Initial assessment of eleven pharmaceuticals using the EMEA guideline in Norway

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Preface

The substances used in pharmaceuticals are subject to an extensive testing and evaluation for potential health effects. There is, however, limited knowledge of the environmental impact of release of pharmaceutical compounds to the environment.

NIVA was commissioned by SFT to perform a preliminary environmental risk assessment according to the EMEA guideline for a suite of eleven pharmaceutical compounds.

An extensive literature search and subsequent quality screening identified that one of the pharmaceuticals (cyclophosphamide) had not been tested for toxicity to aquatic organisms. The toxicity of cyclophosphamide was therefore tested both in an algal test and a *Daphnia* reproduction test. The risk quotients were calculated for all the selected pharmaceutical compounds.

At NIVA, Torsten Källqvist has been responsible for the toxicity tests, while Merete Grung has been responsible for the literature search and calculations of risk quotients. Torsten Källqvist was responsible for quality assurance, and the project was lead by Kevin Thomas.

Oslo, December 2006

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1 Abstract

On commission from Norwegian Pollution Control Authority, NIVA has performed an environmental risk assessment of the following compounds according to the guideline recommended by the European Agency for the Evaluation of Medicinal Products (EMEA):

- Paracetamol
- Ibuprofen
- Metoprolol
- Diclofenac
- Tetracycline
- Ciprofloxacin
- Trimethoprim
- Sulfamethoxazole
- Cefuroxime
- Ethinylestradiol
- Cyclophosphamide

The EMEA guideline was initially compared with the European Unions Technical Guidance Document (TGD) for environmental risk assessment. The EMEA guideline is more specific than the TGD, focusing on the scenarios related to human pharmaceutical consumption and release.

Predicted environmental concentrations (PECs) were calculated according to both the EMEA guideline and a conventional model for comparison. For the conventional model, based on the quantity released, the sales in 2005 from all wholesalers in Norway were collected. Available acute and chronic toxicity data were collected from the literature.

The toxicity of cyclophosphamide has not been previously reported in the literature, so therefore a 72 hour toxicity test on the green alga *Pseudokirchneriella subcapitata* and a reproduction test on *Daphnia magna* were performed. The EC50 value for *P. subcapitata* was > 100mg/L, while the NOEC value for *D. magna* reproduction was 56 mg/L. These data and those obtained following an extensive literature search and subsequent quality screening was used to derive predicted no effect concentrations (PNEC).

Risk quotients (PEC/PNEC) were then calculated for all 11 pharmaceutical compounds. The risk quotients were above 1 for diclofenac, tetracycline, ciprofloxacin, sulfamethoxazole and ethinylestradiol according to the EMEA guideline. The difference between the models for estimating PECs is discussed in the report, as well as comparisons with measured environmental concentrations (MECs) for four of the selected pharmaceuticals.

2 Norsk sammendrag

På oppdrag fra Statens forurensningstilsyn (SFT) har NIVA utført en risikovurdering etter risikoveilederen til European Agency for the Evaluation of Medicinal Products (EMEA) for følgende forbindelser:

- Paracetamol
- Ibuprofen
- Metoprolol
- Diclofenac
- Tetracycline
- Ciprofloxacin
- Trimethoprim
- Sulfamethoxazole
- Cefuroxime
- Ethinylestradiol
- Cyclophosphamide

EMEAs risikoveileder ble sammenlignet med European Unions Technical Guidance Document (TGD) for miljørelaterte risikovurderinger. EMEAs risikoveileder er mer spesifikk enn TGD, og fokuserer på effekter av tilførsler av legemidler til miljøet.

Estimerte miljøkonsentrasjoner (PEC) ble beregnet både etter EMEAs veileder, samt etter en konvensjonell metode for sammenligning. Den konvensjonelle modellen for å estimere PEC er basert på salgstall for alle legemidler i løpet av 2005 fra grossist (tall fra Nasjonalt folkehelseinstitutt). Tilgjengelige akutte og kroniske toksitetsdata ble hentet fra vitenskapelig litteratur.

For cyclophosphamide ble det ikke funnet noen toksisitetsdata i tilgjengelig vitenskapelig litteratur, så en 72 timers vekstinhibisjonstest på *Pseudokirchneriella subcapitata* og en reproduksjonstest på *Dapnia magna* ble utført. EC50 for *P. subcapitata* ble bestemt til >100 mg/L, mens NOEC for reproduksjon på *D. magna* ble bestemt til 56 mg/L. Disse dataene, sammen med data fra et stort litteratursøk som også ble vurdert med hensyn til kvalitet, dannet grunnlaget for bestemmelse av PNEC (predicted no effect concentration).

Risikokvotienten (PEC/PNEC) ble så bestemt for de 11 utvalgte legemidlene. Risikokvotienten var over 1 for diclofenac, tetracycline, ciprofloxacin, sulfamethoxazole og ethinylestradiol etter EMEAs risikoveileder. De ulike modellene for beregning av PEC blir diskutert i rapporten, det samme gjelder en sammenligning med målte miljøkonsentrasjoner for fire av de utvalgte legemidlene.

3 Introduction

Pharmaceutical substances have over the past years become an environmental concern in modern society. Sewage treatment plants (STPs) have been identified as the major source of environmental discharge for these compounds ([1]Figure 1). As a consequence, variable quantities of pharmaceuticals can reach surface waters, groundwaters and sediments, resulting in concentrations ranging from nanograms to micrograms per liter. Pharmaceuticals can be degraded in the environment by biotic and/or abiotic processes, but may cause persistent exposure due to their continuous infusion into aquatic media via STP effluents. In addition, pharmaceuticals have an intrinsic property to cause a biological effect. Therefore, the risk they present to the environment cannot be ruled out.

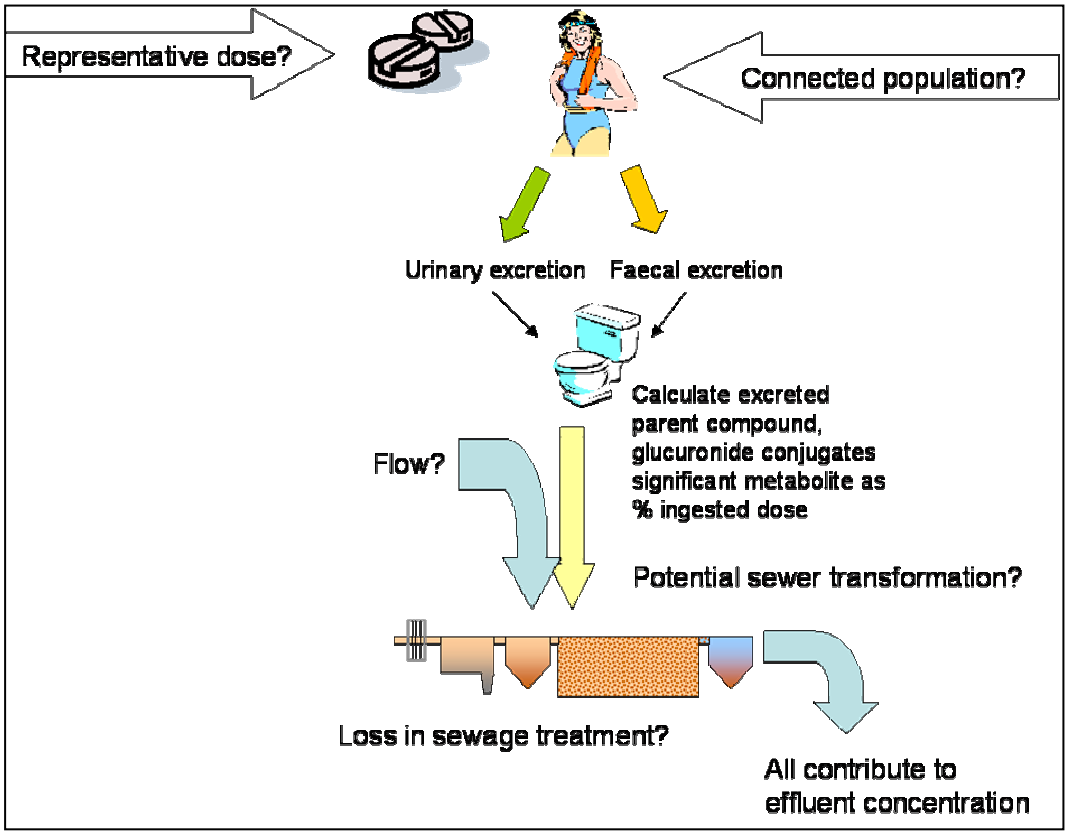

Figure 1 Typical pathway of pharmaceutical compound into the aquatic environment

Procedures for conducting environmental risk assessment (ERA) on pharmaceuticals are under development, or are in effect in Europe and United States. The Committee for Medicinal Products for human use (CMPH) of the Euroopean Medicines Evaluation Agency (EMEA) has published guidelines for ERA which will come into effect on the 1st December 2006 [2]. An ERA is required for all new marketing authorisation applications for medicinal products. An evaluation of the environmental impact should also be made if there is an increase in the environmental exposure, e.g. a new indication may result in a significant increase in the extent of the use. In essence, this procedure follows the general principle of the ERA procedures as applied to existing and new conventional chemicals in Europe (EU TGD) [3].

4 EMEA guideline on the environmental risk assessment of medicinal products for human use

The EMEA guideline [2] describes how to evaluate the potential risks of the medicinal product to the environment. Interestingly, whatever the impact the medicinal product has on the environment, this should not constitute a criterion for refusal of a marketing authorisation (Chapter 2). The guideline is focussed only on the environmental risks associated with the use of medicinal products, not arising from storage, disposal, synthesis or manufacture of medicinal products. This does probably does not constitute a big problem in Norway, where the release to the environment mainly comes from consumption. The guideline describes a stepwise tiered procedure for ERA. The general principles of the approach are presented in Table 1.

In addition, the guideline states that certain substances such as highly lipophilic compounds and potential endocrine disruptors may need to be addressed irrespective of the quantity released into the environment. Pharmaceutical substances with a log K_{ow} >4.5 should be screened for persistence, bioaccumulation and toxicity (PBT) according to the EU TGD.

Table 1. The general principles of the EMEA guidelines

Stage in evaluation	Stage in risk assessment	Objective	Method	Test Data requirement
Phase I	Pre-screening	Estimation of exposure	Action limit	Consumption data, log Kow
Phase II Tier A	Screening	Initial prediction of risk	Risk assessment	Base set aquatic toxicology and fate
Phase II Tier B	Extended	Substance and compartment-specific refinement and risk assessment	Risk assessment	Extended data set on emission, fate and effects

The calculation of the predicted environmental concentration (PEC) in Phase I is restricted to the aquatic compartment and is determined by the use of the following formula:

$$\mathrm{PEC}_{\text{surface water}}\left(\mathrm{mg\,L^{-1}}\right) = \frac{\mathrm{DOSEai} \times \mathrm{Fpen}}{\mathrm{WASTEW}_{\text{inhab}} \times \mathrm{DILUTION}}$$

Where:
$PEC_{Surface\ Water}$ = Predicted environmental concentration for surface water
DOSEai= Maiximum daily dose consumed per inhabitant
Fpen= Market penetration factor of active ingredient
$WASTEW_{inhab}$= Volume of wastewater generated per inhabitant
DILUTION= Dilution of effluent in recipient

For all of the parameters except DOSEai, a recommended value is given within the guideline. The DOSEai can be found in the pharmacopoeia of the region in question, and the maximal daily dose should be used. Fpen, the percentage of market penetration, has a default value of 0.01 (assuming that 1% of the population are treated daily with the drug – based on a wide range of individual market penetration factors). The guideline states that he applicant may use the default value or refine the Fpen by providing reasonably justified market penetration data, e.g. based on published epidemiological data. The volume of wastewater generated per inhabitant per day ($WASTEW_{inhab}$) is set to 200 L inh^{-1} d^{-1}, and the dilution factor is set to 10.

