ENVIRONMENT PROTECTION AUTHORITY

Risks from endocrine disrupting substances in the South Australian aquatic environment

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South Australia

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GLOSSARY

Androgen	Steroid hormone with masculising properties produced by the testes in male vertebrates and also, in smaller amounts, by the ovaries and adrenal cortex in female mammals.
APEs	Industrial surfactants called alkylphenol polyethoxylates (includes nonylphenols, octylphenols, pentaphenols, butylphenols).
APVMA	Australian Pesticides and Veterinary Medicines Authority is responsible for administering the national permit process for agricultural and veterinary chemicals <www.apvma.gov.au/>.
Dioxin	Dioxins are a group of persistent chlorinated chemical compounds, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), which share similar structures, properties and toxicity.
EDCs	Endocrine disrupting chemicals refer to a range of chemical substances that impact or alter the endocrine system in animals.
Endocrine	The term endocrine means to secrete internally and is generally applied to the glands in animals that release secretions into the blood.
Endocrine glands	Ductless glands present in animals that make hormones and are secreted directly into the circulating blood.
Hazard	A hazard refers to the possibility of an adverse effect occurring due to the presence of chemicals in the environment. A hazardous event is an incident or situation that can lead to the presence of a hazard (eg what can happen and how).
Hormone	Chemical messengers secreted from endocrine glands into the blood that trigger an effect in some other part of the body.
Imposex	The development of male sex organs in females.
Limit of detection	The lowest level at which a chemical can be measured in a sample by an analytical method.
LOEC	Lowest observed effect concentration.
Oestrogen	Steroid hormones with feminising properties produced mainly by the ovaries and placenta of female vertebrates but also by the testes and adrenal cortex in male vertebrates.
PCBs	Polychlorinated biphenyls are a group of chlorinated chemical compounds that have similar chemical structure, properties and toxicity to some dioxins.
PNEC	Predicted no effect concentration.
Risk	A risk refers to the probability (likelihood) and consequence (severity) of a hazard causing environmental harm. Risk in this context includes risk assessment, risk management and risk communication.
Risk assessment	Involves hazard identification and risk estimation that includes an assessment of the 'likelihood' and 'consequence' of any identified hazard. This allows an estimation of the level of risk (negligible, low, moderate or high) that can then be used to develop risk management strategies.

Toxicity	The degree to which a substance can harm humans, animals or plants. Acute toxicity involves harmful effects (usually death) to an organism through a single or short-term exposure. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful, sub-lethal effects over an extended period (eg reduction in growth, reproductive success, altered hormonal levels) to the exposed organism.
Units	milligrams (mg/L) or 0.001g
	micrograms (μg/L) or 0.000 001g
	nanograms (ng/L) or 0.000 000 001g
	picograms (pg/L) or 0.000 000 000 001g
WWTP	Waste water treatment plant

1 INTRODUCTION

In recent years there has been increasing concern among the scientific community and general public about the possible adverse effects that may result from exposure to a group of natural and synthetic (man-made) chemicals that have the potential to alter the normal functioning of the endocrine system in wildlife and humans (eg Damstra *et al* 2002; Lintelmann *et al* 2003; Roberson 2003). A large number of substances from a wide range of chemical classes have been implicated and subsequently touted as possible endocrine disrupting chemicals (EDCs), including industrial chemicals, historical and currently used pesticides, heavy metals, pharmaceuticals, and different types of natural hormones and substances produced by plants and animals.

The endocrine system is a critical component of body function in animals and consists of the glands that secrete hormones into the bloodstream, that then act as chemical messengers to trigger an effect in some other part of the body. This includes the pituitary, thyroid and adrenal glands, and the male and female reproductive systems, all of which release hormones into the blood. Collectively, these glands and their hormones regulate processes such as reproduction, growth, development, aspects of behaviour that include responses to stress, and maintenance of blood pressure and heart rate (eg Keith 1997; Damstra *et al* 2002; Manning 2005). While plants lack an endocrine system, they have a chemical signalling system that allows them to produce chemicals to elicit a response to herbivore grazing or infection by bacteria and fungi; it is thus possible that some plant processes may also be adversely affected by EDCs (Manning 2005).

Endocrine disrupting chemicals are thought to cause an adverse effect in an organism, its offspring or local population, by a number of possible pathways. EDCs may mimic the sex hormones and promote similar responses to them, or they may block the activities of the sex hormones oestrogen or androgen. The 'key-lock' mechanism is often used to describe the way in which hormones interact with receptors to trigger an effect; EDCs may act by sending the body a different message by altering or blocking the hormone (key) or the intended receptor (lock). EDCs may also disturb the signalling system before the hormone reaches the receptor by altering essential protein production in the body, producing abnormal hormone levels or several other complex pathways described in Manning (2005). The effects may only be obvious at the tissue or hormonal levels in individuals exposed to specific EDCs, or may be more significant and lead to changes at the population level [cf. studies cited in Manning (2005) for more information].

The evidence for endocrine disruption includes the results from both field and laboratory studies that have shown that the growth, reproduction and development of many species, including mammals, birds, fish, frogs and invertebrates may be affected by the presence of EDCs in the environment. Adverse effects have included developmental abnormalities and feminisation of alligators in Florida following an organochlorine pesticide spill, feminisation of fish by waste water treatment plants and paper mill discharges, and imposex (development of male sex organs in females) of molluscs from exposure to organotin antifoulant paints (Damstra *et al* 2002; Lintelmann *et al* 2003; Manning 2005). The latter also led to the localised extinction of dogwhelks in some industrialised harbours in the United Kingdom (Bryan *et al* 1988). The increased incidence of certain endocrine-related human diseases has also focused attention on the risks posed by exposure to chemicals that have the potential to

cause effects at very low levels (Damstra *et al* 2002). As a result, the threat posed by any chemical in the environment that can affect endocrine systems is potentially very serious.

However, the fact that humans and wildlife are exposed to such chemicals does not mean that there will be impacts because much still depends on the concentration and duration of exposure, the timing of exposure (eg whether embryonic, early development or adult) and degree of tissue harm in affected individuals. The spatial area affected by EDCs (eg small-scale effects at 10s of metres compared to large-scale effects at 10s of kilometres or greater) will also determine if the impact is likely to be trivial or more significant and requiring further management action by regulators, polluters and perhaps the community.

This document summarises the current state of knowledge about the likely sources of endocrine disruptor chemicals, based largely on work carried out in North America and Europe, and identifies possible areas of risk to the South Australian environment. This review focuses on environmental sources and risks, and only summarises some of the major issues relating to human health; readers interested in human risks should refer to the publication by Damstra *et al* (2002) on the World Health Organization website <www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/>; for specific information relating to Australian drinking water issues and risks; the National Health and Medical Research Council website <www.nhmrc.gov.au>; and the publication by Falconer *et al* (2006) provides suitably detailed coverage.

2 BACKGROUND

The process of endocrine disruption was first described in the scientific literature in the 1930s (Dodds & Lawson 1938) but the wider effects of EDCs on wildlife and human health were first summarised in Carson (1962) and, later still, gained widespread attention with the publication of the book *Our Stolen Future* by Colborn *et al* (1996). More recently, the International Programme on Chemical Safety of the World Health Organization/United Nations Environment Programme/International Labour Organization released the report *Global Assessment of the State-of-the-Science of Endocrine Disruptors*, which presents an extensive and detailed global assessment of the then current state of scientific knowledge related to environmental endocrine disruption (Damstra *et al* 2002).

According to Damstra *et al* (2002) there are many chemicals that are released into the environment that have some degree of hormonal activity, including:

- intentionally released products (eg some pesticides are known or suspected EDCs, and mixtures of EDCs released to the environment from waste water treatment plants and industry also cause a range of adverse endocrine disrupting effects on wildlife)
- unintentionally released at some point in the life cycle of a chemical (eg during manufacturing, use or disposal, and from everyday use of pharmaceuticals and chemicals by domestic and commercial consumers)
- unintentionally formed as by-products in a variety of industrial or combustion processes (eg dioxin-like chemicals)
- unintentionally released via leakage from landfill sites or from the disposal and use of sewage sludge on agricultural lands
- naturally occurring EDCs such as phyto-oestrogens and fungal oestrogens (eg isoflavonoid phyto-oestrogens in soy and legumes, lignanes in grains and many fruits and vegetables, coumestans in clover and alfalfa).

