FASS

Activelle®

Novo Nordisk

Filmdragerad tablett 1 mg/0,5 mg
(vit, rund, 6 mm, märkt NOVO 288)

Östrogen och gestagen, kombinationspreparat - systemisk effekt

Aktiva substanser:
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 hög potential att bioackumuleras.

Detaljerad miljöinformation

Environmental risk assessment of estrogens in pharmaceutical products marketed by Novo Nordisk in Sweden in 2016

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

1. The active pharmaceutical ingredients (API)

17β-estradiol is metabolized during human metabolism into the major transformation products estrone, estriol, estrone sulfate and estrone glucoronide (Ref. 28, 45, 60).

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

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

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

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

2. Calculation of the risk quotient (PEC/PNEC)

2.1 Sold amount in Sweden

The total amount of estradiol (hemihydrate and valerat) sold in Sweden in 2016 was 38.0 kg API based on Quintiles IMS/LIF Health sales data.

2.2 Calculation of PEC in surface water

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

$PEC = 1.5*10^{-6}*A*(100-R)$ µg/L, where

A = Total amount of API (kg) sold in Sweden in a given year. 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 38.0 kg = 12.54 kg
- Estrone: 54% of 38.0 kg = 20.52 kg
- Estriol: 13% of 38.0 kg = 4.94 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 waste water treatment plant in accordance with the principles of the EU TGD 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 waste water 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 feces. Adler et al. (ref. 9) 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 waste water 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. 9) 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.

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

- PEC for 17β-estradiol: $1.5 * 10^{-6} * 12.54 * (100-40) = 0.0011$ µg/L
- PEC for estrone: $1.5 * 10^{-6} * 20.52 * (100-8) = 0.0028$ µg/L
- PEC for estriol: $1.5 * 10^{-6} * 4.94 * (100) = 0.00074$ µg/L

2.3 Calculation of PNEC in surface water

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

2.3.1 17β-estradiol

A proposed EQS (PNEC) value has been derived for the substance (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 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. 45) 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 were 6^{o}C 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

2.3.2 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. 56).

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.

2.3.3 Estriol

As for 17β-estradiol and estrone, the mode of action for estriole 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. 46; 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.

2.3.4 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

2.4 Calculation of the risk quotient (PEC/PNEC)

The following risk quotient PEC/PNEC can be calculated:

- PEC/PNEC for 17β-estradiol: 0.0011/0.0004 = 2.75
- PEC/PNEC for estrone: 0.0028/0.0005 = 5.6
- PEC/PNEC for estriol: 0.00073/0.0047 = 0.15

The total risk quotient for 17β-estradiol, estrone and estriol is thus 8.5.

3. Information about degradation, bioaccumulation and PBT/vPvB

3.1. 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. 27). 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 (freswater) was found (ref. 78). Thus, 17β-estradiol is found to be biodegradable in both marine and fresh water. In addition, an activated sludge tests (OECD 302, ref. 2) show that 17β-estradiol is inherently biodegradable under aerobic conditions. Therefore, 17β-estradiol and its metabolites are assessed not to fulfil the criteria of persistence in the aquatic environment.

3.2. Bioaccumulation of 17β-estradiol, estrone and estriol

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. 75**
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 waste water outlet at the secondary sedimentation tank (for up to 141 days). Concentrations in waste water and fish were measured.**	**Ref. 50**
17β-estradiol (E2)	**BCF**	**174**	**Male fathead minnow, plasma**	**Method: no standard**	**Ref. 44**

				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.	
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) and a control was applied in the uptake study. No BCF	Ref. 64

				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. 79
Estrone (E1)	Log Pow	3.43	n-octanol	Calculation	Ref. 75
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 waste water outlet at the secondary sedimentation tank (for up to 141 days). Concentrations in waste water and fish were measured.	Ref. 50
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 lower concentration of E1 (40mg/L) and uptake in the D. magna and	Ref. 35

				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. 79
Estriol (E3)	Log Pow	2.81	n-octanol	Calculation	Ref. 75
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. 79

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. 30, 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. 30, 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.

