Global Water Research Coalition

Endocrine Disrupting Compounds

Priority List of EDCs

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IWA affiliate

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Global Water Research Coalition: Cooperation for worldwide water knowledge, innovation and progress

GWRC is a non-profit organization that serves as the collaborative mechanism for water research. The product the GWRC offers its members is water research information and knowledge. The Coalition will focus on water supply and wastewater issues and renewable water resources: the urban water cycle.

The members of the GWRC are: the Awwa Research Foundation (US), CRC Water Quality and Treatment (Australia), Kiwa (Netherlands), Sues Environment- CIRSEE (France), Stowa - Foundation for Applied Water Research (Netherlands), DVGW – TZW Water Technology Center (Germany), UK Water Industry Research (UK), Veolia - Anjou Recherché (France), Water Environment Research Foundation (US), Water Research Commission (South Africa), WaterReuse Foundation and the Water Services Association of Australia.
These organizations are all in charge of a national research program addressing the different parts of the water cycle. They have provided the impetus, credibility, and initial funding for the GWRC. Each brings a unique set of skills and knowledge to the Coalition. Through its member organisations GWRC represents the interests and needs of 500 million consumers.

The Global Water Research Coalition is affiliated with the International Water Association (IWA). The GWRC was officially formed in April 2002 with the signing of the partnership agreement at the International Water Association 3rd World Water Congress in Melbourne. With the US Environmental Protection Agency a partnership agreement was signed in July 2003.

Endocrine Disrupting Compounds

Priority List of EDCs

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Disclaimer

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GLOSSARY OF DEFINITIONS

Endocrine Disrupting Compound: (WHO definition)
An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an organism, or its progeny, or (sub) populations.

Source water:
Source water is defined as water coming from a natural source ie. water from rivers, dams, streams and fountains. Borehole water is also included in this category.

Drinking Water:
Under the term drinking water is understood water which is treated and intended for human consumption

Tap Water:
This is water taken at the consumer point of the treated water distribution system.

Receiving water: This is water in a river or other body of water into which an effluent is discharged.

Surface water: This refers to river water from the source through to the drinking water intake.

Waste Water:
This is defined as:
1. Untreated waste water: Water used and/or polluted by humans by agricultural or industrial activity.
2. Treated waste water: Water at the exit of a purification plant.

Raw Water:
Untreated water

NOEL
Acronym for 'No observed effect level'.

ELISA
Acronym for 'Enzyme-linked Immunosorbant analysis'.

SUMMARY

There has been increasing concern regarding substances in the environment that could impact on the endocrine systems of wildlife and man. The data that initiated the concern relate to fish, amphibians, reptiles and, to a lesser extent, birds exposed to anthropogenic chemicals through the aquatic environment. Significant changes were observed in the reproductive organs of alligators and turtles exposed to a mixture of persistent pesticides in a Florida Lake while changes in the reproductive organs of male fish exposed to treated sewage effluent were also observed. The changes were shown to be mediated through effects on the endocrine system, which is a complex hormonal mechanism for control of the development and physiological status of animals, particularly vertebrates. These findings also resulted in concern for possible impacts on humans through exposure to endocrine disrupting substances from drinking water derived from primarily from surface sources.

The cost of monitoring the entire spectrum of potential EDCs in water and water-related media would be prohibitive and not all endocrine disrupting substances are likely to be present in aquatic systems. It was considered to be appropriate to compile a priority list of EDCs which would provide a basis for credible analytical determination of EDCs in water.

A preliminary list of compounds was prepared on the basis of information received from the members of GWRC and submitted to the workshop of GWRC members held in South Africa, October 2002 for further refinement. The groups of chemicals considered for inclusion in the list, were: hormones, pesticides and herbicides, industrial chemicals like alkyl phenols, phthalates and polychlorinated biphenyl compounds (PCBs), and heavy metals.

After elaboration all the information the participants agreed on a EDC Priority List to be used in future joint activities. Yet, the present priority list is considered to be dynamic and compounds may be added or deleted as more information becomes available.

1 Introduction

There are presently in excess of 4000 compounds that are reported to show endocrine disrupting properties, primarily in relation to estrogen and estrogenic effects, and this list is expanding as researchers investigate the properties of more compounds. The relative potency of these compounds varies depending on the type of methodology used to determine activity, for example E-screen, Yeast cell test or in vivo animal studies. In different species, differing levels of potency have also been observed. Aquatic fauna seem to be more susceptible to the effects of EDCs than other species, possibly because of the nature of exposure and the more labile nature of the reproductive system of many of these organisms.

Although humans and wildlife are exposed to chemicals via different pathways, water potentially forms an important route for the distribution of EDCs in the environment and for exposure to EDCs. Humans and wildlife drink water, crops are irrigated and food is prepared with or in water. However, the direct uptake of EDCs from drinking water is considered to be relatively low in comparison to other sources such as food. This is largely because most of the most potent EDCs are lipophilic and preferentially adsorb onto particulate matter and sediment, or onto sludge in waste treatment.

The cost of monitoring the entire spectrum of potential EDCs in water and water-related media would be prohibitive and not all endocrine disrupting substances are likely to be present in aquatic systems. It was considered to be appropriate to compile a priority list of EDC compounds which would provide a basis for credible analytical determination of EDCs in water, particularly drinking water. However, such a list must remain flexible in order to take new scientific data into account, and this list should be regarded as a starting point for the GWRC research initiative. In order to maximize available resources, the use of a priority list of EDCs is, therefore, recommended as a basis for a global monitoring programme for EDCs in water systems.

2 Criteria for the inclusion of a substance on the GWRC priority list of EDCs

The following criteria were selected according to which a specific compound would be included or omitted from this list following debate and consensus agreement:

1. The substance should possess EDC properties.
   The substance should show activity in accepted biochemical or other in vitro tests and/or in vivo animal studies.

2. *Use:*
   There must be clear evidence of past or present use and banned substances should not be excluded from the list. Some of the persistent organic pollutants such as organochlorine pesticides (DDT, Dieldrin) may not be in use in most of the member countries, but are still applied in Malaria-infected areas, and could be transported to elsewhere. They are also highly persistent in the environment. The use of PCBs has been largely phased out, but because of the long environmental half-life of these compounds, they are still present in the environment. As indicated above, the breakdown products of some chemicals are more potent than the parent substance, e.g. alkylphenol polyethoxylates.

3. *Persistence:*
   Clear evidence that the substance is sufficiently persistent in the environment for there to be significant exposure.

4. *Substances to be included might occur naturally, be synthetic or anthropogenic:*
   Compounds such as hormones, phyto-estrogens and heavy metals should be considered even though they are not synthetic.

5. *Substances should be of concern to at least two of the GWRC members*: The GWRC EDC programme is intended to address global concerns.

6. *Water solubility/mobility:*
   The water solubility/mobility of compounds should be taken into account especially when monitoring drinking/purified water. Lipophilic compounds such as organochlorine pesticides and PCB's adhere to solid particles and may be found in large amounts in environmental water and sewage sludge, but are easily removed in drinking water treatment processes.
   It should be kept in mind, however, that these compounds have long half-lives and can bio accumulate in aquatic fauna, and predators on aquatic fauna, and so should not be removed from the list.