2.1 Problems with showing effects from exposure to EDCs

Whether these known or suspected EDCs actually cause any adverse effects on humans and the environment is for the most part unknown for the majority of chemicals. According to Falconer *et al* (2003), widespread, low-level environmental exposure to EDCs has not yet been conclusively demonstrated to cause harm to humans. The US Environment Protection Agency or USEPA (1997) also formed the view that with a few exceptions, a causal relationship between exposure to a specific chemical or mixture of chemicals and an adverse effect from endocrine disruption in humans or populations of animals has not been established. This is because there are many problems associated with carrying out sufficiently detailed exposure studies to assess and demonstrate the causal relationship between exposure to EDCs and an effect, either on some aspect of human health or adverse wildlife response. For example, exposure studies are typically designed to show the nature and extent of contact with a chemical under different conditions and involve concentrations in the water, air or food as well as concentrations in living organisms (eg presence in the blood, urine, breast milk). They are very costly to carry out because they must assess the magnitude, duration and frequency of exposure and make some estimate of the number of individual's involved (Damstra *et al* 2002).

Showing cause-and-effect relationships is even more difficult for effluent mixtures from waste water treatment plants and paper mills, where synergistic (ie additive) and antagonistic (ie inhibitory) effects usually occur, and isolating specific chemicals responsible for causing adverse effects has remained elusive to this day (eg Hewitt & Servos 2001).

Despite this uncertainty in showing clear cause-and-effect relationships, many environmental protection agencies, research institutions and water authorities around the world are funding work aimed at identifying chemicals in water, testing treatment options, monitoring pollutant levels in natural waters and in discharges from industry, and initiating screening and other laboratory and field tests and studies to determine whether suspected EDCs in water pose an adverse risk to people, aquatic life and wildlife.

2.2 Recent directions with the review and assessment of chemicals in Australia

There are around 50,000 industrial, agricultural and veterinary chemicals available for use in Australia (EPHC 2006). Most were developed and used in the 1900–1970s, during which time there were few limitations on their usage and little consideration of the environmental consequences in using such chemicals. As a result, there are many parts of our landscape that have been contaminated by past applications of chemicals, particularly from historically used pesticides and their breakdown products, industrial chemicals or metals. These include persistent chemicals that have the potential to bio-accumulate in humans and animals, and others that are capable of causing endocrine disrupting effects.

However, since the early 1990s, new chemicals have undergone an assessment of their potential environmental and health impacts, and many of the older chemicals have been revised and, in some cases, phased out of production by industry and/or regulation through the Australian Pesticides and Veterinary Medicines Authority (APVMA) permit process (for further details on the national registration scheme see <www.apvma.gov.au/>). The ratification of two international agreements relating to the trade of certain hazardous chemicals in 2004 has led to many products being voluntarily withdrawn from the market, or actions by the APVMA to either impose additional restrictions on the use of certain chemicals or withdrawing permits for specific chemicals.

The Rotterdam Convention on the Prior Informed Consent (PIC) procedure for hazardous chemicals and pesticides in international trade has resulted in the following substances, that had toxicant and endocrine disrupting properties, no longer being used in Australia: pentachlorophenol, benomyl, lindane and parathion. For further details on the controls relating to the export of PIC listed chemicals refer to <www.daff.gov.au>.

The Stockholm Convention on Persistent Organic Pollutants (POPs) listed a number of chemicals that are prohibited from being imported, exported, manufactured and used, including known and suspected endocrine disruptors among the following pesticides: aldrin, dieldrin, DDT, endrin, chlordane, heptachlor, mirex and hexachlorobenzene (see <www.pops.int> for further information and updates on the list of banned chemicals).

Chemical use in Australia is also regulated through some additional national approving bodies that include:

- National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is responsible for the regulation of non-agricultural chemicals such as industrial chemicals
- Therapeutic Goods Administration (TGA) is responsible for registering pharmaceuticals and medicines

- Food Standards Australia and New Zealand (FSANZ) is responsible for developing standards for food related issues (EPHC 2006).

Importantly, a national framework for chemicals management in Australia has recently been proposed as a way of providing a nationally consistent approach to environmental chemicals management, and would include the environmental impacts of chemicals (EPHC 2006); this would also include issues relating to the management of many of the EDCs described in this report.

2.3 Overseas screening and research

Considerable effort is currently underway in the USA and Canada, in particular, to develop screening and testing methods and models for EDCs (Huet 2000; Fenner-Crisp *et al* 2000; Parrott *et al* 2001; Roberson 2003). For example, the USEPA plans to screen 15,000 chemicals for their possible effects as endocrine disruptors in wildlife and humans. The US Geological Survey is currently carrying out a national reconnaissance of hormones, pharmaceuticals and other organic waste water contaminants (cf. Kolpin *et al* 2002; Focazio *et al* 2003) from surface and groundwater sources of drinking water across the USA.

Some countries have carried out surveys in major rivers for some newly identified endocrine disrupting chemicals that include steroids and surfactants such as nonylphenols (Naylor *et al* 1992; Blackburn *et al* 1999; Ahel *et al* 2000). Further research on the occurrence of EDCs in effluents from sewage treatment plants, pulp and paper mills, and natural waters is also building on the knowledge-base about the types of chemicals, their concentrations, removal by various treatment processes, environmental effects and in some cases oestrogenic activity (eg Harries *et al* 1996, 1998; Lagana *et al* 2004; Manning 2005).

2.4 Research in Australia

By comparison with work overseas, relatively few studies have been carried out in Australia to identify the sources of EDCs, their concentrations and behaviour in our aquatic environments, and on assessing their risks and effects to ecosystems and humans (Ying & Kookana 2002). A number of published studies have, however, described endocrine disruption in Australia, including:

- imposex of molluscs in harbours due to exposure to tributyltin (TBT) in antifouling paints (Nais *et al* 1993; Daly & Fabris 1993; Burt & Ebell 1995)
- egg-shell thinning and decreased breeding success in raptors related to high organochlorine pesticide residues (Falkenberg *et al* 1994)
- decreased fertility of sheep due to the presence of phyto-oestrogen in pasture grasses (Adams 1998)
- reproductive effects on male mosquitofish exposed to sewage effluent (Batty & Lim 1999).

Endocrine disruption has, however, been widely recognised as a significant water quality issue for Australia as it has been raised in a number of recent initiatives that include:

- a national forum by the Australian Academy of Science (1998)
- listed as a water quality issue by Sydney Water (2000)

- assessed in reviews of the potential for EDCs to contaminate drinking water in Australia by Falconer *et al* (2003, 2006)
- reviewed in relation to reclaimed water use in Australia (Ying *et al* 2004)
- discussed at a Commonwealth Scientific and Industrial Research Organisation (CSIRO) symposium and workshop in Canberra in 2004, with a focus on ecological considerations in the Australasian environment
- most recently, the Interact 2006 conference held in Perth during September 2006 had three sessions dedicated to current research into EDCs in Australia <www.promaco.com.au/conference/2006/raci/left.htm>.

In South Australia, two recent collaborative studies between the CSIRO and EPA have focused attention on the concentrations, effects and potential risks posed by specific sources of EDCs to the environment. They include:

- an industry supported assessment of responses to pulp and paper mill effluent discharged into Lake Bonney SE (Kumar *et al* 2006)

and

- a survey of oestrogen concentrations from dairy sheds, irrigation channels and ambient (background) monitoring sites from streams throughout the southern part of the state (EPA & CSIRO data, publication in preparation).

CSIRO and Land and Water Australia have also been undertaking the project 'Endocrine Disrupting Chemicals in the Australian Riverine Environment' which is focused on assessing the risks from waste water treatment plant (WWTP) discharges to four dry temperate (South Australia), two cool temperate (ACT) and five warm subtropical (Queensland) streams. Sewage effluent is considered to be the major source of EDCs to the environment (Damstra *et al* 2002) and it is anticipated that this research will help clarify the concentrations, fate, exposure and effects of selected EDCs to the Australian environment, and may lead to recommendations for managing discharges to our waterways into the future. The managers of the major WWTPs in South Australia, SA Water Corporation and United Water, are supporting this research, as is the EPA as the state's major environmental regulator.

