FASS

Activelle®

Novo Nordisk

Filmdragerad tablett 1 mg/0,5 mg
(vit, rund, bikonvex, 6 mm i diameter, märkt NOVO 288 på ena sidan och med en apistjur på andra sidan)

Östrogen och gestagen, kombinationspreparat - systemisk effekt

Aktiva substanser (i bokstavsordning):
Estradiol
Noretisteron

ATC-kod:
G03FA01

Läkemedel från Novo Nordisk omfattas av Läkemedelsförsäkringen.

Miljöpåverkan

Estradiol

Miljörisk: Användning av estradiol har bedömts medföra medelhög risk för miljöpåverkan.
Nedbrytning: Estradiol bryts ned långsamt i miljön.
Bioackumulering: Estradiol har låg potential att bioackumuleras.

Detaljerad miljöinformation

Environmental risk assessment of estrogens in pharmaceutical products marketed by Novo Nordisk in Sweden in 2024

1. 17β-estradiol and its main metabolites estrone and estriol

Environmental risk: Use of 17β-estradiol has been considered to result in a moderate environmental risk. Both 17β-estradiol and its two main metabolites estrone and estriol are considered.

Degradation: 17β-estradiol is slowly degraded in the environment.

Bioaccumulation: 17β-estradiol is assessed not to have a high potential for bioaccumulation. The two main metabolites, estrone and estriol are considered to have a low poten-tial for bioaccumulation.

PBT/vPvB: Neither 17β-estradiol nor its two main metabolites are considered to be PBT/vPvB substances.

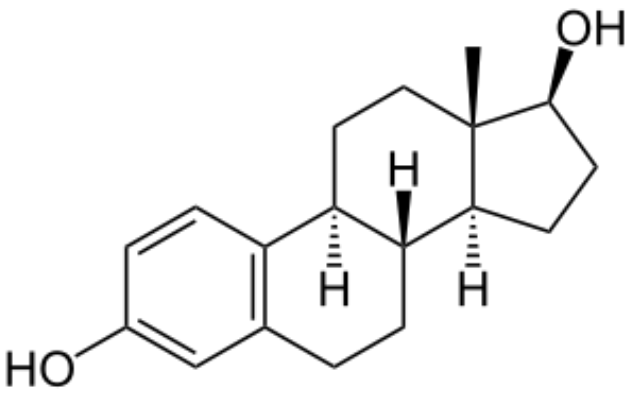
Detailed background information

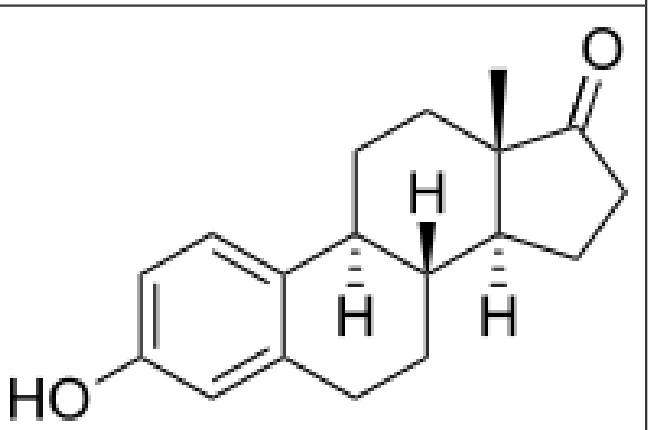
2. The active pharmaceutical ingredients (API)

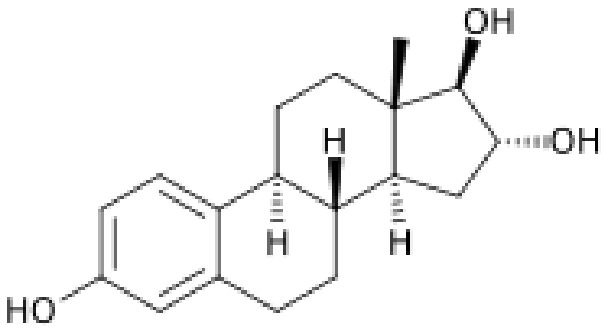
17β-estradiol is used for hormone replacement therapy of women with menopause complications.

17β-estradiol is metabolized during human metabolism into the major transformation products estrone, estriol, estrone sulfate and estrone glucoronide (Ref. 31, 48, 65).

17β-estradiol, estrone and estriol are natural estrogens which belong to the class of steroid hormones. 17β-estradiol is the primary female sex hormone and estrone is the primary metabolite of 17β-estradiol.

Chemical name	**17β-estradiol (E2)**
CAS no.	50-28-2
Molecular structure	
Molecular formula	$C_{18}H_{24}O_2$
Molecular weight	272.38 g/mol

Chemical name	**Estrone (E1)**
CAS no.	53-16-7
Molecular structure	
Molecular formula	$C_{18}H_{22}O_2$
Molecular weight	270.37 g/mol

Chemical name	**Estriol (E3)**
CAS no.	50-27-1
Molecular structure	
Molecular formula	$C_{18}H_{24}O_3$
Molecular weight	288.38 g/mol

3. Environmental Risk classification (PEC/PNEC ratio)

3.1 Predicted Environmental Concentration (PEC)

PEC (Predicted Environmental Concentration) is calculated according to the following formula:

PEC = $(A*10^9*(100-R))/(365*P*V*D*100) = 1.37*10^{-6}*A*(100-R)$ µg/L, where

A = Total amount of API (kg) sold in Sweden in a given year. The total amount of estradiol (hemihydrate 28.5563 and valerat 16.8744) sold in Sweden in 2022 was 45.43 kg API based on IQVIA/LIF sales data (Ref. 10). Reduction of **A** may be justified based on metabolism data. It can be assumed that 17β-estradiol is metabolised in the female body and excreted as 33% 17β-estradiol, 54% Estrone and 13% Estriol (Ref. 5), so A is set to:

- 17β-estradiol: 33% of 45.43 kg = 14.99 kg
- Estrone: 54% of 45.43 kg = 24.53 kg
- Estriol: 13% of 45.43 kg = 5.91 kg

R = Removal rate (%) due to loss by adsorption to sludge particles, by volatilization, hydrolysis or biodegradation. R = 0 if no data is available. The removal rates are based on estimation of distribution of estrogens in a municipal wastewater treatment plant in accordance with the principles of the EU TGD (Ref. 10), and by use of the program SimpleTreat 3.0, which estimates the relative distribution of chemicals to each compartment: effluent, sludge and air. The following removal rates (R) in wastewater treatment plants are estimated (Ref. 5):

- 17β-estradiol: 40% ; Conjugated 17β-estradiol: 6-8%. 17β-estradiol is excreted by mammals as glucuronide or sulfate conjugates in urine or in the unmetabolized form in faeces. Adler et al. (Ref. 12) reported that 50% of 17β-estradiol and 58% of estrone were conjugated in raw sewage. Furthermore, they found by measurement that 87% of the non-conjungated 17β-estradiol was removed in wastwater treatment plant and 47% of the conjungated 17β-estradiol was removed. Overall, a measured removal of 67% was found for 17β-estradiol and its conjugates. Thus, it is considered conservative to keep the SimpleTreat estimated removal for 17β-estradiol of 40%.
- Estrone: 8%; conjugated estrone: 0%. Adler et al. (Ref. 12) measured that 55% of the estrone was removed whereas a slightly higher concentration of the conjugated in the effluent than in the effluent

was found (approximately 7.5 ng/L conjugate in the inlet and 8 ng/L conjugate in the outlet). Overall, a measured removal of 19% was found for estrone and its conjugates. Thus, it is considered conservative to keep the SimpleTreat estimated removal for estrone of 8%.

- Estriol: 2%; conjugates: 0%. Thus, an overall removal for estriol of 0% is assumed here.

P = number of inhabitants in Sweden = $10 * 10^6$ (Ref.1)
V (L/day) = volume of wastewater per capital and day = 200 (ECHA default) (Ref. 11)
D = factor for dilution of wastewater by surface water flow = 10 (ECHA default) (Ref. 11)

On this basis the following PECs in surface water can be calculated:

- PEC for 17β-estradiol: $1.37 * 10^{-6} * 14.99 * (100-40) = 0.0012$ µg/L
- PEC for estrone: $1.37 * 10^{-6} * 24.53 * (100-8) = 0.0031$ µg/L
- PEC for estriol: $1.37 * 10^{-6} * 5.91 * (100) = 0.00081$ µg/L

3.2 Predicted No Effect Concentration (PNEC)

Available eco-toxicological data for 17β-estradiol, estrone and estriol and the derivation of PNEC-values is presented in this section.

3.2.1 17β-estradiol

A proposed EU EQS (PNEC) value has been derived for the 17β-estradiol (Ref. 7) in connection with setting 17β-estradiol on a short-list of 19 possible new priority substances for the Water Frame Directive (Ref. 6). The data used for the derivation of the EQS-value is presented in Appendix together with the derivation, and only a short overview of the derivation is given here.

Knowledge of the mode of action of 17β-estradiol - and strongly supported by the acute and chronic test toxicity data (see Appendix) - suggests that fish and amphibians are likely to be the most sensitive organisms. This is supported by the available chronic toxicity data which indicates that fish are particularly sensitive to 17β-estradiol. Two studies were located on amphibians with LOECs in the range of 1000-2740 ng/l reported for *Rana pipens* and *Xenopus laevis*. These LOECs are far above the NOECs for fish. Therefore, a SSD (Species Sensitivity Distribution) was derived for 17β-estradiol based on data for the most sensitive taxonomic groups, fish - expecting that chronic fish data used for the derivation of an SSD would also be protective of the other less sensitive group.

