6.4. Results for pharmaceuticals for human use

The detailed results for the pharmaceuticals are summarised in Table 10. Data set I corresponds to Data set II and therefore only the results of data set II and III are given.
Evaluation of data set II (91 substances) revealed that fish was the most sensitive species for 13 (14.3%) substances, which would result in a reduction of 71.5% in the number of fish used. In the most restrictive data set III, for 56 out of the 68 substances acute algal EC50 and daphnid EC50 values were lower than or equal to the fish LC50 data meaning that in 12 (17.6%) cases fish was the most sensitive species. Based on the total number of fish used in acute LC50 testing (42 fish per test) versus the total number of fish calculated for the step-down approach (10 fish per step-down test), this leads to a 70.2% reduction in the number of fish used.
If one considers that LC50 results reported >100mg/L might be derived from a limit test, then the reduction in the number of fish used applying the step-down approach would be slightly lower, namely 67.7 and 68.0% for the data sets II and III, respectively.

Table 10: Results for pharmaceuticals for human use

	Data set II 91 substances	Data set III 68 substances
Endpoint selected to set the UTC [%]		
Algae (72hEC50)	46.3	60.7
Daphnia (48hEC50)	33.5	38.2
Algae equal to daphnia result	20.2	1.1
Most sensitive endpoint [%]		
Algae (72hEC50)	39.3	54.5
Daphnia (48hEC50)	23.2	29.6
Algae equal to daphnia result	1.1	0.7
Fish equal to daphnia/algae UTC result	21.5	0.5
Fish (96hLC50)	14.9	14.7
No. of step-down tests needed [No. of substances]		
1 step (threshold test)	76	54
2 steps	14	13
5 steps	1	1
Estimated numbers of fish used in acute toxicity test *LC50 test* LC50 and the limit test considered	 3,822 3,374	 2,856 2,660
Calculated numbers of fish used with the step-down approach	1,090	850
Estimated reduction of fish used [%] Step-down approach/LC50 test Step-down approach/LC50 and the limit test considered	 71.5 67.7	 70.2 68.0

7. Discussion and conclusions

The step-down approach was applied to 1,439 substances extracted from the NCD, 316 substances of the IUCLID database, 68 plant protection products and metabolites, and 91 pharmaceuticals for human use. Since not all of the results of the algae, daphnia and fish tests were given as precise values, we used more and more stringent selection criteria and consequently created three data sets for each group of substances, where the corresponding data sets I had the lowest and data sets III the highest precision.

Regarding the calculation of the achievable reduction, we assumed that the LC50 96h test was performed at five concentrations with seven fish per concentration and seven fish per control. We did not consider that up to 10 fish can be used per concentration or that additional fish might have been used for range finding testing preceding the definitive LC50 test.
In a second evaluation, we considered that the limit test (using 14 instead of 42 fish) might have been carried out for all of the substances with imprecise LC50 96h values >100 mg/L. This consideration mainly had an impact on data sets I, since these contain most of the imprecise values and the estimated reduction of number of fish used decreased (see Table 6).

The possible reductions for data sets II and III for all substances are quite similar and more realistic than the ones for data sets I, since we only evaluated substances for which at least the result of the daphnia or algae test was given as a precise value.

During the course of this study the question arose whether the dilution factor of 3.2 chosen by Hutchinson et al (2003) based on the experience at AstraZeneca is suitable or whether it would increase the uncertainty of the LC50 values compared to generally used dilution factor of 2.2.
Our calculations of the number of threshold/step-down tests needed are based on dilution factor 3.2 but we performed for the NCD an additional calculation using dilution factor 2.2. The total number of tests needed only slightly increases and the overall reduction of number of fish used has hardly been affected (Table 11).

Table 11: Comparison of the number of threshold/step-down tests needed with dilution factor 2.2 and dilution factor 3.2 – New Chemical Database and calculation of possible reduction (%) according to the figures given in Table 7

NCD	Data set I		Data set II		Data set III	
Dilution factor	**3.2**	**2.2**	**3.2**	**2.2**	**3.2**	**2.2**
No steps						
1	915	915	719	719	401	401
2	477	447	94	81	72	63
3	33	42	24	23	19	16
4	12	22	10	16	3	13
5	2	9	1	4	1	1
6	0	4	0	5	0	2
total no of tests	**2026**	**2092**	**1024**	**1064**	**619**	**644**
Numbers of animals: threshold/step-down approach						
10/test	20,260	20,920	10,240	10,640	6,190	6,440
14/test*	28,364	29,288	14,156	14,440	8,666	9,016
Numbers of animals current testing strategy						
LC50	60,438		35,616		20,832	
limit test considered	43,638		27,580		18,144	
Possible reduction (%)						
10/test vs LC50	66.5	65.4	71.2	70.1	70.3	69.1
10/test vs limit test considered	53.6	52.1	62.9	61.4	65.9	64.5
14/test vs LC50	53.1	51.5	60.3	59.5	58.4	56.7
14/test vs limit test considered	35.0	32.9	48.7	47.6	52.2	50.3

* = This calculation was also carried out assuming that 7 test and 7 control fish would be used in the threshold/step down tests (see below)

In addition, the question came up, how many fish should be used in the threshold test and subsequent step-down tests. Are the 5 test and 5 control fish (1 fish could die in the control) proposed by Hutchinson et al (2003) (which correspond to the range finding test) enough to achieve a significant confidence that the LC50 is greater than the concentration used in the threshold/step-down test? Table 12 shows that a significant confidence of 96.88% can only be achieved, if none of the test and control fish dies. In this context, it should be kept in mind that OECD TG 203; Annex V C.1 stipulates a confidence of at least 99% for the limit test, i.e. if 7 test and 7 control fish used, none of the fish should die.

Table 12: Percentage of confidence that LC50 is greater than the Upper Threshold Concentration (and subsequent step-down concentrations)

Group size	Confidence (%)		
	Mortality		
	0	1	2
4	*n.s. 93.75*	-	-
5	96.88	*n.s. (81.25)*	-
6	98.44	*n.s. (89.06)*	-
7	99.22	*n.s. (93.75)*	-
8	99.61	96.48	*n.s. (85.55)*
9	99.8	98.05	*n.s. (82.02)*
10	99.9	98.93	*n.s. (94.53)*

n.s. = not significant

Even if 7 test and 7 control fish would be used in the threshold (and subsequent step-down) tests our calculations (Table 13) still indicate a remarkable reduction.