The present action limit for further investigation is set at a PEC of 0.01 µg/L. This means that in general, a DOSEai greater than 2 mg (maiximum daily dose consumed per inhabitant) will initiate a Phase II environmental fate and effects analysis.

4.1. Phase II Tier A

In Pase II, Tier A, a screening data set provides information on the physico-chemical properties and on the fate of a substance in the environment. The key physico-chemical properties are degradation, determined by using a ready biodegradability test and the organic carbon-water partition coefficient (K_{oc}). If the substance is not readily biodegradable then the transformation studies in sediments should be conducted. A high K_{oc} value usually means that the substance is retained in the STP and possibly reaches the terrestrial environment through the land spreading of sewage sludge.

The recommended physico-chemical tests as well as the aquatic effect studies in this tier are summarised in the Table 2.

Table 2 Physical-chemical, fate and effects studies recommended in Phase II Tier A

Study type	Recommended test protocol
Adsorption – desorption using a batch equilibrium method	OECD 106/OECD 121/OPPTS 835.110
Ready biodegradability test	OECD 301
Aerobic and anaerobic transformation in aquatic sediment systems	OECD 308
Activated sludge respiration inhibition test	OECD 209
Algae, growth inhibition test	OECD 201
Dapnia sp. reproduction test	OECD 211
Fish, early life stage toxicity test	OECD 210

Three standard long-term toxicity tests on fish, *Daphnia* and algae are proposed to determine the predicted no-effect concentration (PNEC – the environmental level at which no adverse effect on aquatic ecosystem function is to be expected). Short-term testing is generally not applicable for human pharmaceuticals since continuous exposure of the aquatic environment via STP effluents is assumed.

Blue-green algae (*Cyanophyta*) are recommended for effects testing of antimicrobials, as they are more sensitive indicator organisms than green algae (OECD 201), along with the activated sludge respiration inhibition test (OECD 209).

The calculation of the PNEC is based upon:

- $PNEC_{water}$ is calculated from the lowest NOEC result
- $PNEC_{microorganism}$ is based on the NOEC of the anti-microbial effect study
- $PNEC_{groundwater}$ is based on the NOEC result of the test with *Daphnia*
- $PEC_{groundwater} = 0.25 \cdot PEC_{Surface\ Water}$ for compounds with K_{oc} <10000 L Kg^{-1} that are not ready biodegradable or DT_{90} is >3 days.

To calculate the PNEC, an assessment factor (AF) of 10 is applied to the NOEC (no observed effect concentration). The AF is an expression of the degree of uncertainty in the extrapolation from the test data on a limited number of species to the actual environment.

At the end of Phase II Tier A, information from the screening data set are available comprising long-term toxicity data for algae, *Daphnia* and fish; data on microbial inhibition; and information on the rate of adsorption (K_{oc}). The $PEC_{Surface\ Water}$ has been refined with information on the predicted sales of the product. Different outcomes of Tier A are outlined, both for water and groundwater.

- $PEC_{Surface\ Water}/PNEC_{Water} < 1$: further testing not necessary
- $PEC_{Surface\ Water}/PNEC_{Water} > 1$: further evaluation necessary in Tier B (preferably fate of drug)
- $PEC_{Groundwater}/PNEC_{Groundwater} > 1$: further evaluation on fate of drug
- $PEC_{Surfacewater}/PNEC_{Miicroorganism} > 0.1$: further evaluation of fate and effects of drug on microorganisms
- Log Kow > 3 : testing of the bioconcentration factor should be considered
- If the compound is not readily biodegradable, special care must be taken if the affinity for the drug substance to bind to sewage sludge is high (K_{oc} >10000 L kg^{-1}) or the results from the water sediment study demonstrate significant shifting of the drug substance to the sediment (>10% of the substance at any time point after or at 14 days is present in sediment)

4.2. Phase II Tier B

If in Tier A the potential for the medicinal product to harm the environment has been identified, than a Tier B assessment should be conducted. In Tier B the PEC or PNEC is further refined according to the outcome of Phase II Tier A (see above).

4.2.1. Extended fate evaluation

For further refinement of PEC, the following test can be used for determining the fate of the drug:

- STP modelling (e.g. SimpleTreat)
- Adsorption of substances to sludge (OECD 106)
- Biodegradability(OECD 301)

The refined risk assessment may be performed using the refined PEC and PNEC for the parent compound, as well as using the dedicated PEC and PNEC for relevant (≥ 10% of amount excreted) metabolic fractions.

4.2.2. Extended effects analysis

The below mentioned effect studies are relevant depending on the Tier A outcome:

- Effects on sediment dwelling organism (OECD 308)
- Standardised tests on single microbial species
- Terrestrial environmental fate and effect (OECD 307, 216, 208, 207 and ISO 11267)

At the end of Phase II Tier B, information from the refined data comprises:

- route(s) of excretion
- qualitative and quantitative information of excreted compounds
- possibly additional long-term toxicity data
- additional data on microbial inhibition
- additional information on the biodegradability of the substance

4.2.3. Precautionary and safety measures to be taken

When the possibility of environmental risks cannot be excluded, precautionary and safety measures may consist of :

- An indication of potential risks presented by the medicinal product for the environment
- Product labelling for patient use, product storage and disposal

The labelling should aim at minimising the quantity discharged into the environment by appropriated mitigation measures. Via the labelling, the patients are encouraged to not dispose of unused medicines via wastewater or household waste.

5 Technical Guidance Document on Risk Assessment (TGD)

Risk assessment for the environment according to the EU TGD [3] follows the pattern described in Figure 2. The TGD is a general guideline for risk assessment for chemicals, and does not describe pharmaceuticals in particular.

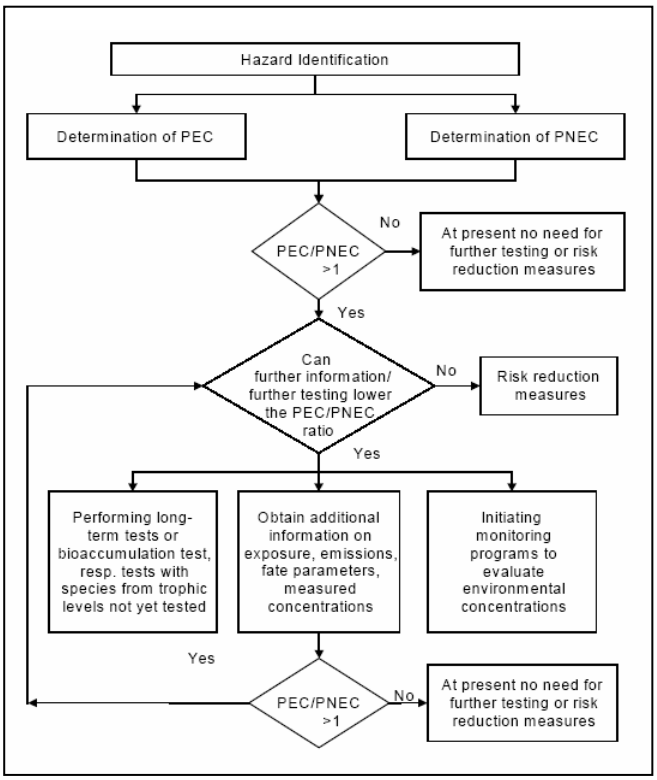

Figure 2 General procedure for environmental risk assessment according to the EU TGD

5.1. Estimation of PEC

Calculation of PEC follows the scheme indicated in Figure 3.

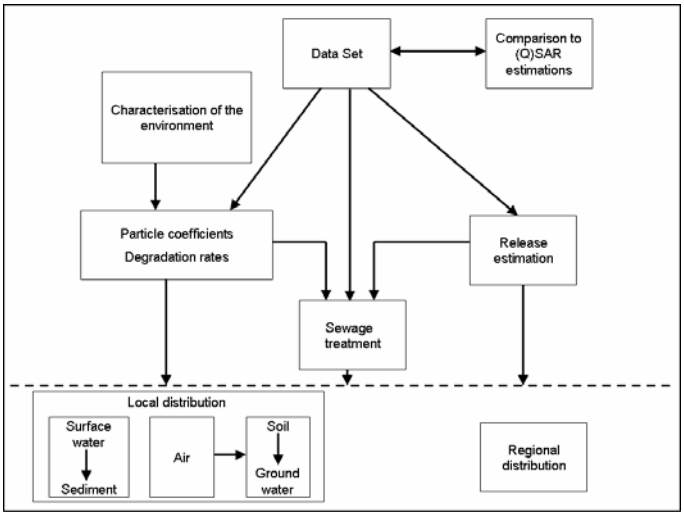

Figure 3. Calculation of PEC according to the EU TGD

For pharmaceutical products the PEC will mainly be determined after release to the environment from private use, or use in hospitals. In the TGD, measured environmental concentrations are preferred to determine the PEC. For new compounds however, modelled concentrations can be used. To estimate the PEC, the estimation of release to the environment (ch. 2.3.3 in the TGD), partition coefficients (ch. 2.3.5) and degradation rates (ch. 2.3.6) are essential.

For pharmaceutical products, the most relevant partition coefficients are those between water and sludge/sediment/soil/suspended matter. Adsorption to solid surfaces is the main partitioning process that drives distribution in soil, surface waters, and sediments. The adsorption of a substance to soil, sediment, suspended matter and sludge can be obtained or estimated from:

- direct measurement
- simulation testing
- K_{oc} measured by adsorption studies (EC C18; OECD 106, 2000a)
- K_{oc} measured by the HPLC-method (EC C19; OECD 121, 2001a)
- adsorption control within an inherent biodegradability test
- if no Koc is available, it may be estimated from K_{ow}

Degradation can consist of hydrolysis, photolysis or biodegradation, the last being most relevant to pharmaceutical compounds. The biodegradation takes place either in the STP or in water/soil/sediment. The assessment of biodegradability and/or removal in sewage treatment plants should preferably be based on results from tests simulating the conditions in treatment plants. Such a test may be the OECD 303 test (2001b) or equivalent.

The ready biodegradability tests that are currently used are aimed at measuring the ultimate biodegradability of a substance. They do not give a quantitative estimate of the removal percentage in a wastewater treatment plant. In order to make use of the biodegradation test results, it is necessary to assign rate constants (0.3-1 $k \cdot h^{-1}$) to the results of the standard tests for use in STP-models. Examples of ready biodegradability tests are:

- Ready biodegradability testing (28 d) (92/69/ EU Annex V C.4 A-F)
- OECD 301A-F (1992f)

By use of inherent biodegradability tests, a lower rate constant is assigned (0-0.1 $k \cdot h^{-1}$). Examples of such tests are:

- Inherent biodegradability testing (28d) (87/302/EEC)
- Zahn-Wellens test (EU Annex V C.9, OECD 302B, 1992g)
- MITI-II test (OECD 302C, 1981d)

Degradation in surface water/soil/sediment is estimated by the following tests:

- ISO 11734 guideline (ISO 1995) (anaerobic biodegradation)
- Draft ISO/DIS 14952-1 (organic substances at low concentration in surface waters) (basis of a draft OECD guideline “Simulation test – Aerobic mineralisation in surface water”
- OECD 307 “Aerobic and anaerobic transformation in soil” (OECD, 2000b; EU Annex V draft C.23)
- OECD 308 “Aerobic and anaerobic transformation in aquatic sediment systems” (OECD, 2000c; EU Annex V draft C.24) are available.

5.1.1. STP treatment

It is assumed that wastewater will pass through a STP before being discharged into the environment. It is also assumed that 80% of the wastewater is treated in a biological STP and the remaining 20% released directly into surface waters. This is not the case in Norway, where only 31% of the Norwegian STPs are using a biological step in their treatment (numbers from 2005, www.ssb.no). This means that most of the Norwegian wastewater is treated less than is the case for the rest of Europe, and as assumed in the TGD.

The percentage removal in STPs should preferably be based upon measured influent and effluent concentrations.

