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Body of Evidence

The effects of chlorine on human health

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The effects of chlorine on human health

This summary text is based on the full report "Body Burden: Chlorine's effects on the human body 1995" written by Michelle Allsopp, Pat Costner and Paul Johnston.

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Abstract

Over the last 50 years, thousands of organochlorine chemicals have been produced in vast quantities by industry for uses ranging from the production of plastics, pesticides, and bleaching of pulp and paper, to constituents in toothpastes and cosmetics. Numerous organochlorines are also produced unintentionally as by-products of such industrial process which utilise chlorines.

Many industrially produced organochlorines are very stable and highly lipophilic so they accumulate in the environment and in the tissues of animals, reaching the highest levels in animals at the top of food chains. As a consequence of the persistent and bioaccumulative properties of such organochlorines, and the global circulation of these compounds, many have become ubiquitous in the global environment and in the tissues of animals and humans.

The introduction of chlorine substituents into organic compounds generally leads to increased chemical and biological reactivity and so to increased toxicity. Some organochlorines which pollute the environment are highly toxic to living organisms. Such organochlorines have been implicated in severe population declines of various wildlife species of fish, reptiles, birds and mammals. Accidental or occupational exposure to relatively high levels of organochlorines in humans has been linked with many toxic effects. Although there is generally a lack of data on the toxicity of many organochlorines, there is now a growing body of evidence from animal and human studies which suggests that some organochlorines may be causing detrimental health effects in humans at levels currently found in the environment.

Organochlorines have been associated with causing a wide range of toxic effects in animals and humans, including effects on the reproductive, nervous and immune systems, toxicity to the liver and kidney, and cancer. The mechanisms by which organochlorines cause these effects are thought to include disruption of the endocrine (hormone) system, and subtle alteration of the levels of certain enzymes, cells of the immune system and chemicals involved in the transmission of nerve impulses. Endocrine disruption is of particular concern because hormones not only control many functions in the body, but are essential for normal development of the embryo and fetus. Disruption of the endocrine system during this period of development can lead to permanent effects on the offspring. Indeed, the developing embryo/foetus is the most sensitive life stage to toxic effects of organochlorines.

In some regions of the world in the general population, pre-natal exposure to organochlorines has been associated with decreased birth size, reduced intellectual ability and effects on behaviour. These effects persisted as children aged, and may have been caused by endocrine disruption during development. Some disorders of the human reproductive system have increased considerably during the last 50 years, and it is possible that this could be due to exposure to endocrine-disrupting chemicals in the environment.

This report discusses some of the scientific evidence on the effects of organochlorines on human health. Particular emphasis is given to developmental and reproductive effects because adverse effects on these processes threaten the future well being of wildlife and humans. The report discusses that current regulatory policies on organochlorines have failed to protect human health and the environment. An alternative strategy is proposed which involves global action for a complete phase out of organochlorine compounds.

Contents

1 Introduction

One of the more notable consequences of technological development in the Twentieth Century is the dispersal throughout the global environment of thousands upon thousands of chemicals that are solely the products of human endeavour. Only a fraction of these are made for commerce; the overwhelming majority are unwanted and often uncharacterized by-products of manufacture, use and disposal.

Among these 'xenobiotic' chemicals, one group stands out: organochlorines, chemicals that have at least one chlorine-carbon bond in their structure. The fate, occurrence and effects of organochlorines were summarized as follows in the conclusions of a seminar conducted jointly by the Commission of the European Communities and the associations of Europe's largest chemical manufacturers and waste disposal firms in 1989: (CEC et al. 1989)

'The vast majority of these compounds does not occur naturally, is rather persistent and can harm the environment more or less severely [sic]. An important part of... [the] compounds produced may remain in use for a shorter or longer period, but will finally appear in the environment.'

Today, organochlorines produced for commerce and those created unintentionally can be found in the air, lakes, oceans, soils, sediments, and animals – including humans – in every region of the planet. The effects of these substances on the environment, particularly on human health, has emerged as one of the most compelling scientific, political and economic issues of our time.

1.1 A Brief History of Organochlorines

The first manmade organochlorines were undoubtedly accidental by-products of the synthesis and use of elemental chlorine in the late 18th century. With the development of the technology for its mass production, elemental chlorine displaced the use of sour milk and sunlight for the bleaching of cotton cloth (Salzberg 1991).

From these humble beginnings, the chloralkali industry has grown into a massive enterprise that manufactures more than 360 billion kilograms per year of elemental chlorine (Shelley 1990). The industry's growth has been accompanied by *'widespread occupational diseases and environmental damage,'* according to an American Chemical Society publication (Salzberg 1991).

About one-third of elemental chlorine goes into the production of only one organochlorine, polyvinyl chloride (PVC) (Johnston and Troendle 1993, Hileman et al. 1994). Most of the remainder is used in manufacturing some 11,000 other organochlorines. PVC is used, of course, in plastic products. The other commercial organochlorines are used in products ranging from pesticides, solvents, lubricants and refrigerants (Johnston and McCrea 1992) to soaps, shampoos, deodorants, and cosmetics (Steinkjer and Braathen 1988) and even toothpastes and mouthrinses (Jenkins et al. 1993).

During the manufacture, use and disposal of these commercial products, large numbers of unwanted organochlorine by-products are created. Very few of these accidental chemicals have been characterised. They are known, however, to include some of the most persistent and toxicologically potent of the organochlorines: the 'dioxins' – the polychlorinated dibenzo-p-dioxins

(PCDDs) and dibenzofurans (PCDFs) – as well as polychlorinated biphenyls (PCBs), polychlorinated benzenes, etc. (Costner et al. 1995)

Approximately 1,200 compounds which contain chlorine or its related elements bromine or fluorine (which are known collectively as organohalogens) are created by some living organisms and other natural processes (Gribble 1994; Gribble 1995). These compounds have been isolated from bacteria, fungi, marine algae and a few have been found in higher plants and animals. Naturally produced organochlorines are not persistent (Henscler 1994), and nearly all are produced in very small quantities. One exception, chloromethane, the simplest of the organochlorines, is primarily produced by marine algae at the rate of 5 million tons per year (Rasmussen et al. 1980). It is thought that this organochlorine may play a part in regulating the ozone layer (Lovelock 1975).

In general, little is known about the functions of naturally produced organohalogens. Those which have been studied appear to be involved in defense mechanisms or play a role as chemical messengers in various biochemical pathways. The diverse array of compounds which are produced naturally suggests they are of great biological importance, but our understanding of these compounds is presently very limited (Neidleman and Geigert 1986).

1.2 Organochlorines in the Environment

The stability that makes organochlorines so attractive to the chemical industry is one of the qualities that also makes them hazardous to public health and the environment. Even the simpler organochlorines can remain intact in some parts of the environment for hundreds and even thousands of years (Jeffers et al. 1989).

The breakdown, by both industrial and natural processes, of synthetic organochlorines typically yields a second generation of organochlorines. Some of these newly-created metabolites and by-products are more toxic and persistent than the original substances. For example, burning simple organochlorines can produce dioxins, which include some of the most toxic and persistent organochlorines yet identified.

Once persistent organochlorines become airborne through either direct emission or volatilization, they can become global pollutants. The more volatile of these chemicals – for example, the chlorofluorocarbons (CFCs) and the solvent, carbon tetrachloride – rise into the upper atmosphere where they deplete the stratospheric ozone layer. The less volatile persistent organochlorines – for example, the dioxins, PCBs, organochlorine pesticides (e.g., chlordane, dichlorodiphenyltrichloroethane (DDT), and hexachlorocyclohexane) – can be carried around the planet before falling to its surface (Iwata et al. 1993; Kannan et al. 1989; Longanathan and Kannan 1994); Travis et al. 1989). In this way, many organochlorine chemicals have become ubiquitous throughout the global environment.

Organochlorine chemicals are preferentially deposited in colder regions, such as those of the northern lakes and seas and the polar and sub-polar regions (Iwata et al. 1989). Through this 'global distillation' process, organochlorines released in the tropics are eventually deposited in colder regions (Goldberg 1975). Global distillation is a factor in the unexpectedly

higher concentrations of organochlorines that have been observed in the air, seawater, (Hargrave et al. 1988; Patton et al. 1989)), precipitation (Gregor and Gummer 1989), plankton (Bidleman et al. 1989), wild animals (Norstron et al. 1988; Muir et al. 1988), and people of the Arctic region (Dewailly et al. 1993a).

1.3 Organochlorines in the Food Web

Persistent organochlorines do not dissolve readily in water; they are fat-soluble. Consequently, they build up (bioaccumulate) in the fats and oils in the environment, which occur primarily in the tissues of living organisms. Through a process known as biomagnification, organochlorines reach the highest levels in tissues of animals at the top of food chains. Even small releases of such chemicals lead to high concentrations in the tissues of birds, fish and animals, including people.

The processes of bioaccumulation and biomagnification are illustrated by the following example: algae and other small aquatic organisms accumulate PCBs at concentrations hundreds of times higher than those in the surrounding water; small fish eat the algae, accumulating even higher PCB concentrations; PCBs levels increase higher still in the larger fish that feed on the smaller fish. Near the top of the food web, among predatory birds like herring gulls, PCB concentrations in their eggs can reach concentrations 25 million times greater than the PCB levels in the water. (Colborn et al. 1990)

1.4 Organochlorines in People: At the Top the Food Web

Long-term exposure to even very low doses of persistent organochlorines lead to the accumulation of these chemicals in people and other animals. For people, exposure to persistent organochlorines is primarily the result of bioaccumulation and biomagnification through the food web.

Several studies indicate that Europeans and North Americans acquire more than 90 percent of their daily intake of dioxins and similar compounds from ingestion of fish, beef and dairy products (Rappe, 1992; Henry et al. 1992). Vegetarians, who eat no meat or fish, have considerably lower exposures (eg. Prachar et al. 1994).

Food-borne persistent organochlorines are readily absorbed into body tissues. Once absorbed, these chemicals are relatively impervious to the body's processes for detoxifying and excreting naturally-occurring toxins, just as they are resistant to degradation in the greater environment.

Since they are not readily detoxified and excreted, these fat-soluble chemicals partition into the fatty tissues of the body from which they are released only very slowly (Hall 1992). For example, the rate of release for the most well-known of the dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has been estimated to be as low as 4.4 percent per year (Wolfe et al. 1994). However, faster rates of release occur during periods of starvation and during lactation (Thomas and Colborn 1992).

Body burdens of specific persistent organochlorines vary regionally, depending on patterns of

production and use. For example, in countries where DDT is still manufactured and used, people tend to have higher concentrations of this pesticide in their tissues than those among people living in countries where DDT use is banned or severely restricted.

People in industrialised countries generally carry higher body burdens of industrially-derived chemicals, such as PCBs and dioxins. This is shown in Table 1.1, which lists the average concentrations of some organochlorine chemicals in breast milk and the countries where levels are notably high (Thomas and Colborn 1992).

As discussed earlier, global distillation may contribute to an underlying trend of increased organochlorine body burdens in the higher, colder latitudes. Due to this phenomenon, environmental deposition and associated body burdens can be expected to continue to increase in colder regions for the foreseeable future, even if all organochlorine releases are immediately halted.

1.4.1 Most Highly Exposed Populations

Certain segments of the population are subject to especially high organochlorine exposures, depending on factors such as age, diet, occupation or place of residence. In general, the most highly exposed of these are embryos, foetuses and nursing infants.

1.4.2 Embryos and Foetuses

Persistent organochlorines in the mother's body pass through the placenta to the embryo and foetus. Among such chemicals identified in placental tissue and cord blood are DDT and its metabolite DDE, hexachlorobenzene (HCB), PCBs, and dioxins (PCDDs and PCDFs) (eg. Jacobson et al. 1984, Ando et al. 1986, Kanja et al. 1992, Koopman-Esseboom et al. 1994).

There are little or no data describing the extent of mother-to-foetus transfers of organochlorines in humans. However, in animals, concentrations of some persistent organochlorines in unborn offspring have been found to range from only a fraction of a percent to as much as 300 percent of than those in the mother (e.g., Abbott et al. 1989; Hagenmaier et al. 1990; Korte et al. 1990). Transfer efficiency depends on many factors, including time during the gestation period and the specific chemical.

1.4.3 Nursing Infants

Organochlorines also pass through the mother's breast milk to the nursing infant (Hall 1992). Some of the persistent organochlorines found in breast milk are as follows: PCBs, dioxins, DDT, DDE, dieldrin, HCB, hexachlorocyclohexane (HCH), heptachlor, chlordane, tris 4-chlorophenyl methanol and methane (TCP methanol and TCP methane (eg. Galetin-Smith et al. 1990, Skaare and Polder 1990, Kanja et al. 1992, Stevens et al. 1993, Rahman et al. 1993, Koopman-Esseboom et al. 1994, Furst et al. 1994).

While breastfeeding increases the infant's body burden of organochlorines, it decreases the body burden of the nursing mothers. For example, in a study of Rhesus monkeys, the mothers shed from 17-44 % of their TCDD burden, while their nursing infants accumulated TCDD concentrations that were more than 400 percent higher than those of their mothers (Bowman et al. 1989). Similarly, studies of nursing women have found that organochlorine levels decline with successive

lactations, the number of children, and the duration of breast feeding (eg. Skaare and Polder 1990; Beck et al. 1994). It has been estimated that an infant which is breast fed for one year will receive between 4 and 12% of its total lifetime exposure to dioxins (US EPA 1994).

1.4.4 Subsistence Fishers and Farmers

For some groups and individuals, fish consumption is a major pathway of exposure. For example, among the Inuit people of Arctic Quebec in Canada, for whom fish and sea mammals are major food sources, breast milk carries levels of dioxins and PCBs that are 3 to 7 times higher than those in milk from a non-Inuit control group (Dewailly et al. 1994).

Sports fishers from the north shore of the Gulf of the St. Lawerence River (Dewailly et al. 1994) and individuals who consume fish from the Great Lakes carry particularly high body burdens of organochlorines, including PCBs and dioxins (Fein et al. 1984). In another example, people of the Faroe Islands, who consume pilot whale meat as part of their diet, are estimated to have organochlorine exposure equivalent to that from eating fish from the Great Lakes (Simmonds and Johnston 1994). Also high fish consumers near the Baltic Sea in Sweden were found to have dioxin levels about 3 times greater than those of nonconsumers (Svensson et al. 1991).

Farmers who raise and consume their livestock have the potential for elevated exposures, specifically those located within the vicinity of organochlorines sources. For example, elevated concentrations of dioxins and related organochlorines have been documented in milk produced near municipal and industrial waste incinerators, as well as other industries (e.g., Liem et al. 1991, de Jong et al. 1992, Lassek et al. 1993, Beck et al. 1990).

1.4.5 Industrial Workers and Communities Surrounding Industrial Facilities

Workers in various industries can be exposed to elevated levels of various persistent organochlorines. For example, studies have found elevated levels of dioxins in the tissues of chemical workers involved in the manufacture of chlorophenolic compounds as well as in workers at municipal waste incinerators (e.g., Luotamo et al. 1993; Schecter et al. 1991; Connally et al. 1990)

Also, people living in communities near facilities that manufacture or dispose of organochlorines may have elevated exposures due to releases during routine operation, accidents or illegal disposal. Among the more well-known of such communities are Seveso, Italy, and Times Beach, Missouri, which are well described by Schecter (1994).

1.5 Effects of Bans on Organochlorine Body Burdens

Because of the extreme longevity and global circulation of persistent organochlorines, regional bans of specific chemicals can provide only limited protection for the populations of the regions enacting the bans. However, banning specific organochlorines in a region can result in some reduction in the body burden of these chemicals in the local population.

In Western Australia, all uses of the following organochlorine pesticides were banned: HCB, in 1972; DDT and dieldrin, by 1987. Subsequently, concentrations of these chemicals in samples of breast milk and adipose tissues from that area declined, as shown in Table 1.2 (Stevens et al. 1993).

Also, after uses of HCB, HCH, DDT, DDE, and dieldrin were restricted in Germany, their concentrations in breast milk declined, as shown in Table 1.3 (Furst et al. 1994). The tissue levels of DDE, the major break down product of DDT, have decreased at a slower rate than other compounds and now appear to have stopped declining to any extent (Furst et al. 1994). In both cases, some portion of the reductions in body burdens of these organochlorines may also be attributed to the banning of many of these same chemicals in other countries during the same time periods.

After the worldwide cessation of commercial PCB production during the 1970s, PCB concentrations in breast milk initially declined. However, such concentrations have apparently now reached a plateau (Furst et al. 1994; Frank et al. 1993). This levelling is due to the extreme persistence of PCBs and to the large remaining quantities that are deposited in sediments, buried in landfills and otherwise available for continued circulation through the global environment.

Banning production and use of only some of the more obviously problematic, commercially-produced organochlorines will not result in significant reductions in releases and subsequent body burdens of the majority of persistent organochlorines. Most of these, such as the dioxins, are not intentionally produced but are unwanted by-products of the manufacture, use and disposal of other organochlorines.

1.6 Human Health Effects of Organochlorines

Research on the health effects of organochlorines has, until recently, focused mainly on cancer and on acutely toxic effects following a single high dose exposure. Such studies have identified numerous organochlorines that act as carcinogens with a wide range of potencies.

Populations of some countries already carry tissue concentrations of certain persistent organochlorines that are associated with elevated cancer rates. For example, the average body burden of dioxin and related chemicals among U.S. citizens has been estimated to pose a risk of cancer death ranging from one in 1,000 to one in 10,000 (USEPA 1994).

More recently, researchers have linked organochlorine exposure to a wide array of non-cancer effects, which can be broadly categorized as follows: reproductive disorders, malfunction of the nervous system, diabetes, suppression of the immune system, disruption of the endocrine system (thymus, thyroid, ovaries, testes, etc.); and reproductive/developmental disorders.

Specific outcomes associated with these effects include reduced fertility; intellectual and attentional deficits; increased susceptibility to bacterial, viral, parasitic, and neoplastic disease; diminished intellectual, emotional and physical capabilities associated with hormonal imbalances; and irreversible abnormalities of the brains, immune systems and reproductive organs of offspring due to pre- and postnatal exposures.

In some parts of the world, average body burdens of certain organochlorines have already reached the levels at which some of these effects occur (Allsopp 1994). As shown in Table 1.4, average concentrations of dioxin now found among the populations of the U.S. and Europe are at or near those levels associated with lower levels of testosterone, the hormone that influences male sexual

characteristics and the libido (sexual drive) in both men and women; altered glucose tolerance, a symptom of diabetes; and changes in the immune system (USEPA 1994). All of these effects have far reaching social, political and economic implications.

In a recent reassessment of the scientific literature on dioxins and related compounds by the US EPA (1994), it was stated:

Subtle changes in enzyme activity indicating liver changes, in levels of circulating reproductive hormones in males, in reduced glucose tolerance potentially indicative of risk of diabetes, and in cellular changes related to immune function suggest the potential for adverse impacts on human metabolism, reproductive biology, and immune competence at or within one order of magnitude of average back-ground body burden levels... Individuals at the high end of the general population range may be experiencing some of these effects. Some more highly exposed members of the population may be at risk for frankly adverse effects including developmental toxicity, reduced reproductive capacity based on decreased sperm counts and potential for increased fetal death, higher probability of experiencing endometriosis, reduced ability to withstand an immunological challenge and others. [EPA 1994]

With organochlorine body burdens at or near levels that may be affecting adults, the consequences for embryos, foetuses and nursing infants may be even more critical. Levels of exposure that cause no discernible effects in adults may cause irreversible harm to the developing foetus and newborn (Thomas and Colborn 1992).

In pre- and postnatal exposure, the magnitude of toxic effects often depends on timing. For example, exposure of pregnant rats to a small dose of dioxin on the fifteenth day of pregnancy (the day when sexual differentiation begins in the foetuses) resulted in demasculinization and feminization of the male offspring. Specific effects included reduced sperm counts, smaller testicles and reduced anogenital distances (see eg. Peterson et al. 1993).

Some studies suggest that pre- and postnatal exposure to persistent organochlorines can have equally profound effects in humans. For example, lower birth weight, slower postnatal growth and reduced short-term memory have been noted among the children of women who consumed moderate amounts of organochlorine-contaminated fish from Lake Michigan (Fein et al. 1984, Jacobson et al. 1990a, Jacobson et al. 1992). Fish consumption, levels of chemicals in maternal blood and birth defects were associated primarily with PCBs, however other organochlorine contaminants in the fish may have also been responsible.

In another study, children of women who lived near dioxin contaminated areas during their pregnancies were found to have abnormal brain function and altered immune systems. These effects, which were noted during early adolescence, are apparently irreversible. (Smoger et al. 1993)

A growing body of scientific evidence attests to the extreme sensitivity of embryos, fetuses and nursing infants to the effects of the persistent organochlorines passed to them from the bodies of their mothers. This is of great concern because effects on the next generation, and especially disturbances of the reproductive system, rapidly threaten populations as a whole.

1.7 How Organochlorines Exert Toxic Effects

Organochlorine chemicals exert many toxic effects on animals and humans including reproductive and developmental toxicities, carciongenicity, neurotoxicity and immunotoxicity. The majority of these effects appear to be caused by the ability of organochlorines to alter the levels of certain hormones, enzymes, growth factors and neurotransmitters (chemicals involved with the transmission of nerve impulses) in the body. Many effects of organochlorines appear to be mediated largely by receptors, especially steroid hormone receptors and the aromatic hydrocarbon receptor (Ah receptor).

The presence of a carbon-chloride bond affects the chemical properties of organic compounds and also has consequences on their toxicological behaviour. Indeed, it has been deduced that the introduction of chlorine substituents into organic compounds generally leads to increased chemical and biochemical reactivity and therefore to increased toxicity. For example, introduction of chlorine substituents makes organic compounds more lipophilic which facilitates interactions with hydrophobic sites such as those in enzymes, promoting reactions with enzymes (Henschler 1994).

Steroid Hormones and Receptors

A hormone is a chemical substance produced by the body which has a specific regulatory effect on the activity of certain cells or organ(s) in the body. One way in which signals from inside and outside the body are interpreted is through molecules called receptors. Receptors are present both inside and on the surface of cells. Molecules such as hormones bind to receptors and the resulting complex then triggers a certain biochemical response. Such a biochemical response can ultimately result in functional changes in cells. The binding of molecules to receptors is very specific such that only a particular sort of molecule can bind to a particular type of receptor.

There is one class of receptors called the nuclear hormone receptors which can interact with certain genes. The many receptors in this class recognise steroid hormones. When such a hormone binds to one of these receptors, the resulting 'hormone-receptor complex' which is formed binds to specific regions of DNA in the cell nucleus. This appears to alter gene expression which can lead to functional alterations in cells, tissues and organs. Thus, by acting through receptors, steroid hormones can result in diverse changes in the body (McLachlan et al. 1992).

Steroid hormones include hormones produced by the adrenal cortex (part of the adrenal glands), testis, ovary and placenta. Table 1.5 shows the steroid hormones which are produced by different organs. Steroid hormones include the female sex hormones oestrogen and progesterone and the male sex hormone testosterone, (the main functions of which are the development and maintenance of genetalia and secondary sex characteristics); the adrenal hormones glucocorticoid and mineralocorticoid; thyroid hormone (thyroxine), vitamins (vitamin D), and retinoic acid (Hall and Besser 1989).

Some organochlorine compounds have been shown to disrupt endocrine (hormonal) function in the body. These endocrine-disrupting chemicals can alter the levels of hormones in the body, and in particular affect steroid hormones. Such changes in the levels of hormones can occur if an organochlorine chemical binds to a specific hormone receptor. The organochlorine may then either 'mimic' the hormone or block the normal biological response by occupying the the receptor site.

Alternatively, organochlorines may be able to react directly or indirectly with the hormone structure to alter it, change the pattern of hormone synthesis, or modulate the number of hormone receptors and their affinities for specific molecules. PCBs for example are known or suspected to express biological effects through most, if not all, of these mechanisms (McKinney and Waller 1994). Since hormones regulate many fundamental processes in the body including reproductive functions and processes of development, any alterations by exogenous organochlorines could adversely affect these bodily functions.

Besides steroid hormones, organochlorine compounds have been shown to affect levels of thyroid hormones in the body, which are involved in processes of growth and development. Also, hormones produced by the pituitary gland in the brain called follicle stimulating hormone (FSH) and luteinising (LH) hormone can be affected by organochlorines (McLachlan et al 1992). These hormones are involved in the regulation of reproductive processes including the development of eggs (ova) and sperm (spermatozoa), development of secondary sex characteristics and regulation of other hormones (Hall and Besser 1989).

