**Scientific Committee on Health, Environmental and Emerging Risks**
**SCHEER**

# Scientific Opinion on "Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive"

# 17-Alpha-Ethinylestradiol (EE2), Beta-Estradiol (E2) and Estrone (E1)

The SCHEER adopted this document
by written procedure on 1st March 2022

## ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this opinion. The members of the Working Group are:

The SCHEER members:
Marian Scott (Chair), Marco Vighi (Rapporteur), Thomas Backhaus, Teresa Borges, Raquel Duarte Davidson, Peter Hoet, Pim de Voogt

External Experts:
Andrew Johnson, Jan Linders

Acknowledgements
Demosthenes Panagiotakos, Theo Vermeire

All Declarations of Working Group members are available at the following webpage:
Register of Commission expert groups and other similar entities (europa.eu)

This Opinion has been subject to a commenting period of four weeks after its initial publication (from 22 November 2022 to 22 December 2021). Comments received during this period were considered by the SCHEER. For this Opinion, a change was made in section 6.3.2, deleting the reference to single species lab studies as argument against lowering of the assessment factor.

**Keywords**: estrogens, 17-alpha ethinylestradiol, beta estradiol, and estrone, Water Framework Directive, environmental quality standards

**Opinion to be cited as**:
SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Preliminary Opinion on "Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive", 17-alpha-ethinylestradiol (EE2), Beta-Estradiol (E2) and Estrone (E1), 1st March 2022.

**About the Scientific Committees (2022-2026)**
Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems that may pose an actual or potential threat.
These two committees are the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER). The Scientific Committees review and evaluate relevant scientific data and assess potential risks. Each Committee has top independent scientists from all over the world who are committed to working in the public interest.
In addition, the Commission relies upon the work of other Union bodies, such as the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

**SCHEER**
This Committee, on request of Commission services, provides Opinions on questions concerning health, environmental and emerging risks. The Committees addresses questions on:
- health and environmental risks related to pollutants in the environmental media and other biological and physical factors in relation to air quality, water, waste and soils.
- complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health, for example antimicrobial resistance, nanotechnologies, medical devices and physical hazards such as noise and electromagnetic fields.

**SCHEER members**
Roberto Bertollini, Teresa Borges, Wim de Jong, Pim de Voogt, Raquel Duarte-Davidson, Peter Hoet, Rodica Mariana Ion, Renate Kraetke, Demosthenes Panagiotakos, Ana Proykova, Theo Samaras, Marian Scott, Emanuela Testai, Marco Vighi, Sergey Zacharov

**Contact**
European Commission
DG Health and Food Safety
Directorate C: Public Health
Unit C2: Health information and integration in all policies
L-2920 Luxembourg
SANTE-C2-SCHEER@ec.europa.eu

©European Union, 2023

PDF ISSN 2467-4559 ISBN 978-92-68-06318-7 doi:10.2875/286676 EW-CA-23-030-EN-N

The Opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The Opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/policy/index_en.htm

## ABSTRACT

The dossier on Environmental Quality Standards for "Estrogens" was reviewed by the SCHEER according to the general mandate on EQS dossiers.

The SCHEER endorses that the **MAC-$QS_{fw,eco}$** for estrogens was not derived because of the chronic exposure pattern and the large acute to chronic ratios observed.

EE2

The SCHEER is of the opinion that in the deterministic approach an assessment factor (AF) of 50 should be used, instead of 10, which would lead to the **AA-$QS_{fw,eco}$ of 0.0032 ng $L^{-1}$**. In the SSD approach, the SCHEER does not agree with lowering the default additional assessment factor to 4. According to the SCHEER, this gives a **AA-$QS_{fw,eco}$ of 0.017 ng $L^{-1}$ .** The SCHEER endorses the additional assessment factor of 2 for marine organisms. However, for marine water, data for only one additional typically marine taxonomic group is available (echinoderm species). In that case an extra AF of 5 is applied in addition to the AF of 2, to deal with residual uncertainty. Therefore the SCHEER proposes to set a value of **0.0016 ng $L^{-1}$** for the **AA-$QS_{sw,eco}$.**

The SCHEER cannot endorse the $QS_{sed}$ of $84.10^{-7}$ mg $Kg^{-1}$ $_{ww}$ because of uncertainties in the dossier.

Although the SCHEER endorses the AF of 30 and the BCF of 610 used for the derivation of the $QS_{biota,secpois}$, it cannot support the $QS_{biota,secpois}$ derived because default values for the energy content of feed are not reported.

