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Review of Endocrine Disrupting Chemicals (EDCs)

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Important Disclaimer:

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Abbreviations used in the report

APE	alkylphenol ethoxylate
BPA	bisphenol A
DDD	(1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE	(1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT	(1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
DES	diethylstilbestrol
E1	estrone
E2	17β-estradiol
EA	Environment Australia
EE2	17α-ethinyl estradiol
EDC	endocrine disrupting chemical
EU	European Union
GEDRI	Global Endocrine Disruptor Research Inventory
IFCS	Intergovernmental Forum on Chemical Safety
MF	microfiltration
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NP	nonylphenol
NSW	New South Wales
OECD	Organization for Economic Cooperation and Development
OP	octylphenol
PAC	powdered activated carbon
PARSCOM	Paris Commission
PBB	polybrominated biphenyl
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PPCP	pharmaceutical and personal care product
QLD	Queensland
SA	South Australia
TBT	tributyltin
TGA	Therapeutics Goods Administration
UF	ultrafiltration
US EPA	US Environmental Protection Agency
USGS	US Geological Survey
WEAO	Water Environment Association of Ontario

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Executive summary

CSIRO was commissioned by Meat and Livestock Australia for a short-term study to provide a strategic review of the issue of Endocrine disrupting chemicals (EDCs) and its likely implications for the meat processing industry.

The specific objectives of the project were to (i) review current knowledge, and the position of key International and Australian authorities, concerning endocrine disruptors; (ii) identify EDCs that may be associated with red meat processing industry; (iii) summarise the treatment technologies for the removal of EDCs from waste stream; and (iv) to suggest priority areas for research that would benefit the meat processing industry to deal with the issue.

State of knowledge on EDCs

In recent years, compelling evidence has been accumulated showing that certain chemicals (e.g. estradiols, nonylphenol, bisphenol A, PCBs and some pesticides) at elevated concentrations can cause disruption to endocrine systems and hormonal control of development in aquatic organisms and wildlife (Hayes et al. 2002; Damstra et al. 2002). These chemicals have been termed as endocrine disrupting chemicals (EDCs).

Evidence on the effects of exposure to endocrine disrupting chemicals on wildlife is substantial, including some reports from Australia. Observed endocrine disruption effects include imposex of molluscs by organotin compounds; developmental abnormalities, demasculisation and feminisation of alligators in Florida by organochlorines; feminisation of fish by waste water effluent from sewage treatment plants and paper mills; hermaphrodism in frogs from pesticides such as atrazine. In contrast, while some published reports suggest endocrine disruption effects on human health such as decrease in semen quality and increase in cancer (testicular and breast cancer) rates, a causal relation between exposure to chemicals and adverse health effect in humans has not been firmly established, except in a few cases such as DES (a synthetic hormone) causing reproductive and developmental problems.

Position of national and international agencies

Australia is one of the over 140 governments and associated partners that are members of the Intergovernmental Forum on Chemical Safety (IFCS). A report by IFCS on the “Global assessment of the state-of-science of environmental endocrine disruptors”, concluded that while there is now sufficient evidence demonstrating the effect of EDCs on certain wildlife species, the cause and effect relation is not conclusive for human health (Damstra et al. 2002).

USA, Japan and several European countries are investing massive resources towards better understanding the EDC issue. Since US congress included endocrine disruption in the amended Safe Drinking Water Act in 1996, USEPA established a screening program for a large number of potential EDCs. Simultaneously, a national monitoring program for EDCs and pharmaceutical chemicals in rural and urban streams across USA is being carried out by US Geological Survey (USGS). The USEPA and the Organization of Economic and Cooperative Development (OECD) are developing tiered procedures for rapid testing and assessment of EDCs. From Europe, a number of studies have reported the presence of estrogenic compounds and associated endocrine disruption in aquatic organisms in freshwater and marine environments. European union and USA are developing programs on transatlantic co-operation in human and environmental health issues such as EDCs. EDCs are clearly a high priority research issue, internationally.

Populist literature and community concern

The community concerns and the populist literature on EDCs has grown immensely since the publication of the book “Our Stolen Future" (Theo Colborn et al. 1996). Currently media interest in EDCs is high and a range of EDC related issues are being widely covered, the extent of which can be judged from the EDCs press link on the homepage of Our Stolen Future (http://www.ourstolenfuture.org/). In Australia, the issue of EDCs is of current media interest, (The

Australian April 2002, Australian Doctor October 2002; ABC Radio National Earthbeat June 2003). ABC Radio National ran a series to two half-hourly programs on EDCs under the Earthbeat program in June/July 2003.

Potential EDCs in meat processing industry

A wide variety of chemicals are known or suspected EDCs. These include pesticides, persistent organochlorines, alkyl phenols, heavy metals, phytoestrogens, and synthetic and natural hormones. Among the potential EDCs that may be associated with meat processing supply chain, the two most important groups include alkylphenols in detergents and cleaning agents (e.g. nonylphenol based surfactants) and hormones (e.g. excretion of natural hormones produced by animals or synthetic hormones used as growth promoters). While the levels of alkylphenols in wastewater from meat processing plants are expected to be much higher (ug/L) than hormones (ng/L), their potency as EDCs is much lower (approximately 10-5) than hormones. Both groups of chemicals are well known EDCs and research about their endocrine disruption effect on wildlife (in vitro) is conclusive. Other chemicals such as pthalates and pesticides may also be present in the waste stream.

Treatment technology

Little attention has so far been given to the removal of EDC's in the treatment methods currently used. However, indications are that commonly used physical solid/liquid separation and biological methods may remove between 51 and 90% of the total estrogenic activity during biological wastewater treatment.

While a range of technologies may be of potential use in the meat processing industry, a combination of advanced oxidation (chemical or photochemical) and tight membrane filtration (such as reverse osmosis) may provide the optimum removal for the greatest variety of EDCs. However, both processes are energy-intensive and consequently expensive to operate. An alternative could involve anaerobic/aerobic treatment of the high strength wastewater using a submerged membrane bioreactor with advanced oxidation treatment of the permeate to ensure maximum degradation of any EDCs transported through the membrane unit.

Priority research area for meat processing industry

Despite the fact that currently there are no regulatory or policy guidelines, many governmental and private agencies are taking proactive measures to deal with EDCs. The following areas are recommended for priority action.

- The meat industry should initially focus on the risks associated with potential release of alkylphenols (nonylphenols and octylphenols) and hormones through wastewater in the environment. Desktop studies together with some strategic monitoring of the levels of the target EDCs in waste stream from meat processing plants are desirable.
- The effectiveness of current treatment processes and the extent of breakdown of EDCs in the receiving environments (land or water) need to be established.
- The industry should develop an inventory of the current use of alkylphenol ethoxylate based cleaning agents and explore the feasibility for replacement of nonylphenol based products.
- The extent of use of hormones as growth promoting substances in Australia and their likely endocrine disruption impact on the environment need to be considered.
- The industry should invest in R& D on (i) development of rapid screening tools for detection of EDCs and (ii) development of suitable treatment technology for removal of EDCs from wastewater.

Introduction

CSIRO was commissioned by Meat and Livestock Australia for a short-term study to provide a strategic review of the issue of Endocrine disrupting chemicals (EDCs) and its likely implications for the meat processing industry.

The specific objective of the project was to review current knowledge, and the position of key International and Australian authorities, concerning endocrine disruptors in general, and more specifically, to evaluate the risk they pose to the red meat industry

The terms of reference for the study were as follows:

1. Perform a concise review of international technical, scientific and populist literature concerning the topic and identify the position of major environmental groups;

2. Hold discussions with appropriate environmental and health agencies in Australia to identify and summarise their current and likely future position on the issue of endocrine disruptors.

3. Identify any endocrine disruptors known and/or likely to be used or present in the meat processing supply chain (hormones, detergent ingredients, etc) and summarise the current scientific position on these chemicals. This includes evaluating the extent to which the science is speculative, controversial or simply incomplete.

4. Summarise the performance of clean technology, best practice and treatment technologies or practices in removing, or reducing endocrine disruptor concentrations in waste streams, both solid and liquid.