Table 13: Summary of possible reduction (%) in the number of fish needed for new and existing chemicals, plant protection products and pharmaceuticals if 14 fish are used in step-down approach

		Data Set I	Data Set II	Data Set III
NCD	Number of substances	**1439**	**848**	**496**
	LC50	53.1 (58.4)	60.3	58.4
	Limit test considered	35.0 (42.3)	48.7	52.2
IUCLID	Number of substances	**316**	**269**	**206**
	LC50	53.1 (53.8)	52.4	53.9
	Limit test considered	46.3 (47.1)	47.9	51.0
PPP	Number of substances	**68**	**47**	**28**
	LC50	57.8 (61.8)	63.8	61.9
	Limit test considered	44.2 (49.4)	56.4	60.0
PHARM	Number of substances	-	**91**	**68**
	LC50	-	60.1	58.3
	Limit test considered	-	54.8	55.3

[a] *Values in the brackets correspond to results of evaluation when imprecise fish LC50 values above 100 mg/L (where UTC=LC50) are taken into consideration, assuming that one step-down test would be sufficient in those cases (see 5.4).*

A further option would be to perform a full LC50 test when fish mortality occurs in the threshold test at UTC concentration. This would correspond to the requirement for the limit test in OECD 203/Annex V C.1. where a full LC50 test should be performed when mortality is observed. It might be interesting in this context to discuss the necessity of a precise LC50 value for classification and labeling and risk assessment or whether the approach chosen for acute oral toxicity (Annex V B.1bis/B.1tris) might be applicable also for acute fish toxicity testing.

Another point of discussion was our assumption that if UTC < LC50 only the threshold test would be performed. Especially when the values are in the same range, our assumption might underestimate the number of necessary tests. It was not possible to investigate this in depth since we only had the LC50 values but no information on the steepness of the dose-response curve. Nevertheless, we re-evaluated NCD data set III and multiplied the UTC (and subsequent step-down concentration by factor 10) and only when LC50 > 10*UTC/step-down concentration the testing was stopped. This resulted in 1,191 tests instead of 619 (results are given in table 14). This is a very conservative approach and it should be kept in mind that even with 5 test and 5 control fish, the LC50 would be greater than UTC/step-down concentration with 96.88% confidence (Table 12) if no mortality occurs.

In addition, the reduction of number of fish used has been estimated for the NCD data set III using several hypotheses on the number of fish that could have been used according to the current requirements and the step-down approach (Table 14). The results show that for these hypotheses the achievable reduction in the number of fish lies between 8.1% and 79.5%.

If the step-down approach would become part of the regulations, it should be evaluated whether the application of humane endpoints (refinement), i.e. terminating the threshold/step-down tests when fish die or show severe clinical signs as proposed by Hutchinson et al (2003) or at least humanely kill moribund fish, could be implemented. In addition, it should be investigated whether the same group of control fish could be used for the needed series of step-down tests.

Our overall conclusion is that the step-down approach would lead to a considerable reduction in number of fish used for regulatory acute aquatic toxicity testing, retaining the same quality of information needed for the classification and labelling and risk assessment, thereby contributing to animal welfare and to reduction in time and costs.

Table 14: Possible reduction (%) in fish needed for NCD data set III based on 4 hypotheses concerning the number of fish used when substances were tested according to the current strategy and on 5 for the proposed testing strategy

Current strategy	Total number of fish used in current strategy↓	Step-down approach			Threshold test at UTC, if mortality occurs LC50 with 42 fish	
		10 fish/test (e)	14 fish/test (f)	14 fish/test + factor 10 for LC50 - UTC/steps comparison	10 fish/test (i)	14 fish/test (j)
Total number of fish used in proposed strategy→		6,190	8,666	16,674	8,950	10,934
LC50 – 42 fish (a)	20,832	70.3	58.4	20.0	57.0	47.5
limit test – 14 fish LC50 – 42 fish (b)	18,144	65.9	52.2	**8.1**	50.7	39.7
LC50 – 60 fish (c)	29,760	**79.2**	70.9	44.0	64.2	57.5
limit test – 14 fish LC50 – 60 fish (d)	25,344	75.6	65.8	34.2	57.9	50.1

To estimate the number of fish that could have been use according to the current requirements four hypotheses were made:

a) All of the substances were tested with the LC50 test using 42 fish
b) Substances where LC50>100 mg/L is reported were tested with the limit test using14 fish and the others were test with the LC50 test using 42 fish
c) All of the substances were tested with the LC50 test using 60 fish
d) Substances where LC50>100 mg/L is reported were tested with the limit test using14 fish and the others were test with the LC50 test using 60 fish.

To estimate the number of fish that could be used according to the step-down approach five hypotheses were made:

e) All of the substances were tested with the step-down approach using 10 fish/test
f) All of the substances were tested with the step-down approach using 14 fish/test
g) All of the substances were tested with the step-down approach using 14 fish/test + factor 10 for LC50- UTC/steps comparison
h) If mortality occurred in the threshold test (10 fish) at UTC, a LC50 test using 42 fish was performed
i) If mortality occurred in the threshold test (14 fish) at UTC, a LC50 test using 42 fish was performed

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Annex

The annex gives an overview on the data files going with the report, the abbreviations and colour codes used in these files:

1. Files

Extracted data = complete data set extracted from the database are included plus the selected data sets according to the selection criteria.
Data set I, II, III for database NCD, IUCLID and PPP
Data set II, III for database PHARM

For NCD additional evaluations were performed and are available in extra Workbooks (Table 11, 14)

colour codes are used in the extracted data files, see 3)

Reduction calculations = summaries of the calculations and achievable reductions are provided for NCD, IUCLID, PPP, PHARM for 10/14 fish per threshold/step-down test and for evaluation "a" (see below abbreviation and 5.4 and 5.5 of the report; not for PHARM). Several calculations (Table 14) were only performed for NCD.

2. Abbreviations:

Algae EbC50 72h, The result of standard algae test (OECD 201) reported as algae biomass also marked as "b" or "Ab" in Excel

Algae ErC50 72h, The result of standard algae test (OECD 201) reported as algae growth rate also marked as "r" or "Ar" in Excel

Daphnia EC50 48h, The result of standard Daphnia test (OECD 202) reported as immobilisation of Daphnia

ECB, European Chemicals Bureau

Evaluation "a", Evaluation, where the probability that one test instead of two would be enough in cases where the fish LC50 is an imprecise value greater than 100. This evaluation was only performed for datasets I, where signs (>, <) where not taken into consideration.

Factor 3.2, Dilution factor allowing the semilogaritmic step-down concentration series

Fish LC50 96h, The result of standard acute fish test (OECD 203) reported as lethality of fish

IUCLID, The International Uniform ChemicaL Information Database (existing chemicals)

Limit test, The fish test which is performed at one concentration using 7-10 test and 7-10 fish. This test is usually performed at 100 mg/L, when toxicity is not expected from all data available or at the saturation concentration when the substance is poorly soluble.

Most sensitive, The test species which is the most sensitive among all test species (the lowest E(L)C50 value).

NCD, New Chemicals Database

PPP, Plant Protection Products (active substances and metabolites are considered in this study)

Step1, Fish LC50 result is compared to the UTC. When fish LC50 is greater we assume that this concentration would not be toxic for fish. The test procedure is stopped. When fish

LC50 is lower than UTC the toxicity for fish at this concentration is expected. The test procedure continues at lower concentration (Step-down).

Step2, Step down concentration at which step-down threshold test is performed when toxicity was observed in Step1 test

Step3, Further, step-down tests (Step 4...) are performed at lower concentrations when toxicity is observed in the considered step

Step-down approach, The testing strategy where fish tests are performed at different concentrations subsequently and not in parallel. Testing is performed at lower concentration (Dilution 1, 2...) only in case when toxicity was observed in the previous step.