The SCHEER cannot endorse the $QS_{biota,hh}$: the AF of 100 is not supported. It is not clear how the value of 0.0609 ug $Kg^{-1}$ was derived as reported in Table 7.1. In addition, the $QS_{biota,\ hh}$ food should be compared with the $QS_{biota,\ secpois.}$ This step was not reported but would result in a standard driven by $QS_{biota,secpois}$., being the lower value.

The SCHEER cannot support the calculation of the $QS_{dw,hh}$ since two different values of ADI have been used.

E2

The SCHEER endorses the **AA-$QS_{fw,eco}$** in the deterministic approach. In the SSD approach, the SCHEER does not agree with lowering the default assessment factor to 3. Applying the default assessment factor of 5 will give a **AA-$QS_{fw,eco}$ of 0.18 ng $L^{-1}$.** The SCHEER endorses the additional assessment factor of 2 for marine organisms. However, no data for typically marine taxonomic groups are available. In that case an extra AF of 10 is applied in addition to the AF of 2, to deal with residual uncertainty. Therefore the SCHEER proposes to set a value of **0.009 ng $L^{-1}$ for** the **AA-$QS_{sw,eco}$ .**

The SCHEER cannot endorse the $QS_{sed}$ of $13.10^{-5}$ mg $Kg^{-1}{}_{ww}$ because of uncertainties in the dossier.

No $QS_{biota,secpois}$ was derived because of a missing BMF value. It is not clear why the default value of 1 was not used as was done for EE2. The SCHEER endorses the AF of 30 and the BCF of 6.5 selected for the derivation of the $QS_{biota,secpois}$. Default values for the energy content of feed are not reported.

The $QS_{biota,hh}$ was correctly derived to be **5.2 ug $Kg^{-1}$ $_{wwt}$ fish**. It is not clear how the value of 3.04 ug $Kg^{-1}$ $_{bw}$ was derived as reported in Table 7.1. In addition, the $QS_{biota,\ hh}$ food should be compared with the $QS_{biota,\ secpois.}$ This step was not reported but would result in a standard driven by $QS_{biota,secpois}$., being the lower value.

According to the SCHEER, it does not seem appropriate to set drinking water limits for E2 and E1, since these hormones have been consumed in milk and dairy products by humans for centuries. The SCHEER agrees with the technical derivation.

E1

The SCHEER agrees with the approach to derive a preliminary EQS based on relative potency of the estrogens EE2, E2 and E1. The SCHEER acknowledges that the environmental and human standards of estrone are difficult to establish due to the lack of fully qualifying long-term studies with E1.

## TABLE OF CONTENTS

# 1. BACKGROUND

Article 16 of the Water Framework Directive (WFD, 2000/60/EC) requires the Commission to identify Priority Substances among those presenting significant risk to or via the aquatic environment, and to set EU Environmental Quality Standards (EQS) for those substances in water, sediment and/or biota. In 2001, a first list of 33 Priority Substances was adopted (Decision 2455/2001) and in 2008, the EQS for those substances were established (Directive 2008/105/EC or EQS Directive, EQSD). WFD Article 16 requires the Commission to periodically review the list. The first review led to a Commission proposal in 2011, resulting in the adoption of a revised list in 2013 containing an additional 12 Priority Substances. Technical work to support a second review has been underway for some time, and several substances have been identified as possible candidate Priority Substances. The Commission will be drafting a legislative proposal, with the aim of presenting it to the Council and the Parliament sometime around mid-2022.

The technical work has been supported by the Working Group (WG) Chemicals under the Common Implementation Strategy for the WFD. The WG is chaired by DG Environment and consists of experts from Member States, EFTA countries, candidate countries and several European umbrella organisations representing a wide range of interests (industry, agriculture, water, environment, etc.).

Experts nominated by WG Members (operating as individual substance Expert Groups and through the Sub-Group on Review of Priority Substances, SG-R) have been deriving EQS for the possible candidate substances and have produced draft EQS for most of them. In some cases, a consensus has been reached, but in some others, there is disagreement about one or other component of the draft dossier. EQS for a number of existing priority substances are also currently being revised.

The EQS derivation has been carried out in accordance with the Technical Guidance Document on Deriving EQS (TGD-EQS) reviewed by the SCHEER[1].