In compiling the priority list the following limitations were considered.

a. *Effects*
   Potential effects on humans and animals, particularly aquatic animals, should be taken into account.

b. *Controls*
   The ability to control a substance in the environment should not be used as a reason to include or exclude it from the priority list. Some substances such as heavy metals and phyto-estrogens occur naturally in the environment and may be virtually impossible to control at source. They may, however, be controlled through drinking water purification should this be considered necessary.

c. *The inability to detect an EDC should not exclude it from the list*
   The lack of a currently available analytical procedure to determine the presence and/or level of a substance at the appropriate detection limit should not be an obstacle to include the substance on the list. It is recommended that new methods be developed in order to overcome this knowledge gap.

3 Priority EDCs

3.1 The EDC Priority List

Based on the information submitted by GWRC members, a preliminary list of EDCs was compiled. The summary of available analytical methods and their use by members are listed in Annex A. Detailed information regarding data from the UK is presented in Annex B 'Endocrine disrupting chemicals in the environment' taken from the UKWIR study Endocrine Disrupting Chemicals in wastewater: a review of occurrence and removal (2001).
The groups of chemicals considered for inclusion in the list, were:
- hormones
- pesticides and herbicides
- industrial chemicals like alkyl phenols, phthalates and polychlorinated biphenyl compounds (PCBs)
- heavy metals.

The preliminary priority list of endocrine disrupting compounds was discussed at the GWRC workshop in Pretoria (October 2002).
Table 3.1 reflects the EDC Priority List as agreed by the members. The present priority list is considered to be dynamic and compounds may be added or deleted as more information becomes available.

Table 3.1 Priority list of EDCs	
Hormones 17ß-Estradiol Estriol Estrone 17a-Ethinylestradiol	**Heavy Metals:** Cadmium
Pesticides and herbicides DDT, DDE, DDD Dieldrin, Aldrin, Endrin, Isodrin a-Endosulphan, ß-Endosulphan, Endosulphan-sulphate Heptachlor, Heptachlor epoxide Lindane (?-BHC) Vinclozolin Parathion Atrazine Simazine Terbutylazine 2.4-D Metoxychlor Tributyltin Cyhexitin	**Industrial Chemicals** PCB (total) Glycol ethers p-Nonylphenol p-Octylphenol Phthalates: DEPH, DBP Bisphenol A

During the discussion a working list was compiled that included additional substances suggested by members, but for which not all the information about the criteria for inclusion was available at the time of the workshop. More information was subsequently gathered on these compounds and some were then added to the priority list. Those not included did not meet the criterion of being relevant to at least two of the members.
Furthermore a number of chemicals where listed as possible candidates:

Glyphosate	Amitrole
2,4-dichlorophenol	Kepone
DPCP (1,2-dibromo-3-chloropropane)	
Chlordecone	ß-BHC
Arsenic	Chrome VI

These substances are all reported to have shown endocrine disrupting properties, but it remains uncertain whether they meet all the criteria for inclusion.

The EDC priority list addresses compounds which have been reported to interact with hormones and receptors associated with the reproductive system, particularly estrogens. Other EDCs which are reported to interact with other hormones or organ systems should also be considered when appropriate data are available. These chemicals may be added in future, when more information becomes available.

3.2 Relative potency of EDCs and limits of the detection

During the compilation of the priority list it became clear that additional knowledge was required on the potency of these compounds. The potency of compounds showing endocrine disrupting effects becomes an important issue when determining appropriate detection limits for chemical analysis. The potency is also important in the assessing risks to exposed populations. No risk assessment can be carried out without suitable dose response data.
A list of potencies was compiled from the available literature (Table 3.2).

Table 3.2: List of relative potency of EDCs compared to 17ß-Estradiol
(17ß-Estradiol shows detectable activity at 0.03ng/liter water)

Compound	Relative Potency	Literature Reference
17 ß-Estradiol	1	
Estrone	0.09^{-1}	Coldham 1997
17a-Ethinylestradiol	0.9-1.2	Coldham 1997
Estriol	0.08-0.8	Coldham 1997
DES	1 0.7	Leffers *et al.*2001 Coldham 1997
Zearanol	1 0.8	Leffers *et al*.2001 Coldham 1997
Genitein	$1x10^{-6}$ $1x10^{-4}$	Leffers *et al.*2001 Coldham 1997
BPA	$1x10^{-7}$ $1x10^{-3}$ $2x10^{-4}$ $5x10^{-4}$	Leffers *et al.* 2001 Report Nordic Council of Ministers Review UK Water Research Ltd.2001 Coldham 1997
p-Nonyl phenol	$1x10^{-5}$ $1x10^{-4}$ $7x10^{-3}$	Report Nordic Council of Ministers Jobling and Sumpter 1996, Metcalf *et al* 2001 Review UK Water Research Ltd. 2001
p-Octyl phenol	$1x10^{-4}$ $1.5x10^{-3}$	Report Nordic Council of Ministers Review UK Water Researcj Ltd. 2001 Routledge 1997
NP2EO	$1x10^{-5}$	Report Nordic Council of Ministers
NP9EO	$1x10^{-6}$	Report Nordic Council of Ministers
BBP	$1x10^{-5}$ $1x10^{-5}$ $1x10^{-4}$	Report Nordic Council of Ministers Coldham 1997 Van Wezel *et al.* 2000
DBP	$1x10^{-6}$ $1x10^{-5}$	Report Nordic Council of Ministers Review UK Water Research Ltd. 2000, Van Wezel *et al.* 2000
DEHP	$1x10^{-5}$	Review UK Water Research Ltd. 2001
DDT	$1x10^{-7}$	Coldham 1997
DDE	$1x10^{-7}$	Coldham 1997
Methoxychlor	$1x10^{-4}$	Coldham 1997
PCB	$1x10^{-2}$-1x 10^{-4}	Coldham 1997 (Depending on conjener)

Detection limits were calculated from these data (Table 3.3). There is considerable variation in the potency of different compounds compared to the most potent hormones .
Potency data were not available for all substances on the priority list and further data on these substances are needed.

Coldham,N.G.,Environmental Health Perspectives **105** (7),1997
Jobling, S., and Sumpter, J.P. Aquatc Toxicology, **27**, 361 – 372 1993
Leffers *et al.,* Human Reproduction **16** (5): 1037 -1045,2001.
Metcalf, C.D. *et.al.*,Environmental Toxicology and Chemistry, **20**(2),297 – 308,2001
Report of Nordic Council of Ministeres, Tema Nord ,580, 1996
Report of UK Water Industry Research Limited., Ref.No.02/TX/04/5,2001
Routledge,E.J., Ph.D. Thesis, Brunel University.,1997
Van Wezel,A.P.,*et.al.*,Ecotoxicology and Environmental Safety,**46**(3),305-321,2000.

Table 3.3 Detection limits calculated from literature information on relative potency

Compound	Limits of Detection
17ß-Estradiol	0.03ng/l
Estriol	0.04ng/l
17a-Ethinylestradiol	0.03ng/l
Estrone	0.03ng/l
?- Nonyl phenol	0.2µg/l
?-Octyl phenol	0.05µg/l
BBP	3.03µg/l
DBP	3.0µg/l
DEHP	3.0µg/l
DDT	30µg/l
DDE	30µg/l
Methoxychlor	0.03µg/l
PCB	0.003µg/l

The workshop also decided that analysis of all the EDCs in the priority list was not necessary for all types of matrices. The substances considered important for each of the matrices are shown in Table 3.4.