3 MECHANISMS OF ENDOCRINE DISRUPTION

There are at least five mechanisms by which environmental contaminants may disrupt the endocrine system:

- some chemicals may be similar enough in structure to hormones to be able to bind to cellular receptors that are designed to be targets for natural hormones; this can cause unpredictable and abnormal cell responses. Those chemicals that act like the sex hormones oestrogen and androgen are called environmental oestrogens and androgens, respectively
- some contaminants may block the receptors described above and prevent hormones from binding with them, resulting in impaired cell responses. Chemicals that block or antagonise the sex hormones are called anti-oestrogens or anti-androgens
- some contaminants may induce the creation of new receptor sites in the cell, leading to an increased hormonal effect on cell activity
- contaminants may interact directly or indirectly with natural hormones, thereby changing the signals from hormones and altering cell activity
- contaminants may disrupt the natural production of hormones by endocrine glands and other tissues, resulting in an abnormal concentration of hormones in an organism.

Further details about how chemicals disrupt the endocrine system are given in Damstra *et al* (2002) and Manning (2005).

4 SOURCES OF POTENTIAL EDCS TO THE SOUTH AUSTRALIAN ENVIRONMENT

There are a large number of man-made and natural chemicals that have endocrine disrupting properties including various pesticides (eg organochlorine insecticides, some herbicides and fungicides); industrial chemicals and pollutants [eg dioxins, polychlorinated biphenyls (PCBs), phthalate plasticisers, phenols, alkylphenol surfactants (APEs), polynuclear aromatic hydrocarbons (PAHs)); heavy metals (eg cadmium, lead, mercury, arsenic]; pharmaceuticals and synthetic hormones (eg the now banned diethylstilbestrol (DES) and widely used female contraceptive pill 17α–ethinylestradiol); natural phyto-oestrogens from plants, and oestrogen and testosterone excreted by people and other animals.

Appendix 1 provides a list of selected chemicals that have been found or suspected to be capable of disrupting the endocrine system and may therefore be of relevance to the South Australian environment. The appendix has been modified from Ying & Kookana (2002) and omitted the pesticides and chemicals that have no current approvals for use. It should be noted that many historically used pesticides and chemicals are occasionally detected in low concentrations in the state as part of the monitoring programs conducted by the EPA and others (eg CSIRO, SA Water, NRM agencies). However, in the absence of any new sources these disused chemicals are expected to degrade into inert components over time. Hence, only those chemicals with current sources of actual or potential endocrine disrupting properties have been included in Appendix 1, along with details of their usage and likely sources into aquatic environments in South Australia.

The EPA regularly conducts a number of monitoring programs and studies on the condition of waters in the state (see <www.epa.sa.gov.au/water_quality.html>), and has included metals, pesticides, PCBs and natural oestrogens within the suite of water quality parameters investigated. This work has helped determine background levels in waters and sediments, and identify areas with elevated concentrations that may be at risk from excess chemicals accumulating in the environment due to human activities in the landscape. For example, the EPA has data and published reports that show:

- locations with elevated metals such as the northern Spencer Gulf from industrial discharges
- Dawesley Creek in the Mount Lofty Ranges impacted by acid mine leachate and associated heavy metals from a disused pyrites mine
- high pesticide concentrations in Cox Creek, Lenswood Creek, and other streams from horticultural and other agricultural activities
- pulp and paper mill impacts at Lake Bonney SE
- risks posed to dolphins, fish and sediments from PCBs and heavy metals discharged from industry into the Port River.

There are also a range of other impact assessment studies and monitoring programs carried out by other government departments and agencies in relation to discharges to inland and coastal waters, generally under license with the EPA. Those with clear relevance to endocrine disruption potential are the various WWTP discharges that typically have elevated metal and phenol concentrations in effluents discharged to the environment, often at toxicant concentrations and potentially able to cause chronic endocrine effects. Other contaminants

such as PCBs and PAHs do not appear to be present at concentrations that are likely to cause toxicant effects (EPA, unpubl. data). However, the capacity to show cause-and-effect responses in the environment from individual chemicals in complex mixtures such as sewage remains elusive, particularly where acute and chronic toxicity responses may occur in addition to the often more subtle effects from endocrine disruption. The work by CSIRO described above in Section 2.4 is expected to clarify the latter effect and provide guidance in managing the risks posed by the presence of potentially hazardous chemicals from WWTPs.

4.1 Major sources of EDCs that require further work

It is important to note that there is nothing particularly different about chemical usage in South Australia compared to elsewhere in the country, or in overseas developed countries, so there are likely to be many similarities in terms of the chemicals that are detected in the environment, as well as their concentrations, fate and effects**.** With this in mind, Ying & Kookana (2002) considered the following priority areas required further work to clarify the risks from EDCs to the Australian environment:

- domestic sewage discharges and the fate and effects from mixtures of chemicals to stream and marine environments. Also related to this is the ongoing application of biosolids to agricultural lands as a soil conditioner/fertiliser and potential effects on soil microbes, as well as chemicals entrained in any agricultural runoff, or leaching to local groundwater
- intensive agriculture due to the widespread use of pesticides over much of the state, and potential to impact on streams, lakes, marine and groundwater habitats from the runoff or leaching of pesticides and associated surfactants
- livestock wastes and associated release of natural hormones from animals to the environment. In 2003, the livestock in Australia included 9.9×10^7 sheep, 8.5×10^7 poultry, 2.7×10^7 cattle and 2.7×10^6 pigs (Australian Bureau of Statistics, Year Book Australia, 2005 cited from <www.abs.gov.au>), so there is considerable potential for livestock to impact on local waterbodies wherever they concentrate near waters
- industrial waste effluents and mining sites. This includes paper and pulp mills that use chlorinated bleaches and compounds, as well as surfactants and phytosterols that may impact on fish and other organisms when discharged to streams, lakes or coastal environments; wool scouring factories that use high concentrations of detergents; and mine sites that leach heavy metals and acid into nearby creeks and groundwater systems
- contaminated industrial sites throughout the urban environment of all major cities and towns. They may include sites contaminated by waste, historical and current pesticide applications and discharges, PCBs, PAHs, heavy metals and other pollutants. Much of this is probably confined to localised soils under current and historical dumps, although local groundwater and depositional areas of nearby urban drains and streams may be impacted by high concentrations of chemicals
- long range transport of organic pollutants through the atmosphere, in recognition of the ubiquitous presence of PCBs, phthalates, organochlorines, PAHs and metals throughout the environment, even in remote locations well away from obvious sources.

There is also concern that some natural and synthetic hormone growth promoting agents (eg trenbolone acetate) that are used to increase the growth, feed conversion efficiency and carcass leanness of beef cattle (DHA 2003), have been shown to demonstrate androgenic

effects, and may also reach the aquatic environment in concentrated runoff from intensive agricultural activities (Manning 2005). In addition, implants containing 17β–estradiol, progesterone, testosterone, trenbolone and zeranol are all registered for use as hormone growth promotants in cattle in all states and territories (DHA 2003). As a result, beef cattle can generally be assumed to pose a risk of adding considerable loads of oestrogenic and androgenic hormones and chemicals to waterways in their excrement, particularly wherever large numbers of animals have direct access to wetlands, rivers and streams.

There are nearly 200 natural plant chemicals called phyto-oestrogens and myco-oestrogens that have some oestrogenic potential; the former are present in whole grains, fibres and soy products, while the latter are produced by fungi growing on cereal crops and other plants (Hewitt & Servos 2001; Manning 2005). These chemicals may cause some adverse effects wherever large concentrations reach rivers and streams, estuaries or marine habitats from human and animal waste. However, the concentrations detected in the major environmental sources from waste water treatment plants and paper mill effluents from overseas studies are generally considered to be too low to cause the adverse responses occasionally seen in wild fish, so their environmental significance remains largely unknown (eg Hewitt & Servos 2001).