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

3.3. PBT/vPvB assessment

Persistence:
In a 100 days simulation study of 17β-estradiol (OECD Test Method no. 308) with a nominal concentrations 0.36 µg/L of unlabelled and 1.1 µg/L ^{14}C-labelled E2, an aerobic mineralisation (marine) of 61±1% respectively 62±3% mineralisation (freswater) was found (ref. 78). Thus, 17β-estradiol is found to be biodegradable in both marine and fresh water. In addition, an activated sludge tests (OECD 302, ref. 2) show that 17β-estradiol is inherently biodegradable under aerobic conditions. Therefore, 17β-estradiol and its metabolites are assessed not to fulfil the criteria of persistence in the aquatic environment.

Bioaccumulation:
In addition, it was concluded in section 4.2 that neither 17β-estradiol nor its metabolites are assessed to have a high potential for bioaccumulation.

Environmental Toxicity:
According to ref. 1, the "T" criteria for toxicity is: "Chronic NOEC < 0.01 µg/L"

The overall critical environmental toxicological effect from exposure to 17β-estradiol, estrone and estriol is the potential to affect population sustainability, e.g. reproductive output, hatching and fertilisation success. The induction of vitellogenin in fish – which may cause a change in sex from male to female - is an indicator of this effect.

The lowest effect concentration for 17β-estradiol is a 35-50 d NOEC of 0.5 ng/l (ref. 45) for the trout *Onchorhynchus mykiss*. This chronic NOEC for 17β-estradiol is significantly lower than the T-criteria. 17β-estradiol is thus regarded as toxic to aquatic organisms.

The NOEC for estrone for induction of vitellogenin and sex ratio for *Danio rerio* is measured at 36 ng/L, which is above the "T" criteria for toxicity.

The NOEC for estriol for induction of vitellogenin and sex ratio for *Oryzias latipes* is measured at 47 ng/L, which is above the "T" criteria for toxicity.

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

4. Environmental risk classification of estrogens

In conclusion:

- The total risk quotient for 17β-estradiol, estrone and estriol is 8.5.
- 17β-estradiol, estrone and estriol do not meet the criteria for classification as a PBT or vPvB substance.

Based on the calculated risk quotients 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.

5. References

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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. 23
Estrone	ISO 9509	62,5–1.000 µg/L 2 hrs	Inhibition of nitrification rate	> 172 µg/L	Ref. 24

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 (freswater)	Ref. 78
17β-estradiol	OECD Test Method no. 301D: “Closed Bottle Test”	1.64 mg/L 28 days	3.5-9.8 % of ThoD	Ref. 26
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. 27

* 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. 25

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. 61
Invertebrate	*Acartia tonsa*	Mortality	48 h	EC50	>1000	2	Ref. 10
Fish	*Cyprinus carpio*	VTG induction in hepatocytes	3 d	EC50	24.52	2	Ref. 62