# 2. TERMS OF REFERENCE

DG Environment now seeks the opinion of the SCHEER on the draft EQS for the proposed Priority Substances and the revised EQS for a number of existing Priority Substances. The SCHEER is asked to provide an Opinion for each substance. We ask that the SCHEER focus on:

1. whether the EQS have been correctly and appropriately derived, in the light of the available information and the TGD-EQS;

2. whether the most critical EQS (in terms of impact on environment/health) have been correctly identified.

Where there is disagreement between experts in working groups on Chemicals or when there are other unresolved issues, we ask that the SCHEER consider additional points, identified in the cover note(s).

For each substance, a comprehensive EQS dossier is or will be available. DG Environment is providing three EQS dossiers ahead of the 3-4 March SCHEER Plenary and expects to provide most of the remaining dossiers over the next three months. The dossiers contain much more information than simply the draft EQS; the SCHEER is asked to focus on the latter.

In some cases, especially where additional points are raised, additional documents may be provided. Some of the studies referred to in the dossiers are not publicly available. If the SCHEER needs to see these studies, it is invited to please contact DG Environment.

[1] https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/ba6810cd-e611-4f72-9902-f0d8867a2a6b/details

The SCHEER is asked to consider the two generic questions in the request, as well as the following additional points on which the Sub-Group on Review of Priority Substances (SG-R) has put a specific question.

*On 17-Alpha-Ethinylestradiol (EE2)*
The SCHEER is asked to consider the two generic questions in the request, as well as the following additional points on which the Subgroup on Review of Priority Substances (SG-R) has put a specific question. The SCHEER's Opinion is requested on whether to use the reduced Assessment Factors (AF) for the Species Sensitivity Distributions (SSD) to derive the Annual Average (AA) EQS for 17-Alpha-Ethinylestradiol. The suggested AF for freshwater is 4 with an additional AF of 2 for the marine environment.

The reasons for the reduced AF are laid out in the dossier in detail, in brief:

- EE2 is a synthetic hormone designed solely with an estrogenic mode of action (MoA). There are several Life Cycle studies available, conducted with the most sensitive taxa. On the other side, these are single species lab studies only. And there are indications from two studies (Zha et al. 2008 and Kidd et al. 2007) that effects are long term effects on populations.

The additional AF of 2 (instead of the regular AF of 10) for the marine environment is also reflecting the fact, that the MoA is extremely specific and no difference in the sensitivity of freshwater and marine species is known nor expected. However, EFPIA is suggesting to use an AF if 2 instead of 4 for determining the $EQS_{fw}$.

*On Beta-Estradiol (E2) and Estrone (E1)*
The SCHEER's Opinion is requested on whether to use the reduced Assessment Factors (AF) for the Species Sensitivity Distributions (SSD) to derive the Annual Average (AA) EQS for Estradiol. The suggested AF for freshwater is 3 with an additional AF of 2 for the marine environment. The reasons for the reduced AF are laid out in the dossier in detail.

In brief:

- The estrogenic Mode of Action (MoA) is the best known for this substance. It is clearly receptor mediated, with the vertebrate sexual endocrine cascade as by far the most sensitive. The effects of E2 are well studied and there is no scientific evidence (including biomarker studies) that indicate that marine fish species are more sensitive to E2 compared to freshwater species. Bosker et al. (2017) conducted a semi-quantitative review on estrogens and suggested that responses occurred at lower doses under freshwater compared to saline conditions.

The additional AF of 2 (instead of the regular AF of 10) for the marine environment is also reflecting the fact that the MoA is extremely specific and no difference in the sensitivity of freshwater and marine species is known nor expected. However, EFPIA is suggesting using an AF of 2 instead of 3 for determining the $EQS_{fw}$.

There are no specific open questions within the expert group for Estrone. But there are still some doubts on the EQS derivation:

The lack of sufficient reliable ecotoxicological effects information prevents the establishment of a robust EQS. Considering the limited data available a very high assessment factor would have to be applied, which would result in an overprotective EQS. Therefore, it is proposed to use an alternative approach to evaluate *in vitro* relative receptor binding affinity and transactivation activity of EE2, E2, and E1.

# 3. OPINION

Specific comments on the different sections of the dossier are listed below.

## Section 6.3.1 Derivation of the MAC-QS$_{fw, eco}$

### 17-Alpha-Ethinylestradiol (EE2)

The MAC$_{fw,eco}$ was not derived. It was considered irrelevant in view of the chronic exposure pattern and the large observed acute to chronic ratios The SCHEER agrees.