The lowest no observed effect concentration for 17β-estradiol is a 35-50 d NOEC of 0.5 ng/l (Ref. 48) for the trout (*Onchorhynchus mykiss*). The observed effects were sperm volume, sperm density and fertilization success. The study was not carried out according to a guideline. Experiments took place in four identical flow-through 0.5 m^3 tanks (three replicates and one control - each tank with 10 males and 3 females of approximate same size). Water inflow temperature was 6^oC and air saturation of water was >90%. Fish were kept under natural photoperiod (experiments were carried out in Kreuzstein in Sankt Gilgen, Upper Austria during December – January).

Overall, reliable chronic NOEC values were available for 11 species of fish and the SSD was based on these 11 fish species (Ref. 7). The HC5 for the SSD was found at 0.8 ng/l. Based on the available dataset and the knowledge of the mode of action, an assessment factor of 2 was considered appropriate. This gives an AA-EQS of 0.4 ng/l.

This derivation of the AA-EQS was reviewed by SCHER (Ref. 8). Both the reliability and the ecological relevance of the endpoints and taxonomic groups were considered. Overall, the SCHER supported the proposed AA-EQS of 0.4 ng/l for 17β-estradiol.

In conclusion, a PNEC of 0.4 ng/L is used for 17β-estradiol

3.2.2 Estrone

A PNEC-value has been derived for estrone in connection with setting the substance (together with 17β-estradiol) on a short-list of 19 possible new priority substances for the Water Frame Directive (Ref. 6). A well-accepted EU PNEC for estrone has been derived at 3.6 ng/l (Ref. 59).

Environmental toxicity data for estrone has been collected and are presented in the annex.

As for 17β-estradiol, the mode of action for estrone suggests that fish and amphibians are likely to be the most sensitive organisms. Based on available data, fish is found to be the most sensitive species to estrone. A NOEC for estrone of 36 ng/l was obtained in 40-day study with *Danio rerio* (according to OECD Draft Test Guideline: A 40-day Juvenile Zebrafish Assay for screening of Endocrine Disrupting Chemicals), and a NOEC for estrone of 5 ng/l was obtained in a 90-day study (no guideline followed, fish specie: *Oryzias latipes*, effects measured: Organ weight in relationship to body weight; hatch, Vitellogenin 1 mRNA).

As for 17β-estradiol, the mode of action for estrone is well-known and fish is the most sensitive species. Therefore, an assessment factor of 10 for the chronic fish toxicity data is considered justified.

Using an assessment factor of 10, a PNEC of 0.5 ng/L was obtained.

3.2.3 Estriol

As for 17β-estradiol and estrone, the mode of action for estriol is well-known and fish is the most sensitive species. Therefore, an assessment factor of 10 for the chronic fish toxicity data is considered justified.

The No Observed Effect Concentration (NOEC) for induction of vitellogenin, which is considered a chronic eco-toxicity test, is found at 0.0465 µg/l for estriol (Ref. 49; not-a guideline study; test species *Oryzias latipes*, duration of study 90 days, temperature: 25 ± 1 °C, three replicates and one control; 30 embryos per replicate).

Using an assessment factor of 10, a PNEC of 4.7 ng/L was obtained.

3.2.4 Derived PNECs

PNEC for the three APIs in surface water is:

- PNEC for 17β-estradiol: 0.0004 µg/L
- PNEC for estrone: 0.0005 µg/L
- PNEC for estriol: 0.0047 µg/L

3.3 Calculation of the risk quotient (PEC/PNEC)

The following risk quotient PEC/PNEC can be calculated:

- PEC/PNEC for 17β-estradiol: 0.0012/0.0004 = 3.0
- PEC/PNEC for estrone: 0.0031/0.0005 = 6.2
- PEC/PNEC for estriol: 0.00081/0.0047 = 0.17

The total PEC/PNEC ratio for 17β-estradiol, estrone and estriol is thus 9.4.

Based on the calculated PEC/PNEC ratios and information about degradation, bioaccumulation and eco-toxicity of 17β-estradiol, estrone and estriol the following environmental risk phrase should be applied to pharmaceutical products with estrogens according to the criteria in the FASS.se guidelines (Ref. 1):

”Use of pharmaceutical products with estrogens has been considered to result in moderate environmental risk”
This risk phrase is according to the FASS.se guidelines applicable for risk quotients in the interval: 1 < PEC/PNEC ≤ 10.

4. Biotic degradation

4.1. Degradation of 17β-estradiol
Activated sludge test according to OECD guideline no. 302A has shown that 17β-estradiol is inherently biodegradable under aerobic conditions in activated sludge (Ref. 30). 17β-estradiol is thus slowly degraded in the environment. In a 100 days simulation study of 17β-estradiol (OECD Test Method no. 308), an aerobic mineralisation (marine) of 61±1% respectively 62±3% mineralisation (freshwater) was found (Ref. 86). Thus, 17β-estradiol is found to be biodegradable in both marine and freshwater. In addition, an activated sludge tests (OECD 302, Ref. 2) show that 17β-estradiol is inherently biodegradable under aerobic conditions.

4.2. Abiotic degradation
Hydrolysis:
No data available

Photolysis:
No data available

5. Bioaccumulation

According to the FASS.se guidelines (Ref. 1), substances with Log Pow ≥ 4 or BCF ≥ 500 are considered to have high potential for bioaccumulation. Valid BCF-data has prevalence above log Pow data. One limitation in the use of log Pow for the estimation of the bioaccumulation potential is that metabolism within the test organism is not considered.

The following data on bioaccumulation are retrieved from the literature and calculations:

Substance	Parameter	Result	Specie	Method	Reference
17β-estradiol (E2)	**log Pow**	**3.94**	**n-octanol**	**Calculation**	**Ref. 82**
17β-estradiol (E2)	**BCF**	**38 (day 21); 43 (day 81); 45 (day 141)**	**High-back crucian carp (*Carassius auratus*)**	**No standard followed. 200 juvenile caged fish were exposed to wastewater outlet at the secondary sedimentation tank (for up to 141 days).**	**Ref. 53**

				Concentrations in wastewater and fish were measured.	
17β-estradiol (E2)	BCF	174	Male fathead minnow, plasma	Method: no standard followed. Male and female fathead minnow were to 17β-oestradiol for 19 days at nominal concentrations that ranged from 27.2-2740 ng l-1. Tissues were collected and the concentration in the plasma was measured. The estimated BCF was 174 in males based on the relationship between waterborne and plasma 17β-oestradiol concentrations in surviving fish from all treatments.	Ref. 47
17β-estradiol (E2)	BCF	6.5	Larvae and juvenile flounder	Method: no standard followed. The estradiol uptake (through 48 hours) and depuration (through 48 hours) was studied both for larvae and juvenile flounders. Five test concentrations (between 4nM and 1000 nM)	Ref. 69

				and a control was applied in the uptake study. No BCF could be established for females	
17β-estradiol (E2)	log Klip,w	Varied between 2.29 (vesicle including cholesterol)-3.79 (vesicle including unsaturated acyl chains).	Three types of synthetic membrane liposomes were tested.	Method: no standard followed. The partitioning between water and the synthetic membrane liposomes were measured by equilibrium dialysis	Ref. 87
Estrone (E1)	Log Pow	3.43	n-octanol	Calculation	Ref. 82
Estrone (E1)	BCF	35 (day 21); 29 (day 81); 35 (day 141)	High-back crucian carp (*Carassius auratus*)	No standard followed. 200 juvenile caged fish were exposed to wastewater outlet at the secondary sedimentation tank (for up to 141 days). Concentrations in wastewater and fish were measured.	Ref. 53
Estrone (E1)	BCF	241/278 (4hr), 229 (16 hr), 165 24 hr	*Daphnia magna*	No standard followed. Uptake of E1 by the D. magna. was measured at 4, 16, and 24 h and the final concentration of E1 in the pond water was analyzed by LC/MS at each time point. The experiment was repeated at a	Ref. 38

				lower concentration of E1 (40mg/L) and uptake in the D. magna and concentration of E1 in the water was determined after 4 h. All bioconcentration experiments were carried out in triplicate.	
	log Klip,w	Varied between 2.45 (vesicle including cholesterol)-3.92 (vesicle including unsaturated acyl chains).	Three types of synthetic membrane liposomes were tested.	Method: no standard followed. The partitioning between water and the synthetic membrane liposomes were measured by equilibrium dialysis	Ref. 87
Estriol (E3)	Log Pow	2.81	n-octanol	Calculation	Ref. 82
Estriol (E3)	log Klip,w	Varied between 0.179 (vesicle including cholesterol)-0.96 (vesicle including unsaturated acyl chains).	Three types of synthetic membrane liposomes were tested.	Method: no standard followed. The partitioning between water and the synthetic membrane liposomes were measured by equilibrium dialysis	Ref. 87

It is noted that 17β-estradiol has a calculated log Pow slightly below but close to the cut-off value of 4. It can be mentioned that a logPow slightly above 4 (4.01) has been measured (Ref. 33, method not reported). Several measured BCFs are available for 17β-estradiol – all well below the cut-off value of 500. Therefore, 17β-estradiol is assessed not to have a high potential for bioaccumulation.

Both estrone and estriol have calculated log Pow well below 4. Actually, measured log Pow values are available for the two substances showing a log Pow of 3.13 respectively 2.45 (Ref. 33, method not reported). In addition, a BCF well below 100 is measured for estrone in the fish “high-back crucian carp”. Thus, both substances are considered to have a low potential for bioaccumulation.

Of some interest to note is the measured partitioning between water and synthetic membrane liposomes – mimicking biological specie-of the three substances. The partitioning of 17β-estradiol and estrone is on the

very same level – whereas the partitioning of estriol to the membrane liposomes is much lower. This is in agreement with the calculated log Pow-values.

Overall, it is assessed that 17β-estradiol, estrone and estriol all have a low potential for bioaccumulation.

6. PBT/vPvB assessment

Considering all three aspects, 17β-estradiol, estrone and estriol do not meet the criteria for classification as a PBT or vPvB substance.

