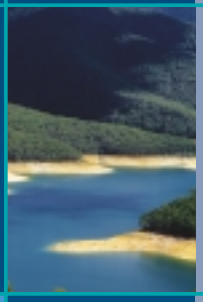
The Cooperative Research Centre for
Water Quality and Treatment

Endocrine Disruptors
in the Context of Australian Drinking Water





Occasional Paper 7: Endocrine Disruptors

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Cooperative Research Centre for Water Quality and Treatment
Private Mail Bag 3
Salisbury SA 5108
AUSTRALIA

Telephone: +61 8 8259 0211
Fax: +61 8 8259 0228
E-mail: crc@sawater.com.au
Web site: www.waterquality.crc.org.au

Occasional Paper 7: Review of Endocrine Disruptors in the Context of Australian Drinking Water

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REVIEW OF ENDOCRINE DISRUPTORS IN THE CONTEXT OF AUSTRALIAN DRINKING WATER

Ian R Falconer[1], Michael R Moore[2], Heather F Chapman[3] and Geetha Ranmuthugala[4]

1. Department of Clinical and Experimental Pharmacology, University of Adelaide Medical School, Adelaide, South Australia

2. National Research Centre for Environmental Toxicology, University of Queensland & Queensland Health Scientific Services, Kessels Rd., Coopers Plains, Queensland 4109

3. School of Environmental Engineering, Griffith University, Nathan Campus, Queensland 4111

4. National Centre for Epidemiology and Population Health, Australian National University, Australian Capital Territory

Executive summary

This review was commissioned by the CRC for Water Quality and Treatment to provide an overview of the effects of endocrine disrupting chemicals (EDCs) and their potential to contaminate drinking water in Australia. The particular issue for the water industry is the provision of safe drinking water from sources of varying quality. It was therefore necessary to review the current knowledge on the human health effects of exposure to EDCs in drinking water and the environment. Knowledge of the concentrations of EDCs in the environment is needed to determine the possible level of exposure. As the majority of potent endocrine-disrupting chemicals (natural and synthetic estrogens in particular) enter the water system from sewage discharge in Australia, the efficiency of removal of these compounds by wastewater treatment works is an important issue for consideration.

The ability to monitor EDCs in the environment, including water supplies, depends on effective methodologies, which will accurately and reproducibly quantitate EDCs at the concentrations occurring in the different environmental media. As estrogens are biologically active at nanomolar concentrations, considerable sensitivity is required in measurement and this has led to the development of biomarkers to detect hormonal activity below the present limits of detection. The ecological impacts of EDCs are an issue in receiving waters, whether fresh or marine but will only be considered briefly in this review. Limited information is available from Australia, although North American and European data show clear impacts on aquatic fauna.

The WHO/IPCS recently released their "Global Assessment of the State of the Science of Endocrine Disruptors" which can be accessed through the WHO website. This presents an extensive and detailed assessment, which is not duplicated here. The intention of the authors of this review is to provide a compact survey with relevance to the Australian water industry and public health.

The overall conclusion from the epidemiological data on adverse effects on human health from EDCs is that general, low level environmental exposure to EDCs has not yet been demonstrated to cause harm. For example the broad-scale population epidemiology of, for example, male infertility has not provided a coherent link to environmental EDCs.

However pharmacological dosing with oestrogens (particularly diethylstilbestrol), accidental and occupational exposure to agricultural chemicals, industrial chemical accidents and accidental consumption of contaminated foodstuffs have been clearly associated with harm. The most vulnerable stage of development in humans is in early pregnancy, but this vulnerability extends to the foetus in later pregnancy and early postnatal life. These effects may not be directly linked to endocrine changes, but may arise from chemical toxicity to developing tissues. Endocrine disruption is recognised as a mechanism for potential harm, leading to a range of possible adverse effects, to be considered with effects from other toxic mechanisms.

Neurological injury in embryonic or foetal life may be among the areas most sensitive to toxicant exposures. Direct effects on adult reproduction, and adverse effects on bodily homeostasis require higher concentrations of toxicants or EDCs. This relates to the differences between the organisational or tissue differentiating functions of hormones, which are very sensitive, and the activation or metabolic functions of hormones that require higher concentrations for effect.

The concentrations of EDCs in domestic wastewater discharge may cause changes in the aquatic fauna in the discharge plume, but are many orders of magnitude lower than the concentrations likely to cause detectable health effects in humans if this water is a component in reuse for drinking water. This may not be the case for heavy industrial or agricultural chemical discharges where discharge concentrations may potentially reach pharmacologically active levels. Australia currently benefits from supply of largely single-use drinking water, with most wastewater discharges to the marine environment, which is not the case in Europe. However the low river flows in inland and coastal rivers due to irrigation use and low rainfall enable pollutants to accumulate, which become an issue for examination with respect to country towns which rely on drinking water pumped from rivers. This also applies to the summer supply for the metropolitan area of Adelaide, the capital of South Australia, which has a limited winter supply of reservoir water collected from a partially degraded catchment. The remaining drinking water requirement for Adelaide is now pumped from the lower reaches of the Murray River. Melbourne, Sydney and Brisbane are more fortunate, with large reservoir supplies and substantial catchment protection.

Recommendations

- We recommend Australian monitoring of the international literature on EDCs for evidence of human health effects, efficacy of drinking and wastewater treatments in removing specific EDCs and for methods for monitoring EDCs and ecological effects.

- Monitoring at selected locations of known and potential ECDs (hormonally active chemicals, agricultural chemicals and their breakdown products with potential EDC activity) in rivers downstream of wastewater discharges and irrigation areas, especially where town drinking water supplies are drawn from these rivers. We recommend parallel ecological monitoring at these locations.

- Monitoring of selected Australian drinking water and wastewater treatment processes for EDC removal, using both chemical and biomarker methods as screening tools, is recommended.

- Chemicals suspected of having endocrine-disruptor activity occur in industrial effluent, sewage discharge and agricultural run-off, and ultimately end up in the ocean. Thus the marine environment is potentially at risk. While this is not directly relevant to the drinking water industry, potential environmental impacts on coastal marine fauna could usefully be investigated.

- On the basis of the large amount of EDC research in progress in the USA, the EU and Japan, it is not recommended that Australia embark on major programs in this field. However it is necessary that we investigate how the emerging technologies in this field may be developed and validated for use in Australia. Evaluation of advances in EDC monitoring methodology, in sewage treatments for EDC removal, risk mitigation technologies for agricultural pesticide and intensive animal industry contaminants and models for the fate of EDCs in the environment are relevant areas for investigation.

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Abbreviations

AP	Alkyl phenol
APE	Alkyl phenol ethoxylate
CDC	US Centers for Disease Control
CRC	Cooperative Research Center
DEHP	Di-2-ethylhexyl phthalate
DES	Diethylstilboestrol
EAT	Endocrine, androgen and thyroid
EDC	Endocrine disrupting compound
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EEQ	Estrogen equivalent
EHP	Environmental Health Perspectives
ER	Estrogen receptor
ERBA	Estrogen receptor binding assay
FAO	Food and Agriculture Organization
ICCVAM	Interagency Coordinating Committee for the Validation of Alternative Methods
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
IUPHAR	International Union of Pharmacology
IUTOX	International Union of Toxicology
MCL	USEPA Maximum Contaminant Level
NIEHS	National Institute of Environmental Health Sciences
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
OECD	Organization for Economic Cooperation and Development
OWC	Organic wastewater contaminant
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxins
PCDF	Polychlorinated dibenzofurans
QSAR	Quantitative Structure Activity Relationship
TBT	Tributyl tin
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TDS	Testicular dysgenesis syndrome
TEQ	Toxic Equivalent
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

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1.0 Introduction

The first attempt in Australia to nationally review the emerging issue of endocrine disrupting chemicals was undertaken by the Australasian Society for Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) in a symposium titled "Endocrine disrupting chemicals: An emerging health and environmental issue?" held on 2nd December 1997 (ASCEPT, 1997). This was quickly followed by the Australian Academy of Science in 1998. Their report 'Endocrine disruption; Australia's role in an international issue' discussed the key areas of public and environmental health, toxicity measurement, regulation and risk assessment. In April 1998 an Australian regulatory agency EDC policy document was posted on Environment Australia's website (Environment Australia, 1998).

At that time the USEPA and the OECD had initiated major research programs into endocrine disruptors, and the main emphasis in Australia since that time has been to monitor international outcomes, while undertaking small scale research. Also prior to the Academy meeting, the IUPAC/IUPHAR/IUTOX '*White book*' on Natural and Anthropogenic Environmental Estrogens was launched in December 1997 in Canberra. It drew attention to the scientific basis for risk assessment arising from the presence of these materials in the human environment (IUPAC, 1998). A more specific evaluation was also undertaken in the special Supplement to Environmental Health Perspectives in 2002 in discussions of the impact of endocrine disruptors on brain development and behaviour (EHP, 2002). The substantial current advances in international research and measurement of endocrine disrupting chemicals in the environment have been very recently reviewed in the WHO/IPCS 'Global Assessment of the State of the Science of Endocrine Disruptors' (WHO/IPCS, 2002). This comprehensive account is a key reference to the present situation and has formed one of the bases of this more limited review in the context of Australian drinking water supplies.

1.1 Background

There has been growing concern that environmental chemicals have the potential to cause adverse health effects on a wide range of species including humans (Colborn *et al.*1996; Kavlock *et al.* 1996; Sharara *et al.* 1998; Sonnenschein and Soto, 1998, Colborn 2002). During the last few decades a number of studies have implicated environmental chemicals with adverse changes in human health, while others have not shown any relationship. Increased rates of testicular and breast cancers and possible foetal abnormalities due to exposure to certain compounds have been reported as well as a decline in quality and quantity of sperm (Gill *et al.* 1976; Nollar *et al.* 1990; Sharpe and Skakkebaek 1993; Sharara *et al.* 1998). It has been hypothesised that these health problems may be caused by compounds in the environment that can interfere with the endocrine system. Clear evidence of adverse effects on the endocrine system was shown following pharmaceutical use of the synthetic estrogen diethylstilboestrol (DES) given to women to prevent miscarriage. Children of mothers who took the drug have shown higher than normal rates of cancers associated with reproductive organs and malformation of these organs (Gill *et al.,* 1979; Nollar *et al.,* 1990). These effects were associated with *in utero* exposure at early developmental stages. However the weight of evidence for endocrine disruption in humans in relation to environmental chemicals is limited and the issue remains controversial (Degen and Bolt, 2000; Safe, 2000), notwithstanding the arguments put forward in 'Our stolen future' (Colborn *et al*., 1996, 2002). While there is documented evidence of impacts to wildlife downstream of sewage discharges (Gagne *et al*., 2002), in order to enable quantification of risk to humans it is essential to determine if adverse effects are likely to occur in response to actual environment concentrations. In the absence of sound scientific data, opinions and decisions will be made based on the perceived risk, which is highly subjective, and will vary considerably among the community, governments and utility managers.

The endocrine system is highly complex and regulates a variety of bodily functions including development, growth and reproduction by a communication network using chemical messengers (hormones) and feedback mechanisms. The endocrine glands secrete hormones directly into the bloodstream to control tissues and organs. The hormones primarily responsible for development, growth and reproduction are secreted by the hypothalamus in the base of the brain, the anterior part of the pituitary gland, the gonads and the thyroid gland. Hormones with major functions in homeostasis (the maintenance of normal body function in varying circumstances) are those from the pancreas-insulin and glucagon, adrenal cortical steroids, posterior pituitary hormones, thyroid and parathyroid hormones, and adrenal medullary hormones. Responsive tissues in the body have cell membrane or cell nucleus receptors that respond to the presence of specific hormones. All vertebrates and some invertebrates have an endocrine system (Barton and Anderson, 1998; Tyler *et al*, 1998).

The comparative vulnerability of mammalian systems to exogenous compounds varies significantly. The tissues most sensitive to adverse effects appear to be those of the early developing embryo and foetus, which can respond to very low concentrations of natural or synthetic agents. At this time the role of a hormone is organisational, acting on tissue development. The developmental effects on the foetus generated during pregnancy may be retained into adult life as adverse changes to structure or function of organs Teratogenesis caused by exogenous compounds can be expressed in many ways, one of which is in reproductive defects. The next most sensitive mammalian systems are those concerned with reproductive function in adults, hence the success of oral estrogen dosage for

contraceptive purposes. The order of magnitude for dosage in adults to obtain a pharmacological effect (or an activational response) on reproduction is substantially higher than the dose needed to affect the foetus. To adversely affect homeostasis, growth or wellbeing by exogenous compounds requires even higher dosages. These issues are discussed further in the section on human epidemiology.