### Beta-estradiol (E2)

The MAC$_{fw,eco}$ was not derived. It was considered irrelevant in view of the chronic exposure pattern and the large observed acute to chronic ratios. The SCHEER agrees.

### Estrone (E1)

The MAC$_{fw,eco}$ was not derived. It was considered irrelevant in view of the chronic exposure pattern and the large observed acute to chronic ratios. The SCHEER agrees.

## Section 6.3.2 Derivation of the AA-QS$_{fw,eco}$

### 17-Alpha-Ethinylestradiol (EE2)

A comprehensive data set on chronic toxicity is available. Although the saltwater data set is much more limited than the freshwater set, it is agreed that the available data do not point at a clear difference in sensitivity. Therefore, pooling in the derivation of the AA-QS is endorsed by the SCHEER.

*Deterministic approach*

In the dossier, the lowest NOEC value considered is 0.16 ng $L^{-1}$ which is correctly extrapolated from a chronic study on the fathead minnow with an LOEC of 0.32 ng $L^{-1}$ (Parrott and Blunt, 2005). As noted in the dossier, a lower adverse effect level is available in the data set (0.2 ng $L^{-1}$). However, in this study the NOEC could not be derived by extrapolation as there was complete spawning failure of exposed females at the concentrations tested down to 0.2 ng $L^{-1}$. Nevertheless, this study shows that apparently the species tested, *Gobiocypris rarus*, is more sensitive than *Pimephales promelas.* In this case the Guidance advises to use an assessment factor of 50 on the NOEC to consider any interspecies variation in sensitivity. In the dossier a factor of 10 is used.

The SCHEER is of the opinion that following the EU's own guidelines an assessment factor of 50 should be used instead of 10 which would lead to the **AA-QS$_{fw,eco}$ of 0.0032 ng $L^{-1}$** for freshwater. There might be scientific reasons to lower the factor of 50 to 10 but this has to be motivated in the dossier.

*SSD approach*

In the dossier, it is concluded that the requirements for an SSD approach are not met since data on higher plants are missing. The SCHEER agrees that the SSD approach can still be applied since plants indeed are not expected to be sensitive to estrogens and will have negligible influence on the probability distribution. The SSD can be based on the lowest NOEC for each species of those taxa that are expected to be particularly sensitive, fish and amphibia, as done in this dossier. This results in the **HC5 of 0.087 ng $L^{-1}$.**

The SCHEER does not agree with lowering the default assessment factor to 4. The considerations given do not justify this reduction, especially the uncertainties in the data package (minimum number, evidence for generational effects and long-term effects on populations). According to the SCHEER, this gives a **AA-QS$_{fw,eco}$ of 0.017 ng $L^{-1}$** .

With regard to marine organisms, the Guidance prescribes an assessment factor of 1-5 on the HC5. Since there is no apparent difference in sensitivity between freshwater and saltwater species and this is not expected for estrogens the *additional*[2] assessment factor of 2, accounting for the higher diversity in marine species, seems reasonable. **The AA-QS$_{sw, eco}$ will then be 0.008 ng L$^{-1}$.**

For marine water, data for one additional typically marine taxonomic group is available (echinoderm species). When there is only one additional marine taxonomic group in the dataset, an AF of 5 is applied in addition to the AF of 2 to deal with residual uncertainty. Therefore the SCHEER proposes to set a value of **0.0016 ng L$^{-1}$** for the **AA-QS$_{sw,eco}$.** In the dossier, no additional factor is applied.

**Beta-estradiol (E2)**

*Deterministic approach*

The lowest effect concentration is a NOEC of 2.9 **ng L$^{-1}$** reported by Seki et al., (2005) for the Japanese medaka (*Oryzias latipes*). The SCHEER endorses this study as well as the assessment factor of 10 used.