Finally, humic substances that cause most of the yellow-brown colour in our rivers and streams, have recently been identified as the cause of hormone-like effects (ie slight feminisation in fish) with increasing concentration; further work continues in order to confirm this effect (Steinberg *et al* 2004). This finding suggests that there are likely to be natural sources of chemicals that have the potential to cause at least some of the endocrine disrupting effects seen with synthetic substances. As a result, natural sources of chemicals also need to be considered when identifying potential hazards and risks from any discharge and release of EDCs to the environment from human activities.

4.2 Progress on identifying concentrations and effects from specific EDC sources in SA

There are some clear lines of investigation that require further work to help determine whether the sources of EDCs to the environment are significant. Some of the major chemicals and high-risk locations in South Australia have been included in Appendix 2. They include locations receiving WWTP discharges and sludge; urban sites and contaminated sediments receiving stormwater and wastes from industrial suburbs in the Adelaide region; Lake Bonney SE and related sites receiving pulp and paper mill discharges; and locations with concentrated agricultural runoff or significant irrigated horticultural areas. Similar sites at risk of showing adverse effects from EDCs in the environment were described for the Canadian landscape by Hewitt & Servos (2001). There is no evidence in the more recent literature considered in this review that points to alternative high-risk areas as a source of adverse effects in the environment.

It is important to note that progress has been made in recent years in relation to monitoring and assessing the effects from effluents discharged from pulp and paper mills, WWTPs and other industries, as well as monitoring levels of heavy metals, pesticides, PCBs and natural hormones in a range of waters in South Australia. This work will contribute towards evaluating the risks posed by these sources to the environment, and may lead to the development of management strategies to avoid or minimise any adverse environmental hazards in the future. However, for risk assessments to be meaningful, research needs to demonstrate whether adverse effects seen at the tissue level in laboratory experiments can be translated to effects

at the population and community level among aquatic life and wildlife in the field. Understanding the way in which chemicals cause effects, for both specific chemicals and mixtures of chemicals, is also needed for regulatory agencies to be able to adequately identify and, where appropriate and possible, recommend and take actions to reduce or prevent risks to the environment from chemicals with endocrine disrupting properties.

4.2.1 Pulp and paper mills

Endocrine disrupting effects from the only pulp and paper mills in the state have been investigated in a joint study funded by government agencies and the current mills' owner, Kimberly-Clark Australia, in the Millicent area in the South East. An evaluation of the discharge from the mills and samples taken from the receiving waters of Lake Bonney was conducted in 2004, and the results from yeast assays[1] showed strong anti-oestrogenic and low androgenic and oestrogenic responses in the effluent from the mill, whereas the lake water in summer showed weak oestrogenic and androgenic responses; lake samples in winter did not show any endocrine disrupting effects due to dilution from winter rains (Kumar *et al* 2006).

In 2005, in response to these findings, the EPA included studies of fish communities in the lake to the existing monitoring program that had focused on aquatic macro-invertebrate and water quality monitoring, to help clarify whether chemicals were preventing fish breeding and impacting on fish health. Incorporation of population assessments of fish living in waters receiving inputs of EDCs has only been reported from a few studies in Canada and Sweden but is required to assess whether the reproductive fitness of fish exposed to EDCs in their natural environment is being compromised (Damstra *et al* 2002; Mills & Chichester 2005).

Preliminary findings to date show that a diverse fish assemblage consisting of young and mature age classes are present in Lake Bonney, including, threatened and rare species at both the national and state levels. Monitoring will continue to determine whether there are any obvious biological signs of endocrine disruption occurring in the lake and may extend to caged fish exposure studies to supplement the fish assemblage monitoring program. Early indications suggest that there is sufficient dilution in Lake Bonney to allow fish and other organisms to survive and, in some cases, thrive in this receiving environment for pulp and paper mill effluent in South Australia (Kumar *et al* 2006; EPA, unpubl. data).

The EPA has also recently evaluated the potential for dioxins and furans to impact on biota in the lake, despite the fact that the major source from the pulp and paper mills to the lake comes from chlorine bleaching, and that form of bleaching ceased in the SE mills in 1991. Not surprisingly, the World Health Organization toxic equivalents concentration (middle bound) for lake water (3.5 pg/L or 0.0000000000035 g/L) and sediments (2.9–5.9 pg/g) were low and comparable to other estuarine habitats in Australia, and not at levels that would cause environmental harm (cf ANZECC & ARMCANZ 2000; Müller *et al* 2004; EPA, unpubl. results; Lake Bonney Coast Care Community Group, unpubl. data). Two species of fish taken from the lake had concentrations of dioxin, furan and dioxin-like PCBs less than the 6 pg/g (fresh weight) action level proposed for NSW and Victoria (Victorian EPA 2007); the European Union has a maximum level for fish fillets and fish products of 8 pg/g fresh weight (EUSC 2001). Concentrations ranged from 4.14–4.5 pg/g fresh weight for Yellow-eyed Mullet and 1.97–2.18

[1] These widely used laboratory tests involve yeast cells that have been modified to harbour either the human oestrogen and androgen receptors, such that when exposed to oestrogenic or androgenic substances the yeast cells express a chemical that liberates a red colour (Routledge & Sumpter 1996).

pg/g fresh weight for Common Galaxias, using the upper bound values from whole fish samples, which represent very conservative values to evaluate the risk from the consumption of fish from the lake (EPA, unpublished 2007 data). The same pattern has been noted from studies in Canada and elsewhere in the world, wherever the pulp and paper sector has reduced or eliminated chlorinated discharges to the environment (Hewitt & Servos 2001). Consequently, dioxins and furans have not been included in Appendix 2 as potential EDCs associated with this industry in SA because any concentration of historically discharged chemicals will remain buried in the sediments of the lake and continue to be degraded by microbial processes in the future.

The EPA and CSIRO have also assessed the concentrations of plant sterols in Lake Bonney sediments and fish and the results for all analytes studied were low (<0.01 ug/g detection levels for the fish and most sediments up to 6.6 ug/g total phytosterols for a lake sediment sample) and unremarkable when compared to overseas data, and not at levels that should cause environmental harm [cf. Manning (2005) and references cited in relation to pulp mills].

Consequently, the chemical or chemicals present in the effluent from the pulp and paper mills in the South East that produced the endocrine related effects in the laboratory tests reported in Kumar *et al* (2006) remains unknown. Such uncertainty is consistent with findings for all other pulp and paper mills studied throughout the world, whereby the source of endocrine disruption has remained elusive despite extensive (and expensive) attempts to identify the chemicals that cause toxic and endocrine effects (Hewitt & Servos 2001; Manning 2005). This is the reason why biological monitoring is important because it can determine if adverse effects are actually occurring in resident populations of plants and animals. If effects occur, then identifying the type of chemical(s) responsible remains an important field of future research, including work aimed at possible treatment options to remove offending chemicals. Alternatively, if no effects are occurring, then it is questionable whether resources should be spent on finding chemicals that cause laboratory effects that cannot be implicated in whole population effects in receiving waters.

4.2.2 Waste water treatment plants (WWTPs)

CSIRO have been coordinating a three-year study (2005–2007) on the fate, exposure and effects of effluent from various waste water treatment plants around the country, including four from South Australia. This work will provide a comprehensive dataset on the concentrations and effects from exposure studies of native fish and yeast assays to discharges from the Bird-in-Hand, Hahndorf, Heathfield and Victor Harbor WWTPs, as well as some additional supplementary data from the Bolivar WWTP (Dr R Kookana, CSIRO, pers. comm.). Preliminary data indicates that there is nothing remarkable about these discharges in terms of concentrations of hormones and other EDCs (eg non-ionic surfactants, bisphenol A, phthalates) in waste water or the laboratory effects data compared to similar studies from overseas discharges (Dr R Kookana and Dr A Kumar, CSIRO, pers. comm.). Dilution in the environment appears to minimise the chance for adverse effects to the wider environment, but discharges of concentrated waste water to streams during low to no flow conditions and the application of reclaimed waste water for irrigation and biosolids for agricultural applications are areas requiring further work.

4.2.3 Background water quality monitoring programs

The EPA's ambient water quality monitoring program and other targeted studies also provide data and knowledge about sites throughout the state that are impacted by the most elevated concentrations of pesticides, metals and hormones from agricultural practices and contaminants from industry and urbanisation. These studies also incorporate an assessment of the aquatic macro-invertebrate communities in inland waters and either seagrass or reef health assessments for coastal and marine waters, to provide evidence that the receiving waters of the state are not showing any obvious unexpected impacts that could be related to an environmental stressor (eg endocrine disruption or toxicant effect). Some of the known high-risk locations have been included in Appendix 2.