Table 3.4 Proposed list of EDCs to be monitored according to respective matrices						
Compound	**Waste water influent**	**Waste water effluent**	**Source water**	**Drinking water**	**Sewage Sludge**	**Sediment suspended matter**
17ß-Estradiol	X	X	X	X	X	X
Estriol	X	X	X	X	X	X
Estrone	X	X	X	X	X	X
17a-Ethninyl Estradiol	X	X	X	X	X	X
Amitrol			X	X		
Vinclozolin			X	X		
Parathion			X	X		
DDT,DDE,DDD	(x)	(x)	X	(x)		X
Dieldrin, Aldrin, Endrin	(x)	(x)	X	(x)		X
Endosulphan	(x)	(x)	X	(x)		X
Heptachlor, heptachlor epoxide	(x)	(x)	X	(x)		X
Lindane (?-BHC)	(x)	(x)	X	(x)		X
Tributyltin			X	X		X
Atrazine			X	X		
Simazine			X	X		
Terbutylazine			X	X		
2,4-D			X			
Metoxychlor	X	X	X			X
PCB	X	X	X	X		
Styrene,toluene,Xylene	X	X	X	X		
p-Nonyl phenol	X	X	X	X	X	X
p-Nonyl phenol ethoxylates	X	X	X	X	X	X
p-Octyl phenol	X	X	X	X	X	X
p-Octylphenol-ethoxylates	X	X	X	X	X	X
DEPH	X	X	X	X	X	X
DBP	X	X	X	X	X	X
BBP	X	X	X	X	X	X
Bisphenol A	X	X	X	X		
Cadmium	X	X	X	X	X	X

Different comparative potencies may also be apparent in different bioassays. A comparison is made by Gutendorf et.al., (Toxicology 166 (2001) 79 – 89) of the potency of EDCs determined by different bioassays. The differences are summarized in Table 3.5.

Table 3.5 Relative potencies (E_2=1) of ER-agnostic compounds determined as ED_{50}-values from dose response curves

Compound	MVLN cells	HGELN cells	E-Screen	Binding to ER-a	Binding to ER-ß
17ß-Estradiol	1	1	1	1	1
Estriol	0.083	04	0.071	0.07	0.26
Estrone	0.01	0.056	0.01	0.007	0.065
Ethinylestradiol	1.25	5.71	1.25	1.16	1.44
Diethylstibesterol	1.25	8.9	2.5	1.75	1.3
4-Nonylphenol	$1.25x10^{-5}$	$8.0x10^{-5}$	$1.3x10^{-5}$	$1.75x10^{-4}$	$2.3x10^{-3}$
4-Octylphenol	$8.33x10^{-5}$	$8.0x10^{-4}$	$1.0x10^{-4}$	$7.0x10^{-4}$	$6.5x10^{-3}$
Bisphenol A	$2.5x10^{-5}$	$1.9x10^{-4}$	$2.5x10^{-5}$	$2.3x10^{-4}$	$2.6x10^{-3}$
Genistein	$1.32x10^{-4}$	$8.0x10^{-4}$	$1.3x10^{-5}$	$1.0x10^{-4}$	0.032
ß-Sitosterol	$1.0x10^{-4}$	$7.3x10^{-4}$	$9.6x10^{-5}$	$8.75x10^{-4}$	0.016
Coumasterol	$1.25x10^{-3}$	$1.0x10^{-3}$	$1.1x10^{-4}$	$1.17x10^{-3}$	0.022
Tamoxifen	$8.33x10^{-6}$	$7.1x10^{-7}$	$4.0x10^{-5}$	0.023	0.054

4 Conclusions

A preliminary list of compounds was prepared on the basis of information received from the members of GWRC and submitted to the workshop of GWRC members held in South Africa, October 2002 for further refinement.
After elaboration all the information the participants agreed on a EDC Priority List to be used in future joint activities. Yet, the present priority list is considered to be dynamic and compounds may be added or deleted as more information becomes available.

Based on the gathered information of relative potencies of the listed EDC the desired detection limits were calculated. But the relative potency may differ according to the bio-assay used and this must be taken into consideration when using these data for the determination of detection limits in chemical analysis.

Annex A. Analytical methods used and monitoring of EDCs in water by GWRC members.

Compound	In use	Tested in waste water	Tested in receiving water	Tested in drinking water	Test method eg. GC-MS, HPLC	Limit of detection
DDT,DDE,DDD	- - - RSA - -	- - UK - GER -	USA FR UK RSA GER AUST	- FR UK RSA GER -	GC/ECD GC-MS GC-MS GC-MS GC/ECD GC/µEDC	0.003µg/l 0.02- 0.04µg/l 0.01µg/l 0.01µg/l 0.1µg/l 0.005µg/l
Lindane	- - UK RSA - -	- - UK - GER -	USA FR UK - GER AUST	USA FR UK RSA GER -	- GC-MS GC-MS GC-MS GC/ECD GC/µECD	- 0.02-0.04 µg/l 0/01µg/l 0.01µg/l 0.01µg/l 0.005µg/l
Dieldrin	- - - RSA - -	- - UK RSA GER -	USA FR UK RSA GER AUST	- FR UK RSA GER -	APHA GC-MS GC-MS GC-MS GC/ECD GC/µECD	0.025µg/l 0.02- 0.04µg/l .006µg/l 0.01µg/l 0.01µg/l 0.001µg/l
Heptachlor	- - - RSA - -	- - UK RSA GER -	- FR UK RSA GER AUST	USA FR UK RSA GER -	GC-MS GC/ECD GC-MS GC-MS GC/ECD GC/µECD	0.025µg/l 0.02- 0.04µg/l 0.006µg/l 0.01µg/l 0.01µg/l 0.005µg/l
Endosulfan	- - - RSA - AUSR	- - UK RSA GER -	USA FR UK RSA GER AUST	- FR - - GER AUST	GC/ECD GC-MS GC-MS GC-MS GC/ECD GC/µECD	0.008-µg/l 0.04µg/l 0.006µg/l 0.01µg/l 0.01µg/l 0.005µg/l
Toxaphene	-	-	USA	USA	GC/MS	0.001µg/l
PCB	- - RSA	USA - RSA	USA FR RSA	USA FR RSA	GC-MS GC/MS GC/ECD	- - -
Atrazine	USA - UK RSA - AUST	- - UK RSA GER -	USA FR UK RSA GER AUST	USA FR UK RSA GER -	LC-MS GC-MS HPLC GC-MS GC-MS GC-MS	- 0.02µg/l 0.01µg/l 0.1µg/l 0.01µg/l 0.05µg/l -