*SSD approach*

The SCHEER agrees that the SSD can be based on the lowest NOEC for fish being the species of the taxa that is expected to be particularly sensitive. This results in the **HC5 of 0.90 ng L$^{-1}$.**

The SCHEER does not agree with lowering the default assessment factor to 3. The considerations given do not justify this reduction, especially the uncertainties in the data package (minimum number, evidence for generational effects and long-term effects on populations). Applying the default assessment factor of 5 will give a **AA-QS$_{fw,eco}$ of 0.18 ng L$^{-1}$ .**

Regarding marine organisms, the Guidance prescribes an assessment factor of 1-5 on the HC5. Since there is no evidence that saltwater species are more sensitive, rather the opposite, the *additional*[3] assessment factor of 2, accounting for the higher diversity in marine species seems reasonable. **The AA-QS$_{sw, eco}$ will then be 0.09 ng L$^{-1}$.**

No data for typically marine taxonomic groups are available. When there is no additional typically marine taxonomic group in the dataset, an AF of 10 is applied in addition to the AF of 2 to deal with residual uncertainty. Therefore the SCHEER proposes to set a value of **0.009 ng L$^{-1}$ for the AA-QS$_{sw,eco}$ .** In the dossier, no additional factor is applied.

**Estrone (E1)**

The lack of sufficient reliable ecotoxicological effects information prevented the establishment of a robust EQS. Considering the limited data available in the dossier a very high assessment factor would have to be applied, which would result in an overprotective EQS. Therefore, in the dossier existing literature was evaluated to estimate whether comparative data of EE2, E2, and E1 allow to derive a preliminary EQS based on relative potency. Data analysed were receptor binding and transactivation studies *in vitro,* VTG induction in various life stages of fish and some *in vivo* tests with apical endpoints.

The conclusions in the dossier are not fully endorsed by the SCHEER. The receptor binding and transactivation studies indeed showed a large span of potency factors between E2 and

[2] It is noted that the additional mandate assumes that EFPIA is suggesting using an AF of 2 *instead of* 'the regular AF of10' for determining the EQS$_{fw}$ while this AF *is additional*.

[3] It is noted that the additional mandate assumes that EFPIA is suggesting using an AF of 2 *instead of* 4 for determining the EQS$_{fw}$ while this AF *is additional*.

E1 *in vitro*. They run from similar potencies to potency factors of up to 100, when E2 is compared with E1. However, the UK Environmental Agency determined a lower potency for E1 compared to E2 (Williams et al., 2008). Therefore, the SCHEER agrees with the approach.

## Section 6.4 Derivation of the $QS_{sediment}$

### 17-Alpha-Ethinylestradiol (EE2)

Due to the lack of sediment toxicity data equilibrium, in the dossier, partitioning was applied to derive the $QS_{sed}$ for ethinyl estradiol. This is endorsed by the SCHEER. In this calculation a $K_{oc}$ of 3.4 was used. It is not clear how this value was derived from the available ranges (2.91-4.68 and 3.21-5.44) and the GLP-study result of 3.66 (Schering et al., 1993a). This should be clarified. The defaults have been applied correctly, but the value used for $QS_{weco}$ (0.035 **ng $L^{-1}$)** is not the value derived in Section 6.3.2.2 of the dossier (0.023 ng $L^{-1}$). Therefore, the SCHEER cannot endorse the $QS_{sed}$ of $84.10^{-7}$ mg $Kg^{-1}$ ww.

### Beta-estradiol (E2)

Due to the lack of sediment toxicity data equilibrium, in the dossier, partitioning was applied to derive the $QS_{sed}$ for beta-estradiol. This is endorsed by the SCHEER. The defaults have been applied correctly, but the value used for $QS_{water,eco}$ (0.53 ng $L^{-1}$ ) is not the value derived in Section 6.3.2.2 of the dossier (0.29 ng.$L^{-1}$). Therefore, the SCHEER cannot endorse the $QS_{sed}$ of $13.10^{-5}$ mg $Kg^{-1}$ ww.

## Section 7.2. Secondary Poisoning

### 17-Alpha-Ethinylestradiol (EE2)

A NOAEL of 0.1 µg $Kg^{-1}$ $d^{-1}$ was used for the derivation of the $QS_{biota,secpois,}$ being the lowest reported (subchronic) effect concentration. The SCHEER is unable to verify this value and has to assume that this value is correct. The calculation of the energy normalised concentration of EE2 is also considered correct. Next, the $QS_{biota,secpois}$ was calculated using the formula:

$$C_{food\ item}\ [\mathrm{mg/kg_{ww}}] = C_{energy\ normalised}\ [\mathrm{mg/k}] \cdot Energycontent_{fooditem,fw}$$

This formula apparently was applied for bivalves and fish. However, the values applied for the energy content of these feed items was not reported. Therefore, the calculated concentrations in these critical food items cannot be verified, nor the proposed value of the $QS_{biota,secpois}$.

