MINISTRY OF ENVIRONMENT
PROVINCE OF BRITISH COLUMBIA

Water Quality Guidelines for Pharmaceutically-active-Compounds (PhACs): 17α-ethinylestradiol (EE2)

Technical Appendix

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

BAF – Bioaccumulation Factor
BCF - Bioconcentration Factor
EC – Effect concentration
EDC – Endocrine disrupting compounds
E1- estrone
E2 – 17β-estradiol
17α-E2 – 17α-estradiol
EE2 – 17α-ethinylestradiol
HC – Hazard concentration
LC – Lethal concentration
LOEC – Lowest observable effect concentration
MATC – Maximum acceptable toxicant concentration
NOEC – No observable effect concentration
SSD – Species sensitivity distribution
VTG - vitellogenin
WWTP – Wastewater treatment plant

1.0 Introduction

Pharmaceutically-active-compounds (PhACs) refer to substances or mixtures of substances that are manufactured or sold for use in the diagnosis, treatment, mitigation, or prevention of a disease, disorder, or abnormal physical state, or its symptoms in humans and animals (Canadian Food and Drugs Act 2006). They can be prescription or non-prescription. PhACs and their metabolites can find their way into the aquatic environment through several avenues including: excretion from human and animal systems; leaching from landfill, manure or biosolids applications; and improper disposal (i.e. flushing them down the toilet). In some instances, some pharmaceutical compounds have been measured at concentrations that may cause adverse effects to resident populations in the aquatic environment. In a recent review on the risks of PhACs to the environment, Enick and Moore (2007) noted serious environmental concerns because they are:

- ubiquitous and globally distributed;
- specifically designed or discovered to alter biological functions;
- associated with a wide range of side effects in non-target organisms; and
- chronically toxic at concentrations found in the environment.

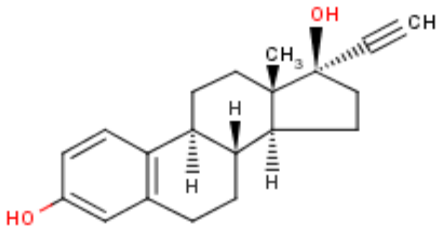
Although many PhACs have been detected in the environment, estrogens and hormone regulators (e.g., estradiol) appear to be most studied in the literature, with regards to their adverse effects in aquatic life. As a result, these compounds are the focus of this report.

2.0 Chemical and Biological Characteristics of the Steroidal Estrogens

Ethinylestradiol (or 17α-ethinylestradiol; CAS Number: 57-63-6; $C_{20}H_{24}O_2$; molecular weight = 296.403; Figure 1) is a synthetic hormone, which is a derivative of the natural hormone estradiol. Ethinylestradiol is an orally bio-active estrogen used in almost all modern formulations of combined oral contraceptive pills and is one of the most commonly used medications. Ethinylestradiol was the first orally active synthetic steroidal estrogen, synthesized in 1938 by Hans Herloff Inhoffen and Walter Hohlweg at Schering AG in Berlin (Petrow 1970)

17α-ethinylestradiol (EE2) is an odourless compound that is white to creamy white in colour. It is sparingly soluble in ethanol (1 part in 6 parts of ethanol), but has relatively low solubility in water (4.8-11.3 mg L^{-1} at 27 °C). It has a relatively high octanol-water partitioning coefficient (Kow =3.67 – 4.2) which causes it to be persistent and preferentially attach to the organic matter in the aquatic environment (NAS undated, Snyder et al. 2008, Lai et al. 2002b).

Figure 1: Chemical structure of ethinylestradiol



17α-ethinylestradiol EE2 ($C_{20}H_{24}O_2$)

The biological half-life of a substance is the time it takes for a substance (drug, radioactive nuclide, or other) to lose half of its pharmacologic, physiologic, or radiologic activity. The half-lives of EE2 and 17β-estradiol (E2) are approximately 33 ±13 hours and 13 hours, respectively.

Lai et al. (2002a) have reviewed the fate of the natural (i.e. E2) and synthetic (i.e. EE2) hormones in the aquatic environment. Because of their relatively high octanol/water partition coefficients (Kow =3.67 to 4.2), these estrogens bind rapidly to suspended solids. In natural river waters, the estrogens degraded in water under aerobic conditions; however, they appeared to be unaffected when incubated under anaerobic conditions. The half-lives of E2 and EE2 were reported to be 3 to 27 days and 46 days, respectively, at 20 °C under aerobic conditions.

In studying the bioaccumulation potential of natural and synthetic estrogens, Lai et al. (2002b) predicted the bioaccumulation of steroidal estrogens in all organisms in the river systems (i.e., plankton, invertebrates, and fish) using a food-web model. The bioaccumulation factor (BAF) for EE2 ranged from a low value of 33 for a benthic invertebrate to a maximum of 332 for a fish. The maximum BAF (332) was found in the fish at the lowest trophic level. These investigators also noted that their BAF predicted from the food web model was generally smaller than the bioconcentration factor (BCF) of 15849 for EE2 calculated using the simpler Kow –BCF relationships.

Both natural hormones (i.e. estrone (E1), 17α-estradiol (17α-E2), 17β-estradiol (E2)) and synthetic hormones (EE2) have the potential to behave like endocrine disrupting compounds (EDC) in the environment. EDCs can cause reproductive disturbances in fish,

including reduced fertility, masculinization of females and feminization of males (Vethaak et al. 2006).