Synthetic chemicals and exogenous natural hormones may affect the health of organisms by disrupting the action of their endocrine system. These so-called endocrine disrupting compounds (EDCs) have been defined by the US Environmental Protection Agency (USEPA) as exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of natural ligands in the body responsible for the maintenance of homeostasis and regulation of developmental processes (Kavlock *et al*. 1996).

A clearer definition from the WHO/IPCS (2002) is 'An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequentially causes adverse health effects in an intact organism, it's progeny or (sub) population'. EDCs can be classified into a number of categories that may not be mutually exclusive, as one EDC may exhibit one or more of these effects (Sonnenschein and Soto, 1998; WWF, 1999):

- Mimic endogenous hormones (agonists)
- Block the effect of endogenous hormones (antagonists)
- Modify the number of receptors (stimulators)
- Modify the response of receptors to their ligands
- Modify hormone metabolism
- Deactivate enzyme function
- Interfere with hormone production/synthesis

EDCs can be regarded as a subset of toxicants in general, which exert harmful effects on living organisms. Toxic responses are seen in many tissues, including those that perform an endocrine function, so that there is no clear distinction between a toxic action that does or does not affect the endocrine system. EDCs can act primarily through cell membrane processes, cell metabolic processes or gene action. There is a need to assess the role of endocrine disruptors relative to other environmental stressors and environmental toxicants. However most studies of EDCs have been limited to evaluation of estrogens and estrogen-like compounds alone (IUPAC, 1998).

1.2 Scope of this review

This review is intended to provide an Australian context to the issue of endocrine disrupter chemicals, focussing on their potential to contaminate drinking water supplies in Australia. Hence only a limited background to the issues of endocrine disruption is included. EDCs in drinking water sources are of concern internationally through their potential to adversely affect human health. The majority of EDCs affecting drinking water sources in Europe (for example) arise from sewage, therefore wastewater treatment techniques to remove EDCs from treatment discharge are relevant to water re-use. This issue may be of relevance to Australia in the near future. Methodological developments are important to provide effective monitoring data on the very low concentrations that may be encountered and have been considered in this review. The research needs of the Australian water supply industry on EDCs are significantly lessened by the large output from studies underway in the USA and Europe, however several issues require attention in Australia based on the geography of the population and distribution of agriculture. These are evaluated in this report.

1.3 Endocrine disruptors and their sources-

Anthropogenic chemicals found experimentally to have endocrine disrupting properties include pesticides such as DDT and its products of degradation (Kelce *et al*, 1995), industrial chemicals such as PCBs, and phthalate plasticizers (Jobling *et al*. 1995), dioxins and dioxin–like compounds, the antifouling agent tributyltin (TBT), detergents or their breakdown products such as nonylphenol (USEPA, 1997) and pharmaceuticals such as the contraceptive pill (17 β-ethinylestradiol). Contamination of the environment or of drinking water may occur from such simple routes as use of manufactured plastic articles. For example plasticisers have been detected widely in surface waters in the USA (Kolpin *et al*, 2002). Di -2-ethylhexyl phthalate (DEHP) is a manufactured chemical used as one of several plasticisers in polyvinyl chloride (PVC) which is widely used for water pipes. DEHP has been frequently detected in surface waters, ground water and finished water (ie drinking water after treatment) in the USA and is included as one of the chemicals which has been classified as a priority pollutant. The USEPA have set the MCL at 6 parts per billion (6 ppb) for drinking water (USEPA, 2001). DEHP is biodegradable in water but tends to accumulate in sediments where it is relatively persistent. Diethyl phthalate (DEP) is a synthetic compound also used to increase the flexibility of plastic and is additionally used in cosmetics, insecticides and asprin (USEPA,2001). It too is used in PVC water pipe manufacture with the possibility of leaching into drinking water.

Naturally-occurring chemicals

A range of naturally occurring compounds have been demonstrated to have endocrine-disrupting activity, which include the so-called phytoestrogens. Phytoestrogens (from subterranean clover) were scientifically reported in the 1940's to cause severe infertility in sheep grazing in Western Australia, and remain an agricultural problem (Adams, 1998). Wild populations are also affected by phytosteroids (naturally occurring plant compounds

related chemically to steroid hormones) as illustrated by the masculinisation of female fish due to exposure to β-sitosterol from pulp paper mill effluent (Howell *et al,* 1980).

In addition to the phytoestrogens there are numerous compounds used in herbal remedies which have the capacity to have endocrine activity, such as the various terpenoids found in products like neem, a pharmacologically active extract from an Indian tree (*Azadirachta indica*) which can used for contraception and inducing abortion (National Research Development Corporation of India, 2003).

Natural hormones excreted by people and livestock (including 17β-estradiol, estrone and testosterone) can also interact with the endocrine systems of consumers when released into the environment (Health Council of the Netherlands, 1999). There is potential for high concentrations of these substances in surface waters along with veterinary pharmaceuticals in areas of intensive livestock production, including feedlots.

A representative list of endocrine disrupting chemicals is provided as Table 1.3.1

Toxic and endocrine disrupting chemicals can enter the environment from a wide range of routes, for example direct discharge to land and water; emissions to air from motor vehicles and from incineration of organic materials; use of pharmaceuticals and chemicals by householders, agriculture and industry; accidental spills and releases of compounds, and indirect diffuse sources such as stormwater run-off. Researchers were first alerted to endocrine dysfunction due to aquatic contamination through a variety of reproductive changes observed in different species of fish sampled immediately down-stream from sewage treatment plant outfalls. (Batty and Lim, 1999; Bortone and Davis 1994).

Table 1.3.1 Endocrine Disrupting Chemicals, representative list

Synthetic origin		
	Hormones	17α-Ethinylestradiol
		Diethylstilbestrol
	Herbicides	Atrazine
		Simazine
		Metoxychlor
		2,4-D
	Insecticides	DDT
		Dieldrin
		Endosulphan
		Lindane
	Industrial chemicals	
		Phthalates
		Bisphenol A
		p-nonylphenol
		PCB's
		Tributyl tin
Biological origin		
	Hormones	17β-estradiol
		Estriol
		Estrone
		Progesterone
		Testosterone
	Plant secondary metabolites	
		Sesquiterpenes
		Phytosterols

1.4 Exposure issues

There are numerous natural and anthropogenic compounds in the environment that are developmentally or hormonally active if consumed in sufficient quantities by people. Some of these are persistent in the environment, and some are not. The lipophilic (fat soluble) compounds tend to be more persistent, whereas other more soluble compounds may only be present in the environment for short periods of time. However even the less persistent compounds may remain in the environment long enough to cause adverse effects at a critical stage of development of a foetus (WHO/IPCS, 2002). Many lipophilic EDCs are highly persistent and accumulate in animal tissues, particularly in body fat. There may be long latency periods between exposure and manifestation of a response, which may be triggered by a changed physiological condition. For drinking water safety, if compounds are not water-soluble it is unlikely that they will represent a problem in drinking water supplies unless their endocrine activity exists at concentrations much greater than those of natural hormonal compounds, or they are carried adsorbed to fine particles.

Most of the more definitive studies on chemically mediated effects have been conducted on highly exposed groups in various occupations or from accidental exposure (Garry *et al.* 2002; Mocarelli *et al.* 1996). Only limited exposure information is available from lower level exposures in part due to limitations associated with analytical sensitivity. Our knowledge of the exposure of wildlife and humans remains limited.

It is considered that many of the potential EDCs enter the human body via ingestion, as components of pharmaceutical products such as contraceptive pills and estrogen replacement therapy and through food. Phytoestrogens are a normal component of the diet. Mycoestrogens (eg. zearalenone) and phytoestrogens from plants (eg. genistein and its analogues) exert a greater biological impact than do the synthetic chemicals, other than stilbestrol (Table 1.4.1). This does not imply that the synthetic estrogens are not important. Rather it emphasizes that exposure assessments need to consider both the magnitude of the exposure and relative potency of the array of EDCs that may be encountered. A 1998 estimate of the comparative intake of estrogens and their biological equivalents indicated that oral contraceptives would be the main source of exposure to estrogen equivalents in females using these pharmaceuticals (Table 1.4.2).

Table 1.4.1 Estrogenic equivalents (EEQs[1]) of EDCs compared to 17βestradiol

Compound	Type of test	EEQ	Reference
Diethylstilbestrol	MCF-7 bioassay	10	Soto *et al* (1992)
17βestradiol	MCF-7 bioassay	1	Soto *et al* (1992)
Zearalenone	MCF-7 bioassay	0.04	Mayr *et al* (1992)
Genistein	MCF-7 bioassay	0.0008	Welshons *et al* (1990)
4-tert-Octylphenol	*in vitro* rainbow trout hepatocyte bioassay	0.000370	Jobling and Sumpter (1993)
Nonyl Phenol	MCF-7 bioassay	0.000003	Soto *et al* (1992)
o,p' -DDT	MCF-7 bioassay	0.000001	Soto *et al* (1992)

[1] Quantification of estrogenicity. EEQ (estrogen equivalent) = $EC50_{ESTRADIOL}/EC50_{SAMPLE}$

Table 1.4.2 Estimated daily doses in females in terms of estradiol equivalents (EEQ[1]) (Pugh and Moore, 1998)

Compound/s	**EEQ**
Contraceptives	16 675
Hormone replacement therapy	3 350
Plants and food	102
17βestradiol	1
Organochlorins	0.0000025

[1] Quantification of estrogenicity. EEQ (estrogen equivalent) = $EC50_{ESTRADIOL}/EC50_{SAMPLE}$

2.0 Hazard assessment

2.1 Epidemiology of Exposure to Potentially Endocrine Disrupting Compounds

"Epidemiology is the study of the distribution and determinants of disease frequency in man" (MacMahon and Pugh, 1970). Epidemiological research aims to evaluate scientific hypotheses examining associations between specific exposures and outcomes (disease). Risk of developing a condition or disease is assessed at a population level and not at an individual level. While epidemiological investigations provide a risk estimate (i.e. gives an estimate of magnitude of increased risk to an exposed individual as opposed to a non-exposed individual), these studies are not able to directly attribute the outcome in an individual to a particular exposure. An assessment is made whether an association is statistically significant at a population level based on probability or confidence intervals. A common method of expressing the outcome of an epidemiological investigation is as an 'Odds Ratio', that is the ratio of risk of an exposed group developing an adverse outcome or disease compared to the risk for an un-exposed control group of individuals.

There are many limitations in epidemiology when it comes to examining associations between environmental contaminants and health outcomes. The limitations are better explained if examined by study design. The four main study designs used when examining casual associations are ecological, case-control, cohort, and intervention trial. Ecological studies examine exposure at a population level, not allowing for individual variations in exposure. This exposure is then linked to outcome data also collected at a population level. The evidence for association between exposure and outcome that is obtained from an ecological study is not conclusive and is often used as a hypothesis generating exercise. This study design does not allow for other factors that may be associated with exposure and outcome, but that are not directly in the casual pathway, i.e. confounders and / or effect modifiers. Many of the studies on potential reproductive effects of endocrine disrupting compounds fall into this design category.

The next level up in strength of evidence comes from case-control studies. These are retrospective studies that are conducted by identifying a group with the outcome of interest (disease etc.) and another group without the outcome of interest. Both groups are interviewed to identify common exposures in the exposed group that have not occurred in the control group. The major limitation associated with this study is recall bias. It is likely that there will be differential recall of exposure in the two groups, with individuals with the health outcome of interest more likely to recall exposure to potentially harmful substances than the control group. This is a commonly used study design when examining rare outcomes such as cancer that may be related to environmental, therapeutic or occupational exposures.

Cohort studies are a stronger study design because they may be conducted prospectively (or retrospectively). Study groups are selected based on exposure levels, and followed-up for outcome in the separate groups. This design does not suffer from recall bias when exposure is assessed prospectively. However, this study design is not commonly used in environmental epidemiology. Firstly, long latency periods require follow-up over many years. Maintaining a study cohort over many years can be logistically challenging and expensive. Secondly, large numbers are required to ensure adequate numbers of outcomes are achieved. Retrospective cohort studies are however useful and have been conducted when opportunities such as the Seveso accident arise (Mocarelli *et al*., 2000). This population provides a cohort of highly exposed individuals who have had biological samples taken for exposure assessment at the time of accident. This cohort can be accessed years later for determining health effects.