Threshold test, The fish test which is usually performed in the range finding procedure before the definitive test is performed. Five fish are used in test concentration and five in the negative control.

UTC, The Upper Threshold Concentration is the highest concentration at which the fish threshold test should be performed. It is derived from algae and daphnia test results. Among the two values the lowest is selected and is equal to the UTC.

3. Colour codes for extracted data

BROWN, Substances to be excluded

DARK GREEN, Values where the result in the data base is reported as =100mg/L. Applicable only for data sets I.

DARK YELLOW, cells with imprecise signs/values greater than (>)

GRAY, cells with imprecise signs/values greater than (>)

PALE YELLOW, Header row

PALE YELLOW, Important cases (Comments included)

PURPLE, Fish LC50 is the lowest test result among all tested species

RED, Exceptional cases (Comments included)

RED, Last line in the data set

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Are pharmaceuticals potent environmental pollutants? Part I: Environmental risk assessments of selected active pharmaceutical ingredients

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Abstract

As part of achieving national environmental goals, the Swedish Government commissioned an official report from the Swedish Medical Products Agency on environmental effects of pharmaceuticals. Considering half-lives/biodegradability, environmental occurrence, and Swedish sales statistics, 27 active pharmaceutical ingredients were selected for environmental hazard and risk assessments. Although there were large data gaps for many of the compounds, nine ingredients were identified as dangerous for the aquatic environment. Only the sex hormones oestradiol and ethinyloestradiol were considered to be associated with possible aquatic environmental risks. We conclude that risk for acute toxic effects in the environment with the current use of active pharmaceutical ingredients is unlikely. Chronic environmental toxic effects, however, cannot be excluded due to lack of chronic ecotoxicity data. Measures to reduce potential environmental impact posed by pharmaceutical products must be based on knowledge on chronic ecotoxic effects of both active pharmaceutical ingredients as well as excipients. We believe that the impact pharmaceuticals have on the environment should be further studied and be given greater attention such that informed assessments of hazards as well as risks can be done.

Keywords: Swedish government commission; Pharmaceuticals; Environmental hazards and risk assessments; Ecotoxicity

1. Introduction

The occurrence of pharmaceuticals in the environment is a growing concern (Richardson and Bowron, 1985; Halling-Sørensen et al., 1998; Ternes, 1998; Daughton and Ternes, 1999; Kümmerer et al., 2000a; Heberer, 2002). Pharmaceutical ingredients, including their metabolites and conjugates, are mainly excreted in urine or faeces. Hence, they enter municipal sewage treatment systems where they can be degraded, adsorbed to sewage sludge, or eventually diluted into surface water. Compounds that adsorb to sludge can reach the terrestrial environment when sludge is used as an agricultural fertilizer. Agricultural land can also be exposed when manure from medicated in-house reared animals is spread. Pharmaceuticals used in animals raised on pastures are excreted directly to the grassland. Pharmaceuticals entering the terrestrial environment can reach surface water and groundwater. In addition, pharmaceuticals used in aquaculture are released directly into surface water. Hence, many

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active pharmaceutical ingredients (APIs) for human and animal use are found in samples from surface waters, ground waters, and drinking water reservoirs. The interest in the occurrence of pharmaceuticals in the environment is ever increasing and the numbers of reports on measurable concentrations of pharmaceuticals in authentic samples or reviews on pharmaceuticals found in the environment are growing (Zuccato et al., 2000; Ternes, 2001; Zwiener et al., 2001; la Farré et al., 2001; Bruno et al., 2001; Kolpin et al., 2002; Calamari et al., 2003; Ferrari et al., 2003; Thomas and Hilton, 2004; Ashton et al., 2004; Wiegel et al., 2004). Analytical techniques are developed and monitoring programs are initiated. Papers dealing with the environmental fate (such as sorption and mobility in soil, removal through water-treatment processes, biodegradation, and photodegradation) of APIs are also beginning to emerge (Steger-Hartmann et al., 1997; Kümmerer et al., 2000a,b; Rabølle and Spliid, 2000; Oppel et al., 2004; Stackelberg et al., 2004).

Despite these numerous reports on environmental occurrence of APIs at levels in the range of ng to low μg/L, the environmental significance, pertaining to environmental effects, is largely unknown. As an exception, the synthetic oestrogen ethinyloestradiol is well known for its potential for endocrine disruptive and reproductive effects. Several papers describe such effects after exposure to either natural or synthetic oestrogens in laboratory settings. For instance, exposure to 0.005 μg/L of ethinyloestradiol delays embryonic development in zebrafish (Kime and Nash, 1999) and vitellogenin induction in rainbow trout is observed at sub ng/L levels (0.0001 μg/L) (Purdom et al., 1994). In the wild, induction of vitellogenin in male and/or juvenile rainbow trout downstream sewage treatment plants (STPs), indicative of oestrogenic activity in the STP effluent, has been reported in several studies originating from various countries, including Sweden (Larsson et al., 1999). Fish displaying intersex characteristics, leading to reduced reproductive success, are commonly found downstream STPs in the UK (Jobling et al., 2002a,b). These effects are likely to affect the population dynamics locally. Similar effects can be induced by oestrogens, including ethinyloestradiol (Länge et al., 2001). Effects observed in the environment, however, are difficult to assign to a specific compound as mixtures with possible overlapping and/or interactive properties are likely to be present.

An unusual route of environmental exposure to an API was recently revealed when excessive use of diclofenac was forwarded as the cause in the decline of a vulture population in Pakistan (Oaks et al., 2004). Autopsy of diseased animals suggested that the cause of death was acute renal failure, which was ascribed to diclofenac exposure. In Pakistan, diclofenac for veterinary use is widely marketed over the counter and livestock are frequently treated with diclofenac (Oaks et al., 2004). The primary food source for the vultures in Pakistan is dead domestic livestock that are left for scavengers, thus introducing a large pool of diclofenac into their diet.

Another example is the veterinary antiparasitic compound ivermectin. It is excreted in faeces where it is known to affect non-target organisms such as dung-beetles and dung-flies. In addition, ivermectin is extremely toxic to the freshwater crustacean *Daphnia magna*. When used as intended, however, ivermectin is not considered as an environmental problem as it is strongly bound to soil and its effects are exerted only locally (Bloom and Matheson, 1993).

It is desirable to be able to predict a compound's potential to cause adverse effects in the environment before these are observed. The probability of a compound to cause undesired environmental effects can be estimated in an environmental risk assessment (ERA). An application for the marketing authorisation for a medicinal product for human use in the European Union (EU) shall include an ERA (Council Directive 2001/83/EC as amended by Council Directive 2004/27/EC), implying that the pharmaceutical industry and the competent authorities may have access to unpublished environmental data.