3.0 Sources, occurrence and behaviour in the environment

A considerable quantity of hormones is excreted by humans and livestock. These hormones find their way to surface and ground waters through sewage treatment plants, septic systems, and through runoff and leaching from agricultural lands. Wastewater originating from industrial sources, such as synthetic hormone production facilities, also contribute to the hormone load in the environment.

In a survey of natural hormones (17α-E2, E2, and E1) and a synthetic hormone (EE2) in an aquatic environment in the Netherlands, Belfroid et al. (2006) noted that concentrations of 17α-E2, E2 and EE2 in surface waters were mostly below method detection limits (MDL) 0.3 ng L^{-1} for 17α-E2 and EE2, and 0.8 ng L^{-1} for E2). E1 was observed in about 45% of all collected water samples (MDL for estrone was 0.3 ng L^{-1}). Estrogenic hormones were not observed in the rain water (MDL <0.6 ng L^{-1}).

In an earlier study, Belfroid et al. (1999) reported concentrations of E2, 17α-E2, and EE2 in samples from the rivers Rhine (maximum of 5.5, 1.1, and 0.4 ng L^{-1}, respectively) and Meuse (maximum of 2.8, 3.0, and 4.3 ng L^{-1}, respectively). All other locations were below the MDLs.

Much higher concentrations of hormones were reported by Pojana et al. (2004) in a study on a shallow coastal lagoon of Venice. Concentrations ranging from <2 to 85 ng L^{-1} (E1), <1 to 51 ng L^{-1} (E2), and <2 to 75 ng L^{-1} (EE2) were found. This was not surprising since the lagoon was subject to anthropogenic sources of pollution at many locations, including untreated sewage. Similarly, Shen et al. (2001) reported concentrations ranging from 1.6 to 15.5 ng L^{-1} for E1, and 5.7 to 30.8 ng L^{-1} for EE2 for a lake in China.

Untreated sewage discharges contribute most to the environment load of estrogenic hormones. In untreated municipal wastewater in the Netherlands, de Voogt et al. (2006) reported concentrations ranging from <0.7 to 15 ng L^{-1} for 17α-E2 (median= 4.9 ng L^{-1}; MDL =0.8 ng L^{-1}), 17 to 150 ng L^{-1} for E2 (median= 36.5 ng L^{-1}; MDL= 0.8 ng L^{-1}), 20 to 130 ng L^{-1} for E1 (median= 12 ng L^{-1}; MDL= 0.3 ng L^{-1}), and <0.3 to 5.9 ng L^{-1} EE2 (mean= 3.2 ng L^{-1}; MDL= 0.8 ng L^{-1}). However, the concentration in the effluent post treatment were much lower at <0.4 ng L^{-1} for 17α-E2, <0.8 ng L^{-1} for E2, <0.3 – 11 ng L^{-1} for E1 (median= 3.4 ng L^{-1}), and <0.3 – 5.9 ng L^{-1} for EE2 (median= 3.2 ng L^{-1}). It appears that while E2 and 17α-E2 removal by the sewage treatment plant was quite efficient, the removal of E1 and EE2 hormones was variable and incomplete.

In a German study, estrogens were measured in more than 50% of the investigated effluents. The maximum values ranged between 0.6 and 35 ng L^{-1} for EE2, between 0.6 and 43 ng L^{-1} for E2, and between 18 and 130 ng L^{-1} for E1 (Karbe et al. 2006).

In a survey of wastewater treatment plants (WWTP) and drinking water supply systems in Calgary, Alberta, Chen et al. (2006) measured 1.5 ng L^{-1} of E2 in the Bonnybrook WWTP effluent whereas no E2 was detected in the Fish Creek WWTP effluent. The reverse was the case for EE2, which was not detected in the Bonnybrook WWTP effluent but was found in the Fish Creek WWTP effluent (8.5 ng L^{-1}). These investigators also found that steroidal estrogens (e.g. E1, E2, 17α-E2, and EE2) were not detected in the upstream surface water (source water) or in the downstream surface water of Calgary. Dilution and sorption on to suspended solids were thought to be the cause of their absence in the downstream given the fact that steroid estrogens cannot be entirely removed by WWTPs.

Fernandez et al. (2007) monitored steroidal estrogens in the primary and final effluents of one of the largest trickling filtration solid contact (TF/SC) municipal WWTP in North America, serving a suburban population of 740,000 (city not named in the reference). EE2 was found to be on the average <1 ng L^{-1} in the effluent samples with the exception of one extreme value of 131 ng L^{-1}; however, the levels of E2 averaged 5.5 ng L^{-1}.

Campbell et al. (2006) measured estrogenic activity in effluent collected weekly from Annacis Island WWTP of the Metro Vancouver Regional District (MVRD) (formerly Greater Vancouver Regional District). The levels of EE2 in some samples were >170 ng L^{-1}. Also, the maximum concentration of E2 exceeded 25 ng L^{-1}, and the concentration of E1 ranged between 1 and 54 ng L^{-1}. It was also noted that the steroidal estrogen levels measured in the Annacis Island effluent were relatively much higher than those reported in Europe (e.g. U.K., Germany). This difference was attributed to the fact that the Annacis Island WWTP was primarily designed to remove total suspended solids to reduce biological oxygen demand levels, and is therefore not designed to remove steroidal estrogens.