5. Recommend priority areas for research that would benefit the meat processing industry in respect of the issue.

These terms of reference are addressed in following sections, each one in a separate chapter. In chapter 1, a short review of scientific literature on EDCs has been provided. An extensive list of references has been provided for further reading. In this chapter, the authors have kept the scope of the study broad to provide information on a wide range of classes of compounds that fall in the EDC category. In chapter 2, the position of Australian environmental and health agencies has been provided. This is based on authors' on-going interactions (meetings, workshops) with these agencies over the last year. For the sake of confidentiality, no particular reference has been made to Meat and Livestock Australia in these meetings. To identify the EDCs likely to be associated with the meat industry (Chapter 3), authors have focussed on meat processing plants only. While the chapter does not consider the waste stream from intensively managed systems such as feedlots, it is recognised that animals would be kept in holding yards temporarily at the meat processing facility. A review of current waste treatment technologies has been provided by Prof Waite in chapter 4, in which emerging promising technologies have been mentioned.

In chapter 5, we have identified some priority research areas for the meat processing industry and made some recommendations for initiation on research and monitoring in some areas.

Chapter 1: A concise review of literature on EDCs[1]

For decades, hormone-like effects of chemicals have been observed in fish, wildlife and humans, at levels of exposure that in many cases exceed the normal environmental concentrations (Colborn et al, 1993; Guillette et al. 1994; Kavlock et al. 1996; Jobling et al. 1998; Sonnenschein and Soto, 1998, Damstra et al*.* 2002).

In recent years, compelling evidence has been accumulated showing that certain chemicals (e.g. estradiols, nonylphenol, bisphenol A, PCBs and some pesticides) at elevated concentrations can cause disruption to endocrine systems and hormonal control of development in aquatic organisms and wildlife (Hayes *et al.* 2002; Damstra *et al.* 2002). These chemicals are termed as endocrine disrupting chemicals (EDCs).

Evidence on the effects of exposure to endocrine disrupting chemicals on wildlife is mounting. These include imposex of molluscs by organotin compounds (Alzieu, 2000; Gibbs et al., 1990; Horiguchi et al., 1994); developmental abnormalities, demasculisation and feminisation of alligators in Florida by organochlorins (Guillette et al., 1994 and 2000); feminisation of fish by waste water effluent from sewage treatment plants and paper mills (Jobling et al., 1998; Bortone et al., 1989); hermaphrodism in frogs from pesticides such as atrazine (Hayes et al. 2002).

There are also reports that human testicular and breast cancer rates have been increasing during the last four decades, especially in developed countries (Brown et al., 1986; Hakulinen et al., 1986; Adami et al., 1994; Feuer, 1995; Moller, 1993; Ries et al., 1991; Wolff et al., 1993). However, except in a few cases (e.g. DES), a causal relation between exposure to chemicals and adverse health effect in humans has not been firmly established.

Endocrine disruption effects reported so far in Australia include:

- abnormal reproductive and developmental functions in offspring of women who took DES and thalidomide (Colborn et al., 1996),
- imposex of molluscs in harbours caused by TBT in antifouling paints (Daly and Fabris, 1993; Kohn and Almasi, 1993; Burt and Ebell, 1995),
- reduced gonopodium size of male mosquitofish (*Gambusia a. holbrooki*) exposed to sewage effluent in NSW (Batty and Lim, 1999),
- decreased fertility of sheep in WA caused by phytoestrogen in pasture grasses (Bennetts et al., 1946; Adams, 1998) and
- decreased breeding success of the peregrine falcon in South Australia being associated with high organochlorine residues (Falkenberg et al., 1994).

However, the presence of EDCs in the Australian riverine environment and their implications are not clear (Lim et al. 2000; Ying and Kookana, 2002).

What are EDCs?

An endocrine disruptor is “an exogenous substance or mixture that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (Damstra et al. 2002).

From reports in literature, a wide range of chemicals have been found or suspected to be capable of disrupting the endocrine systems (Table 1). The list of EDCs include:

1 Partly based on reviews recently published by the authors

- pesticides (e.g. DDT, vinclozolin, TBT, atrazine),
- persistent organochlorines (e.g. PCBs, dioxins and furans),
- alkyl phenols (e.g. nonylphenol and octylphenol),
- heavy metals (e.g. cadmium, lead, mercury),
- phytoestrogens (e.g. isoflavoids, lignans, β-sitosterol), and
- synthetic and natural hormones (e.g. β-estradiol, ethinyl estradiol).

A comprehensive list of EDCs, as compiled by Ying and Kookana (2002) from different sources is provided in Table 1. Many of these compounds have little in common structurally or in terms of their chemical properties (Figure 1), but evoke agonist (similar) or antagonist responses, possibly through comparable mechanisms of action.

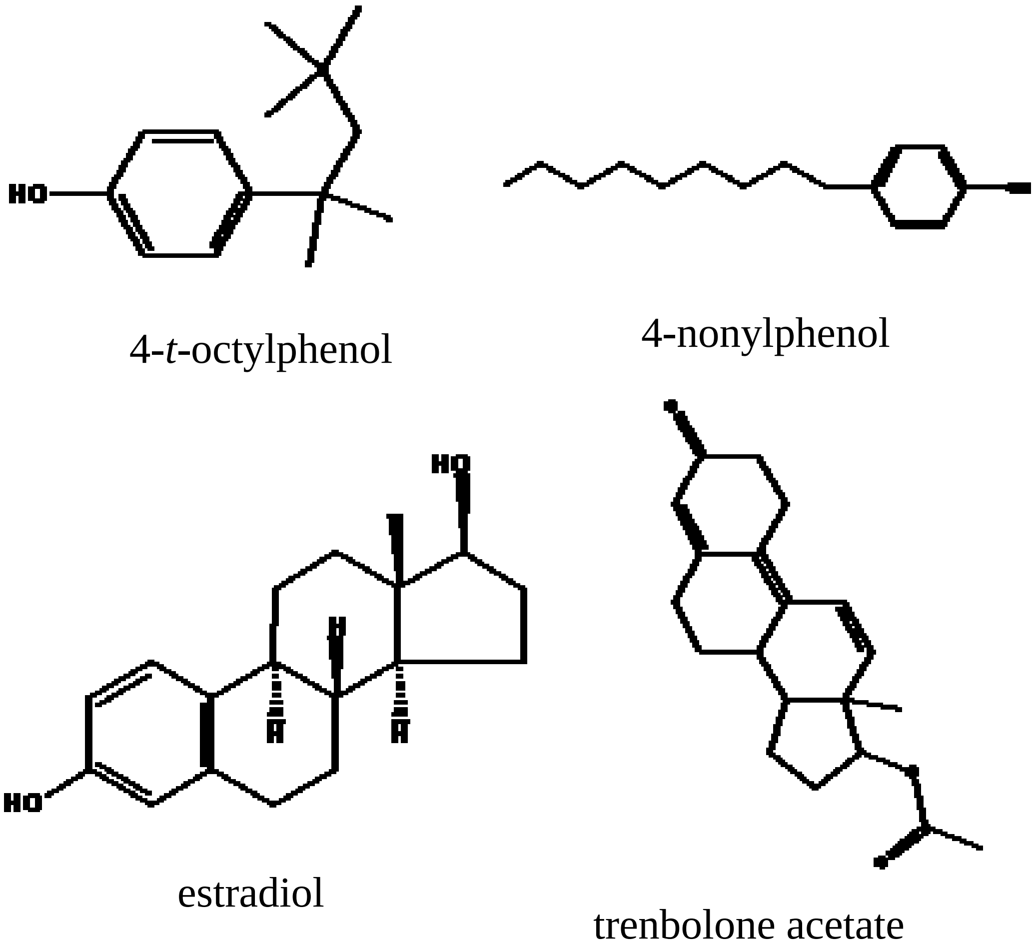

Figure 1. Chemical structure of four common EDCs.