Intervention trials are usually not feasible in environmental epidemiology for ethical reasons, for example the harm that could be caused by deliberate exposure of a group to a potentially damaging environment.

2.2 Endocrine disruptors and human health

The following sections provide an overview of the human health effects associated with endocrine disruptors. Data has been evaluated for occupational exposures, accidental poisonings and overall environmental exposure. An extended discussion of this issue is presented in the WHO/IPCS Global Assessment of the State of the Science of Endocrine Disruptors(WHO/IPCS, 2002).

2.3 Human reproductive system

The following sections summarise literature examining the effects of hormone disrupting compounds on reproduction in humans.

2.3.1 Sexual development and behaviour

In humans and other mammals, the developing foetus does not differentiate into male or female until about the seventh week of embryonic life. The presence of the Y chromosome lays the groundwork for a male foetus by initiating the development of the testes, but it is the male hormone produced by the testes that eventually results in a foetus with male appearance. In the absence of testosterone, or interference with this hormone, the baby, although male in genetic composition, will have external characteristics of a female or have ambiguous genitals (Colborn *et al*., 1996). Sex hormones also influence behaviour. Disruption of hormone messages during the critical time of development may result in abnormal sexual appearance or behaviour.

2.3.2 Birth defects

The prescription of the drug diethylstilbestrol (DES), a synthetic estrogen, was popular in the 1940s to late 1960s for a variety of situations including the prevention of miscarriages, to ensure bigger and stronger babies, suppression of lactation, as a morning-after pill, for alleviation of menopausal symptoms, treatment of gonorrhoea in children and to stunt growth of overly tall teenage girls (Colborn *et al*., 1996). DES was also used by farmers to to produce fattened chickens, cows and other livestock for human consumption. The popularity of DES was despite experimental studies in rats in the 1930s suggesting that extra doses of estrogen in pregnancy could result in sexual deformities in the offspring (Colborn *et al*., 1996). By the 1970's, there was evidence emerging linking the use of DES during pregnancy to the development of clear cell carcinoma of the vagina in the offspring of women who had taken DES during pregnancy. Shortly after, severe deformities of the reproductive tract of these children were reported (Herbst and Bern, 1981).

Exposure to estrogens *in utero* may be associated with increasing incidence of reproductive abnormalities in the male (Sharpe and Skakkebaek, 1993). There are reports of cryptorchidism, hypospadias, higher risk of testicular cancer, and fertility problems (abnormal sperm and low sperm counts), in male offspring born to mothers using DES antenatally (Henderson *et al*., 1976) Gill *et al*., 1977), (Stillman, 1982), (Toppari *et al*., 1996). Birth defects have also been shown to be associated with pesticide exposure. The Red River Valley is an agricultural community in Minnesota, USA, with extensive use of pesticides. An initial study of birth defect frequencies showed a higher age adjusted rate compared with urban areas of Minnesota (Garry *et al*., 1996). A cross-sectional study undertaken to investigate further, showed that pregnancies conceived in spring (when herbicides are used) resulted in significantly more birth defects than conceptions occurring in any other season. Conceptions in spring had 7.6% birth anomalies compared to 3.3%, 3.7%, and 4.0% in fall, winter, and summer, respectively (Garry *et al*., 2002). The same study reports a significantly higher occurrence of central nervous system or neurobehavioural sequelae (Odds Ratio=3.6; Confidence Interval 1.35 – 9.65).

Experimental studies on animals have shown the potential for EDCs to cause reproductive tract abnormalities, as has pharmacological dosing of women with stilbestrol, and pesticide exposure. These issues require further study.

2.3.3 Changed sex ratio

The male:female ratio, also known as the sex ratio, has been on the decline since the 1950s in Denmark, and more recently (i.e. since the 1970s) in Canada and the USA (Davis *et al*., 1998). Similar trends have also been shown in Sweden, Germany, Norway and Finland. However an increase in sex ratio has been reported for Italy, Greece and the Netherlands (WHO/IPCS, 2002)

It has been hypothesised that environmental estrogens are responsible for the recent decline in the human sex ratio (WHO/IPCS, 2002, p.60). A study analysed data from Finland where meticulous records on births have been reportedly maintained by churches and subsequently by Statistics Finland (Vartianinen *et al*., 1999). Proportions of male newborn infants in Finland between the years 1751 and 1997 were analysed by linear regression. This study reports that the decrease in male:female ratio preceded plausible exposure to agricultural or industrial environmental estrogens by several decades, thereby not supporting the hypothesis.

Demonstration of a war–time peak in the sex ratio has been a feature of more than one study (Vartianinen *et al*., 1999), (van den Broek, 1997), (Bromen and Jockel, 1997), (Parazzini *et al*., 1997), generating an alternative hypothesis of a fundamental physiological process that responds to periods of crisis, which may be mediated by a neuroendocrine pathway (Vartianinen *et al*., 1999).

Several other factors have been examined for influence on the human sex ratio. James, (1987) in his review of the literature examining the variation in the human sex ratio, identifies that hormonally induced ovulation and artificial insemination results in an excess of female children. Apart from the fact that only normal births were included in the review, details of search criteria were not provided in the article. Race was also identified as a variable that contributes to variation with the ratio being lower among black populations. This phenomenon has been noted in USA, UK, South Africa, and the West Indies, where the black and white populations coexist. Sex ratio has also been shown to be higher in the oriental populations based on studies in Korea, Japan, and US births to Chinese or Filipino parents. Sex ratio in twins is slightly higher, although not significantly so. Women who smoke, develop toxaemia during pregnancy, or have been diagnosed with multiple sclerosis, are significantly more likely to have a higher sex ratio, while males taking the nematocide (intestinal worm treatment) dibromochloropropane (DBCP), have a significantly lower ratio. (James, 1987; 2001).

Effect of pesticides on the sex ratio has been investigated in several studies. In Italy, low sex ratio was reported following the Seveso accident resulting in exposure to high concentrations of TCDD (Mocarelli *et al*., 1996). A second more detailed study by this group also reported lower sex ratio with increasing concentration of TCDD in serum of fathers (p=0.008) (Mocarelli *et al*.,2000). The effect on sex ratio was not demonstrated following exposures to PCBs and dibenzofurans (PCDFs) in Yusho, Japan, and Yuchecng, Taiwan. These studies did not examine exposure specific to exposed fathers with unexposed mothers as opposed to unexposed fathers and exposed mothers (Rogan *et al*. 1999; Yashimura *et al*., 2001).

A cross sectional study conducted in an agricultural community in Minnesota, USA, reported that M:F ratio was reduced by occupational exposure of the male parent to fungicide (Garry *et al*., 2002).

The decreased sex ratio in post-war industrialised countries is discussed by Jongbloet and others in relation to conception and loss of pathological conceptuses (Jongbloet *et al*., 2001; 2002). The authors present an argument that the turning point of the sex ratio preceded industrialisation and the introduction of pesticides and hormonal drugs.

At present there is no clear evidence that increases in EDC's in the general environment are related to changes in sex ratios.

2.3.4 Male reproductive health

The potential importance of environmental exposures to chemicals in relation to male reproductive health has been the subject of much investigation and debate. In particular occupational exposure to chemicals, including pesticides, was identified as a possible hazard (Sharpe, 1995), (Tas *et al*., 1996), (De Celis *et al*., 2000), (Week, 2001) (Dallinga *et al*., 2002). Estrogenic activity of environmental pollutants was investigated as a possible contributor to declining male reproductive health (Sharpe and Skakkebaek, 1993), (Jensen *et al*., 1995), (Safe, 1995), (Toppari *et al*., 1996), (Daston *et al*., 1997), (Halweil, 1999). Effects of concern in male reproductive health included decline in sperm quality, increase in testicular cancer, cryptorchidism, hypospadia and testicular dysgenesis syndrome (TDS (Moller *et al*., 1996).

Much of the data shows considerable variation between populations and studies and is the subject of controversy. The following discussion attempts to provide a balanced picture.

2.3.5 Sperm count and fertility

Examination of semen for sperm count, morphology and motility is common practice as an indicator of male fertility. A meta-analysis reviewing literature spanning a 50 year period in the mid 20th century reported a decline in sperm count (Carlsen *et al*., 1992). Human studies published from 1930 to August 1991 were identified and reviewed. This review of 61 publications showed a "significant decline in mean sperm count from 113x10^6/ml in 1940 to 66x10^6/ml in 1990 among men without a history of infertility" (Carlsen *et al*., 1992). Evidence for decreasing sperm quality continued to be published following this review. A study from the United Kingdom showed a 2.1% reduction in sperm concentration per year (Irvine *et al*., 1996). A study of semen samples donated to a sperm bank in Paris between1973 and 1992 showed a 2.6% fall in sperm concentration per year (Auger *et al*., 1995). Analysis of semen samples of the male partner of couples having in vitro fertilisation done for tubal infertility, showed a reduction in sperm counts in men born in France since 1950 (DeMouzon *et al*., 1996).

At the same time, worldwide decline in sperm quality was being disputed (Bromwich *et al*., 1994), (Olsen *et al*., 1995), (Becker and Berhane, 1997), (Swan *et al*., 1997), (Heinz, 1999), (Swan *et al*., 2000). A time series analysis of sperm concentration in semen samples collected between 1977 and 1992 from fertile men aged 20 – 45 years living in the Toulouse area in France did not show a change with time, when adjusted for age of donor (Bujan *et al*., 1996). A study of 22,759 men studied for infertility in North-eastern Spain from 1960 to 1996, reported a 1% yearly decline in sperm count, which was not significant. The authors concluded that their study does not support a decline in sperm quality (Andolz *et al*.,1999). Further support for no decline was emerging from USA (Fisch *et al*., 1996), (Paulsen *et al*., 1996), and Finland (Vierula *et al*., 1996).

The American study also reported differences in sperm concentrations between locations within the USA. New York had the highest sperm concentration (131.5±3.5x10^6/mL) and California the lowest (72.7±3.1x10^6/mL) (Fisch *et al*., 1996). Geographical variation in sperm counts was also reported in other studies from France (Auger and Jouannet, 1997), and Canada (Younglai *et al*., 1998). Inconsistency between studies in selection of study subjects and sample collection procedures, together with geographical variation, contribute to the uncertainty regarding declining sperm quality.

The recent WHO/IPCS Global Assessment concluded that no case had been made for a worldwide decline in sperm count or a direct association with environmental endocrine disruptors.

With increasing debate on changing sperm quality, there was also discussion on suitability of sperm quality as a measure of male reproductive health. It was argued that in order to get a proper indicator of the effect on male reproductive health over time, there was a need to examine trends in reproductive function in the general population (Sherins, 1995). A survey conducted in 1996 of a representative sample of the British population assessed the time to pregnancy for births conceived during 1960-93 (Joffe, 2000). If sperm quality was representative of fertility, a reduction in fertility (measured by time to pregnancy) is expected with time. On the contrary, this survey reported an increase in fertility in recent decades.

The effects of specific chemicals and pesticides on fertility have also been studied. A Norwegian study published in 1997 reported a higher proportion of late-term abortions among farmers wives (Kristensen *et al*., 1997). The same year, Savitz *et al*. (1997) reported on a study in Ontario investigating the relationship between

male pesticide exposure and pregnancy outcome. Increased miscarriage was found to be associated with exposure to thiocarbamates, carbaryl and mixed exposures. Preterm delivery was associated with atrazine and 2,4-D exposure. No correlation was observed between exposure to pesticides and the occurrence of pre-term delivery or small for gestation age children in Denmark (Larsen *et al*., 1998).

In a study conducted among Danish farmers, the time to pregnancy was compared between farmers using pesticides and those not using pesticides (organic farmers). The fecundability ratio (conception rate of the exposed compared to control group) was 1.03 (95% CI 0.75 to 1.40), suggesting no effect of pesticide exposure on sperm quality and quantity, and conception (Larsen *et al*., 1998). The same research group reported no significant difference in semen quality between organic and traditional farmers (Larsen *et al*., 1999).

Another study of 836 couples seeking in-vitro fertilisation treatment also showed that paternal exposure to pesticides was significantly associated with lower numbers of fertilised oocytes in the in-vitro fertilisation (IVF) program (Tielemans *et al*., 1999). Compared to couples with no paternal exposure, those with confirmed paternal exposure had an OR of 0.38 (95% CI 0.19-0.78). The suggested reason was that paternal exposure to pesticides resulted in decreased ability of the sperm to fertilise in-vitro. No particular group of pesticides was identified because most participants were exposed to multiple pesticides. Results from a pilot study published recently suggests an association between exposure to PCBs and p,p'-DDE and sperm quality (sperm concentration, morphology, and motility) (Hauser *et al*., 2002).