Compound	In use	Tested in waste water	Tested in receiving water	Tested in drinking water	Test method eg. GC-MS, HPLC	Limit of detection
Simazine	USA FR UK RSA GER -	- - - RSA GER -	USA FR UK RSA GER -	USA FR UK RSA GER -	- GC-MS HPLC GC-MS GC-MS -	- 0.02µg/l 0.01µg/l 0.1µg/l 0.03µg/l -
Terbutylazine	- FR UK RSA GER -	- - - RSA GER -	- FR - RSA GER -	USA FR - RSA GER -	- GC-MS - GC-MS GC-MS -	- 0.02µg/l - 0.1µg/l 0.03µg/l -
2'4'-D	USA - UK RSA GER AUST	- - - RSA GER -	USA - UK RSA GER AUST	USA - UK RSA GER -	- - HPLC GC-MS GC-MS LC-MS	- - 0.01µg/l 0.1µg/l 0.05µg/l 0.01µg/l
Metoxychlor	USA - - RSA - -	- - UK RSA - -	USA - UK RSA - -	USA - - RSA - -	GC/ECD - HPLC GC-MS - -	0.01µg/l - 0.01µg/l 0.1µg/l - -
Deltamethrin	USA FR UK RSA GER -	- - UK RSA GER -	USA FR - RSA GER-	- FR - RSA GER -	LC-MS GC-MS - GC- MS,HPLC GC/ECD -	- 0.02µg/l - 0.1µg/l 0.01µg/l -
p-Nonyl phenol Ethoxy-Late	USA FR UK RSA GER-	USA - UK - GER	USA - UK RSA GER	- - - - --	- - - HPLC --	- - - 0.01µg/l --
p-Octyl phenol Ethoxy-Late	USA FR UK - GER-	USA - UK - GER-	USA - - - GER	- - - - --	- - - - --	- - - - --

Compound	In use	Tested in waste water	Tested in receiving water	Tested in drinking water	Test method eg. GC-MS, HPLC	Limit of detection
Bisphenol A	USA FR UK RSA GER-	USA - UK - GER-	USA - UK - GER-	- - UK - GER-	- - HPLC - GC-MS-	- - 5µg/l - 0.005µg/l-
Cadmium	N/A	FR UK RSA	FR UK RSA	FR UK RSA	ICP-AES/AA AA ICP	10.0 /0.5µg/l 0.3µg/l 5µg/l
Mercury	N/A	FR UK RSA	FR - RSA	FR UK RSA	Atomic Fluor AA AA	0.25µg/l 0.1µg/l 2µg/l
Arsenic	N/A	FR - RSA	FR - RSA	FR UK RSA	ICP-AES,AA AA Hg/ICP	50/5µg/l 1µg/l 5µg/l
Lead	N/A	FR - RSA	FR - RSA	FR UK RSA	ICP-AES/AA AA ICP	50/5µg/l 1µg/l 30µg/l
Natural and synthetic hormones						
17ß-Estradiol	N/A	USA FR UK	USA FR UK	- FR UK	LC-MS GC-MS GC-MS-MS	1ng/l 0.04ng/l 0.3ng/l
Ethinyl- estradiol	N/A	USA FR	USA FR	- FR	GC-MS GC-MS	0.02ng/l 0.32ng/l
Estrone	N/A	USA FR	USA FR	- FR	Immino assay GC-MS	- 0.04ng/l
Estriol	N/A	USA FR	USA FR	- FR	- GC-MS	- 0.08ng/l

Annex B. Endocrine disrupting chemicals in the environment

2.1 Which chmicals should be considered?

The selection of chemicals to be included in this review has largely been based on the UK Environment Agency's strategy on endocrine disruptors in the environment, launched in 2000 (Environment Agency 2000). This strategy identified a number of chemicals that would require priority action in terms of reducing their emissions to the environment. Further evaluation of these was undertaken in order to identify those which were of particular importance both in terms of a) their endocrine disrupting activity and potential environmental impact and b) their likely presence in domestic sewage. Potential future European and scientific developments were also considered to identify whether additional chemicals should also be included.
The chemicals considered were:

- steroid oestrogens
- alkylphenol polyethoxylates and their metabolites
- phthalate esters
- bisphenol A, and
- certain pesticides and polychlorinated biphenyl compounds

The published data on the sources and effects of these chemicals are discussed below in Sections 2.2 to 2.6 and the selection of chemicals for further inclusion in this review is summarised in Section 2.7.

2.2 Steroid oestrogens

The primary function of the natural steroid oestrogen hormones is in the development and function of the female sex organs. They include oestrone (E1), 17β-oestradiol (E2) and oestriol (E3), of which 17β-oestradiol is the most biologically active regarding oestrogenic activity. A synthetic steroid, 17α-ethinyloestradiol (EE2), is an active ingredient in the oral contraceptive pill and is one of the most biologically active steroid oestrogens known (Martingdale 1993). The chemical structures of these steroid oestrogens are similar (Figure 2.1).