An AF of 30 was applied for the derivation of the standard. Although the study used was a multigenerational study and not a 90-day study as reported in the dossier, the AF of 30 is endorsed since the study used was not a chronic study. The SCHEER also agrees with the BCF of 610.

### Beta-estradiol (E2)

A NOAEL of 2.5 µg $Kg^{-1}$ $d^{-1}$ from a 90-day study with male rats was used for the derivation of the $QS_{biota,secpois,}$ being the lowest reported (sub-chronic) effect concentration. This value could not be verified by the JRC and the SCHEER is also unable to verify this value. The calculation of the energy normalised concentration of E2 is considered correct. Next, the $QS_{biota,secpois}$ was calculated using the formula:

$$C_{food\ item}\ [\mathrm{mg/kg_{ww}}] = C_{energy\ normalised}\ [\mathrm{mg/k}] \cdot Energycontent_{fooditem,fw}$$

This formula apparently was applied for bivalves and fish. However, the values applied for the energy content of these feed items was not reported. Therefore, the calculated concentrations in these critical food items cannot be verified, nor the proposed value of the $QS_{biota,secpois}$.

An AF of 30 was applied for the derivation of the standard. This value is endorsed since the study used was not a chronic study. The SCHEER also agrees with the selection of the BCF value of 6.5 reported for whole fish. No QS was derived in view of the lack of a value for the BMF. It is not clear why the default value of 1 was not used as was done for EE2.

## Section 7.3. Human health

### 17-Alpha-Ethinylestradiol (EE2)

17 alpha-ethinylestradiol (EE2) exposure via dietary consumption e.g., fishery products or drinking water, has shown some significant health effects, mostly in animal studies while some evidence suggests the same toxic effect in human subjects. It has been suggested to be a potential endocrine disruptor affecting both sex hormones and sex organs and, it may affect the reproductive system, as well as infant development. Hormonal effects include increased levels of Sex Hormone Binding Globulin (SHBG) and decreased levels of Corticosteroid Binding Globulin (CBG) and Follicle Stimulating Hormone (FSH) in exposure to high doses of EE2. Moreover, some studies in humans have reported of gynecomastia in both male and female infants whose mothers took EE2 while nursing. EE2 is also a known human carcinogen linked to endometrial, ovarian, and breast cancers. To date, a large number of studies have reported EE2 widespread in aquatic systems with reported detection levels typically at low concentrations (<1 ng $L^{-1}$) in surface waters (Huggett et al., 2003; Mouatassim-Souali et al., 2003; Rodgers-Gray et al., 2000; Laurenson et al, 2014). However, there are numerous studies that have reported no significant association of the intake of synthetic estrogens, among them EE2, with adverse health effects in humans (Caldwell et al, 2012, Wise et al, 2012).

The $QS_{biota,hh}$ was derived to be 0.12 ug $Kg_{ww}^{-1}$ fish. The SCHEER does not agree with the AF 100 since the critical study was not a chronic study and this warrants an extra factor of 3 on the subchronic result (as was done in the derivation of the $QS_{biota,secpois}$). Next, it is not clear how the value of 0.0609 ug $Kg^{-1}$ was derived as reported in Table 7.1.

The SCHEER would require justification of why the steps prescribed in the Guidance were not described in the dossier. Once a $QS_{biota,hh}$ food has been estimated, it needs to be established whether secondary poisoning of wildlife or for protection of human health should "drive" the biota standard. To do this, the $QS_{biota,\ hh}$ food should be compared with the $QS_{biota,\ secpois.}$ This step was not reported but would result in a standard driven by $QS_{biota,secpois}$., being the lower value.

As it is reported in the JRC Report (see *Appendix* 5) no details of existing thresholds for EE2 in drinking water were located. Moreover, thresholds have not been derived by either the EU or WHO. However, according to the EQS guidance a provisional drinking water standard should be derived using the following formula:

$$QS_{dw,hh}\ [\mu g\ Kg^{-1}] = (\ 0.2 \times TL_{hh}\ [\mu g\ Kg^{-1}\ d^{-1}] \times bw\ ) / Uptake_{dw}$$

The default values for *bw* and $Uptake_{dw}$ are 70 Kg and 2 Litres respectively. The SCHEER cannot support the calculation since two different values of ADI have been used (0.001 µg $Kg^{-1}$ $d^{-1}$ in section 7.1.1 and 0.007 µg $Kg^{-1}$ $d^{-1}$ in section 7.1.2) and no justification for the difference was given.

