure to dioxin. The Lancet 348: 409.

Moller H. (1996). Change in male:female ratio among newborn infants in Demark. The Lancet 348: 828-829.

Morse *D.C., Koeter H.B.W.M.*, Smits van Prooijen A.E. and Brouwer A. (1992). Interference of polychlorinated biphenyls in thyroid hormone metabolism: possible neurotoxic consequences in fetal and neonatal rats. Chemosphere 25 (1-2): 165-168.

Nagel S.C., vom Saal F.S., Thayer K.A., Dhar M.G., Boechler M., Welshon W.V. (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. Environmental Health Perspectives 105 (1) 70-76.

Nerin C., Cacho J. and Gancedo P. (1993). Plasticizers from printing inks in a selection of food packagings and their migration to food. Food Additives and Contaminants 10 (4): 453-460.

Olsen J., Hemminki K., Ahlborg G., Bjerkedal T., Kyyronen P., Taskinen H., Lindbohm M.L., Heinonen O.P., Brandt L., Kolstad H., Halvorsen B.A. and Egenaes J. (1990). Low birth weight, congenital malformations, and spontaneous abortions among dry cleaning workers in Scandanavia. Scand J. Work Environ. Health 16: 163-168. (Cited in Aggazzotti et al. 1994).

Oslo and Paris Commisions (1992). Oslo and Paris Conventions for the Prevention of Marine Pollution. Final Declaration of the Ministerial Meeting of the Oslo and Paris Commisssions. Paris 21-22 September 1992.

Oslo and Paris Commisions (1997). Oslo and Paris Conventions for the Prevention of Marine Pollution Joint Meeting of the Oslo and Paris Commissions (OSPAR) Brussels: 2-5 September.

Pacyna J.M. (1997). Sources of heavy metal pollution in the Arctic. The AMAP International Symposium on Environmental Pollution in the Arctic. (Extended Abstracts volume1: 140-141). Tromso, Norway, June 1-5, 1997.

Pajarinen J., Laippala P., Penttila A. and Karhunen P.J. (1997). Incidence of disorders of spermatogenesis in middle aged Finnish men, 1981-91: two necropsy series. British Medical Journal 314: 13-18.

van der Pal-de Bruin K.M., Verloove-Vanhorick S.P. and Roeleveld N. (1997). Change in male:female ratio among newborn babies in Netherlands. The Lancet 349: 62.

PARCOM (1992). Recommendation 92/8 on Nonylphenol-Ethoxylates.

Paulsen C.A., Berman N.G. and Wang C. (1996). Data from men in the greater Seattle area reveals no downward trend in semen quality: further evidence tht deterioration of semen quality is not geographically uniform. Fertility and Sterility 65 (5): 1015-1020.

Patlak M. (1997). A testing deadline for endocrine disrupters. Environmental Science and Technology 30 (12): 540A-544A.

Pluim H.J., Koppe J.G., Olie K., van der Slikke J.W., Kok J.H., Vulsma T., van Tijn D. and de Vijlder J.J.M. (1992). Effects of dioxins on thyroid fuction in newborn babies. The Lancet 339:1303

Porterfield S.P. (1994). Vunerability of the developing brain to thyroid abnormalities: Environmental insults to the thyroid system. Environmental Health Perspectives 102 (Suppl. 2):125-130.

Purdom C.E., Hardiman P.A., Bye V.J., Eno N.C., Tyler C.R. and Sumpter J.P. (1994). Estrogenic effects of effluents from sewage treatment works. Chemistry and Ecology 8: 275-285.

Rehana T. and Rao P.R. (1992). Effect of DDT on the immune system in Swiss Albino mice during adult and perinatal exposure:humoral responses. Bull. Environ. Contam. Toxicol. 48: 535-540.

Reijnders P.J.H (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324: 456-457.

Reijnders P.J.H. and Brasseur S.M.J.M (1992). Xenobiotic induced hormonal and associated developmental disorders in marine organisms and related effects in humans; an overview. In: Colborn T and Clement C. (eds.). Chemically-Induced

Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc., Princeton, New Jersey: 159-174.

Ren L., Meldahl A. and Lech J.J. (1996). Dimethyl formamide (DMFA) and ethylene gylcol (EG) are estrogenic in rainbow trout. Chemico-Biological Interactions 102: 63-67.