Raw influent (untreated) and effluent (treated) samples were collected by Nelson et al. (2007) at the 5 WWTP operated by MVRD. Among 14 target endocrine disrupting compounds (EDCs), 4 steroidal and 3 industrial EDCs were detected. E1 and E2 were found in all 5 influents (3.3 to 8.4 ng L^{-1} and 0.2 to 1.9 ng L^{-1}, respectively) and 5 effluents (1.3 to 27.2 ng L^{-1} and 0.1 to 11.2 ng L^{-1}), respectively. EE2 was not detected in any of the 10 samples. These investigators also noted that levels of E1 were relatively higher than that of E2 due to biological conversion of E2 to E1.

Steroidal estrogens go through a transformation in the environment and in the effluent in contact with activated sludge. In a batch experiment spiked with 1 ng mL^{-1}, E2 showed a half-life of about 0.2 h with nearly all being converted to E1. E1 was removed more slowly with a half-life of 1.5 h at 20 °C under conditions similar to that of the E1 batch experiment. It was also found that the conjugates of estrogens were cleaved in wastewater systems, and E2 was oxidized into E1. EE2 was persistent throughout wastewater treament. In addition to the transformation under oxic conditions, sorption of estrogens onto sludge contributes to their elimination from the water phase. About 5% of

the natural estrogens E1 and E2 left the sewage treatment plant sorbed onto the digested sludge (Ternes et al. 1999, Karbe et al. 2006).

EE2 is considered to be more potent than E1 and E2. Even though EE2 is persistent (Ternes et al. 1999), its absence in the WWTP samples, as noted above by Nelson et al. (2007) in the MVRD study, is not unexpected. For instance, Baronti et al. (2000) has shown that mean EE2 concentration in WWTP influent and effluent were 3 ng L^{-1} and 0.4 ng L^{-1}, respectively (a 90% removal rate by the WWTP). Leyton et al. (2000) also reported as much as 80% of the EE2 in wastewater may be bound to the sewage sludge and thus removed from the aqueous phase. It is, therefore, likely that the sorption of EE2 to solids has caused the reduction of EE2 in WWTP effluent samples.

The discussion to follow will focus on the effects of EE2 on the aquatic environment for the purpose of developing a water quality guideline to protect aquatic life in British Columbia. The selection of EE2, in part, was due to the fact that it is a man-made synthetic estrogen with a reasonable amount of data for guideline development. Future work will include assessing the feasibility of developing guidelines for compounds with similar modes of action (MOA) (e.g., estradiol, estrone) through the use of genomics. Total estrogenicity (expressed as E2 equivalents) could be determined by summing concentrations of individual compounds after adjusting by the compound's estrogenic potency (Feruichi et al. 2004). The U.S. Environmental Protection Agency (USEPA) Science Advisory Board (SAB) reviewed the White Paper on *Aquatic Life Water Quality Criteria for Contaminants of Emerging Concern* and suggested that mixtures of contaminants with comparable MOA may result in higher effective concentrations than would be expected with a single contaminant; it is important that aquatic life criteria account for aquatic organisms being exposed to mixtures of these chemicals (USEPA SAB 2008).

Ecotoxicogenomics is an emerging approach to help predict the impacts of contaminants of emerging concern (Poynton and Vulpe 2009). DNA microarrays can help to identify biomarkers of exposure to contaminants and identify genes, proteins, or metabolites, which are altered depending on the pollutant's specific MOA. Phenotypic anchoring (the ability to demonstrate that a molecular event causes or is associated with a toxicological outcome or disease state) provides an important line of evidence for linking genomics with traditional toxicological endpoints (Poynton and Vulpe 2009).

4.0 Aquatic Life Effects

4.1 Effects on freshwater organisms

Endocrine disrupting substances such as EE2, have the potential to adversely affect the sensitive hormone pathways that regulate reproductive functions. In aquatic organisms, for example, the adverse effects may be expressed in terms of reduced fertility and egg production in female fish or reduced gonad size and feminization of male fish. Exposure

to EE2 may also result in a variety of other effects that include: induced production of vitellogenin in male fish, changes in the sex ratio of progeny, and alterations in gene expressions (Tilton et al. 2005, Jobling et al. 2002, Larkin et al. 2003, Denslow et al. 2001, Metcalfe et al. 2001).

Table 1 lists the effects of EE2 on various aquatic organisms. These results show that EE2 toxicity varied over a wide range of concentrations (about 6 orders of magnitude); in essence, toxicity is a function of the type of aquatic organism, its life stage, length of exposure to the contaminant, and the end point used in the study. In general, while aquatic plants appear to be relatively more tolerant, fish are the most sensitive aquatic organisms to the effects of EE2. Some of the most sensitive species are discussed below.