ERA procedures for pharmaceuticals, based on principles already applied in ERAs of chemicals, have been developed for regulatory purposes in the EU. In order to arrive at reliable ERAs, adequate data on the environmental exposure and the ecotoxic potency of APIs are needed. An ERA in accordance with regulatory guidelines, such as the draft Note for Guidance (NfG) (CPMP/SWP/4447/00 draft), released by the European Medicines Agency (EMEA), and the Technical Guidance Document (TGD) in Support of Commissions Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commissions Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances (CEC, 1996), is a step-wise tiered procedure starting with rough estimates and progressing to more elaborate refined methods if a potential risk cannot be excluded. Hence, the assessment may be terminated either when sufficient information is available indicating that the product/compound is unlikely to represent an environmental risk or when a

risk has been identified and sufficiently characterised. A risk quotient (RQ) is usually calculated from a predicted (or measured) environmental concentration (PEC or MEC, respectively) and a predicted no effect concentration (PNEC). When the concentration of a compound in a particular environmental compartment is unknown, it can be predicted from a combination of estimates of the amount of consumption or sales, expected route of entry into the environment, and physico-chemical properties (Struijs et al., 1991). In higher tiers, modelled or known fate in the environment (such as biodegradability, bioconcentration, and adsorption to soil or sediment) is taken into account. A PNEC is obtained by dividing the lowest no observed effect concentration (NOEC) for the most sensitive species with an appropriate safety factor. Thus, an ERA requires acute and chronic ecotoxicity data for standard test organisms such as algae, *Daphnia*, and fish, i.e. species from at least three trophic levels. Accordingly, the outcome of an ERA is dependent on amount used, environmental fate, and the ecotoxicity of the compound in question.

In December 2002, as part of the work of achieving national environmental goals, the Swedish Government commissioned an official report from the Swedish Medical Products Agency on the environmental effects of pharmaceuticals. The report was to include a risk assessment of environmental effects based on the occurrence of the products in the environment in relation to their current sales volumes. There are 1200 APIs used in some 7600 products on the market in Sweden. Environmental monitoring data for APIs from Sweden is, however, scarce (Larsson et al., 1999; Roberto et al., 2003; Ferrari et al., 2003; Johansson et al., 2003). Uniquely, in Sweden, Apoteket AB is the sole retailer of all pharmaceuticals, including over the counter products. Therefore, reliable sales statistics from the Swedish market are available for use in PEC calculations. Sufficient ecotoxicity data for ERAs is available in the literature only for a small fraction of the APIs on the Swedish market, hampering PNEC calculations.

The objective of this study was to perform environmental hazard and risk assessments for selected active pharmaceutical ingredients based on Swedish retail figures and existing environmental data, either disclosed by the pharmaceutical industry or available in the literature. ERAs of selected APIs were, as far as possible, performed according to the draft NfG on ERA of medicinal products for human use (2003 version) taking into account all products containing the compound in question.

2. Material and methods

2.1. Collection of environmental data from the pharmaceutical industry

The pharmaceutical industry was asked to disclose available data (ecotoxicity, biodegradation, bioaccumulation, and relevant physico-chemical data) for all APIs in products approved for marketing in Sweden.

2.2. Collection of volume data

The numbers of defined daily doses (DDD) of the 100 most sold APIs for human use in Sweden in 2002 were obtained from Apoteket AB, the sole Swedish retailer of pharmaceuticals. This information was combined with information on the content of the API in each pharmaceutical product from the Medical Products Agency database to obtain the total weight sold per year for each API.

2.3. Selection of active pharmaceutical substances

Twenty-seven APIs occurring both on the list of the 100 most sold APIs for human use (based on DDDs), and reported as observed in the environment, or otherwise highlighted in the literature, were selected for environmental hazard and risk assessments. Data submitted by the pharmaceutical industry was also screened for compounds with properties indicating persistence, potential for bioaccumulation, and high ecotoxicity.

2.4. Environmental hazard assessments

Environmental hazard assessments and classifications were done according to criteria in Annex VI to the European Community legislation on classification and labelling (Directive 67/548/EEC). Briefly, classifications are made on the basis of experimental data for acute aquatic toxicity, degradation, and the *n*-octanol–water partitioning coefficient (K_{ow}) or bioconcentration factor (BCF), when available. Compounds are classified as "dangerous for the aquatic environment" and assigned risk phrases in accordance with the following criteria:

R50—"Very toxic to aquatic organisms": Acute toxicity (LC_{50} (fish), or EC_{50} (daphnia), or IC50 (algae)) ≤ 1 mg/L.
R50 and R53—"Very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic

environment": Acute toxicity ≤ 1 mg/L, and the compound is not readily degradable, or log $K_{ow} \geq 3$ (unless experimentally derived BCF ≤ 100).
R51—"Toxic to aquatic organisms": Acute toxicity > 1 mg/L and ≤ 10 mg/L.
R51 and 53—"Toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment". Acute toxicity > 1 mg/L and ≤ 10 mg/L, and the compound is not readily degradable, or log $K_{ow} \geq 3$ (unless experimentally derived BCF ≤ 100).
R52 and 53—"Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment": Acute toxicity > 10 mg/L and ≤ 100 mg/L and the compound is not readily degradable, or log $K_{ow} \geq 3$ (unless experimentally derived BCF ≤ 100).
R53—"May cause long-term adverse effects in the aquatic environment": Solubility less than 1 mg/L, and not readily degradable, and log $K_{ow} \geq 3$ (unless experimentally derived BCF ≤ 100).

2.5. Environmental risk assessments

ERAs were, as far as possible, done according to the draft Note for Guidance (NfG) on environmental risk assessment of medicinal products for human use (CPMP/SWP/4447/00 draft), released by the European Medicines Agency (EMEA) in July 2003. In January 2005, EMEA released a new version of the draft NfG, renamed Guideline on the environmental risk assessment of medicinal products for human use (CHMP/SWP4447/00 draft), for consultation available at www.emea.eu.int/pdfs/human/swp/444700en.pdf.

2.5.1. Prediction of environmental concentrations, Phase I

"Worst case" concentrations of active pharmaceutical ingredients in surface water ("worst case"-$PEC_{SURFACE\ WATER}$) were calculated by the equation:

$$\text{"Worst case"} - PEC_{SURFACE\ WATER} = DOSEai * Fpen / (WASTEWinhab * DILUTION * 100)$$

DOSEai is the highest recommended daily dose of the API in question, Fpen is the percentage of market penetration, and 100 is a correction for percentage. In an application for market authorisation, a default value of 1% is used for new products. In this study, the sold amount of API (all products containing the API included, Table 1) per inhabitant, calculated from Apoteket AB's sales statistics in Sweden for 2002 was used as a measure of DOSEai * Fpen/100. This provided for partly refined PEC-values already at this stage. WASTEWinhab is the amount of wastewater used per inhabitant per day and DILUTION is the dilution of sewage water in surface water. In the first tier, default values of 200 L wastewater per inhabitant per day and 10-fold dilution were used.

It was assumed that the entire amount of each product sold was consumed and that the predicted consumed amount was evenly distributed over the year and throughout the population of some 9 million inhabitants in Sweden. Further, as a conservative approach, the compounds were assumed not to be metabolised in humans or biodegraded in STPs. The sewage system was considered the main route of entry for the API into surface water. These calculations render highly conservative, probably overestimated "worst case"-$PEC_{SURFACE\ WATER}$, which would not be expected to reflect realistic environmental concentrations and should only be used as a rough estimate and as a priority setting tool.