The occupational exposure of agricultural workers to pesticides is clearly of concern for reproductive health. Further study is required to pinpoint effects due to particular pesticides and determine exposure levels with detrimental effects. On a world basis there does not appear to be clear evidence of a fertility decline in the recent past.

2.3.6 Hypospadias and cryptorchidism

Hypospadia (the urethra opens on the underside of the penis) and cryptorchidism (undescended testis) are two developmental abnormalities that have been studied in relation to environmental exposures. The incidence of these developmental defects in male children is believed to be increasing, but the role of endocrine disruptors in these abnormalities has not been well established. (Chilvers *et al*., 1984; Group, 1986; Campbell *et al*., 1987, 1991) quoted in Moline *et al*., 2000 and Paulozzi, 1999. A Danish study reports a statistically significant increase in the occurrence of cryptorchidism in sons of female gardeners, suggesting an association with prenatal exposure to pesticides (Weidner *et al*., 1998). There was no association with male gardeners. Hyposapadias were not associated with sons of male or female gardeners.

2.3.7 Stillbirth

The evidence for stillbirth with maternal exposure to pesticides during pregnancy is consistent with risk estimates varying from Odds Ratios (OR) of 1.3 to 5.6 (Vaughan *et al*., 1984), (McDonald *et al*., 1988), (Taha and Gray, 1993), (Pastore *et al*., 1997).

2.3.8 Endometriosis

This is a condition in which endometrial glands and stroma occur in locations outside the uterine lining -for example in the ovary, fallopian tubes and peritoneal cavity. The growth of this tissue is estrogen-stimulated, and occurs in approximately 14% of women (WHO/IPCS, 2002). Exposure to organochlorines has been associated with occurrence of endometriosis, but the evidence in humans is not conclusive. A prospective case-control study in Belgium, where dioxin pollution and incidence of endometriosis are high, did not show a statistically significant association between exposure to dioxin – like compounds and occurrence or severity of endometriosis (Pauwels *et al*., 2001). A previous study conducted in Belgium had reported high frequency of endometriosis implicating pollution and dioxins (Koninckx *et al*., 1994). A study in Israel of 79 women being investigated for infertility, reported detection of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in 18% of 44 cases as opposed to 3% of 35 controls (p=0.04) (Mayani *et al*., 1997). Concentration of dioxin in blood of those with endometriosis ranged from 0.7 to 1.2 ppt while the only control had a blood concentration of 0.4 ppt. A correlation was not observed between dioxin concentration in blood and severity of endometriosis. A population-based historical cohort study was conducted in Seveso, Italy, 20 years after the factory explosion which resulted in "highest TCDD levels known in a human population" (Eskenazi *et al*., 2002). A total of 19 women with endometriosis were identified together with 277 non-diseased women. The median serum TCDD levels for cases and controls were 61.0 ppt and 49.0 ppt, respectively. Although a doubling of risk was observed in this study, this was not statistically significant. The small number of cases in the study may have limited the power to detect a significant effect. A study conducted in USA examined concentrations of TCDD and 21 other polychlorinated dibenzo-p-dioxins in 15 cases and geographically matched controls. This study, using a small sample, was not able to demonstrate an association between exposure to these chemicals and occurrence of endometriosis ((Boyd *et al*., 1995) quoted in (Eskenazi *et al*., 2002) and (Birnbaum and Cummings, 2002)). A Canadian study using a much larger sample (86 cases and 70 controls) was also not able to demonstrate a difference in the concentration of 14 common PCBs

in cases and controls. No significant linear trends were observed for endometriosis with increasing concentration of organochlorine (Lebel *et al*., 1998).

Whether endometriosis is related to exposure to organochlorines or dioxins remains to be clarified.

2.4 Cancer

Endocrine disrupting compounds in the environment, including natural and synthetic hormones, have a possible role in the growth of hormonally dependent cancers such as breast, testicular and prostate cancers. This section provides an overview of epidemiological studies investigating associations of these cancers with endocrine disruptors.

2.4.1 Breast cancer

Several risk factors for breast cancer have been identified and established, the main components being lifetime endogenous estrogen exposure and genetic predisposition. However, these risk factors explain only about half of breast cancer risk which has generated the hypothesis that hormonally active substances in the environment that affect breast cell proliferation, may affect the risk of breast cancer (Snedeker, 2001). Estrogens consumed in oral contraceptives and in hormone replacement therapy are overwhelmingly the main exogenous sources of exposure.

Dichlorodiphenyltrichloroethane (DDT), an organochlorine pesticide classified as a known human carcinogen, and the most prevalent of its breakdown products, dichlorodiphenyldichloroethylene (DDE), are two substances that have shown to have some endocrine disrupting effects in experimental animals (IARC, 1997), (Scribner and Mottet, 1981), (Edmunds *et al*., 2000), (Vom Saal *et al*., 1995). A review undertaken by Snedeker of the epidemiological studies on DDT and DDE and breast cancer risk identifies that of the 27 studies reviewed, only six studies showed an association between DDE and breast cancer risk [Table 1, Snedeker, 2001]. Three of the six studies showing an association had sample sizes of less than 25, with a fourth having less than 50 cases. These studies did not adequately control for confounding factors. The WHO/IPCS (2002) review did not draw any conclusion from present data, the majority of studies showing no association between DDT, DDE and breast cancer.

Dieldrin is another organochlorine pesticide. This compound was found to be associated with increased breast cancer risk in a well conducted prospective nested case control study involving women enrolled in the Copenhagen City Heart Study (Hoyer *et al*., 1998). Blood samples collected from patients approximately 20 years before being diagnosed with breast cancer, were subsequently tested for a range of organochlorine compounds. Dieldrin was found to be the only organochlorine compound associated with breast cancer (Hoyer *et al*., 1998). There was a doubling of risk among those in the highest quartile of exposure compared to those in the lowest quartile of exposure (OR = 2.05, 95% CI 1.17 to 3.57). Following up the same group of women showed that women with the highest exposure based on bloods collected approximately 20 years prior to developing breast cancer, had a significantly higher risk of dying from cancer compared to those in the lowest quartile of exposure (RR=2.78, 95% CI 1.38 TO 5.59).

This positive association between dieldrin and breast cancer risk was not demonstrated in a study conducted in USA in which dieldrin was detected in 56.2% of breast cancer cases and 61.8% controls (Dorgan *et al*., 1999). Dieldrin was not detected in adipose tissue samples in 5 breast cancer cases and 5 controls in another USA study sample (Djordjevic *et al*., 1994). Without further investigation, there is insufficient data to draw any conclusions on the association between dieldrin and breast cancer risk.

Dieldrin, for which estrogenic activity has not been well established (Snedeker, 2001), has also been investigated for its role in endometrial cancer, as part of an investigation of estrogenic organochlorine pesticides (Sturgeon *et al*., 1998). In this case control study of 90 cases and 90 controls, dieldrin was the only organochlorine for which the risk of endometrial cancer was increased, but this increase was not statistically significant. This association needs to be further investigated prior to drawing definitive conclusions

A recent publication examined the association between serum TCDD levels and breast cancer risk in the population living in Seveso during the accident in 1976 (Warner *et al*., 2002). Of the 981 women participating, 15 (1.5%) women had been diagnosed with breast cancer. Serum TCDD for these cases ranged from 13 to 1,960 pg/ml. Serum TCDD concentration was found to be significantly correlated to breast cancer incidence in this population.

2.4.2 Testicular and prostate cancer

There is increasing literature showing a rise in the incidence of testicular cancer (Nethersell *et al*., 1984), (Osterlind, 1986), (Adami *et al*., 1986), (Spitz *et al*., 1986), (Brown *et al*., 1986), (Boyle *et al*., 1987), (Stone *et al*., 1991), (Wilkinson *et al*., 1992). This increase in the incidence is believed to be too large to be attributed to improved diagnosis and surveillance, given that the detection is still by physical examination for a testicular mass (Moline *et al*., 2000). Testicular cancer has been linked to pre and post-natal exposures to DES and other estrogens and antiandrogens (Scottenfeld *et al*., 1980), (Arai *et al*., 1983), (Depue *et al*., 1983), (Moss *et al*., 1986), (Osterlind, 1986), (Henderson *et al*., 1988), (Prener *et al*., 1992), (Coco and

Benichou, 1998). Workplace exposures to hydrocarbons and polyvinyl chlorides, and the use of pesticides have also been associated with testicular cancer (Foley *et al*., 1995), (Hardell *et al*., 1997), (Mills, 1998). Testicular cancer is also associated with poorer semen quality and sub-fertility (Moller and Skakkebaek, 1999), (Moline *et al*., 2000). These reports support the need for further investigation of the relationship between exogenous hormone and endocrine disrupting compound exposure and testicular cancers.

Prostate cancer, while a significant cause of male mortality and a cancer responsive to androgens, has no identified association with EDCs in the environment despite many studies exploring possible relationships (WHO/IPCS, 2002).

While a causal link has not been established between environmental EDCs and cancer in any organ (WHO/IPCS, 2002), the potential for EDC action at critical developmental stages of life (for example pre-natally and during puberty) which predisposes to later cancer growth, requires further consideration.

2.5 Neural Development

Development of the nervous system, including learning and behaviour, is influenced by complex interaction between multiple factors such as genetic composition, and social, physical, and chemical environment. Adverse effects of exposure to toxic chemicals in the environment on the developing nervous system are reported in the scientific literature, though the complexity of response and difficulties of monitoring of exposure lead to problems with interpretation. Schittler *et al*., (2000) in their report titled 'In Harm's Way: Toxic Threat to Child Development', review and analyse relevant literature on the contribution of toxic chemicals often found in the environment to neurodevelopmental disorders. This report, although not limited to endocrine disruptors, identifies the importance of scrutinising exposure to toxic chemicals because prevention is possible with lifelong benefits through child development. The overview on neural development and toxicity given here substantially draws from this report.

There are a wide variety of neuro-developmental disorders that can occur. These include academic disorders (i.e. learning disorders such as in mathematics, reading or writing as well as attention deficit disorders), pervasive developmental disorders (i.e. social impairment, restricted behaviour and interests, with or without language deficits), and behavioural disorders (i.e. disruptive or aggressive behaviour).

Development of the neural system involves an organised process of cell proliferation, migration, differentiation, synapse formation, followed by consolidation of neural circuits. The endocrine disruptors may affect neurodevelopment by altering the function or metabolism of hormones, either intra- uterine or during early childhood, with subsequent interferences at one or more of the development stages. The thyroid hormones (thyroxine and triiodothyronine) play an important role in neural proliferation and differentiation. Even transient or minor alterations in thyroxine levels or interference in their action can lead to impaired intelligence. Other hormones that are important in brain development are growth hormone, adrenal steroids and sex hormones. In addition to hormones, neurotransmitters, neurotrophins and growth factors regulate neural development and function. Neurotransmitters and their receptor location and density develop during early (intra-uterine and neonatal) life. Non-endocrine disrupting compounds which are neurotoxic can also adversely affect neurological development (Spencer and Schaumburg, 2000).

There are several chemicals that have been identified as having adverse effects on neural development. Whether this is through endocrine disruption or neurotoxicity is not clear. Dioxins and polychlorinated biphenyls (PCBs) are particularly implicated. PCBs were widely used in lubricants, coatings, and insulation material in electrical transformers (Schettler *et al*., 2000). Production of PCBs is now banned in the USA and several other countries, however they do not breakdown in the environment and still persist in landfills and hazardous waste sites, leaching into streams. Exposure of humans to dioxins and PCBs is largely from dietary sources (SCOOP, 2000). Studies in monkeys have shown that exposure to dioxins at concentrations similar to estimated current human exposure results in adverse health effects such as increased rate of abortions, and impaired learning and altered social behaviour in offspring (Schettler *et al*., 2000). Dioxin and PCBs concentrate in fat and occur in breast milk as a result of fat mobilisation in lactation. Breast fed children are therefore potentially highly exposed, with exposure in children breast fed for more than a year far exceeding advised limits for chronic exposure (WHO/IPCS, 2002). The transmission of these chemicals from mother to foetus during pregnancy and to infant during breast feeding means high levels of exposure at a time that the nervous system is at a stage of rapid development and is therefore highly vulnerable to adverse effects. The mechanism of action of these compounds is not well understood. These chemicals bind to the aryl hydrocarbon receptor (AhR), with the resulting complex then binding to DNA, impacting on the production of various growth factors, hormones and hormone receptors. Effects are also mediated through interference with thyroid hormone. The effects may present as low IQ, learning disability, hyperactivity, and decreased thyroid hormone in the child (WHO/IPCS,2002).