Among invertebrates, the lowest observed effect level (LOEL) was observed for a variety of zooplankton in a vertebrate-free still-water microcosm during 4 weeks of pre-application, 6 weeks of dosing via controlled release, and a 12 weeks post-treatment period. In the treated microcosms, time-weighted average EE2 concentrations ranged between 7 and 220 ng L^{-1}. The abundance of cladocerans, copepods and rotifers declined during the test. The most affected groups were the offspring of cladocerans and copepods (e.g. *Daphnia longispina* and *Chydorus sphaericus*) (Schramm et al. 2007). These authors also noted that the time-weighted average concentration of 7 ng L^{-1}, where first effects on the plankton community were found in their study, was much lower than the estimated HC_5 of 2,000 ng EE2 L^{-1} (a species sensitivity distribution (SSD)-based hazard concentration predicted to cause stress to only 5% of the organisms in the ecosystem at this concentration) and the predicted no effect concentration (PNEC) of 200 ng EE2 L^{-1} (estimated from an application factor-based approach by applying a safety factor to the LOEC).

In a lab-based comparative study, Jobling et al. (2003) demonstrated that EE2, known to be estrogenic and cause reproductive effects in fish (*Pimephales promelas)*, also affected embryo production in snail (*Potamopyrgus antipodarum*). They reported increased egg-laying in fish and increased embryo production in snails at low exposure concentrations of EE2 (0.1 – 10 ng L^{-1} in fish and 25 ng L^{-1} in snails). Stimulatory effects of EE2 on egg production occurred in fish up to an exposure dose of 3 ng L^{-1} followed by inhibitory effects at higher doses, and cessation of egg production at 100 ng L^{-1}. In snails, however, there was enhanced embryo production, even at EE2 concentrations of 100 ng L^{-1} above the control.

In a life-cycle test, Parrott and Blunt (2005) exposed fathead minnow (*P. promelas*) eggs, 48 h after fertilization, to nominal EE2 concentrations of 0.32 and 0.96 ng L^{-1} and measured concentrations of 3.5, 9.6, and 23 ng EE2 L^{-1}. The fish were observed through the larval, juvenile, and adult stages. No significant changes were observed in fry or juvenile growth from 8 to 30 days post-hatch (dph). An ovipositor index (a female secondary sex characteristic) was the most sensitive early response at 60 dph in fish exposed to >3.5 ng L^{-1}. Continuation of the EE2 exposure until 150 dph significantly decreased egg fertilization and sex ratio (skewed toward females) at the lowest

concentration of 0.32 ng EE2 L^{-1}. The next most sensitive end point was demasculinization (decreased male secondary sex characteristic index) of males exposed to 0.96 ng EE2 L^{-1}. These investigators concluded that the reproductive end points and external sex characteristics measured in mature fish at 150 dph were more sensitive, with a response threshold of EE2 ranging from 0.32 to 0.96 ng L^{-1}.

Similar results were found by Lange et al. (2001) in a complete life-cycle experiment performed with fathead minnow (*P. promelas*) over a 289 day exposure period. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) for gonad histology were 4 and 1 ng L^{-1}, respectively. No testicular tissue could be found in any fish exposed to EE2 concentrations of 4 ng L^{-1} at any age; while the control fish and fish at $\leq$1.0 ng L^{-1} became sexually mature after 120 dph.

Based on the observations made by Lange at al. (2001) above, Grist et al. (2003) argued that an exposure to 4 ng L^{-1} could lead to the extinction of fathead minnow population in an EE2 contaminated environment. With this in mind, Grist et al. (2003) reanalyzed the survival and reproduction data from Lange et al. (2001) to determine the effects of EE2 on the intrinsic rate of population growth. Their results yielded ECr100 values (the concentration estimated to reduce intrinsic growth rate to zero) of 3.1 ng L^{-1} (linear model) and 3.4 ng L^{-1}. These values were comparable to a maximum acceptable toxicant concentration (MATC) of 2 ng L^{-1} for feminization of exposed fish calculated by Lange et al. (2001). Grist et al. (2003) concluded that reduction in population growth rate with increasing concentration occurred more through EE2 acting to reduce fertility than affecting survival rates.

To investigate the impacts on reproductive success and mechanism of disruption, Nash et al. (2004) exposed a breeding population of zebrafish (*Danio rerio*) over multiple generations to EE2. Life-long exposure of 5 ng L^{-1} in the F1 generation caused a 56% reduction in fecundity and complete population failure with no fertilization. However, the same level of exposure for up to 40 days in mature adults in the parental F0 generation had no effect on reproductive success. These investigators attributed infertility in the F1 generation, after life-long exposure to 5 ng L^{-1} of EE2, to disturbed sexual differentiation, with males having no functional testes and no, or undifferentiated intersex gonads. They also observed that the F1 males had a reduced vitellogenic response compared to the F0 males, suggesting an acclimation to EE2 exposure.

Table 1: Toxicity of a steroidal estrogen: 17α-ethinylestradiol (EE2)