Ah Receptor

Much work has been carried out on the toxicity of dioxins and furans (PCDD/Fs), especially the most potent congener TCDD. Studies have shown that dioxins appear to exert effects on the body disrupting endocrine function, including steroid hormones. *in vitro* and *in vivo* studies have also revealed that some effects of dioxin appear to be mediated through another receptor – the aromatic hydrocarbon receptor (Ah-receptor). Some compounds which occur naturally in the environment, particularly in plants, have an affinity for this receptor, and it is possible that the Ah receptor evolved as a substrate-inducible system to metabolise lipophilic substances in the diet (US EPA 1994a).

By binding to the Ah-receptor, dioxin may evoke a cascade of different biochemical and cellular responses. As is the case with steroid hormone receptors, if dioxin binds to the Ah-receptor, a complex is formed which can bind to DNA and affect the activity of certain genes. The effects of dioxin which have been experimentally demonstrated to be mediated through the Ah receptor include induction of cytochrome p450 enzymes in the liver, wasting syndrome (weight loss) and hepatotoxicity (liver toxicity). Other effects of dioxins include thymic atrophy, neurotoxicity, dermal toxicity, immunotoxicity, decreased vitamin A levels, altered lipid metabolism and reproductive and developmental toxicity. It is thought that many of these biological effects may also be mediated by the Ah-receptor although the evidence for this is not yet fully clear (US EPA 1994).

In addition to PCDD/Fs which exert toxic effects through the Ah-receptor, some PCBs (namely coplanar PCBs and their mono-ortho coplanar derivatives) can bind to and act through this mechanism (Safe 1994). Results of recent studies indicate that polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs) may also act through the same receptor mechanisms as dioxins (PCDD/Fs), (Mennear and Lee 1994). In addition, there are a number of other organochlorine chemicals which are known to cause similar effects to dioxins or have structures which mean they may act through an Ah-receptor mechanism (see table 2.5), (Giesy et al. 1994).

1.8 Regulations and Risk Assessment

There are two basic strategies for protecting people from the effects of persistent organochlorines: prevention and regulation. Prevention – for example, banning production and use of some of the less persistent pesticides – has successfully stopped their release into the environment and, with the degradation of stores in the environment, ended human exposures. Regulations do not stop releases of persistent organochlorines so they do not stop human exposures. Regulations establish the rates or quantities that can be legally released.

Other than a limited number of bans, virtually all governments follow a limited regulatory strategy, setting allowable rates of release for a fraction of the persistent organochlorines and allowing unlimited release of the remainder. Allowable limits are typically established one organochlorine at a time, one source at a time, and one medium (air, soil and water) at a time. Beginning with the U.S. in 1954, governments have moved toward the use of risk assessment as the means for selecting the rates of release to be allowed under the limited regulatory strategy. Risk assessment is a process which attempts to provide a quantitative, or numerical, measure of human health impacts caused by a specific pollutant. In risk assessment, the determination of allowable releases of a toxic chemical requires the simultaneous establishment of acceptable rates of deaths or disease incidence.

Risk assessment is, at its core, a series of mathematical equations which are used to estimate the emissions of a chemical, human exposure to the chemical and finally the health effects from such exposure. For example, in a risk assessment of the dioxin emitted from an incinerator stack, the equations are supposed to describe the fate of the dioxin – from the time it is emitted from the stack to the time that it disrupts some critical process in some particular cells in the bodies of its human 'receptors' – with sufficient accuracy to predict the number of these people who will develop, some 20 to 30 years later, a disease, typically a fatal cancer (Silbergeld 1993), caused by the quantity of dioxin that was estimated to have escaped from the incinerator stack.

After all the relevant available factors are inserted in the array of mathematical equations and their solutions calculated, the final number – the number of cancer deaths associated with the estimated quantity of dioxin emitted – is then used to establish allowable dioxin emissions, as determined by the rate of deaths or disease incidence that is deemed socially and politically acceptable.

The factors that must be reduced to a mathematical expression are both quite numerous and extremely complex. They include, for example, the weather (wind, rain, snow, light, heat, cold, etc.) and its effects on the dioxin; the movement of the dioxin through the environment toward its human 'receptors,' including its passage through the food web (uptake by fish, deposition on forage and subsequent uptake in beef and dairy cattle, etc.; its inhalation, ingestion and absorption by the human receptors; and its subsequent interactions within bodies – the systems, organs and cells – of these people.

Although risk assessment is a scientific process, in practice it depends on highly uncertain and subjective assumptions at every stage of the mathematical modelling process (e.g., Ginsburg 1993; Perera 1987; Smith et al. 1993). It is inevitably subject to the personal and political predelictions of its practitioners and interpreters (Silbergeld 1993). Indeed, the former director of the US EPA warned of the subjective nature of the process when describing risk assessment:

'We should remember that risk assessment data can be like a captured spy: if you torture it long enough, it will tell you anything you want to know' (Ruskelshaus 1984).

One of the major limitations of the risk assessment process is that estimates of health effects are based entirely on one endpoint – the risk of developing cancer (Silbergeld 1993). However, many chemicals, including some organochlorines, cause other toxicities such as reproductive and developmental effects, or effects on the nervous and immune system at lower levels of exposure than cancer. Also, more subtle effects such as endocrine disruption, which may lead to many adverse health effects, are not considered in risk assessment. Risk assessment therefore assumes that the calculation for cancer is sufficient to protect people from all other effects. There is no scientific basis for this assumption (Ginsburg 1993). As a consequence of restricting the focus to carcinogenicity, risk assessment may therefore greatly underestimate the true impacts on human health from a particular chemical, leading to permissible releases of chemicals into the environment which may be detrimental to human health.

A further limitation of risk assessment is that it is unable to accommodate the real world situation of multiple chemical exposures of varying doses and duration (Silbergeld 1993). For example, risk assessments only consider one chemical at a time, but humans are exposed to numerous different chemicals in the environment. The toxicity of chemicals together may be additive or or even synergistic (ie. greater than additive). In addition, both the degree and type of chemicals to which people are exposed is very varied in different areas and risk assessment does not take this into account.

One of the greatest sources of uncertainty in risk assessment is the estimation of health risks. For example, a recent evaluation of the carcinogenic potential of perchloroethylene demonstrated that the risk factor could vary by a factor of nearly 2000 depending on the animal studies which were chosen and assumptions on human metabolism used in the calculation (Ginsburg 1993). In addition, the cancer risk calculation does not take into account the variation in sensitivity to chemicals among people and the variability between adults and children (Perera 1987).

A further danger to health from current regulatory methods is that many chemicals which are produced have undergone little and often no toxicity testing. Such chemicals for which there is only weak epidemiology data or toxicologically untested chemicals are essentially regulated as though they are safe (Smith et al. 1993).

Obviously, the process of risk assessment is fraught with uncertainties, plagued by limitations, highly subjective, and vulnerable to manipulation. By adopting this approach, government agencies, such as the U.S. Environmental Protection Agency, become locked into attempting to control releases of toxic chemicals rather than achieving better protection of public health by controlling their production and generation (Ginsburg 1993).

The problems of risk assessment can be avoided through an alternative approach: the precautionary principle, which requires that chemicals are not be discharged into the environment until they are proven to be harmless. In contrast, the current regulatory approach is based on the assumption that all chemicals are harmless until proven harmful and attempts to determine how much of each toxic chemical humans can be exposed to before there are detrimental health effects.

This is often conducted without adequate toxicological data and possibly without an adequate understanding of toxicological processes. The precautionary approach however, avoids problems deriving from the limitations of our understanding from toxicology by removing the assumption that a safe level of a particular compound or compounds can be estimated (Stairs and Johnston 1991). The precautionary approach is now being adopted at both national and international levels (see section 5).

1.9 Summary

Public health and the environment must be protected from the effects of the persistent organochlorines.

A growing body of evidence suggests that people already carry levels of persistent organochlorines in their bodies that are sufficient to affect the health of mature adults and to cause irreversible changes in the mental and physical capabilities of the children currently being conceived, born and breastfed. The probability of such health effects is even higher among the many people who have higher exposures to these pollutants because of their diets, lifestyles, occupations, and places of residence.

The existing body of knowledge of the fate, occurrence and effects of persistent organochlorines suggests strongly that regulation of persistent organochlorines – the establishment of legally acceptable releases into the environment based on the assumption that such releases are harmless – is incapable of protecting public health and the environment.

Prevailing regulatory schemes seek to limit releases of toxic chemicals into the environment to quantities that are thought to be harmless, based either on risk assessments or the notion that dilution and degradation in the environment serves as an adequate safeguard for public health. This regulatory approach is incapable of protecting the public and the environment from the effects of persistent organochlorines due to the following factors: their longevity in the environment as well as the human body; their tendency to bioaccumulate and biomagnify through the food chain; and the state of knowledge of the fate, occurrence and effects of these chemicals in the environment as a whole, and in human bodies in particular, that is currently so limited as to preclude the construction of an array of mathematical equations that, taken together, provide an adequate representation of these almost infinitely complex and, consequently, incompletely characterized interactions.

Prevention is the only remaining option.

If there is no threshold below which harm does not occur, which increasingly appears to be the case for persistent organochlorines both within the environment as a whole and within the bodies of human beings in particular, then the release of these chemicals must be prevented. Existing technologies are incapable of preventing the escape and dispersal into the environment of the persistent organochlorines that are produced for commercial use as well as those formed as by-products during manufacture, use and disposal. Moreover, with existing technologies, the manufacture, use and disposal of non-persistent organochlorines is accompanied by the formation and release of persistent organochlorines. In summary, as long as any organochlorines are produced, some persistent organochlorines will be released into the environment.

2 Health Effects of Organochlorine Chemicals: Reproduction and Development

Many organochlorine chemicals which have been released into the environment in the past 50-60 years are known to disrupt endocrine function. Those organochlorine chemicals which have been shown to disrupt hormones are shown in table 2.1. Steroid hormones in particular seem to be affected. Steroid hormones play a large role in regulating developmental and reproductive processes. Thus disruption of these hormones could potentially lead to effects on reproduction and development. It is now evident from animal, wildlife and human studies that many organochlorines have been associated with adverse effects on reproduction and development. Organs that are particularly at risk during development from maternal exposure to organochlorines are male and female reproductive systems eg. mammary glands, uterus, cervix and vagina in females, seminal vesicles, prostate, epididymides and testes in males, as well as the skeleton, thyroid, liver, kidney and immune system. The developmental stages of life have been shown to be particularly sensitive to effects from organochlorine chemicals, and permanent effects may result from exposure. Effects may be immediately apparent, such as deformities in the offspring or may not be fully or obviously expressed until the offspring reaches maturity (Colborn et al. 1993).

There is much evidence which suggests that organochlorines are responsible for breeding problems in many wildlife populations in areas contaminated with these chemicals. Many of these effects on reproduction and development have been confirmed in animal experiments. There is also some information which suggests that similar effects may be occurring in humans.

This section deals with both developmental effects associated with pre- and postnatal exposure to organochlorines and effects on adult reproductive systems. It is organised into sections on effects on[1] the male and[2] the female reproductive systems,[3] other developmental effects, and[4] effects on wildlife populations.

2.1 Disorders of the Male Reproductive System

2.1.1 Introduction

In recent years it has been shown that the male fertility appears to be declining. The frequency with which a male factor is responsible for a couple's infertility has increased in recent years from about 10% to 25% (Lancet 1995). This observation has been heightened by other studies demonstrating male reproductive problems.

Recent studies have revealed that over the past 30-50 years, there has been an alarming increase in the incidence of disorders of reproductive organs in men in several different countries. Such disorders include a fall in sperm count (eg. Carlsen et al. 1992) and an increase in the incidence of testicular cancer. Increases in the incidence of hypospadias (urethral abnormalities) and crytorchidism (undescended testicles) may have also occurred (Giwercman and Skakkebaek 1992, reviewed by Danish EPA 1995). It is thought that all of these conditions can arise during fetal development and may therefore have a common cause. The most likely causes are a change in environmental factors or lifestyle rather than an change in genetic factors because the changes are recent and are apparent in many countries (Sharpe and Skakkebaek 1993).

There is clear evidence that development of the male reproductive tract during fetal life in humans is sensitive to the levels of a female sex hormone called oestrogen. Levels of oestrogen which are

higher than normal can result in reduced sperm counts and an increased incidence of cryptorchidism and hypospadias in male offspring. In the last 50 years there has been a massive increase in the number and quantity of manmade chemicals which have been released into the environment. Some of these chemicals, including a number of organochlorines, are known to mimic oestrogen when present in the body. Since the development of the male reproductive system is very sensitive oestrogen levels, it could be adversely affected by exposure to 'oestrogenic' chemicals. It has therefore been hypothesised that oestrogenic chemicals may be partly responsible for the increasing disorders of the male reproductive tract in humans over the last 50 years, coinciding with the release of these chemicals into the environment (Sharpe and Skakkebaek 1993).

Recent animal studies have also shown that exposure to dioxin *in utero* can cause similar adverse effects on the male reproductive system, including reduced sperm counts. Dioxin is not an oestrogenic chemical, but may cause the disorders by a different mechanism, possibly by affecting growth factors in the urogenital system (Gray et al. 1995). Since exposure to dioxins (a by-product of the chlorine industry) has increased considerably over the last 50 years and they are now ubiquitous in the environment and in human tissues, it is possible that exposure to dioxins may also be partly responsible for the increasing disorders of the male reproductive system.

Since the above disorders of the male reproductive system have a common origin in fetal life or childhood and most do not become evident until adulthood, the increase in disorders seen today would have originated 20-40 years ago. The prevalence of such defects in male babies born today will therefore not become manifest for another 20-40 years more (Danish EPA 1995). This is obviously of great concern and factors causing the problems need to be addressed.

This section discusses changes in human exposure to oestrogens and oestrogenic chemicals in recent years; the effects of oestrogens and oestrogenic chemicals on male development; studies which show evidence of the increasing disorders of the male reproductive system over the last 50 years; and evidence that prenatal exposure to organochlorine chemicals may be involved in the increasing disorders and effects of organochlorines on adult male reproductive health.

2.1.2 Exposure to Oestrogens and Oestrogenic Chemicals

In the last 50 years there have been several changes in our lifestyle which may have altered our exposure to oestrogens and oestrogen-like chemicals. The extent of exposure will probably be variable in different countries and between individuals depending on the validity and effect of the sources listed below (Sharpe and Skakkebaek 1993).

Diet

In some countries in recent years there has been an increased tendency to consume a low fibre-high fat diet rather than a high fibre-low fat diet. Women who eat such a diet will possibly be exposed to more of the oestrogens in her own body (endogenous oestrogens). This is because in order to excrete oestrogen from the body, it is excreted into the bile. However, it can then be reabsorbed into the gut and is more readily absorbed into a gut which only contains a low amount of fibre. There is no evidence that this would increase exposure to a male fetus in a pregnant woman enough to cause reproductive tract abnormalities. However, there is evidence that links the occurrence of cryptorchidism and testicular cancer to high levels of endogenous oestrogen in the mother (Sharpe and Skakkebaek 1993).

Synthetic Oestrogens

Apart from our own endogenous oestrogens, there are a number of exogenous oestrogens from the environment to which humans may be exposed. Synthetic oestrogens (orally active anabolic oestrogens) were manufactured and given to farming livestock from the 1950's for 20-30 years. These were banned from use in Europe in 1981, but the level of human exposure from these oestrogens from the 1950's to the 70's remains unknown (Sharpe and Skakkebaek 1993).

Another synthetic oestrogen which has been used increasingly in the past 20-40 years is the oral contraceptive pill. This changes levels of oestrogen in the body only while it is being taken. There are reports that it has been detected in water, but there is only limited information on its detection in drinking water. One report from Germany suggested that levels of synthetic oestrogens in drinking water originating from wells or springs was extremely low (Rurainski et al. 1977).

Natural Oestrogens

Besides oestrogens which are deliberately manufactured, there are a number of naturally occurring oestrogens to which we are exposed. In developed countries there is generally a high consumption of dairy products. Milk from dairy cows contains oestrogens from the cow itself (mainly oestrogen sulphate). This is destroyed by the process to make babymilk powder, but it is not clear whether oestrogen sulphate from milk can be absorbed into the gut of an adult or child (Sharpe and Skakkebaek 1993). The levels of oestrogen sulphate present in milk are however extremely low and it has been suggested that oral exposure from cow's milk is unimportant (Gurr 1993).

Many plants and fungi also contain weakly oestrogenic compounds. These are known as phytooestrogens and mycotoxin oestrogens respectively. One of the richest sources of phytooestrogens is in soya. The consumption of soya has vastly increased in the last 20 years. However, rather than being detrimental, recent research shows that the oestrogens in soy protein, called isoflavones, may be beneficial to health. Experiments on rats have shown that dietary soya has a chemopreventative effect on mammary tumours. Little evidence for prevention of cancers exists in humans, but countries where the intake of natural dietary oestrogens is higher eg. Japan, have a low incidence of breast and prostate cancer (Makela et al. 1994). Rye also contains phyto-oestrogens. It has been suggested that rye may protect against colon cancer because the incidence of colon cancer is lower where rye bread forms a staple part of the diet (Ginsburg 1994).

It has been hypothesised that the cancer preventative action of phytooestrogens in oestrogen-related cancers like breast and prostate cancer may be caused by an antioestrogenic action. This could involve blocking the action of endogenous oestrogens or competition for endogenous oestrogen sites. However, recent *in vitro* work on some common dietary oestrogens has shown no evidence of anitoestrogenicity in these compounds, only evidence of oestrogenic action. It was therefore suggested that the cancer preventative action may be mediated by other mechanisms not related to oestrogenicity (Makela et al. 1994).

Oestrogenic Chemicals

The environment has been polluted with many chemicals in the last 50-60 years which may disrupt endocrine function including weakly oestrogenic chemicals. At present, only a small number of chemicals have been tested for such effects. Those organochlorines which have been shown to be oestrogenic either by *in vitro* or in vivo experiments are illustrated in table 2.2 and include

methoxychlor, chlordecone (kepone), B-hexachlorocyclohexane, dicofol, some PCBs, DDE, (Davis et al. 1993, Birnbaum 1994), DDT (both isomers, p,p'-DDT and o,p'-DDT) (Bustos et al. 1988), and endosulphan, toxaphene and dieldrin (Soto et al. 1994). Moreover, *in vitro* work has shown that these oestrogenic chemicals may act cumulatively (ie. when mixed together they induce oestrogenic responses at concentrations lower than those required when each compound is administered alone), (Soto et al. 1994). Recently, alkylphenolic compounds such as nonylphenol, which are widely used as industrial detergents in many countries, and also in paints, herbicides and pesticides, have been isolated from sewage treatment works and river water in the UK and shown to be oestrogenic (White et al. 1994).

An important point which must be stressed here is that the number of studies which have identified hormone-disrupting chemicals is very limited at present. This means that most of the chemical pollutants in the environment have not yet been tested for such effects, so the hormone-disrupting capacity for the vast majority of chemicals is not known.

2.1.3 How Oestrogens Work

Natural endogenous oestrogens exert their regulatory effects on the body by binding to oestrogen receptors. However, exogenous oestrogenic chemicals can also interact with the oestrogen receptors and by doing so they elicit many of the same cellular effects as natural oestrogen. This can be considered as a kind of hormone mimicry in which these exogenous chemicals cause specific responses which are normally only triggered by natural oestrogens. Oestrogenic chemicals may also work by altering oestrogen metabolism and by modulating the number of oestrogen hormone receptors and/or their binding affinities. Since oestrogens play a crucial role in controlling reproduction in females and to a lesser extent in males, and are involved in foetal development, interference by exogenous oestrogens can have far reaching effects on the body (McLachlan et al 1992, White et al. 1994).

A protein in the blood called sex hormone binding globulin (SHBG) binds to approximately 95% of the circulating natural endogenous oestrogen, which renders it biologically inactive. However, many synthetic oestrogens are effective in lower doses than endogenous oestrogen, because they are not bound to SHBG (Danish EPA 1995). Even though oestrogenic chemicals are weak oestrogens, it is possible that they may exert relatively powerful effects because they do not bind to SHBG.

Exogenous oestrogens may have adverse effects on the health of adults or mature animals. Much concern however is focused on the effects of these chemicals on the developmental stages of life which appear to be particularly susceptible to changes in oestrogen levels.

2.1.4 Development of the Male

Normal Male Development

In most mammals and in humans, male sexual development takes place *in utero* in the presence of large quantities of oestrogen. This prenatal development includes the differentiation of the testes, production of the male hormone testosterone and the masculinisation of the internal and external genitalia (Mittwoch et al. 1993).

Early in sexual development the mammalian fetus exists with gonads genetically determined to

either become an ovary or testes with the rudiments of both the male and female genital tract co-existing (McLachlan et al. 1992). When testes develop in the male fetus they do so faster than the development of ovaries in the female fetus. The testes then start to produce testosterone. It seems that this faster development of the testes and subsequent production of testosterone is vital for successful development of the male. This is because the fetus is exposed to high levels of oestrogen from the placenta, and if testosterone is not produced at the correct time during development, the male genital system is in danger of becoming 'demasculinised' (loss of male sexual characteristics) as a consequence of the high levels of oestrogen. Thus in mammals and humans, testes develop faster than ovaries to ensure the production of testosterone which is needed for the successful development of male sex organs (Mittwoch et al. 1993).

Effects of exposure to oestrogenic chemicals on fetal development in experimental animals
Experiments on rats and mice have shown that exposure to exogenous oestrogens during pregnancy results in demasculinisation/feminisation of the genital system in male offspring. These effects, include failure of testicular descent into the scrotum (cryptorchidism) and retention of the female genital tract in an animal which is genetically a male (McLachlan 1992). Other effects include a reduction in sperm count and testicular weight, and an increase in the incidence of hypospadias (a developmental anomaly where the urethra opens on the underside of the penis or in the space between the scrotum and the anus). The mechanism by which sperm count is reduced has been studied in rats (Sharpe 1993) and is explained below.

In mammals and humans, the production of sperm (spermatogenesis) is regulated by cells in the testes called Sertoli cells. Each Sertoli cell supports the development of germ cells (immature cells) into spermatozoa (sperm). However, the number of spermatozoa supported by each Sertoli cell is finite, which means that the maximum attainable sperm production is limited by the total number of Sertoli cells present. This is the key factor which explains why sperm count is reduced in male animals when they are exposed to exogenous oestrogens *in utero*. An increased oestrogen level alters the multiplication of Sertoli cells in fetal life, which results in less Sertoli cells in the animal when it is born. The development of Sertoli cells themselves takes place *in utero* and their number becomes 'fixed' such that no more can be formed shortly after birth. Consequently if Sertoli cell number is reduced by exposure to oestrogens *in utero*, sperm production is also reduced in the animal for the rest of its life (Sharpe 1993, Sharpe and Skakkebaek 1993).

Studies in rats have shown that Sertoli cell multiplication starts around 19-20 days of gestation and ceases around day 15 of postnatal life. A hormone called follicle stimulating hormone (FSH) is very important in controlling Sertoli cell multiplication during this time period. If FSH is reduced before day 15 of postnatal life, it causes a reduction in the number of Sertoli cells and a consequent drop in sperm count in adult life.

During fetal life and up to day 15 of prenatal life, FSH stays at a constant level, after which it increases. FSH controls the multiplication of the Sertoli cells and also stimulates the cells to secrete oestrogen. In turn, this oestrogen keeps the levels of FSH in check. This occurs until day 15 of postnal life when the matured Sertoli cells lose the ability to secrete oestrogen. The question is what happens if more oestrogen is added during development *in utero* or just after birth. The answer is that more oestrogen upsets the balance of oestrogen already present causing a suppression in the level of FSH. As a consequence of reduced FSH, Sertoli cell number, sperm

count and testicular weight will be reduced. Experiments with rats have shown that if the mother is exposed to oestrogens during pregnancy or if a male pup is exposed on day 4 of life, the level of FSH is suppressed causing a reduction in Sertoli cell number, sperm count and testicular weight, though the formation and production of sperm (spermatogenesis) is normal.