Table 1. List of suspected/known endocrine disrupting chemicals (EDCs) compiled from literature by Ying and Kookana (2002)

Classification	Endocrine disrupting chemicals	
Pesticides	2,4-D	Kepone (Chlordecone)
	Atrazine	Lindane
	Benomyl	Malathion
	Carbaryl	Mancozeb
	Cypermethrin	Methomyl
	Chlordane (γ-HCH)	Methoxychlor
	DDT and its metabolites	Mirex
	Dicofol	Parathion
	Dieldrin/Aldrin	Pentachlorophenol
	Endosulfan	Permethrin
	Endrin	Simazine
	Heptachlor	Toxaphene
	Hexachlorobenzene (HCB)	Trifluralin
	Iprodione	Vinclozolin
Organohalogens	Dioxins and furans	PBBs and PBDEs
	PCBs	2,4-Dichlorophenol
Alkylphenols	Nonylphenols	Nonylphenol ethoxylates
	Octylphenols	Octylphenol ethoxylates
	Pentaphenols	Butylphenols
Heavy metals	Cadmium	Mercury
	Lead	Arsenic
Organotins	Tributyltin (TBT)	Triphenyltin (TPhT)
Phthalates	Di-ethylhexyl phthalate	Di-hexyl phthalate
	Butyl benzyl phthalate	Di-propyl phthalate
	Di-n-butyl phthalate	Dicyclohexyl phthalate
	Di-n-pentyl phthalate	Diethyl phthalate
Natural Hormones	17β-Estradiol	Estriol
	Estrone	Testosterone
Pharmaceuticals	Ethinyl estradiol	Tamoxifen
	Mestranol	Diethylstilbestrol (DES)
Phytoestrogens	Isoflavonoids	Zearalenone
	Coumestans	β-sitosterol
	Lignans	
Phenols	Bisphenol A	Bisphenol F
Aromatic hydrocarbons	Benzo(a)pyrene	Anthracene
	Benz(a)anthracene	Pyrene
	Benzo(b/h)fluoranthene	Phenanthrene
	6-hydroxy-chrysene	n-Butyl benzene

Before discussing the mechanisms of endocrine disruption, it is pertinent to describe the endocrine system briefly.

Endocrine system

An endocrine system is found in nearly all animals, including mammals, non-mammalian vertebrates (e.g. fish, amphibians, reptiles and birds) and invertebrates (e.g. snails, lobsters, insects and other species). Along with the nervous system, the endocrine system is one of the two communication systems that regulate all responses and functions of the body.

The endocrine system consists of glands and the hormones they produce that guide the development, growth, reproduction and behaviour of humans and animals. The major endocrine glands of the body include the pituitary, thyroid, parathyroids, adrenals, pancreas, pineal gland and gonads (ovaries in females and testes in males).

Hormones are biochemicals that are produced by endocrine glands in one part of the body, travel through the bloodstream and cause responses in other parts of the body. They act as chemical messengers and interact with specific receptors in cells to trigger responses and prompt normal biological functions such as growth, reproduction and development.

Hormones generally fall into four main categories: (1) amino acid derivatives, (2) proteins, (3) steroids and (4) eicosanoids (Lister and van der Kraak, 2001). The unifying nature of hormone action is the presence of receptors on target cells, which bind a specific hormone with high affinity and stereospecificity. Steroid and thyroid hormones act by entering target cells and stimulating specific genes. All other hormones bind to receptors on the cell surface and activate second-messenger molecules within the target cells. The body has hundreds of different kinds of receptors; each one is designed to receive a particular kind of chemical signal. The hormone and its receptor have a 'lock-and-key' relationship. The binding of the hormone with the receptor triggers the production of particular proteins that 'turn on' the biological activity associated with the hormone.

Subtle effects on the endocrine system can result in changes in growth, development or behaviour that can affect the organism itself, or the next generation (Guillette et al., 1996; vom Saal et al, 1997; Palanza et al., 1999). Hormones play a crucial role in the proper development of the growing fetus. Embryos and fetuses are especially sensitive at particular times to low doses of endocrine disruptors (Guillette et al., 1996; vom Saal et al, 1997; Palanza et al., 1999). Substances that have no effect in an adult can become poisonous in the developing embryo. The timing of exposure may be more important than the dose. The ultimate effects of endocrine disruption might not be seen until later in life or even until the next generation (Colborn et al., 1996; US EPA, 1997).

Mechanisms of endocrine disruption

There are several ways that chemicals can interfere with the endocrine system (Sonnenschein and Soto, 1998). They can mimic or block natural hormones, alter hormonal levels and thus affect the functions that these hormones control. Less direct interferences involve alteration of the body's ability to produce hormones, interference with the ways hormones travel through the body and changes in numbers of receptors. Regardless of the situation, having too much or too little of the hormones it needs may cause the endocrine system to function inappropriately.

EDCs can cause endocrine disruption through a range of mechanisms by acting as:

(1) environmental estrogens, e.g. methoxychlor, bisphenol A;

(2) environmental antiestrogens, e.g. dioxin, endosulfan;

(3) environmental antiandrogens, e.g. vinclozolin, DDE, Kraft mill effluent;

(4) toxicants that reduce steroid hormone levels, e.g. fenarimol and other fungicides, endosulfan;

(5) toxicants that affect reproduction primarily through effects on the central nervous system, e.g. dithiocarbamate;

(6) toxicants that affect hormone status, e.g. cadmium, benzidine-based dyes (Ying and Kookana, 2002).

Potency and environmental fate of EDCs

The potency of the endocrine disruptors i.e. their effectiveness in binding with the receptor and turning-on the response, varies greatly among various classes of EDCs (Table 2). Most EDCs have very low potency, as their chemistry is significantly different from the hormones they mimic. In addition to potency, the potential for a hormone-like effect depends on dose. For most of the endocrine disruptors, the dose-response relationship has not yet been established, especially at the low dose range, and this may differ from species to species.

Table 2 Potency of selected EDCs (relative to Estradiol) measured by E-screen

(Gutendorf and Westendorf,2001).

Compound	E-Screen Potency
Estradiol (E2)	1
Estrone (E1)	0.01
Ethinyl Estradiol (EE2)	1.25
Nonylphenol (NP)	1.3E-5
Octylphenol (OP)	1.0E-4
Bisphenol A (BPA)	2.3E-4
Sitosterol	9.6E-5

The risk of endocrine disruptors to humans and wildlife also depends on their behaviour and fate in the environment. Given the wide range of the compounds that can act as EDCs, their fate in the environment varies greatly with the nature of compound in question. While 17β estradiol is generally found at ng/L levels in the environment, shows shorter persistence in water (less than a week) and moderate affinity for sorption on organic carbon (K_{oc}) (Ying et al., 2002a), in contrast, endosulfan pesticide is usually detected at µg/L level in waterways, shows much longer persistent in water (50 days half-life) and has much higher K_{oc}. The high value of K_{oc}, representing greater sorption affinity for organic carbon, indicates the potential association of the compound with water or sediments. During treatment process a compound with high K_{oc} value (e.g. nonylphenol) is likely to settle out rather with sludge than remaining in wastewater.

The environmental fate and behaviour of EDCs vary greatly with temperature, and oxidation state of the media and as a result, their breakdown rates and persistence are different in different environmental compartments such as water, sediment and air. Even the rate of removal of EDCs during the treatment process in the sewage treatment plants has been found to depend on several factors including chemical nature of the compound, the treatment technology and climatic conditions (Schäfer et al. 2002).

Sources of EDCs in Australian Environment

The EDCs are released in the environment from a wide variety of sources such as domestic sewage disposal, intensive agriculture, industrial wastes, mining activity, and landfills. Suspected EDCs can now be found in every compartment of our environment (air, water, soil, sediment and biota), in industrial products and household items and even in the food we consume. They are often found in mixtures, such as effluents from sewage treatment plants, paper mills and textile factories. However, It is not clear whether the components in a mixture act additively, synergistically or antagonistically.

The important sources of EDCs in Australia include humans, animals, agriculture, mining and other industries. These are briefly discussed below.

Human sources through domestic sewage effluents

Humans contribute to the load of EDCs in the wastewater stream both directly and indirectly. We not only use synthetic EDCs in our daily life (e.g. detergents, pharmaceuticals) but also excrete natural hormones from our body, which are released into the environment through wastewater disposal (Ying et al., 2002a and b). For example, 17β estradiol is the main female sex hormone produced by ovarian cells whereas the 17α-ethynylestradiol (EE2) is a synthetic hormone, and is used in contraceptive pills. Both find their way into the environment. The daily excretion rates of natural estrogens for women varies with age and other conditions, and the average is estimated to be in the lower microgram range, with a maximum of 64 µg/day reported for estriol (Ternes *et al.*, 1999). On the other hand the oral contraceptive contains between 30 and 50 µg of EE2 per pill. Consequently, sewage effluents and biosolids are known to contain hormones and various other classes of chemicals known to be endocrine disruptors. These include hormone steroids, surfactant degradation products (e.g. nonylphenol), organohalogens (e.g. PCBs, chlorophenols), phthalates, PAHs, heavy metals (e.g. Hg, Pb, Cd) and pharmaceutical residues (Lim *et al.,* 2000).