If estimation of the removal of the substance in a STP is not available, simulation tests can be used:

- OECD guideline on simulation testing of aerobic sewage treatment (OECD, 2001b)
- Coupled Units Test (OECD, 1981b).
- DOC (Dissolved Organic Carbon) and/or COD (Chemical Oxygen Demand) (removability is determined by monitoring the changes)
- ISO/DIS 14952-1
- Draft OECD (2001d)
- OECD 307 (soil, 2000b)
- Draft EU Annex V C.23
- OECD 308 (sediment, 2000c)
- Draft EU Annex V C.24

If there are no measured data available, the degree of removal can be estimated by means of a wastewater treatment plant model using log K_{ow} (K_{oc} or more specific partition coefficients can also be used).

5.2. Estimation of PNEC

For the aquatic environment, a PNEC is derived that ensures overall protection of the environment if not exceeded. The size of the assessment factor (AF) depends on the confidence with which a PNEC can be derived from the available data. This confidence increases if data are available on the toxicity to organisms at a number of trophic levels, taxonomic groups and with lifestyles representing various feeding strategies. Thus lower AFs can be used with larger and more relevant datasets than the base-set data.

When only short-term toxicity data are available, an AF of 1000 will be applied to the lowest L(E)C50 of the relevant available toxicity data, irrespective of whether or not the species tested is a standard test organism (See table 3 for AFs). A lower assessment factor will be applied on the lowest NOEC derived in long-term tests with a relevant test organism.

The algal growth inhibition test of the base-set is, in principle, a multigeneration test. However, for the purposes of applying the appropriate assessment factors, the EC50 is treated as a short-term toxicity value.

Table 3 Assessment factors to derive a PNEC

Available data	Assessment factor
At least one short term L(E)C50 from each of three tropic levels of the base set (fish, *Daphnia* and algae)	1000
One long-term NOEC (either fish or Dapnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50
Long-term NOECs from at least three species (normally fish, Dapnia and algae)representing three tropic levels	10
Species sensitivity distribution method	5-1
Field data or model ecosystems	Reviewed case to case

5.3. Refinement of PNEC: Stategy for further testing

To refine the PNEC, the TGD gives a strategy for further testing. This consists of the following tests:

- Fish early-life stage toxicity test (OECD 210, 1992h)
- Fish, short-term toxicity test on embryo and sac-fry stages (EU Annex V C.15, OECD 212, 1998c)
- Fish, juvenile growth test (EU Annex V C.14, OECD 215, 2000d)
- Fish, prolonged toxicity test, 14-day study (OECD 204, 1984c)
- *Daphnia magna* reproduction test (EU Annex V C.20, OECD 211, 1998b)
- Algae toxicity test (EU Annex V C3, OECD 201, 1984a)

Table 4 Decision table for aquatic toxicity testing when results from a full base set (FBS) using an assessment factor on the lowest L(E)C50 show that PEC/PNEC>1

Variation in base set data	Further testing	Data available for assessment after testing	Assessment factor
No significant difference between the L(E)C50 values of fish, *Daphnia* or algae	Long-term fish test + long-term Dapnia test + determination of NOEC algae	FBS + algae + Dapnia + fish	10
Fish LC50 more than 10 times lower than L(E)C50 of *Daphnia* and algae	Long-term fish test + determination of NOEC algae If S/L* ratio for fish > 20: long-term *Daphnia* test	FBS + algae + fish FBS + algae + fish + *Daphnia*	50 10
Daphnia L(E)C50 more than 10 times lower than L(E)C50 of fish and algae	Long-term Dapnia test + determination of NOEC algae If S/L ratio for Daphnia > 20: long-term fish test	FBS + algae + Daphnia FBS + algae + fish + Daphnia	50 10
Algae L(E)C50 more than 10 times lower than L(E)C50 of fish and Daphnia	Test on other alga species + long-term fish/Daphnia test	FBS + two algae + fish/Daphnia	10
Fish LC50 more than 10 times higher than L(E)C50 of Dapnia and algae	Long-term Daphnia test + determination of NOEC algae If S/L ratio for Daphnia >20; long-term fish test	FBS + algae + Daphnia FBS + algae + fish + Daphnia	50 10
Daphnia L(E)C50 more than 10 times higher than L(E)C50 of fish and algae	Long-term fish test + determination of NOEC algae If S/L ratio for fish >20; long-term Daphnia test	FBS + algae + fish FBS + algae + fish + Daphnia	50 10
Algae L(E)C50 more than 10 times higher than L(E)C50 of fish and Daphnia	Long-term Daphnia test + long-term fish test + determination of NOEC algae	FBS + algae + fish + Daphnia	10

* S/L refers to the short-term to long-term ratio, i.e. the ratio between the L(E)C50 from a short-term test and the NOEC from a long-term test

5.4. Summary: TGD and EMEA comparison

The EMEA guideline has taken the essence of the TGD and used the specific elements required for the assessment of a specific group of compounds (i.e. pharmaceuticals). The guidance provided by the EMEA guidelines is therefore very specific to the scenario related to the main inputs of human pharmaceuticals (Figure 1). The clearest example of this is the use of long-term chronic bioassay test data for the generation of pharmaceutical specific PNEC since it is widely accepted that pharmaceutical compounds represent a greater chronic than acute risk.

The initial PEC estimation is also quite simplistic with an action limit set to PEC 0.01 µg/L. This means that in general, a DOSEai of more than 2 mg (maximum daily dose consumed per inhabitant) will initiate a Phase II environmental fate and effect analysis. This approach to PEC generation and a more detailed approach may be more suitable in individual countries. The simplistic PEC estimation in the EMEA guideline reflects the situation common in EU countries, that statistics about use of pharmaceuticals in the community is sparse. This is in contrast to the situation in Norway where legislation is that all wholesales in Norway are reported to the Norwegian Institute of Public Health.

As stated within the TGD, measured environmental concentrations will always provide a better assessment of what volumes of a substance are entering the aquatic environment. This will be evaluated for the SFT selected pharmaceuticals in the next Chapter of this report.

6 Environmental Risk Assessment of selected compounds according to EMEA guidelines

On assignment from SFT, NIVA has performed a risk assessment of selected compounds according to the EMEA guidelines. The suite of compounds were selected by SFT, and consisted of the following pharmaceutical compounds:

- Paracetamol (analgesic, ATC codes N02AA59 (in combination with codeine), N02AC54 (in combination with dextropropoxyphene) and N02BE01)
- Ibuprofen (analgesic, anti-inflammatory and antirheumatic product, ATC codes M01AE01 and M02AA13)
- Metoprolol (heart medicine, betablocking agent ATC code C07AB02)
- Diclofenac (anti-inflammatory and antirheumatic product, ATC codes D11AX, M01AB05 and S01BC03)
- Tetracycline (antibiotic, ATC code J01AA07)
- Ciprofloxacin (antibiotic, ATC codes J01MA02, S01AX13 and S02AA15)
- Trimethoprim (antibiotic, ATC codes J01EA01 and J01 EE01)
- Sulfamethoxazole (antibiotic, ATC code J01EE01)
- Cefuroxime (antibiotic, ATC code J01DC02)
- Ethinylestradiol (hormonal contraceptive, ATC codes G03AB04, G03AB03, G03AA12, G03AA07, G03AA13, G03AA09, G03HB01
- Cyclophosphamide (antitumor, antineoplastic agent ATC code L01AA01)

The ATC codes (the Anatomical Therapeutic Chemical Classification System) is used for the classification of drugs. It is controlled by the WHO Collaborating Centre for Drug Statistics Methodology. Drugs are divided into different groups according to the organ or system on which they act and/or their therapeutic and chemical characteristics.

6.1. EMEA Phase I assessment of SFT prioritised pharmaceuticals

6.1.1. Estimation of PECs according to EMEA guideline

The basic physico-chemical properties and EMEA Phase I data required as well as the calculated PEC are presented in Table 5. Diclofenac has a log K_{ow} greater than 4.5, and therefore needs to be screened for persistence, bioaccumulation and toxicity (PBT) according to the EU TGD.

The PECs of the suite of pharmaceutical compounds listed by SFT were estimated according to the EMEA guidelines. The DOSEai was found in the Norwegian pharmacopoeia (www.felleskatalogen.no). The DOSEai for some of the pharmaceuticals is higher than the defined daily dose (DDD) [4] for the same compounds which are given for comparison. The use of the maximal dose is recommended by the guideline. A comment on which drug has been the basis of the DOSEai has been made in the "Comment" column. For some compounds, the DDD is not assigned. For ethinylestradiol the DOSEai was calculated by assuming an intake of one tablet containing ethinylestradiol every day (hormonal contraceptives normally consist of 28 tablets where 7 of those contain no drugs). The PEC was estimated according to the EMEA guideline equation (this report, chapter 2), assuming a market penetration of 1% for all the compounds.

Table 5 Estimation of PEC according to the EMEA guidelines

Substance	DOSEai (mg)	Log K_{ow}	PKa	**PEC (EMEA) (µg/L)**	DDD [4] mg	PEC (Pharmatreat) (µg/L)	PEC EMEA/PEC Pharm	Comment
Paracetamol	3000	0.46	9.5	**15**	3000	43.97	0.3	
Ibuprofen	1200	3.97	4.4	**6**	1200	8.44	0.7	
Metoprolol	800	1.88	9.6	**4**	150	1.77	2.3	Metoprolol™ (hypertyreosis)
Diclofenac	450	**4.51**	4.2	**2.25**	100	0.5	4.5	Voltaren™
Tetracycline	1000	-1.19	3.3	**5**	1000	0.33	15	
Ciprofloxacin	1500	0.4	6.4	**7.5**	1000	0.28	27	
Trimethoprim	400	0.91	7.12	**2**	400	0.17	12	
Sulfamethoxazole	2400	0.89		**12**	na	0.07	171	Bactrim™
Cefuroxime	9000			**45**	500-3000 (O-P)	0.03	1500	Cefuroxim™ (meningitis)
Ethinylestradiol	0.035	4.15		**0.0002**	na	0.00060	0.3	Synfase™
Cyclophosphamide	500	0.63		**2.5**	na	0.00027	9259	Sendoxan™ (individual treatment)

na: not assigned

For all the compounds except ethinylestradiol, the PEC is above the action limit of 0.01 µg/L. Since ethinylestradiol has endocrine disrupting properties, this pharmaceutical still has to be further evaluated.

6.1.2. Other model of estimating PECs

The PECs estimated by NIVA in the NFR funded SIP "Pharmatreat" are also given in the table for comparison. Data were collected in collaboration with the Norwegian Public Health Institute for all drug sales data from all wholesalers in Norway during 2005. The wholesales statistics database includes complete national figures from wholesalers to pharmacies, hospitals and non-pharmacy outlets. The data collected contained information of the number of defined daily doses (DDD) sold for each drug; and also the quantity of packages sold. Information on the DDD for each pharmaceutical in each ATC code (Anatomical Therapeutic Chemical Classification System controlled by WHO) was collected [4] to calculate the amount of drug (in kilograms) sold during 2005. For pharmaceuticals appearing in more than one ATC code, and consequently possibly having different DDD for each ATC code, data was summarised. For pharmaceuticals without DDD, the amount sold (in kilograms) was estimated based on information from the Norwegian pharmacopoeia (www.felleskatalogen.no).

Having estimated the amount sold during 2005, the predicted environmental concentration (PEC) in water was calculated according to the following equation [5]:

$$\mathrm{PEC\,(Pharmatreat)_{surface\ water}}\ \left(\mathrm{gL^{-1}}\right) = \frac{A \times (1 - R/100)}{365 \times P \times V \times D}$$

Where:

A= predicted amount used per year in the relevant geographic area (kg)
R= removal rate (due to loss by adsorption to sludge particles by volatilisation, by hydrolysis, by biodegradation or other specific, naturally occurring processes
P= number of inhabitants of the geographic area considered
V= volume of wastewater per capita and day ($m^{3)}$, normally between 0.15 and 0.3 m^3 in EU
D= Dilution of waste water by surface water flow (average factor 10)

The wastewater per capita was estimated to 190 L, and a dilution factor of 10 was used. The WTW removal rate was set to 0 (worst case scenario). The PEC was estimated for approximately 200 compounds, based on frequency of use and estimated risk associated with the compound.