In humans, regulation of Sertoli cell proliferation may be very similar. The multiplication of Sertoli cells is also controlled by FSH during prenatal and neonatal life. However, the limited data available suggests that the number may also increase during puberty, so the potential for adverse effects on Sertoli cell multiplication may be longer than in the rat. However, it is not yet known whether decreased Sertoli cell number occurs as a result of oestrogen exposure in humans or whether this is the reason for decreased sperm counts in humans (Danish EPA 1995).

Effects of exposure to oestrogenic chemicals on fetal development in humans

The above findings from work with animals are very relevant to humans because in both man and other animals, comparable physical changes in male offspring can be induced by inappropriate exposure *in utero* to oestrogens (Sharpe 1993). This conclusion is largely based on evidence from an unfortunate experience in which many women were treated with a synthetic oestrogen called diethystilbestrol (DES). Between 1945 and 1971, DES was given to more than five million women to prevent miscarriages and pregnancy complications, but it was banned in 1971 after it was linked toincreases in a rare vaginal cancer. Further investigation of the effects of DES revealed that *in utero* exposure resulted in a much greater incidence of cryptorchidsm and hypospadias in male boys. In addition, when they reached adulthood, many of them had reduced sperm counts. The same outcome was achieved in the male offspring when DES was administered to pregnant rats and mice. Therefore, inappropriate exposure to oestrogens *in utero* in animals causes comparable defects in man, including a fall in sperm counts (Sharpe 1993, Sharpe and Skakkebaek 1993).

Since an increase in oestrogen levels during pregnancy can lead to disorders of the male reproductive system in humans and animals, it is possible that exposure of the general population to oestrogenic chemicals in the environment may lead to the same effect. This is discussed below.

2.1.5 Falling Sperm Counts in Men

Several recent studies have been consistent in demonstrating a decrease in sperm counts over the last 20-50 years.

In 1992, Carlsen et al. published a systematic review and analysis of every study in the literature between 1938 and 1991 which reported on sperm counts in men who did not have a history of infertility. The review covered a total of 61 studies from various countries with data on 14947 men. Results showed that there was a highly significant drop in the mean sperm count of 42% ($p<0.0001$) between 1940 (sperm count = 113x10 6 /ml) and 1990 (sperm count = 66x10 6 /ml). In addition, semen volume was also found to decrease over the same time period from 3.40ml to 2.75ml ($p=0.027$), which meant that total sperm count has actually fallen by more than 50%. The study concluded that the decline in semen quality over the past 50 years may reflect an overall reduction in male fertility, since sperm count is to some extent correlated with male fertility.

The above study has been criticised on some of the statistical methods which were used (Farrow 1994, Bromwich et al. 1994). In reply, the authors agreed that the analysis could lead to artefacts in

the data, but concluded that data do show that sperm counts are now lower than they were in the 1940s and '50s (Keiding 1994).

Another criticism of the study pointed out that the main decline in sperm counts occurred before 1970 and since then sperm counts increased slightly. However, results of three recent studies have clearly refuted this (Irvine 1994, Auger et al. 1995, Van Waeleghem et al. 1994) with two of the studies showing that sperm counts have decreased over the last 20 years and at an alarming rate of approximately 2% per year (Auger et al. 1995, Van Waeleghem et al. 1994).

To address the issue of whether sperm count has decreased in recent years as a result of factors affecting male development, Irvine et al. (1994) investigated whether the trend in semen quality was related to time of birth. Data on 3729 semen samples submitted by a large group of semen donors who were born between 1940 and 1969 were examined. Results showed there was a significant ($p<0.0005$) fall in median sperm counts from 128 x 10 6 /ml in men born in the 1940's to 75 x 10P 6 /ml in men born in the late 1960's. This study therefore supported the results of Carlsen et al. (1992).

Studies by Auger et al. (1995) and Van Waeleghem et al. (1994) also provide evidence that sperm count has decreased in recent years as function of age. The study by Auger et al. (1995), reviewed semen characteristics of 1351 fertile sperm donors aged between 19 and 39 who had attended a centre in Paris during the last 20 years. It revealed that the mean concentration of sperm decreased by 2.1% per year from 89 x 10 6 / ml in 1973, to 60 x 10 6 / ml in 1992. The study by Van Waeleghem also looked at semen characteristics in 360 consecutive candidate sperm donors over the last 17 years in Belgium, in which 90% of the donors were aged between 21 and 30. In both studies, not only the sperm count declined at an average rate of 2% per year, but there was also a significant deterioration in sperm characteristics with time. These changes included a reduction in sperm motility as well as an increase in the proportion of abnormal sperm. For example, Auger et al. (1995) reported the percentage of motile sperm decreased by 0.6% per year and the percentage of normal sperm decreased by 0.5% per year (both $p<0.001$). The characteristics of motility and numbers of normal and abnormal sperm are closely related to fertility potential. The decline in sperm counts and in quality of the sperm could reflect impairment of sperm production and possibly Sertoli cells. Results from these studies are of great concern because if the decline in sperm quality and sperm count is occurring in the general population at the same rate as in these studies, the proportion of men with fertility problems will continually increase (Auger et al. 1995).

2.1.6 Other Effects on the Male Reproductive System

Apart from the apparent decline in sperm counts over the last 30-50 years, the incidence of other disorders of the male reproductive system has also increased. Testicular germ cell cancer is now the most common malignancy among young males in the Western World. The Danish Cancer registry has collected reliable data since 1943 on the incidence of testicular germ cell cancer, and clearly shows that the disease increased 3-4 fold between the 1940s and 1980s. Studies based on data from cancer registries in other countries including the UK, the Nordic and Baltic countries, Australia, New Zealand and the USA have also reported significant increases in the incidence of testicular cancer (reviewed by Danish EPA 1995). Together these studies provide convincing evidence that there is a worldwide trend towards an increased incidence in testicular cancer.

Other disorders of the male reproductive system may be increasing. Evidence from studies in Britain suggests that the prevalence of cryptorchidism may have doubled since the 1950s and studies in other countries also suggest an increase. The incidence of hypospadias has been reported to increase in Britain, Sweden, Norway, Denmark and Hungary (reviewed by Giwercman et al. 1993, and Danish EPA 1995). There appears to be considerable geographic variation in the prevalence of hypospadias within and between different countries (Danish EPA 1995).

The disorders of the male reproductive system discussed above are closely associated with each other. For example, the risk of testicular cancer is significantly increased in men with a history of crytorchidism. Also a significant proportion of men with testicular cancer have impaired spermatogenesis (sperm production). Finally, spermatogenesis is generally impaired in maldescended testes (Giwercman et al. 1993). Since it is believed that all of these testicular disorders can arise in during fetal development/childhood it is likely that some common factors could be responsible for the increased incidence of testicular cancer, cryptorchidism, hypospadias and a decrease in sperm counts. Such remarkable changes in occurrence of these disorders over a relatively short period of time is probably caused by environmental factors rather than by genetic factors (Carlsen et al. 1992).

2.1.7 Are Organochlorine Chemicals Responsible for the Increased Incidence of of the Male Reproductive System?

There are a number of factors which could be involved in the increased incidence in male reproductive disorders. Smoking and drinking habits as well as sexual behaviour have markedly changed over the last 60 years. More promiscuous sexual activity can increase the risk of contracting sexually transmitted diseases which often result in infections of the genital tract, causing lower sperm counts. There is no conclusive evidence that smoking reduces sperm counts in men, but smoking during pregnancy can have an adverse effect on the gonads of the fetus. Alcohol consumption is known to have adverse effects on spermatogenesis in excessive amounts, but not in moderate amounts. Also, ionising radiation is known to have an adverse effect on semen quality (Giwercman et al. 1993).

Although the above factors may have contributed to increased disorders of the male reproductive system in recent years, the increase in testicular cancer incidence appears to be occurring on a worldwide scale and increases in other disorders are reported to be occurring in many countries. These increases cannot therefore be explained simply by changes in lifestyle, because not all countries have changed to the same extent. Moreover, since there has been an increase in several disorders of the male reproductive system, and it is thought that these disorders can all arise during fetal life, there is probably a common prenatally acting factor involved in causing these effects. A prime suspect for this is environmental chemicals which can cause endocrine disruption during prenatal life resulting in disorders after birth (Giwercman et al. 1993).

Certain organochlorine chemicals which are present in the environment are known to disrupt hormones during fetal life and can result in such disorders of the male reproductive system. Oestrogenic chemicals are of particular concern in this regard. In addition, dioxin may produce similar adverse effects on the male reproductive system, possibly by affecting growth factors involved in growth and development of the male urogenital system. All of these chemicals may therefore be partly or wholly responsible for the rise in adverse effects on the male reproductive

system over the past 50 years. In addition, some organochlorine compounds have also been shown to affect sperm count and reproductive hormones during adult life.

Further evidence which suggests that organochlorine chemicals can adversely effect the male reproductive system both in fetal/childhood and adult life are discussed below.

2.1.8 Effects of Prenatal Exposure to Organochlorines

Increased levels of oestrogens during pregnancy are known to cause disorders of the male reproductive system. Much of the evidence that synthetic exogenous oestrogens can cause these disorders both in laboratory animals and in humans comes from studies following *in utero* exposure to DES as discussed in section 2.1.4. It is therefore plausible that oestrogenic chemicals could have similar effects on the developing male.

In Taiwan in 1978-9 (Yucheng incident), many people were poisoned when they consumed rice which was contaminated with PCBs and dioxins. A study has been carried out on the sexual development of children born to women who were pregnant at the time of the incident (Guo et al. 1993). Results showed that the penis size of boys aged 11-14 was significantly shorter than the penises of matched control children. It is thought probable that this effect is due to *in utero* exposure to PCBs, which are oestrogenic.

Dioxins are not oestrogenic chemicals (in fact they can exert anti-oestrogenic effects), but prenatal exposure to dioxins in animals nevertheless results in similar male reproductive disorders as exposure to oestrogenic chemicals. For example, exposure of pregnant rats to relatively low levels of dioxin (TCDD) which did not cause maternal toxicity, resulted in a number of permanent alterations in their male offspring, including a reduction in sperm count, and reduction in testicular weight and accessory sex organs (reviewed by Peterson et al. 1993). In a recent experiment these effects were shown to occur after only a single very low dose of dioxin (TCDD) (64ng/kg) given at a critical time (on day 15) of pregnancy. No effect on other organ systems has ever been observed at such a low dose as this and the study concluded that the male reproductive system is highly sensitive to perinatal (prenatal and lactational) exposure of dioxins (Mably et al. 1992).

It was suggested that the above effects on the male reproductive system may result from altered levels of hormones during development, such as reduced testosterone levels. Sexual behaviour of offspring was also affected (it was demasculinised and feminised) by low dose exposure to dioxin on day 15 of pregnancy. In this case the regulation of luteinizing hormone (LH) was found to be altered. It was suggested that changes in LH, lowered testosterone levels and possibly also other mechanisms could impair the sexual differentiation of the central nervous system, causing the changes in sexual behaviour (Peterson et al. 1992).

A similar but more detailed study than those carried out by Mably et al. (1992) on prenatal dioxin exposure in both rats and hamsters has recently been reported (Gray et al. 1995). This study also demonstrated that prenatal exposure to relatively low levels of dioxin, which caused no maternal toxicity, did alter the reproductive development in male progeny in rats and hamsters. Results showed that exposure on day 15 of gestation to 1μg TCDD/kg in rats or on da 11 to 2μg TCDD/kg in hamsters caused a reduction in ejaculated sperm count of at least 58% and a reduction in size of accessory sex organs.

However, unlike studies by Mably et al. (1992) in which testosterone levels were reduced in male offspring, in the more detailed study by Gray et al. (1995), both the levels of serum testosterone and testosterone production were not affected by dioxin exposure. Also, there was no change in numbers of androgen (male sex hormone) receptors in the sex glands. These results fundamentally change previous explanations of the mechanisms by which dioxin exerts adverse effects on the male reproductive system. For example, it was previously thought that reductions in levels of male sex hormones including testosterone could be responsible for adverse reproductive effects, and anti-oestrogenic effects on the central nervous system could partly account for changes in sexual behaviour (Peterson et al. 1992). Results from the study by Gray et al. (1995) however do not support evidence for such mechaniams and an alternative hypothesis has been proposed. Rather than acting on steroid hormone levels or their receptors, it is possible that TCDD alters morphological sex differentiation by altering the levels of growth factors and receptors involved in cell proliferation and differentiation within the urogenital system (including progenitors of the epidydymis, seminal vesicle, prostate, external genitalia and kidneys). This could occur in a manner similar to that reported for the uteric epithelium of mice which were prenatally exposed to a single dose of TCDD. This exposure induced hyperplasia of the epithelial cells in embryonic ureters and altered regulation of epidermal growth factor (EGF) receptors (Abbot and Birnbaum 1990).

In a recent paper, Safe (1995) suggested that exposure to oestrogenic chemicals in the environment was probably not responsible for increasing disorders of the male reproductive tract. Safe hypothesised that on balance, the natural and manmade oestrogens and anti-oestrogens to which humans are exposed, in effect cancel each other out, and that humans are actually exposed to more anti-oestrogenic substances overall. However, at a time when the oestrogenicity of the majority of manmade chemicals has not even be tested, and it is questionable whether natural oestrogens from plants exert antioestrogenic activity (eg. Makela et al 1994), it is impossible to calculate the combined effects of human exposure to natural and manmade oestrogenic chemicals. The level of exposure to oestrogenic chemicals is not yet known (Danish EPA 1995). In addition, other scientists (eg. R. Peterson and L.E. Gray) are quoted as being sceptical about Safe's hypothesis because they do not think that oestrogenic and anti-oestrogenic chemicals, both of which modulate hormones, will simply cancel each other out (Stone 1994). For example, effects on the male reproductive system from exposure to oestrogenic organochlorines or anti-oestrogenic dioxin result in the same adverse effects, probably because they are mediated by different mechanisms.

2.1.9 Effect of Exposure to Organochlorines in Adult Males

There could be one or more different mechanisms by which organochlorine chemicals adversely effect male fertility in adults. Animal experiments have shown that organochlorines may interfere with reproductive hormones involved in testosterone metabolism and this would affect mechanisms of male reproduction (Pines et al. 1987). Alternatively, enhancement of oestrogen activity or direct damage to the testicular cells and semen may be caused by the organochlorine compounds. Semen motility may also be adversely effected by organochlorines. For example, Bush et al. (1986) found a association between semen motility and the concentration of some PCB's in infertile males.

Several studies have reported that occupational exposure to certain organochlorine chemicals has led to detrimental effects on male fertility. Rupa et al. (1991) conducted a study on a the fertility of

1016 male workers who were exposed to a mixture of organochlorine pesticides in cotton fields in India. The workers who were selected for study were engaged in mixing and spraying pesticides from July to March every year for durations ranging between 1 and 20 years. They were exposed to OC pesticides such as DDT, BHC and endosulphan as well as some organophosphorous pesticides. The study divided the workers into smokers and non-smokers since smoking can have adverse effects on human health. These two groups were compared to matched controls from the same socioeconomic group who had not been occupationally exposed to pesticides. Statistical analysis showed that there was a significant decrease in the numbers of fertile males in the group exposed to pesticides compared to controls. In the non smoking group, the number of fertile males was 83.67% compared with 96.45% in control subjects, and in the smoking group 76.68% compared to 92.50%. Moreover, when outcomes on the children of the workers were considered, there was a significant increase in other reproductive disorders eg. still births, neonatal deaths and congenital defects born to the exposed fathers. Such adverse reproductive effects indicate the presence of chromosomal damage in the germ cells from which sperm is produced. The study illustrates that exposure to such mixtures of pesticides can have adverse effects both on adult male fertility and on the offspring of exposed males, probably due to chromosomal damage to germ cells.

Dibromochloropropane

It has been scientifically proven that the organochlorine pesticide dibromochloropropane (DBCP) is has caused many cases of infertility in males who have been occupationally exposed to the compound (see eg. Whorton and Foliart 1983). DBCP was used as a soil fumigant from the mid-1950s and became very widely used in the mid-1970's until it was banned in 1979 by the US EPA. The first report that this compound caused infertility in men was in a California pesticide formulation plant in 1977. Initially, a group of 5 workers were investigated after they became aware that none of them had recently been able to father children. All 5 men were found to have low sperm counts. This prompted a further investigation of other workers at the plant. Results showed there was a very strong relationship between the duration of DBCP exposure and sperm count such that lower sperm counts were found in men who had been exposed for the longest times. Several studies were subsequently undertaken at various DBCP producing plants and amongst agricultural workers using the chemical. A summary of results from some of these studies are presented in table 2.2. In these surveys, workers had been exposed to various amounts of DBCP for different durations. However, all the studies revealed that occupational exposure to DBCP has disastrous effects on testicular function. For example 15.7% of the exposed workers were azoospermic (no sperm count) while the expected number from 500 workers would normally be 5% at the most. Similarly, 22.1% were oligospermic (low sperm count of <20 million per ml), but the maximum expected number would be 9-10%.

The exact mechanisms by which DBCP exerts adverse effects on the male reproductive system are unknown, but it is clear that it damages and destroys the germ cells that produce sperm. This results in a reduction in the numbers and motility of sperm which causes infertility. Sperm counts have recovered in some of the men affected by DBCP, but others remain sterile.

The true scale of the problem from the use of DBCP is even worse than results shown by studies in North and Central America and Israel given in table 2.3. High quantities of DBCP were used on the Atlantic banana growing regions of Costa Rica between 1971-1978 and workers were typically exposed to the chemical for long durations. By mid-1990, approximately 1500 male workers from

these banana plantations were medically diagnosed as infertile as a result of exposure to DBCP. Moreover, physicians estimate there are about 1000 other cases in the country which have not yet been reported. About 60 to 70% of all sterile victims recorded were azoospermic (no sperm count) and the rest are oligospermic (low sperm count of <20 million per ml). Since male virility has a very high social value in Latin America, the incident has provoked other psychological and social effects including mental depression (in over half the patients), impotence and an increase in divorce because of sterility (Thrupp 1991).

There are also economic and policy implications. By 1988 compensation payments from the country's nationalised health insurance company had been given to approximately 500 workers ranging from about one sixth to two thirds of the average annual salary. However, between 1983 and 1990 about 400 workers filed law suits against Dow Chemical and Shell Oil in the U.S who supplied the DBCP. This is because information from animal experiments on the extreme toxicity of DBCP on fertility were initially concealed by the companies and inadequate safety warnings were printed on labels of DBCP marketed for agricultural use. The pesticide companies have already lost about 10 lawsuits in the U.S on failure to warn against hazards of DBCP, plus pay offs 50 victims in out of court settlements. The losses of lawsuits from Costa Rica would constitute a large financial burden to the chemical companies (Thrupp 1991).

Dioxins

Dioxins have been shown to have adverse effects on the male reproductive system in animals and humans both after exposure and during adult life. Experiments on rats and other laboratory animals have shown that exposure of the adult male to relatively high amounts of dioxins results in a number of effects on the male reproductive system. These include changes in the levels of male reproductive hormones such as a reduction in testosterone, decreased spermatogenesis and fertility and reduced weights of the testis and accessory sex organ (reviewed by Peterson et al. 1993).

In humans, studies have also shown that occupational exposure to dioxins may lead to adverse effects on the adult male reproductive system. For example, in the Vietnam War, Agent Orange was contaminated with dioxins and men who participated in the aerial spraying of this chemical were thus exposed to dioxins. It was later found that testicular size was reduced in some of these men, and this effect was related to the amount of dioxins in their blood (Wolfe et al. 1992). A recent study on chemical workers who were occupationally exposed to dioxins found changes in three reproductive hormones, namely a decrease in testosterone and increases in FSH and luteinising hormone (LH). Altered levels in all three of the hormones were significantly related to dioxin levels in the blood of the workers, which suggests that dioxin may alter the levels of reproductive hormones in adult men (Egeland et al. 1994).

Other Organochlorines

One study has reported that exposure to organochlorines in the general population may affect male fertility (Pines et al.1987). 29 men from Israel who had at least a 5 year history of infertility of an unknown cause were studied alongside a control group of fertile men. Results showed that males who had fertility problems generally had higher levels of organochlorines in their blood than the control group. There was a significant increase in levels of DDT, DDD and DDE, as well as lindane and some PCBs (tetra and penta-CBs). Associations were also found between the blood levels of organochlorine chemicals and the levels of two reproductive hormones which are known

to be involved with testosterone metabolism. Although this was a small study and larger studies on the general population would be needed for conclusive evidence, the results are of concern because they show that long-term, low level exposures of the general population to various organochlorine compounds may interfere with the biological processes responsible for male fertility.

2.1.10 Summary

In summary, some organochlorines have been shown to adversely affect the reproductive system in occupationally exposed men. Moreover, it is highly plausible that prenatal exposure to oestrogenic chemicals and other organochlorines, such as dioxin and related compounds, may be partly responsible for the increasing disorders of the male reproductive system. There is a growing concern among scientists relating to this subject and also other possible health effects from exposure to such chemicals. For example, a recent international conference entitled Oestrogens in the Environment III: Global Health Implications was held in Washington D.C., January 9-11, 1994. Furthermore, the Danish EPA (1995) recently released a specific report on the subject entitled Male Reproductive Health and Environmental Chemicals with Oestrogenic Effects.

2.2 Disorders of the Female Reproductive System

2.2.1 Introduction

Women in industrialised countries now reach puberty (menarche) earlier and may experience menopause later. Lactation is often of much reduced frequency and duration. In addition, the incidence of some reproductive cancers is increasing, including breast cancer and vaginal/cervical cancer. The incidence of endometriosis is also increasing and the age of onset is decreasing. It is possible that many of these conditions may be associated with the elevated exposure to endocrine-disrupting chemicals, particularly oestrogenic chemicals, during development or in adult life (Whitten 1992, Eaton et al. 1994).

For example, women who were exposed to the synthetic oestrogen DES *in utero* have suffered an increased incidence of vaginal and cervical cancer in adulthood and also had increased fertility problems (Hines 1992). In a recent study on 20 women with unexplained infertility, 8 had elevated blood levels of lindane (>100 ng/L) and/or pentachlorophenol (>25ug/L). This may have caused disturbances in reproductive hormones and led to the problems with fertility (Gerhard et al. 1991). Finally a study on levels of organochlorines in breast milk showed that higher levels of organochlorines were associated with shorter lactation periods (see Hunter and Kelsey 1993). In this section, current evidence for associations of organochlorines with breast cancer, age at menarche and endometriosis are discussed.

2.2.2 Breast Cancer

Breast cancer is a major worldwide public health problem, representing between 3 and 5% of all deaths in developed countries and 1-3 % in developed countries. Although traditionally considered as a disease of advanced lifestyle and affluence, the incidence in developing countries is also high. For example, in 1980 breast cancer accounted for 22.9% of all cancers diagnosed in developed countries and 14.2% of all cancers diagnosed in developing countries (reviewed by Boyle 1991).

The incidence of breast cancer has continually risen both in industrialised and developing countries in recent years. It is now the leading cause of death from cancer in women in the USA (El-Bayoumy 1992). Breast cancer mortality has increased at an estimated 1% per year since the 1940's in the USA, even allowing for increased detection rates by mammography. Between 1982 and 1986 the incidence rose even more sharply by 4%. Increases have occurred among all age groups, although the disease is most prevalent in women between the ages of 40 and 55 (Harris et al. 1992).

Risk Factors

The known risk factors for breast cancer include age, age at menarche, menopause and first full-time pregnancy, total dietary calorie intake, family history of breast cancer alcohol intake, obesity and radiation exposure (Harris et al. 1992). Oral contraceptive use may possibly increase the risk in young women (Boyle 1991). At best, these risk factors only account for 30% of all cases of breast cancer. Interestingly, there is experimental and human evidence which shows that some of these risk factors are linked with total lifetime exposure to reproductive hormones. For example, studies indicate that the greater the total lifetime exposure to oestrogen, the greater the risk for breast cancer. Thus, elevated levels of oestrogen can increase the risk of breast cancer. Since it is known that manmade oestrogenic chemicals in the environment can mimic natural endogenous oestrogens or interfere with their metabolism, it has been hypothesised that such chemicals may increase the risk of breast cancer (Davis et al. 1993).