In terms of PCBs and dioxins, two studies provide data that indicates the risk to the South Australian environment from past discharges of these chemicals is low and present little concern for this state. A study of PCBs and heavy metals in dolphins, fish and sediment in the Port River and elsewhere in the state showed that the levels in the blubber of dolphins were well below most of the concentrations reported in the recent scientific literature (SA EPA 2000). The fish tested had metal concentrations that met food standards, and were comparable to levels in fish from other parts of the state. The sediments showed some elevated PCB and metal locations associated with historical and possibly some recent contamination that will form the basis for further investigations by the EPA.

Müller *et al* (2004) included samples from the Torrens River, River Murray, Franklin Harbor and Coffin Bay in the national dioxin study recently carried out in Australia. The results showed that the sediments had low concentrations of dioxin-like chemicals compared to other states, which were generally at levels considered too low to cause adverse effects. Sampling of three species of fish from the Adelaide area showed very low levels but the results from bivalve sampling at Coffin Bay yielded anomalous results, with one sample of oysters exceeding a level that warranted further investigation. A subsequent analysis of an additional sample from the same location showed much lower results. The overall message from the study indicated that the background concentrations from sites sampled in Australia were low and not likely to present a widespread concern. However, as noted above, there are hot-spots in the environment (eg parts of the Port River) where concentrated point-source discharges or deposits of waste occur and have the potential to cause adverse effects on the local environment.

Recent work in 2006 on oestrogen concentrations in the environment from dairying and grazing land-uses in South Australia indicates that most agricultural streams have concentrations of 17β–oestradiol in the range 0.3–3.9 ng/L, that are generally higher than the predicted no effect concentration (PNEC) for fish of 1 ng/L (Appendix 3). Similarly, another oestrogen called oestrone has been recorded at concentrations of 0.06–14 ng/L for surface streams whereas the PNEC is around 3–5 ng/L. Even higher values were also recorded from drains receiving dairy waste water, suggesting localised effects from concentrated waste from livestock operations may be possible. However, the high organic loading of such receiving environments and associated low dissolved oxygen concentrations would tend to select for air-breathing, tolerant pest species such as introduced carp and goldfish compared to more sensitive native species. Complementary work on yeast assays indicates that while oestrogen is present in agricultural streams there are also anti-oestrogenic chemicals present that may mask the potential adverse effects posed by the oestrogen, or provide additional effects on resident fauna (CSIRO and EPA, unpubl. data). Further work focusing on fish communities in

high-risk habitats may be needed once all the results from this study have been analysed and published. Some questions that could be investigated include:

- Is there a higher incidence of intersex or unusual sex ratio in fish from locations with elevated oestrogen concentrations compared to a reference condition from well vegetated catchments with limited to no livestock grazing?
- Is reproductive success of fish impaired in streams with the higher oestrogen concentrations?
- Are rare and endangered fish species limited to catchments and habitats with low oestrogen concentrations and limited grazing and human sources of oestrogen?

One of the most conclusive endrocrine disrupting relationships currently recorded is that of tributyltin (TBT) and its impacts on marine invertebrates. As noted earlier, there have been numerous studies showing imposex in marine molluscs due to nanogram per litre (ng/L) concentrations of TBT in waters near marinas and harbours (Bryan, 1986; IPCS, 1990). In 2004 the Australian Government initiated a program in response to the banning of TBT as an antifoulant on ships, called 'The Tributyltin (TBT) Analysis Protocol Development and Current Contamination Assessment'. The Port River, Boston Bay and Coffin Bay were sampled from South Australia as part of this study and TBT was detected in bivalves and, to a lesser extent, in sediment on all three sites. While this project was not designed as an ambient program, it demonstrated that locations frequented by large vessels have significant concentrations of TBT (and its metabolites) at levels that could potentially be causing an impact on surrounding populations of sensitive organisms. These sites are expected to be re-sampled again in 2013 to look at the impact of the ban over time, with the expectation that concentrations will decline over time.

Nias *et al* (1993) have shown that significant populations of the gastropod *Lepsiella vinosa* have been affected by imposex throughout the Port River and Barker Inlet in South Australia. However, they were not able to link these occurrences wholly to TBT effects due to the presence of other potential EDCs (metals, hydrocarbons, etc) in this industrialised port and associated habitats.

5 EXPOSURE PATHWAYS

Wildlife can be exposed to EDCs by a number of pathways, including water, air, soil and sediments, and the food organisms eat. In terms of aquatic organisms, the major routes of exposure are absorption of dissolved chemicals in the water through the gills or gut, ingestion of sediment and consumption of contaminated food (Manning 2005).

Some EDCs are present in the environment at very low concentrations that may be measured in ng/L (ie 0.000000001 g/L) or lower, have low persistence and moderate mobility (eg 17β–estradiol), whereas others such as nonylphenol are present in ug/L (eg 0.000001 g/L), are more persistent, have low mobility in water and bind readily to sediments (Ying *et al* 2004). Consequently, the risk of EDCs to animals (and humans) is dependent on the concentration, fate and behaviour of these chemicals in the environment, as well as on the manner in which wildlife are exposed to these chemicals.

A number of other factors also contribute to the risks from EDCs including:

- synergistic or antagonistic effects from combinations of chemicals entering the environment (as invariably happens with most discharges and stormwater runoff from urban, industrial and agricultural areas)
- the degree of dilution in the receiving environment and particularly locations where there is limited dilution and the highest concentrations of chemicals and possibly impacts on resident animals may occur (eg lowland streams and estuaries)
- the time required before environmental exposure leads to adverse biological outcomes (eg sensitive life stages such as eggs and larvae cannot avoid long-term exposure compared to more tolerant and mobile adult stages of fish and other animals that can move elsewhere).

Further discussion about issues relating to wildlife and human exposure from EDCs can be found in Damstra *et al* (2002).

6 DOES THE PRESENCE OF EDCS MATTER? WHAT ARE THE RISKS TO OUR ENVIRONMENT?

There has been a steady growth in the use of risk assessment approaches to identify and manage water quality in Australia and internationally largely due to recognition that the presence of particular chemicals and other environmental stressors does not always mean that adverse biological effects will follow.

The most recent national water quality guidelines for fresh and marine waters have incorporated an ecological risk-based approach in developing guidelines for different types of ecosystems and issues (ANZECC & ARMCANZ 2000). They include a process for determining the level of risk posed by stressors and toxicants (eg chemicals) to the survival and health of aquatic ecosystems. The guidelines also describe key performance indicators and trigger levels or concentrations that indicate that there is a significant risk that adverse biological affects may occur (Hart *et al* 1999). However it is important to emphasise that the listed concentrations are estimations of hazard and not values that, if exceeded, indicate harm will necessarily occur. If exceeded, values are intended to *trigger* the incorporation of additional information or further investigation to determine whether or not a real risk to the environment exists and, where possible, to adjust the trigger values into regional, local or site-specific guidelines (ANZECC & ARMCANZ 2000).

The guidelines recommend the use of decision frameworks for chemical stressors and contaminants that involve a series of decisions aimed at assessing whether there is some risk of an impact occurring. Establishing decision frameworks for ecological risk assessment can involve determining whether there are modifying effects in the environment that affect bioavailability, toxicological studies or direct toxicity assessment relevant to the site in question, or field biological assessments.

Consequently, the process of ecological risk assessment helps determine if chemicals are likely to have an adverse impact on environmental values (eg populations and communities of animals and plants), and includes a consideration of both the likelihood (or frequency) and consequence (or severity) of a particular problem. For example, a low-risk evaluation would probably be appropriate if an extremely toxic substance was discharged to the environment but was then contained so that no actual exposure occurred to organisms in receiving waters. However, if a less hazardous substance was released in large volumes it could result in a major impact on the receiving environment; that would then represent a high risk to the environment. It is important to note, however, that unlike human health risk assessment, that seeks to identify risks to individuals, ecological risk assessments aim to characterise risks to populations, species and ecosystems.