According to the draft NfG, a Phase II environmental effect analysis needs to be performed only when "worst case"-$PEC_{SURFACE\ WATER}$ exceeds 0.01 μg/L. Nevertheless, we chose to assess all selected APIs according to Phase II regardless of the outcome of the Phase I assessment.

2.5.2. Prediction of environmental concentrations, Phase II

In Phase II, Tier A, the $PEC_{SURFACE\ WATER}$ is refined with an estimation of the market penetration factor based on health care statistics and/or epidemiological data. In the present study, refinement according to Tier A was not necessary since actual sales statistics were used for "worst case"-PEC-calculations in Phase I. Refinement of the $PEC_{SURFACE\ WATER}$ by applying Simple Treat 3.1 modelling (Struijs et al., 1991), in accordance with Tier B was, however, done at this stage of the assessment. Simple Treat 3.1 is an exposure model based on the principles given in the TGD (CEC, 1996), and is recommended by EMEA for higher tier modelling. Simple Treat 3.1 models the steady-state concentrations in sewage water, sludge, or air after passage through a modern STP consisting of a primary settler, an aeration tank, and a solids–liquid separator. For a generic assessment, default values for a hypothetical European STP are used to generate a refined local $PEC_{SEWAGE\ WATER}$ and a default dilution factor of 10 is applied to obtain a refined local $PEC_{SURFACE\ WATER}$.

Table 1
Selected active pharmaceutical ingredients, sold amounts in Sweden 2002, and estimated fractions entering the sewage

Compound	Therapeutic use	No. of approved products in Sweden	No. of sold DDDs	Sales amount (kg)	Estimated fraction entering the sewage (%)
Atenolol	β_1-Adrenergic blocking	19	59961126	4500	100
Cyclofosfamide	Antineoplastic	2	Not on the top 100 list	26	20
Dextropropoxyphene	Analgesic	7	17609300	1800	100
Diazepam	Tranquilizer	13	18635653	183	100
Diclofenac	Analgesic Anti-inflammatory Antipyretic	38	27583523	3960	100
Enalapril	ACE-inhibitor Antihypertensive	67	71408563	1200	100
Ethinyloestradiol	Oestrogen	42	96873252	6.4	100
Furosemid	Diuretic	25	173942872	6960	100
Hydrochlorothiazide	Diuretic	102	36266666	1770	100
Ibuprofen	Analgesic Anti-inflammatory Antipyretic	25	46351919	68200	100
Ifosfamide	Antineoplastic	1	Not on the top 100 list	12	20
Ketoprofen	Analgesic Anti-inflammatory Antipyretic	27	10477073	62700	85
Metformin	Antidiabetic	16	26247795	81900	100
Metoprolol	β_1-Adrenergic blocking	55	63503488	18900	100
Naproxen	Analgesic Anti-inflammatory Antipyretic	17	27472056	13700	100
Norethisterone	Progestin	34	27494292	50	100
Oestradiol	Oestrogen	112	36439617	153	100
Oestriol	Oestrogen	14	26889214	38	100
Oxazepam	Tranquilizer	8	12837257	642	100
Oxytetracycline	Antibiotic	17	Not on the top 100 list	293	100
Paracetamol	Analgesic Antipyretic	53	107403852	418000	100
Ranitidin	Gastric secretory inhibitor Histamine, H_2-receptor antagonist	42	15314781	8360	100
Salbutamol	Bronchodilator β_2-Sympathomimetic	23	16800345	107	100
Simvastatin	Antihyperlipidemic	63	95345198	1430	80
Terbutalin	Antiasthmatic β_2-Sympathomimetic	38	31285280	81	100
Tetracycline	Antibiotic	7	Not on the top 100 list	2400	100
Warfarin	Anticoagulant	2	20144288	137	100

2.6. Local emission scenario

Release estimations based on number of inhabitants, sewage flow, and dilution in surface water specific for the local scenario of Stockholm, Sweden, were chosen rather than the hypothetical European average, thus generating site-specific refined local $PEC_{SEWAGE\ WATER}$ and $PEC_{SURFACE\ WATER}$ values. In Stockholm, 1.5 million inhabitants are connected to three major STPs, Käppala (534000 inhabitants), Henriksdal (695000 inhabitants), and Bromma (286000 inhabitants), releasing effluent sewage waters into the Stockholm archipelago (www.stockholmvatten.se/indexie.htm and www.kappala.se/tekniska.htm). The sewage flow per inhabitant and day are 375 L (Käppala), 347 L (Henriksdal), and 430 L (Bromma).

The amount of an API entering the sewage system was estimated by the total sale per day per inhabitant connected to each STP, multiplied with the fraction of parent compound excreted after metabolism. Metabolites lacking environmental and/or ecotoxicity data were regarded as parent compound and added to this fraction. Thus, a compound excreted to less than 1% as parent compound could still be calculated as 100%

parent compound. Only publicly available information on metabolism and excretion (e.g. information based on the summary of product characteristics (SmPC) published by the Swedish Association of the Pharmaceutical Industry at www.fass.se) and/or information submitted by the pharmaceutical industry were taken into account. When the API data set was incomplete it was assumed that 100% was excreted as the parent API, thus maintaining a conservative approach.

Default values provided in the Simple Treat 3.1 model were used for defining the operating conditions for the STPs, with the exceptions of choosing bubble aeration and setting the water temperature to 10 °C. Physico-chemical input data were molecular weight, *n*-octanol–water partitioning coefficient (K_{ow}), vapour pressure, water solubility, and acid–base dissociation constants. Data submitted by the pharmaceutical industry were used when available or calculated with the Estimation Programs Interface Suite™ (EPI Suite™) when lacking (data not shown). A degradation rate constant was assigned to each API based on available information on biodegradation (Table 3). Paracetamol was assigned a degradation rate constant of 0.1/h, due to available data on inherent biodegradability (Richardson and Bowron, 1985). The other APIs were assigned a degradation rate constant of zero in the Simple Treat 3.1 model, due to lack of biodegradation data or data indicating a compound not to be biodegradable.

A site-specific dilution factor, rather than the default of 10, was applied to the modelled $PEC_{SEWAGE\ WATER}$ in order to obtain a refined local $PEC_{SURFACE\ WATER}$. A realistic site-specific regional $PEC_{SURFACE\ WATER}$ could be obtained by applying a catchment area model such as the Geo-referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER) (Schowanek and Webb, 2002) or the Pharmaceutical Assessment and Transport Evaluation (PhATE) (Anderson et al., 2004), provided that data for the actual catchment area is available. For simplicity, the combined effluents from the three STPs (560 000 m^3/day) were considered as one, and were assumed to be diluted into 14 millions m^3/day of water from the lake Mälaren (average outflow available at www.vasteras.se/malarensvattenvardsforbund/miljon.htm) resulting in a site-specific dilution factor of 25. The background concentration of the API in the recipient water was assumed to be negligible.