Fish	*Oncorhynchus mykiss*	Mortality	96 h	LC50	>500	1	Ref. 60
Fish	*Oncorhynchus mykiss*	VTG induction in hepatocytes	3 d	EC50	7.08	2	Ref. 62
Fish	*Oryzias latipes*	Egg and embryo mortality	72 h	LC50	460	2	Ref. 41
Fish	*Oryzias latipes*	Adult	72 h	LC50	3500	2	Ref. 41
Long-term data							
Algae	*Desmodesmus subspicatus*	Growth	72 h	NOEC	>3100	1	Ref. 61
Algae	*Pseudokirchneriella subcapitata*	Growth (OECD 201, GLP)	72 h	NOEC	>523	2	Ref. 77
Arthropoda	*Balanus amphrite*	larval colonization	2 d	NOEC	=0.1	2	Ref. 11
Invertebrate	*Acartia tonsa*	development	5 d	EC10	370	2	Ref. 10
Invertebrate	*Acartia tonsa*	development	5 d	EC50	720	2	Ref. 10
Invertebrate	*Acartia tonsa*	Reproduction GLP, Not a guideline study;	21 d	NOEC	>368	2	Ref. 13
Invertebrate	*Ceriodaphnia dubia*	reproduction	7 d	NOEC	=10000	2	Ref. 70
Copepoda	*Nitocra spinipes*	reproduction	18 d	NOEC	≥160	2	Ref. 14
Copepoda	*Tisbe battagliai*	reproduction	21 d	NOEC	≥100	2	Ref. 34
Amphibien	*Xenopus laevis*	feminization	84 d	LOEC	2.74	2	Ref. 42
Amphibien	*Rana pipiens*	Intersex	162 d	LOEC	≤1	2	Ref. 51
Fish	*Cyprinodon variegatus*	Proportion of viable eggs F1 and F2	280 d	LOEC	0.04	2	Ref. 16
Fish	*Cyprinodon variegatus*	Proportion of viable eggs F1 and F2	280 d	NOEC	0.01	2	Ref. 16
Fish	*Danio rerio*	altered gonadal histology, sex ratio	21 d	LOEC	0.1	2	Ref. 15

Fish	*Danio rerio*	altered gonadal histology, sex ratio	21 d	NOEC	0.025	2	Ref. 15
Fish	*Danio rerio*	altered gonadal histology, secondary sexual characteristics	21 d	NOEC	0.005	2	Ref. 15
Fish	*Danio rerio*	reproduction	200 d	NOEC	≤0.005	2	Ref. 53
Fish	*Danio rerio*	Egg number in the clutch and hatching	21 d	NOEC	0.087	2	Ref. 66
Fish	*Gabiocypris rarus*	sex ratio	21 d	LOEC	0.025	2	Ref. 48
Fish	*Gabiocypris rarus*	sex ratio	21 d	NOEC	0.005	2	Ref. 48
Fish	*Gambusia holbrooki*	reproductive success	84 d	LOEC	0.02	2	Ref. 28
Fish	*Gambusia holbrooki*	reproductive success	84 d	NOEC	0.1	2	Ref. 28
Fish	*Melanotaenia fluviatilis*	egg production	14 d	LOEC	0.3	2	Ref. 58
Fish	*Melanotaenia fluviatilis*	egg production	14 d	NOEC	0.1	2	Ref. 58
Fish	*Oncorhynchus mykiss*	Sperm volume, sperm density and fertilization success	35-50 d	LOEC	0.001	2	Ref. 45
Fish	*Oncorhynchus mykiss*	Sperm volume, sperm density and fertilization success	35-50 d	NOEC	0.0005	2	Ref. 45
Fish	*Oryzias javanicus*	Fertility of the eggs	187 d	LOEC	0.016	2	Ref. 37
Fish	*Oryzias javanicus*	Fertility of the eggs	187 d	NOEC	0.0095	2	Ref. 37
Fish	*Oryzias latipes*	Gender shift (testis-ova)	90 d	LOEC	0.1	2	Ref. 52
Fish	*Oryzias latipes*	Gender shift (testis-ova)	90 d	NOEC	0.01	2	Ref. 52