In terms of applying a full risk assessment towards EDCs in South Australia, it is important to note that there are significant limitations to our current knowledge that prevent a complete evaluation of risk to the environment from being made at this time. This is because the majority of suspected EDCs have limited to no available data on the amount of any specific chemical or combination of chemicals needed to cause a particular adverse biological effect. In many cases, the available data is only from laboratory studies based on evaluating cellular and tissue responses. Data on subsequent effects for whole individuals, much less populations in the field, is generally lacking which presents a serious dilemma because it is not clear if these effects would be observed in environmentally relevant concentrations for long enough exposure periods to cause declines in populations of aquatic life and wildlife. Another serious

problem is that there have been few studies that have shown a credible cause-and-effect relationship between the presence of a chemical in the environment and adverse responses seen in the field; particularly where numerous chemical and non-chemical stressors may be responsible either alone or in combination in mixtures. This means that any estimation of the likelihood of harm can only be based on using the precautionary principle argument for whatever reliable data is available, and acknowledging that any assessments have a degree of speculation associated with them. The fact that many EDCs produce biological effects at trace (ug/L) and ultra-trace (ng/L and pg/L) concentrations obviously makes this particularly difficult in terms of detection and showing cause-and-effect relationships in the field with these types of substances.

Given the fact that large volumes of potential and known EDCs have been discharged and used throughout much of the landscape in SA during both historical and current day usage, focusing work on the most likely sources of large concentrations containing mixtures of EDCs (eg WWTPs and pulp and paper mills) appears to be a sensible line of investigation for assessing whether adverse biological effects consistent with endocrine disruption are evident in nearby receiving environments. Confirming the concentrations of relevant chemicals will provide valuable background data to help characterise the risk posed by chemicals in the environment but a more effective field of study would incorporate biological effects monitoring in the receiving environment, with particular attention on endocrine disruption effects that could include measures of the growth, sex ratios and/or population persistence for sensitive species.

Sumpter & Johnson (2005) highlight the fact that problems with natural and steroidal oestrogens in the environment were not proposed by anyone actually suggesting there may be a problem with these chemicals; it was because multiple lines of evidence pointed at these chemicals *after* problems were noted in fish populations. These authors go on to suggest that the aim should not be to assess the impact of each particular chemical in the environment but to determine if the collective mixture of chemicals is, or is not, adversely affecting biodiversity and its sustainability in ecosystems. And in any evaluation of impacts from discharges (and spills) to the environment, it should also be emphasised that there are often other significant adverse biological effects from adding waste water to natural water that may be as, or more significant, than endocrine disruption effects. For example, major losses and changes in biological communities may result from the effects of discharges of waste water that cause major reductions in dissolved oxygen levels or significant pH and salinity changes, temperature extremes, excessive nutrient loadings, or some sort of acute and/or chronic toxicant response. Careful study designs are needed to allow the effects of endocrine disruption to be assessed along with other adverse physical and chemical effects that are invariably associated with human sources of waste water disposed into the environment.

It appears that sewage effluent the world over contains a number of potential EDCs at concentrations that have the *potential* to impact on aquatic wildlife (cf. Harries *et al* 1998 and references therein). They include natural hormones (17β–estradiol and estrone) and the more potent (up to 20 times) synthetic oestrogens (17α–ethynylestradiol) at concentrations <1 ng/L–308 ng/L and <1 ng/L–10 ng/L, respectively; phyto-oestrogens (eg isoflavonoids) ranging from 3–83 ng/L; and industrial chemicals with oestrogenic activity such as nonylphenol (up to 1 ug/L) and bisphenol A (13–36 ng/L)(cf. Ying *et al* 2002; Manning 2005). It is also important to note that many other chemicals known to possess weak oestrogenic activity (eg phthalates, pesticides such as dieldrin, atrazine, simazine) are also frequently

detected in sewage effluents, and others no doubt remain to be identified but may contribute to adverse effects on biota inhabiting receiving environments.

The concentrations of natural hormones in receiving waters where sewage is discharged have been described from studies in the USA, various European countries and Japan. Most were in the 1–10 ng/L range but occasional extremes have been noted, including up to 93 ng/L of 17β–estradiol, 112 ng/L of estrone, and 800 ng/L of 17α–ethinylestradiol (Koplin *et al* 2002; Manning 2005).

The UK Environment Agency has reviewed the available data and proposed several predicted no effect concentrations (PNEC) for the protection of freshwater life and marine life from these hormones: 0.001 ug/L for 17β–estradiol, 0.003–0.005 ug/L for estrone, and 0.0001 ug/L for 17α–ethinylestradiol (Young *et al* 2002). In the absence of any Australian guideline values for these chemicals, it appears that these concentrations could be used in South Australia as preliminary thresholds beyond which some adverse environmental effects may potentially occur. Similarly, the European Chemicals Bureau has recommended that levels of alkylphenol polyethoxylates (eg nonylphenols and octylphenols) below 0.33 ug/L are unlikely to have adverse effects in fish because they adsorb onto particles and accumulate in sediments where they are then slowly broken down by microbial processes (Manning 2005). So, again, in the absence of any alternative approach, this value can be used as a trigger level to highlight areas at risk from elevated concentrations of these industrial chemicals in the environment.

A summary of the predicted no effect and lowest effect concentrations for these and other well-studied EDCs is included in Appendix 3. The values cited provide a series of low-risk trigger levels that could be used to describe areas where continued monitoring may be suitable for low-risk environments, but for high-risk instances where the concentrations (PNEC values in the appendix) are exceeded, then further ecosystem specific investigation may be needed to determine whether ecosystem impacts have occurred.

7 CONCLUSION

There are a number of actions that need to be carried out to ensure that the risks posed by EDCs to the South Australian environment are effectively assessed and if needed, managed, to prevent harm to the environment and human population. The most significant step will involve focusing attention on those reaches of streams and coastal and marine environments where the most concentrated wastes from known sources are likely to occur. The next and most important step will involve work to determine whether any effects are actually adversely impacting on the local wildlife. This approach has been discussed in part by Damstra *et al* (2002) and advocated by Sumpter & Johnson (2005), and directs attention away from endlessly measuring chemicals to focusing on evaluating whether harm is occurring to the environment.

Given our limited knowledge about the presence of different EDCs in our waters, there may be value in carrying out a short-term (1–2 years) environmental study of surface and groundwaters throughout the state to provide data on the environmental concentrations and loads of EDCs in our waters, thereby allowing the potential human and environmental health risks to be more accurately assessed. This could include chemical monitoring of ambient and targeted waters (eg WWTP discharges, urban and industrialised land uses, agricultural and control sites from national parks and areas with significant remnant native vegetation) and biological effects monitoring (eg laboratory and field exposure experiments). Collectively, this data could be used to evaluate thresholds or guideline values for the protection of humans and wildlife in a risk assessment that can also incorporate synergistic actions of oestrogenic EDCs for specific waters in the state. Such a study design would subsequently require risk management of all major sources of EDCs to the environment and include an evaluation of treatment options to effectively remove any EDCs that occur in high enough concentrations to pose a risk to humans or wildlife [cf. Bursch *et al* (2004) for a description of a similar study design for Austria].

Based on the data from a range of overseas studies and more limited studies in Australia, it appears that the risks to humans from exposure to EDCs via drinking water within Australia are likely to be negligible because the concentrations of EDCs are invariably below those considered to result in harm to human health (Falconer *et al* 2003). This is partly the result of single-use drinking water supplies, modern filtration and treatment processes and because most waste water discharges occur into lowland streams and the marine environment and not into the catchments of drinking water supply reservoirs. The discharge from the Hahndorf WWTP into the Mt Bold-Happy Valley reservoir system in the Onkaparinga River catchment is, however, an obvious exception and the subject of a current research study involving CSIRO and the water authorities (Dr R Kookana, CSIRO pers. comm.).

