



Sixth
Framework
Programme

KNAPPE

Knowledge and Need Assessment on Pharmaceutical Products in Environmental Waters

Contract n° 036864

Operative commencement date of the project: February 1st 2007

Final date of the project: September 30th 2008

Deliverable number : D4.3

Typology of PPs with regard to ERA procedure

The deliverable authors are responsible for the content

AUTHOR:	Besse JP
AFFILIATION:	
ADDRESS:	
TEL.:	3 bis quai Chauveau, CP 220, 69336 LYON cedex 09 FRANCE
EMAIL:	jeanne.garric@cemagref.fr / jean-philippe.besse@cemagref.fr
FURTHER AUTHORS:	Garric J

Document Information

DOCUMENT TYPE	<i>report</i>
DOCUMENT NAME:	<i>Typology of PPs with regard to ERA procedure</i>
REVISION:	<i>1</i>
REV.DATE:	
CLASSIFICATION:	
STATUS:	

STATUS, CONFIDENTIALITY AND ACCESSIBILITY

Status			Confidentiality			Accessibility	
S0	Approved/Released		R0	General public	x	Work-space	
S1	Reviewed		R1	Restricted to SWIFT6WFD members		Internet	x
S2	Pending for review		R2	Restricted to European. Commission		Paper	
S3	Draft for comments						
S4	Under preparation						

Table of content

Acknowledgements	1
Executive Summary	2
Introduction	3
Review of environmental risk assessment and prioritization strategies for pharmaceuticals	5
I. Existing regulatory strategies to assess the environmental risk of human pharmaceuticals:	
.....	5
I.1. European Medicines Agency (EMA) guideline:	5
I.2. FDA risk assessment methodology for human pharmaceuticals (Figure 2):	8
II. Review of prioritization strategies and risk assessment for human pharmaceuticals in different European countries:	10
Example of prioritization strategy	22
I. Exposure assessment:	23
II. Effect assessment:	24
III. Results and discussion:	26
Discussion	27
I Exposure assessment:	27
I.1. Accuracy of PEC calculation with field measurements:	27
I.2. Consumption amounts:	27
I.3. Uncertainties and limitations in the exposure assessment:	29
I.4. Calculation of PEC for other compartments:	33
II. Effect assessment:	34
II.1. Ecotoxicological data:	34
II.2. Pharmacological data:	39
Conclusion	43
References	45
Annexes	52

Acknowledgements

Pr. Pierre Lanteri and Mr Jean-Yves Gauvrit (Université Claude Bernard-Lyon 1 UMR 5180, Sciences Analytiques) for their help on QSAR and on the evaluation of the EPISuite modelling software.

Executive Summary

The objective of this work was to gather the information about the existing prioritization schemes implemented in different countries to propose lists of pharmaceutical of environmental concern.

This document consists in three parts. In the first one, from a literature review we present the existing regulatory strategies and the previous work conducted with regard to environmental risk assessment (ERA) and/or prioritization of human pharmaceuticals. Most of the studies implemented in Europe use the European Medicines Agency (EMA) guideline for ERA as a basis of their work. This review highlights the fact that chronic ecotoxicological data are lacking, which makes hard to implement accurate ERAs.

The second part gives an example of such a prioritization strategy, using ecotoxicological, but also human pharmacological and physico-chemical data to bypass the lack of ecotoxicological data. As a result of this strategy, a priority list of pharmaceuticals (parent compounds and metabolites) relevant for the French situation is given.

In the third part of the document, the different criteria used by several authors to build lists of priority pharmaceuticals, are discussed. Parameters influencing quantities of pharmaceuticals reaching the aquatic environment and reviewed and discussed and so are the parameters proposed to assess the environmental biological effects of human pharmaceuticals on aquatic species.

Introduction

It is now recognized that pharmaceutical compounds reach the environment and can be considered as environmental contaminants. As a wide range of drugs including antibiotics, antiphlogistics, blood lipid-lowering agents, antiepileptics, β -blockers, hormones modulators and so on, have been found in the effluents and surface waters of several countries, there is a growing need to assess their environmental risk. Consequently in Europe, monitoring pharmaceuticals in the surface water and/or ground water is becoming mandatory, within the water framework directive.

Prior to implement a monitoring program in the aquatic environment, there is a need to rank pharmaceuticals according to their environmental relevance (e.g. their presence in the environment and their potential for ecotoxicological effects), because of the high number of molecules used as marketed human drugs. Indeed, it is not conceivable to search for all molecules in the environment and test all of them for ecotoxicity. Therefore, a methodology needs to be developed to select for priority molecules. Priority molecules can be defined as molecules for which a monitoring strategy and eventually ecotoxicological assays are to be implemented. Similarly to the model used in the existing regulatory approaches for the assessment of the environmental risk of pharmaceuticals, the prioritization of pharmaceuticals can be based on risk quotients, as described in the current European Medicine Evaluation Agency (EMA 2006) guideline for environmental risk assessment (ERA) for pharmaceuticals. To build such risk ratios, PEC (predicted environmental concentration) and PNEC (predicted no effect concentration) values are required. However, although it is possible to estimate the PEC for a number of compounds, ecotoxicological data are too scarce to calculate PNEC values for most of the pharmaceuticals currently in use (Fent et al., 2006; Crane et al., 2006; Carlsson et al., 2006).