A recent review of published literature examining exposure to PCBs and neurological development in children suggest an adverse association (Ribas-Fito *et al*., 2001). A total of 29 publications sourced from Medline, conference proceedings, and citations in identified reports were reviewed. These publications dated from 1979 to 2000, and reported on seven independent populations.

Three were from the USA (North Caroline, Michigan, and Oswego), with the remaining four being from Japan, Taiwan, The Netherlands, and Germany. These studies were also included in another review of hormonally active agents in the environment (NRC, 2000). Effects on newborns (<1 month of age) were examined separately to mental, motor, and behavioural effects on children aged over 3 months to 11 years. All studies were prospective, they varied in selection of study samples with three of the seven studies (North Carolina, The Netherlands and Germany) being conducted with children from the general community. The Japanese and Taiwan studies selected children born to women highly exposed to PCBs through accidental contamination of rice cooking oil. The Michigan and Oswego studies chose children born to women who ate contaminated fish. Measurement of exposure also varied making it difficult to consolidate the findings. Five of the seven studies measured PCBs in biological samples (cord blood and maternal serum for pre-natal exposure), milk, or child's serum (for post- natal exposure)). The remaining two studies (the Japanese and Taiwan studies, both of children born to women highly exposed to PCBs) had serum levels analysed only in a sub-set of the study sample. The Japanese study had the highest mean PCB concentration detected in a biological sample, being 6.0 ng/ml in serum. The mean concentration in serum in the Taiwan study was 0.99 ng/ml. The four studies which examined cord blood had mean PCB levels of 0.38 (The Netherlands), 0.52 (Oswego), 0.55 (Germany), and 2.5 ng/ml (Michigan) detected. The three studies from the USA and the study from The Netherlands examined neonatal effects. In two of the studies (North Carolina and The Netherlands), PCBs in breast milk were associated with altered neural development (hypotonicity and hyporeflexia – North Caroline, and reduced neurological optimality – The Netherlands). In both studies, PCB levels in cord blood were not associated with adverse neural effects.

The Taiwan study conducted on children born to highly exposed women showed negative effects of prenatal exposure on mental development, with the effect being significant in several age groups, especially less than 7 years of age. The Michigan study of children born to women eating contaminated fish, reported significant mental impairment in the early stages(6-7 months) as well as at 11 years of age. The Netherlands study of the general community reported a significant negative effect at 4 years of age. The North Carolina study on the general community showed a non-significant reduction at 1 year, but no effects at 3, 4 , or 5 years of age. The German study did not report a negative effect on mental development.

Psychomotor development was significantly reduced at the early ages (3 months to 1 year) with prenatal exposure to PCBs in the Taiwan, The Netherlands, and North Carolina studies. The Taiwan study also showed significantly negative effects on motor development at 2 years of age.

The Taiwan and Michigan studies examined behavioural development, with prenatal exposure. Negative effect of exposure on behavioural scales was reported in the Taiwan study while the Michigan study did not demonstrate a negative association. The Japanese study, while not using standardised tests, provided supportive data of decreased neurodevelopment in 127 exposed children.

Four studies (North Carolina, Michigan, The Netherlands, and Germany) examined the effects of post-natal exposure, based mostly on PCB levels in breast milk. The evidence for an adverse relationship between PCB levels in breast milk and neurodevelopment was not strong (WHO/IPCS,2002).

The above review suggests that exposure to PCBs, particularly with prenatal exposure, is associated with long-term or permanent adverse effects on neurological development in children including psychomotor development delays, cognitive impairment and behaviour abnormalities. The evidence for adverse effects from postnatal exposure through breast milk is not conclusive. This association between exposure to endocrine disruptors and adverse effects on neurodevelopment needs to be further evaluated with studies using large-scale longitudinal studies using a standardised study design and measures of exposure and outcome. In the studies described the organochlorine intake was through contaminated food, with no evidence of intake from drinking water.

Pesticides such as the organophosphates (e.g. diazinon, chlorpyrifos and organochlorines (e.g. DDT) have also been suggested as having the potential to adversely affect neurological development in humans. While these effects are unlikely to be related to endocrine dysfunction, it is relevant to consider them in a context of environmental contaminants. Exposure of mice to DDT at a critical development stage has been shown to result in altered brain structure and function. Studies examining effects on neural development in humans are few. A study conducted in Mexico comparing children aged 4 –5 years living in an area where pesticides were widely used, with a similar group of children not exposed to pesticides has shown significant differences in neurological development (Guillette *et al*., 1998). The children in the exposed area were found to have reduced stamina, gross and fine eye-hand coordination, 30-minute memory, and ability to draw a person. The two groups did not differ in physical growth. In a previous study of the exposed community, concentrations of a variety of pesticides in cord blood and in maternal milk (measured 1-month post partum), including lindane, heptachlor, benzene hexachloride, aldrin, endrin and ΣDDE, exceeded WHO / FAO established limits. A cohort study of 50 adult females living in Mexico City measured DDT metabolites in maternal milk, and estimated DDT intake for infants. This study estimated that 6% of breast fed babies had DDT daily intakes exceeding the WHO / FAO recommendation of 0.005 mg/kg, putting them at risk of adverse health effects

(Torres-Arreola *et al*., 1999). Prenatal exposure to DDT concentrations as low as 0.9 ppm has been shown to result in diminished reflexes and reduced muscle tone (Rogan *et al*., 1986).

The mechanism of action of organophosphates as pesticides is by inhibition of acetylcholinesterase, which is responsible for breaking down the neurotransmitter acetylcholine. Inhibiting breakdown of this neurotransmitter during neural development interferes with cell replication and differentiation, as well as reducing neurite outgrowths from neurons. Organophosphates such as chlorpyrifos decrease DNA synthesis, affecting the number of cells in the developing brain(Schettler *et al.* 2000). The organochlorines interfere with nerve cell function and alter the excitability of nerve cells. They also alter neuroreceptor levels in selected sites of the brain, thus influencing behaviour. Direct effects of pesticides acting through the endocrine system are not well established (Cocco, 2002).

The potential for human intake of insecticides is greatest through occupational exposure, which is the risk most thoroughly established by epidemiological studies. As a consequence of the high volumes of these chemicals used in irrigation agriculture, the possibility also exists for the contamination of waterways, with potential for adverse effects on water consumers and the natural ecology. These adverse effects may be due to toxicity unrelated to endocrine disruption.

2.6 Immune System

Based on studies in animals, the US EPA assessed dioxin to be harmful to the immune system, even at low doses (USEPA, 1994). Temporary exposure at a critical stage in the development of the immune system has been identified as having an adverse impact on the immune system. Dioxin can also stimulate the immune system resulting in hypersensitivity, allergies, and autoimmune conditions (Montague, 1994).

A retrospective cohort study compared 158 chemical workers exposed to dioxin during a chemical reactor spill in Germany in 1953, with 161 unexposed workers (Zober *et al*., 1994). This study reported an 18% increase in total illness episodes among the exposed group (p=0.002). Total respiratory disease increased by 35%, while there was more than a two-fold increase in episodes of infectious and parasitic disease, per 100 person years. The authors state that the increase in upper respiratory tract infections and parasitic infections (among other conditions) is "consistent with reduced host resistance… " (Zober *et al*., 1994). However, the authors alert the readers to the fact that the results were derived from insurance data, and that the exposed group may have accessed more health services due to greater awareness and health concern following exposure.

There is some epidemiological evidence supporting the compromise of the immune system with exposure to pesticides, followed by recovery. A study conducted in Aberdeen, North Carolina, investigated the effects of living near a pesticide dump-site containing organochlorine pesticides, volatile organic compounds and metals, on the immune system (Vine *et al*., 2000). The first phase of the study, a telephone survey of 1642 adults living in Aberdeen and several nearby communities, identified higher likelihood of reporting of herpes zoster among those aged 18-40 years, with a RR of 2.2 (95% CI = 1.0 –4.0) (Arndt *et al*., 1999). There was also an increased risk reported for Aberdeen residents before 1985 compared with those living in Aberdeen after 1985. These results suggested a possible association between exposure to the pesticides from the dump-site and a compromised immune system. The second phase, conducted from 1994-1996, examined levels of immune markers in these communities (Vine *et al*., 2000), (Vine *et al*., 2001). The only pesticide detected in the blood of 302 study participants was DDE. The authors conclude that "although some statistically significant differences in immune markers were noted with respect to location of residence near the dump sites, the magnitude of the effects are of uncertain clinical significance." The authors offer an explanation for immune marker levels in the later study being within normal range in most cases, by the recovery of the immune system.

There is also supporting evidence for immunocompromise with perinatal exposure to organochlorines in the environment. A higher incidence of respiratory symptoms were reported in the first six months after birth of children born to women who had ingested PCB contaminated rice oil in Taiwan (Rogan *et al*., 1988). Children of exposed mothers also had a higher incidence of middle ear infections (Chao *et al*., 1997). Children born to mothers with high levels of organochlorine compounds in breast milk were found to be associated with more acute otitis media in the first years of life (Dewailly *et al*., 2000). A Dutch research group reported associations of prenatal exposure to PCBs and dioxins with changes in T-cell lymphocyte populations in infants (Weisglas-Kuperus *et al*., 1995). An effect on health status could not be demonstrated.

2.7 Limitations in epidemiological information and future needs

Exposure to toxic chemicals, including those with endocrine activity, can result in adverse structural and functional changes in reproductive, endocrine, metabolic, immunological, and neurological systems, with long-term impact on human health. Unlike genetic influences on health, harmful compounds and other substances occurring in the environment can be minimised or removed. For this reason, with emerging literature on potential health hazards associated with environmental exposures to toxic chemicals and pharmaceuticals, there is an increased expectation of regulatory authorities to

set safety standards and monitor the occurrence of these substances in the environment. Setting of safety standards for food and drinking water is in place in Australia and developed countries generally. Implementation of safety standards, for EDCs that have demonstrable adverse effects on the population, can be expected to reduce health risks associated with exposure to these potentially harmful substances. Selection of which EDCs should be targeted for regulation in Australia, and at what concentrations, will require considerable expert scrutiny. The regulatory and advisory criteria in Australia and elsewhere are subject to ongoing revision, including the rolling revision of the drinking water guidelines issued by the National Health and Medical Research Council of Australia.

The setting of safe guideline values for pharmaceutical and chemical exposure requires specific risk estimates for toxicity. The evidence of toxicity for many of the anthropogenic compounds in the environment is based on animal studies, which are then used to extrapolate risks to humans. There are problems associated with this practice, which are accommodated through the setting of safety factors. Humans and experimental animals such as rats and mice are different in body mass and physiology, which may result in different responses to toxins. Also, humans are rarely, if at all, exposed to single compounds in the environment. It is more likely that exposure is to a complex mixture, and the effects of one compound may synergistically or antagonistically affect the end result of exposure to another compound. Experimental evidence from laboratory testing is normally based on testing of single compounds, not mixtures. As a result of these and other issues, the safety/risk factors that are used in the calculation of safe human intakes are therefore conservative and may generally overestimate risk. Thus the safe intake levels so determined are likely to be well below the amount that would cause any detectable injury. It is however historically the case that revisions of safe exposure levels, including the recent revision of the acceptable arsenic concentration in drinking water in the USA, have been to reduce the acceptable exposure.

The ideal method for determining safe levels of intake of toxic or endocrine disrupting compounds from the environment is through quantitative human epidemiological data. This is enormously difficult, due to inconsistencies in study design and lack of accurate data, including exposure and outcome measurement. It is recognised that measurement of exposure alone has huge limitations in environmental epidemiology. Exposure is often estimated using existing (usually incomplete) data from environmental monitoring, which does not allow for individual variations in exposure. Questionnaires are often used in an attempt to quantify exposure at an individual level but are faced with issues of recalling contact or exposure over several years. Advances in identification of biological markers of exposure now enable 'snapshots' of exposure but may not provide a true estimate of past exposure. The conduct of prospective studies of exposed populations, using accurate and reliable exposure measures, will help overcome some of these issues. "A comprehensive approach to exposure assessment is also important" particularly when exposure to complex mixtures are likely (Moline *et al*., 2000). These methods need to be developed and standardised.