In terms of future directions relating to EDCs in SA, the EPA should carry out the following steps:

- continue to monitor the international literature on EDCs for evidence of human and wildlife health effects; methods for monitoring EDCs and evaluating their ecological effects; and the efficacy of new waste water treatments in removing specific EDCs. This is important given the large investment of research funds in the USA, Canada, European Union and Japan into the identification and assessment of risks posed by EDCs, and as already noted, the fact that most developed countries are faced with managing the same sorts of chemicals that are released into the environment by human activities at the same

sorts of concentration ranges. The recently proposed national framework for chemicals management in Australia would provide a logical means to improve the sharing and integration of environment agency knowledge in the assessment and regulation of chemicals (EPHC 2006)

- coordinate monitoring at targeted sites of known or potential EDCs in streams and coastal sites that receive licensed discharges of treated waste water. The low rainfall and associated low river flows and limited flushing of estuarine habitats throughout most of the state provides the potential for pollutants from discharges (eg point source waste water from industry and diffuse runoff from agriculture) to accumulate (EPHC 2006). Similarly, many of the state's coastal environments do not mix and dilute thoroughly during tidal cycles (Pattiaratchi & Jones 2005), making it possible for discharges from sewage treatment works to remain concentrated and cause adverse effects around outfalls. Chemical characterisation studies aimed at understanding the concentrations and fate of chemicals in receiving waters would help assess the ongoing risks from continued discharges to designated waters in the state. Complementary biological monitoring should be carried out to clarify if an ecological effect is evident and significant [cf. lesson 1 described by Sumpter & Johnson (2005)], determine what is causing the effect and why, and thereby help direct attention at specific chemicals that require further treatment aimed at avoiding or mitigating adverse impacts in the future
- continue to work with CSIRO and other research organisations with an interest in EDCs to ensure the knowledge gained from any studies carried out in SA is widely disseminated and locally relevant. Future work may contribute towards improved methods for monitoring EDCs, or suggest alternative sewage treatment approaches to reduce the potential adverse effects from EDCs discharged to the environment, or risk mitigation strategies for specific agricultural pesticides or perhaps specific intensive animal keeping industries
- continue to evaluate any unusual biological observation that indicates some sort of pollutant may be having an adverse effect on an animal group in the aquatic environment. This includes surface waters such as streams, urban and industrial wetlands, and coastal marine fauna, and also groundwater when our knowledge of underground fauna improves in the future. Given the wide range of chemicals and substances that can affect natural waters, as well as the effects from flooding and drought, it will be important to develop an understanding of the natural variation in ecosystem health that may result from differences in the physical (eg temperature, dissolved oxygen, turbidity, flow), chemical (eg nutrients, colour, pH, endocrine disruptor), toxicant (eg ammonia, sulfide, organic contaminant) and biological (eg microbes) composition of our state's waters if we are to truly protect them adequately.

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APPENDIX 1 KNOWN AND SUSPECTED EDCS FOR AQUATIC ENVIRONMENTS IN SOUTH AUSTRALIA

Modified from Hewitt & Servos (2001) and Ying & Kookana (2002)

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
Pesticides	2,4-D	Defoliant and post-emergent herbicide	Suspected	Agricultural runoff	Keith (1997)
	Atrazine	S-triazine herbicide: selective herbicide used for weed control in agriculture	Promotes breast cancer in laboratory rats; immunotoxic effects	Agricultural runoff, urban stormwater	Keith (1997)
	Carbaryl	Contact insecticide	Suspected	Agricultural runoff	Keith (1997)
	Chlorpyrifos	Non-systemic, broad range, organophosphate insecticide (acetylcholine esterase inhibitor)	Suspected	Agricultural runoff	Keith (1997)
	Cypermethrin	Synthetic pyrethroid insecticide effective as contact pesticide, barrier treatment or repellent. *APVMA permits for SA expired in June 2006*	Suspected	Agricultural runoff, stormwater runoff	Keith (1997)
	Endosulfan	Contact organochlorine insecticide	Environmental anti-oestrogen; reduces steroid hormone levels	Agricultural runoff	Depledge & Billinghurst (1999)
	Mancozeb	Contact fungicide in the dithiocarbamate chemical group, used on a wide range of legume, vegetable and pulse crops	Suspected; contains manganese that may impact on the pituitary gland and at the level of gonadal steroid production	Agricultural runoff	Keith (1997)

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
	Methomyl	Carbamate insecticide (contact and stomach action) for control of moths (also thrips and nematodes)	Suspected	Agricultural runoff	Keith (1997)
	Permethrin	Synthetic pyrethroid insecticide. *APVMA permits expired in SA in June 2006*	Suspected	Agricultural runoff	Manning (2005)
	Simazine	Pre-emergent triazine herbicide	Suspected	Agricultural runoff, stormwater runoff, WWTP discharges	Keith (1997)
	Trifluralin	Pre-emergent herbicide	Suspected	Agricultural runoff	Keith (1997)
Industrial chemicals and pollutants Organo-halogens	Dioxins and furans (PCDD/PCDFs)	Present in a variety of pesticides, the wood preservative pentachlorophenol and chlorinated phenols. Also formed during the combustion process, including bushfires, the burning of fossil fuels, wood, garbage and motor vehicles	Environmental anti-oestrogen	Emissions to air from industry and fires, and diffuse deposition to waters. Also possibly in WWTPs receiving trade waste from industry (eg foundries and manufacturers of chlorinated products), industrialised harbours	Depledge & Billinghurst (1999)
	PCBs, PBBs and PBDEs	Chlorinated biphenyl isomers used in making electrical and hydraulic equipment, dielectric fluids, fire retardants, heat transfer agents	Oestrogenic and thyroid hormone effects	Industrialised harbours, WWTPs and contaminated stormwater from historical industrial use. Now banned from production in Australia.	Keith (1997); Hewitt & Servos (2001)

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
	2,4-Dichlorophenol and perhaps various other phenolics (eg 4-chloro-2 methylphenol)	Used in organic synthesis and in the making of 2,4-D. Also a wood preservative, antiseptic and seed disinfectant	Suspected	Agricultural runoff, WWTP discharges	Keith (1997); Manning (2005)
	Tributyltin (TBT)	Anti-fouling paint applied to boats. *APVMA cancelled registration for this product in 2003.*	Unknown mechanism of action but TBT causes imposex in female dogwhelks and other molluscs	Slipways, marinas, harbours and ports	Smith (1981)
Phthalates	Di-2-ethylhexyl phthalate, Butyl benzyl phthalate, Di-n-butyl phthalate, Di-n-pentyl phthalate, Di-hexyl phthalate, Di-propyl phthalate, Dicyclohexyl phthalate, Diethyl phthalate	Plasticisers (makes plastics flexible) for polyvinyl chloride (PVC), resins, toys, food wraps, containers; also used in heat-seal coatings, solvents, cosmetics, glues, explosives, inert ingredient in pesticides	Suspected environmental oestrogens	WWTP discharges, contaminated urban runoff, localised groundwater under municipal tips and industrial sites	Keith (1997)
Phenols	Bisphenol A, and perhaps Bisphenol F	Plasticiser used in the manufacture of polymers, epoxy resins (lining food cans and water pipes), dyes, polycarbonates, fungicides, flame retardants, rubber, antioxidants, plastic dental fillings	Environmental oestrogens and thyroid hormone antagonists	WWTP discharges, contaminated urban stormwater	Depledge & Billinghurst (1999); Zoeller *et al* (2005)

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
Non-ionic surfactants/ alkylphenol polyethoxy-lates (APEs)	Nonylphenols, octylphenols, pentaphenols, butylphenols	Breakdown products of various alkylphenol ethoxylates, which are surfactants used in industrial and domestic detergents (eg washing wool), also in some paint and pesticides	Oestrogen mimics	WWTPs, biosolids applications to agricultural lands, contaminated urban stormwater, industrial sites	Jobling & Sumpter (1993); White *et al* (1994)
Polynuclear aromatic hydrocarbons (PAHs)	Benzo(a)pyrene, Benz(a)anthracene, Benzo(b/k)fluor-anthene	Found in petroleum, wax and smoke	Suspected; possible environmental oestrogens	Contaminated industrial sediments, WWTPs, contaminated urban stormwater	Keith (1997)
	Anthracene	Dyestuffs	Suspected	Contaminated industrial sediments, WWTPs, contaminated urban stormwater	Keith (1997)
	Pyrene	Occurs in coal tar and used in biochemical research and in making many other PAHs. Results from the incomplete combustion of wood and fossil fuels.	Suspected	Contaminated industrial sediments, WWTPs, contaminated urban stormwater	Keith (1997)
	Phenanthrene	Used in the synthesis of dyestuffs and explosives. Results from the incomplete combustion of wood and fossil fuels	Suspected	Contaminated industrial sediments, WWTPs, contaminated urban stormwater	Keith (1997)