7. References

General references

1. Environmental classification of pharmaceuticals at FASS – Guidance for pharmaceutical companies 2012 v.3.0.
2. D'Ascenzo G., A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, et al. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ, 301 (2003), pp. 199-209
3. DHI (2001): Litteratur-review over økotoksikologiske data for østradiol og østron. November 2001. Udført af DHI. (only in Danish)
4. DHI (2003): Summary of selected investigations performed for Novo Nordisk A/S - Steroid hormones. October 2003. Prepared by DHI.
5. DHI (2003): Fate and effects of humanly excreted estrogens - 17 β-estradiol, estrone, estriol and ethinylestradiol. October 2003. Prepared by DHI.
6. European Union (2013). “Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy”.
7. EU (2011): Beta-estradiol EQS dossier 2011.
8. SCHER (Scientific Committee on Health and Environmental Risks) (2011). OPINION ON "CHEMICALS AND THE WATER FRAMEWORK DIRECTIVE: DRAFT ENVIRONMENTAL QUALITY STANDARDS" 17β-estradiol (E2) SCHER adopted this opinion at its 12th plenary on 30 March 2011.
9. ECHA, European Chemicals Agency. 2008 Guidance on information requirements and chemical safety assessment.
10. 10. ECHA (2016): Guidance on information requirements and Chemical Safety Assessment. Chapter R.16: Environmental exposure assessment. Version 3.0.
11. 8.11. IQVIA/LIF (2021): kg consumption 2020.

Data references

12. Adler P., Th. Steger-Hartmann, W. Kalbfuß (2001): Vorkommen natürlicher und synthetischer östrogener Steroide in Wässern des süd-und mitteldeutschen Raumes. Acta Hydrochim. Hydrobiol, 29 (2001), pp. 227-241
13. Andersen H R, Wollenberger L, Halling-Sørensen B, Kusk K O (2001): "Development of copepod nauplii to copepodites - a parameter for chronic toxicity including endocrine disruption." Environmental Toxicology and Chemistry 20(12): 2821-2829.
14. Billinghurst Z, Clare A S, Fileman T, McEvoy J, Readman J, Depledge M.H. (1998): "Inhibition of barnacle settlement by the environmental oestrogen 4-nonylphenol and the natural oestrogen 17-beta-oestradiol." Marine Pollution Bulletin 36(10): 833-839.
15. Bjerregaard, P., P.R. Hansen, K.J. Larsen, C. Erratico, B. Korsgaard, and H. Holbech(2008):Vitellogenin as a Biomarker for Estrogenic Effects in Brown Trout, Salmo trutta: Laboratory and Field Investigations. Environ. Toxicol. Chem.27(11): 2387-2396
16. Bjørnestad E (2002): Chronic toxicity test of 17 beta-Estradiol (CAS No. 50-28-2) with the crustacean Acartia tonsa. Rapport fra DHI Vand & Miljø.

17. Breitholtz M und Bengtsson B E (2001): "Oestrogens have no Hormonal Effect on the Development and Reproduction of the Harpacticoid Copepod Nitocra spinipes." Marine Pollution Bulletin 42(10): 879-886.
18. Brion F, Tyler C R, Palazzi X, Laillet B, Porcher J M, Garric J, Flammarion P (2004): "Impacts of 17-beta-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (Danio rerio)." Aquatic Toxicology 68(3): 193-217.
19. Cripe G M, Hemmer B L, Goodman L R, Fournie J W, Raimondo S, Vennari J C, Danner R L, Smith K, Manfredonia B R, Kulaw D H, Hemmer M J (2009): "Multigenerational exposure of the estuarine sheepshead minnow (Cyprinodon variegatus) to 17-beta-estradiol. I. Organism-level effects over three generations." Environmental Toxicology and Chemistry 28(11): 2397-2408.
20. Dammann,A.A., N.W. Shappell, S.E. Bartell, and H.L. Schoenfuss(2011):Comparing Biological Effects and Potencies of Estrone and 17beta-Estradiol in Mature Fathead Minnows, Pimephales promelas. Aquat. Toxicol.105(3/4): 559-568
21. Ghekiere,A., T. Verslycke, and C. Janssen(2006):Effects of Methoprene, Nonylphenol, and Estrone on the Vitellogenesis of the Mysid Neomysis integer. Gen. Comp. Endocrinol.147(2): 190-195
22. DHI (2002): Algal growth inhibition test of ß-Estradiol with the micro alga Pseudokirchneriella subcapitata. 2002.06.17. Prepared by DHI.
23. DHI (2002): Algal growth inhibition test of Estrone with the micro alga Pseudokirchneriella subcapitata. 2002.06.27. Prepared by DHI.
24. DHI (2002): Chronic toxicity test of ß-Estradiol [CAS no. 50-28-2] with the crustacean Acartia tonsa. 2002.06.28. Prepared by DHI.
25. DHI (2002): Zebra fish chronic toxicity test with Estrone [CAS no. 53-16-7]. 2002.08.30. Prepared by DHI.
26. DHI (2002): Nitrification inhibition test of ß-Estradiol with activated sludge. 2002.07.03. Prepared by DHI.
27. DHI (2002): Nitrification inhibition test of Estrone with activated sludge. 2002.07.04. Prepared by DHI.
28. DHI (2002): Enchytraeus albidus chronic toxicity test with ß-Estradiol. 2002.07.05. Prepared by DHI.
29. DHI (2002): Ready Biodegradability – Closed Bottle Test with Estradiol. 2002.07.12. Prepared by DHI.
30. DHI (2002): Activated Sludge Biodegradability Simulation Test with Estradiol. 2002.07.12. Prepared by DHI.
31. Doyle C J und Lim R P (2005): Sexual behavior and impregnation success of adult male mosquitofish following exposure to 17-beta-estradiol. Ecotoxicology and Environmental Safety 61 :392–397.
32. Hirai N, Nanba A, Koshio M, Kondo T, Morita M, Tatarazako N (2006): Feminization of Japanese medaka (Oryzias latipes) exposed to 17β-estradiol: Formation of testis-ova and sex-transformation during early-ontogeny. Aquatic Toxicology 77 (1):78-86.
33. Hansch C., Leo A. and Hoekman D. (1995). Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC., American Chemical Society.
34. Hobkirk R., Mellor J.D. and Nilsen M. (1975). In vitro metabolism of 17beta-estradiol by human liver tissue. Can. J. Biochem. 53, : 903-906.
35. Holbech,H., K. Kinnberg, G.I. Petersen, P. Jackson, K. Hylland, L. Norrgren, and P. Bjerregaard(2006):Detection of Endocrine Disrupters: Evaluation of a Fish Sexual Development Test (FSDT). Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol.144(1): 57-66
36. Huang Bin, Wenwen Sun,Xiaoman Li, Jingliang Liu, Qiang Li, Renmin Wang, Xuejun Pan (2015): Effects and bioaccumulation of 17β-estradioland 17α-ethynylestradiol following long-term exposure in crucian carp. Ecotoxicology and Environmental Safety 112, 169–176
37. Hutchinson, T.H., N.A. Pounds, M. Hampel & T.D. Williams (1999): Impact of natural and synthetic steroids on the survival, development and reproduction of marine copepods (*Tisbe battagliai*). The science of the Total Environment 233: 167-179
38. Gomes Rachel L., L.Hannah E. Deacon, Ka M. Lai, Jason W. Birkett, Mark D. Scrimshaw And John N. Lester (2004): Assessment Of The Bioaccumulation Of Estrone In Daphnia Magna
39. Environmental Toxicology and Chemistry, Vol. 23, No. 1, pp. 105–108, 2004

40. Imai S, Koyama J, Fujii K (2005): Effects of 17b-estradiol on reproduction of Java medaka (Oryzias javanicus), a new test fish. Mar Poll Bull 51: 708–714.
41. Imai S, Koyama J, Fujii K. 2007. Effects of estrone on full life cycle of Java medaka(Oryzias javanicus), a newmarine test fish. Environ Toxicol Chem 26:726–731.
42. Jukosky J A Watzin M C, Leiter J C (2008a): The effects of environmentally relevant mixtures of estrogens on Japanese medaka (Oryzias latipes) reproduction. Aquatic Toxicology 86:323–331.
43. Kang I J, Yokota H, Oshima Y, Tsuruda Y, Yamaguchi T, Maeda M, Imada N, Tadokoro H, Honjo T (2002): Effect of 17-beta-estradiol on the reproduction of Japanese medaka (Oryzias latipes). Chemosphere 47(1): 71-80.
44. Kashiwada et al. (2002): Fish test for endocrine disruption and estimation of water quality of Japanese rivers. Water Research 36: 2161-2166.
45. Kloas W, Lutz I and Einspanier R (1999): Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. Science of The Total Environment 225: 59-68.
46. Kramer V J, Miles-Richardson S, Pierens S L, Giesy J P (1998): Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (Pimephales promelas) exposed to waterborne 17-beta-estradiol. Aquatic Toxicology 40(4): 335-360.
47. Kramer V.J., Miles-Richardson S., Pierens S.L. and Giesy J.P. (1998). "Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (Pimephales promelas) Exposed to waterborne 17[beta]-estradiol." Aquatic Toxicology 40(4): 335-360
48. Lahnsteiner F, Berger B, Kletzl M, Weismann T (2006): Effect of 17β-estradiol on gamete quality and maturation in two salmonid species. Aquatic Toxicology. 79:124–131.
49. Lei,B., J. Kang, Y. Yu, J. Zha, W. Li, Z. Wang, Y. Wang, and Y. Wen(2014):Long-Term Exposure Investigating the Estrogenic Potency of Estriol in Japanese Medaka (Oryzias latipes). Comp. Biochem. Physiol. C Toxicol. Pharmacol.160:86-92
50. Lei,B., Y. Wen, X. Wang, J. Zha, W. Li, Z. Wang, Y. Sun, J. Kang, and Y. Wang(2013):Effects of Estrone on the Early Life Stages and Expression of Vitellogenin and Estrogen Receptor Genes of Japanese Medaka (Oryzias latipes). Chemosphere93(6): 1104-1110
51. Liao T, Guo Q L, Jin S W, Cheng W, Xu Y(2009): Comparative responses in rare minnow exposed to 17β-estradiol during different life stages, Fish Physiol. Biochem. 35: 341–349.
52. Lievertz R.W. (1987). Pharmacology and pharmacokinetics of estrogens. Am. J. Obstet. Gynecol. 156:1289-1293.
53. Liu Jingliang , Renmin Wang, Bin Huang, Chan Lin, Jiali Zhou, Xuejun Pan (2012):Biological effects and bioaccumulation of steroidal and phenolic endocrine disrupting chemicals in high-back crucian carp exposed to wastewater treatment plant effluents. Environmental Pollution 162 (2012) 325-331
54. Mackenzie C A, Berrill M, Metcalfe C, Pauli B D (2003): Gonadal differentiation in frogs exposed to estrogenic and antiestrogenic compounds. Environmental Toxicology and Chemistry. Volume 22, Issue 10: 2466–2475
55. Metcalfe C D, Metcalfe T L, Kiparissis Y, Koenig B, Khan C, Hughes R J, Croley T R, March R E , Thomas P. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 20(2): 297-308.
56. Nash J P, Kime D E, van der Ven L T M , Wester P W , Brion F , Maack G, Stahlschmidt-Allner P. and Tyler C.R. (2004): Long-Term Exposure to Environmental Concentrations of the Pharmaceutical Ethynylestradiol Causes Reproductive Failure in Fish. Environmental Health Perspectives 112(17): 1725-1733.
57. Nimrod A C und Benson W H (1998): Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. Aquatic Toxicology 44(1-2): 141-156.
58. Notch,E.G., and G.D. Mayer(2013):Impact of Environmental Estrogens on Nucleotide Excision Repair Gene Expression in Embryonic Zebrafish. Comp. Biochem. Physiol. C Toxicol. Pharmacol.157(4): 361-365
59. Oekotoxzentrum, Eawag (2011): Proposed PNEC value for Estrone.