Rice D.C. (1995). Neurotoxicity of lead, methylmercury, and PCBs in relation to the Great Lakes. Environmental Health Perspectives 103 (9): 71-87.

Rier S.E., Martin D.C., Bowman R.E., Dmowski W.P. and Becker J.L. (1993). Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlordibenzo-p-dioxin. Fundamental and Applied Toxicology 21: 433-441.

Rier S.E., Martin D.C., Bowman R.E. and Becker J.L. (1995). Immunoresponsiveness in endometriosis: implications of estrogenic toxicants. Environmental Health Perspectives 103 (Suppl. 7): 151-156.

Rivero-Rodriguez L., Borja-Aburto V.H., Santos-Burgoa C., Waliszewskiy S., Rios C. and Cruz V. (1997). Exposure assessment for workers applying DDT to control malaria in Veracruz, Mexico. Environmental Health Perspectives 105 (1): 98-101.

Rogan W.J. and Gladen B.C. (1992). Neurotoxicology of PCBs and related compounds. Neurotoxicolgy 13: 27-36.

Ross P.S., de Swart R.L., Reijnders P.J.H., Van Loveren H., Vos J.G. and Osterhaus A.D.M.E. (1995). Contaminant-related suppression of delayed-type hypersensitivity and antibody reponses in harbor seals fed herring from the Baltic Sea. Environmental Health Perspectives 103 (2): 162-167.

Safe S. and Krishnan V. (1995). Chlorinated hydrocarbons: estrogens and antiestrogens. Toxicology Letters 82/83: 731-736.

Santodonato J. (1997). Review of the estrogenic and antiestrogenic activity of polycyclic aromatic hydrocarbons: relationship to carcinogenicity. Chemosphere 34 (4): 835-848.

Santti R., Newbold R.R., Makela S., Pylkkanen L. and McLachlan J. (1994). Developmental estrogenization and prostatic neoplasia. The Prostate 24: 67-78.

Savitz D.A. (1994). Re: Breast cancer and serum organochlorines: prospective study among white, black, and Asian women. J. Natl. Cancer Inst. 86: 1256.

Schecter A. Exposure Assessment: Measurement of Dioxins and Related Chemicals in Human Tissues. In: Dioxins and Health. A Schecter (eds.). Plenum Press. New York and London.

Schwaiger J., Worle B., Bauer C., Braunbeck T., Islinger M., KalbfusW. and Negele R.D. Toxic effects of nonylphenol on rainbow trout (*Oncorynchus mykiss*) and common carp (*Carpio carpio*) with special emphasis on estrogenicity. (Abstract). Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands, 6-10 April.

Seegal R.F. and Shain W (1992). Neurotoxicity of polychlorinated biphenyls. The role of ortho-substituted congeners in altering neurochemical function. The Vulnerable Brain and Environmental Risks, vol 2: Toxins in food: R.L Isaacson and K.F. Jensen (eds.). Pleunum Press, New York: 169-195.

Seo B-W., Li M-H., Hansen L.G., Moore R.W., Peterson R.E. and Schantz S.L. (1995). Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. Toxicology Letters 78: 253-262.

Shane B.S. (1994). 'Introduction to Ecotoxicology', in 'Basic Environmental Toxicology', eds. L.G. Cockerham and B.S. Shane, CRC Press, Boca Raton, FL, 1994, ch. 1, pp. 3-10.

Sharman M., Read W.A., Castle L. and Gilbert J. (1994). Levels of di-(2-ethylhexyl)phthalate and total phthalate esters in milk, cream, butter and cheese. Food additives and contaminants 11 (3): 375-385.

Sharpe R.M. and Skakkebaek N.E. (1993). Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341:1392-1395.

Sharpe R M., Fisher J.S., Millar M.M., Jobling S. and Sumpter J. (1995). Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environmental Health Perspectives 103 (12): 1136-1143.

Simmonds M.P., Johnston P.A. and French M.C. (1993). Organochlorine and mercury contamination in United Kingdom seals. Veterinary Record 132: 291-295.

Skaare J.U. and Polder A. (1990). Polychlorinated biphenyls and organochlorine pesticides in milk of Norweigen women during lactation. Arch. Environ. Contam. Toxicol. 19: 640-645.