2.6.1. Prediction of no effect concentration (PNEC)

In accordance with the NfG and the TGD, compound-specific PNEC values were derived from the lowest available acute LC_{50}-, EC_{50}-, or IC_{50}-value divided by the assessment factor 1000. When a chronic NOEC-value for the most sensitive species was available it was used for PNEC calculations using the assessment factor 100. If chronic NOECs for two trophic levels were available the assessment factor 50 was used and if NOECs for three trophic levels were available the assessment factor 10 was used (Table 4).

2.6.2. Risk characterisation

A risk quotient (RQ=PEC/PNEC) for the aquatic environment was calculated from the refined $PEC_{SURFACE\ WATER}$ and the respective PNEC-value.

3. Results and discussion

Twenty-seven APIs for human use were selected for environmental hazard and risk assessments in accordance with standardised methodologies. Ecotoxicity data found in the literature for the selected APIs are given in Table 2. Significant data gaps were present for many of the compounds. Some of the gaps in the literature data set were filled with the assistance of the Swedish Association of the Pharmaceutical Industry and individual pharmaceutical companies (Table 3). Preliminary ERAs could be done for 24 out of 27 selected APIs (Table 4). Nine APIs were classified as dangerous for the aquatic environment but only the sex hormones oestradiol and ethinyloestradiol were identified as possible aquatic environmental risks.

3.1. How ecotoxic are active pharmaceutical ingredients?

The acute EC_{50}-values for the 24 APIs differed markedly, from a few μg/L for the antibiotics in algae to more than 1000 mg/L for some of the APIs in fish. Few chronic NOEC-values were available and the opportunity to reduce the assessment factor when calculating PNEC-values was low (Tables 2, 3, and 4). Risk assessments based on chronic ecotoxicity data were possible for only four APIs, i.e. the oestrogens and diclofenac. The derived PNEC-values were in the interval 0.00002 to 984 μg/L with the sex hormones having the lowest PNEC-values.

Among the 27 selected APIs, diclofenac, ethinyloestradiol, ibuprofen, metoprolol, norethisterone, oxytetracycline, and paracetamol were, based on inherent properties, classified as "dangerous for the aquatic environment" (Table 4). Norethisterone was classified as "very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment" (R50/53). Oxytetracycline was assessed as "very toxic to aquatic organisms" (R50). Ethinyloestradiol and ibu-

profen were classified as "toxic to the aquatic environment and may cause long-term adverse effects in the aquatic environment" (R51/53). Diclofenac and metoprolol were classified as "harmful to the aquatic environment and may cause long-term adverse effects in the aquatic environment" (R52/53). Seventeen ingredients had incomplete data sets and environmental hazard assessments and classifications were therefore not possible. Only furosemid, hydrochlorothiazide, and terbutalin were classified as "not dangerous for the aquatic environment".

The number of papers reporting on laboratory aquatic ecotoxicity testing of APIs is growing. A common strategy for the selection for ecotoxicity testing seems to be the most prescribed drugs or pharmaceuticals. For instance, 10 pharmaceuticals commonly prescribed in the UK (ibuprofen, paracetamol, acetylsalicylic acid, amoxicillin, bendroflumethiazide, furosemide, atenolol, diazepam, digoxin, and amlodipine) were selected for acute and chronic ecotoxicity testing in the cnidarian *Hydra vulgaris* (Pascoe et al., 2003). No treatment related acute effects on survival at concentrations up to 10 mg/L were observed. For amlodipine significantly shortened tentacles and contracted bodies were observed at 1 mg/L. Chronic exposure at concentrations of 10 μg/L of diazepam, digoxin, or amlodipine resulted in inhibited polyp regeneration.

Ten frequently used pharmaceutical compounds (clofibric acid, carbamazepine, ibuprofen, diclofenac, naproxen, captopril, metformin, propranolol, and metoprolol) were investigated for acute toxicity in the crustacean *D. magna* and the green algae *Desmodesmus subspicatus*. In addition, chronic toxicity was assessed in the aquatic higher plant *Lemna minor*. EC_{50}-values were generally between 10 and 100 mg/L (Cleuvers, 2003). Lower EC_{50}-values (5–8 mg/ L) were observed only for diclofenac, propranolol and metoprolol. The most sensitive species was *L. minor*, conceivably because the *Lemna* test is a chronic test.

Other reports focus on a specific class of pharmaceuticals. Brain et al. (2004) recently assessed the tions are one to two orders of magnitude higher than observed environmental concentrations. Still, the authors were of the opinion that a significant ecological impact is possible at observed environmental concentrations. A growth reduction exceeding 20% is considered ecologically relevant in aquatic higher plants and the EC_{25} and EC_{10} values were close to measured environmental concentrations.

The acute effects of some β-blockers (metoprolol, nadolol, and propranolol) were investigated in two crustaceans (*Ceriodaphnia dubia* and *D. magna*), a sediment living amphipod (*Hyalella azteca*), and the Japanese ricefish (*Oryzias latipes*) (Huggett et al., 2002). No toxic effects were observed for nadolol in any of the investigated species. The most potent compound was propranolol, with an LC_{50}-value of 0.8 mg/L in the most sensitive species *C. dubia*. For metoprolol, the corresponding value was 8.8 mg/L. The only compound having acute toxic effects on Japanese ricefish was propranolol with an LC_{50}-value of 24.3 mg/L. The chronic effects of propranolol were also investigated. The most sensitive invertebrate was the amphipod *H. azteca* for which reduced reproduction was observed at 0.1 mg/L. The corresponding value for *C. dubia* was 0.24 mg/L. In fish, the numbers of produced and hatched eggs were reduced after 4-week exposure to propranolol at 5 μg/L. Following 2-week exposure to propranolol at only 1 μg/L, alterations in plasma oestradiol and testosterone levels were observed.

Aquatic acute and chronic toxicities of some antibiotics for veterinary use (metronidazole, olaquindox, oxolinic acid, oxytetracycline, streptomycin, sulfadiazine, tetracycline, tiamulin, and tylosin) were investigated in *D. magna* (Wollenberger et al., 2000). In the acute toxicity study, the most potent compound was oxolinic acid (EC_{50}=4.6 mg/L). The EC_{50}-values for the other compounds were between 40 and 680 mg/L. In the chronic reproduction test, the values were generally one order of magnitude lower. Oxolinic acid was because of its common use as a feed additive in fish farming considered as a potential environmental

Table 2
Ecotoxicity data found in the literature for some of the selected active pharmaceutical ingredients