60. Panter, G.H., R.S. Thompson & J.P. Sumpter (1998): Adverse reproductive effects in male fathead minnows (Pimephales promelas) exposed to environmentally relevant concentrations of the natural oestrogenes, oestradiol and oestrone. Aquatic toxicology 42: 243-253
61. Pollino C A, Georgiades E,. Holdway D A (2007): Use Of The Australian Crimson-Spotted Rainbowfish (Melanotaenia Fluviatilis) As A Model Test Species For Investigating The Effects Of Endocrine Disruptors. Environmental Toxicology and Chemistry, Vol. 26, No. 10: 2171–2178
62. Robinson C D, Brown E, Craft J A, Davies I A, Megginson C, Miller C, Moffat C F (2007): Bioindicators and reproductive effects of prolonged 17-beta-oestradiol exposure in a marine fish, the sand goby (Pomatoschistus minutus). Aquatic Toxicology 81: 397–408.
63. Roepke T A, Snyder M J, Cherr G N (2005): Estradiol and endocrine disrupting compounds adversely affect development of sea urchin embryos at environmentally relevant concentrations. Aquatic Toxicology 71:155–173.
64. Routledge E J, Sheahan D, Desbrow C, Brighty G C, Waldock M, Sumpter J P (1998): Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. Environmental Science and Technology 32: 1559-1565.
65. Schering AG (1995): Acute toxicity of 17beta-estradiol with the rainbow trout. Report A05662.
66. Schering AG (2002). Growth inhibition test with estradiol (ZK 5018) on the green algae Desmodesmus subspicatus. Report A30506.
67. Segner H, Navas J M, Schäfers C, Wenzel A (2003): Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo. Ecotoxicology and Environmental Safety 54:315-322.
68. Slaunwhite R.W., Kirdani R.Y. and Sandberg A.A. (1973). Metabolic aspects of estrogens in man. In: R.O. Greep and E.B. Astwood (Eds.). Handbook of Physiology. Section 7: Endocrinology, Vol. 2, Female Reproductive System, part 1, Chapter 21, Washington DC, American Physiology Society. pp. 485-523.
69. Specker and Chandler (2003). Methodology for estradiol treatment in marine larval and juvenile fish: uptake and clearance in summer flounder. Aquaculture, 217, 663-672.
70. Schering AG (2002). Growth inhibition test with estradiol (ZK 5018) on the green algae Desmodesmus subspicatus. Report A30506.
71. Seki M., Yokota H., Maeda M. and Kobayashi K. (2005). "Fish full life-cycle testing for 17beta-estradiol on medaka (Oryzias latipes)." Environmental Toxicology and Chemistry 24(5): 1259-1266.
72. Shappell N W, Hyndman K M, Bartell S E, Schoenfuss H L (2010): Comparative biological effects and potency of 17-alpha- and 17-beta-estradiol in fathead minnows. Aquatic Toxicology:100: 1–8.
73. Shioda T und Wakabayashi M. (2000): Effect of certain chemicals on the reproduction of medaka (Oryzias latipes). Chemosphere 40(3): 239-243.
74. Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M, Magara Y (2001): "Water Science and Technology. 43 2:109-116.
75. Tatarazako N, Takao Y, Kishi K, Onikura N, Arizono K, Iguchi T. (2002): Styrene dimers and trimers affect reproduction of daphnid (Ceriodaphnia dubia)." Chemosphere 48(6): 597-601.
76. Thorpe, K.L., T.H. Hutchinson, M.J Hetherudge, M. Scholtze, J.P Sumpter & C. Tyler (2001): Assessing the Biological Potency of Binary Mixtures of Environmental Estrogens using Vitellogenin Induction in Juvenile Rainbow Trout (oncorhynchus mykiss). Environ. Sci Technol. 35: 2476-2481
77. Thorpe K.L. Thomas H., Malcolm J.H., Martin S., P. Sumpter & And C.R. Tyler (2001): Assessing the Biological Potency of binary mixtures of Environmental Estrogens using Vitellogenin Induction in Juvenile Rainbow Trout. Environ. Sci. Technol. 2001, 35, 2476-2481. Environ Sci Technol. 2003;37(6):1142-9.
78. Thorpe K L, Benstead R, Hutchinson T H, Cummings R I, Tyler C R (2003): Reproductive effects of exposure to oestrone in the fathead minnow. Fish Physiology and Biochemistry 28: 451–452.
79. Thorpe K L, Cummings R I, Hutchinson T H, Scholze M, Brighty G, Sumpter J P, Tyler C R (2003):Relative Potencies and Combination Effects of Steroidal Estrogens in Fish.

80. Thorpe KL, Benstead R, Hutchinson TH, Tyler CR. 2007. Associations between altered vitellogenin concentrations and adverse health effects in fathead minnow (Pimephales promelas). Aquat Toxicol (Amst) 85:175–183.
81. Toft G und Battrup E (2003): Altered sexual characteristics in guppies (Poecilia reticulata) exposed to 17b-estradiol and 4-tert-octylphenol during sexual development. Ecotoxicology and Environmental Safety 56: 228–237.
82. US EPA (2012): EpiSuite
83. Van den Belt K, Berckmans P, Vangenechten C, Verheyen R, Witters H 2004): Comparative study on the in vitro and in vivo estrogenic potencies of 17β-estradiol, estrone, 17α-ethynylestradiol and nonylphenol. Aquat Toxicol 66(2):183-185.
84. Van der Ven LTM, Van den Brandhof E-J, Vos HJ, Wester PW (2007) Effects of the estrogen agonist 17β-Estradiol and antagonist tamoxifen in a partial life-cycle assay with zebrafish (Danio rerio). Environ Tox Chem 26(1):92-99.
85. Winther-Nielsen M (2002): Algal growth inhibition test of 17-beta-Estradiol with micro alga Pseudokirchneriella subcapitata. Rapport fra DHI - Institut for Vand & Miljø.
86. Winther-Nielsen (2011): Aerobic transformation of 17β-estradiol in aquatic sediment systems. DHI GLP report. 2011.03.31
87. Yamamoto Hiroshi and Howard M. Liljestrand (2004): Partitioning of Selected Estrogenic Compounds between Synthetic Membrane Vesicles and Water: Effects of Lipid Components.

Appendix

Nitrification inhibition test with activated sludge:

Substance	Method	Concentration & Exposure time	Effect parameter	EC20	Reference
17β-estradiol	ISO 9509	62,5–1.000 µg/L 2 hrs	Inhibition of nitrification rate	> 918 µg/L	Ref. 26
Estrone	ISO 9509	62,5–1.000 µg/L 2 hrs	Inhibition of nitrification rate	> 172 µg/L	Ref. 27

The studies did not show significant inhibition of the nitrification rate in activated sludge at the tested concentrations.

Biodegradation test of 17β-estradiol:

Substance	Method	Concentration & Exposure time	Result	Reference
17β-estradiol (E2)	OECD Test Method no. 308: “Aerobic transformation of 17β-estradiol in aquatic sediment systems”	Nominal concentrations 0.36 µg/L and 1.1 µg/L of unlabelled and 14C-labelled E2, respectively 100 days	61±1% mineralisation (marine) 62±3% mineralisation (freshwater)	Ref. 86
17β-estradiol	OECD Test Method no. 301D: “Closed Bottle Test”	1.64 mg/L 28 days	3.5-9.8 % of ThoD	Ref. 29

Substance	Method	Concentration & Exposure time	Result	Reference
17β-estradiol (E2)	OECD Guideline no. 302A: “Inherent Biodegradability: Modified SCAS Test” and “Activated Sludge Biodegradability Simulation Test”	Ca. 20 µg/L Aerobic: 48 hrs Anoxic: 8 days	Aerobic: See below * Anoxic: No significant degradation	Ref. 30

* Results according to OECD Guideline no. 302A:

- The total ^{14}C-concentration decreased by 70% of the initial added ^{14}C within the first 45 minutes of the test period
- During the first 45 minutes of the test period, a 1. order rate constant was estimated at 2.2 ± 0.2 $L*day^{-1}*gSS^{-1}$ for the total test substance concentrations > 2.5 µg E2/L
- During the test period from 3-48 hours, a 1. order rate constant was estimated at 0.031 ± 0.003 $L*day^{-1}*gSS^{-1}$ for the total test substance concentrations < 2.5 µg E2/L

On basis of the biodegradation test results it can be concluded that:

- 17 β-estradiol is not readily degradable under closed bottle conditions since the minimum requirement BOD = 60% of ThOD within 10 days is not fulfilled.
- 17 β-estradiol is inherently biodegradable under aerobic conditions but not under anoxic conditions in activated sludge simulation.