There are also two other risk factors for breast cancer which are as yet unproven. Increased fat consumption in the diet may increase the risk (eg. Bradlow and Fishman 1993), and breast feeding may decrease the risk. It is well known that consumption of a fatty diet results in a higher intake of organochlorine chemicals and that lactation reduces the body burden of these chemicals. If exposure to organochlorine chemicals does increase the chance of developing breast cancer, this could partly explain the possible risk factors of high fat consumption in the diet and protective effects of breast feeding (Dewailly et al. 1993)

Mechanisms of Breast Cancer Development

A number of organochlorine compounds including DDT and symmetrical triazine herbicides eg. simazine and chlortriazine and atrazine have been shown to induce or promote breast cancer in laboratory animals (see eg. Scribner and Mottet 1981, Stevens et al. 1994, Wetzel et al. 1994). The mechanisms by which such environmental oestrogens could increase the risk of breast cancer include alteration of oestrogen metabolism or mimicking oestrogens by binding to oestrogen receptors. Alternatively, these chemicals may operate completely outside of oestrogen pathways as direct carcinogens (through metabolism by cytochrome p450 enzymes and affecting certain genes, see section 2.1), (Davies et al. 1993).

Oestrogenic compounds may increase breast cancer risk by binding to and acting through oestrogen receptors, thereby mimicking natural oestrogens. Experimental evidence shows that elevated levels of oestrogen can promote breast cell proliferation which can lead to breast cancer. Human studies also show that an increase in exposure to exogenous oestrogens can increase the risk of breast cancer. For example, oestrogen replacement therapy in women may increase the risk of breast cancer by about 30% after 15 years of use (Steinburg et al. 1991). Similarly, women who were given the synthetic oestrogen DES also have an increased risk of developing breast cancer (reviewed by Boyle 1991).

A recent epidemiology study has confirmed observations from previous studies that elevated levels of endogenous oestrogens increase the risk of developing breast cancer. This study monitored women whose endogenous oestrogen levels fell in the normal range. Those women who subsequently developed breast cancer tended to show higher endogenous levels of oestrogens and a lower percent of oestrogen bound to sex hormone-binding globulin than women who remained free of cancer (Toniolo et al. 1995). The implications of this study suggest that factors which increase endogenous oestrogen production or reduce the binding of estradiol to SHBG may increase a woman's risk of developing breast cancer. Oestrogenic chemical pollutants in the environment are possible suspects because they can interfere with oestrogen levels in the body.

Recent studies have shown that oestrogen metabolism may be affected by environmental oestrogens, and in turn this effect on metabolism could contribute to the development of breast cancer. There is evidence that oestrogen is metabolised (broken down) in the body by two mutually exclusive pathways, pathway I and pathway II. In pathway 1, oestrogen is metabolised to 2-hydroxyestrone (2-OHE1), a compound which is known to inhibit breast cell proliferation. This compound may therefore protect against breast cancer because it inhibits breast cell growth. However, pathway II yields a different compound called 16a-hydroxyestrone (16a-OHE1) which enhances breast cell growth (by virtue of its ability to bind covalently to the oestrogen receptor), and may contribute to the development of breast cancer. It has been demonstrated that 16a-OHE1 is genotoxic (causes DNA damage) to cultures of breast cells *in vitro*. Animal studies have shown that the proportion of 16-OHE1 is increased in breast cancer, which means that the ratio of 16a-OHE1/2-OHE1 is elevated. There is also evidence that the levels of 16a-OHE1 are elevated by 50% in human breast cancers. Thus it is probable that increased levels of 16a-OHE1 and are associated with an increased risk of developing breast cancer (Davies et al. 1993, Bradlow et al. 1995, in prep.).

A recent study investigated the effect of various organochlorine compounds on oestrogen metabolism in breast cancer cells *in vitro* (Bradlow et al. 1995, in prep.). The study showed that the organochlorine pesticides atrazine, lindane, DDT, DDE, hexachloride, kepone and endosulphans I and II, and PCBs significantly increased the the proportion of 16-OHE1 and consequently the ratio of 16a-OHE1/2-OHE1 was increased. Moreover, the chemicals increased the ratio by a similar or even greater extent than dimethylbenzanthracene (DMBA), a known carcinogen. The greatest effects were observed with o,p'-DDE, followed by atrazine and DDT. It is therefore possible that some organochlorines may increase the risk of developing breast cancer by affecting oestrogen metabolism such that the ratio of 16-OHE1/2-OHE1 is elevated. This argument is further strengthened by dietary studies in animals which show that that feeding with substances that elevate 2-OHE1 have a protective effect against breast cancer and colon cancer. In humans, consumption of food such as cabbage which stimulates the 2-OHE1 pathway may also have a protective effect (Bradlow et al. 1995, in prep.).

Epidemiology Studies

Data from epidemiology studies is summarised in table 2.4. Several studies have provided evidence which suggests that organochlorine oestrogenic chemicals may be linked to breast cancer. A study by Mussala-Rauhamaa et al.(1990), found that breast tissue from breast cancer patients had significantly higher levels of B-hexachlorocyclohexane (HCH) than tissue from women who did not have the disease by about 40% (average levels 0.13 versus 0.08 ppb). Two other studies have linked DDT and PCBs with breast cancer (Falck et al 1992, Wolff et al. 1993). The study by Falck

et al. (1992) measured the concentrations of various organochlorines in samples of breast tissue taken from 20 women with breast cancer. The results were compared to samples of breast tissue taken from 20 women without the disease who were matched for age, and other relevant characteristics. The breast fat of women with cancer had about 40% more DDE (the major metabolite of DDT) and PCBs (ie. bis(4-chlorophenyl)-1,1 dichloroethane, and bis(4-chlorophenyl)-1,1,1 trichloroethane) than the controls. These results were statistically significant and suggested that these oestrogenic chemicals may have a role in the genesis of breast cancer.

In the above study (Falck et al. 1992), the authors reported that it was not known whether the higher breast tissue levels among cancer cases may be due to a redistribution of chemicals to the breast during the disease process. A recent study partly overcame this problem, by sampling blood instead of breast tissue and taking the samples shortly before cancer was diagnosed (Wolff et al. 1993). This was acceptable because blood levels of organochlorines are known to reflect levels found in tissues. Unlike previous studies, this study also adjusted for known breast cancer risks. The study determined the levels of DDE and PCBs in stored blood specimens from women who were part of a study cohort of 14290 women which had been set up between 1985 and 1991. Levels of environmental oestrogenic chemicals were measured in blood from 58 of the women who developed breast cancer within 6 months of entering the study. These were compared to blood from 171 well matched control subjects from the cohort who did not develop breast cancer during the study period. The results showed that mean levels of DDE and PCBs were higher for breast cancer patients than for control subjects, but were only statistically significant for DDE. It was calculated that women with such raised levels of DDE were 4 times more likely to develop breast cancer.

A small study involving 20 women with malignant breast cancer and 17 women with benign breast diseases showed that blood levels and breast tissue levels of organochlorines were higher in the women with breast cancer (Dewailly et al. 1993). HCB levels were significantly higher in blood in breast cancer cases compared to controls (316 vs. 25 ng/L, $p=0.02$). DDE levels were also higher in blood and breast tissue of women with cancer but the difference was not statistically significant. The difference was, however, significant ($p<0.01$) in breast tissue for women with hormone responsive breast cancer (oestrogen receptor positive, ER+) compared to controls or women with non-hormone responsive cancer (oestrogen receptor negative ER–).

A similar but larger study than that undertaken by Wolff et al. (1993) was carried out by Krieger et al. (1994). However, this study found no link with blood levels of organochlorines and breast cancer. In the San Francisco Bay Area between 1964 and 1971, thousands of women had a multiphasic health investigation during which a blood sample was taken and stored frozen. The health of the women was followed up until the end of 1990. 150 women from this group (50 white, 50 black and 50 Asian) who had been diagnosed with breast cancer more than 6 months (an average of 14 years) after the initial multiphasic health investigation, were selected for Krieger's study. This study was different from previous studies because blood samples were taken before 1972 when DDT was banned in the U.S and people were exposed to higher environmental levels. It also provided the opportunity to use blood samples taken well before breast cancer diagnosis, which is important because it negates the possibility that cancer may cause changes in the body which could elevate organochlorine levels. Concentrations of DDE and PCBs were measured in the blood samples from the 150 women with breast cancer and compared with healthy matched control subjects. No

association was found between the levels of DDE or PCBs and the risk of breast cancer. However, the results of one epidemiology study alone cannot be considered as definitive evidence for any disease and the authors suggested that much more research is needed before a link with environmental chemicals and breast cancer can be proved more conclusively.

A reanalysis of this study has revealed that when the racial/ethnic groups of women were considered separately from one another, a different result was obtained (Savitz 1994). Higher organochlorine levels were found to be associated with a 2-3 fold increased risk of breast cancer in black and white women. However, no such associations were found in Asian women. This result is consistent with previous studies which show that Asian women may have dietary or genetic protective factors for breast cancer and therefore have lower levels of the disease.

The original study by Krieger et al. (1994), and another previous study (Unger et al. 1984), found no relationship between exposure to organochlorines and breast cancer. Also, even though the study by Mussalo-Rauhamaa found an association between beta-hexachlorocyclohexane levels in breast tissue and breast cancer, no association was found with DDT or DDE as was the case in other studies. Although there is evidence from epidemiology studies which suggests an association between breast cancer and exposure to organochlorines, results from all the studies are not consistent, and therefore, as yet cannot prove or disprove the hypothesis that organochlorines cause breast cancer. A recent combined analysis of published epidemiology studies concluded that PCBs probably did not increase the risk of breast cancer, but more research would be needed to confirm whether DDT or DDE was related to an increased risk (Key and Reeves 1994). The general consensus among researchers in this field is that because the hypothesis is very plausible and is supported by evidence from animal studies and some epidemiological evidence, more epidemiology and laboratory studies need to be carried out to find the answer (Taubes 1994, Key and Reeves 1994). Krieger's group are already in the process of doing another much larger study (Taubes 1994).

Other studies have suggested a link between exposure to organochlorines and breast cancer. Elevated breast cancer rates were detected in women exposed to high levels of PCBs and dioxins in Japan in the Yusho incident (Kuratsune et al. 1987). A large scale geographical study in Israel found an association between a decrease in population exposure to organochlorine chemicals and a decrease in mortality rate from breast cancer, but did not use any direct measurements of exposure (Westin and Richter 1990). During 1976-1984, the rate of mortality from breast cancer in Israel decreased by 8%, but rates in other European countries at the time continued to increase. Even more unusual was that this decrease was caused by a fall in the death rate of more than 30% in premenopausal women (<44 years of age) and not in postmenopausal women whose death rate continued to increase. In an attempt to solve this anomaly, risk factors for breast cancer were investigated. However, the risk factors leading to a higher expected breast cancer incidence all got worse. For example, alcohol consumption increased, age at first birth increased, intake of dietary fat increased and intake of fibre decreased. Based on these findings, breast cancer incidence would be expected to increase and not to decrease.

Since it was younger women who were affected and the decreased mortality was not due to a change in risk factors, an explanation for the anomaly pointed to a dramatic change in lifestyle or the environment. One change in the environment did offer a possible explanation. In 1978, the

organochlorines lindane and hexachlorocyclohexane were banned from use in dairy farming in Israel, and the use of DDT was restricted. For at least 10 years prior to 1978, hexachlorocyclohexane, lindane and DDE were present in extremely high levels in cow's milk, the mean concentrations being 1000, 17 and 5 times greater than those found in cow's milk in the USA. The dramatic reduction in the use of these organochlorines would have resulted in a decrease in exposure of the general population. Since organochlorines can act as cancer promoters it is a estimated that a reduction in exposure to these chemicals could result in a fall in mortality rate in a period as short as 10 years. This study suggests that organochlorines are linked to breast cancer but cannot be considered as conclusive evidence because no direct measurements of exposure to organochlorines in humans were carried out.

Prenatal Exposure

Increased concentrations of oestrogens in adult life are known to increase the risk of breast cancer. It has been hypothesised that increased concentrations of oestrogens during pregnancy may also increase the risk of breast cancer in daughters (Trichpoulos 1990). There is some evidence to support this view. For example, dizygotic twins generally have higher rates of breast cancer, and their mothers are known to have higher levels of oestrogen during pregnancy. Also, a recent epidemiological study investigated the association between breast cancer and the incidence of pre-eclampsia (pregnancy induced hypertension) in the mothers (Ekbom et al. 1992). Pre-eclampsia is a condition where the concentrations of oestrogens during pregnancy are substantially reduced. The study found that daughters born to women with pre-eclampsia had a significantly reduced risk of developing breast cancer, which suggested that levels of oestrogen during pregnancy do affect the risk factors for breast cancer.

Summary

In summary, increased levels of oestrogens in women are known to increase the risk of developing breast cancer and the hypothesis that an extra burden from environmental oestrogens may also increase the risk is very plausible. Recent *in vitro* studies have shown that organochlorines may affect oestrogen metabolism in a way that may increase the risk of developing breast cancer,(ie. by increasing 16-OHE1/2-OHE1 ratio). DDE, the main metabolite of DDT, was found to have the greatest effect. This is of particular interest because several epidemiology studies and the Israeli breast cancer study have also suggested that DDT may play a role in increasing the risk of developing breast cancer. There is also some evidence that triazines and HCH could be associated with breast cancer and this is also consistent the *in vitro* study on oestrogen metabolism.

It should be noted that a recent paper by Safe (1995) has suggested that it is not plausible that industrial oestrogenic chemicals pose a breast cancer risk, particularly DDE, because it is not oestrogenic. However, other studies, for example, Krieger et al. (1994) have cited that DDE is oestrogenic. In addition, as previously discussed, DDE has been found to affect oestrogen metabolism in a way that may increase the risk of breast cancer (Bradlow et al. 1995, in prep.).

Results from experimental, animal and epidemiology studies considered together do suggest that exposure to some organochlorines, especially DDE, could be associated with an increased risk of developing breast cancer. However, results from epidemiology studies do show inconsistencies. More epidemiology studies are needed for conclusive evidence, but the current evidence is certainly a matter for concern.

2.2.3 Age at Menarche

The age at which women reach puberty (menarche) is predetermined during early development. Onset of puberty in both humans and mammals is accompanied by major changes in reproductive hormones (including an increase in a hormone called luteinizing hormone, LH), and maturation of a certain region of the brain (the hypothalmus), which releases some of these reproductive hormones. Experiments on rodents has shown that elevated levels of oestrogens during pregnancy or before puberty accelerates these processes, causing earlier onset of first estrous (equivalent to menarche in humans), (Whitten 1992).

It is known that oestrogen levels in women also influence the possibility of giving birth to twins. Those twins born as a result of a double ovulation are called dizygotic twins. It is known that such multiple ovulation is associated with elevated levels luteinizing hormone (LH). It is interesting that Japanese women have lower LH levels and correspondingly lower rate of twinning than European women. A possible explanation is that Japanese women are exposed to more isoflavones (a type of plant oestrogen) in their diet. Since isoflavones reduce the level of endogenous oestrogens in the body, it is possible there is a link between lower exposure to oestrogens and reductions in dizygotic twinning (Whitten 1992).

The age at menarche has declined from an average of about 17 years two centuries ago to the current average of 12.8 years (Harris et al. 1992). Records of menarcheal age indicate that that it declined about 0.3 years per decade from 1880 until the 1960-70s in most modern industrialised countries (Marshall and Tanner 1986). It is evident that the periods in which menarcheal age declined are mirrored by declines in the numbers of dizygotic twins who were born in the same groups of women. Declines in twinning rates are taken to indicate declining fecundity and perhaps toxic exposure. However, declining age of menarche has been taken to indicate improved nutrition and public health. Since both twinning and menarcheal age are developmental effects a generalised improvement in nutrition cannot explain both. It has therefore been suggested that only changes in reproductive hormone function, like the changes induced by oestrogens in experiments with rodents discussed above, can readily explain these coordinated changes (Whitten 1992). Thus it is possible that declining age at menarche in humans during the last century and the corresponding declines in the number of twins born, reflects an increase in exposure to oestrogens. Such an increase could be explained by our increased exposure to oestrogenic chemicals in the environment (Whitten 1992).

2.2.4 Endometriosis

Endometriosis is characterised by growth and proliferation of endometrial cells outside the uterus. Common sites of endometrial cell growth observed with this condition include the ovary, bladder, intestine and pelvic peritoneum. Although endometriosis is considered to be a benign disease, both mild and severe forms are associated with infertility and chronic pain. Endometriosis is only found in humans and non-human primates. Its prevalence in society is unknown but studies in the USA estimate endometriosis occurs in 10% of reproductive-age women (Rier et al. 1993).

Two studies on rhesus monkeys have shown that exposure to PCBs (Campbell et al. 1985), or dioxin (TCDD) (Rier et al 1993) increase the risk of developing endometriosis. These organochlorines are known to affect the immune system, and it is thought that immune mechanisms may be involved in the endometriosis disease process. In addition, dioxin may modulate various hormone receptor

systems which play a role in uterine function, and may alter the action of oestrogen in reproductive organs.

Rier et al. (1993) undertook a long-term study on the health effects of chronic dioxin exposure in rhesus monkeys. For a period of 4 years between 1977 and 1982 one group of rhesus monkeys were fed 25ppt dioxin in their diet, a second group were fed 5ppt dioxin and a third group acted as controls and were given no dioxin. Ten years after the end of dioxin treatment the animals were examined for the presence of endometriosis. Results showed that chronic exposure to dioxin was directly correlated with a significant increased incidence in the development of endometriosis. 5 out of 7 animals (71%) exposed to 25ppt dioxin and 3 out of 7 animals (43%) exposed to 5ppt dioxin had moderate to severe endometriosis compared to 33% of animals in the control group. The differences were statistically significant ($p<0.05$) in both groups of exposed animals.

This study is of particular concern because endometriosis was observed at very low chronic doses of dioxin. In fact the dose was 7-8 times lower than the no adverse effect level of 1000pg/kg/d proposed by WHO indicating that this guideline may not be protective of human health. Moreover, the effects occurred at an estimated body burden of only 27ng/kg, which is within an order of magnitude of current human body burdens (estimated as 5-10 ng/kg, EPA 1994b). Further studies in the USA are now underway, including an investigation by the National Institute of Environmental Health Sciences to determine blood levels of dioxins and PCBs in women diagnosed with endometriosis.

2.3 Developmental Effects

2.3.1 Susceptibility of the fetus to toxic insult in general

One of the main concerns of fetal pathology is that the cumulative effect of hundreds of organochlorine and other environmental pollutants, each one of which individually is undetectable or indeed unknown, may be causing damage to developing offspring.

Because the fetus is growing at a rapid rate and is immature in a number of functional aspects, it is more susceptible to damage from a toxic insult of a given size than an adult (South West Environmental Protection Agency 1995). The reasons for this are varied:

- The placenta selectively uptakes certain chemicals from the maternal blood stream.
- There is little body fat in the fetus and therefore no protective reservoir for fat soluble toxins such as organochlorines, as in adults. This automatically leads to higher circulating levels of toxic substances than in adults.
- Enzyme systems are immature and of low capacity, so the fetus can only detoxify toxic substances at a low rate and is exposed to high levels for a long time.
- Cell multiplication in the fetus is high. Dividing cells are more susceptible to injury from chemical and physical insults. Damage to the dividing cells of a developing organ may cause severe deformity.

- The fetus has a higher metabolic rate and therefore less (or no) cells in the resting state. Therefore the build up of intermediate toxic metabolites for a given body weight will be greater.
- Fetal kidneys cannot concentrate urine as well as adults
- The blood-brain barrier is not fully formed.

2.3.2 Fetal Death

There is evidence from animal experiments and epidemiology studies that exposure to certain organochlorines can result in fetal death and spontaneous abortion. Much of the evidence in humans comes from women who have been occupationally exposed to these chemicals in the dry cleaning and pharmaceutical industries, and in agriculture. Recent data on occupational exposure of men also suggests that abnormalities in sperm caused by exposure to organochlorines may result in spontaneous abortion.

Animal Experiments

Exposure to dioxin (TCDD) during pregnancy causes fetal death in monkeys and other small laboratory mammals (Peterson 1993). Prenatal exposure to PCBs has been found to cause fetal malformations (teratogenic) in laboratory mammals (Shane 1989). Similarly exposure to the organochlorine perchloroethylene (tetrachloroethylene) which is used in the dry cleaning industry causes fetal death in rats and growth disturbances in mice (Schwetz et al. 1975). Organochlorine pesticides such as lindane caused fetal death in mice (Sircar and Lahri 1989), and aldrin and p,p'-DDT caused fetal death in beagle dogs (Saxena and Siddiqui 1983). As with other developmental effects, it appears that there is a critical period during pregnancy when the fetus is more susceptible to chemical induced toxicity which causes fetal death (Peterson 1993).

Human Studies

Epidemiology studies have reported that exposure to certain organochlorines in humans can increase the incidence of spontaneous abortion during the first 3 months of pregnancy. In the dry cleaning industry, women are exposed to relatively high atmospheric concentrations of the solvent perchloroethylene. A study among female workers in the industry in Finland revealed that exposure to perchloroethylene was associated with significantly greater incidence (3.6 times, $p<0.05$) of spontaneous abortion during the first 3 months of their pregnancy compared to unexposed women (Kyyronen et al. 1989). An earlier study in Finland (Hemminki et al. 1980) also reported a greater incidence of spontaneous abortion (10.14%) after exposure to dry cleaning chemicals compared to the general population (5.52%). A study in Italy showed a four-fold increase in the percentage of spontaneous abortions among women in the dry cleaning industry, but this result was not significant because only a small number of women were studied (Bosco et al. 1987). One study on workers in Sweden did not find an association between spontaneous abortion and exposure to perchloroethylene (Ahlborg 1988). However, other epidemiology studies discussed here together with evidence from animal studies suggests that exposure perchloroethylene may cause an increase in the incidence of spontaneous abortions.

Exposure to organic solvents in general, including the organochlorines tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane was found to be significantly associated with

spontaneous abortion in female workers (Lindbohm et al. 1990). A study on Finnish workers employed in the pharmaceutical industry between 1973 and 1981 revealed that exposure to organic solvents, particularly methylene chloride, was associated with an increased risk in spontaneous abortion. For example, exposure to 4 or more solvents was associated with a 3.5 fold increased risk in spontaneous abortion, which was statistically significant (odds ratio 3.5, p=0.05), and exposure to methylene chloride was associated with a 2.3-fold increase in risk of borderline significance (odds ratio 2.3, p=0.06). Furthermore the risk increased with increasing frequency of exposure to the solvents. The study also found an elevated risk for spontaneous abortion after exposure to oestrogens when controlling for the effects of other chemicals (odds ratio 4.2, p=0.05), although the number of women involved was small (Taskinen et. al. 1986).

There have been reports of increased spontaneous abortion in relation to exposure to organochlorine pesticides. A case report of a woman who was 16 weeks pregnant and swallowed 250ml of the pesticide lindane in a suicide attempt resulted in the fetal death (Konje et al. 1992). This chemical is used in agriculture and also as a 1% solution for the treatment of lice and scabies. As a result of this case study and because lindane causes fetal death in mice, it was suggested that the topical application for pest treatment during the first trimester of pregnancy should be avoided.

Exposure of male workers to DBCP in Israel may have caused an increase in spontaneous abortions experienced by their wives. 6.6% of women married to workers who were not exposed to DBCP in this region experienced spontaneous abortion compared to 19% of pregnancies in women married to exposed workers (Whorton and Foliart 1983). Similarly in India, there was an increased incidence in spontaneous abortion in women married to workers who were exposed to various organochlorine pesticides (Rupa et al. 1990). It is thought that spontaneous abortion in these cases is probably caused by genetic damage to the germ cells in the men from which sperm is produced.

One study in India investigated the levels of some organochlorine chemicals in maternal blood and umbilical cord blood from women experiencing stillbirths and livebirths. The levels of aldrin and p,p'-DDT in all samples from 9 stillbirth cases were significantly higher than in 27 matched control livebirth samples. For example, the mean level of aldrin and p,p'-DDT in maternal blood of stillborn cases was 33.3 ppb and 62.9 ppb respectively compared to levels in livebirths of 5.6ppb and 9.2 ppb ($p<0.01$). The authors concluded that a possible association with high levels of these pesticides and fetal death existed (Saxena and Siddiqui 1983).

Finally there is some evidence that exposure to dioxins and PCBs cause spontaneous abortion. In Japan and Taiwan in the Yusho and Yucheng incidents, many people consumed rice contaminated with PCBs and dioxins. An increase in fetal mortality was recorded amongst women who were pregnant at the time of the incidents (Peterson 1993).