6.1.3. Comparison of EMEA PEC estimation to other model PEC estimation

The literature model [5] of estimating the PEC may be more closely linked to the true emission numbers, assuming that people buying pharmaceutical compound actually use these compounds. From the ERA in Sweden in 2004 [6] data indicate that 90% of pharmaceuticals sold in Sweden is consumed, so this seems to be a fair assumption.

From the comparison of the two estimated PECs, it can be seen that the PEC (EMEA) for a number of pharmaceuticals is quite similar to PEC (Pharmatreat), however for others there are significant differences. For example, the PEC (EMEA) provided lower estimations than the PEC (Pharmatreat) for paracetamol, ibuprofen and ethinylestradiol. These three compounds are frequent use pharmaceuticals with a market penetration of more than 1%.

Numbers from the Public Health Institute indicate that the actual market penetration percentages for the three compounds are 2.6% (this number is for (ATC code N02BE01 (paracetamol alone), in addition comes drugs with paracetamol in combination with other drugs), 3.6% (ibuprofen as tablets, in addition ibuprofen in gel preparations) and 4.3% (ethinylestradiol) respectively. For the estimation of market penetration data for ethinylestradiol, data were summarised regarding the following ATC-codes: G03AB04 (Synfase), G03AB03 (Trionetta), G03AA12 (Yasmin), G03AA07 (Loette 28 and Microgynon), G03AA13 (Evra), G03AA09 (Marvelon and Mercilon), G03HB01 (Diane and Feminil Sandoz).

For the less frequently used pharmaceuticals, the PEC EMEA is higher than the PEC (Pharmatreat). This is particularly true for the anticancer agent cyclophosphamide, which is certainly not used by 1% of the population. The difference between the two PECs for the antibacterial cefuroxime is also large. Comparisons with measured environmental concentrations (MEC) for this suite of substances from another SFT commissioned project (SFT contract 6006125) will provide further information on the accuracy of the different PEC estimation approaches used.

However, for the estimation of a new drug on the market, the use of a market penetration of 1% seems reasonable, and will give an indication of which PEC to expect after introduction onto the market.

6.2. EMEA Phase II Tier A assessment of SFT prioritised pharmaceuticals

A literature search was performed regarding the physical-chemical tests and aquatic effects studies recommended by EMEA (Table 2). This search utilised data from both published and non-published (e.g. Boucard, Pers. Comm.) sources. The results are shown in Table 6.

6.2.1. Physico-chemical properties, fate and effects

For all the selected pharmaceutical substances a literature value for the ready biodegradability or removal rate in STP system was available. These data are presented in Table 6. Since removal rates are evaluated as a good method in the TGD, the results of such studies are used in this report. The results for paracetamol and ibuprofen all indicate that these compounds are readily biodegradable with low persistence. Regarding diclofenac, two reports on its removal rates in STPs show that the removal rate is quite low, 39% in one case and 22% in another. The biodegradability of diclofenac is 93% when performed in soil, not in sludge systems. For the rest of the compounds, the results show that the biodegradation of these compounds is relatively low.

The sorption behaviour (K_d or K_{oc}) of all compounds apart from cyclophosphamide has been reported. All compounds with the exception of tetracycline exhibited a low affinity for organic carbon. Since also tetracycline is below the limit of log Koc < 4, the environmental assessment of the pharmaceutical compounds in terrestrial systems is therefore not necessary.

Table 6 Available physico-chemical properties, fate and effects of substances according to Phase II Tier A EMEA guidelines

Substance	Adsorption using a batch equilibrium method				Ready Biodegradability test		Transformation sediment systems		Algae growth inhibition test		*Daphnia* sp. reproduction test		Early life stage tox.test (fish)		Respiration inbibition test	
	K_d abs	Ref	K_{oc}	Ref	%transformation or DT_{50} (d)	Ref	DT_{50} (d)	Ref	EC_{50} (mg L^{-1})	Ref	EC_{50} (mg L^{-1})	Ref	mg L^{-1}	Ref	IC_{50} (mg L^{-1})	Ref
Paracetamol	36-45 1-10% 64-53%	[7] [8] [9]	62	[8]	Low persist. 57% 99%	[9] [10] [11]	3.1	[9]	134	[10]	50[a] 9.2[a]	[10] [12]	19[a]	[10]		
Ibuprofen	6-64 10-20% 17-9% 453	[7] [8] [9]	324	[8]	<LOQ 28 d >90%[b] 10-60%	[9] [13] [14]	<6	[9]	0.001 7.1 EC_5 72.9 NOEC 1	[15] [16] [17] [46]	9.06[a] EC_5 58.4[a] NOEC 20	[16] [17] [18]	NOEC 1.02 173	[19] [16]		
Metoprolol	9.6-37.6[c] 3-8%	[8]	2803[c]		0%[b] <10%[b]	[20] [13]	Negligible				77.5 63.9[a] 8.8[a,d] LOEC 6	[21] [22] [22] [23]	>100 944	[22] [24]		
Diclofenac	4-10 164.5[f] 0.45[g]	[7] [25] [25]	2310	[25]	93% 39%[b] 22%[b]	[7] [13] [20]			EC_5 44.2 NOEC 10	[17] [26]	41[a,d] EC_5 10 NOEC 10 NOEC 1[d]	[21] [17] [18] [26]	4 1[a] 0.0005 LOEC 0.005 LOEC 0.001	[26] [27] [28] [29] [30]		
Tetracycline	8400 1140-1620	[31] [32]	6059	[33]	0-62% 2%	[34] [35]			16 2.2 0.09	[36] [37] [37]	44.8 EC_{10} 29.4	[38] [38]	220	[39]	Time curve 1-10	[34] [40]
Ciprofloxacin	430 417	[33] [41]	61000[e]	[33]	0% 1.6-2.5	[42] [41]			0.017 0.005	[43] [41]	>10[a] NOEC 60[a]	[43] [41]	NOEC 100[a]	[41]	0.08	[33]
Trimethoprim	76	[41]			22-41 d 4%	[41] [35]			110 16	[41] [44]	123[a]	[41]	NOEC 100[a]	[41]	>100	[40]
Sulfamethoxazole	37.6 0.23	[25] [25]	530	[25]	0 % 4%	[45] [35]			NOEC 0.0059 EC_{10} 17, EC_{50} 81 0.52	[26] [46] [47]	NOEC 0.25[d] 0.21[d] 25.2[a]	[26] [47] [47]	NOEC >8 NOEC 1000	[26] [47]	>100 256	[40] [33]
Cefuroxime			1,09-1,19	[48]	28% -1%	[48] [35]			NOEL 91	[48]	>1000	[48]	NOEL 120[a]	[48]	>100 >100	[40] [48]
Ethinylestradiol	40% 691	[49] [50]			20%	[51]	1%	[49]	0.84	[16]	0.1	[52]	1.6 0.000001	[16] [53]		
Cyclophosphamide					0% 0%	[54] [55]	Negligible		>100	This study	NOEC 56	This study				

[a] Acute toxicity data [b] Removal rate STP [c] data for propranolol [d] Other invertebrate [e] Soil
[f] high organic content [g] low organic content

Table 7 Refined PEC according to Phase II guidelines and determined PEC/PNEC ratios

EMEA	PEC EMEA (µl/L)	Suspended solids (mg/L)	Kd L/kg	Refined PECads	Biodeg. Max (%)	Biodeg. Min (%)	Refined PEC min	Refined PEC max	Lowest NOEC/EC50 (mg/L)	AF	PNEC µg/L	PEC min./ PNEC	PECmax/ PNEC max
Paracetamol	15	17,0	36	14,91	0,99	0,57	0,1491	6,4108	9,2	1000	9,2	0,016	0,70
Ibuprofen	6	17,0	453	5,57	0,90	0,1	0,5571	5,0139	1	50	20	0,028	0,25
Metoprolol	4	17,0	37	3,97	0,10	0	3,5775	3,9750	3,1	100	31	0,12	0,13
Diclofenac	2,25	17,0	165	2,19	0,93	0,22	0,1532	1,7071	0,00115	10	0,115	**1,3**	**15**
Tetracycline	5	17,0	8 400	2,06	0,62	0	0,7825	2,0593	0,09	1000	0,09	**8,7**	**23**
Ciprofloxacin	7,5	17,0	417	7,00	0	0	7,0035	7,0035	0,005	1000	0,005	**1401**	**1401**
Trimethoprim	2	17,0	76	1,97	0,04		1,8955	1,9745	16	1000	16	0,12	0,12
Sulfamethoxazole	12	17,0	38	11,92	0,04	0	11,4461	11,9230	0,0059	50	0,118	**97**	**101**
Cefuroxime	45	17,0		45,00	0,28	0	32,4000	45,0000	91	1000	91	0,36	0,49
Ethinylestradiol	0,000175	17,0	691	0,00	0,20		0,0001	0,0002	0,000001	10	0,0001	**1,3**	**1,6**
Cyclophosphamide	2,5	17,0		2,50	0,00	0	2,5000	2,5000	56	50	1120	0,002	0,002
Pharmatreat	PEC Pharmatreat												
Paracetamol	43,97	17,0	36	43,70	0,99	0,57	0,4370	18,7921	9,2	1000	9,2	0,048	**2,0**
Ibuprofen	8,44	17,0	453	7,84	0,90	0,1	0,7837	7,0529	1	50	20	0,039	0,35
Metoprolol	1,77	17,0	37	1,76	0,10	0	1,5830	1,7589	3,1	100	31	0,051	0,057
Diclofenac	0,5	17,0	165	0,49	0,93	0,22	0,0340	0,3794	0,00115	10	0,115	0,30	**3,3**
Tetracycline	0,33	17,0	8 400	0,14	0,62	0	0,0516	0,1359	0,09	1000	0,09	0,57	**1,5**
Ciprofloxacin	0,28	17,0	417	0,26	0	0	0,2615	0,2615	0,005	1000	0,005	**52**	**52**
Trimethoprim	0,17	17,0	76	0,17	0,04	0	0,1611	0,1678	16	1000	16	0,010	0,010
Sulfamethoxazole	0,07	17,0	38	0,07	0,04	0	0,0668	0,0696	0,0059	50	0,118	0,57	0,59
Cefuroxime	0,03	17,0		0,03	0,28	0	0,0216	0,0300	91	1000	91	0,0002	0,0003
Ethinylestradiol	0,00060	17,0	691	0,00	0,20	0	0,0004	0,0005	0,000001	10	0,0001	**4,3**	**5,4**
Cyclophosphamide	0,00027	17,0		0,00	0,00	0	0,0003	0,0003	56	50	1120	0,0000002	0,0000002

$PEC_{Ads:}$ Refinement made based upon partition coefficient (Kd), AF: Assessment Factor.