Fish	*Oryzias latipes*	total study	90 d	LOEC	0.004	3	Ref. 52
Fish	*Oryzias latipes*	total study	90 d	NOEC	0.0004	3	Ref. 52
Fish	*Oryzias latipes*	feminization	200-300 d	NOEC	0.1	2	Ref. 69
Fish	*Oryzias latipes*	reduced fertility	59 d	NOEC	0.0029	2	Ref. 66
Fish	*Oryzias latipes*	feminization	28 d	LOEC	≤0.01	2	Ref. 54
Fish	*Oryzias latipes*	number of eggs	14 d	NOEC	0.272	2	Ref. 68
Fish	*Oryzias latipes*	reduced fertility	21 d	NOEC	0.227	2	Ref. 40
Fish	*Oryzias latipes*	Hatching time	20 d	NOEC	0.034	2	Ref. 29
Fish	*Oryzias latipes*	various reproduction endpoints	14 d	NOEC	0.379	3	Ref. 39
Fish	*Pimephales promelas*	Feminization and weight gain	91 d	LOEC	0.0279	1	Ref. 60
Fish	*Pimephales promelas*	Feminization and weight gain	91 d	NOEC	>0.008	1	Ref. 60
Fish	*Pimephales promelas*	reduced egg production	19 d	EC10	0.0066	2	Ref. 43
Fish	*Pimephales promelas*	reproduction, reduced egg production	21 d	NOEC	0.044	3	Ref. 78
Fish	*Poecilia reticulata*	Feminization (GSI, sex ratio)	90 d	LOEC	0.5	2	Ref. 74
Fish	*Poecilia reticulata*	Feminization (GSI, sex ratio)	90 d	NOEC	0.1	2	Ref. 74
Fish	*Pomatoschistus minutus*	reproduction	240 d	NOEC	0.097	2	Ref. 59
Fish	*Thymallus thymallus*	Sperm volume, motility of sperm	50 d	LOEC	≥0.001	2	Ref. 45

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.

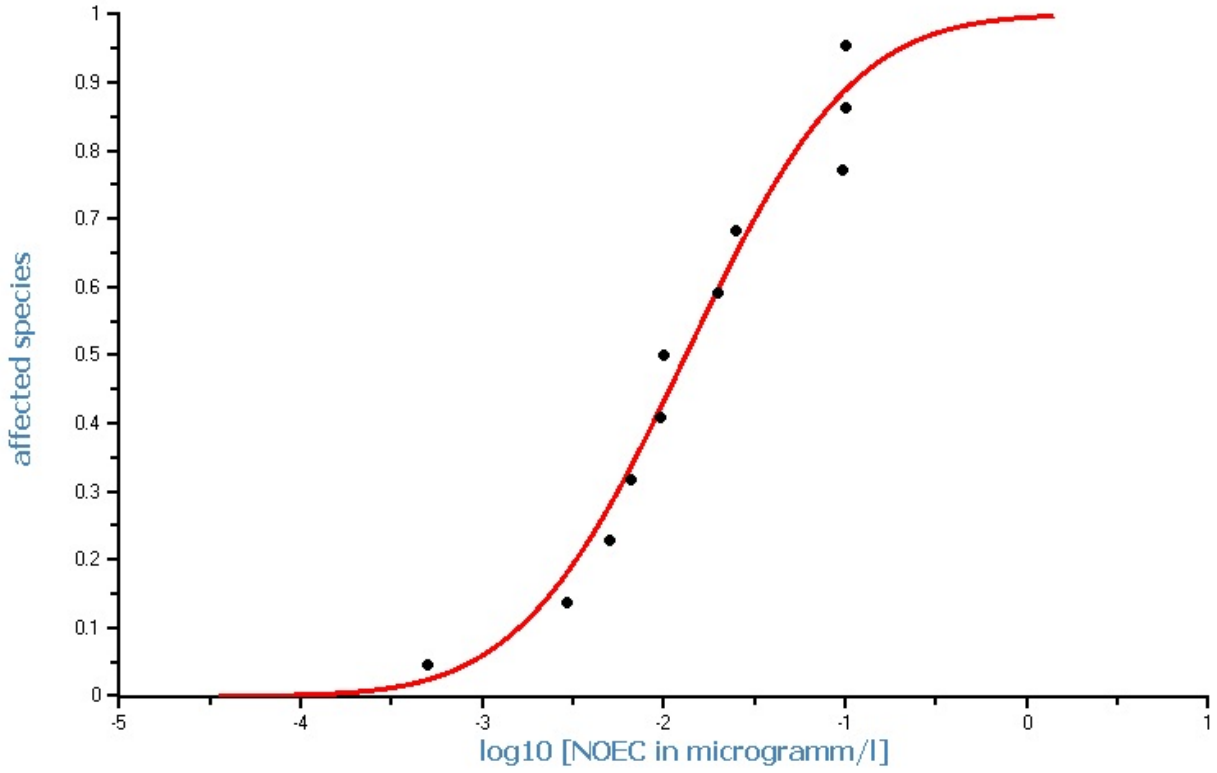
- a fish species
- a second family in the phylum Chordata
- a crustacean
- an insect
- a family in a phylum other than Arthropoda or Chordata
- a family in any order of insect or any phylum not represented
- algae
- a higher plant