### Beta-estradiol (E2)

Whilst it may at first seem reasonable to set limits on the human intake of E2 (and E1, EE2) it should be recalled that these are both present in very high concentrations in dairy products (Pape-Zambito et al., 2010). Thus, these hormones have been consumed in milk and dairy products by humans for centuries. It does not seem appropriate therefore to set drinking water limits. Beta-estradiol (E2) is a natural estrogen that may exert the endocrine- disrupting

effect and cause serious problems for the aquatic organisms, animals in many aquatic systems, as well as humans. In particular, for human the health consequences of E2 intake above the safe thresholds are numerous: Firstly, it has been associated with increased rates of breast cancer in women and prostate cancer in men (Moore et al., 2016; Nelles et al., 2011; Trevino et al., 2015. Moreover, E2 in food and water could induce premature menopause in mature women and cause virilization in young girls, thus affecting the reproductive potential of women. Furthermore, there is evidence that estrogens and especially E2 has been associated with decreased sperm quality and sperm count and decreased male fertility (Bolong et al., 2009; Sumpter and Jobling, 2013). Other adverse effects include changes in serum level of testosterone hormone, preterm birth and intrauterine growth restriction, genitourinary disorders including hypospadias, cryptorchidism, decreased fatal testosterone level, recurrent abortion, polycystic ovary syndrome, genital anomalies, testicular cancer, birth weight loss, endometriosis, early puberty, obesity, and menstrual dysfunction (Balabanic et al, 2011). The $QS_{biota,hh}$ was correctly derived to be 5.2 ug $Kg^{-1}$ $_{ww}$ fish. Next, it is not clear how the value of 3.04 ug $Kg^{-1}$ $_{bw}$ was derived as reported in Table 7.1.

As before, the SCHEER would like to see once a $QS_{biota,hh}$ food has been estimated, , the establishment of whether secondary poisoning of wildlife or for protection of human health should 'drive' the biota standard. To do this, the $QS_{biota,\ hh}$ food should be compared with the $QS_{biota,\ secpois.}$ This step was not reported but would result in a standard driven by $QS_{biota,secpois}$., being the lower value.

No details of existing thresholds for beta-estradiol in drinking water have been suggested. However, according to the EQS guidance a provisional drinking water standard should be derived using the following formula:

$$QS_{dw,hh}\ [\mu g\ Kg^{-1}] = (\ 0.2 \times TL_{hh}\ [\mu g\ Kg^{-1}\ d^{-1}] \times bw\ ) / Uptake_{dw}$$

The default values for *bw* and $Uptake_{dw}$ are 70 Kg and 2 Litres respectively. The $TL_{hh}$ refers to an available ADI or TDI. Using the ADI of 0.05 µg $Kg^{-1}$ $_{bw}$ per day derived by WHO (2000) gives a $QS_{dw,hh}$ for beta-estradiol of 0.3 µg $L^{-1}$. The SCHEER agrees with this technical derivation; however, as noted above, the SCHEER recommends to discuss drinking water limits in relation to dietary exposures.

**Estrone (E1)**

Estrone (E1) is responsible for female sexual development and function. E1 enters the environment through the human and animal excretions. The practise of spreading poultry and cattle waste in farming has been implemented in the contamination of groundwaters with E1. Environmental E1 intake can occur through the consumption of foods that contain estrone naturally, such as meat, eggs, milk, and yogurt or drinking contaminated water or taking hormonal medication that contains estrone as an ingredient (Ying et al., 2002). The endocrine disrupting properties of E1 are the key mechanism of action of the substance. The effects of low or high estrone levels are not yet well known. Women with breast cancer or men who are being treated to reduce testosterone levels need to monitor their estrone level. Excess estrone levels have been associated with breast and endometrial cancer. Women with low estrone levels are also prone to develop osteoporosis. Moreover, adverse effects for prescribed doses of estrone are also including cardiovascular disorders, stroke and dementia (National Library of Medicine, 2013). However, the SCHEER acknowledges that the environmental and human standards of estrone are difficult to establish due to the lack of fully qualifying long-term studies with E1.