The loading of domestic wastewater with EDCs is expected to be greater around the major centres of population around Australia. However, since sewage effluent is increasingly being used as a source of irrigation, it also serves as a source in riverine environments in peri-urban and rural areas. In Australia, more than 5000 ML of sewage effluents are generated every day from municipal sewage treatment plants. Most of the sewage effluents are directly discharged to aquatic and marine environments and in 2000 only 11% was reused (Dillon, 2001). However, more than 70% of the biosolids are reused as fertilizer on land, and the chemicals in biosolids may be persistent and may lead to runoff to surface water or leach into groundwater.

Animals and Livestock wastes

The population of livestock in Australia is much larger than the human population. Indeed there are at least 10 livestock animals to every human being in Australia. The total number of livestock animals in Australia include sheep - 115×10^6, cattle -26.5×10^6, pigs - 2.63×10^6 and poultry -93.6×10^6, plus other animals. Livestock wastes contain not only nutrients (N and P) but also hormone steroids excreted from animals. Little is known about the excretion rates of hormones by various animals, and their loading on the riverine environment in Australia in intensive livestock production areas. Indeed, the high density of livestock in certain areas, such as at feedlots and piggeries, can constitute a potentially significant source of EDCs and veterinary pharmaceuticals in the environments.

Intensive agriculture

Intensive agriculture is another important source of endocrine disruptors in Australia. Pesticides have been and are widely used in Australian crops. Many of them are potential endocrine disruptors such as atrazine, trifluralin, endosulfan, vinclozolin, DDT, lindane. And, many pesticide products also contain surfactants, which could degrade into alkyl phenols, such as nonylphenol. Pesticides have been a concern due to their bioactive properties and extensive application in rural and urban environment. In addition, the biosolids use in agriculture as a fertilizer and wastewater use for irrigation can add to the sources of EDCs linked to agriculture.

Industrial waste effluents and mining sites

Australian industries also release wastes that contain endocrine disruptors. Effluents from paper and pulp mills discharge into rivers, lakes and coastal environment in Australia. Those effluents may contain chlorinated compounds (chlorophenols, dioxins), surfactants (nonylphenol) and/or phytosterols, which could affect fish in the receiving water. Australia is one of the most important wool producers in the world. Wool scouring uses large quantities of detergents during the process and the waste effluent contains very high concentrations of surfactants and their degradation products such as nonylphenol. Heavy metals from mining activities have become a problem in some areas, especially in older lead, zinc, copper, gold and silver mines. Mine-wastes constitute a potential source of EDC contamination to the environment, where heavy metals and acid are released.

Chapter 2: Current Position of Various National and International Agencies on EDCs

The following section summarizes the current position of various national and international agencies on the issue of endocrine disrupting chemicals (EDCs) in the environment. The notes are based on personal communications and information published in literature/web.

International Agencies

In April 2000, a meeting of the environment ministers of the G8 group of industrialized countries listed EDCs as one of the high priorities and called for a furtherance of knowledge acquisition on EDCs through jointly planned and implemented projects and international information sharing (Loder, 2000).

Inter-governmental Forum on Chemical Safety

The Intergovernmental Forum on Chemical Safety (IFCS) is the key international group established in 1994, to implement the Agenda 21, Chapter 19 (Environmentally Sound Management of Chemicals). Australia is one of the over 140 governments and associated partners that are members of the IFCS. Inter-governmental Forum of Chemical Safety (IFCS). The IFCS considered EDCs at the meeting at Ottawa, Canada in 1997, and identified EDCs as an emerging issue. The IFCS has taken the lead in coordinating the 'Global Endocrine Disruptor Research Inventory' (GEDRI) and the development of the 'Global Assessment of the State-of-the-Science of Endocrine Disruptors'. Their recent report on the "Global assessment of the state-of-science of environmental endocrine disruptors", which can be downloaded from - http://www.who.int/pcs/pcs_new.html), concluded that there is now sufficient evidence demonstrating the effect of EDCs on certain wildlife species. However, for human health, the cause and effect relation is not conclusive, except in some circumstances of occupational exposure (Damstra et al. 2002). Consequently, due to considerable uncertainties, it remains a priority research issue. Australian agencies are in agreement with the assessment of IFCS.

Environmental Protection Agency of USA and US Geological Survey

Considering the importance of EDC issue, US congress included endocrine disruption in amended Safe Drinking Water Act in 1996. As a result of this USEPA formed an Advisory Committee (EDSTAC) to report to the Congress and a screening program was established for a large number of chemicals. This screening program continues. Simultaneously, a national monitoring program for pharmaceutical chemicals and EDCs in rural and urban streams across whole of USA is being carried out by US Geological Survey (USGS). Detection of hormones and other EDCs at trace levels has been published by Kolpin et al. (2002).

Recent monitoring studies by USGS on antibiotics in waste streams from piggeries and animal feedlots located in six states of USA have revealed that 95% of samples contained one or more of three commonly found antibiotics (chlortetracycline, sulfamethazine, lincomycin) (Meyer et al 2003, Personal communication). Both EDC and pharmaceutical and personal care products (PPCP) are also associated with biosolids, and can be released in the terrestrial environment.

USEPA is placing a considerable emphasis on minimizing the adverse effect of PPCP on the environment and has initiated an environmental stewardship program called "The Green Pharmacy" (http://www.epa.gov/nerlsesd1/chemistry/ppcp/greenpharmacy.htm). Clearly, there is a high degree of awareness of EDCs and PCPP related environment issues in the USA, both of which are relevant to meat processors in Australia.

Surveys of some new emerging endocrine disrupting chemicals (e.g. nonylphenol and steroids) in major rivers of some countries have been undertaken (e.g. Naylor et al., 1992; Blackburn et al., 1999; Ahel et al., 2000; Tabata et al., 2001). The US EPA and the Organization of Economic and Cooperative Development (OECD) have invested considerable resources to develop tiered procedures for the testing and assessment of EDCs (Fenner-Crisp et al., 2000; Huet, 2000; Parrott et al., 2001). The US EPA planned to screen 15,000 chemicals for their possible effects as endocrine disruptors in animals and humans (Macilwain, 1998).

European Union

In Europe, there is relatively higher sensitivity towards the EDC issues, "including hormones in meat". There have been considerable discussions during the mid nineties between EU and USA, on the issue of the use of hormones for animal growth. The EC Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) has the opinion that, because no safe threshold could be established for any of the hormones used for animal growth, exposure to even small traces in meat carries a risk, and that of the various susceptible risk groups, pre-pubertal children is the group of greatest concern because of the extremely low level of endogenous production of hormones by pre-pubertal children. Consequently, it was proposed to the EU Council and the European Parliament in 2000 to definitively ban the use of 17β-estradiol and its ester-like derivatives in farm animals and to maintain provisionally the prohibition for growth promotion of all other substances having an estrogenic, gestagenic or androgenic effect until more complete scientific information becomes available. EC is also seeking data from USA, Australia and New Zealand on hormones in beef. Several scientific studies supported by EC are in progress.

The Environment Agency of the United Kingdom believes that "some fish populations are at risk of exposure to steroid oestrogens in rivers that receive sewage effluents. Where this occurs in sufficient quantities, these substances will feminize male fish (both their early-life stage development and throughout their life). This compromises their ability to reproduce. These harmful effects have implications for the sustainability of fish populations. And, because fish are considered a sensitive indicator, also for the wider environment" (EA 2002).

Water Environment Association of Ontario

Water Environment Association of Ontario **(**WEAO) published the finding of a review and stakeholders concern regarding contaminants in wastewater and biosolids in April 2001 (WEAO, 2001). This report places EDCs (especially estrogenic hormones) in category II, for which currently there is insufficient data to assess the risks.

Australian Agencies

Several Australian agencies are interested in the endocrine disruption issue. The Australian Academy of Science organised a forum "Endocrine disruption: Australia's role in an international issue" in April 1998 and emphasized the need to work out the risks, and the ways that the chemicals affect humans and wildlife, and also the levels which are hazardous in Australian environment (Australian Academy of Science, 1998). Some Australian Industry sectors have already acknowledged the public concern about the potential health effect through exposure to endocrine disrupting chemicals. Sydney Water listed endocrine disruption as one of the water quality issue in its five-year drinking water quality management plan (Sydney Water, 2000). In the plan, Sydney Water will support research in this area through its research partnership arrangements and closely monitor the research conducted worldwide.