The US National Research Council, commissioned by the US Environmental Protection Agency (EPA), US Centre for Disease Control (CDC), and the US Congress, conducted an independent review of existing literature on hormonally active agents in the environment, and made several recommendations (NRC, 2000). In view of the evidence from animal studies supporting reproductive and developmental abnormalities, and the inadequacy of human data, longitudinal studies were recommended in human populations exposed to endocrine disruptors, to assess developmental milestones from conception through adulthood. The recommendation includes the use of standardised criteria to assess various aspects of development. Studies on the immunological effects of endocrine disruptors were also recommended, particularly for individuals exposed prenatally (i.e. whose mothers were exposed during pregnancy). Further investigation of the association between exposure to endocrine disruptors and development of various cancers was recommended, with the conduct of appropriately designed case-control and retrospective cohort studies. Measurement of internal dose (concentration of the substance in blood or adipose tissue) while conducting these studies is recommended. Further long-term studies of populations exposed to endocrine disruptors were recommended to assess the effect of age and structure of population as well as the effect of altering population size. Because of the long-term nature of these investigations, and delays in establishing criteria for compounds of interest, exposure assessment methods and reference populations, it is likely to be a decade or more before human epidemiology has a major role in setting safe guideline levels of human exposure.

3.0 The Evidence for Effects on Wildlife

There is available evidence that some compounds disrupt the reproduction and development of wildlife. A spill in 1980 of the pesticide dicofol contaminated with DDT metabolites was associated with severe developmental effects on alligators and turtles in Lake Apopka in Florida, particularly on production and hatching, juvenile survival and normal development of reproductive systems (Guillette 1995; USEPA 1997). Exposure of alligator eggs to estrogen at a temperature that would normally produce males has been shown to produce females. Reptiles as a taxonomic group have received little attention from an ecotoxicological perspective, however it is clear that there are some developmental processes in reptiles that are susceptible to endocrine disruptors. It is unclear however,

how widespread the problem is (WHO/IPCS, 2002). It was recently reported that there is insufficient evidence to implicate EDCs as causative agents in the decline of amphibian populations and species, nor sufficient evidence to link environmental contamination to frog malformations (WHO/IPCS, 2002). However in a more recent article in Nature there was a report that exposure of frogs (*Rana pipiens*) to the herbicide atrazine has resulted in gonadal abnormalities in male frogs (Anon, 2002).

Reduced fertility in some fish species has been observed (Bortone and Davis, 1994). The effects range from the masculinisation of female fish (Denton *et al.,* 1985), the feminisation of male fish (Gimeno *et al.,* 1998a; Gimeno *et al.,* 1998b; Batty and Lim, 1999) and problems with spawning time (Kramer *et al.,* 1998). Alkyl phenols (e.g. nonylphenol and octylphenol), which are breakdown products of alkylphenol polyethoxylates used as industrial surfactants and bases for household products, have been linked with estrogenic effects in fish (Jobling *et al*. 1996). Delayed ovulation in some female rodents has been linked to a disruption of the pituitary gland function due to exposure to steroid compounds (Goldman *et al.,* 1998). The problems with eggshell thinning in some of the top avian predators in North America received much public debate a few decades ago.

Invertebrates account for roughly 95% of the known species of animals on our planet, yet our knowledge of their endocrinology is limited. The best understood invertebrate endocrine system is that of the insects, owing to their economic, ecological and agricultural importance (de Fur *et al*, 1999). Identification of the hormones controlling insect growth and metabolism has permitted the synthesis of compounds that mimic, block, or otherwise interact with the target insect. Examples are pyriproxyfen (juvenile hormone agonist) and tebufenozide (an ecdysteroid which controls moulting).

Among the aquatic invertebrates one of the best-studied groups is the decapod crustacean due to their commercial and recreational importance. Moulting and reproduction is highly coordinated in some crustaceans, accordingly ecdysteroids can have a significant impact on their reproductive success by virtue of their role in the moulting process (de Fur *et al*, 1999).

Tributyltin (TBT), which was formerly widely used as an anti-fouling paint for ship hulls, is well known for causing imposex (development of both female and male characteristics in reproductive tissues) in female gastropods (Sharara *et al.,* 1998).

Among agricultural chemicals in current use are atrazine and related herbicides such as simazine; endosulfan, methoxychlor and fenithrothion (pesticides) and vinclozolin and ketoconazole (fungicides). Simazine has been demonstrated to impair reproduction and moult frequency in the water flea *Daphnia Pulex* at 4-20mg/L (Fitzmayer *et al*, 1982). Atrazine is a herbicide with neuroendocrine effects on reproduction and mammary tumour growth in rodents and reproduction in fish (WHO/IPCS, 2002).

Xenobiotics can act as endocrine disruptors without affecting hormone binding, by modulating endogenous hormone levels. Fenitrothion, Vinclozolin and Ketoconazole are fungicides with anti-androgen effects on mammals, by inhibiting key enzymes of androgen synthesis (WHO/IPCS, 2002). Methoxychlor is an organochlorine pesticide used against a range of pests and methoxychlor and/or its metabolite HDPE is believed to have estrogenic effects in mice (USEPA, 2001). In this case contamination of drinking water is regarded as a potential health risk and the USEPA advises that water containing more than 0.05 mg/L of the parent compound and metabolite should not be consumed by children (or 0.2 mg/L by adults) (USEPA, 2001).

A more extended discussion of the impacts of pesticides on the environment is to be found in the recent comprehensive report 'Pesticide use in Australia' by the Australian Academy of Technological Sciences (Radcliffe, 2002). The extent of pesticide use in Australia is not precisely known, though estimates for the amount of active compound applied annually for each of the most-used chemicals ranges from 500 tonnes/annum to 15,000 tonnes/annum, with the majority of pesticides with possible EDC activity used at 1000 to 3000 tonnes/annum

The basic mammalian toxicology of these compounds has been thoroughly investigated in order for them to be registered for use in Australia. As a consequence their toxic effects, and the dose levels at which these occur, are known for test animal species (National Regulatory Authority, 2003). Specific endocrine effects may not be as well characterised, particularly in wildlife exposures. The toxicological assessments may need extension into endocrine and related impacts to clarify EDC-like actions of pesticides.

4.0 EDCs in Surface and Discharge Waters

4.1 Surface Waters

Monitoring of surface waters in Australia has so far not specifically addressed endocrine disruptors. There are many associated variables for any chemical discharged to surface waters including frequency of discharge, concentrations in the discharge itself and in the receiving environment and toxicological effects. During the last few decades, the impact of chemical pollution has focused on well-known "priority" pollutants, including pesticides, especially those displaying persistence in the environment. Monitoring of streams and rivers in intensively used irrigated agricultural regions in Australia has widely

detected pesticides (Radcliffe, 2002) This is likely to be only one piece of a larger puzzle (Daughton and Ternes, 1999). Other surface water contaminants may include pharmaceutical, veterinary and illicit drugs, ingredients in cosmetics, food supplements and other personal care products together with their respective metabolites and transformation products. There is no data available explicitly for EDCs in Australian freshwater, apart from studies of wastewater carried out on an experimental basis

The United States Geological Society (USGS) recently surveyed 139 streams across 30 states in the USA. This survey was biased towards streams susceptible to contamination (ie. downstream of intense urbanisation and livestock production). Organic wastewater contaminants (OWC) were detected during this study in 80% of the streams sampled (Kolpin *et al*, 2002). Of the steroids and hormones in this survey the percentage detection of the steroids and hormones in these waters ranged from 1.4% (equilin) to 85.7% (coprostanol). Other frequently detected contaminants in order of frequency of detection included non-prescription drugs, insect repellents, detergent metabolites, disinfectants, plasticisers, fire retardants, antibiotics, insecticides, PAHs, reproductive hormones, prescription drugs, antioxidants, fragrances and solvents (Kolpin *et al*, 2002). While there is no reason to assume that this type of contamination is occurring in Australia, the study reported by the USGS demonstrates the type of contaminants that may occur in surface waters, under certain conditions. Recently there has been recognition that components of personal care products (eg. triclosan) and veterinary pharmaceuticals such as antibiotics may be present in water and some of these have known EDC properties (Klaassen, 2001).

4.2 Estrogens in treated sewage water

Water suppliers use a variety of treatment processes to remove contaminants from drinking water. Individual processes may be arranged as a series in a sequence appropriate for the contaminants found in the source water (USEPA 2001). Commonly used processes include flocculation, sedimentation, filtration and disinfection of surface water. The USEPA recently recommended granular activated carbon to remove DDT, PCBs, endosulfan, methoxychlor, diethylphthalate (DEP), diethylhexyl phthalate (DEHP) and Bisphenol A from drinking water (USEPA, 2001). There are wide differences in treatment plants capability to remove EDCs.

Natural hormones including estrogens can be released into the environment via effluent and from sources such as animal feed lots. Studies in the UK have shown that the hormones 17βestradiol, 17αethinyl estradiol and estrone although excreted in an inactive conjugate, can be degraded in sewage treatment plants to release the active steroid hormone (Desbrow *et al*, 1998). In Australia concentrations from <5ng/L (the limit of reporting) to 19ng/L of 17βestradiol, and up to 78ng/L of estrone have been detected in secondary treated effluent (Table 4.2.1). The pharmaceutical 17αethinylestradiol, the active ingredient in birth control pills was <5ng/L in all samples analysed. At the Landsborough Water Reclamation Plant in Queensland, the estrogen hormones were not detectable at a limit of reporting of 5ng/L after tertiary treatment (sand filtration, ozonation and UV disinfection) (Chapman 2002a), however these hormones are known to be biologically active at <1ng/L in laboratory test systems (Koerner *et al*, 1999). Concentrations up to 11ng/L of 17βestradiol, 9ng/L of 17αethinyl estradiol and 17ng/L of estrone have been reported for treated effluent from the UK (Table 4.2.2). Estrogenic effects in wild fish in the United Kingdom have been shown to be widespread in UK rivers (Jobling *et al*, 1998). There has been one report of this type in Australia (Batty and Lim, 1999).

Table 4.2.1 Concentrations (ug/L) of estrogens from wastewater treatment/reclamation plants in Queensland, Australia in 2000-2002[1]

Sampling date	Location	Treatment stage	17βestradiol	ethinylestradiol	estrone
17 Apr 2000	Landsborough WRP, Qld	influent	0.020	<0.005	<0.005
19 Jul 2000	Landsborough WRP, Qld	influent	0.018	<0.005	0.078
04 Sept 2002	Landsborough WRP, Qld	influent	0.320	<0.005	0.011
04 Sept 2002	Landsborough WRP, Qld	bioreactor	0.006	<0.005	<0.005
04 Sept 2002	Landsborough WRP, Qld	N/DN[2]	0.009	<0.005	<0.005
17 Apr 2000	Landsborough WRP, Qld	clarifier	<0.005	<0.005	<0.005
19 Jul 2000	Landsborough WRP, Qld	clarifier	<0.005	<0.005	<0.005
28 Jul 2002	Landsborough WRP, Qld	clarifier	0.019	<0.005	0.046
04 Sept 2002	Landsborough WRP, Qld	clarifier	0.012	<0.005	<0.005
04 Sept 2002	Landsborough WRP, Qld	sandfilter	0.009	<0.005	<0.005
04 Sept 2002	Landsborough WRP, Qld	ozone	0.007	<0.005	<0.005
17 Apr 2000	Landsborough WRP, Qld	UV	<0.005	<0.005	<0.005
19 Jul 2000	Landsborough WRP, Qld	UV	<0.005	<0.005	<0.005
04 Sept 2002	Landsborough WRP, Qld	UV	<0.005	<0.005	<0.005
22 Jul 2002	Cooroy STP, Qld	secondary effluent	0.011	<0.005	0.046
22 Jul 2002	Cooroy STP, Qld	chlorinated effluent	0.011	<0.005	0.013
22 Jul 2002	Oxley STP, Qld	secondary effluent	<0.005	<0.005	0.054
28 Jul 2002	Maleny STP, Qld	secondary effluent	<0.005	<0.005	<0.005
27 May 2002	Carole Park STP, Qld	secondary effluent	<0.005	<0.005	0.046
17 Apr 2000	Kawana STP, Qld	secondary	<0.005	<0.005	<0.005

[1] Chapman, Heather (unpublished data)

[2] N/DN Nitrification/denifrification unit

Table 4.2.2 Concentrations of estrogens in sewage effluent in Europe and Nth America (ng/L)

Treatment type	County of origin	17βestradiol	17α ethinyl-estradiol	estrone
activated sludge system[1]	Netherlands	0.09	<lor	4.5
activated sludge system[1]	Netherlands	<lor	<lor	0.4
Clarification and aeration[1]	Germany	<lor	1	9
clarification and aeration[1]	Canada	6	9	3
treated effluent[1]	Britain	11	0.73	17.3

[1] Desbrow *et al* (1998 Table 1) or limit of reporting

5.0 Methodologies for Detection and Monitoring of EDCs

5.1 Chemical analytical techniques

Established analytical methods are available for many of the compounds implicated as endocrine disrupting compounds (EDCs). Most developed countries have established regulatory authorities and requirements for chemical analysis and methods for testing food or the environment (WHO/IPCS, 2002). The methods are well developed for chemicals such as pesticides, metals, industrial chemicals and PCBs. However for some of the other EDCs such as hormones, drugs, and personal care products the analytical methods are less well developed. Some of these substances (eg. pharmaceuticals) are designed to be biologically active at trace concentrations (parts per trillion) and may exert an effect in the environment below the routine limit of reporting using standardized analytical methods (Geisy *et al*., 2002). There are potentially significant classes of compounds that are not studied in detail due to a lack of suitable instrumental techniques or analytical standards. Chemical analyses can also be costly and time consuming. Characteristic of these are the dioxins which include the polychlorinated dibenzodioxins and the polychlorinated dibenzofurans. These are relatively water insoluble but all of which have biological activity at very low environmental concentration.