When the all the data from animal and human studies are considered, there is much evidence which suggests that some organochlorine chemicals are associated with fetal death in humans.

2.3.3 Growth Retardation and Reduced Birth Size

Exposure to industrial pollutants in the environment, including organochlorines, has been associated with growth retardation of the fetus in the womb, a condition known as intrauterine growth retardation (IUGR). Studies have shown that IUGR is associated with cot deaths (Sudden

Infant Death Syndrome (SIDS). In addition, one study has shown that among those individuals born with IUGR who survive infancy, there is a reduced adult life expectancy due to an increased death rate from heart disease (Barker 1989).

Recent work has shown that IUGR in human infants is associated with a highly significant reduction in the number of nephrons (the basic filtration unit) in the kidneys (Hinchliffe et al. 1992). While normal infants and adults have about 1,000,000 nephrons per kidney, cases of IUGR which have been studied demonstrate a nephron population of between 300,000 and 600,000. A recent study has shown similar findings in 70% of the cases of SIDS which have been examined (Hinchliffe et al. 1991). This work indicates that there is a critical period during development when nephrons are created. Any outside influence, such as exposure to toxic chemicals, which disturbs the growth of the embryo may disturb this process and lead to a permanent deficit of nephrons.

There is evidence from animal studies and epidemiology studies that some organochlorines are associated with growth retardation in the foetus. Both animal experiments and epidemiology studies that prenatal exposure to PCBs is associated with reduced birth size. Prenatal exposure to PCBs in pregnant rats, mice and rhesus monkeys causes reduced birth weight in the offspring.

Studies on pregnant women who were exposed to PCBs and dioxins in Japan (Yusho) and Taiwan (Yucheng) after eating contaminated rice, revealed that their children were born prematurely and had a reduced birth weight (Funatsu et al 1972, Wong et al. 1981). The reduction in birth weight was found to be more pronounced in female babies than in males.

Several epidemiology studies have investigated birth size in the general population around the Great Lakes area. The Lakes are polluted with PCBs and other organochlorine chemicals. Consumption of PCB-contaminated fish from Lake Michigan has been found to be related to PCB concentrations in maternal serum and milk. In turn, maternal serum levels of PCBs reflect serum levels in the umbilical cord, which represents a source of exposure to the fetus. Most of the studies in the Great Lakes area have found that maternal exposure to PCBs through eating contaminated fish is associated with a reduction in birth size in newborn children (Swain 1991).

For example, Fein et al.(1984) studied 242 children born between 1980 and 1981 to women who had previously consumed a moderate amount of Lake Michigan fish. Birth size in the newborns was compared to a control group of children whose mothers had not consumed Lake Michigan fish. The study reported that exposure to PCBs, measured by fish consumption and levels in umbilical cord serum, was related to lower birth weights and smaller head circumference in newborn infants, and a shorter gestation period. Exposed infants were significantly lighter (160-190grams, $p<0.05$) than controls and their heads were significantly smaller (0.6-0.7cm, $p<0.01$).

Evidence from animal studies, from the Yucheng incident and from the Lake Michigan studies also indicates that growth retardation caused by *in utero* exposure to PCBs persists beyond the newborn period. A follow-up study of children from the Lake Michigan study showed that the growth retardation was still apparent at the age of 4 (Jacobson and Jacobson 1990). The most highly exposed children (assessed by umbilical cord serum level of 5ng/ml or more) weighed on average 1.8 kg less than the least exposed children. The result was statistically significant in girls where the weight difference was 2.2 kg ($p=0.038$). The weight difference was similar in boys (1.7kg)

but this was not statistically significant. The growth retardation of girls was also more pronounced in the Yucheng study (Rogan 1988), and their height was significantly shorter (2.8cm p=0.02) than control at the ages 11-14 (Guo et al. 1993). Similarly, a study of PCB exposure in the general population of Japan also revealed that more highly exposed infant females lagged in weight and height at birth and for sometime afterwards (Hayashi et al. 1985).

In sum, it is apparent from animal and epidemiology studies that *in utero* exposure to PCBs is associated with reduced birth size in infants and this growth retardation may persist in children.

2.3.4 Effects on Muscle and Bone

In Holland, a study on 200 women and their babies in the general population from three different areas was initiated by the Dutch government to investigate whether exposure to dioxins and PCBs were having any developmental health effects on babies. This followed reports in 1989 that cow's milk in the Lickbaert area had been highly contaminated with dioxins and PCBs from an incinerator in Rotterdam (Liem et al. 1991). A previous study had also reported that women in the Netherlands had high levels of these chemicals in their breast milk (WHO 1989), and subsequently it was found that levels were higher in industrialised areas (Koopman-Esseboom 1994a). The study monitored exposure of the babies by measuring the concentration of dioxins and PCBs in breast milk and umbilical cord. Results showed that at both ages of 10 days and 3 months, babies who were more highly exposed to dioxin had a lower quality of muscle tone, which means that their ability to use their muscles was reduced. More highly exposed babies also had less developed reflexes. The muscle tone effects were not apparent at 18 months but the children will be tested again at the age of 3 and 7 (Brouwer et al. 1995, in press).

Some studies in animals have shown that PCBs and the pesticide lindane (gamma-hexachlorocyclohexanae) can affect calcium metabolism. This can lead to reduced bone density and bone strength in young animals (eg. Andrews 1989, Andrews and Grey 1990). Studies in humans on such effects are very limited. Skull abnormalities were reported in some of the children born after the Yusho incident in Japan where people had consumed rice contaminated with PCBs and dioxins. These abnormalities may have been the result of irregular calcification (Miller 1985).

2.3.5 Effects on the Nervous System and on Behaviour

The brain, like other organs, also has a strict timetable for development and if this is perturbed, a permanent deficit will result. Several epidemiology studies on children exposed *in utero* to organochlorine chemicals show that their behaviour, including some aspects of intelligence may be affected as a result. The mechanisms which cause disturbances in development of the nervous system and brain are not fully understood, but are thought to be partly under the control of reproductive and thyroid hormones. The balance of these hormones during development can be disturbed by some organochlorine chemicals. Such disturbances may be partly responsible for the changes in behaviour observed in children who were prenatally exposed to organochlorines.

2.3.5.1 Hormonal Effects on Development of the Nervous System

Sexual Differences in the Brain

In birds, mammals and humans there are some differences between males and females in the structure and function of the brain. These differences account for the distinctive differences in

sexual behaviour between males and females. In humans the differences are also thought to be responsible for other brain functions which bear no obvious relation to reproduction, but which are different in men and women, such as verbal fluency and certain types of visuospatial ability (Hines 1992, Dohler and Jarzab 1992).

The development of the sexually different areas of the brain in the fetus and infant are controlled by various reproductive hormones and other chemicals in the nervous system (neurotransmitters). Animal experiments have shown that if the balance of these hormones is altered during development, the sexual behaviour of the animal can be affected. For example, higher than normal levels of oestrogen during development has dramatic and permanent influences on brain structure and function. If female rats are prenatally exposed to DES or other oestrogens it causes them to have a masculine sexual behaviour pattern throughout their life. This is called 'masculinisation' of behaviour. It occurs because increased levels of oestrogen cause the sexually different region of the brain to develop the structural characteristics of a male brain. The same effect occurs if the rats are exposed to elevated levels of testosterone, because this hormone is broken down to oestrogen in this region of the brain.

It is possible that exposure to environmental oestrogens could affect the sexual development of the brain in humans (Hines 1992, Dohler and Jarzab 1992). In some studies on behaviour and learning in children who were exposed *in utero* to organochlorine compounds, girls were often more affected than boys. This difference may be partly due to the disturbance of reproductive hormones during development of the brain.

The role of thyroid hormones in development of the nervous system

Thyroid hormones are essential for normal development of the nervous system in humans. They control the growth rate, and maturation of neurons (nerves), and also the organisation of neurons in some regions of the brain. If thyroid function is disturbed in adult life the nervous system may be impaired, but this can usually be reversed with appropriate treatment. However, disturbances during the critical period when the nervous system is developing can produce irreversible neurological damage, and possibly permanent changes in thyroid function. In humans this critical period begins *in utero* and extends until 2 years of age. Conditions that result from thyroid hormone deficiencies in fetal life in humans include endemic cretinism and congenital hypothyroidism. Both conditions often are characterised by mental retardation and speech disorders which can be very severe in endemic cretinism (Porterfield 1994).

Animal experiments have shown that dioxins and PCBs can block the action of thyroid hormones. These chemicals are similar in structure to thyroid hormones and as a result they can bind to the same receptors, including the Ah receptor and the thyroid hormone receptor. They can also bind to a protein in the blood which normally binds to thyroid hormone (called T4) and transports it around the body. The result of these actions is that dioxins and PCBs either mimic or decrease the biological action of the thyroid hormones. Either effect could have disastrous consequences during development as exemplified by the thyroid hormone disorders described above which lead to permanent brain damage. Even in nontoxic doses it is possible that low levels of dioxins and PCBs can alter neurological function by their effects on thyroid hormone action (Porterfield 1994).

One study investigated the levels of thyroid hormones in 38 healthy newborn babies in the

Netherlands because there had been reports that dioxin levels in human breast milk were high in this country, as well as in Belgium and the UK (Pluim et al. 1993). The infants were divided into a high and low exposure groups based on the levels of dioxins measured in the breast milk of the mothers. Thyroid hormone levels were measured at birth and at 1 and 11 weeks after breast feeding. A comparison of the two groups revealed that levels of two hormones (t4 and TSH) were significantly higher in the high exposure group than the low exposure group. Such differences in these hormones are consistent with results from animal experiments. It was concluded that the differences in thyroid hormone concentrations in the two groups was probably due to an increased exposure to dioxins *in utero* and possibly from breastmilk (Pluim et al. 1992 and 1993).

Accidental exposure to dioxins and PCBs in pregnant women resulted in behavioural disorders and learning difficulties in their children (see below). Although the mechanism which causes this toxicity to the nervous system is unknown, current evidence does suggest that the effect could be partly or entirely a result of effects on thyroid hormones (Porterfield).

There is also evidence that PCBs may be toxic to the developing nervous system because they affect the level of a chemical in the nervous system called dopamine which is involved in the transmission of nerve signals in the brain (Seegal and Shain 1992). *in vitro* studies on extracts of chemicals from salmon in the Great Lakes showed that PCBs from the fish, but not other organochlorines, caused reductions of dopamine concentrations experimentally (Seegal et al. 1991). PCBs are also thought to be toxic to the developing nervous system because they decrease the receptors of another chemical involved in the transmission of nerve impulses called acetylcholine (Seegal and Shain 1992).

2.3.5.2 Effects on Cognitive Function and Behaviour

Exposure to certain PCBs and dioxins during fetal life has been shown to produce behavioural effects in humans and animals. This toxic effect on the nervous system and brain is very complex leading to various behavioural problems such as reduced intelligence, learning difficulties, memory problems, hyperactivity and under-activity. These outcomes are dependent on several factors including the time in development when exposure occurred, the specific chemicals involved, the dosage of exposure and probably the species (Porterfield 1994).

Much of the evidence for behavioural effects in humans comes from studies on children born to women who were exposed to PCBs and dioxins in the Yucheng incident in Taiwan during 1978-9 (see eg. Chen et al. 1992, Lai et al. 1994, Guo et al. 1994). These studies followed the development of children born to women who were pregnant during the poisoning incident and also those who were born to exposed women up to 12 years after the incident. The children's cognitive development was assessed by various intelligence tests which were given to them every year. From 18 months of age up to 7 years of age, scores on these tests were consistently and statistically significantly lower for the Yu-Cheng children at each age level compared with an unexposed control group of children. This is very troubling because it appears that the impairment of intellectual function in these children at a young age does not improve over time as they get older, which means that damage could be permanent. In addition, children born up to 12 years after the incident were as affected or even more affected than those born only one year after the incident despite the fact that the levels of toxins in the women had declined (Chen et al. 1992, Guo et al. 1994). One study also reported that girls were more badly affected than boys which may be due to

disturbances in sex hormones following prenatal exposure to PCBs/dioxins.

Studies on the Yu-Cheng children up to the age of 12 have also found that they suffer from mildly disordered behaviour and higher activity levels than unexposed children. As with intellectual (cognitive) development the behavioural problems persisted as the children aged (see eg. Yu et al. 1994).

The effects on behaviour of prenatal exposure to PCBs as a result of consuming contaminated fish from the Great Lakes has also been studied. The offspring of pregnant rats fed on a diet of salmon from Lake Ontario showed altered behaviour compared to those fed on non-contaminated salmon. These experiments proved that it was the contaminants in the fish which caused behaviour changes in the rats (Daly H.B. 1992). It is more difficult to prove such a relationship in human studies, but a series of studies on children born to women who ate contaminated fish from Lake Michigan do suggest that PCBs in the fish are associated with behavioural problems in the children. In behavioural tests at the ages of 3 days, 7 months and 4 years the children had significantly lower scores compared to children whose mothers did not eat contaminated fish (Jacobson et al. 1985, Jacobson and Jacobson 1993). Tests at 4 years of age revealed that prenatal exposure to PCBs as a result of eating the fish, was associated with small but significant deficits in short-term memory and speed of information processing (thinking). Although the magnitude of the associations was modest, the results are worrying because even relatively subtle deficits in short-term memory and speed of information processing may have a significant impact on the child's ability to master basic reading and arithmetic skills in school (Jacobson and Jacobson 1993).

Finally, a recent study has used a different approach to investigate developmental effects in exposed children by measuring physiological changes in nerves of the brain (neurophysiological functioning). The study investigated 14 children who were born between 1977 and 1983 to mothers who resided in the dioxin-contaminated environment of Times Beach, Missouri. Times Beach was contaminated with dioxin (TCDD) during the 1970's when contaminated waste oil was sprayed on roads and at many horse arenas for dust control. The children tested were found to have abnormal brain measures (neurophysiological dysfunction) in an area of the brain which is thought to affect intellectual processes. The effect was more pronounced in the girls than the boys and was statistically significant in the girls (Cantor et al. 1993)

In sum, it is evident that *in utero* exposure to PCBs and dioxins causes adverse effects on behaviour in laboratory animals and in humans. In humans, this observation is also supported by recent findings that in growth retarded human fetuses and neonates (eg. as seen with YuCheng and Michigan neonates), there is a reduced number of brain cells (Ansari et al. 1994). Studies on YuCheng children revealed they scored significantly lower on intelligence tests and had mildly disordered behaviour problems which all persisted into childhood. Studies on women who consumed fish from Lake Michigan suggest that some members of the general population are already exposed to sufficient quantities of contaminants to cause an effect in their children. It is possible that effects of these chemicals on thyroid hormones during development may lead to changes in development of the nervous system which cause the behavioural effects. In addition, in some cases females appeared to be more adversely affected than males which could have been due to the organochlorines interfering with reproductive hormones during development of the brain.

2.3.6 Effects on the Immune System

The function of the immune system is to maintain health. The immune system consists of a network of specialised cells which mount responses to foreign substances in the body to prevent infection and disease. Toxic chemicals may cause either suppression of immune system cells or may lead to an increase in immune system cells. Suppression of the immune system can result in an increase in the incidence of infectious diseases and some types of cancer. Conversely, an increase in immune system cells may lead to an increase in autoimmune diseases (a condition caused by an immune response which is directed against the bodies own cells/tissues).

There is evider.ce from both animal and human studies that prenatal exposure to some organochlorines and the synthetic oestrogen diethylstilbestrol (DES) can have a detrimental effect on the immune system. However, in general this whole area is under-researched. Presently, there are only limited models for studying the immunotoxicty of organochlorine chemicals and consequently there are large gaps in the data.

There is evidence from animal and human studies that prenatal exposure to some organochlorines and the synthetic oestrogen diethylstilbestrol (DES) can have a detrimental effect on the immune system. Some of these studies are discussed below.

Prenatal exposure to dioxin in mice causes immunotoxicity (reviewed by US EPA 1994a). Studies on mice have also shown that perinatal exposure to DES can significantly and permanently affect the immune system. The severity of the immune defects detected often increase with age. The severity of the immune defects detected often increase with age. Major changes have been found in the functioning of some of the cells of the immune system (T cells, B cells and natural killer cells). Also, the animals may be more susceptible to the development of tumours and less able to withstand the stress of experimental manipulations such as surgery (Blair 1992a).

DES was given to women as a treatment to prevent miscarriages between 1945 and 1971. Several studies on individuals who were exposed to DES *in utero* suggest that changes in the immune system may have occurred. Altered function of T cells and natural killer cells has been reported in a limited number of subjects. In addition, an increased lifetime prevalence of autoimmune diseases and a 3-fold increase in rheumatic fever has been demonstrated in exposed individuals (Blair 1992b). This observation was strengthened by analysis of blood samples of exposed individuals which revealed a significant difference in factors of the immune system (increased red blood cell antigens and immunoglobulin class IgA) which are associated with autoimmune diseases. Overall, data from the human studies indicates that individuals who had prenatal exposure to the synthetic oestrogen DES do not have severe defects in immune system function but do have effects, such as an increase in autoimmune diseases and rheumatic fever, which are consistent with defects in immune system regulation. It is not known whether such effects will get worse with age as seen in animal experiments (Blair 1992a).

There are only a few studies on immune system effects of organochlorines in humans. Infants who were exposed to high levels of PCBs and dioxins *in utero* during the Yucheng incident in Taiwan were found to experience colds and bronchitis more frequently than non-exposed infants, but no differences in immune system cells in older children aged between 7 and 9 were detected (Lan et al. 1990). A study was also carried out on 15 children aged between 9 and 14 who were born to women

who lived in a dioxin (TCDD) contaminated environment at Times Beach, Missouri during and subsequent to pregnancy. The children were found to have significant changes in the numbers of several types of cells (lymphocytes) of the immune system. The results were consistent with previous animal studies and showed that perinatal exposure to dioxin can cause deficiencies in the immune system that persist for 10 years or more (Smoger et al. 1993). Finally, high levels of PCBs and dioxins have been found in the breast milk of Inuit women which is due to the consumption of a diet of mainly fish and sea mammals. A recent study has reported that Inuit neonates up to one year old had increased episodes of acute otitis media (infected inflammation of the middle ear), which related to the levels of dioxins and PCBs found in breast milk. These results implied that an increase in the incidence of otitis media could be due to deficiencies in the immune system caused by exposure to PCBs and dioxins (Dewailly et al. 1993).

2.3.7 Summary of Developmental and Reproductive Effects in Humans

There has been an increased incidence of male and female reproductive disorders over the last 50 years. It is now suspected that these effects may be due to an increased exposure to oestrogenic and other endocrine-disrupting chemical pollutants in the environment, including many organochlorines. The fetus and neonate are particularly sensitive to toxic insult and many of the observed effects may be resulting from prenatal exposure to such chemicals. Occupational exposure in adults to some organochlorine chemicals has also been associated with effects on the reproductive system.

Prenatal exposure to organochlorine chemicals, which may exert their effects by disrupting endocrine function, has been associated with other developmental effects in humans. Children born to mothers who ate organochlorine contaminated fish from the Great Lakes, and children from the XuCheng disaster whose mothers ate rice contaminated with PCBs and dioxins, suffered growth retardation and neurobehavioural effects with have persisted with age. In fact, based on current breast milk concentrations in the USA, it is estimated that 5% and possibly more of the babies are exposed to quantities of PCBs sufficient to cause neurological effects (Colborn et al. 1993).

A full report on developmental toxicity from exposure to polyhalogenated aromatic hydrocarbons, (organochlorines and related substances) in animals and humans was recently commissioned by the Dutch government (Brouwer et al. 1995, in press). The report concludes that functional developmental effects observed in experimental animals can persist into adulthood and include; neurobehavioural effects (impairment of cognitive functioning, altered neuromotor and sexual behaviour), developmental reproductive effects and neurochemical effects (including reduced brain and circulating thyroid hormone levels). The report discusses the fact that comparable effects are found in humans, concluding that prenatal and lactational exposure to PCBs and dioxins in humans can produce developmental effects, including low birth weight, lower scores in cognitive tests, psychomotor changes and alterations in plasma thyroid hormone levels. It was noted that although effects on neurobehavioural development were relatively subtle with no evidence of gross malformation or retardation, the effects may nevertheless have implications at a population level.

2.4 Developmental and Reproductive Effects on Wildlife

2.4.1 Introduction

In the last 50 years there have been severe, often sudden declines in the populations of many wildlife species which have been related to contamination of the environment with industrial chemicals (Wingspread 1993). Many of these declines have been specifically linked with the release of organochlorine chemicals into the environment.

The Great Lakes have been heavily contaminated with a multitude of organochlorine chemicals. Studies in this region over the last 20-30 years have found declines in many wildlife species which are associated with organochlorine pollutants. However, the problem is not isolated to this region, but is occurring on a global scale (Wingspread Conference 1993).

Reproductive and developmental disorders resulting from exposure to organochlorine chemicals have been reported to occur in fish, reptiles, birds and mammals. It has been postulated that disturbances in the early phase of reproductive cycles are linked to steroid hormone imbalances caused by organochlorines (Reijnders and Brasseur 1992). Exposure to organochlorines which disrupt hormones has been associated with many effects including decreased fertility in fish, birds and mammals, decreased hatching success in fish, birds and turtles, demasculinisation and feminisation of male fish, birds and mammals and decreased viability of offspring (Colborn et al. 1993). Some of these disorders may be due to oestrogenic effects of certain organochlorines (Bergeron et al. 1994). In some cases specific organochlorines have been linked to adverse effects, but in many cases it is probable that effects are due to exposure to multiple organochlorines (eg. Giesy et al. 1994).

This section discusses examples of reproductive and developmental effects in fish, reptiles, birds and mammals which have been associated with exposure to organochlorine chemicals.

2.4.2 Fish

Organochlorines have been linked with increased embryo mortality and embryo deformities in fish. For example experiments have shown that fertilised fish eggs of zebrafish (Brachydanio rerio) exposed to lindane and atrazine, exhibited increased deformities and decreased growth in the embryos and a reduction in the survival of juveniles which hatched (Gorge and Nagel 1990). Studies on lake charr (trout) in the Great Lakes have demonstrated much evidence which suggested that high embryo mortality in this species was related to organochlorines. The problems appeared to be largely due to maternally transfered PCBs, and have continued in recent years even though many contaminants in Great Lakes fish have decreased over the last 20 years. Reproductive problems have also been reported in stocks of Pacific coho salmon in Lake Erie. Between 1980 and 1990 the rates of survival to hatch in these fish decreased considerably, ranging from as high as 48% in 1981 to less than 5% in 1990. In addition, there was a high prevalence of embryo deformities and poor expression of secondary sexual characteristics in males. Studies on these fish have shown that the reproductive problems are probably related to environmental contaminants (reviewed by Leatherland 1992).

Studies on Great Lakes Pacific salmon stocks over a number of years have shown that 100% of these fish have enlarged thyroids which cannot be explained by low iodine content in the lakes.

When these fish were fed to rats, the animals also developed thyroid enlargement, providing evidence of environmental anti-thyroid substances in the Great Lakes food chain. It is probable that environmental chemicals in the water are responsible for these effects but it is not yet clear which ones (reviewed by Leatherland 1992).

Breeding difficulties in several species of sea fish have also have been associated with exposure to organochlorines including PCBs, DDT and DDE (see eg. Johnston and McCrea 1992).

2.4.3 Reptiles

Many reptilian species have temperature-dependent sex determination, which means that the temperature at which the egg is incubated determines the sex of the offspring. Application of oestrogen to the eggshells of such species during the developmental period of sexual differentiation, can counteract the effects of temperatures that normally produce male hatchlings and instead induce ovarian development. A recent study on the red-eared slider turtle (Trachemys scripta) was conducted to determine the effect of PCBs on sex determination (Bergeron et al. 1994). In this species, warm egg incubation temperatures (eg. 31°C) produce all female hatchlings and cold temperatures (eg. 26°C) produce all male hatchlings. The study showed that spotting the eggs with oestrogenic PCBs had the same effects as oestrogen causing a reduction in male offspring and increased female offspring. Moreover, the PCB levels that disrupted sex differentiation in the turtles were comparable to average levels of PCBs found in human breast milk in industrialised countries (Bergeron et al. 1994).