Environment Australia (EA) and Therapeutics Goods Administration (TGA) under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) has significant input into the existing industrial chemicals programs of the OECD, and represent Australia on the IFCS and its assessment programs. Environment Australia also released a paper in April 1998 on EDCs, recognizing the need for basic science and sound data including the environmental fate and bioavailability of EDCs. The paper recognized that the risks of EDCs to ecosystem, animal and human health under Australian conditions are unclear. Australia supports the recommendations of the IFCS (see above) and has been actively participating in surveys and meetings within the OECD Chemicals Program. A recent initiative of EA on this issue is the National Dioxin Program under which a national level monitoring study of dioxin levels in the environment is being conducted. Environment Australia is keeping an active link with international agencies on this issue.

Currently the regulatory agencies of Australia believe that “further work is needed to refine and clarify the measurement of trends in human reproductive health. The scope of research on wildlife should be extended to cover a range of populations, not just those affected by point sources of pollution. To ensure responsible chemical regulation, the greatest benefit will derive from providing input where expertise and resources allow and from utilizing international efforts to guide Australia's national activities and decisions”. Australia is in touch with scientific developments and is committed to national and international opportunities for risk reduction related to EDCs.

NSW EPA and state regulatory agencies have been keeping a “Watching Brief” on the EDC issue. In addition, NSW EPA has carried out a literature review and also is actively researching this aspect in collaboration with the University of Technology, Sydney. The EPA of South Australia is planning to carry out a risk assessment to develop a strategy to address the EDC issue appropriately.

Water supply and reuse industries in Australia are particularly concerned about the EDC issue and are responding in various ways. ***Sydney Water*** has included EDC as an issue in its 5-year Drinking Water Quality Management Plan. ***SA Water*** has recently completed a review of literature on EDCs and is developing R&D program through the CRC for Water Quality and Treatment.

The Nature Conservation Council of NSW published a paper on chemicals in sewage including EDCs (Randall and Abood, 2001) and recommended improvement of effluent quality by reducing pollutant loads at the source; limiting the disposal of effluent to receiving water bodies, including groundwater resources; and improving Sewage Treatment Plants to improve the quality of effluent. The identification of contamination pathways and the need to increase the barriers between contaminants and human and natural environments was seen to be crucial in this respect. It called in the long term for the reduction of oral hormone in-take by humans and animals, including reducing the use of supplement growth hormones in animal feed and human food stocks.

Environment Protection and Heritage Ministerial Council established a high-level task force to scope the issues associated with EDC and other chemicals. The council recognised the need for a national approach to consideration of environmental issues in the management and regulation of chemicals.

Since the publication of the book "*Our Stolen Future*" (Theo Colborn *et al*. 1996), public concerns about the adverse effects of chemicals on reproductive systems of wildlife and human beings have grown immensely. Media coverage, such as in *TIME* (October 30, 2000 issue), in which links between exposure to certain chemicals and endocrine disruption in humans (early puberty in girls) is hinted, has further enhanced these concerns. USGS has just published (Kolpin *et al.* 2002) the results from a monitoring study on 139 streams in heavily populated areas of US, reporting 80% of streams contaminated with trace levels of pharmaceuticals, hormones and organic wastewater contaminants. *The Australian* (March 23-24, 2002), covered this under the headlines “Alarm as drugs flood waterways”.

In Australia, the issue of EDCs is of current media interest, (The Australian April 2002, *Australian Doctor* October 2002; *ABC Radio National Earthbeat* June 2003). ABC Radio National ran a series to two half-hourly programs on EDCs under the Earthbeat program in June/July 2003.

Chapter 3: Endocrine disruptors known and/or likely to be used or present in the meat processing supply chain

The chemicals that may be associated with meat processing supply chain include synthetic (such as detergent and cleaning agents) and natural chemicals (such as hormones excreted by animals). Some of these are known to be endocrine disrupting chemicals. These chemicals may be released in the environment through the waste streams originating from the meat processing units. A schematic diagram of the various form of wastes and their potential disposal forms have been depicted in the following diagram (Figure 2).

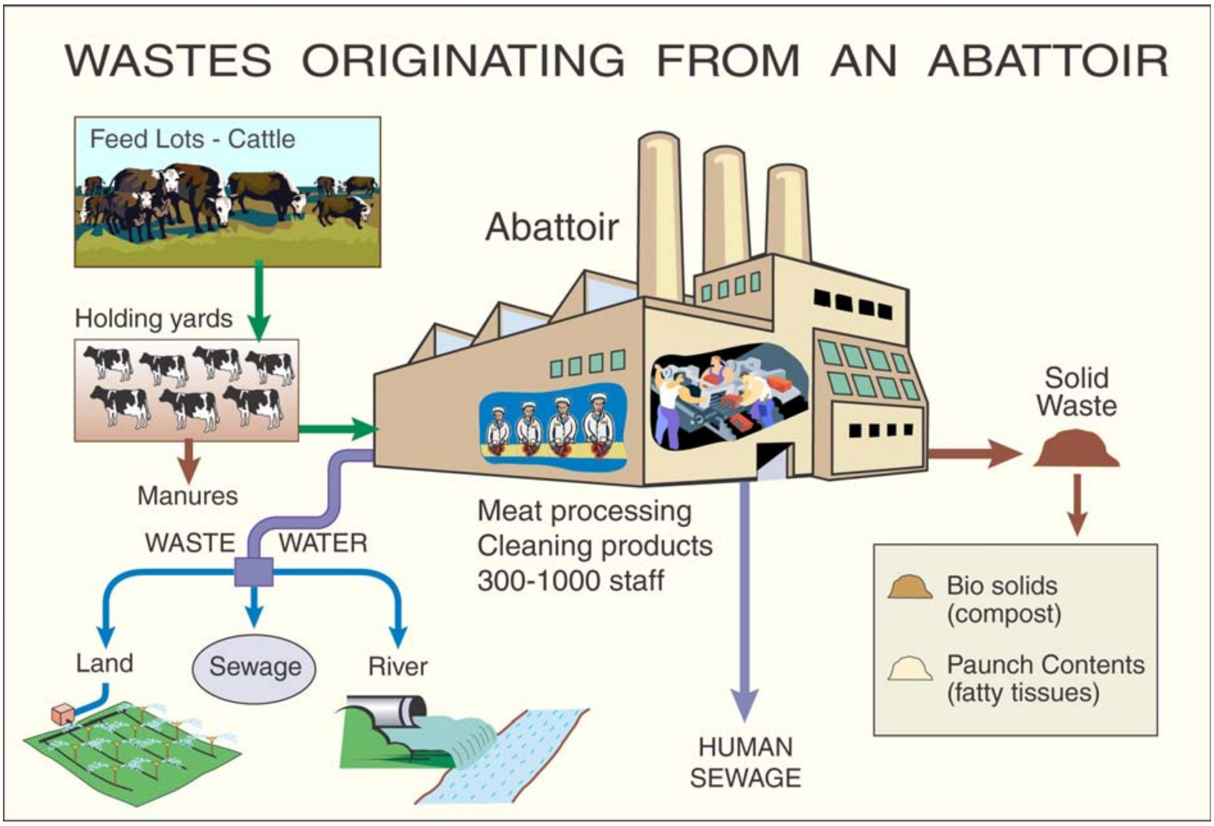

Figure 2. A schematic diagram of wastes associated with meat processing.

The specific chemicals belonging to various classes and the current scientific position of these chemicals is summarized in Table 3, and are also briefly discussed below.

Detergents and surfactants

Due to hygiene requirements at the meat processing plant, detergents constitutes the most important group of chemicals that is used daily in meat processing. The alkylphenol ethoxylates (APEs) surfactants present in detergents, especially nonylphenol and octylphenol based APEs, are of main concern from the point of views of their direct toxicity and endocrine disruption effect. The breakdown products (nonylphenols and octylphenol) of these APEs are weakly estrogenic compounds. For their potency relative to hormones, refer to Table 2. This led to policy measures and a voluntary ban on APE use in household cleaning products and restrictions on industrial applications, starting from 1995 in many European countries.