Many potential endocrine disruptors exist as mixtures. Individual compounds within mixtures may vary greatly in estrogenic potency and may interact with each other in an unpredictable manner. While this may not be such a problem for a simple matrix containing only a few well-defined contaminants, in the majority of cases there will be too many chemical components to easily identify those that are hormonally active. Mixtures of various organic wastewater contaminants (OWC) were commonly found during the USGS survey of streams in the USA. Seventy five percent of the streams sampled having more than one of the OWCs identified (Kolpin *et al*, 2002).

5.2 Screening and testing programs

The USEPA Science Policy Council's interim position on endocrine-disrupting compounds was presented in a report published by the USEPA in 1997 (USEPA 1997). EPA chartered a scientific advisory committee under the Federal Advisory Committee to provide advice and recommendations on a strategy for determining whether pesticides and other substances may modify the normal functioning of human and wildlife endocrine, or hormonal systems and cause developmental, behavioural, and reproductive problems (USEPA, 2000). The advisory committee, Endocrine Disruptor Screening and Testing Committee (EDSTAC) address effects on both wildlife and humans. Following this recommendation, EPA announced the establishment of the Endocrine Disruptor Screening Program (EDSP) in August 1998.

The USEPA is using a tiered approach for (1) identifying substances which have the potential to interfere with the endocrine system and (2) to confirm the potential and characterize the effects (USEPA, 2000). The EDSP scope includes

- Effects on humans and wildlife
- Effects on estrogen, androgen and thyroid (EAT) hormone related processes
- Evaluation of chemical substances

The Tier 1 screening includes a utero-trophic screen, a Hersberger screen (male rodent-based tests for androgenic activity), a rodent pubertal female screen, a rodent pubertal male screen, estrogen and androgen receptor reporter gene screens, a fish reproduction screen and a frog metamorphosis screen. Tier 2 includes a two-generation mammalian reproduction and development test and a mysid shrimp reproduction test (USEPA, 2000). USEPA validation work is being conducted in close liaison with the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) established by the National Toxicology Program under the auspices of the National Institute of Environmental Health Sciences (NIEHS).

5.3 Alternate test method development

New and revised toxicological testing methods are being developed around the world incorporating molecular and cellular biology and these hold promise for reducing whole animal testing. Biological methods can be used as screens to determine if EDC-active compounds are present in a given environmental sample (ICPS, 2002). These can be carried out with chemical methods to establish cause and effect and to quantify the EDCs present (Cech *et al*. 1998). The majority of tests developed so far include *in vitro* bioassays for assessing estrogenic and anti estrogenic substances and *in vivo* methods using fish or wildlife. Ideally a battery of screens should be used to address a range of different mechanisms of endocrine disruption (Matthews *et al*, 2000). Tests are also being developed to detect androgens and anti androgens, thyroid active chemicals and compounds that interfere with steroid bio-synthesis and metabolism (WHO/ICPS, 2002).

5.4 Biomarkers

In vitro and *in vivo* bioassays are useful techniques for the determination of receptor-mediated activities in environmental samples containing complex mixtures of contaminants. The bioassays determine contamination by pollutants with specific modes of action (Geisy *et al*, 2002) and also integrate possible interaction between compounds. Extracts from various matrices can be tested

to evaluate their biological activity and identify those samples that require further investigation using resource intensive analytical techniques. *In vitro* and *in vivo* bioassays offer a rapid, sensitive and relatively inexpensive solution to some of the limitations of instrumental analysis. Methods that rely on biological activity are finding increasing utility as screening tools, because the chemical nature of the endocrine disrupting sample may be unknown and the biological method may be the best (or only) indicator of EDC activity.

5.4.1 Receptor binding assays

Receptor binding assays measure binding of agonists or antagonists to a specific cellular receptor. This provides assessment of endocrine disrupting activity at the molecular level of biological organisation. Thus, this represents the first level of signal transduction of these hormones to modulate the expression of specific genes. The potency of compounds is dictated by their relative affinity to these receptors. Receptor binding affinity measurements can then be used to indicate the potential of specific compounds or mixtures of compounds to act as EDCs. The concentrations able to be detected for some of these tests can be in the picogram per litre (pg/L) range (Soto *et al*, 1995). An estrogen-receptor binding assay (ERBA) using sheep uterus estrogen receptors has recently been used to derive estradiol equivalents (EEQ) from sewage effluent from the Landsborough Water Reclamation Plant, Queensland (Table 5.3.1) (Leusch *et al*, 2002)

5.4.2 Estrogen receptor activation assays

ER activation depends on the ability of estrogens to induce cellular responses in target organs such as fish liver cells (hepatocyte bioassay) (Stephensen *et al*, 1998) or human breast cancer cells such as MCF-7 cells (Koerner *et al*, 1999). When primary cell cultures are used, a particular protein, such as vitellogenin which is a protein unique to egg development, can be measured as the endpoint. One of the advantages of using whole cell bioassays is the ability to differentiate a hormone agonist from a hormone antagonist (both of which may show receptor affinity). Cell proliferation can also be induced at very low concentrations of estrogenic substances. The E-screen, using MCF-7 human breast cancer cells has recently been conducted using effluent extracts from the Landsborough Water Reclamation Plant, Queensland to quantify the removal of EDCs (based on net estrogenic activity) by various tertiary treatments (Table 5.3.1) (Leusch *et al*, 2002).

Table 5.3.1 Results of biomarkers assays indicating estrogenic activity (EEQ) in reclaimed water from Landsborough, Queensland[1,3]

	E-screen[2]	**ERBA[2]**
Treatment	**ER activation expressed as EEQ**	**ER binding assay expressed as EEQ**
Influent	21	50
BioP[5]	6	100
Bioreactor	19	4
Clarifier	3	Not detected
Sand filter	3	Not detected
Ozone	Cytotoxic[4]	Not detected
UV	Not detected	Not detected

1 Landsborough Water Reclamation Plant, Queensland.
2 EEQ (estrogen equivalent) = $EC50_{ESTRADIOL}/EC50_{SAMPLE}$, limit of detection – $_{lod}$ ≤1.5ng/L
3 Leusch *et al*, (2002)
4 Cells died during assay
5 Biological phosphate removal

5.4.3 Whole animal assays

Whole animal assays may also be used as a biomarker of exposure and/or effect. For example in Australia the mosquito fish *Gambusia affinis holbrooki* been used to assess hormonal properties of effluent in Sydney, NSW (Batty and Lim, 1999). The assay for estrogenicity and androgenicity uses morphological changes as the endpoint. In males the anal fin develops under the influence of androgens to form a structure (gonopodium) which is used in sperm transfer. When a male mosquito fish is exposed to high concentrations of estrogens or estrogen mimics, the size of the gonopodium is reduced. This response has been demonstrated downstream of sewage effluent discharge in NSW (Batty and Lim, 1997) although the same response was not observed in mosquito fish inhabiting a small U.S. river receiving treated domestic sewage effluent (Angus *et al*, 2002). The response is likely to be concentration dependent. In the presence of an androgen, the female mosquito fish will develop a fin resembling the gonopodium of the male (Howell *et al*, 1980). These endpoints can be monitored in natural environments as indicators of EDC mediated effects but may not be as sensitive as some of the molecular or cellular endpoints.

6.0 Australian Context

6.1 Pharmaceuticals and natural hormones

From the discussion in the preceding chapters it is apparent that the main sources of EDC's in the rivers and lakes of Europe and North America are sewage effluent and agricultural chemicals. Because of the population density and frequency of inland towns in Europe and North America, many rivers drain catchments carrying ten or more million people. Hence the quantity and variety of pharmacological agents, including EDCs, passing into sewage treatment is very considerable. Some pharmaceuticals are excreted unmetabolised or in conjugates which release the active molecule on sewage treatment. The persistence of some pharmaceuticals in the environment is greater than a year (Halling-Sorensen *et al*., 1998). In the highly populated Po Valley in Italy, a range of pharmaceuticals was measured in river water, sediments and drinking water. The drugs were selected for measurement on the basis that tons/year were used in the river catchment. Seven human pharmaceuticals and one agricultural growth promoter were identified in the Po river water. Below Milan an additional three drugs and two agricultural growth promoters were measured in the river Lambro. Only two pharmaceuticals were found in finished drinking water at the concentrations detectable in these studies, these were clofibric acid and diazepam. Hormonal pharmaceuticals were not reported in this paper, as other studies were in progress on these compounds (Zuccato *et al*., 2000).

In the USA, with a substantial proportion of the population of almost 290 million living in inland cities, a recent survey of streams across the entire country showed the highest concentration of wastewater contaminants coming from detergents, followed by natural faecal steroids and plasticisers. Hormonally active compounds of natural origin- testosterone, 17β-oestradiol and progesterone were present in somewhat smaller concentrations than the pharmaceuticals 17α-ethynyl oestradiol and 19-norethisterone from oral contraceptives. Maximum detected hormone concentrations were lower than 1.0µg/L, and the median concentrations around one tenth of this (Kolpin *et al*., 2002).

Australia, with a population of 20 million, has a very small proportion located on inland rivers, with the largest inland city, Canberra, having a population of 320,000 (Australian Bureau of Statistics, 2001 census). This can be compared to the inland population of the Po River catchment in Italy of over 16 million. The larger Australian inland towns with populations over 60,000, which may potentially cause sewage contamination downstream, are Penrith (178,000), Toowoomba (90,000), Bendigo (90,000) and Ballarat (83,000). All other inland towns are smaller, though Albury and Wodonga together have a population of 76,000 (Australian Bureau of Statistics, 2001 Census).

There are some special cases where contamination of rivers from wastewater may be a significant issue. In particular rivers such as the Hawkesbury in New South Wales there may be no net flow during dry periods, with abstraction for drinking water and irrigation equalling the input of water from wastewater plants. Under these conditions drinking water intakes downstream of Penrith are accessing treated, though largely undiluted, wastewater. Similarly, downstream from the Australian Capital Territory during dry conditions effectively all the flow in the Murrumbidgee River is treated wastewater from Canberra. The situation of drinking water intakes for one city being downstream of wastewater outlets from another occurs frequently in Europe and North America. The main difference is that in most of Europe and North America there are substantial river flows all year round. By contrast low-flow or no-flow conditions occur in many Australian rivers during dry periods. This applies even to the huge Murray-Darling catchment, as the Murray River has been flowing backwards in some sections during 2002, with the Darling River currently not flowing and the river mouth to the sea almost closed.

A further difference between Australia and Europe and North America is the relatively higher proportion of surface water use in Australia, due to the lack of availability of high quality groundwater.