Reproduction test for 17β-estradiol on the earth worm, *Enchytraeus albidus*

Method	Concentration & Exposure time	Effect parameter	NOEC	Reference
OECD Draft Test Guideline 220: “Enchytraeidae Reproduction Test”, March 2000 and in agreement with the existing OECD Guideline No. 220: Enchytraeid Reproduction Test	50–1,000 mg/kg soil d.w. 21 days	Adult mortality Inhibition of reproduction Changes in behaviour and/or morphology	> 1,000 mg/kg	Ref. 28

The study did not show significant effect on neither of the stated parameters at the tested concentrations.

Derivation of PNEC for 17β-estradiol

A suggestion for AA-EQS has been drafted and reviewed (Ref. 7). The below derivation is based on this derivation.

Specie Group	Organism	Effect	Duration	End-Point	Value (µg/L)	KLIMISH Score	Reference
Short Term Data							
Algae	*Desmodesmus subspicatus*	Growth (GLP)	72 h	EC50	>3100	1	Ref. 66
Invertebrate	*Acartia tonsa*	Mortality	48 h	EC50	>1000	2	Ref. 13
Fish	*Cyprinus carpio*	VTG induction in hepatocytes	3 d	EC50	24.52	2	Ref. 67
Fish	*Oncorhynchus mykiss*	Mortality	96 h	LC50	>500	1	Ref. 65
Fish	*Oncorhynchus mykiss*	VTG induction in hepatocytes	3 d	EC50	7.08	2	Ref. 67
Fish	*Oryzias latipes*	Egg and embryo mortality	72 h	LC50	460	2	Ref. 44
Fish	*Oryzias latipes*	Adult	72 h	LC50	3500	2	Ref. 44
Long-term data							
Algae	*Desmodesmus subspicatus*	Growth	72 h	NOEC	>3100	1	Ref. 66
Algae	*Pseudokirchneriella subcapitata*	Growth (OECD 201, GLP)	72 h	NOEC	>523	2	Ref. 85
Arthropoda	*Balanus amphrite*	larval colonization	2 d	NOEC	=0.1	2	Ref. 14
Invertebrate	*Acartia tonsa*	development	5 d	EC10	370	2	Ref. 13
Invertebrate	*Acartia tonsa*	development	5 d	EC50	720	2	Ref. 13
Invertebrate	*Acartia tonsa*	Reproduction GLP, Not a guideline study;	21 d	NOEC	>368	2	Ref. 16
Invertebrate	*Ceriodaphnia dubia*	reproduction	7 d	NOEC	=10000	2	Ref. 75
Copepoda	*Nitocra spinipes*	reproduction	18 d	NOEC	≥160	2	Ref. 17
Copepoda	*Tisbe battagliai*	reproduction	21 d	NOEC	≥100	2	Ref. 37
Amphibien		feminization	84 d	LOEC	2.74	2	Ref. 45

	Xenopus laevis						
Amphibien	*Rana pipiens*	Intersex	162 d	LOEC	≤1	2	Ref. 54
Fish	*Cyprinodon variegatus*	Proportion of viable eggs F1 and F2	280 d	LOEC	0.04	2	Ref. 19
Fish	*Cyprinodon variegatus*	Proportion of viable eggs F1 and F2	280 d	NOEC	0.01	2	Ref. 19
Fish	*Danio rerio*	altered gonadal histology, sex ratio	21 d	LOEC	0.1	2	Ref. 18
Fish	*Danio rerio*	altered gonadal histology, sex ratio	21 d	NOEC	0.025	2	Ref. 18
Fish	*Danio rerio*	altered gonadal histology, secondary sexual characteristics	21 d	NOEC	0.005	2	Ref. 18
Fish	*Danio rerio*	reproduction	200 d	NOEC	≤0.005	2	Ref. 56
Fish	*Danio rerio*	Egg number in the clutch and hatching	21 d	NOEC	0.087	2	Ref. 71
Fish	*Gabiocypris rarus*	sex ratio	21 d	LOEC	0.025	2	Ref. 51
Fish	*Gabiocypris rarus*	sex ratio	21 d	NOEC	0.005	2	Ref. 51
Fish	*Gambusia holbrooki*	reproductive success	84 d	LOEC	0.02	2	Ref. 31
Fish	*Gambusia holbrooki*	reproductive success	84 d	NOEC	0.1	2	Ref. 31
Fish	*Melanotaenia fluviatilis*	egg production	14 d	LOEC	0.3	2	Ref. 61
Fish	*Melanotaenia fluviatilis*	egg production	14 d	NOEC	0.1	2	Ref. 61
Fish	*Oncorhynchus mykiss*	Sperm volume, sperm density and fertilization success	35-50 d	LOEC	0.001	2	Ref. 48
Fish			35-50 d	NOEC	0.0005	2	Ref. 48

	Oncorhynchus mykiss	Sperm volume, sperm density and fertilization success					
Fish	*Oryzias javanicus*	Fertility of the eggs	187 d	LOEC	0.016	2	Ref. 40
Fish	*Oryzias javanicus*	Fertility of the eggs	187 d	NOEC	0.0095	2	Ref. 40
Fish	*Oryzias latipes*	Gender shift (testis-ova)	90 d	LOEC	0.1	2	Ref. 55
Fish	*Oryzias latipes*	Gender shift (testis-ova)	90 d	NOEC	0.01	2	Ref. 55
Fish	*Oryzias latipes*	total study	90 d	LOEC	0.004	3	Ref. 55
Fish	*Oryzias latipes*	total study	90 d	NOEC	0.0004	3	Ref. 55
Fish	*Oryzias latipes*	feminization	200-300 d	NOEC	0.1	2	Ref. 74
Fish	*Oryzias latipes*	reduced fertility	59 d	NOEC	0.0029	2	Ref. 71
Fish	*Oryzias latipes*	feminization	28 d	LOEC	≤0.01	2	Ref. 57
Fish	*Oryzias latipes*	number of eggs	14 d	NOEC	0.272	2	Ref. 73
Fish	*Oryzias latipes*	reduced fertility	21 d	NOEC	0.227	2	Ref. 43
Fish	*Oryzias latipes*	Hatching time	20 d	NOEC	0.034	2	Ref. 32
Fish	*Oryzias latipes*	various reproduction endpoints	14 d	NOEC	0.379	3	Ref. 42
Fish	*Pimephales promelas*	Feminization and weight gain	91 d	LOEC	0.0279	1	Ref. 65
Fish	*Pimephales promelas*	Feminization and weight gain	91 d	NOEC	>0.008	1	Ref. 65
Fish	*Pimephales promelas*	reduced egg production	19 d	EC10	0.0066	2	Ref. 46
Fish	*Pimephales promelas*	reproduction, reduced egg production	21 d	NOEC	0.044	3	Ref. 86
Fish	*Poecilia reticulata*		90 d	LOEC	0.5	2	Ref. 81

		Feminization (GSI, sex ratio)					
Fish	*Poecilia reticulata*	Feminization (GSI, sex ratio)	90 d	NOEC	0.1	2	Ref. 81
Fish	*Pomatoschistus minutus*	reproduction	240 d	NOEC	0.097	2	Ref. 62
Fish	*Thymallus thymallus*	Sperm volume, motility of sperm	50 d	LOEC	≥0.001	2	Ref. 48

Acute effects have been considered of no relevance and therefore no MAC-EQS has been derived.

Chronic toxicity data for 17β-estradiol is available for a range of species including algae, crustaceans, rotifers, amphibians and fish. It is concluded that the critical effect due to exposure of 17β-estradiol and its primary metabolites estrone and estriol is the induction of vitellogenin in fish that may cause a change in sex from male to female.

In order to apply the SSD (Species Sensitivity Distribution) approach the available dataset should preferably contain more than 15, but at least 10 NOECs/EC10s from different species covering at least 8 taxonomic groups. For estimating an AA-EQS freshwater using the SSD approach the following taxa would normally need to be represented, i.e.