European Union (EU) has taken several policy measures to address the environmental risks associated with nonylphenol (NP) and nonylphenol ethoxylates (NPE). EU has recommended that comprehensive phase-outs under Directive 76/769/EEC are applied to those industries which contribute most to the regional concentration and/or for which alternatives to NPE are known to be available. These are industrial, institutional and domestic cleaning, textiles, leathers, agriculture (veterinary medicines), metals, pulp and paper, and cosmetics. It is believed that this measure would eliminate some 70% of the nonylphenol burden on the environment.

In Europe, a voluntary ban on the use of NPE in domestic detergents has been agreed by all the major manufacturers of detergents. Paris Commission (PARCOM) Recommendation 92/8 required signatory countries to achieve the phase out of nonylphenol ethoxylates in domestic detergents by 1995 and in all detergent applications by 2000.

Recently, the EU has adopted the Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). The details of which can be found at the URL:

(http://europa.eu.int/comm/enterprise/chemicals/legislation/markrestr/ongowk/recentmodif.htm).

The directive states: "In order further to protect the environment, the placing on the market and the use of NP and NPE should be restricted for specific uses which result in discharges, emissions or losses to the environment."

Our investigations, albeit limited, revealed that Australian abattoirs do use the nonyl phenol and octyl phenol detergent ingredients in relatively concerning amounts. For example, in meat processing plants, depending upon the size of the processing unit, tens to hundred litres or kilograms of detergents are used daily. The water used during cleaning is also high. For example, as a rough estimate 100L of a detergent products containing NPE are used with a 0.5 to 1 megalitre of water use daily. This can potentially result in afew hundred micrograms of alkylphenols per litre of water (μg/L or parts per billion). There is a need of carrying out a more thorough survey and analysis of current use of NPE products in the industry.

The actual risk of endocrine disruption in an organism through the wastewater discharge in the environment will depend upon several factors, such as wastewater treatment processes, disposal methods, environmental conditions and the exposure pathways and sensitivity of the receptor organisms.

Hormones

The source of hormones is the natural production and excretion of hormones by animals and humans at the processing plant. Animals in the holding yards of the plant and the workers at the unit may constitute a significant source of natural hormones in the waste stream (manure and sewerage) from the processing plant.

While the extent of use of growth promoting hormones in the Australian meat and livestock industry is not clear, this may potentially constitute another source of hormones, (e.g. Trenbolone acetate). Trenbolone acetate has been found to have strong androgenic effect on fish (Ankley et al, 2003).

Hormones being the most potent endocrine disrupters are undoubtedly of significant concern in relation to their potential impact on human and ecosystem health. The debate on European demand on hormone-free meat is well publicised. Hormones in runoff water from lands amended with manures have been noted (e.g. Steele. 1995).

Other chemicals (Pharmaceuticals, Pesticides etc.)

In addition to growth promoting hormones, a range of other Agvet chemicals are used in the livestock industry. These may include veterinary pharmaceutical products for treatment against diseases, pests and parasites (viruses, bacteria, worms, ticks) as well as to keep the processing plant weed and pest free. The industry has well established time intervals (commonly known as Export Slaughter Intervals-ESIs) between the last treatment of an animal with an Agvet chemical and their slaughter for export. Only a small number of Agvet compounds are known or suspected endocrine disruptors (Tables 3). The use of plastic and other materials in packaging may also be a source of chemicals in meat processing plants. Phthalates are a group of compounds that can arise from packaging materials and are classed as endocrine disrupting chemicals. The load of such chemicals in the waste stream is difficult to estimate.

Table 3 Endocrine disrupting chemicals likely to be used/associated with meat processing supply chain

Chemical	Usage	Source	Scientific Position
Surfactants Nonylphenol polyethoxylates Octylphenol polyethoxylates	Cleaning	Wastewater and sludge	• The degradation products nonylphenol and octylphenol from APE surfactants have been known to have weak estrogenic activity by in vitro tests. • They can bioaccumulate in aquatic organisms such as fish and can caused feminisation of male fish. • These effects are well established and widely accepted
Pesticides Methomyl Glyphosate Deltamethrin, Cypermethrin Permethrin Endosulfan	Controlling pests including ticks, mosquito	Manure and wastewater	• Pesticides are designed to be biologically active. Some pesticides like DDT, chlordane and vinclozolin were known to affect reproductive systems in wildlife. • Literature on modern pesticides (including those listed in this table) in terms of their endocrine disrupting effects is incomplete and more research is needed to understand their potential impacts in terms of endocrine disrupting activity.
Hormones Estradiol, Estrone, Estriol Testosterone Zeranol Trenbolone acetate Melengestrol acetate Progesterone	Natural hormones excreted by animals. Synthetic hormones used as growth promoters.	Manure and wastewater	• Hormones, whether natural or synthetic, possess high endocrine disrupting potency. • Feminisation of fish in English rivers were suspected to be casued by hormones in wastewater discharged into the rivers. • In addition to natural hormones excreted by all animals, synthetic hormone drugs are also used as growth promoters in some countries. • While their potency of endocrine disruption is well known, little is known about the fate of those hormones in manure and their possible effects on ecosystem.
Phthalates	Packaging materials	Wastewater and sludge	• Phthalates are widely used in the world. They are almost ubiquitous in the environment. Phthalates are known to have a weak estrogenic potency. Early puberty of girls has been suspected to be casued by phthalates. • Little is known on their effects on wildlife.

Chapter 4: Clean technology, best practice and treatment technologies or practices in removing, or reducing EDCs in waste streams[2]

Simple physical solid/liquid **separation and biological methods** have been widely used to manage liquid and solid wastes generated in the meat and livestock industries. While there has been essentially no attention given to the removal of EDC's in the treatment methods currently used, some insights can be obtained from the results of studies into the fate of EDCs in domestic wastewaters. Thus, between 51 and 90% of the total estrogenic activity has been shown to be removed during biological wastewater treatment. A loose correlation has been observed between sludge age and the oestrogenicity of mixed liquor suspended solids suggesting that treatment facilities may be designed and operated to enhance the removal of estrogenic compounds from the liquid phase.

It has long been known that natural steroidal hormones may be degraded by the microorganisms of wastewater and activated sludge and that some synthetic steroidal compounds exhibit greater overall resistance. For example, in batch aerobic experiments, natural oestrogens have been rapidly eliminated, however the synthetic ethinyloestradiol has proved to be somewhat more persistent. Similar results have been reported for tests simulating aerobic biodegradation in aquifer material, however very poor removal for all compounds was reported under anaerobic conditions.

While adsorptive removal using aluminium and ferric coagulants has been shown to be ineffective for removal of many EDCs, powdered activated carbon (PAC) has been shown to be useful in laboratory-scale batch experiments with removals generally greater than 90% (Snyder *et al.*, 2003). If PAC is used for adsorption of EDCs, it may be separated from solution using relatively porous ultrafiltration (UF) or microfiltration (MF) submerged membranes operating in a low vacuum environment (Chang et al., 2003). EDCs are generally relatively hydrophobic and found to associate with organic particles or organic coatings on surfaces. The hydrophobic nature of EDCs and the variable organic content of subsurface environments may account for the observation that EDCs are variably adsorbable to aquifer materials. It is noteworthy that the adsorbed compounds are not destroyed in the treatment process and have the potential of subsequently being released into the environment from sludge or composts.

Removal of EDCs from liquid streams may also be undertaken by size exclusion using nanofiltration or reverse osmosis membranes. In view of the low molecular weight of most EDCs, it is not surprising that loose nanofiltration membranes have been found to achieve only minor removal of EDCs, while tight nanofiltration achieve moderate to good removal (Snyder *et al.*, 2003). Reverse osmosis will give almost complete removal from solution.

Chemical treatments of organic components of aqueous wastes regularly incorporate a reaction of the trace contaminants with a highly oxidative chemical species. Lab-scale batch experiments have indicated that ozonation and chlorination may each be used for the removal of a range of steroidal EDCs from water (Snyder *et al.*, 2003), however, the removal efficiencies are compound-specific and dependant on operational parameters such as oxidant dose with ozonation generally more effective than chlorination. The presence of hydrogen peroxide (which induces the formation of the hydroxyl radical) further improves the ozonation process.