Table 8 Species used in aquatic effect studies, duration and scheme followed during testing

Substance	Alga				Daphnia			Fish			
	Ref.	Species	Duration	OECD 201	Ref.	Duration	OECD 211	Ref.	Species	Duration	OECD 210
Paracetamol	**[10]**	*Scenedesmus subspicatus*	72 h	201	[10]	?	OECD 202	[10]	*Brachydanio rerio*	48 h	Acute toxicity fish embryos
					[12]	48 h	DIN 38412 part II				
Ibuprofen	**[15]**	*Lemna gibba*	7 d	USEPA 1996							
	[16]	*Skeletonema costatum*	96 h	?	[16]	48 h	Acute tox	[16]	*Lepomis macrochirus*	96 h	Acute toxicity
	[17]	*Scenedesmus subspicatus*	?	EU guideline 1993	[17]	24+48 h	EU 1992				
	[46]	*Lemna gibba*	7 d	ASTM 1998	**[18]**	21 d	US EPA 1994	[19]	*Planorbis carinatus*	21 d	?
Metoprolol					[21] *T. platyurus*	24 h	Kit, crustacean larvae				
					[22]	48 h	US EPA 1991	[22]	*Oryias latipes*	48 h	Acute toxicity
					[22] *C.dubia*	48 h	US EPA 1992	[24]	*?*	?	?
					[23]	18 d ?	US EPA 1994				
Diclofenac	[17]	*Scenedesmus subspicatus*	?	EU guideline 1993	[17]	24+48 h	EU 1992	**[26]**	*Danio rerio*	10 d	ISO 12890
								[27]	*Danio rerio embryos*	96 h	delayed hatching
				kit. crustacean larvae	**[18]**	21 d	US EPA 1994	**[28]**	*Salmo trutta. 18 months*	21 d	various biomarkers measured
					[21] *T. platyurus*	24 h	Kit, crustacean larvae	[29]	*Oncorhynchus mykiss*	28 d	histopathology
	[26]	*Synechococcus leopolensis*	96 h		[26] *C. dubia*	7 d	AFNOR T90-375	**[30]**	*Oncorhynchus mykiss*	28 d	cytological alterations
Tetracycline	**[36]**	*Nitszcia closterium*	72 h	?	**[38]**	21 d	OECD 211	[39]	*Salvelinus namaycush*	96 h	?
	[37]	*Selenastrum capricornutum*	7 d	ISO 1989							
	[37]	*Microcystis aeruginosa*	7 d	ISO 1989							
Ciprofloxacin	**[41]**	*Microcystis aeruginosa*	?	OECD 201	[41]	48 h	OECD 202	[41]	*Brachydanio rerio*	72 h	OECD 203
	[43]	*Microcystis aeruginosa*	5 d	?	[43]	48 h	US EPA 1988				
Trimethoprim	**[41]**	*Salvelinus capricornutum*	?	OECD 201	[41]	48 h	OECD 202	[41]	*Brachydanio rerio*	72 h	OECD 203
	[44]	*Rhodomonas salina*	?	ISO 8692							
Sulfamethoxazole	**[26]**	*Synechococcus leopolensis*	96 h		[26] *C. dubia*	7 d	AFNOR T90-375	[26]	*Danio rerio*	10 d	ISO 12890
	[46]	*Lemna gibba*	7 d	ASTM 1998	[47] *C. dubia*	7 d. young org.					
	[47]	*Pseudokirchneriella subcapitata*	72 h	ISO 8692	[47]	24 h	ISO 6341	[47]	*Danio rerio*	96 h	ISO 7346
Cefuroxime	**[48]**	*Selenastrum capricornutum*	72 h	?	[48]	48 h	?	[48]	*Oncorhyncus mykiss*	96 h	?
Ethinylestradiol	[16]	*Unspecified algae*	?	?				[16]	*Oncorhyncus mykiss*	96 h	?
					[52]	?	OECD 211	**[53]**	*Danio rerio*	From day 20 to 60 days post hatch	Sex ratio diff. observed at given conc

The EMEA guidleine recommends that compounds that are not readily biodegradable should be investigated in a water-sediment study. Data are only available for the two substances which are regarded as readily biodegradable; paracetamol and ibuprofen. The transformation of compounds in sediment systems is beyond the scope of this project, and was therefore not further pursued. However, this does demonstrate that environmental data for certain pharmaceutical compounds, even high use generic pharmaceuticals such as ibuprofen and paracetamol, are not available.

6.2.2. Determination of PNEC

Ecotoxicological data were available for all of the compounds except cyclophosphamide (Table 6). As a part of this project, algal toxicity and *Daphnia* reproduction toxicity were determined for cyclophosphamide (Appendix 1 and 2 respectively). Long-term algal data were available for most of the compounds, for *Daphnia* most of the substances also had reproduction data. Long-term data for fish were only found for diclofenac and ethinylestradiol. These long-term data are based on sex-ratio for ethinylestradiol and histopathology and cytopathology for diclofenac. Hence, the use of the NOEC from these studies must be regarded as conservative, and needs further evaluation. Regarding the algal effects of ibuprofen, we have not used the lowest observed NOEC of 1µg/L due to the fact that Pomati *et al.* [15] did not replace the medium daily, and that the result is substantially lower than other toxicity studies with the same organism [16, 46].

Our evaluation of the toxicity studies, the duration and scheme is given in table 8. For clarity, the long-term references are marked bold in table 8.

The $PNEC_{Surface\ Water}$ are presented in Table 7 and have been calculated from the lowest NOEC or EC value presented in Table 6 using the appropriate assessment factor (AF) as recommended by the TGD (Table 3). The AFs are presented alongside the PNECs in order to present the level of confidence in the available data.

6.2.3. Refinement of PEC

Since all of the PECs were > 0.01 µg L^{-1}, refinement was necessary for all substances (Table 7). This refinement takes into consideration the removal of the pharmaceutical substances through adsorption and degradation. The value for suspended solids used to calculate the $PEC_{Ads.}$ is a value determined in the Oslo area [56]. In Table 7 two PECs are presented; Refined $PEC_{Ads.}$ which is a refinement based upon adsorption to suspended particles and the refined PEC (expressed as Max. and Min.) based upon degradation. The data are presented as max. and min. to account for the variability in the available degradation rates for these compounds (see table 5). It is these PECs that have been used in the final assessment.

6.2.4. Calculation of risk quotients according to EMEA guideline

Risk quotients derived from PEC/PNEC are presented in Table 7. The derived risk quotients for the following substances are below 1 according to estimation by EMEA:

- Paracetamol
- Ibuprofen
- Metropolol
- Trimethoprim
- Cefuroxime
- Cyclophosphamide

For the remaining compounds the following have a risk quotient greater than 1

- Diclofenac
- Tetracycline
- Ciprofloxacin
- Sulfamethoxazole
- Ethinylestradiol

6.2.5. Calculation of risk quotients according to literature model of calculating PEC

Risk quotients derived from PEC/PNEC are presented in Table 7. The derived risk quotients for the following substances are below 1 according to estimation by PEC according to literature model:

- Ibuprofen
- Metropolol
- Trimethoprim
- Sulfamethoxazole
- Cefuroxime
- Cyclophosphamide

For the remaining compounds the following have a risk quotient greater than 1 when using maximal PEC

- Paracetamol
- Diclofenac
- Tetracycline
- Ciprofloxacin
- Ethinylestradiol

6.2.6. Comparison of PEC with MEC

In another SFT commissioned project the measured effluent concentrations of the selected pharmaceuticals have been performed (SFT contract 6006125). The effluent concentrations can be used to generate further refined quasi-MECs in which we will have greater confidence. Comparison of the refined PEC with quasi-MECs provides an indication of how accurate the PEC determinations, including refinements are. The median concentrations of selected compounds were measured in influent and effluent to VEAS during a seven week period from 9/8-2006 to 20/9-2006. The measured median effluent values were converted into quasi-MECs using a dilution factor of 10 (Table 9).

Table 9 Comparison of quasi-MEC and PEC

Substance	PEC (EMEA) (µg/L)	Quasi-MEC† (µg/L)	Quasi-MEC/PEC
Paracetamol	15	0.0002	0.00001
Ibuprofen	6	0.044	0.007
Metoprolol	4	0.065	0.02
Diclofenac	2.25	0.026	0.01

† Median effluent concentration divided by 10 (dilution factor).

The quasi-MECs are considerably lower than the PECs (estimated by both EMEA guideline and literature model) (Table 9). The PECs estimated by the EMEA and literature model are not very different for these four compounds, however considerable differences are observed in the measured concentrations. Paracetamol, which is efficiently removed by STPs is only present at very low concentrations. None of the compounds has a quasi-MEC > PEC which indicates that the PEC models are very conservative and precautionary, however some caution must be used when assessing the data since they are median values for one STP (VEAS, Oslo, Norway).

6.2.7. Summary of the EMEA guideline evaluation of SFT prioritised pharmaceuticals

- Two pharmaceuticals (paracetamol and sulfamethoxazole) had risk quotients which differed depending on the method used to estimate PEC.
- It was difficult to calculate PECs for certain pharmaceuticals due to variable literature biodegradation rates which gave PEC/PNEC ratios both > and < 1.
- For 4 compounds the quasi-MEC was significantly lower than the PECs.

7 Conclusions

- The EMEA guideline provides a tool to assess the risks associated with pharmaceuticals entering the aquatic environment through STPs. This is an important first step.
- When compared to the EU TGD, the EMEA guideline has taken the essence of the TGD and used the specific elements required for the assessment of a specific group of compounds from a specific source (i.e. pharmaceuticals from STPs).
- PECs generated using the EMEA guidelines are conservative and precautionary, even following refinement. The absence of good, reliable and consistent fate data makes PEC refinement difficult. This is very important when the resultant risk quotient is ~1. It may be more suitable in Norway to use a more specific approach due to the good usage data available.
- It may be more suitable to use STP models to refine the PEC since the processes occurring are complex.
- MECs will always provide better data for the calculation of environmental occurrence and therefore we recommend the use of MECs, where possible in pharmaceutical ERA.
- The major weakness in any pharmaceutical ERA is the PNEC. PNECs based on mortality, even when exposure is chronic, may not be sufficient to protect the aquatic environment. Exposure in the environment is likely to be chronic and the effects sub-lethal. We recommend that better strategies are required to deal with this.
- Basic ecotoxicity data are not available for many pharmaceuticals (e.g. cyclophosphamide). The use of basic *Daphnia* and algae data provide data for simple ERA but are not sufficient for safe-guarding against long-term sub-lethal effects since at present the effects are not known.

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TEST REPORT

Cyclophosphamide

Algal growth inhibition test with *Pseudokirchneriella subcapitata*

NIVA Study number: B481a

November 2006

INTRODUCTION

The growth inhibition effect of Cyclophosphamide on the green alga *Pseudokirchneriella subcapitata* has been investigated. The test was performed according to OECD Test Guideline201: Alga, Growth Inhibition Test (1).

The experimental phase of the definitive study was conducted between 21 and 24 November 2006.

MATERIALS AND METHODS

Test material

Identification:	Cyclophosphamide monohydrate
Sample labelling:	CAS 6055-19-2
Purity:	Not declared
Appearance:	Clear, colourless
Date received:	30/10-2006
Storage conditions:	In darkness at 3-7 °C
Expiry date:	Not declared

Test species

Name	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Strain	NIVA strain CHL 1
Source	NIVA culture collection
Stock culture	Cultured in 10 % Z8 medium (2) on reciprocating shaker and continuous light at approximately 22^{o}C.
Inoculation culture	Inoculation culture was set up one day before test initiation in the same medium as used in the test. Incubation conditions were the same as during the test (See 3.7). The cell density in the inoculum culture increased a factor 4.2 during one day.

Test medium

The composition of the test medium (OECD 201) is shown in appendix 1.

Experimental design

The toxicity test was conducted at 7 concentrations of cyclophosphamide (3.2, 5.6, 10, 18, 32, 56 and 100 mg/l). There were with 3 replicates of each concentration and 6 control replicates.

The test cultures were contained in glass vials, covered with plasic film. The culture volume was 10 ml.

Preparation of solutions

The test solutions were prepared from stock solution of 3 g/l of cyclophosphamide in test medium. The stock solution was diluted in test medium to obtain the final concentrations. Test algae from the inoculum culture were added to the solutions to obtain a cell density of approximately $5 \cdot 10^6$ cells/l of *P. subcapitata.* The same amount of test algae was added to the control test medium.

Exposure conditions

The flasks were placed on a reciprocating shaker for continuous agitation. The incubation temperature was maintained at 23±1 ^{o}C with continuous illumination from daylight-type fluorescent tubes, suspended 0.6 meters above the culture vessels and providing 60 µM m^{-2} s^{-1} direct irradiation (PAR)

Observations

pH was measured at the start and at the end of the test

Cell counts in the control cultures were performed after approximately 24, 48 and 72 hours, using a Coulter Multisizer III.