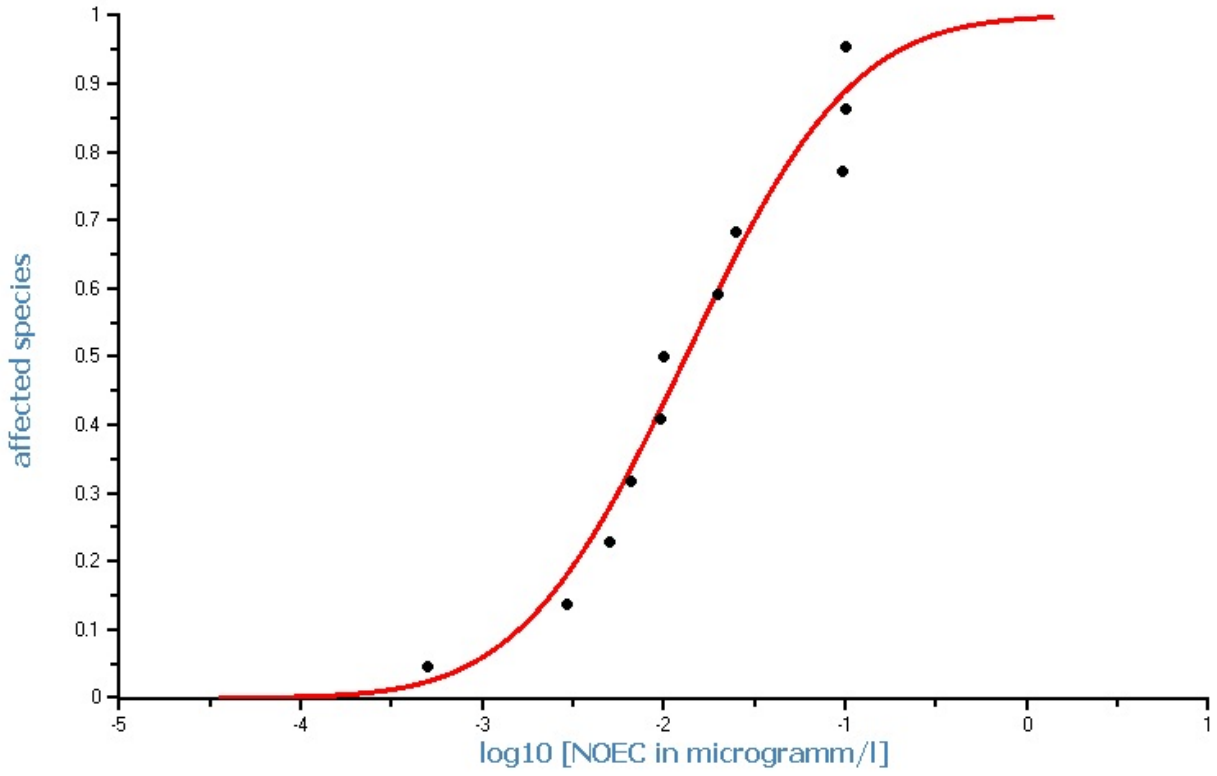
- a fish species
- a second family in the phylum Chordata
- a crustacean
- an insect
- a family in a phylum other than Arthropoda or Chordata
- a family in any order of insect or any phylum not represented
- algae
- a higher plant

The available chronic toxicity dataset for 17β-estradiol does not meet the data requirements for using the SSD approach. However, 17β-estradiol is a naturally occurring hormone and has a specific mode of action with effects on the reproductive physiology of vertebrates. The EU guidance notes that if a chemical is known to have a specific mode of action an SSD can be derived for only those taxa that are expected to be particularly sensitive.

Knowledge of the mode of action of 17β-estradiol suggests that fish and amphibians are likely to be the most sensitive organisms. This is supported by the available chronic toxicity data which indicates that fish are particularly sensitive to 17β-estradiol. Two studies were located on amphibians with LOECs in the range of 1000-2740ng/l reported for Rana pipens and Xenopus laevis. It is therefore proposed that an SSD is derived for β -estradiol based on data for the most sensitive taxonomic groups. It is expected that based on knowledge of the mode of action the chronic fish data the derivation of an SSD based on fish species only should be protective of other less sensitive group.

Reliable chronic NOEC values were available for 11 species of fish. An SSD has therefore been derived based on 11 fish species. For several species a number of different studies have been reported. The EU guidance on the derivation of an SSD indicates that where a number of data points are available for a species a geometric mean should be calculated to propose a single value for a species. This approach is not appropriate for all the available data as the studies are often non-standard and consider a range of endpoints and exposure durations and are therefore not directly comparable. In these cases, the lowest NOEC value is used for a species.

The SSD based on the fish data is shown below. The distribution fit to a log normal distribution.



The HC5 from the above SSD is 0.8 ng/l. An assessment factor in the range of 1-5 should be applied to the HC5 based on the guidance given in the TGD-EQS (E.C., 2011). Based on the available dataset and the knowledge of the mode of action it is considered that an assessment factor of 2 (mode of toxic action is well understood, HC5 has been derived based on data for the most sensitive taxonomic group, a wide range of endpoints and durations including population relevant endpoints such as hatching, fertilisation, changes in sex ratio are included in the dataset) is appropriate for the derivation of the AA-EQS.
This gives a EQS of 0.4 ng/l.

The derivation of the AA-EQS has been reviewed by SCHER (Ref. 8). Both the reliability and the ecological relevance of the endpoints and taxonomic groups have been considered. Overall, the SCHER supports the proposed AA-EQS of 0.4 ng/l.

Derivation of PNEC for estrone

Specie Group	Organism	Effect	Duration	End-Point	Value (µg/L)	KLIMISH Score	Reference
Short Term Data							

Algae	*Pseudokirchneriella subcapitata*	Growth (OECD 201)	72 h	EC50	>451	1	Ref. 71
Crustacean	*Acartia tonsa*	Mortality	48 h	NOEC	≥1000	2	Ref. 13
Crustacean	*Neomysis integer*	Mortality	96 h	LC50	>10000		Ref. 21
Copepoda	*Tisbe battagliai*	Mortality	10 d	LC50	≥100		Ref. 31
Echinoderm	*Strongylocentrotus purpuratus*	Development	96 h	EC50	6,4.4	2	Ref. 63
Long-term data							
Algae	*Pseudokirchneriella subcapitata*	Growth (OECD 201)	72 h	NOEC	≥451	2	Ref. 71
Crustacean	*Acartia tonsa*	Development	5 d	EC10	250	2	Ref. 13
Copepoda	*Tisbe battagliai*	Sex ratio; Re-production (method #1)	21 d	NOEC	≥100	2	Ref. 31
Fish	*Danio rerio*	Vitellogenin induction, sex ratio (OECD Draft Test Guideline: A 40-day Juvenile Zebrafish Assay for screening of Endocrine Disrupting Chemicals)	40 d	NOEC	0.036	2	Ref. 25
Fish	*Danio rerio*	Vitellogenin 1 mRNA; XPA mRNA; XPC mRNA	4 d	NOEC	0.1		Ref. 58
Fish	*Danio rerio*	Ovarian Somatic Index (OSI)	21 d	EC10	0.195	2	Ref. 83
Fish	*Danio rerio*	Vitellogenin induction	21 d	EC10	0.139	2	Ref. 83
Fish	*Oncorhynchus mykiss*	VTG-Induction (adult)	21 d	NOEC	0.048	2	Ref. 64
Fish			14 d	NOEC	0.0032	3	Ref. 77

	Oncorhynchus mykiss	VTG-Induction (adult)					
Fish	*Oryzias latipes*	Feminization		NOEC	0.1		Ref. 55
Fish	*Oryzias latipes*	Imposex, intersex conditions	- d	NOEC	<0.008		Ref. 55
Fish	*Oryzias latipes*	Hatch	15 d	NOEC	0.005		Ref. 49
Fish	*Oryzias latipes*	Vitellogenin 1 mRNA	90 d	NOEC	0.005		Ref. 49
Fish	*Oryzias javanicus*	Time to hatch		NOEC	0.198		Ref. 41
Fish	*Oryzias javanicus*	Number of eggs; number of fertilized eggs, time to hatch	239 d	NOEC	0.484		Ref. 41
Fish	*Pimephales promelas*	Vitellogenin induction (method #2)	21 d	NOEC	0.01	2	Ref. 60
Fish	*Pimephales promelas*	Egg production		NOEC	0.098		Ref. 80
Fish	*Pimephales promelas*	Hatch	4 d	NOEC	0.781		Ref. 80
Fish	*Pimephales promelas*	Organ weight in relationship to body weight; Sexual development; stage; Vacuolization	21 d	NOEC	0.054		Ref. 20
Fish	*Pimephales promelas*	Vitellogenin	4 d	NOEC	0.034		Ref. 80
Fish	*Pimephales promelas*	Vitellogenin	21 d	NOEC	0.054		Ref. 20
Fish	*Pimephales promelas*	Number of eggs	21 d	NOEC	0.307		Ref. 76
Fish	*Pimephales promelas*	Plasma vitellogenin	21 d	NOEC	0.00074		Ref. 77
Fish	*Salmo trutta*	Vitellogenin	10 d	NOEC	0.063		Ref. 21

Method#1: Newly released 24 h old species were exposed to the substance dissolved in sea water. Effects monitored in terms of survival, development and sex ratio after 10 days at 20^{o}C. Adult males and females were then paired and exposures continued to investigate effects on reproductive output after 21 days total exposure.
Method#2: The effects on the plasma vitellogenin level and gonadosomatic index of male fathead minnows (*Pimephales promelas*) was studied in a continuous flow exposure system for 21 days. All fish were acclimated to the test conditions for a period of 24 h before the start of the exposure.

Derivation of PNEC for estriol

Specie Group	Organism	Effect	Duration	End-Point	Value (µg/L)	KLIMISH Score	Reference
Short Term Data							
-	-						
Long-term data							
Fish	*Danio rerio*	Vitellogenin (method#1)	18 d	NOEC	0.3		Ref. 35
Fish	*Danio rerio*	Survival (method#1)	40 d	NOEC	21.7		Ref. 35
Fish	*Danio rerio*	Sex ratio (method#1)	40 d	NOEC	6.7		Ref. 35
Fish	*Oryzias latipes*	Abnormal(method#2)	15 d	NOEC	0.4622		Ref. 49
Fish	*Oryzias latipes*	Hatch (method#2)	15 d	NOEC	0.0465[1]		Ref. 49
Fish	*Oryzias latipes*	Sex ratio (method#2)	30 d	NOEC	4.517		Ref. 49
Fish	*Oryzias latipes*	Vitellogenin 1 mRNA; hatch; Organ weight in relationship to body weight (method#2)	90 d	NOEC	0.0465[1]		Ref. 49
Fish	*Oryzias latipes*	Estrogen receptor alpha mRNA; Organ weight in relationship to body weight (method#2)	90 d	NOEC	4.517		Ref. 49

[1]It was found that the Vtg gene in male medaka fish can be induced by estriol at environmentally relevant concentration of 5 ng/L. However, it was noted that the Vtg mRNA changes are hardly ever reflected in

concomitant changes in functional protein. Therefore, further studies were concluded to be needed to detect more sex hormone pathway gene expressions and functional protein levels to evaluate comprehensively estrogen potency of estriol in fish.