Direct exposure to sunlight has been found to be effective in EDC degradation in some instances with almost complete degradation within 100 hours (Gray & Sedlak, 2003). In natural systems however, effective removal may be limited by the rapid attenuation of the sunlight. UV irradiation in the presence of catalysts such as semiconducting TiO2 have been shown to reduce degradation times for oestradiol to about 3 hours (Ohko *et al.*, 2002).

In summary, it should be noted that EDCs comprise a chemically broad array of compounds and the most suitable treatment technologies will be influenced by the specific target chemicals. In practise, it may be necessary to incorporate a combination of two or more treatment technologies for the effective removal of

[2] By Prof. TD Waite, University of NSW, Sydney.

all EDCs. Once the most appropriate treatment technologies have been selected, optimised design and operational conditions will be crucial.

Current indications are that a combination of advanced oxidation (chemical or photochemical) and tight membrane filtration (such as reverse osmosis) may provide the optimum removal for the greatest variety of EDCs. Both processes are energy-intensive however and consequently expensive to operate but have the advantage of needing only a relatively small amount of space compared to some conventional wastewater treatment technologies. However, this advantage may not be realised since these processes will inevitably require some pre-treatment for highly contaminated wastes. An alternative could involve anaerobic/aerobic treatment of the high strength wastewater using a submerged membrane bioreactor with “advanced oxidation” treatment of the permeate to ensure maximum degradation of any EDCs transported through the membrane unit.

Chapter 5: Recommendations for priority areas for research that may benefit the meat processing industry

Undoubtedly, the scientific evidence and the community concern about the potential adverse impact of EDCs in the environment are growing rapidly, both overseas and in Australia. Despite the fact that currently there are no regulatory or policy guidelines, many governmental and private agencies are taking proactive measures to deal with EDCs. The following areas are suggested for priority research.

(1) Assuming "hormones in meat" is an important issue being addressed already by the industry, the emphasis of EDCs research on the environment should be placed on minimizing any adverse impact of surfactants (alkylphenol ethoxylate detergents) and hormones in waste streams from meat processing on the environment.

(2) Considering significant use of cleaning agents for the hygiene of processing plants, the industry needs to establish the likely concentrations of alkylphenols (nonylphenol and octylphenols polyethoxylates) released in wastewater from processing plants. The maximum levels can be easily estimated from the volume of water and detergent used during cleaning processes. Such an estimate, coupled with strategic and suitable monitoring of the levels of alkyphenols and hormones in waste streams from the treatment process, is needed to assess the actual discharge of the EDCs through waste streams. Waste streams from feedlots and animal holding yards near processing plants need to be analysed for hormone levels.

(3) The effectiveness of current treatment processes and the extent of breakdown of EDCs in receiving environments (land or water) need to be established. This can lead to the identification of treatment systems (treatment method plus environmental conditions) that are effective in removing the target EDCs.

(4) An assessment of the likely exposure pathways and risks to ecosystem health due to the levels of alkylphenols and hormones in waste streams is desirable. The wastewater treatment processes, disposal methods, environmental conditions and the receptor organisms would determine the actual risks. Such an assessment is helpful for addressing both actual and perceived risks.

(5) Considering the European ban on nonylphenol based surfactants and current review by USA, the industry should develop an inventory of the current use of alkylphenol polyethoxylate based cleaning agents and explore the feasibility for replacement of nonylphenol based products.

(6) The extent of use of hormones as growth promoting substances in Australia and their likely endocrine disruption impact on the environment need to be considered.

(7) The industry should invest in developing and optimizing rapid screening tools for EDCs in waste streams from the meat processing industry.

(8) The meat processing industry, in partnership with other industries with similar wastewater issues, should consider directing resources towards research and development of suitable treatment technology for removal of EDCs from wastewater.

References

Adami, H.O., Bergstron, R., Mohner, M., Zatonski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Ziegler, H., Rahu, M., Gurevicius, R. and Stengrevics, A. (1994). Testicular cancer in nine northern European countries. *International Journal of Cancer* **59**, 33-38.

Adams, N.R. (1998). Clover phyto-oestrogens in sheep in Western Australia. *Pure & Applied Chemistry* **70**(9), 1855-1862.

Ahel, M., Molnar, E., Ibric, S. and Giger, W. (2000). Estrogenic metabolites of alkylohenol olyethoxylates in secondary sewage effluents and rivers. *Water Science and Technology* **42**(7-8), 15-22.

Alzieu, C. (2000). Impact of tributyltin on marine invertebrates. *Ecotoxicology* **9**, 71-76.

Australian Academy of Science (1998). Endocrine disruption: Australia's role in an international issue. National Science & Industry Forum Report, April 7, 1998. pp. 1-12. (Canberra, Australia.)

Batty, J. and Lim R. (1999). Morphological and reproductive chracteristics of male mosquitofish (Gambusia affinis holbrooki) inhabitating sewage-contaminated waters in New South Wales, Australia. Archives of Environmental Contamination and Toxicology, 36, 301-307.

Bennetts, H., Underwood, E. and Shier, F. (1946). A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Australian Veterinary Journal* **22**, 2-12.

Blackburn, M.A., Kirby, S.J. and Waldock, M.J. (1999). Concentrations of alkylphenol polyethoxylates entering UK estuaries. *Marine Pollution Bulletin* **38**(2), 109-118.

Bortone, S.A., Davis, W.P. and Bundrick, C.M. (1989). Morphological and behavioural charaters in mosquito fish as potential bioindicators of exposure to kraft mill effluent. *Bulletin of Environmental Contamination Toxicology* **43**, 370-377.

Brown, L.M., Pottern, L.M., Hoover, R.N., Devesa, S.S., Aselton, P. and Flannery, T. (1986). Testicular cancer in the U.S. trends in incidence and mortality. *International Journal of Epidemiology* **15**, 164-170.

Burt, J.S. and Ebell, G.F. (1995). Organic pollutants in mussels and sediments of the coastal waters off Perth, Western Australia. *Marine Pollution Bulletin* **30**(11), 723-732.

Chang' S., Waite; T.D., Ong' P.E.A., Schäfer' A.I. and Fane, A.G. (2003a). Assessment of Trace Estrogenic Contaminants Removal by Coagulant Addition, PAC Adsorption and PAC/MF Processes. Journal of Environmental Engineering (in press).

Colborn, T. vom Saal FS and Soto A. (1993). Developmental effects of endocrine-disrupting chemicals in wild-life and humans. *Environmental Health Perspectives* **101**, 378-384.

Colborn, T., Dumanoski, D. and Myers, J.P. (1996). 'Our Stolen Future'. (Plume/Penguin Book: New York.).

Daly, H. and Fabris, J.G. (1993). An environmental study of tributyltins in Victorian waters. (Environmental Protection Authority. Melbourne, Victoria, 3000. SRS 90/020.)

Damstra, T., Barlow, S., Bergman, A., Kavlock, R. and Van Der Kraak, G. (2002). Global assessment of the state-of-the-science of endocrine disruptors. WHO/PCS/EDC/02/2.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R. and Van Der Kraak, G. (2002). Global assessment of the state-of-the-science of endocrine disruptors. WHO/PCS/EDC/02/2. (*http://www.who.int/pcs/emerg_site/edc/global_edc_TOC.htm*)

Dillon, P.J. (2001). Water Reuse in Australia: Current, future and research. *Australian Water Association Journal Water*, **28 (3)** 18-21.

EA 2002. Managing Chemicals for a Better Environment. The Environment Agency's Strategy, June 2002. UK.

Falkenberg, I.D., Dennis, T.E. and Williams, B.D. (1994). Organochlorine pesticide contamination in three species of raptor and their prey in South Australia. *Wildlife Research* **21**, 163-173.

Fenner-Crisp, P.A., Maciorowski, A.F. and Timm, G.E. (2000). The endocrine disruptor screening program developed by the U.S. Environmental Protection Agency. *Ecotoxicolgy* **9**, 85-91.

Feuer, E.J. (1995). State Bite: Incidence of testicular cancer in US men. *Journal of National Cancer Institute* **87**, 405.

Gibbs, P.E., Bryan, G.W., Pascoe, P.L. and Burt, G.R. (1990). Reproductive abnormalities in female *Ocenebra erinacea* (Gastropoda) resulting from tributyltin-induced imposex. *Journal of Marine Biological Association of UK* **70**, 639-656.

Gray, J. L. and Sedlak, D. L. (2003) Removal of 17β-estradiol and 17α-ethinyl estradiol in engineered treatment wetlands. In: 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Compounds in Water The National Ground Water Association, Minneapolis, Minnesota.