Species	Conc. (ng/L)	Duration	Effect	Effect Endpoint	Reference
Aquatic plants					
S. subspicatus (green algae)	54 000	3d	NOEC	Biomass	Kopf (1997)*
	<100 000	3d	NOEC	Biomass	Lange (2002)*
	<100 000	3d	NOEC	Growth rate	Lange (2002)*
Invertebrates					
P. antipodarum (snail)	100	63d	NOEL	Embryo production	Jobling et al. (2004)*
D. magna	387 000	21d	NOEC	Reproduction	Schweinfurth et al. (1997)*
	100 000	21d	NOEC	Reproduction	Kopf (1997)*
	500000 1000000	21d 21d	NOEC LOEC	Reproduction	Clubbs & Brooks (2007)
B. calyciflorus (rotifer)	202 000	3d	NOEC	Number of females	Radix et al. (2002)*
S. crystalline (cladoceran)	100 000	3generations	NOEC	Reproduction	Jaser et al. (2003)*
C. reticulate (cladoceran)	200 000	3generations	NOEC	Reproduction	Jaser et al. (2003)*
N. spinipes (copepod –marine)	50 000	18d	NOEC	Reproduction	Breitholtz and Bengtsson (2001)*
T. battagiliai (copepod-marine)	>100 000	21d	NOEC	Reproduction, sex ratio, fecundity	Hutchinson et al. (1999)*
G. pulex (amphipod)	100	100d	NOEC	Sex ratio, population size	Watts et al. (2002)*
H. azteca (amphipod)	100	273d	NOEC	Reproduction	Vandenbergh et al. (2003)*
C. tantan	2300000 800000 4100000	10d 10d 10d	EC25 EC10 LC50	Growth Growth Survival	Dussault et al. (2008)
H. azteca	600000 20000 1100000	10d 10d 10d	EC25 EC10 LC50	Growth Growth Survival	Dussault et al. (2008)
Zooplanktons	7-220	4 wk	LOEC	Abundace, diversity	Schramm et al. (2008)
M. cornuarietis (snail)	50 <0.1	180d 180d	NOEC NOEC	Imposex, cogenesis; 'super female'	Schulte-Oehlmann et al. (2004)*
L. stagnalis (pond snail)	50 100	70d 21d	NOEC NOEC	Egg masses, sex ratio, emergence, egg prod.	Segner et al. (2003)*
Hydra vulgaris (Cnidarian-FW)	100 000	42d	NOEC	Sexual reproduction	Pascoe et al. (2002)*

Table 1 (contd.): Toxicity of a steroidal estrogen: 17α-ethinylestradiol (EE2)

Species	Conc. (ng/L)	Duration	Effect	Effect Endpoint	Reference
Amphibians					
Xenopus (Silurana) tropicalis (frog)	<784	42d	NOEC	Sex ratio	Pettersson et al. (2006)*
Rana pipiens *Rana sylvatica*	<1000 <1000	134-162d 76d	NOEC NOEC	Gonad differentiation and sex ratio	Mackenzie et al. (2003)*
Rana pipiens	1482 (or 5 nM)	42d	LOEC	Morphology & development	Hogan et al. (2008)
Rana temporaria (frog)	2.3 27.0	40d 40d	NOEC LOEC	Sex ratio	Pettersson et al. (2007)*
X. (Silurana) tropicalis (frog)	2.0 20.0	32d 32d	NOEC LOEC	Sex ratio	Pettersson et al. (2007)*
Fish					
P. promelas (fathead minnow)	1.0	21d	LOEC	Egg production	Jobling et al. (2004)*
	1.0 4.0 4.0	301d 301d 301d	NOEC NOEC-VTG LOEC	Reproduction (F0)	Lange et al. (2001)*
	1.0 >1.0	28d 28d	NOEC LOEC	Reproduction (F1)	Lange et al. (2001)*
	2.0 3.1-3.4	289d 289d	MATC LOEC	Feminization Intrinsic growth	Grist et al. (2003)
	0.32 1.0	150d 150d	NOEC LOEC	Reproduction	Parrott and Blunt (2005)*
	1.0 3.5	60d 60d	NOEC LOEC	Ovipositor index	Parrott and Blunt (2005)*
	3 0.1 1.0	21d 21d 21d	NOEC NOEC-VTG LOEC-VTG	Egg fertilization	Pawlowski et al. (2004)*
	4.0	14d	LOEC	VTG induction	Brodeur et al. (2005)
D. rerio (zebra fish)	5.0 0.5 50	40d 40d 40d	NOEC NOEC-VTG LOEC (acute)	Reproduction (F0)	Nash (2004)
	0.5 5.0 5.0	210d 210d 210d	NOEC NOEC-VTG LOEC	Reproduction(F1)	Nash (2004)
	3.0 3.0	42d 42d	NOEC NOEC-VTG	Reproduction	Fenske (2005)*
	0.05 1.67	75d 75d	NOEC LOEC	Multiple	Segner et al. (2003)*
	1.67 3.0	28d 28d	NOEC LOEC	Gonad transition	Maack and Segner (2004)*
	1.0 1.0 10.0	90d 90d 90d	NOEC NOEC-VTG LOEC	Sex ratio	Van den Belt et al. (2004)*
	1.0 1.0 10.0	60d 60d 60d	NOEC NOEC-VTG LOEC	Male gametogenesis	Weber et al. (2003)*
	10.0 1.0	60d 60d	NOEC NOEC-VTG	Female gametogenesis	Weber et al. (2003)*

Table 1 (contd.): Toxicity of a steroidal estrogen: 17α-ethinylestradiol (EE2)