Method#1: A Fish Sexual Development Test (FSDT) (an extension of the existing OECD TG 210, fish early life stage toxicity test).
Method#2: Measurement of the impact of estriol on the embryonic development, sex differentiation, growth, and changes of functional genes related to reproduction of medaka (*O. latipes*) exposed to different concentrations of estriol during embryo-larval-, juvenile- and adult life stages. The corresponding time to hatching, hatchability, gross abnormalities, sex ratio, hepatosomatic index (HSI), gonadosomatic index (GSI), and changes of Vtg-I and ERα genes in livers of the fish exposed to estriol for 90 days were determined. Embryos less than 4 h post-fertilization were used in the exposure experiments. The embryos were exposed to nominal estriol concentrations of 5, 50, 500 and 5000 ng/L in charcoal-dechlorinated tap water for 15 days. Each exposure level had 3 replicate test concentrations with 30 embryos per replicate. In addition, solvent controls (SC) were included in the experimental design. The embryos in each group were placed in a glass dish and incubated on a 16:8 h light: dark photoperiod cycle at 25 ± 1 °C. Eighty percent of the test solution was renewed every 24 h. Hatchability, time to hatching and gross abnormalities were recorded. Once hatched, the hatched fry were continuously maintained at the same concentrations for the additional 15 days. After the additional 15 days of exposure, the genetic sex ratio was determined. Ten fish including five females and five males were assigned randomly to a 5-L glass aquarium and duplicate aquaria were used at each exposure level. Fish were continuously exposed to nominal estriol concentrations of 5, 50, 500, and 5000 ng/L and the SC was included in the experiment design. The solution was renewed every 24 h. Treated and control fish were exposed for another 60 days. The entire test duration was 90 days.

Noretisteron

Miljörisk: Användning av noretisteron har bedömts medföra medelhög risk för miljöpåverkan.
Nedbrytning: Noretisteron är potentiellt persistent.
Bioackumulering: Noretisteron har låg potential att bioackumuleras.

Detaljerad miljöinformation

Environmental risk assessment of norethisterone acetate (NETA) in pharmaceutical products marketed in Sweden in 2024

This document includes environmental risk assessment of norethisterone acetate (NETA) in pharmaceutical products marketed in Sweden in 2024. The risk assessment is performed in accordance with the FASS.se guidelines on environmental classification of pharmaceuticals (ref. 1).

1. Norethisterone acetate (NETA)

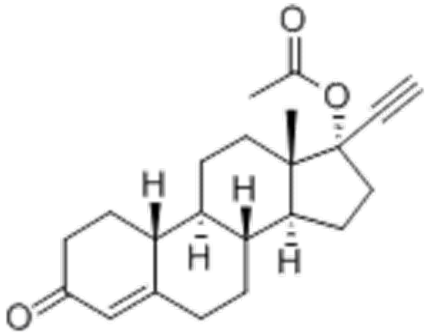
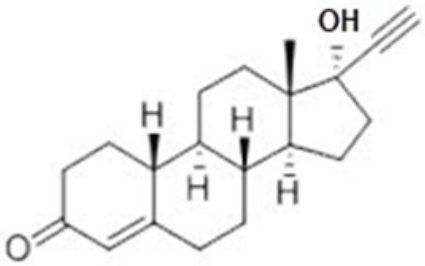
- **Environmental risk:** The risk quotient (PEC/PNEC) for NETA was calculated at 4.7.
- **Degradation:** NETA is potentially persistent in the environment.
- **Bioaccumulation:** NETA has low potential for bioaccumulation.
- **PBT/vPvB assessment:** NETA does not meet the criteria for classification as a PBT or vPvB substance.

Based on the available test data the following environmental risk phrase should be applied to pharmaceutical products containing NETA according to the criteria in ref. 1:

”Use of Norethisterone (acetate) has been considered to result in moderate environmental risk”.

1.1. The active pharmaceutical ingredient

Norethisterone acetate (NETA), also known as norethindrone acetate, is a steroidal progestin that is used as a hormonal contraceptive. It is an acetate ester of norethisterone (NET, CAS no. 68-22-4) which belongs to the class of steroid hormones. As NETA is completely and rapidly deacetylated to NET after oral administration, it is considered very reasonable to assume that the environmental toxicity of NETA can well be assessed by using environmental toxicity data on NET, possibly adjusting for the differences in molar masses by multiplying the effect concentration of NET with 1.14 (molar mass ratio).

Chemical name	Norethisterone Acetate (NETA)	Norethisterone (NET), Norethindrone
CAS no.	51-98-9	68-22-4
Molecular structure		
Molecular formula	$C_{22}H_{28}O_3$	$C_{20}H_{26}O_2$
Molecular weight	340.46 g/mol	299.43 g/mol
Water solubility	4.4 mg/L at 20ºC	5.6 mg/L at 25ºC

2. Environmental Risk Assessment (ERA)

2.1. Predicted Environmental Concentration (PEC)

According to ref. 1, PEC (Predicted Environmental Concentration) in surface water is calculated according to the following formula:

$PEC\ (\mu g/L) = (A*10^9*(100-R))/(365*P*V*D*100) = 1.37*10^{-6}*A*(100-R)$

$PEC_{Surface\ water} = 0.0022\ \mu g/L$

where:

- A = 16.04 kg (total amount of API, including norethisterone (0.5587 kg) and norethisterone acetate (15.4839 kg), sold in Sweden in year 2023, data from IQVIA and provided by LIF). Reduction of A may be justified based on metabolism data.
- R = 0 % removal rate (due to loss by adsorption to sludge particles, by volatilization, hydrolysis or biodegradation). R = 0 if no data is available.
- P = number of inhabitants in Sweden = $10*10^6$
- V (L/day) = volume of wastewater per capital and day = 200 (ECHA default) (Ref. 9)
- D = factor for dilution of wastewater by surface water flow = 10 (ECHA default) (Ref. 9)

Due to lack of data, the calculation of PEC of NETA in surface water is based on the following assumptions:

- no metabolism in the body, even though it is recognised that NETA is primarily excreted as metabolites (see section 5). However, no environmental toxicity data are available for the metabolites, thus the metabolites are assumed equally environmental toxic as NETA.
- no removal in wastewater treatment plants.

2.2. Predicted No Effect Concentration (PNEC)

2.2.1 *Ecotoxicological studies*

Algae (Desmodesmus subspicatus) (Ref. 4):

Acute toxicity

EC_{50} (growth inhibition) = 0.4 mg NETA/L biomass; 0.6 mg NETA/L growth rate (OECD 201)

Chronic toxicity

No data available.

Since EC_{50} < 1 mg/L, NETA is considered to be very toxic to the green algae *Desmodesmus subspicatus*.

Crustacean (Daphnia Magna) (Ref. 2 and 3):

Acute toxicity

EC_{50} 48h (immobilisation) = 4.4 - 4.6 mg NETA/L (OECD 202)

Chronic toxicity

Chronic toxicity of NET was assessed in a semi-static test according to the standard protocol for *Daphnia magna* reproduction test (OECD 211), ref. 14. Daphnids were exposed to three different concentrations of NET: 20, 100 and 500 ppb during 25 days (standard duration 21 days). During the chronic toxicity test, the green algae *Scenedesmus sp.* was supplied with the concentration of $5x10^4$ cells/ml every second day. The number of offspring, reproduction frequency, number of moltings, sex ratio of offspring, and presence of a resting egg were checked as endpoints. No deviations from the controls were observed for the included endpoints at the highest test concentration. Thus, the NOEC was determined at > 500 µg NET/L = (>0.5 mg NET/L).

Since 1 mg/L < EC_{50} ≤ 100 mg NETA/L in the acute toxicity text, NETA is considered to be moderately acute toxic to crustaceans.

Fish:

Acute toxicity:

The DK QSAR database, ref. 15, predicted acute toxicity for NETA: LC50 (Fathead minnow, 96hr): 1.03 mg NETA/L.

This predicted LC50 is the average of two QSAR model predictions: Leadscope (1.02 mg NETA/L) and SciQSAR (1.03 mg NETA/L). Thus, the two models predict very comparable LC50 values.

Chronic toxicity

The below table summarizes identified studies on the chronic toxicity of NET/NETA to fish. All identified studies are carried out for NET. The lowest NOEC is identified at 0.0041 µg NET/L (measured) corresponding to for the 28-days reproductive fish study on effects on fish egg production.