As water becomes more scarce due to population growth and increasing agricultural usage, re-use of wastewater becomes an alternative to single use of surface water. This issue is just as important for coastal cities as inland cities, as most population growth is in the coastal cities and available surface water supplies are already in use. In addition to the potential problems in wastewater of pathogens, heavy metal accumulation, toxic and carcinogenic organic chemicals and salt content, the potential for redistribution of endocrine disrupting compounds is an issue of concern. Monitoring data for endocrine disruptors in wastewater discharge (Tables 4.2.1 and 4.2.2) indicates that several potent estrogenic compounds, ethinyl estradiol and 17β-estradiol for example, occur in wastewater in biologically active concentrations for aquatic organisms. Any adverse effects on human populations at these concentrations remains to be evaluated. Assessment of EDC removal in wastewater treatment and drinking water treatment then becomes essential if re-use for drinking water is to be considered. Widespread current use of solids from wastewater plants as fertilisers for garden and agricultural application also allows the redistribution of EDCs that adsorb to wastewater sludge and may be released and taken up into crops.

6.2 Agricultural and veterinary chemicals

Many banned, and some presently used, agricultural compounds have EDC properties. The banned agricultural chemicals with well-demonstrated adverse effects include DDT, dieldrin and lindane. Organochlorine pesticides have

been used to control a variety of pests since the 1940s (USEPA, 1997). Some of the compounds in this group are capable of interacting with the estrogen receptor while others (eg. p,p'-DDE) cause effects by blocking the androgen receptor (Kelce, 1995). Although DDT is no longer used in most developed countries it is still in use in some developing countries to control the spread of malaria. There is potential for global distribution of these pesticides via the atmosphere (Kallenborn *et al*, 1998).

Agricultural runoff is responsible for the presence of most pesticides found in surface waters, with the concentrations highest after the first storm following application (Dabrowski *et al*, 2002). Agricultural and veterinary products in Australia are regulated by the National Registration Authority (NRA). Assessment is based on known toxicological effects (Chapman, 2002b), however to date few (if any) have been assessed for adverse endocrine effects. Although there is information available on the hormonal potency of the older organochlorine pesticides (eg. p,p' DDE), there is considerably less information for the less persistent organochlorines (endosulfan and methoxychlor), organophosphates, pyrethroids, herbicides and fungicides and the "biorational" insecticides which have been designed to interfere with invertebrate physiology (eg. the juvenile hormone analogue pyriproxyfen).

The EDC activity of the fungicides vinclozolin and ketoconazole relates to their mechanism of action in fungal inhibition, as both are inhibitors of enzymic pathways of steroid metabolism which in mammals result in androgen formation. Hence exposure to sufficient concentration of these compounds can lead to reproductive defects in males (WHO/IPCS,2002)

Many endocrine responses to anthropogenic and 'naturally' occurring EDCs remain complex or elusive. An example is the mycoestrogen zearalenone which can occur during fungal damage to stored grain. This has been shown to act as an ER agonist (Celius *et al*, 1999), to inhibit CYT-P450 3a4 (a metabolic effect) and steroidal hormone actions, to increase the incidence of Leydig cell adenoma in rats (Klaassen, 2001), and can lower serum testosterone levels by inhibiting testosterone synthesis (Rajfer *et al*, 1986). Depending on hormonal potency, in some cases endocrine effects may be trivial compared to the suite of other toxic responses.

The surfactants widely used in agricultural chemical formulations frequently contain nonylphenol derivatives, which are suspected of low-level EDC activity. Human exposure to alkylphenols (AP) or alkylphenol ethoxylates (APE) may occur through drinking water that is drawn from a polluted source. However there is no conclusive evidence that APs or APEs could cause adverse health effects in humans. There are however many reports of alkylphenols causing production of the female-associated liver protein, vitellogenin, in male fish (Jobling, 1995).

The locations of greatest likelihood for human and ecological impacts from agricultural chemicals are the irrigation areas with the highest quantity and frequency of chemical use. Occupational exposures have demonstrated adverse effects worldwide and these are supported by experimental exposure data from laboratory animals. Regulatory requirements may need to be strengthened by including prenatal and immediately post-natal exposure studies on animals, carried through to measurement of adult reproductive performance and neurological capability.

6.3 International comparison of ECD risks

The potential hazard to the Australian population from endocrine disruption in drinking water is very limited. In comparison to the potential problems in underdeveloped countries in which large populations have no access to treated drinking water and wastewaters are released untreated into water courses, Australia has a sophisticated network of both wastewater and drinking water treatment facilities. In comparison to Europe where indirect wastewater re-use is normal, Australia currently has only the single use of drinking water in most locations. Inland towns adjacent to rivers and the city of Adelaide are exceptions, as they use river water for the drinking supply. Rivers are the recipient of wastewater in Australia from most (but not all) inland towns, including Canberra, the largest inland Australian city.

Attention to EDCs and agricultural chemicals in inland watercourses is required, to establish both any potential risk to human populations dependent upon inland waters for drinking water and also risk to the ecosystems. Adelaide's population are the major consumers of inland river water during summer, when Murray River flow is minimal and water use in irrigation maximal. Monitoring of EDC activity, as well as the presently undertaken pesticide monitoring in the lower Murray water, should be routinely undertaken. The same applies to other rivers used routinely for drinking water supply. The potential for contamination of groundwater in aquifers should also be considered and appropriate monitoring undertaken

6.4 Economic considerations

In addition to the direct effects on humans, animals and ecosystems it is of value to keep economic considerations in mind. Impoverishment of ecosystems with consequent diminution in ecosystems potential will have economic consequences on the efficiency of the food web, which ultimately controls the availability of foodstuffs for humans. Such effects are clearly indirect but nonetheless real. A simple example of this is the way in which scavenger organisms affect the quality of the waters flowing into reservoirs. If populations of these organisms are diminished by toxicological events – including EDCs, water quality may suffer. This then can then lead to greater economic costs from pollution through additional treatment to produce

drinking water of appropriate quality for human use. These types of economic consequence are complex and are inter-related to a host of toxic influences, which will include compounds other than EDCs.

7.0 General conclusions and research needs

It is apparent from the information available that adverse effects on the health of human populations have occurred from exposure to toxic and endocrine disrupting chemicals through accidental exposure, occupational exposure and pharmaceutical drug treatment. Consumption of chemically contaminated food has also been shown repeatedly to result in short and long-term harm to health. However the evidence for environmental concentrations of EDCs (in particular) having demonstrable ill effects on human populations is far from clear. As many compounds being investigated for EDC activity are also toxic through other mechanisms, the proportional effects of endocrine disruption may be minimal. The relevance of environmental exposure to EDCs to human health will not be resolved until prospective epidemiological studies, in which exposure is monitored closely, have been continued for a decade or longer. These are in progress elsewhere.

Concentrations of EDCs in wastewater streams have been shown to cause feminisation of male fish. The likely compounds responsible are the natural mammalian and synthetic estrogens, plant origin estrogens and anti-estrogens. These appear to be resistant to primary sewage treatment, and to some secondary and tertiary treatments.

A comparable issue is the concentrations of pesticide residues in rivers downstream of irrigation areas, especially where the river water is then used as a drinking water source (Radcliffe, 2002). The Murray River and its major tributaries are all used as irrigation water supplies and as drinking water sources for the towns along the rivers. Monitoring of pesticides is undertaken downstream of some wastewater treatment discharges, for example in the ACT, but is more important downstream of irrigation areas where hundreds of tons of pesticides are used annually. While some State agencies (eg NSW Land and Water Resources) are carrying out concerted monitoring programs in specific areas (Kookana *et al.*1998), the pattern of monitoring is inconsistent which 'precludes a clear understanding of pesticide exposure in the Australian environment' (Radcliffe, 2002). Because of low flow conditions increasingly common in Australian rivers through drought and water abstraction for agriculture, pesticide residues may be a potential health risk in drinking water through toxicity or through endocrine disruption. Drinking water resources are becoming critical in some towns and cities in Australia as a result of the current drought. For example Yass in NSW is currently on Stage 5 drinking water restrictions with reservoir capacity below 30%. This immediately raises the issue of direct or indirect re-use of wastewater, including groundwater recharge by wastewater. If wastewater reuse as a source of drinking water becomes a necessity for cities in Australia the problem of estrogen removal will require urgent attention from the drinking water supply industry. As there is substantial research internationally into these issues, on-going monitoring of the research outcomes is advisable. It is not recommended that Australia duplicate the research being undertaken within the OEDC framework, though application to the Australian environment will be required.

Measurements of concentrations of EDCs, pesticides and their breakdown products in rivers and of the rates of natural degradation of these compounds in rivers and lakes used for drinking water are required to assess the risk. Monitoring of estrogen discharge and pesticide use in river basins should be routinely undertaken for risk assessment. The health risks may well not relate to endocrine effects, but to adverse impacts of pesticides on other organ systems.

A broad scale survey of EDCs in the Australian environment is probably not required at the present time. There are simply too many compounds and for many substances adequate analytical methods or sensitivity are still not available in Australia. However selected monitoring of EDC activity from anthropogenic sources in rivers downstream of irrigation areas would be useful especially in the lower Murray River. The likelihood of exposure of humans could indicate if intervention is necessary for drinking water. Monitoring capability for EDCs in Australian wastewaters and rivers is however necessary, and should be extended using the methodologies currently being validated in the USA and elsewhere.

It will soon be possible to use biomarkers to test compounds of interest (ie. those in effluents, present in surface waters or reported from other monitoring programs) for hormonal activity. These biological markers for exposure can usefully be validated for use in Australia, prior to any detailed investigation of EDCs in general. When there is clear biomarker evidence of EDC contamination there may be a need to evaluate the constituent compounds. The development of the assessment process should be based on integrated effects, which would include EDCs as well as other toxicological effects.

As the relevant Australian industries are largely subsidiaries of multinational corporations, it is unlikely that pharmaceuticals, industrial chemicals or agricultural pesticides differ greatly between Australia, North America and Europe. The research conducted by the companies, national regulatory bodies and research organisations elsewhere is therefore informative for Australian actions. On-going review of overseas research is essential for Australian understanding of EDCs and relevant national responses.

8.0 Recommendations

- We recommend Australian monitoring of the international literature on EDCs for evidence of human health effects, efficacy of drinking and wastewater treatments in removing specific EDCs and for methods for monitoring EDCs and ecological effects.
- Monitoring at selected locations of known and potential EDCs (hormonally active chemicals, agricultural chemicals and their breakdown products with potential EDC activity) in rivers downstream of wastewater discharges and irrigation areas, especially where town drinking water supplies are drawn from these rivers. We recommend parallel ecological monitoring at these locations..
- Monitoring of selected Australian drinking water and wastewater treatment processes for EDC removal using both chemical and biomarker methods as screening tools is recommended.
- Chemicals suspected of having endocrine-disruptor activity occur in industrial effluent, sewage discharge and agricultural run-off, and ultimately end up in the ocean. Thus the marine environment is potentially at risk. While this is not directly relevant to the drinking water industry, potential environmental impacts on coastal marine fauna could usefully be investigated.
- On the basis of the large amount of EDC research in progress in the USA, the EU and Japan, it is not recommended that Australia embark on major programs in this field. However it is necessary that we investigate how the emerging technologies in this field may be developed and validated for use in Australia. Evaluation of advances in EDC monitoring methodology, in sewage treatments for EDC removal, risk mitigation technologies for agricultural pesticide and intensive animal industry contaminants and models for the fate of EDCs in the environment are relevant areas.

9.0 Useful Web Sites

Australian regulatory policy documents (Environment Australia and National Regulatory Authority)	http://www.ea.gov.au/industry/chemicals/international/pubs/edc.pdf http://www.nicnas.gov.au/foreign/endocrine.htm http://www.nra.gov.au
European Union	http://europa.eu.int/comm/environment/chemicals/pdf/workshop_report.pdf
US Geological Society	http://www.cerc.cr.usgs.gov/endocrine/
Chemicals Evaluation Research Institute, Japan	http://www.cerij.or.jp/ceri_en/index_e.shtml
Environment Canada	http://www.ec.gc.ca/eds/fact/broch_e.htm
US EPA	http://www.epa.gov/scipoly/oscpendo/index.htm
OECD	http://www.oecd.org/EN/document/0,,EN-document-524-14-no-24-6685-0,00.html
International Program on Chemical Safety (ICPS)	http://www.who.int/pcs/
WHO/IPCS	http://www.who.int/pcs/emerg_site/edc/global_edc_ch5.pdf
bioassay method validation	http://iccvam.niehs.nih.gov/methods/endodocs/ed_brd.htm

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CRC for Water Quality and Treatment
Private Mail Bag 3
Salisbury SOUTH AUSTRALIA 5108
Tel: (08) 8259 0211
Fax: (08) 8259 0228
E-mail: crc@sawater.com.au
Web: www.waterquality.crc.org.au

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