Temperature during incubation was recorded on a min./max. thermometer with the sensor placed in water at the level of the test cultures.

The irradiation (PAR) was measured once during the incubation using a unidirectional LiCor quantum sensor.

Verification of test concentrations

A test solutions with nominal concentration 18 mg/l at the start and end of the exposure period was sampled for verification of exposure concentration.

Evaluation of data

Calculation of area under growth curve

The area under growth curve is calculated according to the equation:

(i) $$A = \frac{N_1 - N_0}{2} \times t_1 + \frac{N_1 + N_2 - 2N_0}{2} \times (t_2 - t_1) + ... + \frac{N_{n-1} + N_n - 2N_0}{2} \times (t_n - t_{n-1})$$

where A = area
N_o = Cell concentration at t_o (10^6 l^{-1})
N_1 = Cell concentration at t_1 (10^6 l^{-1})
N_2 = Cell concentration at t_2 (10^6 l^{-1})
N_n = Cell concentration at time t_n (10^6 l^{-1})
t_1 = time of first measurement (hours from start)
t_n = time of nth measurement (hours from start)

Growth rate calculations

The average growth rate for each test concentration is calculated from initial cell concentration and cell concentration after 72 hours using the equation:

(ii) $$\mu = \frac{\ln(N_n) - \ln(N_0)}{t_n - t_0}$$

where the symbols are the same as in equation (i).

Calculation of effect concentrations

For both test endpoints (area under growth curve and growth rate), the percentage of inhibition as compared to the control was calculated for each treatment. The data was analysed for significant differnces between the treatments and the controls.

RESULTS

Validity criteria

	Criterium	Observed
Cell increase in controls after 72 h compared to start	≥16	507
pH increase in controls	≤1.0	0.1
Variation in mean coefficient of variation for section-by-section specific growth rates *(i.e. days 0-1, 1-2 and 2-3)*	<35 %	6.2 %

pH-values

The pH measured in test solutions at the start and end of the test are shown in appendix 3. The pH was 7.8 at the start of the test. Variation in pH in the test solutions during the test was 0.1 pH units.

Verification of test concentrations

The analysis of test solutions have not yet been completed

Algal growth

Cell density in all cultures as measured after 24, 48 and 72 hours are shown in appendix 2.
The growth curves for the test algae in control and test solutions are shown in figure 1. The curves show that growth in the control cultures was almost exponential throughout the exposure period. The growth curves in solutions with different concentrations of cyclophosphamide was very similar to the control, indicating only minor effects of cyclophosphamide on the performance of the algae.

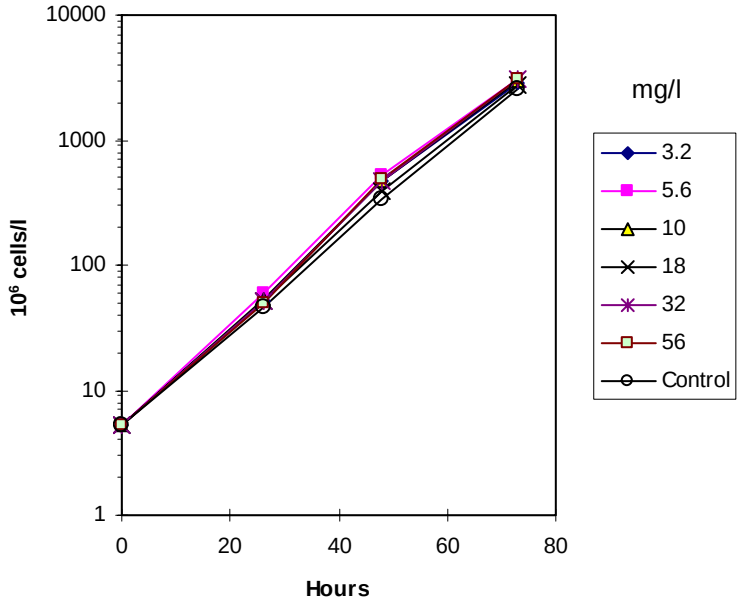

Figure 1. Growth curves for control cultures and various concentrations of the test substance (%). (Mean values of replicates).

Area under growth curve and growth rate calculated according to equation (i) and (ii) are shown in appendix 2. Mean values are presented in Table 2.

Table 2. Calculated endpoints in the algal growth inhibition test (mean values of replicates).

Concentration mg/l	Growth rate d^{-1}			Area under growth curve (10^6 cells × h × l^{-1})		
	Mean	St. d.	% of control	Mean	St. d.	% of control
Control (0)	2.03	0.01	100	40419	1527	100
3.2	2.08	0.01	102	47659	1162	118
5.6	2.10	0.02	103	52573	3397	130
10	2.08	0.03	102	48900	3808	121

18	2.06	0.02	101	44582	2371	110
32	2.10	0.02	103	50914	2546	126
56	2.10	0.02	103	51196	2255	127
100	2.04	0.02	100	42259	2091	105

Effect concentrations

No inhibition of the growth was observed within the concentration range of cyclophosphamide tested (3.2 – 100 mg/l). Hence, the effect concentrations can only be expressed as > 100 mg/l.

CONCLUSION

No inhibition of the growth of the test alga, *Pseudokirchneriella subcapitata*, was observed in the concentration range 3.2 – 100 mg/l of cyclophosphamide. The following effect concentrations for the tobramycin solution for the two test endpoints **growth rate** and **area under growth curve** can be derived:

Endpoint	**Parameter**	**mg/l**
Growth rate	E_rC_{50}	>100
Growth rate	E_rC_{10}	>100
Growth rate	NOEC	≥100
Area under growth curve	E_bC_{50}	>100
Area under growth curve	E_bC_{10}	>100

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Appendix 1: Composition of test medium

Composition of test medium for toxicity test with the green algae *Pseudokirchneriella subcapitata* (ISO 8692)

Salt	**Concentration**
$NaHCO_3$	50 mg/l
NH_4Cl	15 mg/l
$MgCl_2 \cdot 6H_2O$	12 mg/l
$CaCl_2 \cdot 2H_2O$	18 mg/l
$MgSO_4 \cdot 7H_2O$	15 mg/l
KH_2PO_4	1.6 mg/l
$FeCl_3 \cdot 6H_2O$	64 µg/l
$Na_2EDTA.2H_2O$	100 µg/l
H_3BO_3	185 µg/l
$MnCl_2 \cdot 4H_2O$	415 µg/l
$ZnCl_2$	3 µg/l
$CoCl_2 \cdot 6H_2O$	1.5 µg/l
$CuCl_2 \cdot 2H_2O$	0.01 µg/l
$Na_2MoO_4 \cdot 2H_2O$	7 µg/l

APPENDIX 2: Cell density measurements

	Day:	0	1	2	3
	Hours:	0	24	48	72
		10^6 cells/l	10^6 cells/l	10^6 cells/l	10^6 cells/l
Control	a	5	46	325	2719
"	b	5	46	337	2570
"	c	5	44	306	2472
"	d	5	48	342	2422
"	e	5	45	375	2587
"	f	5	44	336	2457
3.2	a	5	50	508	2847
"	b	5	48	430	2830
"	c	5	55	452	2929
5.6	a	5	59	547	3196
"	b	5	59	495	2878
"	c	5	59	545	3295
10	a	5	53	467	2918
"	b	5	56	519	3176
"	c	5	54	449	2706
18	a	5	52	403	2953
"	b	5	50	376	2686
"	c	5	54	378	2661
32	a	5	53	496	3266
"	b	5	53	449	2947
"	c	5	51	465	3129
56	a	5	50	506	3243
"	b	5	50	491	2912
"	c	5	50	479	3144
100	a	5	47	365	2508
"	b	5	48	414	2726
"	c	5	48	392	2507

Calculated average growth rate and area under growth curve.

		growth rate	area under growth curve
Control	a	2.06	42417
"	b	2.04	40837
"	c	2.03	38835
"	d	2.02	39152
"	e	2.04	41918
"	f	2.02	39353
0.0032 %	a	2.07	48414
	b	2.07	46320
	c	2.08	48243
0.0056 %	a	2.11	53909
"	b	2.08	48712
"	c	2.12	55099
0.010 %	a	2.08	48410
"	b	2.11	52929
"	c	2.06	45361
0.018 %	a	2.09	47319
"	b	2.05	43299
"	c	2.05	43130
0.032 %	a	2.12	53441
"	b	2.08	48349
"	c	2.10	50952
0.056 %	a	2.12	53317
"	b	2.08	48827
"	c	2.11	51445
0.10 %	a	2.03	40744
“	b	2.06	44644
“	c	2.03	41390

APPENDIX 3: pH, temperature and light conditions

pH values

Concentration mg/l	start	72 hours
Control	7.8	7.9
3.2		7.9
100	7.8	7.9

Temperature

The following temperatures were recorded during the test:

min.: 22.0 °C max: 24.2 °C

Light conditions

The light measured on the incubator at the level of the cultures was:

Date	direct light (µE m^{-2} s^{-1})
22.11.2006	60

TEST REPORT

Cyclophosphamide

Daphnia magna Reproduction test

NIVA Study number: B481b

November 2006

INTRODUCTION

The effect of cyclophosphamide on the reproduction of *Daphnia magna* has been studied. The objective of the study was to determine the highest concentration causing no reduction of the reproductive output of *D. magna* during 21 days incubation. The procedures were in conformity with OECD Test Guideline 211: *Daphnia magna* Reproduction test. (1).

The experimental phase of the definitive study was conducted between 07.11 and 28.11 2006.

MATERIALS AND METHODS

Test material

Identification:	Cyclophosphamide monohydrate
Sample labelling:	CAS 6055-19-2
Purity:	Not declared
Appearance:	Clear, colourless
Date received:	30/10-2006
Storage conditions:	In darkness at 3-7 °C
Expiry date:	Not declared

Test species

Name	*Daphnia magna*
Strain	Clone A (2)
Source	University of Sheffield, U.K.
Stock culture	Cultured in Elendt M7 medium (3) with continuous feeding with green algae (*Pseudokirchneriella subcapitata*). The culture is maintained at 20 ±1 °C under a 16 h light and 8 h dark regime.
Selection of test animals	Adult *Daphnia magna* were isolated 24 hours prior to initiation of the test. Young daphnids produced overnight (age <24 h) were used in the test.

Dilution water

The dilution water was Elendt M7 medium (3), prepared from reagent grade chemicals and glass-distilled water. The pH of the medium was adjusted to 7.8±0.2 using 1N HCl. The composition of the medium is shown in Appendix 4.

Experimental design

A preliminary acute immobilsation test showed no immobilisation of juvenile D. magna after 48 hours incubation at 100 mg/l. The reproduction test test was conducted with five concentrations of the test material (10, 18, 32, 56 and 100 mg/l). For each test concentration ten animals were held individually in vessels containing approximately 50 ml of the test medium. Ten animals in dilution water served as control. The test was carried out as a semi-static test with renewal of test solutions three times per week.

Preparation of test solutions

Test solutions were prepared by dilution of a stock solution of the test substance containing 600 mg/l in dilution water.

Exposure conditions

The test vessels were kept at at 20 ±3 °C. Light was provided from a fluorescent lamp with a 16h/8h light/dark cycle. The light intensity in the area the test vessels were located was 2-4 µmol m^{-2} s^{-1}.

Feeding

The animals were fed a diet of green algae (*Pseudokirchneriella subcapitata*). The amount of feed per animal was gradually increased from 20×10^6 cells/animal/day at the beginning of the test to 30×10^6 cells/animal/day during the last week of the test. The feeding ratio corresponds to 150-200 µg C/animal/day. Feeding was performed daily except for day 1, 4, 5, 7, 9 and 11 (See Appendix 3). It was confirmed by visual inspection that surplus algae were present those days when no feed was added.