Smoger G.H., Kahn P.C., Rodgers G.C., and Suffin S. (1993). In-utero and postnatal exposure to 2,3,7,8-TCDD in Times Beach, Missouri: 1. Immmunological effects: Lymphocyte phenotype frequencies. In: Dioxin '93, 13th International Symposium on Chlorinated Dioxins and Related Compounds, Vienna. Organohalogen Compounds 13: 345-348.

Soto A.M., Lin T-M., Justicia H., Silvia R.M. and Sonnenschein C. (1992). An in culture" bioassay to assess the estrogenicity of xenobiotics (E-Screen). In: Colborn T. and Clement C., eds. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Princeton, New Jersey: 295-309.

Soto A.M., Chung K.L. and Sonnenschein C. (1994). The pesticides endosulfan,

toxaphene and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environmental Health Perspectives 102 (4): 380-383.

Soto A.M., Sonnenschein C., Chung K.L., Fernandez M.F., Olea N. and Serrano F.O. (1995). The E-Screen Assay as a tool to identify estrogens: an update on estrogenic environmental pollutants.

Sprague J.B. (1991). Environmentally desirable approaches for regulating effluents from pulp mills. Wat. Sci. Techno. 24: 361-371.

Stairs K.C. and Johnston P. (1991). The precautionary action approach to environmental protection. Environ. Poll 1 - ICEP.1: 473-479.

Stevens M.F., Ebell G.F. and Psaila-Savona P. (1993). Organochlorine pesticides in Western Australian nursing mothers. The Medical Journal of Australia. 158: 238-241.

Stevens J.T., Breckenridge C.B., Wetzel L.T., Gillis J.H., Luempert L.G. and Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides. Journal of Toxicology and Environmental Health 43:139-153.

Sullivan F.M. (1993). Impact of the environment on reproduction from conception to parturition. Environmental Health Perspectives Supplements 101 (Suppl. 2): 13-18.

Sumpter J.P. and Jobling S. (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environmental Health Perspectives 103 (Suppl 7): 173-178.

de Swart R.L., Ross P.S., Vos J.G. and Osterhaus A.D.M.E. (1996). Impaired immunity in harbour seals (*Phoca vitulina*) exposed to bioaccumulated environmental contaminants: review of a long-term feeding study. Environmental Health Perspectives 104 (Suppl 4): 823-828.

Tanabe S. (1988). PCB problems in the future: foresight from current knowledge. Environmental Pollution 50: 5-28.

Thomas K.B. and Colborn T. (1992). Organochlorine endocrine disruptors in human tissue. In: Colborn T. and Clement C., (Eds), Chemically-Induced Alterations in Sexual Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Priceton, New Jersey: 365-394.

Thomas V.M. and Spiro T.G. (1996). The US dioxin inventory: are there missing sources? Environmental Science and Technology 30 (2): 82-85.

Tiemessen C.H.J., Evers J.L.H., Bots R.S.G.M. (1996). Tight-fitting underwear and sperm quality. The Lancet 347: 1844-1845.

Toniolo P.G., Levitz M., Zeleniuch-Jacquotte A., Banerjee S., Koenig K.L., Shore R.E., Strax P. and Pasternack B.S. (1995). A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J.Natl. Cancer Inst. 87: 190-197.

Toppari J., Larsen J.C., Christiansen P., Giwercman A., Grandjean P., Guillette L.J., Jegou B., Jensen T.K., Jouannet P., Keiding N., Leffers H., McLachlan J.A., Meyer O., Muller J., Rajpert-De Meyts E., Scheike T., Sharpe R., Sumpter J. and Skakkebaek N.E. (1995). Ministry of Environment and Energy. Denmark. Male reproductive health and environmental chemicals with estrogenic effects. Miljoprojekt nr 290 1995. Copenhagen. Danish Environmental Protection Agency, 1995.

Tryphonas H. (1995). Immunotoxicity of PCBs (Aroclors) in relation to Great Lakes. Environmental Health Perspectives 103 (Suppl 9): 35-46.

US EPA (1994). Health assessment document for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Volume III of III. United States Environmental Protection Agency, Office of Research and Development, Washington DC 204 60. EPA/600/BP-92/001c. June 1994. External Review Draft.

US EPA (1997). Special report on environmental endocrine disruption: An effects assessment analysis. Prepared for the Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. EPA/630/R-96/012 February 1997.