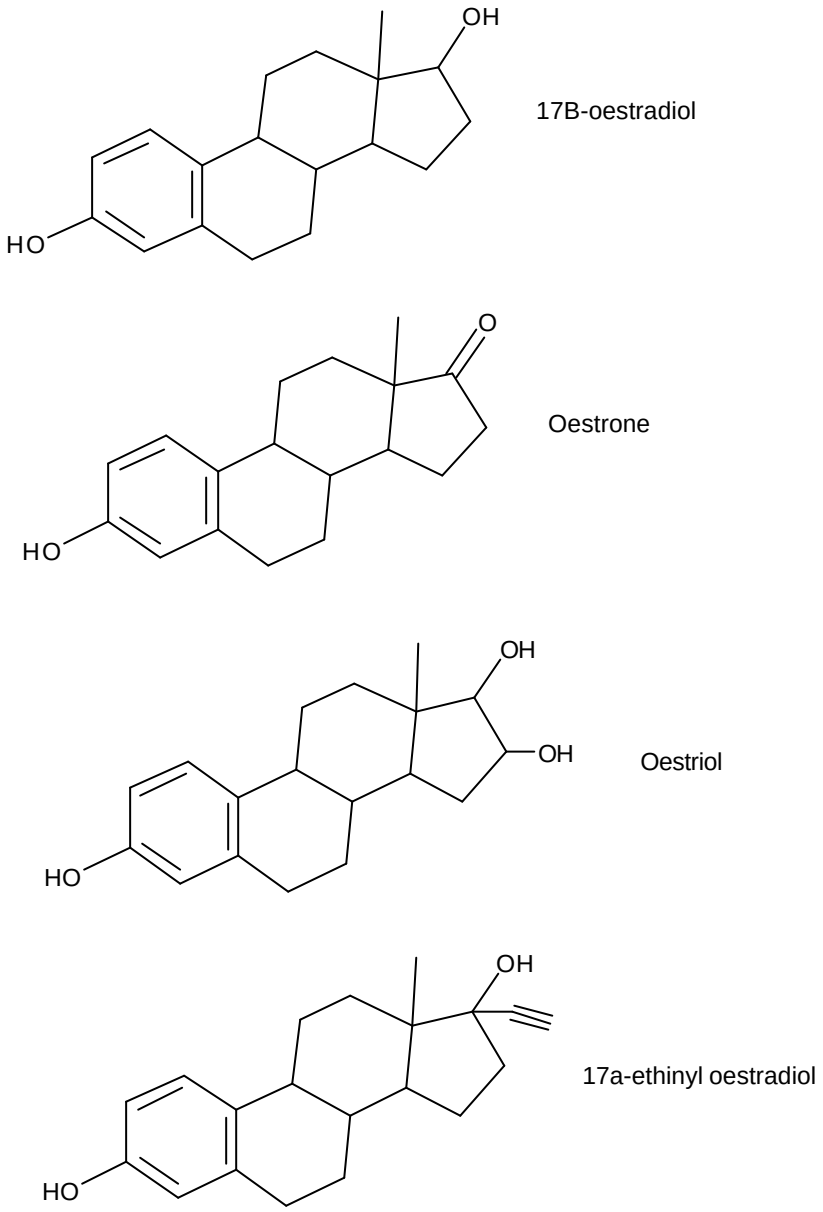

Figure 2.1 Chemical structures of the main steroid oestrogens

Menstranol is another synthetic steroid oestrogen and an active ingredient of oral contraceptives. However, menstranol is rapidly converted to 17α-ethinyloestradiol within the body (Martingdale 1993) and thus excretion of menstranol itself is not considered to be a significant source of oestrogenic activity in sewage.
Naturally occurring steroid oestrogens are present in the urine and excrement of both male and females. The synthetic steroid, 17α-ethinyloestradiol, is excreted during use of oral contraceptive and therapeutic hormone preparations. The proportions of the individual steroids present in sewage are affected by demographic factors such as; the ratio of males to females, age distribution of the female population, oral contraceptive use and the proportion of pregnant females. Other potential sources include wastewater from synthetic steroid manufacture or from formulation and packaging of certain pharmaceutical products. These sources could result in localised releases, either as trade effluent discharges to sewer or as direct discharges of treated effluent to surface water. However, it is understood that

there is no bulk manufacture of 17α-ethinyloestradiol in the UK and that aqueous releases from pharmaceutical packaging plants are small and highly localised. Animal excrement is a potentially significant source of steroid oestrogens in the environment (Blok and Wösten 2000). However, with the possible exception of trade effluents from cattle markets, abattoirs and veterinary premises, animal sources are unlikely to make significant contributions to the oestrogenic properties of domestic sewage when compared with human releases.

The androgenic steroid hormone testosterone is a potential precursor to the oestrogen 17β-oestradiol. This transformation requires the reduction of the ketone group to an alcohol and the removal of a methyl group (Figure 2.2). Whilst this conversion may occur within the human body (Turan 1996), there is no evidence that this process occurs in the sewer following excretion. There is, however, some evidence that testosterone may be removed during sewage treatment. Layton *et al*. (2000) observed over 95% removal from the aqueous phase of ^{14}C-labelled testosterone in activated sludge from four STWs, with most elimination being due to mineralisation rather than accumulation in sludge. Overall, the reported endocrine disruption effects of sewage effluents are oestrogenic rather than androgenic, suggesting that testosterone release to the environment is not generally significant.

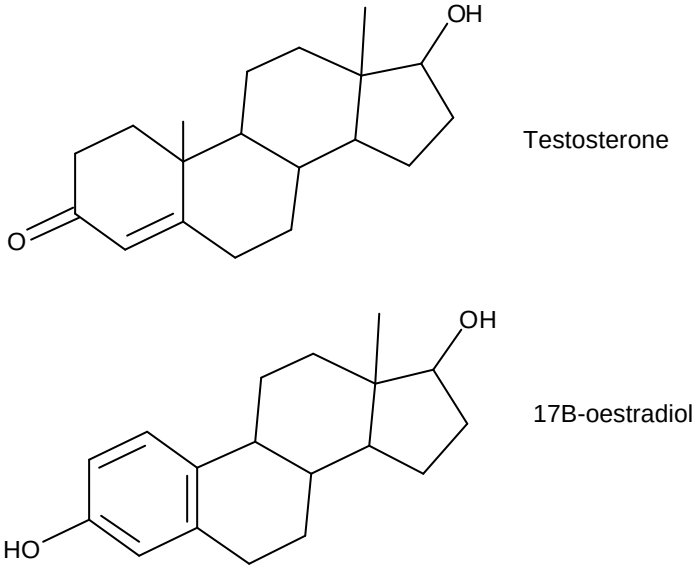

Figure 2.2 Comparison of the chemical structures of 17b-oestradiol and testosterone

Only about 5 to 10% of the natural and synthetic steroid oestrogens are excreted as the free compounds. The majority are excreted as a variety of biologically-inactive glucuronide, sulphate or sulphoglucuronide conjugates (Andrelini *et al*. 1987; Guengerich 1990). Thus, at a first glance, it might not have been expected that these compounds would give rise to concerns regarding endocrine disruption. However, there is increasing evidence that the glucuronide conjugates hydrolyse either prior to entering the STWs or subsequently during treatment, thereby reforming the parent biologically active steroid. This finding has been explained by the activity of the enzyme β-glucuronidase, which is common in bacteria found in sewage (Ternes *et al*. 1999a, Belfroid *et al*. 1999, Panter *et al*. 1999, Wegener *et al*. 2001). Although survey data for glucuronide conjugates in treated sewage

effluent are limited, the data indicate that levels are low or are undetectable (Belfroid *et al* 1999, Huang and Sedlak 2001).
Oestrone is believed to be more commonly excreted as the sulphate conjugate (Andrelini *et al*. 1987), which might be expected to be more persistent since the arylsuphatase enzyme is likely to be less common in the general microbial population (Baronti *et al*., 2000). In laboratory tests oestrone-3-sulphate appeared to be fairly persistent and whilst it was transformed, the free oestrone could not be detected (Hetheridge 2001). The role of de-conjugation in the removal of the steroid oestrogens is discussed in more detail later (Section 4.1.1).
The concentration at which oestrogenic effects are induced in aquatic organisms varies widely and is dependent upon factors such as the test organism, the end-point and the compound tested. However, the oestrogenic activity of these steroid hormones has been demonstrated at concentrations of a few nanogrammes per litre in a variety of bioassays. These bioassays include numerous *in vivo* studies conducted in fish (e.g. Länge *et al*. 2001, Schäfers *et al* 2001, Brion *et al*. 2001, Van den Belt *et al*. 2001, Thorpe *et al*. 2000, Panter *et al*. 1998, Kramer *et al*. 1998, Routledge *et al*. 1998). Toxicity identification and evaluation studies utilising the *in vitro* yeast bioassay have specifically identified 17β-oestradiol, oestrone and 17α-ethinyloestradiol as compounds associated with the main oestrogenic peak in domestic UK effluents (Desbrow *et al*. 1998).
On the basis of the available data, the Environment Agency have identified the steroid oestrogens as being one of the most important groups of EDCs in the effluent from domestic STWs (Environment Agency 2000). Consequently these chemicals are likely to be the subject of future regulatory controls and potential limit values are discussed later (Section 8.1.1). The relative importance of the steroid oestrogens is supported by Körner *et al*. (2000) who concluded that xenobiotic compounds in sewage effluent constituted only 1-4% of the observed oestrogenic activity.