Observations

pH was measured in fresh and used test media at all media renewals. Dissolved oxygen was measured in used test media on day 10, using a WTW OXI Level 2P oxygen meter with CellOx standard electrode.

The animals were observed at least five days per week for survival and production of offspring. From day 12, observation was performed each day. The number of offspring produced by each animal was counted at least five days per week. Aborted eggs or dead offspring were noted.

Verification of test concentrations

Samples of test solutions were taken when freshly prepared on day and when renewed on day ..

Data analysis

The total number of live offspring produced per parent animal alive at the end of the test was calculated. Parent animals that died during the test were excluded from the analysis.
The mean reproductive output across replicates for each concentration and the pooled residual standard deviation were calculated using analysis of variance (ANOVA). The mean for each concentration was compared with the control using t-test assuming unequal variances (EXCEL, p=0.05). This option was used since the assumption of homogeneity did not hold and log-transformation to homogenise variance was not possible for data series containing zero.

The EC_{50} for effect on reproduction was calculated by fitting a logistic curve to the data using CurveExpert software. The following model was used:

$$Y = \frac{c}{1 + \left(\frac{x}{x_0}\right)^b}$$

where:

Y = the total number of juveniles per parent animal alive at the end of the test (calculated for each vessel) and x is the concentration
c = the expected number of juveniles when x=0
x_0 = the EC_{50} in the population
b = the slope parameter

RESULTS

Observations of pH and dissolved oxygen

The pH in new and used test solutions are shown in Appendix 2. The pH range was 7.6-8.0 in new test solutions and 7.6-7.9 at renewal of the solutions.

The concentration of dissolved oxygen was measured in used test solutions after 10 days. The values range from 8.8 in the control to 7.8 at the highest concentration of cyclophosphamide (100 mg/l).

Validity criteria

	Criterion	Observed
Mortality in control	≤ 20 %	0
Mean number of offspring produced per animal surviving at the end of the test in the control	≥ 60	103 (mean)

Verifica tion of test concentrations

The analysis of test solutions have not yet been completed.

Survival of Daphnia magna

All test animals survived in the controls and at all test concentration except 10 mg/l where one of the animals died after 13 days.

Reproduction

The first broods of offspring appeared after 10 days in one replicate at 10 mg/l and two replicates at 18 mg/l. All surviving animals had produced the first brood after 12 days.

The number of live offspring produced by each adult are shown in appendix 1. The data are summarised in table 2. Graphs showing the accumulated production of offspring from each adult of different groups are shown in figures 1- 5.

Table 1. The number of young produced per surviving adult in the different groups.

Concentration (nominal)	Min.	Max.	average	Standard deviation
Control	62	144	103	30
10 mg/l	61	121	88	23
18 mg/l	56	121	85	27
32 mg/l	56	122	90	22
56 mg/l	0	130	82	40
100 mg/l	54	71	63	6.8

Aborted eggs were observed in one animal at 56 mg/l. This animal did not produce any live offspring during the test. The occurrence of aborted eggs are shown in appendix 1.

The average production of offspring was lower than respective controls at all tested concentrations. The t-test showed, however, that the reproductive output was significantly less than the control only at 100 mg/l. Thus the test yields a LOEC = 100 mg/l and NOEC = 56 mg/l.

The EC_{50} derived from the logistic model was 158 mg/l, which is above the highest test concentration. Thus, EC_{50} should be expressed as >100 mg/l.

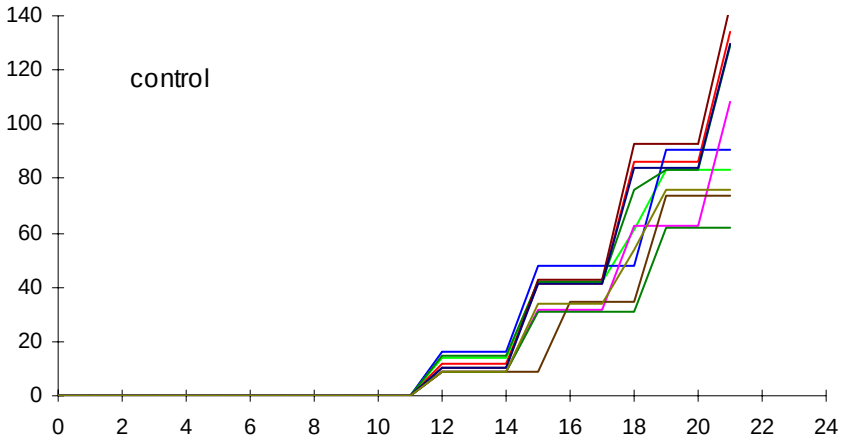

Figure 1. Cumulative production of offspring per parent animal in control.

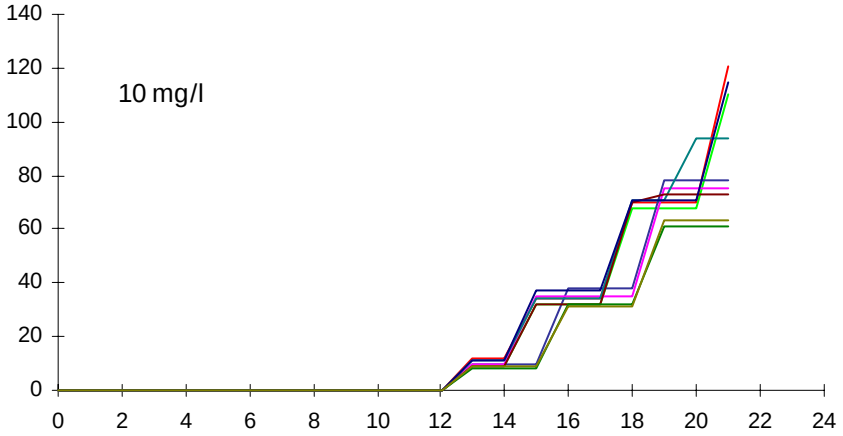

Figure 2. Cumulative production of offspring per parent animal at 10 mg/l.

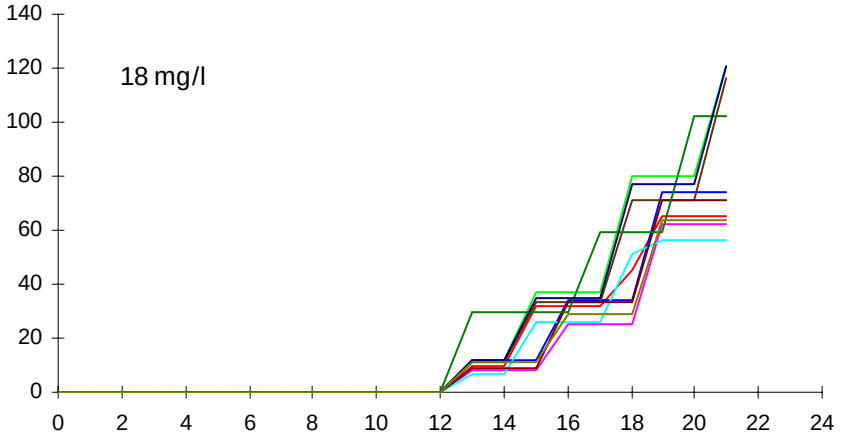

Figure 3. Cumulative production of offspring per parent animal at 18 mg/l.

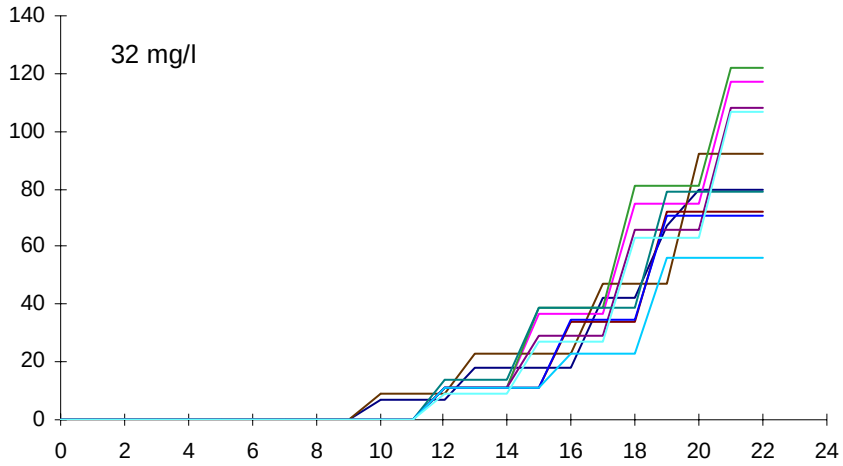

Figure 4. Cumulative production of offspring per parent animal at 32 mg/l.

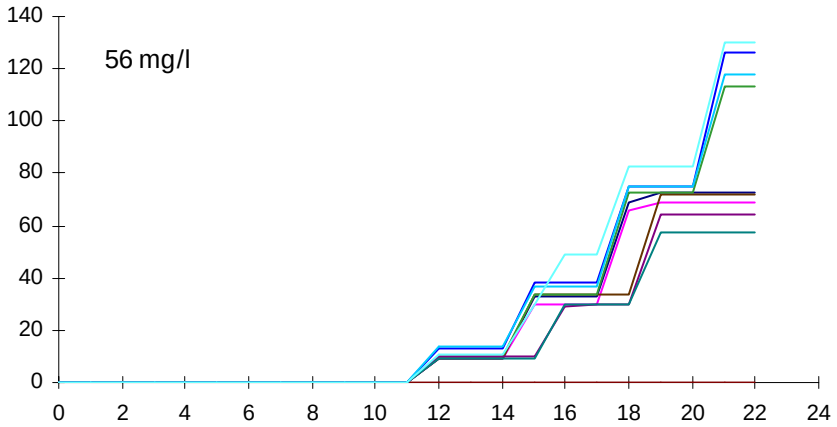

Figure 5. Cumulative production of offspring per parent animal at 56 mg/l.

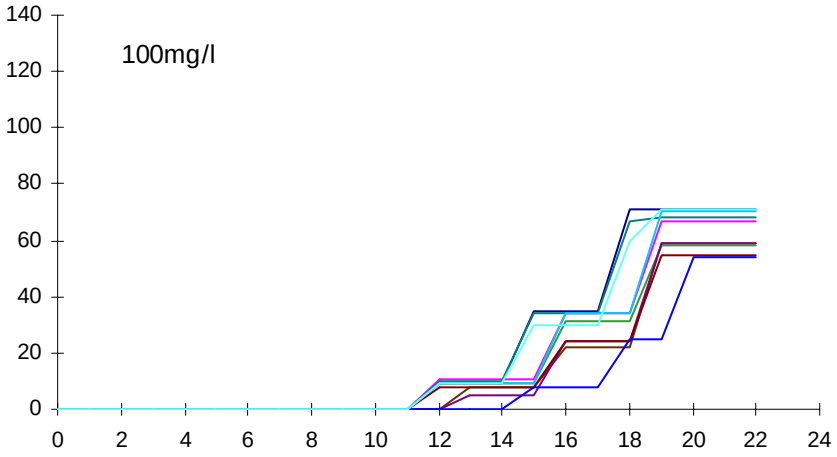

Figure 5. Cumulative production of offspring per parent animal at 100 mg/l.