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. 66
Crustacean	*Acartia tonsa*	Mortality	48 h	NOEC	≥1000	2	Ref. 10
Crustacean	*Neomysis integer*	Mortality	96 h	LC50	>10000		Ref. 18
Copepoda		Mortality	10 d	LC50	≥100		Ref. 28

	Tisbe battagliai						
Echinoderm	*Strongylocentrotus purpuratus*	Development	96 h	EC50	6,4.4	2	Ref. 60
Long-term data							
Algae	*Pseudokirchneriella subcapitata*	Growth (OECD 201)	72 h	NOEC	≥451	2	Ref. 66
Crustacean	*Acartia tonsa*	Development	5 d	EC10	250	2	Ref. 10
Copepoda	*Tisbe battagliai*	Sex ratio; Re-production (method #1)	21 d	NOEC	≥100	2	Ref. 28
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. 22
Fish	*Danio rerio*	Vitellogenin 1 mRNA; XPA mRNA; XPC mRNA	4 d	NOEC	0.1		Ref. 55
Fish	*Danio rerio*	Ovarian Somatic Index (OSI)	21 d	EC10	0.195	2	Ref. 77
Fish	*Danio rerio*	Vitellogenin induction	21 d	EC10	0.139	2	Ref. 77
Fish	*Oncorhynchus mykiss*	VTG-Induction (adult)	21 d	NOEC	0.048	2	Ref. 61
Fish	*Oncorhynchus mykiss*	VTG-Induction (adult)	14 d	NOEC	0.0032	3	Ref. 74
Fish	*Oryzias latipes*	Feminization		NOEC	0.1		Ref. 52
Fish	*Oryzias latipes*	Imposex, intersex conditions	- d	NOEC	<0.008		Ref. 52
Fish		Hatch	15 d	NOEC	0.005		Ref. 46

	Oryzias latipes						
Fish	*Oryzias latipes*	Vitellogenin 1 mRNA	90 d	NOEC	0.005		Ref. 46
Fish	*Oryzias javanicus*	Time to hatch		NOEC	0.198		Ref. 38
Fish	*Oryzias javanicus*	Number of eggs; number of fertilized eggs, time to hatch	239 d	NOEC	0.484		Ref. 38
Fish	*Pimephales promelas*	Vitellogenin induction (method #2)	21 d	NOEC	0.01	2	Ref. 57
Fish	*Pimephales promelas*	Egg production		NOEC	0.098		Ref. 73
Fish	*Pimephales promelas*	Hatch	4 d	NOEC	0.781		Ref. 73
Fish	*Pimephales promelas*	Organ weight in relationship to body weight; Sexual development; stage; Vacuolization	21 d	NOEC	0.054		Ref. 17
Fish	*Pimephales promelas*	Vitellogenin	4 d	NOEC	0.034		Ref. 73
Fish	*Pimephales promelas*	Vitellogenin	21 d	NOEC	0.054		Ref. 17
Fish	*Pimephales promelas*	Number of eggs	21 d	NOEC	0.307		Ref. 73
Fish	*Pimephales promelas*	Plasma vitellogenin	21 d	NOEC	0.00074		Ref. 74
Fish	*Salmo trutta*	Vitellogenin	10 d	NOEC	0.063		Ref. 18