Compound	Test-species	Test-type	Ecotoxicity data	Reference
Atenolol	(Green algae)	*PNEC*, *ECOSAR*	77.7 μg/L[a]	(Jones et al., 2002)
	Hydra vulgaris (cnidaria)	Acute toxicity, 7 days, effect on polypstructure, (LOEC)	10 mg/L (only effective conc.)	(Pascoe et al., 2003)
	Hydra vulgaris (cnidaria)	Acute toxicity, 7 days, effect on polypstructure, (NOEC)	1 mg/L	(Pascoe et al., 2003)
Cyclofosfamide	*Biomphalaria glabrata* (mollusc, freshwater)	Dominant lethal test	Germ cell mutagen	(Nakano et al., 2003)
	(Fish)	EC_{50}, *ECOSAR*	70 mg/L[a]	(Sanderson et al., 2003)
	(Daphnid)	EC_{50}, *ECOSAR*	1795 mg/L[a]	(Sanderson et al., 2003)
	(Algae)	EC_{50}, *ECOSAR*	11 mg/L[a]	(Sanderson et al., 2003)
Dextropropoxyphene	*Brachionus calyciflorus* (most sensitive of 5 invertebrates)	EC_{50}, 24 h (Rotoxkit F)	5.4 mg/L	(Calleja et al., 1994)
	Daphnia magna	EC_{50}, 24 h	24.6 mg/L	(Calleja et al., 1994)
Diazepam	*Daphnia magna* (most sensitive of 5 invertebrates)	EC_{50}, 24 h	14.1 mg/L	(Calleja et al., 1994)
	Daphnia magna	EC_{50}	13.9 mg/L; 4.3 mg/L	(Halling-Sørensen et al., 1998)
Diclofenac	Bird, vulture	LOAEL, dietary intake, renal failure	0.007 mg/kg	(Oaks et al., 2004)
	Vibrio fischeri (bacteria)	EC_{50}, 30 min	11.45 mg/L	(Ferrari et al., 2003)
	Vibrio fischeri (bacteria)	EC_{50}, ToxAlert, 15 min	13.5 mg/L	(la Farré et al., 2001)
	Vibrio fischeri (bacteria)	EC_{50}, Microtox	13.7 mg/L	(la Farré et al., 2001)
	Pseudokirchneriella subcapitata = *S. capricornutum* (green algae)	NOEC, 96 h, growth	10 mg/L	(Ferrari et al., 2003)
	Pseudokirchneriella subcapitata = *S. capricornutum* (green algae)	LOEC, 96 h, growth	20 mg/L	(Ferrari et al., 2003)
	Daphnia magna	EC_{50}, 48 h	224.3 mg/L	(Ferrari et al., 2003)
	Cerodaphnia dubia (crustacean)	EC_{50}, 48 h	22.7 mg/L	(Ferrari et al., 2003)
	Cerodaphnia dubia (crustacean)	NOEC, 7 days, reproduction	1.0 mg/L	(Ferrari et al., 2003)
	Cerodaphnia dubia (crustacean)	LOEC, 7 days, reproduction	2.0 mg/L	(Ferrari et al., 2003)
	Brachionus calyciflorus (rotifer)	NOEC, 48 h, reproduction	12.5 mg/L	(Ferrari et al., 2003)
	Brachionus calyciflorus (rotifer)	LOEC, 48 h, reproduction	25 mg/L	(Ferrari et al., 2003)
	Danio rerio (zebrafish)	NOEC, 10 days, ELS	4 mg/L	(Ferrari et al., 2003)
	Danio rerio (zebrafish)	LOEC, 10 days, ELS	8 mg/L	(Ferrari et al., 2003)
	Desmodesmus subspicatus (green algae)	EC_{50}, 72 h, (sodium salt)	72 mg/L	(Cleuvers, 2003)
	Daphnia magna	EC_{50}, 48 h, (sodium salt)	68 mg/L	(Cleuvers, 2003)
	Lemna minor (aquatic vascular plant)	EC_{50}, 7 days, growth	7.5 mg/L	(Cleuvers, 2003)
Ethinyloestradiol	Invertebrate	NOEC, reproduction	0.01 mg/L	(Halling-Sørensen et al., 1998)
		EC_{50}, reproduction	0.105 mg/L	(Halling-Sørensen et al., 1998)
		EC_{50}, acute	5.7 mg/L	(Halling-Sørensen et al., 1998)
	Algae	EC_{50}	0.84 mg/L	(Halling-Sørensen et al., 1998)
	Oryzias latipes (fish—Japanese ricefish)	LOEL, induced intersex (testis–ova), exposure hatched—90 days	0.00003 μg/L	(Metcalfe et al., 2001)

Ibuprofen	*Lemna gibba* (aquatic vascular plant)	NOEC, 7 days, growth	>1000 μg/L (highest test conc.)	(Brain et al., 2004)
	Vibrio fischeri (bacteria)	EC_{50}, ToxAlert, 15 min	12.1 mg/L	(la Farré et al., 2001)
	Vibrio fischeri (bacteria)	EC_{50}, Microtox	19.1 mg/L	(la Farré et al., 2001)
	Lepomis macrochirus (fish)	LC_{50}, 96 h	173 mg/L	(Halling-Sørensen et al., 1998)
	Lepomis macrochirus (fish)	NOEC, 96 h	10 mg/L	(Halling-Sørensen et al., 1998)
	Daphnia magna	LC_{50}, 48 h	9.06 mg/L	(IUCLID, 2000)
	Daphnia magna	NOEC,48 h	3.37 mg/L	(IUCLID, 2000)
	Skeletonema costatum (algae)	EC_{50}, 96 h	7.1 mg/L	(Halling-Sørensen et al., 1998)
Ketoprofen	(Fish)	EC_{50}, *ECOSAR*	32 mg/L[a]	(Sanderson et al., 2003)
	(Daphnid)	EC_{50}, *ECOSAR*	248 mg/L[a]	(Sanderson et al., 2003)
	(algae)	EC_{50}, *ECOSAR*	164 mg/L[a]	(Sanderson et al., 2003)
	Vibrio fischeri (bacteria)	EC_{50}, Microtox	19.3 mg/L	(la Farré et al., 2001)
	Vibrio fischeri (bacteria)	EC_{50}, ToxAlert 100, 15 min	15.6 mg/L	(la Farré et al., 2001)
Metformin	(Green algae)	*PNEC*, *ECOSAR*	511 μg/L[a]	(Jones et al., 2002)
	(Fish)	EC_{50}, *ECOSAR*	3.32×10^4 mg/L[a]	(Sanderson et al., 2003)
	(Daphnid)	EC_{50}, *ECOSAR*	1345 mg/L[a]	(Sanderson et al., 2003)
	(Algae)	EC_{50}, *ECOSAR*	511 mg/L[a]	(Sanderson et al., 2003)
	Lepomis macrochirus (fish—bluegill sunfish)	LC_{50} (metformin HCL)	>982 mg/L	(Webb, 2001)
	Daphnia spp.	EC_{50} (metformin HCL)	130 mg/L	(Webb, 2001)
Metoprolol	*Hyallela azteca* (amphipod)	LC_{50}, 48 h	>100 mg/L	(Huggett et al., 2002)
	Cerodaphnia dubia (crustacean)	LC_{50}, 48 h	8.8 mg/L	(Huggett et al., 2002)
	Daphnia magna	LC_{50}, 48 h	63.9 mg/L	(Huggett et al., 2002)
	Oryzias latipes (fish—Japanese ricefish)	LC_{50}, 48 h	>100 mg/L	(Huggett et al., 2002)
Naproxen	*Vibrio fischeri* (bacteria)	EC_{50}, ToxAlert 100, 15 min	21.2 mg/L	(la Farré et al., 2001)
	Vibrio fischeri (bacteria)	EC_{50}, Microtox	35 mg/L	(la Farré et al., 2001)
	Lemna gibba (aquatic vascular plant)	LOEC, 7 days , growth	1000 μg/L	(Brain et al., 2004)
Oestradiol	*Oryzias latipes* (fish—Japanese ricefish)	LOEL, induced intersex (testis–ova), exposure hatched—90 days	0.004 μg/L	(Metcalfe et al., 2001)
	Oryzias latipes (fish—Japanese ricefish)	NOEL, induced intersex (testis–ova), exposure hatched—90 days	0.0004 μg/L	(Metcalfe et al., 2001)
Oestriol	*Oryzias latipes* (fish—Japanese ricefish)	LOEL, induced intersex (testis–ova), exposure hatched—90 days	0.75 μg/L	(Metcalfe et al., 2001)
	Oryzias latipes (fish—Japanese ricefish)	NOEL, induced intersex (testis–ova), exposure hatched—90 days	0.075 μg/L	(Metcalfe et al., 2001)
	Oryzias latipes (fish—Japanese ricefish)	LOEL, skewed sex-ratio (significantly more males than females), exposure hatched—90 days	0.01 μg/L	(Metcalfe et al., 2001)