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
Heavy metals	Cadmium	Natural sources and major use as fusible alloy in electroplating; also nickel plating, engraving, Ni-Cd batteries, amalgam in dentistry	Suspected; toxicant that affects hormone status	Runoff from agricultural lands through application of biosolids, runoff or leaching from contaminated industrial sediments to urban stormwater or local groundwater	Depledge & Billinghurst (1999)
	Lead	Natural sources and used in making containers for corrosive liquids, alloys, storage batteries, cable coverings, plumbing, ammunition, glass, paint, petrol, insecticides	Strongly suspected; toxicant that affects hormone status	Runoff from agricultural lands through application of biosolids or pesticides, runoff or leaching from contaminated industrial sediments to urban stormwater or local groundwater	Keith (1997); Depledge & Billinghurst (1999)
	Mercury	Natural sources and used in the recovery of gold from ores, making instruments, lamps and signs, batteries, antifouling paint	Strongly suspected; toxicant that affects hormone status	Runoff from agricultural lands through application of biosolids, runoff or leaching from contaminated industrial sediments to urban stormwater or local groundwater, contaminated sediments near slipways in harbours and ports	Keith (1997); Depledge & Billinghurst (1999)

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
	Arsenic	Natural sources and used as alloying agent, in bronzing, poisons and insecticides	Strongly suspected; toxicant that affects hormone status	Runoff from agricultural lands through application of biosolids or pesticides, runoff or leaching from contaminated industrial sediments to urban stormwater or local groundwater	Keith (1997); Depledge & Billinghurst (1999)
Natural products Natural hormones	17β–Estradiol	Natural hormone excreted continuously by women and men	Natural oestrogen	WWTP discharges and agricultural runoff (from stock)	
	Estrone	Natural hormone produced as breakdown product of 17β –estradiol. Excreted continuously by women and men	Natural oestrogen	WWTP discharges and agricultural runoff (from stock)	
	Estriol	Natural hormone produced as breakdown product of estrone		WWTP discharges and agricultural runoff (from stock)	
	Testosterone	Natural hormone excreted by men and women	Natural androgen	WWTP discharges and agricultural runoff (from stock)	

Classification	Examples of endocrine disrupting chemicals	Chemical use	Mode of endocrine disruption action	Likely sources of input to aquatic environments in SA	Selected reference(s) to endocrine disrupting effects
Phyto-oestrogens and myco-oestrogens	Isoflavonoids, coumestans, lignans	Plant sterols naturally produced in diverse range of plants	Oestrogenic effects	Agricultural runoff, WWTP discharges	Keith (1997); Lagana *et al* (2004) recorded concentrations (<7–384 ng/L) in an Italian WWTP
	β-sitosterol and stigmasterol	Plant sterols naturally produced in diverse range of plants	Oestrogenic and androgenic effects	Agricultural runoff, pulp and paper mill effluent, WWTP discharges	Damstra *et al* (2002)
	Zearalenone and derivative compounds	Toxins produced by several *Fusarium* species of fungi, colonising cereal grain crops	Oestrogenic effects	Agricultural runoff, WWTP discharges, runoff from intensive beef cattle activities (relates to areas wherever zeranol is used as a growth hormone)	Manning (2005); Lagana *et al* (2004) recorded low concentrations (<20 ng/L) in an Italian WWTP
Synthetic chemicals/ pharmaceutic-cals	17α–ethinylestradiol	Synthetic hormone used in the contraceptive pill and in hormone replacement therapy	Synthetic oestrogen	WWTP discharges and municipal dumps	Damstra *et al* (2002)
	Mestranol	Synthetic hormone used in oral contraceptives	Synthetic oestrogen	WWTP discharges	Damstra *et al* (2002)
	Tamoxifen	Anti-cancer medication that blocks the effects of oestrogen in the body	Anti-oestrogenic	WWTP discharges	Ashton *et al* (2004) report detections from WWTP effluent of 0.02–0.04 ug/L

APPENDIX 2 COMPLEX MIXTURES IN SOUTH AUSTRALIAN AQUATIC ENVIRONMENTS KNOWN OR SUSPECTED TO HAVE THE POTENTIAL TO AFFECT ENDOCRINE FUNCTION IN AQUATIC BIOTA

Mixture	Potentially associated chemicals	Possible sites in South Australia affected by EDCs
Pulp and paper mill effluents	Natural wood derivatives Plant sterols	Lake Bonney SE English Gap drain (receives effluent from the mills) The shallow aquifer associated with the drain
Municipal effluents and urban runoff	Non-ionic surfactants (eg alkylphenol ethoxylates or APEs) Natural and synthetic hormones (human, animal and plant) Bisphenol-A PAHs Phthalates Polybrominated diphenyl ethers (PBDPEs) Pesticides	Bolivar WWTP (major urban) discharge to the marine environment Victor Harbor, Heathfield, Hahndorf WWTP discharges (minor urban), Bird-in-Hand WWTP and council STEDS discharges in rural areas to rural streams West Lakes Patawalonga Port River
Contaminated sediments	PAHs PCBs and metabolites PCDD/DFs Heavy metals Phthalates TBT (shipyards only) Pesticides	Wingfield Landfill groundwater Port River (shipyards, drain outfalls, depositional areas) West Lakes Patawalonga Torrens Lake Water supply reservoir sediments and Sturt River flood control dam
Agricultural runoff	Pesticides Heavy metals Natural hormones (eg 17β –estradiol, estrone, stilbenes, flavonoids)	Lower Murray swamps SE drains Disposal evaporation basins on the River Murray floodplain Cox and Lenswood Creeks (horticulture)
WWTP sludge application to agricultural land; waste water reuse schemes	APEs and metabolites Natural and synthetic oestrogens Heavy metals	Sites in Mount Lofty Ranges where sludge has previously been applied (cf. SA Water records) Virginia

APPENDIX 3 PREDICTED NO EFFECT CONCENTRATIONS (PNEC) AND LOWEST TESTED CONCENTRATION AT WHICH AN EFFECT OCCURRED (LOEC) FOR MAJOR EDCS THAT ARE CURRENTLY CONSIDERED PROTECTIVE OF THE ENVIRONMENT.

Also included is the range of results from selected parameters that have been measured from surface waters in South Australia by EPA monitoring programs and in joint work with CSIRO.

Chemical	Measured in SA ambient surface waters (ug/L) or sediment (ug/kg)	PNEC (ug/L)	LOEC (ug/L)	Species affected	References
17β –estradiol (E2)	Surface waters range 0.00027–0.0039; dairy effluent range 0.0022–0.0087	0.001	0.01–1	Fish	Young *et al* (2002); see references cited in Mills & Chichester (2005)
Estrone (E1)	Surface waters range 0.00006–0.014; dairy effluent range 0.0082–0.039	0.003-0.005	0.1–1	Fish	Young *et al* (2002); see references cited in Mills & Chichester (2005)
17α–ethinyl-estradiol (EE2)	–	0.0001	0.0017–0.003	Fish	Young *et al* (2002); Segner *et al* (2003); Mills & Chichester (2005)
Tributyltin	4–85 ug/kg in Port River estuary	0.0004	0.0074–0.0278	Marine invertebrates (eg molluscs)	SA EPA (1997); ANZECC &ARMCANZ (2000); USEPA (2003)
Bisphenol A	Up to 0.02 ug/L in Port River	1.6	10	Fish	Bursch *et al* (2004); Mills & Chichester (2005); CSIRO unpubl. data
Nonylphenol and octylphenol	Pt River up to 0.7 ug/L and WWTP discharges range 1.2–7 ug/L	0.33	0.5–1	Fish and snails	Oehlmann *et al* (2000); European Chemicals Bureau (2002) and Seki (2003) cited in Manning (2005); Mills & Chichester (2005); CSIRO unpubl. data
Atrazine	<0.02–1.94	0.1	0.7	Frogs	ANZECC & ARMCANZ (2000); Hayes *et al* (2002)