Species	Conc. (ng/L)	Duration	Effect	Effect Endpoint	Reference
Fish					
D. rerio (zebra fish)	1.0 1.0 10.0	60d 60d 60d	NOEC NOEC-VTG LOEC	Not specified	Hill and Janz (2003)*
	1.0 1.0 10.0	40d 40d 40d	NOEC NOEC-VTG LOEC	Sex ratio	Orn et al. (2003)*
	1.0	28d	LOEC	Not specified	Keil (2006)*
	25.0 2.5	21d	NOEC NOEC-VTG	Faminization	Islinger et al. (2003)*
	<10.0 >10.0 10.0	60d 60d 60d	NOEC NOEC-VTG LOEC	Sex ratio	Orn et al. (2006)*
	0.31 1.0	75d 75d	NOEC LOEC	Reproduction	Schafers et al. (2007)*
	0.31 1.1	177d 177d	NOEC LOEC	Reproduction (F0)	Schafers et al. (2007)*
	0.36 2.0	162d 162d	NOEC LOEC	Reproduction (F1)	Schafers et al. (2007)*
O. letipes (Japanese medaka)	2 10	120-180d 120-180d	NOEC LOEC	Reproduction	Balch et al. (2004)*
	261 32 488	21d 21d 21d	NOEC NOEC-VTG LOEC	Not specified	Seki et al. (2002)*
	0.2 5.0 500	14d 14d 14d	NOEC NOEC-VTG LOEC	Reproduction and development	Tilton et al. (2005)
	<0.1 0.1	100d 100d	NOEC LOEC	Feminization	Metcalfe et al. (2001)
	1.0 10.0 10.0	60d 60d 60d	NOEC LOEC NOEC	Male sex ratio, 14d egg Male sex ratio, 14d egg Female sex ratio, 14d egg	Scholz et al. (2000)*
C. variegates (sheepshead minnow-marine)	2.0 20.0	59d 59d	NOEC LOEC	Production; hatch; 7d fry-survival	Zillioux et al. (2001)*
	20.0 200	43d 43d	NOEC LOEC	Production; hatch; 7d fry-survival, semen quality	Zillioux et al. (2001)*
O. mykiss (rainbow trout)	<16.0 16.0	62d 62d	NOEC LOEC	Embryo viability	Schultz et al. (2003)*
	11.2	21d	NOEC	GSI	EURAS (2007)*
	2.0	21d	LOEC	Reduced testicular growth (GSI)	Jobling et al. (1996)**
	0.2-1.13 1.0-7.6	14d 14d	NOEC LOEC	VTG induction in female	Thorpe et al. (2003)
P. reticulata (guppy)	44 112	108d 108d	NOEC LOEC	Reproduction; sex ration in male guppy	Kristensen et al. (2005)*
R. rutilus (common roach)	0.3 4.0 4.0	720d 720d 720d	NOEC NOEC-VTG LOEC	Sex reversal	U.K. Environ. Agency (2008)*

Table 1 (contd.): Toxicity of a steroidal estrogen: 17α-ethinylestradiol (EE2)

Species	Conc. (ng/L)	Duration	Effect	Effect Endpoint	Reference
Fish					
A. fulvesens (Lake sturgeon)	60	25d	NOEC	GSI (gonadosomatic index)	Palace et al. (2001)*
Pomatoschistus minutus (sand goby) (marine)	6.0		LOEC	Delayed male maturation, sex behaviour, egg prod.	Robinson et al. (2003)**
M. margarita (pearle dace)	4.5-8.1	3 yr	LOEC	Biochemical, histopa-thology in lake expt.	Palace et al. (2006)
G. aculeatus(three-spined stickleback)	10	12d	NOEC	Nesting behaviour	Brian et al. (2006)

*These data were used by Caldwell et al.(2008) to predict no effect concentration (PNEC), using the species sensitivity distribution (SSD) approach.
**As cited by Enick and Moore (2007)

In a whole-lake experiment, Kidd et al. (2007) demonstrated that chronic exposure of fathead minnows to 5-6 ng L^{-1} of EE2 led to feminization of males through the production of vitellogenin (VTG) mRNA and proteins, impacts on gonadal development (intersex in males and altered oogenesis in females), and a fathead minnow population collapse in the lake.

Segner et al. (2003) reported a LOEC of 1.67 ng L^{-1} for zebrafish exposed to EE2 for both changes in the fertilization success and VTG induction. They suggested that in chronic exposure experiments with zebrafish, the sensitivity of reproductive parameters measured at the organism level is comparable to that of molecular markers such as VTG, although some variation depending upon the (estrogenic) compound tested may occur.

Schafers et al. (2007) also studied zebrafish exposed to EE2 in partial life cycle (PLC) and full life-cycle (FLC) experiments. Fecundity and fertility were evaluated in the fish from fertilization to 75 days post-fertilization (dpf). No effect was evident at concentrations $\leq$0.31 ng L^{-1}. There was delayed spawning and a reduction in fertilization success to 41% of controls at measured concentrations of 1.1 ng L^{-1} and greater. Also, there was no effect of on fecundity at any concentration. FLC exposure of parental (F1) fish from fertilization to 177 dpf also resulted in the same NOEC (0.31 ng L^{-1}) and LOEC (1.1 ng L^{-1}). Life-cycle exposure of the F2 generation continued for 162 dpf at measured concentrations of 0.09, 0.36, and 2 ng L^{-1} of EE2. The NOEC and the LOEC for the F2 generation at 162 dpf was 0.36 and 2 ng EE2 L^{-1}.