Van Waeleghem K., De Clercq N., Vermeulen L., Schoonjans F. and Comhaire F. (1996). Deterioration of sperm quality in young healthy Belgium men. Human Reproduction 11 (2): 325-329.

Vine M.F. (1996). Worldwide decline in semen quality might be due to smoking. British Medical Journal 312: 506.

Vinkelsoe K., Jensen G.H., Johansen E., Carlsen L. and Rastogi S.C. (1997). Migration of phthalates from teething rings. Danish EPA.

vom Saal F.S., Montano M.M. and Wang M.H. (1992). Sexual differentiation in mammals. In: Chemically-induced alterations in sexual and functional development: The wildlife/human connection, Colborn T. and Clement C. (eds.). Princeton Scientific Publishing Co., Inc., Princeton, New Jersey: 17-85.

vom Saal F.S., Nagel S.C., Palanza P., Boechler M., Parmigiani S. and Welshons W.V. (1995). Estrogenic pesticides: binding relative to estradiol in MCF-7 cells and effects of exposure during fetal life on subsequent territorial behaviour in male mice. Toxicology Letters 77: 343-350.

Vonier P.M., Crain D.A., McLachlan J.A., Guillette L.J. and Arnold S.F. (1996). Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environmental Health Perspectives 104 (12): 1318-1322.

Wania F. and Mackay D. (1996). Tracking the distribution of persistent organic pollutants. Environmental Science and Technology 30 (9): 390-396.

Warhurst A.M. (1995). An environmental assessment of alkylphenol ethoxylates and alkylphenols. Published by Friends of the Earth.

Weisglas-Kuperus N., Sas T.C., Koopman-Esseboom C., van der Zwan C., Riddler M.A.J., Boishuizen A., Hooijkaas H. and Sauer P.J.J. (1995). Immunological effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in infants. Pediatric Research 30 (3): 404-410.

Wetzel L.T., Luempert III L.G., Breckenridge M.O., Stevens J.T., Thakur A.K., Extrom P.J. and Eldridge J.C. (1994). Chronic effects of atrazine on estrus and mammary tumor
formation in female Sprague-Dawley and Fischer 344 rats. Journal of Toxicology and Environmental Health 43: 169-182.

White R., Jobling S., Hoare S.A., Sumpter J.P. and Parker M.G. (1994). Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135 (1): 175-182.

Whitten P.L. (1992). Chemical revolution to sexual revolution:historical changes in human reproductive development. In: Colborn T. and Clement C., (Eds.), Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc. Princeton, New Jersey: 311-334.

WHO (1996). Levels of PCBs, PCDDs and PCDFs in human milk. Second round of WHO-coordinated exposure study.World Health Oraganisation European Centre for Environment and Health. Environmental Health in Europe No.3.

Wilcox A.J., Baird D.D., Weinberg C.R., Hornsby P.P. and Herbst A.L. (1995). Fertility in men exposed prenatally to diethylstilbestrol. The New England Journal of Medicine 332 (21): 1411-1416.

Wine R.N., Li L-H., Barnes L.H., Gulati D.K. and Chapin R.E. (1997). Reproductive toxicity of di-n-butylphthalate in a continuous breeding protocol in Sprague-Dawley rats. Environmental Health Perspectivies 105 (1): 102-107.

Wingspread Conference (1991). Statement from the work session on chemically-induced alterations in sexual development: The wildlife/human connection. In: Colborn T., and Clement C., eds. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing Co., Inc, Princton, New Jersey: 1-8.

Wingspread Conference (1993). Statement from the work session on environmentally induced alterations in development: A focus on wildlife. Wingspread conference Centre, Racine, Wisconsin, December 10-12.

Wolff M.S., Toniolo P.G., Lee E.W., Rivera M. and Dubin N. (1993). Blood levels of organochlorine residues and risk of breast cancer. Journal of the National Cancer Institute 85 (8):648-652.

Yu M.L., Hsu C.C., Guo Y.L., Lai T.J., Chen S.J. and Luo J.M. (1994). Disordered behaviour in the early-born Taiwan Yu-Cheng children. Chemosphere 29 (9-11): 2413-2422.

Zielhuis G.A., Gijsen R., Gulden J.W.J. (1989). Menstrual disorders among dry-cleaning workers. Scand. J. Work Environ. Health 15: 238.