2.3 Alkylphenol polyethoxylates and their metabolites

The alkylphenol polyethoxylates (APEOs) are non-ionic surfactants based on ethoxylated derivatives mainly of nonylphenol and octylphenol. For decades, the APEOs (principally the nonylphenol polyethoxylates (NPEOs)) have been economically important as non-ionic surfactants used in a variety of applications. Total UK consumption was reported to be 16000 to 19,000 tonnes/pa, of which approximately 6,500 tonnes was released to the aquatic environment (Blackburn and Waldock, 1995). In an assessment of UK applications of nonylphenol polyethoxylates (Table 2.1), almost half the production was considered to be consumed in industrial cleaning products (47%). The remainder of uses were in paint (15%), agrochemical formulations (12%), coatings and adhesives (8%), textile scouring (6%) and a diverse range of other products (12%), which included photographic, medicinal, fuel additives, cold cleaners for cars, and household cleaners (Whitehouse 2000; Thiele *et al*. 1997).

Table 2.1 Estimated uses of nonylphenol polyethoxylate surfactants (CES, 1993)

Sector	Usage (tonnes p.a.)
Industrial cleaning products	7500-8500
Paint	2000-3000
Agrochemical formulations, for aiding product stability and aids to wetting and penetration	2000
Emulsion polymers e.g. coatings, adhesives	1500
Textiles (scouring*, fibre lubrication, dyeing)	1000
Metal finishing, especially cleaning	1000
Lubricating oils (as nonylphenol polyethoxylates phosphate esters)	600
Other applications e.g. photographic applications, metal working fluids, spermicidal lubricants, fuel additive, hand cleaning gels, dust suppression	100-1000
Total	14500-18500

* a process designed to remove the natural oils present in wool

It is of note that NPEOs have not been used in domestic cleaning products or detergents in the UK since 1976 following their voluntary withdrawal from such products. In 1992, the Paris Commission issued a recommendation to also phase out their use in industrial cleaning products by the year 2000, although this recommendation did not extend to the use of octylphenol polyethoxylates. As a result of pressure from the Environment Agency, the use of all APEOs for wool scouring is being reduced by the textile industry (Whitehouse 2000).

Despite these measures, APEOs continue to remain in widespread use and clearly the quantities received by domestic STWs may vary considerably between locations and over time depending on the local industrial use. Whilst activated sludge treatment has been shown to successfully eliminate the parent compounds, a wide variety of by-products are formed (Ahel *et al*. 1994a,b; Di Corcia *et al*. 1998; Fujita *et al*. 2000) through a process of removal of ethylene oxide (EO) units and oxidation of the terminal OH group. Together, these result in the formation of APEOs with shorter EO chain lengths ('lower' APEOs, especially the alkylphenol mono and diethoxylates) and their carboxylated derivatives. In some cases, compounds which are carboxylated in both the ethylene oxide and alkyl chains are formed (e.g. Di Corcia *et al*, 1998). These may undergo further degradation to form the alkylphenols, nonylphenol and octylphenol which, as explained below, are of greatest significance by virtue of their greater persistence, toxicity and oestrogenicity.

The original compounds, their derivatives and breakdown products are conventionally abbreviated to simplify presentation. The approach to abbreviation adopted here is summarised in Table 2.2 and examples of some chemical structures and their abbreviations are shown in Figure 5.1.

Table 2.2 Abbreviations used here for alkylphenol ethoxylates and metabolites

General name	Abbreviation	Specific example	Abbreviation
Alkylphenol	AP	Nonylphenol	NP
Alkylphenol ethoxylate	APEO	Nonylphenol diethoxylate	NPEO (specifically NP2EO in this case)
Alkylphenol carboxylate	APEC	Nonylphenol monocarboxylate	NPEC (specifically NP1EC in this case)
Alkylphenol carboxylates which are also carboxylated in the alkyl chain	CAPEC	Carboxy nonylphenol diethoxycarboxylate	CA8P2EC

N.B. The prefix 'alkylphenol' is a generic term including both nonylphenol and octylphenol compounds.

Whilst parent APEOs are considered to be biodegradable and of low aquatic toxicity, the metabolites of APEOs have received a great deal of attention regarding their inherent toxicity and persistence. Indeed, it has been shown that their toxicity, persistence and oestrogenic potency increases as the length of the ethoxylate chain decreases. For these reasons, research and regulatory pressure has been increasing on this group of compounds, leading to the development of EQSs for nonylphenol, octylphenol and APEOs (Whitehouse, 1998a,b; 2000). Much of the research has focussed on the potential endocrine disrupting properties of some of the metabolites. In particular, there has been considerable research conducted on the oestrogenic potency of 4-*tert*-nonylphenol and 4-*tert*-octylphenol based on both *in vitro* assays (e.g. Jobling and Sumpter 1993, White *et al*. 1994) and *in vivo* studies in fish (e.g. Routledge and Sumpter 1996). The oestrogenic activity of the 'lower' ethoxylates and carboxylates is less well studied. Nonetheless, the studies to date suggest that these metabolites, 4-*tert*-nonylphenol and 4-*tert*-octylphenol are all of low oestrogenic potency ($\approx 10^{-4}$-10^{-5}) compared to 17β-oestradiol (Jobling and Sumpter 1993, White *et al*. 1994, Routledge and Sumpter 1996, Jobling *et al*. 1996, Metcalfe *et al*. 2001). In particular, Routledge (1997) compared the relative oestrogenic potency of 4-*tert*-nonylphenol (NP), 4-*tert*-octylphenol and the short ethoxylate-chain metabolites of NP, using an *in vitro* assay. Of these 4-*tert*-octylphenol was the most potent (1.5×10^3 less potent than 17β-oestradiol), followed by 4-*tert*-nonylphenol (7×10^3 less potent). However, alkylphenols are a complex mixture of isomers and the technical grade of nonylphenol used in this comparison contains only 76-79% 4-*tert*-nonylphenol (Wheeler *et al*.,1997), which suggests the actual potency to be 5×10^3 less than 17β-oestradiol, approximately 3 times less potent than 4-*tert*-octylphenol. The nonylphenolcarboxylates (NPECs) tested were approximately one order of magnitude less potent, and nonylphenol diethoxylate (NP2EO) two orders of magnitude less potent than 4-*tert*-octylphenol. Probably of greater significance than the oestrogenic effects of most of these APEO metabolites is their acute and chronic toxicities to aquatic life and it is on this basis that EQSs for the APEOs and even the more oestrogenically potent alkylphenols have been derived (see Section 8.1.2). Nevertheless, there is evidence that the alkylphenols produced as a result of biodegradation tend to accumulate in sediments by virtue of their greater hydrophobicity. Thus, it is possible that contamination of sediments by alkylphenols could lead to adverse

effects if concentrations rose to a sufficient level. Currently, no standards for sediment contamination by alkylphenols or APEOs are available.

2.4 Phthalate Esters

In this context the compounds of particular interest are the esters of o-phthalic acid (1,2-benzenedicarboxylic acid) and these are generally described here by the generic term “phthalates”. The phthalates are widely used in the manufacture of plastics because they impart flexibility, transparency and other desirable properties. Although some 20 phthalate esters are in common use, only a small number are produced in large quantities (see Table 2.3). Of particular note is that di(2-ethylhexyl)phthalate (DEHP) is by far the most commonly used of these chemicals, accounting for over half the commercial production (Moore *et al* 2001).