## 4. LIST OF ABBREVIATIONS

| | |
|---|---|
| AA-QS | Annual Average Quality Standard |
| AF | Application Factor |
| AMR | Anti-Microbial Resistance |
| BAF | Bioaccumulation Factor |
| BCF | Bioconcentration Factor |
| BMF | Biomagnification Factor |
| dw | dry weight |
| EQS | Environmental Quality Standards |
| MAC-QS | Maximum Acceptable Concentration Quality Standard |
| NOAEL | No Observed Adverse Effect Level |
| PNEC | Predicted No Effect Concentration |
| TL | Threshold Level |
| ww | wet weight |

## 5. REFERENCES

Balabanic D, Rupnik M, Klemencic AK. Negative impact of endocrine-disrupting compounds on human reproductive health. Reprod Fertil Dev. 2011, 23(3), 403-16.

Bolong N, Ismail A, Salim MR, Matsuura T (2009). A review of the effects of emerging contaminants in wastewater and options for their removal. Desalination, 239, 229-246.

Caldwell DJ, Mastrocco F, Anderson PD, Lange R, Sumpter JP (2012). Predicted-no-effect concentrations for the steroid estrogens estrone, 17 beta-estradiol, estriol, and 17 alpha-ethinylestradiol. Environmental Toxicology and Chemistry 31, 1396-1406.

EC (European Commission) (2018).Technical Guidance for Deriving Environmental Quality Standards. Common Implementation Strategy for the Water Framework Directive. Guidance Document No. 27 Updated version 2018.

Huggett DB, Foran CM, Brooks BW, Weston J, Peterson B, Marsh KE, La Point TW, Schlenk D (2003). Comparison of in vitro and in vivo bioassays for estrogenicity in effluent from North American municipal wastewater facilities Toxicol. Sci., 72, 77-83.

Laurenson JP, Bloom RA, Page S, Sadrieh N (2014). Ethinyl estradiol and other human pharmaceutical estrogens in the aquatic environment: a review of recent risk assessment data. AAPS J., 16, 299-310.

Moore SC, Matthews CE, Shu XO, Yu K, Gail MH, Xu X, Ji B-T, Chow W-H, Cai Q, Li H (2016). Endogenous bstrogens, estrogen metabolites, and breast cancer risk in postmenopausal Chinese women. J. Nat. Cancer Inst., 108.

Mouatassim Souali A, Tamisier-Karolak SL, Perdiz D, Cargouet M, Levi Y (2003). Validation of a quantitative assay using GC/MS for trace determination of free and conjugated estrogens in environmental water samples. J. Sep. Sci., 26, 105-111.

National Library of Medicine. Daily Med. Premarin Drug Label.Updated Sept. 2013.http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=87e2da8d-432c-4ed5-67a1-dc26294b2295.

Nelles JL, Hu W-Y, Prins GS (2011). Estrogen action and prostate cancer. Expert Rev. Endocrinol. Metab 6, 437-451.

Pape-Zambito DA, Roberts RF, Kensinger RS (2010). Estrone and 17 beta-estradiol concentrations in pasteurized-homogenized milk and commercial dairy products. Journal of Dairy Science 93, 2533-2540.

Rodgers-Gray TP, Jobling S, Morris S, Kelly C, Kirby S, Janbakhsh A, Harries JE,. Waldock MJ, Sumpter JP, Tyler CR (2000). Long-term temporal changes in the estrogenic composition of treated sewage effluent and its biological effects on fish. Environ. Sci. Technol. 34, 1521-1528.

Sumpter JP, Jobling S (2013). The occurrence, causes, and consequences of estrogens in the aquatic environment. Environ. Toxicol. Chem. 32, 249-251.

Thorpe KL, Cummings RI, Hutchinson TH, Scholze M, Brighty G, Sumpter JP (2003). Relative potencies and combination effects of steroidal estrogens in fish. Environmental Science & Technology 37, 1142-1149.

Trevino LS, Wang Q, Walker CL (2015). Hypothesis: activation of rapid signalling by environmental estrogens and epigenetic reprogramming in breast cancer. Reprod. Toxicol. 54, 136-140.

Williams RJ, Johnson AC, Keller V, Wells C, Holmes MGR, Young AR (2008). Catchment risk assessment of steroid oestrogens from sewage treatment works. Environment Agency, Bristol, UK. Report SC030275/SR3.

Wise A, O'Brien K, Woodruff T (2011). Are oral contraceptives a significant contributor to the estrogenicity of drinking water? Environ. Sci. Technol. 45, 51-60.

Ying G, Kookana RS, Ru YJ. (2002). Occurrence and fate of hormone steroids in the environment. Env Intl 28, 545.