NOECs of 0.21 to 1.13 ng L^{-1} and LOECs of 1.0 to 7.6 ng L^{-1} were reported by Thorpe et al. (2003) for increases in plasma VTG in female juvenile rainbow trout exposed to EE2 for 14 days in 2 different experiments. These authors also reported median effect concentrations (EC50) of 0.95 ng L^{-1} and 1.8 ng L^{-1} from these experiments.

The lowest observed effect concentration in Table 1 was produced by Metcalfe et al. (2001) who reported a nominal concentration of 0.1 ng L^{-1} as a LOEC for the testis-ova induction (i.e. feminization) in Japanese medaka fish (O*ryzias latipes*) in a 100 day test. This value was based on response of one male fish (out of a total of 60 animals used in the test) which revealed a single oogonium within the testicular tissue. Overall, these authors reported that no testis-ova induction occurred in the fish exposed to10 ng L^{-1} of EE2.

Table 2 lists the NOECs and LOECs of most sensitive fish species taken from Table 1 and Figure 2 shows a plot of the data in Table 2. Only selected (the most sensitive) data were plotted to employ a linear scale (a log scale would be needed to plot all the data which would obscure details in the sensitive range) and to visualize details in the sensitive range. Invertebrates and aquatic plants data were not plotted because these organisms were relatively less sensitive to the effects of EE2. The actual LOECs and NOECs and species plotted are also shown in the summary table accompanying the figure. The purpose of this figure is to show: (i) the variability in the LOEC values in the sensitive range, and (ii) the relationships between LOEC, NOECs, and the recommended EE2 guideline which is rationalized in sections 4.4 and 4.5.

Table 2. No observable effect concentrations (NOEC) and lowest observable effect concentrations (LOEC) for 17α-ethinylestradiol (EE2) for the most sensitive endpoints of several fish species taken from Table 1. (*Note: Data from Table 2 was used to create Figure 2.)*

Species	Endpoint	NOEC (ng L^{-1})	LOEC (ng L^{-1})
P. promelas	Egg production		1
P. promelas	Reproduction (F0)	1	4
P. promelas	Feminization	2	3.1
P. promelas	Ovipositor index	1	3.5
P. promelas	VTG induction	0.1	1
P. promelas	VTG induction		4
D. rerio	Reproduction (F1)	0.5	5
D. rerio	Multiple	0.05	1.67
D. rerio	Gonad transition	1.67	3
D. rerio	Sex ratio	1	10
D. rerio	Male gametogenesis	1	10
D. rerio	Female gametogenesis	1	
D. rerio	Not specified	1	10
D. rerio	Sex ratio	1	10
D. rerio	Not specified		1
D. rerio	Reproduction (F0)	0.31	1.1
D. rerio	Reproduction (F1)	0.36	2
O.latipes	Feminization		0.1
O.latipes	Male sex ratio	1	10
O. mykiss	VTG induction in female	0.2	1

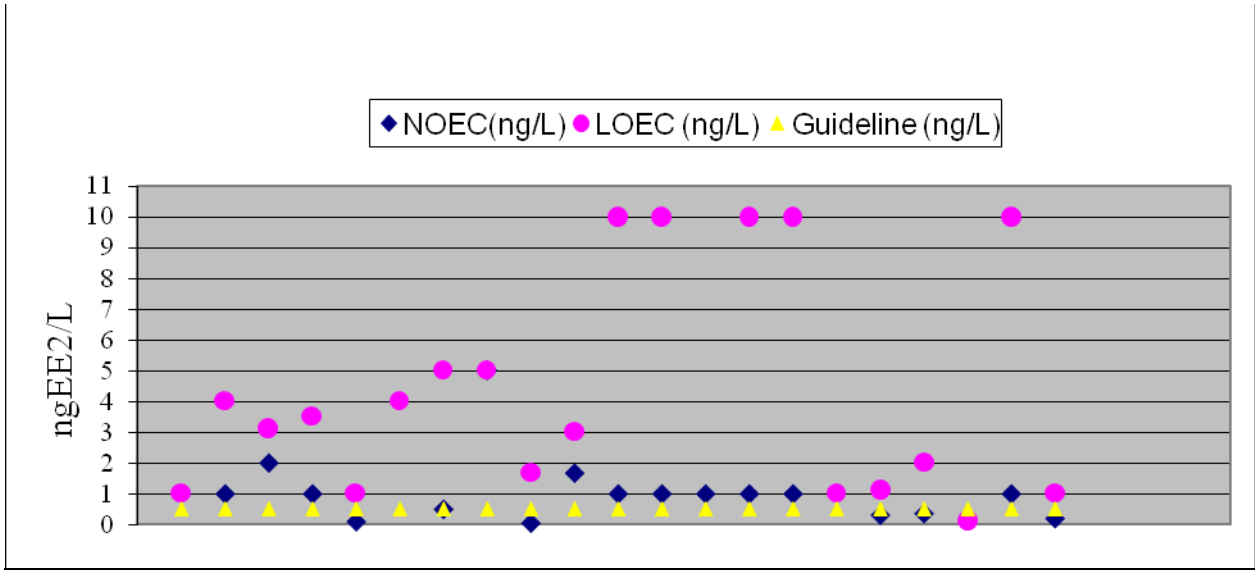


Figure 2. Toxicity of 17α-ethinylestradiol to fish species (data taken from Table 2).