Lake Apopka in Florida is contaminated by a previous extensive spill of the organochlorines dicofol and DDT. During the 1980's there was a progressive decline of the alligator population on the lake which is presently still continuing. The population is now only a tenth of its former size recorded in the 1970's. A recent study on the alligators has found evidence of decreased reproductive ability. Oestrogen levels in female juveniles were twice that of levels in alligators from an unpolluted lake, and they exhibited abnormal ovarian structure. Male juveniles had significantly depressed levels of testosterone (3 times lower than controls), and had poorly organised testes and small phalli. These results strongly suggest that the gonads of juvenile alligators have been permanently altered during development in the egg, so that normal production of sex hormones is not possible making normal sexual differentiation unlikely (Guillette et al. 1994).

2.4.4 Birds

Reproductive abnormalities in wildlife bird populations, have been found to be associated with organochlorine chemicals in the environment. These abnormalities include eggshell thinning, increased embryo mortality and deformities, feminisation of males and abnormal behaviour. These breeding problems have led to massive declines in some bird populations. For example, studies on many fish-eating birds of the Great Lakes have shown that eggs of these birds contain organochlorine pesticides, PCBs and dioxins (PCDDs and PCDFs) at sufficiently high concentrations to cause adverse effects on the birds and their chicks, and has led to population decreases in these birds since the 1950's (Giesy et al. 1994).

The most dramatic decreases of reproductive performance in wild birds was eggshell thinning, caused primarily by DDE, a degradation product of the insecticide DDT (Giesy et al. 1994). In Britain DDE was implicated in eggshell thinning and the subsequent population decline of peregrine

falcons in the 1950's (Gilbertson 1989). Many populations of Great Lakes fish-eating birds were reduced because of decreased survival due to eggshell thinning, including a massive decline in North American Bald eagles (Haliaeetus leucocephalus) in the 1950's and 60's. Despite restrictions on the use of DDT since 1972, embryonic and chick survival along the shore of the Great Lakes is still not adequate to maintain stable populations (see Colborn et al. 1993).

Fox (1992) reviewed the breeding problems of some species of fish-eating birds in North America which have been associated with exposure to organochlorines. Western Gulls (Larus occidentalis) in colonies on the Channel Islands off the coast of southern California, and the Great Lakes populations of Herring Gulls and Caspian Terns (Sterna caspia) suffered from breeding difficulties in the late 1960's and throughout the 1970's. Problems included increased embryonic and chick mortality, growth retardation and deformities and altered behaviour patterns resulting in population declines. Studies of newly hatched chicks collected from Lake Ontario revealed gonads resembling ovaries in male birds. In addition, female-female pairing of gulls were found tending abnormally large clutches of eggs (supernormal clutches). Very few of these eggs were fertile. The female-female pairings may have been a result of abnormal female behaviour. Alternatively, it may have occurred due to embryonic feminisation of males or increased male mortality. The studies on gulls do suggest that feminisation of males was widespread in the most contaminated bird populations during the period of peak organochlorine contamination and that males either died, or were feminised and chose not to attempt to breed. The factors which caused feminisation of male birds are not yet certain, but experiments have shown that that DDT, methoxychlor, dicofol and mirex are oestrogenic in birds. Concentrations of o,p'-DDT (as low as 2ppm) and p,p'-DDE (20 and 100ppm) induce feminisation in male gull embryos (Fox 1992, Colborn et al. 1993).

Since the restriction of use of DDT and other organochlorines, some Great Lakes fish-eating birds have made a recovery in numbers, including the double-crested cormorants and herring gulls. However, some species have not recovered and others such as the common tern and Foster's tern continue to decline in number. This does not appear to be due to egg shell thinning in many cases but instead is due to a suite of other reproductive effects including embryo lethalities and birth defects. The biochemical causes of some of these effects including alterations in the induction of cytochrome p450 enzymes, depletion of liver reserves of retinoids and vitamin A and wasting syndrome, are consistent with the effects which are caused by dioxin and dioxin-like chemicals which exert their effects through the Ah receptor. Consequently, it has been hypothesised that dioxin-like chemicals may be responsible for some of the reproductive effects observed in the birds (Giesy et al 1994). For example, strong correlations have been observed between concentrations of TEQs, (total dioxins and dioxin-like PCBs, measured with a sensitive *in vitro* bioassay), and the rates of deformities in cormorant and Caspian tern chicks.

In addition to these dioxins and dioxin-like PCBs there are a number of other chlorinated or related compounds that are known to cause similar adverse effects or, because of their structure, might be expected to cause effects through Ah-receptor mediated mechanism (see table 2.5). Some of these compounds have been identified in the environment and it has been suggested that these chemicals may also be partly responsible for reproductive abnormalities in birds as well as dioxins and dioxin-like PCBs (Giesy et al. 1994).

In sum, studies on the Great Lakes fish-eating birds strongly suggested that several organochlorine

contaminants were responsible for reproductive abnormalities which have greatly affected bird populations. Eggshell thinning caused by DDT was thought to be responsible for a large part of the damage to the bird populations in the 1950's to the 1970's. There has also been evidence of feminisation of male birds. Current evidence suggests that the continued problems in breeding are caused by the toxic effects of multiple organochlorine compounds in the environment which may exert their effects through the Ah-receptor mechanism (Giesy et al 1994).

2.4.5 Semiaquatic Mammals

Semiaquatic mammals include otters (Lutra lutra, L. canadensis) and American and European mink (Mustela vison and M.lutreola respectively). European otters and European mink populations are in decline, the mink now being an endangered species. It has been suggested that organochlorine pesticides and PCBs may be partly responsible for the decreasing populations (see Lopez-Martin et al. 1994).

Studies on captive mink have shown they are highly sensitive to reproductive impairment induced by organochlorine chemicals. A recent study investigated effects on fetal resorption and growth retardation following prenatal exposure to PCBs. The study suggested embryotoxicity and retarded embryo growth were adversely affected as a result of effects on the oestrogen and progesterone (a sex hormone) receptors (Patnode and Curtis 1994). A preliminary study on 4 wild mink in Spain reported that tissue levels in the mink exceeded levels which produced reproductive effects on captive mink, suggesting that the population decline of these animals may be due to organochlorines in the environment (Lopez-Martin et al. 1994).

2.4.6 Marine Mammals

Predatory marine mammals are exposed to high levels of organochlorines in their diet. For example, organochlorine chemicals including DDT, PCBs, chlordanes, HCH's and PCC's (polychlorinated camphenes eg. toxaphene) have been found in several marine mammals in various parts of the Arctic, namely the fur seal (Callorhinnus ursinus), ringed seal (Phoca hispida), hooded seal (Cystophora cristata), bearded seal (Erignathus barbatus), walrus (Obdobenus rosmarus divergens), beluga (Delphinapterus leucas), porpoise (Phocoena phocoena), narwhal (Monodon monoceros) and polar bear (Ursus maritimus), (Norstrom and Muir 1994).

Reproductive failure in some seal species has been connected with exposure to organochlorine contaminants. In the Wadden Sea, reproductive failure of the common seal (Phoca vitulina) and subsequent population decline has been attributed to PCBs. Studies have shown that the problem was due to failure of reproductive processes around implanation (reviewed by Reijnders and Brasseur 1992). There is also evidence of a disease complex affecting populations of Baltic ringed seals (Phoca hispida) and grey seals (Halichoerus grypus) which involves reproductive failure, bone lesions and adrenal hyperplasia. Skull and bone deformities were observed in highly contaminated animals at the beginning of the 1970's and reproduction was suppressed. The disease complex has been associated with high levels of PCBs and DDTs. Adrenocortical hyperplasia was reported in these seals and it is suggested that disturbance of steroid hormones may be partly responsible for disrupted reproductive performance (reviewed by Reijnders and Brasseur 1992, Norstrom and Muir 1994). At the same time as the reproductive failure in seals was occurring, high levels of DDT residues in Californian sea lions were found to be associated with increased frequency of spontaneous abortion (De Long et al. 1973).

Beluga whales in the St. Lawrence estuary have much greater levels (10-50 times higher) of organochlorine contaminants than belugas in the arctic (Norstrom and Muir 1994). A study of the St. Lawrence population revealed that 79% of female belugas were not breeding compared to only 35% of females examined from the Arctic population. In addition, lesions were detected in mammary glands of 36% of the St. Lawrence females which would have seriously affected their ability to feed if any calves were produced. It has been suggested that organochlorine contaminants are associated with the reproductive impairments observed in the St. Lawerence belugas (see Johston and McCrea 1992).

2.4.7 Terrestrial Mammals

The majority of the remaining (approximately 35 individuals) endangered Florida panthers (Felis concolor coryi) exhibit a number of developmental abnormalities and reproductive defects. Males (n=12) have low ejaculate volumes, low sperm concentrations, poor sperm motility, and an extremely high proportion (92.9%) of sperm with morphological abnormalities. In addition, cryptorchidism has increased exponentially in male cubs since 1975, and male fertility may be a problem. Females have high body burdens of various contaminants including DDE, PCBs, methoxychlor, oxychlordane and t-nonachlor and it has been suggested that the reproductive abnormalities in the panthers may be due to contamination of the mothers by endocrine-disrupting chemicals (reviewed by Danish EPA 1995).

2.5 Conclusions of Reproductive and Developmental Effects on Wildlife and Comparisons with Effects in Humans

Exposure to organochlorine chemicals in the environment has been associated with many deleterious effects on development in wildlife. Maternal exposure before egg production in birds, fish and reptiles, or pregnancy and lactation in mammals, is associated with various effects on the reproductive systems of offspring and other effects such as decreased survival and structural deformities. Such effects may cause decreased fertility and survival, and lead to population declines. These deleterious effects were not reported to occur before 1950 and have been observed in areas known to be contaminated with man-made organochlorine chemicals such as the Great Lakes in North America. The problem is however not isolated, and today many wildlife populations are at risk (Colborn et al. 1993).

In humans and animals alike, studies have shown that the embryo/fetus is the most sensitive lifestage to toxic insult from organochlorine chemicals. Many of the developmental effects in wildlife species appear to be due to disruption of the endocrine system. Some other effects may be mediated through the Ah receptor mechanism. Effects include abnormal sexual development and reproductive disorders, neurotoxic effects (eg.) behavioural abnormalities and immunotoxic effects. These findings have been confirmed in experiments with laboratory animals. In humans, prenatal exposure to the synthetic oestrogen DES has provided a clear model for similar effects caused by increased exposure to oestrogens (Colborn et al. 1993). Moreover, limited evidence from studies on humans exposed prenatally to organochlorines, shows that effects in wildlife populations are similar to effects in humans. Indeed it has been stated that wildlife are reliable sentinels of effects of chemicals on human populations (Wingspread Conference 1993). Examples

of similar reproductive problems in wildlife and humans are given below.

In humans, reproductive abnormalities have occurred in male and female offspring following prenatal exposure to DES. Small phalli were also found in male boys born to women who were exposed to high levels of PCBs and dioxins in the Yucheng disaster. Similar reproductive problems are also evident in many wildlife species. For example, endocrine disruption and abnormal reproductive structures eg. small phalli have been found in declining populations of alligators in some Florida Lakes which were previously contaminated with organochlorines. Recently, it has been revealed that female Florida panthers have high tissue levels of organochlorines and reproductive problems are being found in their offspring. Specifically, male offspring have a very high incidence of cryptorchidism. Males also have low sperm concentrations, poor sperm motility, a high proportion of sperm with morphological abnormalities and male sterility may be a problem. These reproductive problems are clearly comparable to recent findings in the male human population which indicate decreasing sperm counts, sperm quality and reduced fertility (Danish EPA 1995).

It is now becoming apparent that endocrine disruption and comparable effects from exposure to organochlorine chemicals are evident in wildlife and humans. It is therefore now a matter of urgency that the warning signals of adverse effects seen in wildlife populations should be heeded. Present day testing of chemicals for risk assessment does not include long term developmental studies or consider hormone-disrupting effects. The current endpoints in testing (carcinogenicity, acute toxicity and immediate mutagenicity) therefore cannot detect the toxic effects of many organochlorine chemicals. Consequently, the present system of testing chemicals has led to the misconception that hormone-disrupting chemicals as well as organochlorines (eg. dioxins) which may exert developmental toxicities through other mechanisms, do not pose a threat to wildlife, domestic animals or humans (Colborn et al. 1993) In addition, many organochlorines are produced which have undergone little or no toxicity testing and are essentially regulated as though they were safe. Clearly, current regulatory systems have failed to protect wildlife and human health.

3 Cancer

3.1 Introduction: Cancer and environmental chemicals

It has been estimated that 80% of all cancers in the US, and about 50% of cancers in Europe are caused by environmental factors (Moller 1993). These factors include environmental agents, namely chemicals, radiation and viruses and also factors related to lifestyle such as diet, occupation, smoking and alcohol consumption (Moller 1993, Silberhorn et al. 1990). Several classes of chemicals have been found to induce cancer. Such chemicals are called chemical carcinogens, and include some organochlorine compounds.

Development of Cancer

In order to induce cancer, most chemical carcinogens have to be metabolised in the body to another form. This involves conversion of the chemicals by enzymes (eg. cytochrome p450 enzymes) into other forms (electrophiles). These electrophiles may be more reactive than the original chemical. They may react with DNA causing mutations in DNA or activate genes called oncogenes which are associated with the formation of cancer (Parke 1994). Such genetic changes are thought to be critical in the process of cancer development.

The development of cancer is very complex but the first stages can be simplified into phases called initiation and promotion. Initiation involves an initiating agent (eg. a chemical) causing damage to DNA such that it produces a mutation. The second stage, promotion, involves subsequent repeated exposure to a promoter (eg.a chemical) which results in the selective growth of such genetically mutated cell(s). The development of cancers in this way is thought to be a multistage process involving several mutations and many different mechanisms of promotion which govern the growth of these genetically altered cells. Initiation may occur after only a single dose of a chemical carcinogen, but repeated exposure to a promoter is necessary for the promotion phase. Chemicals that can act as initiators and directly damage DNA are known as genotoxic. Those chemicals which are promoters are not genotoxic and cannot usually produce cancer on their own, but can increase the risk of cancer by (eg.) increasing the growth rate of mutated cells. Some organochlorine compounds are genotoxic while others can act as cancer promoters. Long-term exposure to trace amounts of organochlorines such as in the diet or in polluted air may therefore lead to the formation of cancer (Silberhorn et al. 1990, El-Bayoumy 1992).

Cancer development is complex and exposure to a chemical carcinogen may be influenced by a number of other factors. For example, promotion of cancers is modulated by diet, hormonal and environmental factors. A chemical may also enhance or inhibit tumour promotion if there is simultaneous exposure to another carcinogen (Silberhorn et al. 1990).

Evidence for a link between environmental chemicals and cancer

There are several *in vitro* tests which can detect if chemicals can cause genetic damage. Animal experiments also provide important information on the role of chemicals in the induction of particular types of cancer. Together these tests are used to provide an understanding of how environmental chemicals can lead to cancer in humans. Evidence from epidemiology studies in humans is essential if a link between environmental chemicals and cancer is to be found, but inference from animal and *in vitro* data is also necessary to provide conclusive proof of such a link.

The International Agency for Research on Cancer (IARC) has classified many chemicals as known

or suspected human carcinogens based on experimental and epidemiological evidence which is available. Table 3.1 lists commonly known organochlorines chemicals classified by the IARC as known or probable human carcinogens. Numerous organochlorine chemicals which could be carcinogens will not be included in this list because little or no toxicology data exists for them. It is also noteworthy that other regulatory bodies in different countries may list more chemicals as carcinogens than table 3.1, because the toxicological evidence used for such classification systems differs nationally.

3.2 Liver and Intestinal Cancers

There is conclusive evidence from animal experiments that long-term exposure to some mixtures of PCBs induces liver cancer (hepatocellular carcinoma). There is also some evidence that stomach cancers can result from PCB exposure. Most animal and *in vitro* studies show conclusively that some PCBs act as tumour promoters, but a few studies suggest that PCBs may also be genotoxic (reviewed by Silberhorn et al. 1990).

There are a few epidemiology studies in the literature relating to the mortality of humans exposed either accidentally or occupationally to PCBs. Studies of deaths among the people who were exposed to PCBs and dioxins in the Yusho incident showed that the total cancer mortalities (33 observed vs. 15.51 expected) and deaths from liver cancer (9 observed vs. 1.61 expected) in males were significantly higher than expected. Stomach cancer mortality was also elevated (8 observed 5.69 expected) but was not statistically significant. These results were consistent with animal experiments and the study concluded that the poisoning incident may have caused liver cancer (reviewed by Silberhorn et al. 1990).

A study of workers exposed to PCBs at a capacitor factory in the U.S found that the total number of deaths from cancer (62 observed vs. 80 expected) was less than expected, but there was a significant excess in cancers of the liver, gall bladder and biliary tract (5 observed vs. 1.9 expected), (Brown 1987). A similar study at a capacitor factory in Italy reported an a significant excess from the total number of deaths from cancers and a significant increase in cancers of the digestive tract (2 stomach, 2 pancreas, 1 liver, 1 biliary tract vs. a total of 2.2 expected), (Bertazzi et al. 1987). However, another study at a capacitor factory in Sweden did not reveal an excess of cancers or liver cancer, but this study was relatively small (Gustavsson et al. 1986).

As a whole the few epidemiology studies on PCBs are not conclusive. However, they do suggest an association between exposure to PCBs and the development of cancer, particularly liver cancer and cancers of the digestive tract. The epidemiology studies are also consistent with results from animal studies. Consequently the US EPA has classified PCBs as probable human carcinogens and have recommended a concentration of zero in drinking water. Because this is not yet achievable, in 1989 the EPA proposed a maximum contaminant level in water of 0.0005mg/l and calculated this would correspond to an excess lifetime cancer risk of slightly less than 1 in 10,000 (reviewed by Silberhorn et al 1990).

In 1976, an accident in a chemical plant near Seveso, Italy, exposed the local population to high levels of dioxin (2,3,7,8-tetrachlordibenzo-p-dioxin, TCDD). An epidemiology study of persons

residing in the area has found a two-fold excess in liver cancer (4 cases) and cancer of the gallbladder and bile ducts (5 cases), and a 1.8-fold increase in hepatobilary cancers (10 cases). This study suggested an association between exposure to dioxin and these cancers, although the study was limited by only a short latency period of 10 years, which may be an inadequate time for the true number of cancers resulting from the accident to develop (Bertazzi et al. 1993).

Pancreatic Cancer

Studies in animals have found that exposure to DDT increases the risk of developing pancreatic cancer. A recent epidemiology study has found a strong association between exposure to DDT and pancreatic cancer (Garabrant et al. 1992). A chemical company in the US initiated a program in 1971 in which a mortality register of employees at the plant was recorded. In 1987 it became apparent that there was an excess mortality from pancreatic cancer. Following these results a study was conducted to investigate if any chemicals at the plant were associated with these deaths. Subjects were carefully matched to control individuals so that risk factors for pancreatic cancer like smoking were considered. Results showed that there was a strong association between DDT exposure and pancreatic cancer which was not explained by lifestyle or other chemicals at the plant. The risk increased with duration of exposure and the time since first exposure. Compared with non-exposed workers the risk of pancreatic cancer was 4.8 times greater in workers exposed to DDT (relative risk, RR = 4.8: 95% confidence interval = 1.3-17.6). In workers with a mean exposure to DDT of 47 months, the risk was 7.4 times as great than workers with no exposure. The study concluded that heavy prolonged exposure to DDT may cause pancreatic cancer. Other scientists have commented that the study suggests a real association (Malats et al. 1993). However, more than one epidemiology study will be needed for conclusive evidence.

3.3 Respiratory Cancers

Chloromethyl Ethers

Chloromethyl ethers are organochlorine chemicals which are used in the chemical industry. These chemicals contain bis-chloromethyl ether (BCME) as a contaminant which causes respiratory cancers in animals. Epidemiology studies have shown that workers exposed to chloromethyl ethers have an increased risk of respiratory cancers which is thought to be caused largely by BCCM (Gowers et al. 1993). Consequently, the International Agency for Research on Cancer have classified chloromethyl ethers as human carcinogens (see table 3.1).

A general finding of the epidemiology studies is that exposure to chloromethyl ethers results in elevated rates of respiratory cancer among workers but no excesses of other cancers (see eg. DeFonso and Kelton 1976, Pasternack et al. 1977, McCallum et al. 1983, Maher and DeFonso 1987 and Gowers et al. 1993). Studies have shown elevated risks of respiratory cancers in exposed workers compared to unexposed workers of, for example, 2.79-fold, 3.8-fold and 5-fold in studies by Maher and DeFonso 1987, DeFonso and Kelton 1976, and Gowers et al. 1993 respectively. The epidemiology studies also clearly indicate that the risk of respiratory cancers increases with increasing intensity and duration of exposure, and in groups of highly exposed workers greater than 10-fold risks have been reported (eg. Pasternack et al. 1977, Maher and DeFonso 1987).

Other organochlorines

A study of workers at a dye and resin manufacturing plant in the U.S. found an association between lung cancer and exposure to anthraquinone (AQ) and epichlorohydrin (ECH) dyes and chlorine (Barbone et al. 1992). ECH is a carcinogen in experimental animals and is classified as a possible human carcinogen. The study investigated 51 cases of lung cancer which occurred in a group of 2642 men employed at the plant after 1952. The subjects were compared to non-exposed control subjects who were matched for smoking status. There was a 2.4 fold increase in lung cancer cases in workers exposed to the dyes (odds ratio = 2.4;95% confidence interval (CI) 1.1-5.2). In addition, acute exposure to chlorine (in 6 cases) was associated with a 27 fold increase in the rate of lung cancer (OR = 27;95% CI = 3.5-205), and routine exposure to chlorine (in 8 cases) was associated with an 8.9 fold increase (OR = 8.9;95% CI = 2.0-40). The number of individuals exposed to chlorine was however too small to prove a link between exposure and lung cancer. The study concluded that exposure to chlorine and employment in AQ and ECH dye production are associated with lung cancer exposure after adjustment for smoking. However, more research is necessary to clarify whether exposure to these chemicals causes lung cancer.

Other studies have also found an association between exposure to chlorine and lung cancer. For example, in a Norwegian cohort of magnesium production workers with potential exposure to chlorine gas and other chlorinated by-products such as hexachlorobenzene, the relative risk (RR) for lung cancer was 1.8 (95% CI = 1.2-2.5). The excess was particularly evident in individuals who were employed for 10 years or more and with at least 20 years since first employment (Heldaas 1989). A Swedish study of chloralkali workers who were exposed to chlorine reported a 2-fold increase in risk for lung cancer was (RR = 2.0; 95% CI:1.0-3.8), (Barregard 1990). These studies do suggest an association between chlorine exposure and lung cancer, but they could not adequately control for smoking and other occupational exposures including asbestos.

Pulp and paper mill workers are also exposed to chlorine and a number of chlorinated organic compounds formed during the bleaching process including dioxins (PCDDs and PCDFs). A few studies have revealed an excess of lung cancer among pulp and paper workers (eg. Jappinen et al. 1987, Jappinen and Pukkala 1991). A study in Finland found an excess of lung cancer among men working at pulp and paper mills, especially among board mill workers where the incidence of lung cancer was more than twice that expected, (40 observed, 18.1 expected standardised incidence ratio (SIR) 222, 95% CI = 158-302). The risk was further increased after a latency period of 20 years (25 observed, 7.8 expected, SIR 323, 95% CI = 209-476), (Jappinen et al. 1987). Another Finnish study found the incidence of lung cancer was 6 times higher than expected (6.0 observed, 1.0 expected, SIR 6.3;95% CI = 2.3-14) in a study population of 152 workers. In these studies the incidence of cancer was compared with local expected rates rather than using control subjects and consequently results were not adjusted for smoking. However, it is unlikely that smoking alone can explain the excess risk of lung cancer found (Jappinen and Pukkala 1991). A study by Fingerhut et al. (1991) in the USA, investigated cancer mortality in a large group of workers who produced chemicals which were contaminated by dioxins. Mortality from respiratory cancers was significantly increased (1.4-fold) in workers who were exposed to the chemicals for greater than 1 year with at least 20 years latency, (Standardised mortality ratio, SMR = 142; 95% CI = 103-192). This increase was most likely not due to smoking.

In conclusion, chloromethyl ethers are classified as human carcinogens and have been strongly

associated with respiratory cancers following occupational exposure. There is also evidence which strongly suggests that occupational exposure to chlorine, dioxins and other organochlorines in the pulp and paper industry is associated with an increase in the incidence of respiratory cancers.