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. 32
Fish	*Danio rerio*	Survival (method#1)	40 d	NOEC	21.7		Ref. 32
Fish	*Danio rerio*	Sex ratio (method#1)	40 d	NOEC	6.7		Ref. 32
Fish	*Oryzias latipes*	Abnormal(method#2)	15 d	NOEC	0.4622		Ref. 46
Fish	*Oryzias latipes*	Hatch (method#2)	15 d	NOEC	0.0465[1]		Ref. 46
Fish	*Oryzias latipes*	Sex ratio (method#2)	30 d	NOEC	4.517		Ref. 46
Fish	*Oryzias latipes*	Vitellogenin 1 mRNA; hatch; Organ weight in relationship to body weight (method#2)	90 d	NOEC	0.0465[1]		Ref. 46
Fish	*Oryzias latipes*	Estrogen receptor alpha mRNA; Organ weight in relationship to body weight (method#2)	90 d	NOEC	4.517		Ref. 46

[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: Risk för miljöpåverkan av noretisteron kan inte uteslutas då det inte finns tillräckliga ekotoxikologiska data.
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 2016

This document includes environmental risk assessment of norethisterone acetate (NETA) in pharmaceutical products marketed in Sweden in 2016. The risk assessment is performed in accordance with the FASS.se guidelines on environmental classification of pharmaceuticals (ref. 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 which belongs to the class of steroid hormones.

Chemical name **Norethisterone Acetate (NETA)**
CAS no. 51-98-9
Molecular formula $C_{22}H_{28}O_3$
Molecular weight 340.46 g/mol
Water solubility 4.4 mg/L at 20ºC

2. Eco-toxicological data

The following tests have been performed with norethisterone or norethisterone acetate (NETA):
• Immobilisation test on dapnia
• Growth inhibition test on green alga
• Growth inhibition test on bacteria

• Biodegradation
• Octanol/water coefficient

The tests are performed by Schering in accordance with OECD principles for good laboratory practice.

Immobilisation test on dapnia (ref 2 and 3):
Acute immobilization test with norethisterone and NETA on Daphnia magna, 48h:
• No effect was observed at saturated concentration. EC_{50} > 4.4 - 4.6 mg/L.

Method: OECD guidelines for testing of chemicals, no. 202: “Daphnia sp., Acute immobilisation test and reproduction test”.

Growth inhibition test on green alga (ref 4):
Growth inhibition test with NETA on the green alga Desmodesmus subspicatus:
• EC_{50} (biomass): 0.4 mg/L
• EC_{50} (growth rate): 0.6 mg/L

Method: OECD guidelines for testing of chemicals, no. 201: “Alga, growth inhibition test”

Growth inhibition test on bacteria (ref. 5):
Growth inhibition test with norethisterone on the bacterium Pseudomonas putida:
• No inhibition at saturated concentration (ca. 7.8 mg/l)

Method: Schering method no. TX.ME.572.3 and DIN 38412 L8, March 1991.

Biodegradation test (ref. 6 and 7):
Biodegradation test on norethisterone and NETA:
• norethisterone and NETA are not readily biodegradable under “modified Sturm test” conditions since less than 10% of the substance was biodegraded within 28 days.

Method: OECD guideline for testing of chemicals, Ready biodegradability: CO_2-evolution test, no 301B.

Octanol/water coefficient (ref. 8) Method Unknown:
The octanol/water coefficient for NETA has been determined to $LogP_{ow}$ = 3.7. Since $LogP_{ow}$ < 4 it indicates that NETA has low potential for bioaccumulation according to ref. 1.