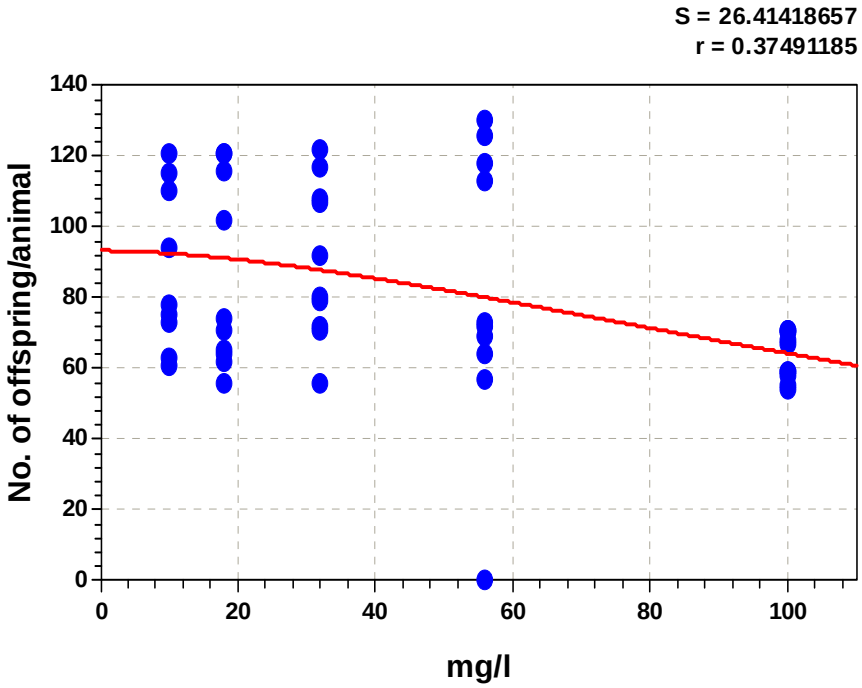

Figure 6. Response plot for effect of cyclophosphamide on the number of offspring per parent animal. The response curve was fitted using a logistic model.

CONCLUSION

The results of the test show that the test substance affects the reproductive output of *Daphnia magna* at concentrations above 56 mg/l (NOEC). The EC_{50} for effect on reproduction was > 100 mg/l. No mortality of parent animals was observed at the highest test concentration (100 mg/l).

REFERENCES.

1. OECD (Organization for Economic Cooperation and Development) 1998: Guidelines for Testing of Chemicals 211, *Daphnia magna* Reproduction Test.

1. Baird, D.J., Barber, I, Bradley, M.C., Soares, A.M.V.M. and Calow, P. 1991: A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Strauss. Ecotoxicology and Environmental Safety Vol. 21, pp. 257-265.

2. Elendt, B.-P. 1990: Selenium deficiency in Crustacea; An ultrastructural approach to antennal damage in *Daphnia magna* Strauss. Protoplasma Vol. 154, pp. 25-33.

Appendix 1

Observation of live offspring

ab= aborted eggs, M= Mortality (parent animal)

Control

Day no:	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**SUM**
1	0	0	12	0	0	29	0	0	45	0	0	48	134
2	0	0	14	0	0	28	0	0	19	22	0	0	83
3	0	0	16	0	0	32	0	0	0	43	0	0	91
4	0	0	15	0	0	27	0	0	34	7	0	46	129
5	0	0	9	0	0	23	0	0	31	0	0	45	108
6	0	0	9	0	0	0	26	0	0	39	0	0	74
7	0	0	10	0	0	33	0	0	50	0	0	51	144
8	0	0	9	0	0	22	0	0	0	31	0	0	62
9	0	0	10	0	0	31	0	0	43	0	0	46	130
10	0	0	9	0	0	25	0	0	20	22	0	0	76
												Mv.	**103**
												St.d.	30

10 mg/l

Day no:	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**SUM**
1	0	0	12	0	0	22	0	0	36	0	0	51	121
2	0	0	9	0	0	23	0	0	36	0	0	42	110
3	0	0	0	M									
4	0	0	10	0	0	0	28	0	0	40	0	0	78
5	0	0	10	0	0	25	0	0	0	40	0	0	75
6	0	0	11	0	0	23	0	0	37	0	23	0	94
7	0	0	9	0	0	23	0	0	38	3	0	0	73
8	0	0	8	0	0	0	24	0	0	29	0	0	61
9	0	0	11	0	0	26	0	0	34	0	0	44	115
10	0	0	9	0	0	0	22	0	0	32	0	0	63
												Mv.	**88**
												St.d.	23

18 mg/l

Day no:	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**SUM**
1	10	11	12	13	14	15	16	17	18	19	20	21	65
2	0	0	10	0	0	22	0	0	13	20	0	0	121
3	0	0	12	0	0	25	0	0	43	0	0	41	74
4	0	0	12	0	0	0	22	0	0	40	0	0	116
5	0	0	11	0	0	22	0	0	38	0	0	45	62
6	0	0	8	0	0	0	17	0	0	37	0	0	56
7	0	0	7	0	0	19	0	0	25	5	0	0	71
8	0	0	9	0	0	0	24	0	0	38	0	0	102
9	12	0	0	18	0	0	0	29	0	0	43	0	121
10	0	0	12	0	0	23	0	0	42	0	0	44	64
												Mv.	**85**
												St.d.	27

32 mg/l

Day no:	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**SUM**
1	7	0	0	11	0	0	0	24	0	25	13	0	80
2	0	0	11	0	0	26	0	0	38	0	0	42	117
3	9	0	0	14	0	0	0	24	0	0	45	0	92
4	0	0	11	0	0	28	0	0	42	0	0	41	122
5	0	0	11	0	0	18	0	0	37	0	0	42	108
6	0	0	11	0	0	0	23	0	0	38	0	0	72
7	0	0	14	0	0	25	0	0	0	40	0	0	79
8	0	0	11	0	0	0	24	0	0	36	0	0	71
9	0	0	11	0	0	0	12	0	0	33	0	0	56
10	0	0	9	0	0	18	0	0	36	0	0	44	107
												Mv.	**90**
												St.d.	22

56 mg/l

Day no:	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**SUM**
1	0	0	9	0	0	24	0	0	36	4	0	0	73
2	0	0	9	0	0	21	0	0	36	3	0	0	69
3	0	0	9	0	0	25	0	0	0	38	0	0	72
4	0	0	10	0	0	24	0	0	39	0	0	40	113
5	0	0	10	0	0	0	19	1	0	34	0	0	64
6	0	0	0	0	0	0	ab	0	0	ab	0	0	0
7	0	0	9	0	0	0	21	0	0	27	0	0	57
8	0	0	13	0	0	25	0	0	37	0	0	51	126
9	0	0	14	0	0	23	0	0	38	0	0	43	118
10	0	0	11	0	0	19	19	0	34	0	0	47	130
												Mv.	**88**
												St.d.	23

100 mg/l

Day no:	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**SUM**
1	0	0	9	0	0	26	0	0	36	0	0	0	71
2	0	0	11	0	0	0	23	0	0	33	0	0	67
3	0	0	0	8	0	0	14	0	0	37	0	0	59
4	0	0	9	0	0	0	22	0	0	27	0	0	58
5	0	0	0	5	0	0	19	0	0	35	0	0	59
6	0	0	8	0	0	0	16	0	0	31	0	0	55
7	0	0	10	0	0	24	0	0	33	1	0	0	68
8	0	0	0	0	0	8	0	0	17	0	29	0	54
9	0	0	9	0	0	0	25	0	0	36	0	0	70
10	0	0	9	0	0	21	0	0	30	11	0	0	71
												Mv.	**63**
												St.d.	7

Appendix 2

pH, dissolved oxygen and temperature

pH values (D= day no.)

Conc.	new start	used D 3	new D 3	used D 6	new D 6	used D10	new D10	used D13	new D13	used D16	new D16	used D19	new D19	used D21
control	7.96	7.91	7.78	7.70	7.94	7.82	7.83	7.79	7.73	7.72	7.83	7.79	7.67	7.58
10 mg/l	7.95	7.86	7.69	7.63	7.92	7.81	7.75	7.74	7.81	7.76	7.93	7.85	7.88	7.76
18 mg/l	7.95	7.87	7.81	7.68	7.92	7.83	7.74	7.61	7.86	7.79	7.92	7.83	7.85	7.63
32 mg/l	7.95	7.83	7.82	7.68	7.91	7.79	7.74	7.69	7.88	7.79	7.78	7.75	7.69	7.61
56 mg/l	7.95	7.91	7.77	7.70	7.92	7.86	7.73	7.62	7.85	7.79	7.67	7.65	7.66	7.60
100 mg/l	7.94	7.73	7.83	7.67	7.93	7.83	7.74	7.71	7.84	7.8	7.79	7.78	7.64	7.59

Dissolved oxygen in used test solutions

Conc.	**Day 10**
Control	8.78
0.10 mg/l	8.76
0.26 mg/l	8.77
0.64 mg/l	8.13
1.6 mg/l	8.04
4.0 mg/l	7.77

Temperature

The following temperatures were recorded during the test:

min: 16.7 °C max: 22.8 °C

Appendix 3

Feeding

Amount of food given to each test animal during the test. The cell numbers of *Pseudokirchneriella subcapitata* are converted to approximate concentration of organic carbon using the conversion factor 7.5×10^{-6} µg/cell.

Day no.	10^6 cells/animal	µg C/animal
0	13	100
1	13	100
2	13	100
3	26	200
6	15	110
7	15	110
8	20	150
9	20	150
10	48	360
12	24	180
13	27	200
14	27	200
15	27	200
16	27	200
17	27	200
18	27	200
19	27	200
20	27	200

Appendix 4

Composition of the dilution medium

The test medium ELENDT M7 is prepared from distilled water and chemicals of p.a. quality. The final composition of the medium is as follows:

Salt	**Concentration**
$CaCl_2 \cdot 2H_2O$	294 mg/l
$MgSO_4 \cdot 7H_2O$	123mg/l
KCl	5.8 mg/l
$NaHCO_3$	64.8 mg/l
$Na_2SiO_3 \cdot 9H_2O$	10.0 mg/l
$NaNO_3$	2.74 mg/l
KH_2PO_4	0.143 mg/l
K_2HPO_4	0.184 mg/l
H_3BO_3	0.714 mg/l
$MnCl_2 \cdot 4H_2O$	90 µg/l
LiCl	76.5 µg/l
RbCl	18 µg/l
$SrCl_2 \cdot 6H_2O$	38µg/l
$Na_2MoO_4 \cdot 2H_2O$	16µg/l
KBr	4.6µg/l
$CuCl_2 \cdot 2H_2O$	4.2 µ/l
$ZnCl_2$	13 µg/l
$CoCl_2 \cdot 6H_2O$	10 µg/l
KI	3.25 µg/l
$Na_2SeO_3 \cdot 5H_2O$	3.33 µg/l
NH_4VO_3	0.56 µg/l
$FeSO_4 \cdot 7H_2O$	249 µg/l
$Na_2EDTA.2H_2O$	625µg/l
Thiamin hydrochloride	750 µg/l
Cyanobalamin (B12)	10µg/l
Biotin	7.5 µg/l

The dilution medium is adjusted to pH 7.8 ±0.2 by addition of HCl or NaOH

Reference:

Elendt, B.-P. 1990: Selenium deficiency in Crustacea; An ultrastructural approach to antennal damage in *Daphnia magna* Strauss. Protoplasma Vol. 154, pp. 25-33.

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Utgiver Statens forurensningstilsyn	Prosjektet er finansiert av Statens forurensningstilsyn

Forfattere	Merete Grung Torsten Källqvist Kevin Thomas
Tittel Initial assessment of eleven pharmaceuticals using the EMEA guideline in Norway Initiell risikovurdering av elleve legemidler etter EMEAs risikoveileder i Norge	

Sammendrag
NIVA har utført en risikovurdering etter EMEAs risikoveileder for elleve legemidler utvalgt av SFT. Tilgjengelige akutte og kroniske tokisitetsdata ble hentet fra vitenskapelig litteratur. En vekstinhibisjonstest på alge, samt reproduksjonstest på Daphnia ble gjennomført for kreftmiddelet cyclophosphamide. EC50 ble bestemt for algetesten, mens en NOEC er bestemt for reproduksjon på Daphnia. Risikokvotienten er på over 1 for diclofenac, tetracycline, ciprofloxacin, sulfamethoxazole og ethinylestradiol etter EMEAs risikoveileder.

4 emneord	4 subject words
Risikovurdering	Risk assessment
EMEA risikoveileder	EMEA guideline
Legemidler	Pharmaceuticals
Toksisitetstesting	Toxicity testing