3.4 Soft Tissue Sarcoma and Non Hodgkin's Lymphoma

Soft tissue sarcoma (STS) is a rare form of cancer, affecting for example only 4 in 100,000 people in England and Wales (Brahams 1992). STS is a general term for tumours which develop from different types of connective tissue in the body, including fat, muscle, peripheral nerve, blood and lymphatic tissue. Several epidemiology studies have suggested an association between increased incidence of STS and occupational exposure to chlorophenols and phenoxyacetic acids, although some studies have found no association. Chlorophenols include the chemicals trichlorophenol, tetrachlorophenol and pentachlorophenol, and phenoxyacetic acids include 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D). These chemicals are known to cause cancer in laboratory animals and are classified as probable human carcinogens by the IARC. It has been suggested that while chlorophenols and phenoxyacetic acids may cause STS, it is also possible that dioxins, which are present as contaminants of some phenoxyacetic acids and all chlorophenols, may be partly responsible for increased rates of STS seen after exposure to these chemicals.

Several epidemiology studies on the incidence of soft tissue sarcoma in relation to exposure to chlorophenols and phenoxyacetic acids have been conducted among workers of various occupations in Sweden (eg. forestry, agriculture, painting, carpentry and saw mills), (eg. Hardell and Sandstrom 1979, Eriksson et al. 1981, Hardell and Eriksson 1988, Eriksson et al. 1990). These studies estimated occupational exposure to chlorophenols and phenoxyacetic acids from questionnaires given to subjects or their families. All the studies found an association between exposure to phenoxyacetic acids and STS whilst 3 of the studies also found an association with exposure to chlorophenols. An approximately 3-fold increase in risk for STS was reported with exposure to these chemicals in the first studies.

In the most recent of the studies (Eriksson et al. 1990), there was a 1.8-fold increase in risk of STS associated with exposure to chlorophenols or phenoxyacetic acids. When the risks of exposure to both chemicals were considered separately, it became apparent that dioxins, present as contaminants of some of the chemicals, may have been partly responsible for the increased incidence of STS. For example, all chlorophenols are contaminated with dioxins and there was a 5-fold risk with high exposure to chlorophenols in this study (RR = 5.25; 95% CI = 1.69-16.34). Not all phenoxyacetic acids however are contaminated with dioxins and the risk of exposure to these compounds was lower than for chlorophenols and not significant (RR = 1.34; 95% CI = 0.7-2.56). Moreover, the phenoxyacetic acid 2,4-T is contaminated with the most potent of the dioxin congeners (TCDD) and if exposure to 2,4-T was not included in the latter calculation the risk was lower (RR = 0.66; 95% CI = 0.18-2.06).

Studies on exposure to dioxins have suggested an association with an increased risk of STS. For example, a study by Fingerhut et al. (1991) reported a significant 9-fold increase in mortality from STS in a group of workers who were exposed to dioxins for at least 1 year with 20 years latency,

although the number of cases was small (3 deaths, SMR = 922; 95% CI = 190-2695). A study on cancer incidence following the Seveso incident found a 2.3-fold increase in the incidence of STS after a latency of 10 years (Bertazzi et al. 1993).

A recent study in Sweden undertaken by the IARC assessed cancer incidence in 270 chlorophenoxy herbicide/chlorophenol production workers who were employed between 1964 and 1978. Analysis of blood from 5 of the workers revealed that even 16-21 years after employment, the concentration of dioxin (TCDD) in their blood was very high (the highest ever found in Sweden) compared to levels in blood of matched control individuals. The study found no link between exposure to phenoxyacetic acids/chlorophenols and an increased incidence of STS or NHL between 1965 and 1993. However, the number of individuals in this study was relatively small and further studies are being conducted on larger numbers of workers (Littorin et al. 1994).

Overall, the results of the Swedish studies suggest that exposure to chlorophenols and phenoxyacetic acids are associated with an increased incidence of STS. Results from studies on exposure to dioxins and the most recent Swedish study (Eriksson et al. 1990) suggest that this may be partly due to contamination of these chemicals with dioxins. The authors suggest that inconsistency in results of other published studies may be explained by the differing amounts of dioxin contaminants in the chemicals. Other studies of workers exposed to phenoxyacetic acids, chlorophenols and dioxins in the chemical industry have also found an association with an increased incidence of STS in the workers (eg. Fox and Collier 1977, Cook 1981, Johnson et al. 1981, Honchar and Halperin 1981). As a consequence of the growing body of evidence which suggests an association between these chemicals and STS and possibly non-Hodgkins lymphoma (see below), pentachlorophenol has been banned in Sweden, Switzerland, Germany, Denmark and Sweden (Reigner et al. 1993).

Non-Hodgkin's Lymphoma

Non-Hodgkins lymphoma (NHL) is a cancer of the lymphatic system. Epidemiology studies suggest that there is a strong association between exposure to phenoxyacetic acids and NHL. There is also some evidence that use of the phenoxyacetic acid 2,4-D may be linked to NHL.

Several studies on have found that a higher risk of NHL is associated with farming (eg. Cantor 1982, Burmeister et. al. 1983, Buesching and Wollstadt 1984). A well designed population-based study in the state of Kansas in the U.S. investigated the incidence of NHL in agricultural workers exposed to phenoxyacetic acid herbicides compared to non-exposed control subjects (Hoar et al. 1986). STS was not found to be associated with exposure, but men exposed to herbicides for more than 20 days per year had a 6-fold increase in the risk of NHL (odds ratio (OR) = 6.0;95% CI = 1.9-19.5). Moreover, the more frequent users who mixed or applied herbicides themselves had an 8-fold increased risk (OR = 8.0; 95% CI = 2.3-27.9). The excesses appeared to be associated with exposure to the herbicide 2,4-D. This phenoxyacetic acid herbicide does not contain the most carcinogenic dioxin congener (TCDD) although it may be contaminated with other less toxic dioxin congeners. The authors concluded that results were consistent with a previous Swedish study in which exposure to phenoxyacetic acids not likely to be contaminated with dioxins were associated with an increased incidence of NHL.

A similar study among agricultural workers in eastern Nebraska, where previous studies had

revealed a 2-fold increase in NHL, found an association between NHL and exposure to the phenoxyacetic acid herbicide 2,4-D (Weisenburger 1990). The risk of developing NHL was 1.5 times higher than for non-exposed workers and the risk increased as the use of the chemical increased. For example, mixing or applying 2,4-D more than 20 days per year was associated with a 3-fold increased risk (OR = 3.3; 95% CI 0.5-22.1). The risk also increased if workers did not change their clothes after using the herbicide.

A study of nearly 70,000 Canadian farmers in the Saskatchewan province of Canada found that herbicide use in 1970 was associated with a 2.2-fold increase in mortality from NHL in farmers (relative risk 2.2; 95% CI = 1.0-4.6). In addition, mortality rose significantly with an increasing number of acres sprayed. Although the study does not address specific herbicides, it indicated that the phenoxyacetic acid 2,4-D accounted for 75% of the weight of all herbicide active ingredients used in agriculture in Saskatchewan in 1970 (Wigle et al. 1990).

Finally, a population study in an area of northern Italy where there had been extensive agricultural use of phenoxyacetic acid herbicides (2,4-D, 2,4,5-T and 4-chloro-2-methylphenoxyacetic acid) since 1950 also found an increased incidence of NHL (Vineis et al. 1991). All cases of STS and NHL reported in the area between 1985 and 1988 were investigated. The study found a higher incidence of NHL in males living in more highly polluted areas. The authors suggested that this increase was most likely due to occupational rather than environmental exposure to phenoxyacetic acid herbicides.

In summary, there is evidence which suggests a strong association between STS and exposure to chlorophenols, phenoxyacetic acids and dioxins in industry. Similarly, evidence from epidemiological studies also suggests a strong association between NHL and exposure to phenoxyacetic acid herbicides, especially from agricultural use. As a result of some of these studies the phenoxyacetic acid herbicide 2,4-D was withdrawn from use in Sweden in 1991. Although some scientists believe that there is an association between exposure to this group of organochlorine chemicals and development of STS and NHL, not all think there is sufficient evidence specifically on 2,4-D to ban it from agricultural use (Ibrahim et al. 1991). Consequently 2,4-D is still a very widely used herbicide in other countries (Sharp and Salleh 1993). It is, however, difficult to prove with epidemiology studies that an individual chemical causes cancer and consequently science usually deals with probabilities rather than proofs. In the case of occupational exposure to chlorophenols and phenoxyacetic acids the weight of evidence does suggest a strong association both soft tissue sarcomas and non-Hodgkins lymphoma. It would therefore seem appropriate to follow the precautionary approach already adopted by a Sweden for 2,4-D and a few European countries for pentachlorophenol, and phase out the use of these chemicals in industry and agriculture.

Aplastic Anaemia

Aplastic anaemia is regarded as a pre-cancerous disorder which affects certain white blood cells and consequently affects immune system functioning. The association between the development of aplastic anaemia and previous exposure to industrial chemicals has been recognised for many years although no individual chemical has been suspected in most cases. A review of the available literature in 1993 confirmed that 280 reported cases of aplastic anaemia were associated with pesticide exposure (Fleming and Timmeny 1993). Regarding exposure to specific organochlorine

chemicals, it was recently noted in several case studies that aplastic anaemia was associated with exposure to lindane (v-hexachlorocyclohexane) or pentachlorophenol, following treatment of timber for woodworm in the home and after occupational exposure (eg. Rugman and Cosstick 1990, Brahams 1992, Brahams 1994).

Lindane is known to cause cancer in animals and according to the WHO report on lindane it is reasonable to regard such a chemical as posing a carcinogenic risk in humans (Rugman and Cosstick 1990). Although there are no epidemiology studies on exposure to organochlorines and aplastic anaemia the available individual case studies do suggest that aplastic anaemia may be caused by exposure to lindane. Legal claims for compensation have been made and one firm of solicitors in the UK reported over 60 cases where injury was claimed as a result of exposure to wood treatment chemicals (Brahams 1992).

3.5 Bladder and Rectal Cancers Associated with Chlorination of Drinking Water

Chlorine (hypochlorite) is added to drinking water in many countries as a disinfectant to prevent infectious diseases. It has proved to be an enormously successful healthcare measure, doubtlessly preventing widespread illness and death. In 1974, it was found that chlorination of water resulted in the formation of many volatile organic chlorinated compounds from reactions of chlorine with organic matter in the water. Most of these by-products are called trihalomethanes (THM) and include chloroform which is known to cause cancer in animals. Chemical analysis of chlorinated water samples has since detected hundreds of other nonvolatile chlorinated substances which only occur at trace levels (<1ppb) much lower than levels of THMs, but are also toxic. Extracts of chlorinated by-products from drinking water have been shown to be genotoxic in a wide range of *in vitro* assays using bacterial, rodent and human cells (Wilcox and Williamson 1986). Over the last 20 years since the discovery of THMs in drinking water, many epidemiology studies have been conducted to investigate whether cancer risk is elevated in humans as a result of chlorination by-products in drinking water (Zieler et al. 1988, Cantor 1994).

Evidence from many of the epidemiology studies has suggested an association between chlorination of drinking water and an increased risk of cancers of the bladder, colon and rectum (Cantor 1994). The studies included ecologic surveys which compared morbidity or mortality rates from cancer in areas which used chlorinated surface water source to areas which used a nonchlorinated ground water source. Several (case-control) studies which matched individuals with control subjects were also performed using death certificate records. Both of these types of study can be subject to many sorts of biases. Nevertheless, data from the studies was consistent in revealing small excesses in bladder, colon and rectal cancers.

A few recent case-control studies have collected more reliable data using interviews rather than death certificates, so that an individual's lifetime exposure to chlorinated water sources could be assessed accurately (eg. Cantor et al. 1987, McGeehin et al. 1993). These studies also found that the risk of developing bladder cancer increased with long-term exposure to chlorinated water, and that the risk increased as duration of exposure increased. It has been suggested by the IARC that there is inadequate information to assess risk for development of cancer in humans from chlorinated

drinking water alone (Dunnick and Melnick 1993). However, the growing body of evidence of toxicological and epidemiological data suggests that the relative risk is likely to be 1.5 to 2 for bladder and rectal cancers (Cantor 1994).

It is not clear which chlorinated by-products in water are responsible for the associated increased risk of cancers. Recent animal studies have suggested that the organic by-products of chlorination, namely THMs, are of greatest concern (Dunnick and Melnick 1993). However, there is also increasing evidence that the nonvolatile chlorinated by-products are carcinogenic and are responsible for a major part of the toxicity (Cantor et al. 1987, Cantor 1994). The US EPA and water authorities in England and Wales have set limits for trihalomethane levels at 100ug/L (or ppb) (Zieler et al. 1988, Water Supply (Water Quality) Regulations 1989). Further limitations on levels requires major shifts in treatment practices. Possible alternatives to chlorination such as ozone treatment and UV treatment are being studied in some countries as a result of animal and epidemiology studies on cancer from water chlorination (Zieler et al. 1988). Some cities and towns in the US and Europe are already using alternative techniques such as ozone and UV treatment.

3.6 Other Cancers

Mortality from all Cancers after Dioxin Exposure

There is conclusive evidence that dioxin (TCDD) causes cancer in animals. Recent epidemiology studies have also shown that exposure to dioxins is strongly associated with an increase in mortality of all cancers considered together (Zober et al. 1990, Manz et al. 1991, Fingerhut et al 1991). These studies reported on mortality from cancers in workers who were occupationally exposed to dioxins. Manz et al. (1991) studied a group of 1583 workers employed who had been exposed to dioxin (TCDD) through employment in a herbicide plant in Germany. Cancer mortality was increased 1.8 times among men with 20 or more years of employment at the plant, compared to other non-exposed workers (Standardised mortality ratio, SMR = 1.82; 95% CI = 0.97-3.11). Workers employed before 1955 were exposed to very high dioxin levels and these men, who had also been employed for at least 20 years, had an even greater increase in cancer mortality risk (SMR = 2.24; 95% CI = 1.19-3.83).

The study by Zober et al. (1990) investigated cancer mortality among 247 workers who were exposed to excessive amounts of dioxin in 1954 after an accident at a chemical plant in Germany. It also revealed a significant increase in total cancer mortality 20 or more years after exposure in individuals who were highly exposed to dioxin (SMR = 2.01; 95% CI = 1.22-3.15). A study by Fingerhut et al. (1991) was performed on a group of 5172 workers who produced chemicals contaminated with dioxin (TCDD). This study found that mortality from all cancers was increased by 1.46 times which was statistically significant (SMR = 1.46; 95% CI = 1.21-1.76).

The epidemiology studies on dioxins have recently been reviewed by the US EPA (US EPA 1994). The review suggests that epidemiological evidence is consistent with experimental studies, and indicates that dioxins are potentially multisite carcinogens. Although the evidence is not considered sufficient to confirm that dioxin causes increased cancer incidence, the EPA concluded that dioxin (TCDD) probably increases cancer mortality of several types.

4 Effects on the Nervous System, Immune system and the Liver and Kidney in Adults

4.1 Neurotoxicity

There is evidence from animal and epidemiology studies that some organochlorine chemicals are toxic to the nervous system. The mechanisms by which these chemicals produce damage is not completely understood, but is believed to be due to interference with various hormones and receptors, as well as enzymes and chemicals (neurotransmitters) which are involved in the transmission of nerve impulses.

PCBs and Dioxins

Many case studies and a few epidemiology studies have recorded neurotoxic effects shortly after exposure to dioxins in occupationally exposed individuals. Symptoms of both peripheral and central nervous system toxicity have been found, in some cases lasting for many years. Symptoms include headache, insomnia, nervousness or irritability, loss of libido, fatigue, depression and anxiety. In one study on a group of 55 workers exposed to dioxin (TCDD) in the production of 2,4,5-T, hexachlorobenzene and pentachlorophenol, severe neurotic symptoms were recorded in 64% of the workers. Forty-four of the workers were tested again 10 years after the initial examination, and 58% of them continued to have neurotic symptoms, showing that neurological damage could persist for long durations.

Studies on Vietnam veterans who were exposed to dioxins and individuals who were exposed to dioxins at Seveso have found some evidence of neurotoxicity, although there did not appear to be any long-term neurologic effects as in exposed occupationally exposed workers (reviewed by US EPA 1994b).

Animal studies have shown that PCBs are toxic to the adult nervous system. Recent studies have shown that the mechanism of this neurotoxicity is largely due to altered levels of a neurotransmitter found in the brain called dopamine. Further *in vitro* studies have found that this probably occurs because PCBs inhibit the synthesis of dopamine by affecting a certain enzyme (reviewed by Seegal and Shain 1992). A recent study on monkeys which were fed PCBs in their diet showed that short term exposure to PCBs had long-lasting effects on dopamine concentrations in the brain (Seegal et al. 1994). For example, the animals were fed PCBs for a period of 20 weeks, following which some were sacrificed immediately and others were observed for an additional period of 24 or 44 weeks with no more exposure to PCBs. The levels of PCBs in the animals brains were significantly lower in animals which were not sacrificed until 24 or 44 weeks after exposure compared to those which were sacrificed immediately. However, the levels of dopamine in the brain were significantly reduced (by about 60%) by the same amount in all of the animals. Thus the initial exposure to PCBs resulted in long-lasting effects on dopamine concentrations in the brain, even though the levels of PCBs in the brain declined over time.

The above experiment has some relevance to workers who are occupationally exposed to PCBs. PCBs are used in the manufacture of transformers and capacitors. Although the levels to which the monkeys were exposed are much higher than would be expected for humans exposed occupationally, the monkeys were exposed for a relatively short period of time compared to many workers. Most importantly, it was found that blood levels of PCBs in the animals (about 1ppm) were virtually identical to levels found in the blood of capacitor workers (about 3ppm), (Seegal et al. 1994). However, there have been few studies on the nervous system effects of PCB exposure in such workers.

Studies in humans have been carried out on workers exposed to PCBs and dioxins (PCDD/PCDFs) following fires involving transformers or capacitors (reviewed by Rogan and Gladen 1992). Two studies showed that workers suffered from peripheral nerve damage (distal axonopathy) following exposure. Another study on firemen who were exposed to these chemicals when extinguishing a fire at a power house showed memory impairment and lower scores on cognitive (intelligence) tests. Finally, in Japan and Taiwan in the Yusho and Yucheng incidents where people consumed rice which was contaminated with PCBs and dioxins, there were signs of nerve damage in some adults who were exposed to the chemicals. The nerve damage was characterised by decreased speed in conduction of nerve signals in the peripheral nervous system (Seegal and Shain 1992).

Hexachlorocyclohexane

Gamma-hexachlorocyclohexane (HCH), (lindane), has been shown to have convulsive effects on the nervous system following acute exposure to humans, but no effects were seen in a study of workers with lower level long term (ie. chronic) exposure (Baumann et al. 1981). However, a recent study of 356 workers at HCH manufacturing plants in India found neurological symptoms in workers (Nigam et al. 1993). Exposure was estimated from blood measurements of HCH (beta-HCH and gamma-HCH). Various symptoms were found among workers including an increase in the incidence of headaches, giddines, malaise and nausea. ECG abnormalities were found in some workers which indicates disturbances in nerve function of the brain. These neurological symptoms were found to be related to the intensity of HCH exposure in the workers and appeared to be caused by recent exposure to HCH. The results of this study are of great concern and further monitoring of the workers was advised to check for signs of severe health impairment.

Parkinson's Disease

Parkinson's disease (PD) is a neurological disorder which is characterised by tremor, decreased mobility and muscular rigidity. The prevalence of this condition, especially early onset Parkinson's disease has increased in industrialised countries in recent years compared with non-industrialised nations. Family history is a known risk factor for PD and it is thought that onset of PD may result from a combination of these genetic factors and exogenous toxins (eg. environmental toxins or particular foods in diet). Several epidemiology studies in different countries have pointed to a connection between exposure to pesticides and PD (reviewed Golbe 1993). Most of these studies consistently show that exposure to pesticides and herbicides is associated with an increased risk in Parkinsons disease, but cannot explain a large percentage of PD cases.

A recent study by Fleming et al. (1994) screened for 16 organochlorine pesticides in postmortem brain samples from 20 PD cases. DDE was found in most of the brains and in matched control brains. However, dieldrin was detected in 6 of the 20 PD brains but none of the control samples. This study does not prove that dieldrin is involved in the onset of PD, but suggests that dieldrin should be investigated as a potential agent causing PD.

Chronic Motor Neuron Disease

Chronic motor neuron disease is a condition which affects motor neurons (ie. nerves/muscles involved with producing movement). It has been associated with various environmental toxins including aluminium, manganese, selenium, lead and mercury. A recent study found that 2 patients with a history of long-term exposure to organochlorine pesticides developed symptoms of chronic motor neuron disease and subsequently died. Measurements of organochlorines in their blood

when after the disease developed revealed there were elevated levels of lindane and heptachlor in blood from one patient and high levels of aldrin in the second patient. The study suggested that cases of motor neuron disease should be investigated further, particularly in Third World countries where workers are not usually adequately protected from pesticides (Fonseca et al. 1993).

4.2 Immunotoxicity

The immune system consists of a network of specialised cells which mount responses to foreign substances in the body to prevent infection and disease. Some organochlorine chemicals are toxic to the immune system. The toxicity may result in a decrease in certain cells of the immune system in which case the host resistance to infection and tumours will be reduced. Alternatively, toxicity may result in an increase in immune system cells which can lead to autoimmune disease (Thomas 1990).

Numerous animal studies have shown that many organochlorine compounds including PCBs (Safe 1994), DDT, dieldrin, dioxins, hexachlorocyclohexane, hexachlorobenzene, chlordane and pentachlorophenol are toxic to the immune system (Thomas 1990, McConnachie et al. 1991 and 1992). Several epidemiology studies have also shown that some of these chemicals are associated with immunotoxicity in adults.

PCBs and Dioxins

Evidence from numerous studies in several different animal species has demonstrated that the immune system is a target for toxicity of dioxins (PCDD/Fs) and PCBs. The mechanisms for this toxicity are not completely understood, but appear to involve direct effects on some cells of the immune system and possibly indirect mechanisms via the endocrine system (US EPA 1994).

Animal studies provide evidence that exposure to low doses of dioxin (TCDD) results in increased susceptability to bacterial, viral, parasitic and neoplastic diseases (US EPA 1994a). Recent studies have shown that these effects occur at extremely low doses. For example, mice which were challenged with influenza virus and subsequently exposed to a single low dose of dioxin (10 ng/kg) significantly increased mortality levels (Burleson et al., submitted). The estimated body burden of the animals at this dose was only 7ng/kg (US EPA 1994a). Other experiments have also shown effects on the immune system at very low levels of dioxin. For example, chronic (long-term) low dose exposure to dioxin in marmoset monkeys demonstrated changes in lymphocytes at body burdens of only 6-8ng/kg (Neubert et al. 1992). Results from such animal experiments are of concern because effects on the immune system are occurring at body burdens similar to, or within an order of magnitude of current human body burden estimates (5-10 ng/kg), (US EPA 1994b).

Epidemiology studies on individuals exposed to dioxins have recorded alterations in the levels of different types of immune cell populations. However, the specific cell populations which are affected and whether they increase or decrease has not generally been consistent from study to study. This may be because appropriate sensitive endpoints for assessing immunotoxicity of dioxins have not yet been well defined (US EPA 1994a and 1994b).

Epidemiology studies following the Yusho and Yucheng incidents in Japan and Taiwan, in which

people had eaten rice contaminated with PCBs and dioxins, showed significant reductions in some types of immune system cells and an increase in respiratory bronchitis and skin infections in exposed individuals. Several studies have found alterations of some types of immune system cells were also recorded in residents who were potentially exposed to dioxin (TCDD) at Times Beach, Missouri, although the changes were not always consistent with each other or with animals studies. (reviewed by Margolick and Vogt 1991, US EPA 1994a).

A follow up study of workers in Germany who were exposed to high concentrations of dioxin (TCDD) after an accident at the BASF plant was recently published (Zober et al. 1994). It showed that workers had experienced increased illness episodes, including a significant increase in infectious and parasitic diseases and upper respiratory tract infections. These results were consistent with reduced resistance to infection due to immunotoxic effects. It is possible that the results were due to bias, but since there were a number of positive associations between exposure and increased disease incidence the associations are most likely real. Another study on these workers also found some lymphocyte cell populations were decreased in these workers (reviewed by EPA 1994b).