Table 2.3 Production rates for high consumption phthalate esters (>2000 tonnes/annum)

Phthalate name	Abbreviation	European consumption (tons/annum)
Di(2-ethylhexyl)phthalate	DEHP	400,000-500,000
Diisononyl phthalate	DINP	100,000-200,000
Diisodecyl phthalate	DIDP	100,000-200,000
Butyl benzyl phthalate	BBP	20,000-50,000
Dibutyl phthalate	DBP	20,000-40,000
Diisobutyl phthalate	DIBP	20,000-40,000
Ditridecyl phthalate	DIBP	3,000-10,000
Diethyl phthalate	DEP	10,000-20,000 (with DMP)
Dimethyl phthalate	DMP	10,000-20,000 (with DEP)

Adapted from Harries *et al* (1997b)

Although the phthalates were not identified as priority compounds in the Environment Agency’s strategy on endocrine disrupters, this group continues to generate significant interest in their potential endocrine-disrupting properties. Indeed, butylbenzyl phthalate (BBP), dibutyl phthalate (DBP) and DEHP have been identified by the EU as endocrine disruptors with high exposure concern (ENDS Daily 10/7/2000). Whilst these chemicals may not be strongly oestrogenic they have been observed at relatively high concentrations in sewage. It is therefore considered appropriate to consider phthalates further here in view of the potential for future regulation.

As the phthalates comprise a large group of compounds, it is important to identify those that are significant in terms of endocrine disrupting properties. Phthalates have been tested for their endocrine-disrupting properties in a variety of *in vitro* assays (which include receptor binding affinity tests, cellular proliferation assays, gene expression tests). However, only a few exhibit weak activity, with the majority demonstrating no activity (e.g. Jobling *et al*. 1995, Harris *et al.* 1997, Soto *et al*. 1995, Bolger *et al*. 1998, Zacharewski *et al*. 1998, Knudsen and Pottinger, 1999, Metcalfe *et al*. 2001, Van Wezel *et al*. 2000, Picard *et al.* 2001).

For those phthalates where endocrine disrupting effects have been observed, the relative potency compared to the natural hormone 17β-oestradiol is low (10^{-4}-10^{-8}). The phthalate esters with the most evidence for oestrogenic activity *in vitro* are

BBP and DBP, their relative potencies being 10^{-4}-10^{-6} and 10^{-5}-10^{-7}, respectively. Studies investigating the oestrogenic activity of DEHP *in vitro* have provided conflicting results, although most have proved negative. Where positive results have been reported, these again indicate a low relative potency (10^{-5}) compared to 17β-oestradiol (Van Wezel *et al*. 2000).

It is recognised that whilst *in vitro* studies are useful as a first screen for identifying chemicals, which should be further, evaluated, *in vivo* tests (i.e. in whole organisms) are more biologically relevant (Moore 2000).

When phthalate esters have been tested for oestrogenic activity *in vivo*, these have almost entirely involved mammalian bioassays. Two commonly used tests are the mouse uterotrophic and vaginal cornification assay, neither of which have provided evidence for oestrogenic activity for a variety of phthalate esters (including DBP, DEHP and BBP) (Zacharewski *et al*. 1998, Milligan *et al*. 1998, Brady *et al*. 1998, Coldham *et al*. 1997).

However, other *in vivo* rodent studies using different protocols (e.g. multi-generation studies) have reported adverse effects on the development of the male reproductive tract following exposure to DBP (Wine *et al*. 1997, Mylchreest and Foster 1998, Ema *et al*. 1996a,b,c), DEHP (Acardia *et al*. 1998, Moore *et al*. 2001) and BBP (Nagao *et al*. 2000). However, these effects were observed following exposure to relatively high doses, making their interpretation with regard to environmental exposure difficult. In general, these studies support the view of an anti-androgenic mode of action (Mylchreest and Foster 1998, Moore *et al*. 2001).

In one *in vivo* rodent study, pregnant female rats were given BBP in the drinking water at 1 mg/l (Sharpe *et al*. 1995). Reduced testicular weight was seen in male offspring at sexual maturity, which suggested that BBP was weakly oestrogenic and at relatively low doses. However, a repeat of the study by Ashby *et al*. (1997) did not reproduce these results. In addition, repeats by Sharpe's own laboratory failed to confirm the original findings, thereby casting further doubt over the validity of the original data (Sharpe *et al*. 1998).

Only two studies were located which have specifically tested the endocrine disrupting potential of phthalate esters in aquatic organisms. Zou and Fingerman (1997) investigated the effect on time to molting in *Daphnia magna* when exposed to high concentrations of diethylphthalate. A concentration of 22.4 mg/l was found to increase the time to molting, whereas no such effect was seen at 11.2 and 5.6 mg/l. Metcalfe *et al*. (2001) exposed early life stages (1 day after hatch) of the Japanese medaka fish to nominal concentrations of up to 5 mg/l of DEHP until the medaka reached approximately 1.5 cm in length (which occurred at 85-110 days post hatch). No evidence of *in vivo* oestrogenic activity was observed based on alteration to sex ratios and the development of testis-ova.

In conclusion, the phthalate esters considered to have the most evidence of endocrine disrupting activity on the basis of both *in vitro* and *in vivo* mammalian tests are BBP, DBP and DEHP. This is reflected by their inclusion on the EU priority list of endocrine disrupters. However, their oestrogenic potency compared to 17β-oestradiol is considered to be weak. Furthermore, studies in aquatic organisms support the view that phthalates are unlikely to pose a significant endocrine disruption risk to aquatic life. In view of the potential regulatory concern these compounds have been considered further in this review.

2.5 Bisphenol A

Bisphenol A (BPA, 4,4'-isopropylidenediphenol) is an intermediate used in the production of polycarbonate and epoxy resins. Polycarbonate plastics are commonly used in the automotive, construction, packaging and electronics industries, whilst epoxy resins are used for surface coatings such as paint, the lining of metal food cans, bottle tops, dental coatings and some linings applied to water mains, as well as the construction and electronics industries. In 1998, the world production of BPA was 1.62 x 10^6 tonnes, of which 0.5 x 10^6 tonnes was produced in Western Europe. Because BPA is used widely in both household and industry, it has the potential to be present in raw sewage and wastewater effluents. BPA is classed as a weak oestrogen in terms of *in vitro* effects. Routledge (1997) analysed a range of potential oestrogens using a yeast oestrogen assay and suggested BPA to be 20,000 times less potent than 17β-oestradiol. Metcalfe (2001) and Takigami (2000), both reported *in vitro* values of approximately 25,000 times less potent using the same yeast oestrogen assay. Islinger *et al.* (1999) again using an *in vitro* assay determined BPA to be 2000 times less potent than 17β-oestradiol, and measurements by Gaido (1997) also using a yeast based receptor assay, indicated BPA to be 15,000 times less potent. These data suggest the oestrogenic potency of BPA to be of the order of 1-2 x 10^4 less than that of 17β-oestradiol.

In vitro assays are useful indicators for relative potency of oestrogenic chemicals, but *in vivo* effects are more relevant. Several examples of *in vivo* effects of BPA on fish, frogs and snails have been reported.