Summary of test results:
The obtained test results indicate that norethisterone/NETA:

• is very toxic to the green alga Desmodesmus subspicatus (since EC_{50} < 1 mg/L)
• has no effect on immobilisation of Daphnia magna
• has no effect on the growth of the bacteria Pseudomonas putida
• is not readily biodegradable
• has low potential for bioaccumulation

3. Calculation of the risk quotient (PEC/PNEC)

3.1. Sold amount in Sweden
The total amount of norethisterone and norethisterone acetate (NETA) sold in Sweden in 2016 was 14.90 kg API based on QuintilesIMS/LIF Health sales data.

3.2. Calculation of PEC in surface water

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

$PEC = 1.5*10^{-6}*A*(100-R)$ µg/L, where

• A = Total amount of API (kg) sold in Sweden in a given year. Reduction of A may be justified based on metabolism data.
• R = Removal rate (%) due to loss by adsorption to sludge particles, by volatilization, hydrolysis or biodegradation. R = 0 if no data is available.

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

• no metabolism of in the body
• no removal in waste water treatment plants.

Without taking removal effects of metabolism and waste water treatment into consideration the following PEC of NETA in surface water can be calculated according to ref. 1:

• $PEC = 1.5*10^{-6}*14.90*(100-0) = 0.00224$ µg/L.

Since $PEC_{Surface\ water}$ is below the action limit 0.01 µg/L stated in ref. 1 it is unlikely that NETA constitutes a significant risk for the environment.

3.3. Calculation of PNEC in surface water

According to ref. 1, calculation of PNEC (Predicted No Effect Concentration) in surface water should be based on eco-toxicological data for three trophic levels. However, it has only been possible to present eco-toxicological data for two trophic levels i.e. green alga and daphnia. Furthermore, it is not known if these organisms are the most sensitive to NETA.

Consequently, it is not possible to calculate a valid PNEC according to the requirement in ref. 1 on basis of the available eco-toxicological data.

3.4. Calculation of the risk quotient (PEC/PNEC)

The risk quotient (PEC/PNEC) cannot be calculated for the reason stated in section 3.3.

4. PBT and vPvB assessment

Persistence:
NETA is not readily biodegradable under “modified Sturm test” conditions since less than 10% of the substance was biodegraded within 28 days (ref. 6 and 7).

According to ref. 1, it cannot be excluded that NETA is potentially persistent in the environment.

Bioaccumulation:
The Log P_{ow} for NETA is estimated to 3.7 (ref. 8).

Since Log $P_{ow} < 4$, it indicates that NETA has low potential for bioaccumulation (ref. 1).

Eco-toxicity:
The obtained results of the performed eco-toxicity tests indicate – as stated in section 2 - that norethisterone/NETA:

• is very toxic to the green alga Desmodesmus subspicatus (since EC_{50} < 1 mg/L)
• has no effect on the immobilisation of Daphnia magna
• has no effect on the growth of the bacteria Pseudomonas putida

Conclusion regarding PBT/vPvB properties:
Considering all three PBT-aspects, NETA does not meet the criteria for classification as a PBT or vPvB substance.

5. Environmental risk classification of NETA

In conclusion:
- A valid risk quotient (PEC/PNEC) for NETA cannot be calculated due to lack of eco-toxicity data.
- NETA:

- is potentially persistent in the environment
- has low potential for bioaccumulation
- is very toxic to green algae
- does not meet the criteria for classification as a PBT or vPvB substance

Since the PEC/PNEC cannot be calculated due to lack of eco-toxicity data the following environmental risk phrase should be applied to pharmaceutical products containing NETA according to the criteria in ref. 1:

"Risk of environmental impact of norethisterone acetate (NETA) cannot be excluded due to lack of eco-toxicity data".

6. References

1) Environmental classification of pharmaceuticals at www.fass.se – Guidance for pharmaceutical companies 2012.
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.