Consumption of fatty fish from the Baltic Sea is an potential source of human exposure to organochlorine compounds including dioxins and PCBs. A Swedish study investigated 23 males from the general population who had a relatively high fish consumption and a control group of 20 males with virtually no fish consumption (Svensson et al. 1993). High fish consumption was found to be associated with a significant reduction in the levels of a type of cell in the immune system called natural killer cells. No changes in other types of cell were found. Natural killer cells are believed to contribute to defense against viruses and cancer as well as regulating the functions of other immune cells (Margolick and Vogt 1991, Thomas 1990). Other studies on individuals accidentally exposed to dioxins however have not found changes in natural killer cells.

Dioxin and AIDS

In the past few years there has been a disconcerting spread of acquired immunodeficiency syndrome (AIDS) and, concomitantly, a continuous increase in the contamination of the environment with toxic chemicals including dioxins. It is possible that the combined action of the human immunodeficiency virus 1 (HIV-1) and dioxin on the immune system may be more deleterious than each one alone, since for example, both appear to have similar targets in the immune system, causing suppression of certain lymphocyte cells (T-cells). Recent studies on the activation of HIV-1 infection now suggest that dioxin (TCDD) stimulates the production of the HIV-1 virus in cultured human cells by activating genes in the virus (Tsyrlov and Pokrovsky 1993, Yao et al. 1995).

In one study (Tsyrlov and Pokrovsy 1993), MT-4 human lymphoid cells were incubated with dioxin (TCDD). After a few hours the cells were then infected with the HIV-1 virus. Results showed that by first adding dioxin to the cells there was an increase in HIV-1 production, compared to when cells were infected with the HIV-1 virus alone.

A recent study (Yao et al. 1995) provides a possible explanation of the mechanisms by which dioxin increases HIV production in cell cultures. Experiments showed that TCDD activates the expression of certain genes of the HIV-1 virus by 2.5-3 fold. (The genetic sequence which was actived by dioxin

chlordane is now banned in the USA it is still exported to other countries. Similarly, PCP is still used in many countries.

Summary

Studies show that organochlorines cause immunotoxic effects in adult animals. Evidence from epidemiology studies is sparse, but existing data suggests that exposure to organochlorines is associated with immunotoxic effects. Accidental exposure or a high dietary exposure to dioxins and PCBs has been associated with alterations in cells of the immune system, and accidental exposure has also been associated with increased respiratory tract infections. Exposure to chlordane and pentachlorophenol in the home following treatment for pests has been associated with long-term alterations in immune system cells.

4.3 Liver and Kidney Toxicity

One of the functions of the liver and kidney is to detoxify substances in the body. Many environmental contaminants are detoxified by liver enzymes and measuring the levels of these enzymes can indicate toxicity to the liver. Some organochlorines are toxic to the liver and kidney in laboratory animals. These include dioxins, the pesticide chlorodecone, tetrachloromethane, bromotrichlormethane (Rao et al. 1990) and dichlorobenzene (Valentovic et al. 1991), which is used in the chemical industry and some chemicals in the home such as insect repellent and toilet deodorizer. There are currently very few studies which have focused specifically on liver and kidney toxicity in humans following exposure to organochlorine chemicals.

A study of workers at HCH manufacturing plants in India found significant changes in liver enzymes which related to the levels of HCH in the blood of the workers and strongly suggested liver injury. The findings were reported to be consistent with another previous study which found liver injury (cirrhosis and chronic hepatitis) in 8 workers who were exposed to HCH and DDT (Nigam et al. 1993).

Water Chlorination

Chlorination of water results in the production of chlorination by-products, some of which are known to be carcinogenic in animals (see section 3.6). These compounds can be absorbed into the body by ingesting water or by inhalation and absorption through the skin whilst bathing/showering (Weisel and Chen 1993). For individuals using chlorinated swimming pools, inhalation is the main route of exposure, and since the chlorinated by-products are volatile, particularly high exposures are experienced by those who swim for a long time under physical strain. Studies on competition swimmers using indoor chlorinated swimming pools have found that chloroform levels were elevated in their blood (Aggazzotti et al. 1990, Aiking et al. 1994). Swimmers in outdoor pools however had similar blood levels of chloroform as non-swimmers (Aiking et al. 1994). In this study, 10 competition swimmers who trained in indoor pools had tests for liver function and kidney function. No subjects had abnormal liver function. However, in the younger swimmers an enzymes related to kidney function (B2-microglobulin) was higher than normal. This indicated that there may be kidney damage and that further investigation was needed because such damage could be problematic in later life. More studies on a larger number of swimmers would be necessary to confirm the findings.

5 Conclusions

5.1 Organochlorines – a unique class

Among organic chemicals, the very presence of a carbon-chlorine bond is generally associated with certain properties:

- stability – increased resistance to degradation;
- toxicity – the ability to interfere with or otherwise impair biological functions; and
- lipophilicity – an increased solubility in fats and oils, which leads to accumulation in living organisms, a major reservoir of fats and oils.

Because of these properties, organochlorines that were produced decades ago are still circulating in the environment today, concentrating in the tissues of both humans and wildlife at levels sufficient to impair health.

Some organochlorines are less persistent, toxic and lipophilicthan others. However, even when these are manufactured, other extremely persistent, toxic and lipophilic organochlorines will be created and released into the environment.

Some organochlorines are less persistent, toxic and lipophilic than others. However, the manufacture, use and disposal of even the least harmful organochlorines is accompanied by the unintentional formation and release of organochlorines that are extremely persistent, toxic and lipophilic. AS LONG AS ANY ORGANOCHLORINE IS MASS PRODUCED, ORGANOCHLORINES THAT ARE PERSISTENT, TOXIC AND LIPOPHILIC WILL BE CREATED AND RELEASED INTO THE ENVIRONMENT.

5.1 Failed Regulatory Strategies

Current national and regional regulations, which seek to control or limit the release of organochlorines, chemical by chemical, have not stopped the threats to human health posed by these chemicals. Persistent organochlorines continue to enter the environment at a rate far greater than the rate at which they are broken down into inorganic substances. In other words, current regulatory schemes are allowing continuing additions of persistent organochlorines to the already overloaded environment.

Even national and regional bans of specific organochlorines can achieve only limited reductions in environmental loadings and resulting human body burdens of the banned chemicals. Global circulation of these persistent chemicals ensures that releases from their production and use at any point on the planet will eventually reach every other part of the planet.

Moreover, due to the global distillation phenomenon, colder regions bear a disproportionate share of the organochlorine burden. Even if all the nations of the colder regions agree jointly to ban production of all organochlorines, organochlorine loadings to these regions will continue as long as production continues in more temperate and tropical regions.

In summary, persistent organochlorines are a global problem that requires a global solution.

5.1.1 Risk Assessment

An increasing number of nations are turning to risk assessment as the basis for setting allowable rates of release for organochlorines. The risk assessment consists of solving a series of mathematical equations that are supposed to represent the physical, chemical and biological behaviour of a toxic chemical: its release from the source; its interactions with wind, rain, heat, light and other environmental factors; its deposition in, movement through and continuing interactions with the environment; its uptake in and movement within the body of a human 'receptor'; and its interactions with and impacts on the many processes taking place within the organs and cells of the human body, including effects such as cancer, that may occur 20-40 years after exposure.

Since many of the chemical's interactions are not addressed and most of those that are addressed are far from completely characterized, the risk assessment's mathematical equations offer, at best, only very remote approximations of a few of the complex interactions of the chemical in the real world. In addition, many of the numerical factors that are inserted in the array of equations in order to arrive at the final calculation of risk are the products of other remote approximations. For example, one key number is the chemical's carcinogenic potency, which has been known to vary by a factor of 2000, depending on which experimental data are used.

Risk estimates are currently calculated for cancer. Other, more subtle effects such as immune suppression, neurotoxicity, reproductive and developmental effects are not addressed. Moreover, sufficient toxicological data for conducting an assessment exists for only about 2 percent of all commercial chemicals.

Like every other method for predicting the future, risk assessment is plagued by lack of essential data and fraught with uncertainties, many of which may not ever be resolved. As a result, risk estimates derived from this procedure are at least as much matters of opinion as they are matters of science, as described by the former director of the U.S. Environmental Protection Agency:

'We should remember that risk assessment data can be like the captured spy: if you torture it long enough, it will tell you anything you want to know.'

5.1.2 Testing All Organochlorines is Not Feasible

At least 11,000 organochlorines are produced commercially. Many thousands more are created as by-products during the manufacture, use and disposal of these initial 11,000.

Only a few of the commercial organochlorines have been tested for carcinogenicity and a few other effects. Fewer still have been tested for their abilities to disrupt the human endocrine system, alter the sexual, neurological and immunological development during pre- and postnatal periods, or any of a long list of equally subtle but potentially devastating effects.

With the exception of dioxins, few of the organochlorine by-products have been tested for these effects. Indeed, the majority have not yet been fully chemically characterized. Further, there has been almost no testing of the effects of exposures to mixtures of organochlorines, such as those usually encountered in environmental and human tissue samples. The costs for doing so are essentially prohibitive. For example, in 1987, the cost of a minimal study of the effects of a mixture of 25 common toxic chemicals was estimated at U.S. $3 trillion (Yang and Rauckman 1987).

Currently, it takes more than five years and hundreds of thousands of dollars to gather basic toxicological data for one single compound. Testing all commercial organochlorines and organochlorine by-products, individually and in their mixtures currently occurring in human tissues, for even a minimal set of the most problematic of their potential effects – carcinohtiicity, mutagenicity, endocrine disruption, developmental effects, immune suppression, and acute toxicity – would require decades and decades not to mention more money than any combination of governments and industries can afford. For some organochlorines, studies suggest that there is no threshold below which no effects occur. For such chemicals as well as those known to be persistent, risk assessment and toxicological testing directed towards establishing acceptable rates of release and exposure are exercises in futility.

5.3 Natural versus Manmade Organochlorines

About 1,200 organochlorines occur in nature as products of a relatively small number of organisms and events, such as volcanic eruptions. Only one – chloromethane produced primarily by marine algae – is produced in large amounts and may well be a natural regulator of the the ozone layer.

Unlike manmade organochlorines, none of the organochlorines produced by organisms are persistent. Consequently, they do not accumulate in the environment or the tissues of living organisms.

The natural occurrence of any number of organochlorines in any quantity does nothing to diminish the impacts on public health and the environment of manmade organochlorines. For example, the natural occurrence of chloromethane and other organohalogens with ozone depleting potential is not regarded by scientists, national governments or even the chemical manufacturers as a defense for the mass production of ozone depletors of organochlorine ozone-depletors.

5.4 Global Contamination requires Global Strategy

As part of the worldwide effort to preserve the protective ozone layer in the upper atmosphere, more than 130 nations have ratified the Montreal Protocol to phase-out the production and use of CFCs and some other ozone depletors. Most industrial nations now forbid the production and open use of PCBs (Barrie 1992), and 70 nations have banned, either partially or fully, the production or use of one or more of the organochlorine pesticides.

However, in the absence of a mechanism for global bans or phaseouts, organochlorines that are banned in some industrialized countries are still being manufactured for use and exported to other nations. Due to the migration of these toxins via air transport, a global instrument offers the only means for protecting the health of ecosystems and humans, especially those in colder regions such as the Arctic and Antarctic.

A voluntary global ban of PCBs has dramatically reduced the production of these compounds and they are now deliberately produced only in strictly controlled environments. However, following an initial decline, PCB levels in the environment appear to have stabilised as they continue to migrate

from sediments and landfills. Current figures for the quantities of PCBs present in waste sites suggest that future migration could theoretically double the historical releases to date from these sources (Johnston 1994)

Toxaphene, another widely restricted organochlorine, is not used in Western Europe and is banned in 27 countries. Nonetheless, high levels of toxaphene in fish in the North Sea and North Atlantic have recently been reported. This has been attributed to the use of toxaphene in cotton growing in the Caribbean region.

The global circulation and deposition of PCBs, toxaphene and other locally and regionally banned organochlorines attest to the failure of local and national regulations in protecting public health and the environment from persistent organochlorines. With the importation to less industrialised countries of technologies that make and use persistent organochlorines, this global problem can be expected to grow.

5.5 Global Ban on Organochlorines

The piecemeal regulations currently in place across the globe address only a small fraction of the organochlorines of greatest concern, which are predominantly the unintentional by-products of the manufacture, use and disposal of commercial organochlorines. The threat posed by organochlorines to the global environment and the people of the world necessitates a global phaseout plan. The major elements of this global strategy are as follows:

- Primacy of the precautionary principle;
- Global and legally binding mechanism to phaseout organochlorine compounds as a class, sector by sector;
- Progressive tax on the production of chlorine; and
- Transition fund for displaced workers and communities as well as for technical and financial assistance to developing countries and small businesses for product substitution.

Precaution as Primacy

At both national and international levels, the precautionary principle is gaining widespread acceptance as the foundation upon which all effective strategies for protecting public health and the environment from the effects of persistent organochlorines and other critical pollutants must be constructed.

The precautionary principle stipulates that substances are not discharged into the environment unless they are proven to be non-deleterious, rather than allowing their release until harm has occurred or potential harm proven.

Legally Binding Instruments

Over the years, the following international political fora have acted on their concerns about organochlorines and their impacts on human health and the environment:

1) The Contracting Parties to the Barcelona Convention agreed in October 1993 as follows:

'[To] reduce and phase out by the years 2005 inputs to the marine environment of toxic, persistent and bioaccumulative substances listed in the LBS Protocol, in particular organohalogen compounds having those characteristics.... "
and:
'[T]o promote measures to reduce inputs into the marine environment and to facilitate the progressive elimination by the year 2005 of substances having proven carcinogenic, teratogenic and/or mutagenic properties in or through the marine environment."

2) The Paris Commission agreed on September 1992 to the following commitment under Article 3 of Annex I on the Prevention and Elimination of Pollution from Land-based Sources:

'... it shall, inter alia, be the duty of the (Paris) Commission to draw up:

(a) plans for the reduction and phasing out of substances that are toxic, persistent and liable to bioaccumulate arising from land-based sources;'

3) In addition, the Paris Conference Ministers adopted a future action plan which calls for substantial reduction of inputs to the maritime area with the aim of their elimination, for substances that are toxic, persistent and liable to bioaccumulate with priority given to the organohalogens. The Paris Ministerial Declaration takes this approach one step further by agreeing, inter alia, to the following:

'Agree that, as a matter of principle for the whole Convention area, discharges and emissions of substances which are toxic, persistent and liable to bioaccumulate, in particular organohalogen substances, and which could reach the marine environment should, regardless of their anthropogenic source, be reduced, by the year 2000, to levels that are not harmful to man or nature – with the aim of their elimination; to this end to implement substantial reduction in those discharges and emissions and where appropriate, to supplement reduction measures with programmes to phase out the use of such substances; and instruct the Commissions to keep under review what timetables this would require;'

4) The Paris Commission have also made recommendations concerning the substitution of organochlorines (OsParCom 1992):

'To adopt further measures for the prohibition of the use of organohalogen substances which are unnecessary for the intended use or process, and do not therefore need to be substituted for. Otherwise to compile a list of processes and substances which are suitable for use as substitutes.'

5) One way forward has been initiated by the International Joint Commission of the Great Lakes (IJC 1994). Their recommendations to the governments of the United States and Canada concluded that in cooperation with other jurisdictions and interests they should take the following steps:

'[C]onsult 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 consider ations must be taken into account in developing the strategies and timetables.'

6) To contribute to the definition of the issue the Commission created a Virtual Elimination Task Force. This group was specifically mandated to provide advice and recommendations about the contents of a virtual elimination strategy and ways in which to implement it. In addition, in their recommendations to the business community, labour and media, The Commission suggested that:

'Labour Unions include in their negotiations the issue of transition to a sustainable economy without persistent substances'.
and
'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.'

7) The Inuit Circumpolar Conference of 1989 called for an international agreement to control the toxic contaminants in the Arctic – most of which reaches the Arctic through long-distance air pollution.

Yet, none of these commitments have resulted in any regulated reduction of chlorinated compounds. Even if regional commitments to eliminate organochlorines were to bear results, the global circulation of these contaminants from other unregulated regions would carry these chemicals pollutants across the globe.

Therefore a global and legally binding instrument to phase out these compounds as a class is required. This instrument must include timelines for phaseout of chlorinated compounds in large sectors where alternatives are proven effective and affordable:pulp and paper, solvents, PVC and pesticides and as a second priority in the area of chlorinated intermediates and water disinfection.

Chlorine Tax/Transition Fund

A tax should be instituted on the chlor-alkali process per ton of chlorine produced. This revenue should be held in a fund to aid the transition to a chlorine-free industrial society. In particular, funds should be used for exploring and demonstrating economically viable alternatives and for easing dislocations among affected workers and communities. In addition, funds should be targeted towards clean production processes to assist developing countries and small businesses make the change.

One initiative to recognize and plan for the transition has come from the Oil, Chemical and Atomic Workers Union in the United States since the nature of the industries that the union representation are ultimately unsustainable. The Superfund for Workers idea stems from the two aims of the union: 1) to protect the jobs, incomes and working conditions of their members and 2) ensure safe jobs and a healthy environment.

The idea builds on the Superfund Act in the United States where a budget is held for chemically contaminated land clean-ups. The Superfund for Workers program advocates education with income support for workers and for workers unable to find work after retraining, a guaranteed annual wage coupled with education. The revenue from the chlorine tax can also be used for demonstration projects and low interest loans to show feasibility of chlorinated solvent substitution such as in dry cleaning sector; government procurement of non-chlorinated products; start up of clean production centres and preferential costing to stimulate the market in clean materials.

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7 Tables

Table 1.1
Human breast milk fat concentrations worldwide

Chemical	*Average Level*	*High Level and Location*
Dieldrin	0.05 ppm	1.78 ppm Australia 1.00 ppm Iraq
Heptachlor & its Epoxide	0.05 ppm	2.50 ppm Spain 0.48 ppm Italy
Chlordane	0.08 ppm	>2.00 ppm Mexico & Iraq
Total DDT	1.00 ppm	>100 ppm Guatemala
HCB	0.10 ppm	7 .00 ppm Greece
Beta-HCH	1.00 ppm	6.50 ppm Chile
Gamma-HCH (lindane)	0.05 ppm	0.89 ppm Italy
Total PCB (Hudson Bay)	1.00 ppm	3.6 ppm Canada
2, 3, 7, 8- TCDD (dioxin)	2.00 ppt	1.45 ppb Vietnam, 1970s

Source: Adapted from Thomas and Colborn 1992

Table 1.2a
Median concentrations of organochlorine pesticides in breast milk*, Western Australia.

Survey	*DDT*	*Dieldrin*	*HCB*
1974	3.60	0.24	2.60
1978	2.00	0.24	0.75
1980	1.20	0.17	0.15
1991	0.8	0.05	0.1

* Breast milk concentrations as mg/kg extractable fat.

Source: Adapted from Stevens et al. (1992)

Table 1.3
Mean values of organochlorine pesticides in breast milk in Germany

Compound	1984	1986	1988	1990	1991
HCB	0.511	0.361	0.316	0.227	0.177
Beta-HCH	0.130	0.112	0.076	0.064	0.056
Gamma-HCH	0.021	0.021	0.015	0.008	0.006
pp-DDE	0.893	0.718	0.637	0.534	0.504
pp-DDT	0.085	0.053	0.038	0.027	0.027
Dieldrin	0.022	0.014	0.018	0.011	0.009

Source: Furst et al. 1994.

Table 1.4
Health effects associated with dioxins in humans and animals

Effect	*Species*	*Estimated Body Burden of Dioxin (ng/kg)*
"Background" level	Human	9
Cancer	Human	109-7000
	Hamster	500
	Mouse	1000
Decreased testosterone (Reproductive hormone)	Human	83
Decreased testis size	Human	14
Altered glucose tolerance	Human	14-110
"Low-dose effects" in animals		
Endometriosis	Monkey	54
Decreased sperm count	Rat	64
Decreased offspring viability	Monkey	270
Enhanced viral susceptability	Mouse	7

Source: Adapted from Anon. (1995).

Table 1.5
Site of production of some steroid hormones

Organ	*Hormone*
Ovary	progesterone oestrogen
Testis	testosterone
Adrenal Cortex	mineralocorticoids glucocorticoids androgens, eg testosterone
Placenta	progesterone oestrogen

Source: M. Allsopp

Table 2.1
Organochlorine chemicals with widespread distribution in the environment reported to have reproductive and hormone-disrupting effects in laboratory animals, wildlife and/or humans

Pesticides and Insecticides

2,4-D
2,4,5-T
Alachlor
Atrazine
Beta-HCH
Chlordane
Dicofol
Dieldrin
DDT and metabolites
Endosulfan
Heptachlor
Lindane (gamma-HCH)
Methoxychlor
Mirex
Nitrofen
Oxychlordane
Toxaphene
Transnonachlor

Nematocides

DBCP

Fungicides

Hexachlorobenzene

Industrial Chemicals

Dioxin (2,3,7,8-TCDD)
PBBs
PCBs
Pentachlorophenol

Source: Colborn et al. 1993

Table 2.2
Organochlorine chemicals which have been shown experimentally either *in vitro* and/or *in vivo* to be oestrogenic

Atrazine
Chlordane
o,p'DDT
p,p'DDT*
Heptachlor
Beta-hexachlorocyclohexane
Kepone (Chlodecone)
Methoxychlor
PCBs

* Bustos et al. 1988

Source: Adapted from Davis et al. 1993.

Table 2.3
Summary of Studies (1977-1978) on workers with variable exposure to DBCP

Study Population	Number of subjects		Number of men with sperm count results(million/ml)	
		•		0.1-9
California factory workers	114	15	8	12
Colorado factory workers	64	5	2	7
Alabama factory workers	71	1	6	5
Arkansas factory workers	86	30	(17)*	(3)
Calafornia DBCP applicators	74	6	8	(7)
Israel factory workers	23	12	6	0
Southeast US DBCP users	53	2	12	8
Mexican factory workers	23	9	(11)	
Totals	508	80 (15.7%)	70 (13.8%)	42 (8.3%)

*Numbers in parenthesis extrapolated from data presented

Source: Whorton and Foliart 1983

Table 2.4
Studies on organochlorines and breast cancer

Reference	*No. of Cases/Controls*	*Associated Organochlorine*
Unger et al.(1984	14/21	No Association
Mussala-Rauhamaa et al. (1990) (p=0.026)	41/33	HCB
Falck et al. (1992) (p<0.05)	20/20	DDE, PCB
Wolff et al. (1993) (p=0.031)	58/171	DDE
Dewailly et al. (1993) (p=0.02)	20/17	HCB
		DDE for ER+vs
Krieger et al.(1994) ER- (p<0.05)	150/150	No Association

Source: M. Allsopp

Table 2.5.
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
Polychlorinated biphenyls
Polychlorinated dibenzo-p-dioxins
Polychlorinated dibenzofurans
Polychlorinated napthalenes
Polychlorinated diphenyltoluenes
Polychlorinated diphenyl ethers
Polychlorinated anisole
Polychlorinated phenoxy anisoles
Polychlorinated xanthenes
Polychlorinated xanthones
Polychlorinated anthracenes
Polychlorinated fluorenes
Polychlorinated dihydroanthracenes
Polychlorinated diphenylmethanes
Polychlorinated phenylxylylethanes
Polychlorinated dibenzothiophenes
Polychlorinated quaterphenyls
Polychlorinated quaterphenyl ethers
Polychlorinated biphenylenes
Polychlorinated thioanthrenes
Polybrominated diphenyl ethers
Polychlorinated azoanthracenes

Source: Giesy et al. (1994)

Table 3.1
Evaluation of carcinogenic risk of chemicals to humans by the international agency for research on cancer (IARC)

a) Organochlorine compounds that are carcinogenic in humans (classified as Group 1)
   Bis(chloromethyl)-ether and chloromethyl methyl ether (technical grade)
   Vinyl chloride

b) Common organochlorine compounds that are probably carcinogenic in humans (classified as Group 2B)
   Chloroform
   Chlorophenols
   Chlorophenoxy herbicides
   DDT
   Dichloromethane
   Hexachlorobenzene
   Hexachlorocyclohexanes
   Mirex
   Polychlorinated Biphenyls (PCBs)*
   2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)
   Tetrachloroethylene
   Toxaphene

* PCBs are classified as group 2A chemicals.

Source: Kipen and Weinstein (1992)