The figure clearly shows that the lowest observed effect concentrations (LOECs) for EE2 can vary within and among species. Hence, some overlap can be expected among NOECs and LOECs when data for various species are combined. The relationship between plotted LOECs and the guideline can be appreciated from explanations rendered in sections 4.4 and 4.5.

4.2 Effects on marine organisms

There were limited data on the toxicity of EE2 on marine organisms. In a 59-day trial with sheepshead minnow (C*yprinodon variegates* Lacepede), an estuarine fish species, Zillioux et al. (2001) reported a NOEC and LOEC of 2 and 20 ng L^{-1} for reproductive success (as determined from egg production from 2 subsequent spawning trials), hatching, and semen quality. Robinson et al. (2003) reported a LOEC of 6 ng L^{-1} causing delayed male maturation, sex behaviour and egg production in sand goby (*Pomatoschistus minutes*).

4.3 Guidelines from Other Jurisdictions

Water quality guidelines to protect freshwater or marine life were not found in the literature. The Canadian Council of Ministers of the Environment (CCME) has not set a water quality guideline to protect aquatic life in the Canadian environment.

Based on the literature data (Table 1), Caldwell et al. (2008) recommended a maximum concentration of 0.35 ng EE2 L^{-1} to protect biota from adverse effects in surface water.

This guideline was predicted from published NOECs using a SSD approach and corresponded to $HC_{5,50}$ (hazard concentration at 50% confidence interval that will only stress 5% of the species tested).

4.4 Recommended Guidelines

For the protection of freshwater aquatic life, it is recommended that the 30-day average concentration of 17α-ethinylestradiol (EE2) in water, based on 5 weekly samples, should not exceed 0.5 ng L^{-1} with no single value to exceed 0.75 ng L^{-1} (no more than 50% above the guideline value). The guideline refers to total concentration of EE2 in an unfiltered sample. Where laboratories filter samples, analysis can be conducted separately on the aqueous and solids and summed together (Hamilton -Axys, personal communication).

As expected, the recommended guideline for EE2 is lower than the most-sensitive LOECs plotted in Figure 2, except in a few instances. The rationale in support of the recommended guideline is presented in section 4.5.

Because of insufficient data, a water quality guideline for the protection of marine life is not recommended at this time.

4.5 Rationale

The recommended guideline is derived from LOEC of 1.0 ng L^{-1} of EE2 for reproduction and egg production (Parrott and Blunt 2005, Jobling et al. 2004, Thorpe et al. 2003). A safety factor of 2 was applied to arrive at the recommended guideline. This is consistent with the British Columbia Ministry of Environment protocol for the derivation of the aquatic life guideline (Singleton et al. 1995). The protocol recommends that a safety factor of 2 to 10 may be employed with the chronic LOEC, based on science and /or professional judgment. Given a narrow spread between measured NOECs and LOECs for the most sensitive species in Table 1, the use of safety factor of 2 was considered appropriate.

The guideline is based on fish species that are resident in British Columbia and North America (fathead minnow, rainbow trout).

Current detection limits at BC laboratories (lowest MDL of 5 ng L^{-1}), presently do not meet our precision objectives of 1/10 guideline value), however laboratories (e.g. Axys and PESC) have indicated that method development could be done to improve the MDLs. Users of this guideline are advised to use the lowest detection limit available at a laboratory in BC, until such time that our precision objective is met (to give an MDL of 0.05 ng L^{-1}).

The recommended guideline is close to the predicted no effect concentration (PNEC) derived by Caldwell et al. (2008) using a SSD approach and published NOECs. This PNEC was not adopted in this document directly because of several factors that may influence the results of the model used:

- It was noted that the results of the SSD could be different depending on the NOEC used; Caldwell et al. (2008) discarded some data and manipulated others to compute some of the NOECs used.
- The NOECs used in the SSD model were literature values which may be influenced by the experimental design (e.g. concentration levels and intervals used in the toxicity study). A statistically based (effect concentration of 10% of individuals) EC_{10} is considered to be a better estimate of the true NOEC.
- The NOEC is not only a function of test conditions but also varies with the end point employed in the toxicity test. Obviously, the PNEC based on NOECs with mixed end points will be different and less desirable than that obtained from NOECs with single end point (e.g. reproductive effects). The PNEC by Caldwell et al. (2008) was based on NOECs with mixed end points.
- SSD is not an approved method of guideline development for BC since it does not follow the guiding principles of protection of the most sensitive species and life stage indefinitely.

There were some data which yielded a lower LOEC (0.1 ng EE2 L^{-1}) than 1.0 ng L^{-1} (Metcalfe et al. 2001 – see Figure 2); however, these data were not used for guideline development because they were considered to be anomalous for reasons as explained in section 4.1.

EE2 concentrations as low as 0.1 ng L^{-1} have been shown to significantly elevate plasma vitellogenin (VTG) in male trout (Purdom et al. 1994 - data not listed in Table 1). This response was not considered in development of the guideline for the following reasons:

(1) The VTG production levels quoted in their paper appear to be in error (e.g., mean VTG concentration of 0.06 µg mL^{-1} in male trout exposed to 0.1 ng EE2 L^{-1} is much greater than the quoted range: 0.01-0.02 µg L^{-1}); and
(2) Although VTG induction in male fish is an excellent biomarker of exposure to estrogens, and while intuitive, the causal linkage to biological effects is limited (Office of Water/Office of Research and Development: OW/ORD 2008).

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