Guillette, L.J., Arnold, S.F. and McLachlan, J.A. (1996). Ecoestrogens and embryos – Is there a scientific basis for concern? *Animal Reproduction Science* **42**, 13-24.

Guillette, L.J., Crain, D.A., Gunderson, M.P., Kools, S.A., Milnes, M.R., Orlando, E.F., Rooney, A.A. and Woodward, A.R. (2000). Alligators and endocrine disrupting contaminants: a current perspective. *American Zoology* **40**, 438-452.

Guillette, L. J., Jr., T. S. Gross, G. R. Masson, J. M. Matter, H. F. Percival, and A. R. Woodward. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile American alligators from contaminated and control lakes in Florida. *Environmental Health Perspectives* **102**, 680–688.

Gutendorf, B. and Westendorf, J. 2001. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology 166, 79-89.

Hakulinen, T., Andersen, A.A., Malker, B., Rikkala, E., Shou, G. and Tulinius, H. (1986). Trends in cancer incidence in the Nordic countries. A collaborative study of the five Nordic Cancer Registries. *Acta Pathologica Microbiologica Immunologica Scandinavia* **94** (Section A), Suppl. 288, 1-151.

Hayes, T.B., Collins A., Lee, M., Mendoza, M. Noriega N, Stuart A.A., Vonk, A. (2002). Hermaphroditic, demasculinised frogs after exposure to the herbicide atrazine at low ecologically relevant doses. PANS, 99:5476-5480.

Horiguchi, T., Shiraishi, H., Shimizu, M. and Morita, M. (1994). Imposex and organotin compounds in *Thais claviger* and *T. bronni* in Japan*. Journal of Marine Biological Association of UK* **74**, 651-669.

Huet, M. (2000). OECD activity on endocrine disrupters test guidelines development. *Ecotoxicology* **9**, 77-84.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G. and Sumpter, J.P. (1998). Widespread sexual disruption in wild fish. *Environmental Science and Technology* **32**, 2498-2506.

Kavlock, J.R., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, M., Sink, T. and Tilson, H.A. (1996). Research needs for risk assessment of health and environmental effects of endocrine disruptors: a report of the US sponsored workshop. *Environmental Health Perspectives* **104** (suppl. 4), 715-740.

Kohn, A.J. and Almasi, K.N. (1993). Imposex in Australian conus*. Journal of Marine Biological Association of UK* **73**, 241-244.

Kolpin DW., Furlong, ET, Meyer, MT, Thurman, EM, Zaugg SD. Barber, LB. and Buxton, HT. (2002). Pharmaceuticals, Hormones and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. *Environmental Science and Technology* **36**:1202-1211

Kookana, R. GG Ying, A Kumar and P Dillon (2002a) Endocrine Disrupting Chemicals (EDCs): Potential Sources and Impacts on the Australian Riverine Environment. Summary of the Workshop held on 28th June 2002 in Adelaide

Kookana, RS, Ying, G-G, Kumar, A and Dillon P. (2002b). Endocrine Disrupting Chemicals- Potential Sources and Impacts on Australian Riverine Environment. Summary of the Workshop held on June 28, 2002. Adelaide. CSIRO Land and Water, Adelaide. (www.clw.csiro.au/staff/RKookana)

Lim, R., Gale, S. and Doyle, C. (2000). Endocrine disrupting compounds in sewage treatment plant (STP) effluent reused in agriculture – is there a concern? In "Watrer Recycling Australia" (P. J. Dillon, ed.). pp. 23-28. CSIRO &AWA, Australia.

Lister, A.L. and Van Der Kraak, G.J. (2001). Endocrine disruption: why is it so complicated? *Water Quality Research Journal of Canada* **36**(2), 175-190.

Loder, N. (2000). Royal Society warns on hormone disrupters. *Nature* **406**, 4.

Macilwain, C. (1998) US panel split on endocrine disruptors. *Nature* **395**, 828.

Moller, H. (1993). Clues to the aetiology of testicular germ cell tumours from descriptive epidemiology. *European Urology* **23**, 8-15.

Naylor, C.G., Mieure, J.P., Adams, W.J., Weeks, J.A., Castaldi, F.J., Ogle, L.D. and Romano, R.R. (1992). Alkylphenol ethoxylates in the environment. *JAOCS* **69**(7), 695-703.

Ohko, Y., Iuchi, K.-i., Niwa, C., Tatsuma, T., Nakashima, T., Iguchi, T., Kubota, Y. and Fujishima, A. (2002) 17-Estradiol degradation by TiO2 photocatalysis as a means of reducing estrogenic activity. *Environ. Sci. Technol.,* **36**(19)*,* 4175-4181.

Palanza, P., Morellini, F., Parmigiani, S. and vom Saal, F. S. (1999). Prenatal exposure to endocrine disrupting chemicals: effects on behavioural development. *Neuroscience and Biobehavioral Reviews* **23**, 1011-1027.

Parrott, J., Wade, M., Timm, G. and Brown, S. (2001). An overview of testing procedures and approaches for identifying endocrine disrupting substances. *Water Quality Research Journal of Canada* **36**(2), 273-291.

Randall, Peter and Greg Abood (2001). Black Water - Grey Areas: An analysis of Sewage Management in NSW. Nature Conservation Council of NSW Inc. June 2001.

Ries, L.A.G., Hankey, B.F. and Miller, B.A. (1991). Cancer statistics review 1973-88. DHEW (NIH) Publ. No. 91-2789. (US Government Printing Office: Washington, DC.)

Schäffer, A.I., Nghiem, L.D. and Waite, T.D. (2002). Removal of natural hormone estrone from aqueous solutions and reverse osmosis. *Environmental Science and Technology* **37**, 182-188.

Sonnenschein, C. and Soto, A.M. (1998). An updated review of environmental estrogen and androgen mimics and antagonists. *Journal of Steroid Biochemistry and Molecular Biology* **65**(1-6), 143-150.

Snyder, S. A., Yoon, Y., Westerhoff, P., Vanderford, B., Pearson, R. and Rexing, D. (2003) Evaluation of conventional and advanced drinking water treatment processes to remove endocrine disruptors and pharmaceutically active compounds: Bench-Scale Results. In: 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Compounds in Water The National Ground Water Association, Minneapolis, Minnesota.

Steele K. (1995). Impact of animal manure and the land water interface. Lewis Publishers, CRC Press, Boca Raton, FL.

Sydney Water (2000). Sydney Water’s 5-year drinking water quality management plan (Discussion paper). Community and Stakeholder Consultation. August 2000. (Sydney, Australia.)

Tabata, A., Kashiwa, S., Ohnishi, Y., Ishikawa, H., Miyamoto, N., Itoh, M. and Magara, Y. (2001). Estrogenic influence of estradiol-17β, *p*-nonylphenol and bisphenol A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. *Water Science and Technology* **43**(2), 109-116.

Ternes T.A., Kreckel P., Mueller J., (1999). Behaviour and occurrence of estrogens in municipal sewage treatment plants – II. Aerobic batch experiments with activated sludge, *The Science of Total Environment*, **225**, 91-99.

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

vom Saal, F.S., Timms, B.G., Montano, M.M., Palanza, P., Thayer, K.A., Nagel, S.C., Dhar, M.D., Ganam, V.K., Parmigiani, S., and Welshons, W.V. (1997). Prostate enlargement in mice due to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 2056-2061.

WEAO. (2001) Fate and significance of contaminants in sewage biosolids applied to agricultural land through literature review and consultation with stakeholders groups. Final Report, April 2001.

Wolff, M.S., Toniolo, P.G., Lee, E.W., Rivera, M. and Dubin, N. (1993). Blood levels of organochlorine residues and risk of breast cancer. *Journal of National Cancer Institute* **85**, 648-662.

Ying, G.G. and Kookana, RS. (2002). Endocrine disruption: an Australian Perspective. *Australian Water Association Journal Water* **29**(9), 42-45.

Ying, G.G., Kookana, RS. and Ru, Y-J. (2002a). Occurrence and fate of hormone steroid in the environment. *Environment International* 28: 545-551.

Ying, G.G. Williams, B. and Kookana, RS. (2002b). Environmental fate of alkylphenols and alkaylphenol ethoxylates – a review. *Environment International* 28: 215-226.