An extensive multigenerational study on fathead minnow was reported in two stages by Sohoni *et al* (2001a and b). This study demonstrated the effect of BPA over three generations starting with sub-adult fish (120 days post hatch). Fish were exposed to BPA at 1, 16, 160, 640 and 1280 μg/l in flow-through systems such that the F1 generation was exposed for their entire life and F2 generation for 60 days post hatch. The total exposure period was 431 days and supporting analysis confirmed nominal test concentrations were maintained for the duration of the study. Several end-points were used to assess the estrogenic nature of BPA, these included egg production and hatchability, measurement of the induction of vitellogenin, inhibition of gonadal growth (as measured by the gonadosomatic index:GSI). In addition, for male fish, the gonad histology included a scoring of the various testicular cell types in order to assess the progression of spermatogenesis. The study concluded that BPA acts as a weak oestrogen to fathead minnow when exposed via water. The overall NOEC for conventional endpoints of survival and growth was 160 μg/l, and for reproduction based on the hatchability of the F2 generation, and for vitellogenin production was 16 μg/l.

However, gonad histology indicated that the proportion of the four different spermatogenesis cell types was affected at the lowest test concentration, (1 μg/l). Progression of spermatogenesis is a relatively new biomarker, which is not fully understood in terms of its ecological significance, particularly when assessing reproduction. It is of note that the hatchability of eggs dosed at test concentrations of 1 and 16 μg/l BPA was unaffected in the study.

A semi-static test system was described by Metcalfe *et al*. (2001) for a 100-day exposure of BPA to 1-day post hatch Japanese medaka at test concentrations of 10 μg/l, 50 μg/l, 100 μg/l and 200 μg/l. The study was carried out with supporting

analysis. Two end points were considered; alteration to sex ratio, and development of testis-ova (an indication of intersex condition). The observed sex ratios for all medaka exposed to all concentrations of BPA were not statistically different from controls. The development of testis-ova was observed in only two medaka exposed to 10 μg/l BPA, this was not observed at higher doses. Male medaka dosed at 50 μg/l and above did however, show morphologic changes including loss of testicular structure and a decrease in the number of spermatazoa. Based on these findings, the author claimed an endocrine disruption end point could be observed in Japanese medaka fish at a concentration of 10 μg/l.

A flow-through study, Yokota (2000) determined the effect on Japanese medaka dosed with BPA at nominal concentrations of 3.2, 16, 80, 400 and 2000 μg/l from fertilised eggs to 60 day post hatch. The authors examined effects on hatchability, growth, and testis development. BPA had no effect on hatchability of the fertilised eggs. The authors used secondary sex characteristics to determine the sex ratio of fish from each dose. They concluded that although this method is not a particularly reliable indicator for medaka, no male fish were observed in the top dose and a skewed sex ratio (3:1 female to male) was observed at 400 μg/l BPA. The histological examination produced similar sex ratio to those described for secondary characteristics, however the development of testis-ova (32%) was only observed in medaka exposed to the top concentration, nominal 2000 μg/l BPA.

Two studies have been reported on Amphibian species *Xenopus* (African clawed frog). Kloas *et al* (1999) exposed 2-3 day old tadpoles to nominal concentrations of BPA at 23 μg/l and 2.3 μg/l for approximately 84 days in a semi-static dosing system. The sex ratio of males to females was determined following exposure and a statistically significant increase in the number of female phenotypes in relation to the controls was observed for *Xenopus* dosed at 23 μg/l. A decreased male:female ratio was also observed in the 2.3 μg/l test group but was not statistically different to the controls.

Pickford *et al* (2001) reported a similar study in an attempt to repeat the original findings of Kloas. The test was initiated with four-day-old larvae and exposure to the test substance commenced approximately 2 days post-hatch. These were exposed to BPA at nominal concentrations of 1, 2.3, 10, 23, 100 and 500 μg/l using a flow-through test system with four replicate test vessels at each concentration and a positive control (17β-oestradiol). The test was terminated at day 90. The sex ratios were assessed and the author concluded that no significant difference from the expected 50:50 sex ratio were observed in any of the test concentrations or the dilution water control. A significant feminisation effect was observed for the positive control group. Additionally, the exposure of larvae to BPA did not result in an increase in gross gonadal abnormalities.

One recent publication on the endocrine effect of BPA on snails, Oehlmann *et al* (2000), is significant because of its conclusions. The authors reported the effect of BPA on two species of prosobranch snails. Adult snails were exposed to nominal concentrations of 1, 5, 25, and 100 μg/l BPA using a semi-static system. The authors referred to effects that they termed 'superfeminisation', which occurred at each test concentration and were statistically significant when compared to the control. The authors concluded that the results demonstrate prosobranchs are sensitive to endocrine disruption at the lowest concentrations of BPA tested (1 μg/l). However, there was no effect on the hatching success of eggs produced after exposure to BPA over the first 5 months of the study which suggests further

studies are needed to determine the accuracy of these findings and relevance of this study.
In summary, endocrine disrupting effects other than the standard end-points of hatchability, gonadal index and vitellogenin production, may be occurring at levels below the 16 µg/l no observable effect concentration (NOEC) reported by Sohina *et al*. (2001a). These effects include disruption to male spermatozoa development in fathead minnow at 1 µg/l and the superfeminisation observed in prosobranch snails at 1 µg/l. However, the ecological significance of these latter effects remains unclear, particularly given the ability of the fish to reproduce and that 16 µg/l BPA had no effect on hatchability.

2.6 Peticides and PCBs

A number of insecticides such as methoxychlor (Soto *et al*. 1995), endosulphan and dieldrin (Soto *et al*. 1994) have been suggested as having endocrine disrupting effects. Similarly the anti-foulant tributyltin (Oehlmann *et al*. 1996; Gülden *et al*. 1998), and industrial waste products such as PCBs (Gülden *et al*. 1998) have been implicated. However, apart from the occasional trade waste, none of these compounds would be considered as significant constituents of domestic sewage influent. It might be tempting to argue that these compounds, which largely enter catchments via diffuse inputs, play a more important role in endocrine disruption than the EDCs present in sewage effluent. However, both caged fish studies (Harries *et al*. 1997a) and those with wild roach (Jobling *et al*. 1998a,b) indicated that the greatest endocrine disruption was associated with fish populations close to sewage effluent discharges. Therefore, in view of the low occurrence in domestic sewage, these compounds have not been considered further in this review.

2.7 Summary

On the basis of the studies briefly reviewed here, steroid oestrogens have been identified as the most likely candidates for future regulatory control. Firstly, they have been demonstrated in several studies to be the more oestrogenically potent components of domestic sewage effluent. In addition, a growing number of laboratory studies in fish have been published which support the view that they display oestrogenic effects at concentrations of a few nanogrammes per litre. Since the compounds are primarily of human origin and cannot easily be controlled at source, emissions to the environment from STWs will be dependent in part upon the degree of treatment given.
Alkylphenols and short-chain ethoxylate metabolites, derived from the widely used alkylphenol polyethoxylates products, represent another group of compounds of particular interest. Although of relatively low oestrogenic potency their occurrence is widespread and some localised impacts have been noted.
Both phthalates and bisphenol A may be found in sewage effluent at low concentrations but, given their weak oestrogenic potency, they are currently of low concern as endocrine disrupting chemicals. Nonetheless, in view of possible future regulatory interest, they have been included in the subsequent review.
Some pesticides, PCBs, and anti-foulant compounds have been shown to have weak oestrogenic properties. However, their occurrence in domestic sewage is relatively low and they would be expected to contribute more through diffuse inputs to the environment rather than through domestic sewage. Consequently, these compounds have not been considered further.

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