(continued on next page)

Table 2 (*continued*)

Compound	Test-species	Test-type	Ecotoxicity data	Reference
Oxytetracycline	*Folsomia fimetaria* (springtail)	EC_{50}, 21 days, reproduction	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Folsomia fimetaria (springtail)	NOEC, 21 days, reproduction	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Folsomia fimetaria (springtail)	NOEC, 21 days, survival adults	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Enchytraeus crypticus (white pot-worm)	EC_{50}, 21 days, reproduction	2701 mg/kg dryweight	(Baguer et al., 2000)
	Enchytraeus crypticus (white pot-worm)	NOEC, 21 days, reproduction	2000 mg/kg dryweight	(Baguer et al., 2000)
	Enchytraeus crypticus (white pot-worm)	LC_{50}, 21 days, survival adults	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Enchytraeus crypticus (white pot-worm)	21 days, survival adults	3000 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	EC_{50}, 21 days, reproduction	4420 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	NOEC, 21 days, reproduction	3000 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	NOEC, 21 days, survival adults	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	EC_{50}, 21 days, hatchability	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	NOEC, 21 days, hatchability	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	EC_{50}, 21 days, growth	>5000 mg/kg dryweight	(Baguer et al., 2000)
	Aporrectodea caliginosa (earthworm)	NOEC, 21 days, growth	3000 mg/kg dryweight	(Baguer et al., 2000)
	Daphnia magna	LOEC, LC_{10} 48 h	100 mg/L (highest test conc.)	(Wollenberger et al., 2000)
	Daphnia magna	EC_{50}, 21 days, reproduction	46.2 mg/L	(Wollenberger et al., 2000)
	Microcystis aeruginosa (cyanobact/blue-green algae)	EC_{50}	0.207 mg/L	(Halling-Sørensen et al., 2000)
	Selenastrum capricornutum (green algae)	EC_{50}	4.5 mg/L	(Halling-Sørensen et al., 2000)
	R. salina (algae)	EC_{50}	1.6 mg/L	(Halling-Sørensen et al., 2000)
	Lemna minor (aquatic vascular plant)	EC_{50}, 7 days, growth, no. of fronds	4.92 mg/L	(Pro et al., 2003)
	Chlorella vulgaris (green algae)	EC_{50}, 48 h	6.4 mg/L	(Pro et al., 2003)
	(Fish)	EC_{50}, *ECOSAR*	1.66×10^5 mg/L[a]	(Sanderson et al., 2003)
	(Daphnid)	EC_{50}, *ECOSAR*	2432 mg/L[a]	(Sanderson et al., 2003)
	(Algae)	EC_{50}, *ECOSAR*	2294 mg/L[a]	(Sanderson et al., 2003)
	Penaeus setiferus (crustacean)	LC_{50}, 24 h	>5 mg/L	(Webb, 2001)
	Morone saxatilis (fish—stripped bass, larvae)	LC_{50}, 24, 48, 72 h (oxytetracycline HCL)	62.5 mg/L	(Webb, 2001)
	Morone saxatilis (fish—stripped bass, fingerling)	LC_{50}, 24, 48, 72, 96 h (oxytetracycline HCL)	150, 125, 100, 75 mg/L	(Webb, 2001)
	Lemna gibba (aquatic vascular plant)	EC_{50}, 7 days, growth	1010 μg/L	(Brain et al., 2004)
	Lemna gibba (aquatic vascular plant)	LOEC, 7 days, growth	1000 μg/L	(Brain et al., 2004)
	Selenastrum capricornutum (green algae)	EC_{50}, 72 h	4.18 mg/L	(De Liguoro et al., 2003)

Paracetamol	*Streptocephalus proboscideus* (most sensitive of 5 invertebrates)	EC_{50}, 24 h (Streptox kit F)	9.2 mg/L	(Calleja et al., 1994)
	Daphnia magna	EC_{50}, 24 h	55.5 mg/L	(Calleja et al., 1994)
Ranitidine	*Daphnia* spp.	EC_{50}	650 mg/L	(Webb, 2001)
Tetracycline	*Daphnia magna*	NOEC, LC_{10} 48 h	340 mg/L (no effect at highest conc.)	(Wollenberger et al., 2000)
	Daphnia magna	EC_{50}, 21 days, reproduction	44.8 mg/L	(Wollenberger et al., 2000)
	Microcystis aeruginosa (cyanobact/blue-green algae)	EC_{50}, 7 days, growth	0.09 mg/L	(Halling-Sørensen et al., 2000)
	Selenastrum capricornutum (green algae)	EC_{50}, 3 days, growth	2.2 mg/L	(Halling-Sørensen et al., 2000)
	(Fish)	EC_{50}, *ECOSAR*	16 mg/L[a]	(Sanderson et al., 2003)
	(Daphnid)	EC_{50}, *ECOSAR*	550 mg/L[a]	(Sanderson et al., 2003)
	(Algae)	EC_{50}, *ECOSAR*	475 mg/L[a]	(Sanderson et al., 2003)
	Nitszcha closterium (marine diatom)	EC_{50}, 72 h	16 mg/L	(Webb, 2001)
	Salvelinus namaycush (fish—lake trout)	LC_{50}, 24/96 h (tetracycline HCL)	220 mg/L	(Webb, 2001)
	Morone saxatilis (fish—stripped bass)	LC_{50}, 24, 48, 96 h (tetracycline HCL)	>182 mg/L	(Webb, 2001)
	Lemna gibba (aquatic vascular plant)	EC_{50}, 7 days, growth	723 μg/L	(Brain et al., 2004)
	Lemna gibba (aquatic vascular plant)	LOEC, 7 days, growth	1000 μg/L	(Brain et al., 2004)
Warfarin	*Photobacterium phosphoreum* (most sensitive of 5 invertebrates)	EC_{50}, 15 min (Microtox)	67 mg/L	(Calleja et al., 1994)

[a] Ecological Structure Activity Relationship value.