Substance	Effects	Result	Specie	Method	Reference
NET	Survival and growth	NOEC (survival): 1.5 µg NET/L	Fathead minnow	Not a guideline study	11

Substance	Effects	Result	Specie	Method	Reference
		NOEC (growth): 0.37 µg NET/L LC50: >14.8 µg NET/L Based on measured concentrations.		Early Life-Stage Toxicity study Survival and growth were used to assess chronic toxicity in a 28 days post hatch test Nominal test concentrations: 10, 1, 0.5, 0.25, and 0.125 µg/L	
NET	ED	NOEC (egg production): 0.0041 µg NET/L (measured), 0.005 µg NET/L (nominal)	Japanese medaka	Not a guideline study Short-term reproductive test over 28 days (semi-static with daily renewal). 42 reproducing fish pairs were selected after a 14 days preexposure period and used in test. The fish pairs were assigned into one of seven exposure concentrations: 1, 5, 25, 125, 625 ng/L NET. Fecundity was monitored daily.	12
NET	ED	NOEC (egg production): <0.0012 µg NET/L (no significant effects were found at 10 ng NET/L, however significant effects were observed at 1 ng NET/L). This makes the	Fathead minnow	Not a guideline study The test took place in sets of tanks - each containing one male and one female fish The experiment consisted of a 21-day pre-exposure period, a 3-day	12

Substance	Effects	Result	Specie	Method	Reference
		interpretation of the study results uncertain, and the study is not included in the PNEC-derivation. NOEC (masculinization of female fish): <0.0012 µg NET/L based on measured concentrations.		transition (when dosing of NET was started to ensure tanks were at steady state), and a further 21 days of exposure to NET. Test concentrations were 1, 10, 100 ng NET/L (6 pairs of fish for each test concentration). Studied effects: spawning and secondary sexual characteristics were also noted, including tubercle (presence/absence) and dorsal fin spot (presence/absence)	
NET	ED	NOEC (plasma, thyroxine): 0.007 - 0.084 µg NET/L NOEC (brain, thyrotropin and corticotropin releasing factor): 0.084 µg NET/L NOEC (brain, thyroid stimulating hormone) 0.007 µg NET/L NOEC (brain, disruption of HPT-axis related genes): 0.007 – 0.81 µg NET/L based on measured concentrations.	*Zebrafish (Danio rerio*)	Adult zebrafish (5 months old) were randomly selected and exposed to solvent control and three nominal concentrations of NET (10, 100 and 1000 ng/L) for 90 days. Each treatment concentration had three replicate tanks, with 8 females and 8 males in each tank. Plasma from pooled blood samples	13

Substance	Effects	Result	Specie	Method	Reference
				from the tail vein from 8 females and 8 males in each replicate was extracted for the determination of thyroid hormone concentrations. The brain and head (containing thyroid follicle, but without brain tissue) from 5 females and 5 males in each replicate were pooled and preserved for subsequent transcriptional analysis.	

Bacteria (Pseudomonas putida) (Ref. 5):
Acute toxicity:
EC_{50} (growth inhibition) = no inhibition at saturated concentration (ca. 7.8 mg NETA/L) (Schering method no. TX.ME.572.3 and DIN 38412 L8, March 1991)

Chronic toxicity
No data available.

The acute toxicity studies showed high acute toxicity of NETA/NET to algae and fish and medium toxicity to crustaceans.

No NOEC for algae is available. As NETA/NET is a hormone, fish is expected to be the most sensitive taxonomic group, which also available data for chronic toxicity indicate. The lowest NOEC for fish is identified at 0.0041 µg NET/L (egg production), which indeed is several factors lower than the NOEC of 0.5 mg NET/L for *Daphnia magna*.

The regulatory default standard AF of 10 was used for the derivation of PNEC, which is applicable when there are chronic aquatic toxicity studies representing the three trophic levels (algae, crustaceans, and fish).

PNEC = 0.0041 µg NET/L×1.14/10 = 0.00047 µg NETA/L.

2.3. Environmental risk classification (PEC/PNEC ratio)
The risk quotient PEC/PNEC was calculated with 0.0022 µg/L / 0.00047 µg/L = 4.7.

Justification of chosen environmental risk phrase:

A risk quotient between 1 and 10 qualifies for the phrase “Use of Norethisterone (acetate) has been considered to result in moderate environmental risk”.

3. Degradation

3.1. Biotic degradation

Ready biodegradability:
Test results in <10 % degradation in 28 days under “modified Sturm test” (OECD 301b) (ref. 6 and 7).

Inherent degradability:
No data available.

Simulation studies:
No data available.

3.2. Abiotic degradation

Hydrolysis:
No data available.

Photolysis:
No data available.

Since less than 10 % was degraded in the biodegradation test, NETA is not readily biodegradable. It cannot be excluded that NETA is potentially persistent in the aquatic environment according to ref. 1.

4. Bioaccumulation

According to the FASS.se guidelines (Ref. 1), substances with Log Pow ≥ 4 or BCF ≥ 500 are considered to have high potential for bioaccumulation. Valid BCF-data has prevalence above log Pow data. One limitation in the use of log Pow for the estimation of the bioaccumulation potential is that metabolism within the test organism is not considered.

The following data on bioaccumulation are retrieved from the literature and calculations:

Substance	Parameter	Result	Specie	Method	Reference
NETA	**Log Pow**	**3.7**	**-**	**Measured**	**8**
NET	Log Pow	2.7	-	Measured, OECD Guideline 117	16
NET	BCF	**Muscle tissue** BCF_k: 7.1 BCF_p: 4.5 Lipid normalized: 186 **Brain tissue** BCF_k: 7.4 BCF_p: 4.9 Lipid normalized: 40 **Gill tissue**	*Channel Catfish (Ictalurus punctatus*)	Measured, flow-through, 7 d uptake period, depuration period 1 week – both shorter than the OECD 305 recommended durations of 28 days uptake dura tion and 14 days depuration durati	10

Substance	Parameter	Result	Specie	Method	Reference
NETA	**Log Pow**	**3.7**	**-**	**Measured**	**8**
		BCF_k: 11 BCF_p: 7.5 Lipid normalized: 74 **Plasma tissue** BCF_k: 13 BCF_p: 11 **Liver tissue** BCF_k: 41 BCF_p: 25 Lipid normalized: 252		on. NAT concentration 100 µg/L at which no effects from NAT was observed. Initial fish loading rate: approx. 25 g fish per L, which is above the OECD 305 recommended loading range of 0.1 – 1 g fish/L. Concentrations measured in both muscle, brain, gill, plasma and liver cells.	
NET	BCF	**Muscle tissue** BCF_k: 2.6 BCF_p: 4.7 **Kidney tissue** BCF_k: 27 BCF_p: 7.5 **Liver tissue** BCF_k: 9.3 BCF_p: 16	*Fathead minnow (Pimephales promelas*)	Measured, flow-through, 28 d uptake period, depuration period 14 days – in agreement with the OECD 305 recommended durationa. NAT concentration 50 µg/L at which no effects from NAT was observed. Initial fish loading rate: approx. 4 g fish per L, which is above the OECD 305 recommended loading range of 0.1 – 1 g fish/L. Concentrations measured in both muscle, brain, gill, plasma and liver cells.	10

Bioconcentration factor (BCF):

No data on measured BCF is found for NETA but for NET, where the BCF for NET has been measured in different tissues in fathead minnow and channel catfish. As NETA is completely and rapidly deacetylated to NET after oral administration, and as NET has a very low measured BCF below 500 of it is considered acceptable to conclude NETA has a low potential for bioaccumulation.

Partitioning coefficient:
The octanol/water coefficient for NETA has been determined to $LogP_{ow}$ = 3.7 (ref. 8).

Since $LogP_{ow}$ < 4 and since the BCF most likely is below 500, NETA is assessed to have a low potential for bioaccumulation according to ref. 1.

5. Excretion

NET/NETA undergoes extensive biotransformation, primarily via reduction, followed by sulfate and glucuronide conjugation. The majority of metabolites in the circulation are approximately equal amounts of sulfates and glucoronides sulfates.

6. PBT and vPvB assessment

Considering all three PBT aspects stated in EU REACH criteria, NETA does not meet the criteria as a PBT or vPvB substance (Ref. 9).

7. References

1. Environmental classification of pharmaceuticals at www.fass.se – Guidance for pharmaceutical companies 2012 v3.0.
2. Research report from Schering, no. X211: Acute immobilization test of norethisterone with *Daphnia magna*, 02 May 1997.
3. Research report from Schering, no. X224 - draft: Acute immobilization test of norethisterone acetate (ZK 5422) with *Daphnia magna*, 23 June 1997.
4. Research report from Schering, no. A08345: Growth inhibition test of norethisterone acetate (ZK 5422) on the green algae *Desmodesmus subspicatus*, 20 January 2004.
5. Research report from Schering, no. X126: Growth inhibition test of norethisterone on the bacterium *Pseudomonas putida*, 12. aug. 1996
6. Research report from Schering, no. X128: Study on the biodegradability of norethisterone in the CO_2 -evolution test (modified Sturm-test), 12 Aug. 1996
7. Research report from Schering, no. X308 – Draft: Study on the biodegradability of norethisterone acetate in the CO_2-evolution test (modified Sturm test), 17 May 1999.
8. Report from Schering, LJ03.
9. ECHA, European Chemicals Agency. 2008 Guidance on information requirements and chemical safety assessment. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm
10. Nallani Gopinath C., Peter M. Paulos, Barney J. Venables, Regina E. Edziyie, Lisa A. Constantine and Duane B. Huggett (2012): Tissue-Specific Uptake and Bioconcentration of the Oral Contraceptive Norethindrone in Two Freshwater Fishes. Arch. Environ. Contam. Toxicol. (2012) 62:306–313. DOI 10.1007/s00244-011-9691-x.
11. Overturf M. D. , C. L. Overturf, D. Baxter, D. N. Hala, L. Constantine, B. Venables and D. B. Huggett (2011): Early Life-Stage Toxicity of Eight Pharmaceuticals to the Fathead Minnow, *Pimephales promelas*. Arch Environ Contam Toxicol (2012) 62:455–464.

12. Paulos P, Runnalls TJ, Nallani G, La Point T, Scott AP, Sumpter JP, Huggett DB. (2010). Reproductive responses in fathead minnow and Japanese medaka following exposure to a synthetic progestin, norethindrone. Aquat Toxicol 99:256–262.
13. Liang Yan-Qiu, Wenqiang Xu, Xingyi Liang, Zhanxin Jing, Chang-Gui Pan and Fei Tian (2020): The synthetic progestin norethindrone causes thyroid endocrine disruption in adult zebrafish. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, Volume 236, October 2020, 108819.
14. Goto,T., and J. Hiromi (2003): Toxicity of 17alpha-Ethynylestradiol and Norethindrone, Constituents of an Oral Contraceptive Pill to the Swimming and Reproduction of Cladoceran Daphnia magna, with Special Reference to Their Synergetic Effect. Mar. Pollut. Bull.47(1-6): 139-142.
15. Danish (Q)SAR database.https://qsardb.food.dtu.dk/db/index.html
16. REACH registration dossier for Norethisterone. https://echa.europa.eu/sv/information-on-chemicals/registered-substances/-/disreg/substance/external/10