A number of studies have been conducted in the ten past years in order to assess the environmental risk of pharmaceuticals and/or to prioritize them. The determination of the exposure is quite similar in all studies but the assessment of the effect differs from a study to another.

As a consequence of the lack of the ecotoxicological data , a number of studies recently focused on the way to assess the biological effects of pharmaceuticals on non-target aquatic organisms by using pharmacological data, quantitative structure relationships (QSAR), or evolutionary conservation of molecular drug targets.

This report is divided in three main parts:

- In a first time, the two main regulatory approaches of ERA for human pharmaceutical are reviewed as well as the most important international studies directed toward ERA or ranking of pharmaceuticals.
- In a second part, an example of a prioritization strategy and a priority list for pharmaceuticals are given.
- Finally in the third part of the document, a critical review of the parameters used in the exposure and in the effect assessment for pharmaceuticals is done.

Review of environmental risk assessment and prioritization strategies for pharmaceuticals

I. Existing regulatory strategies to assess the environmental risk of human pharmaceuticals:

Since pharmaceuticals have been detected in the aquatic environment, there was a growing concern in targeting and quantifying these substances. Consequently, regulatory agencies (EMA, FDA) implemented environmental risk assessment (ERA) strategies to assess the risk for human pharmaceuticals. Here are described the two main existing ERA methodologies which are the main basis of all the following strategies currently developed.

I.1. European Medicines Agency (EMA) guideline:

EMA published in year 2006 a revised guideline aiming at the risk assessment of human pharmaceuticals. The approach is tiered in several phases (Figure 1). In the first tier (phase I) of the assessment, a preliminary conservative Predicted Environmental Concentration (PEC) is calculated, assuming a worst case hypothesis and using the maximum daily dose and the percentage of market penetration as an estimation of the consumption amounts of the selected pharmaceutical (Equation 1). PECs are therefore compared to a threshold value set at 10 ng.L⁻¹.

$$PEC_{surfacewater} = \frac{DOSE_{ai} \times F_{pen}}{WW_{inhab} \times Dilution \times 100}$$

DOSE_{ai} : Maximum daily dose consumed per inhabitant (mg.hab⁻¹.day⁻¹)

F_{pen} : Percentage of market penetration (default value = 1%)

WW_{inhab} : Amount of wastewater per inhabitant per day (l.hab⁻¹.day⁻¹; default value = 200 l)

Dilution : Dilution factor from STP to surface water (default value = 10)

Equation 1 : Phase I PEC for surface water (EMA 2006).

If the $PEC_{SURFACEWATER}$ value is below 10 ng.L^{-1} , and no other environmental concerns are apparent, it is assumed that the medicinal product is unlikely to represent a risk for the environment following its prescribed usage in patients. If the $PEC_{SURFACEWATER}$ value is equal to or above 10 ng.L^{-1} , then a Phase II environmental fate and effect analysis is performed. A refined PEC is calculated taking into account metabolism of pharmaceuticals in human body, removal rates in sewage treatment plants (STP) and adsorption to suspended matter. PNEC values and risk quotients are finally calculated. In some cases, the action limit may not be applicable. As some drug substances may affect the reproduction of vertebrate or lower animals at concentrations lower than 10 ng.L^{-1} , these substances should enter Phase II and a tailored risk assessment strategy should be followed that addresses its specific mechanism of action.

In the second phase, a refined PEC (Equation 2) is calculated and environmental fate and effects on no-target organisms are analysed. In this tier, all relevant data should be included, e.g. data on physico-chemical properties, pharmacodynamics, toxicology, metabolism, persistence of the drug and/or relevant metabolites.

$$PEC_{surface\ water} = \frac{Dose_{ai} \times F_{excreta} \times F_{stp} \times F_{pen}}{WW_{inhab} \times Factor \times Dilution \times 100}$$

F_{excreta}: fraction of the active substance excreted
DOSE_{ai}: maximum daily dose consumed per inhabitant
F_{stp}: fraction of emission directed to surface water
Factor: factor taking the adsorption to suspended matter into account
F_{pen}: market penetration factor with F_{pen}

with
$$F_{pen} = \frac{consumption \times 100}{DDD \times hab \times 365}$$

consumption: consumption of active substance ($\text{mg} \cdot \text{year}^{-1}$)
DDD: defined daily dose (average daily dose per inhabitant)
hab: number of inhabitants

Equation 2 : Refined phase II PEC (EMA 2006).

To assess the risk, aquatic Predicted No Effect Concentrations (PNEC) values are subsequently calculated using available ecotoxicological data. Assessment factors are applied to the data, to take into account the uncertainty in extrapolating laboratory data to environmental considerations.

The assessment factor is set at 10 and is applied to the lowest NOEC (No observable Effect Concentration) value observed. The 2006 EMEA guideline only consider chronic ecotoxicological data and assume that 3 NOEC values (corresponding to three trophic levels: algae, invertebrates and fish) are needed to calculate a PNEC value. If PEC/PNEC ratio is above one for a compound, therefore extended effect analysis are to be conducted (water sediment effects, specific effects on micro-organisms, bioaccumulation study).

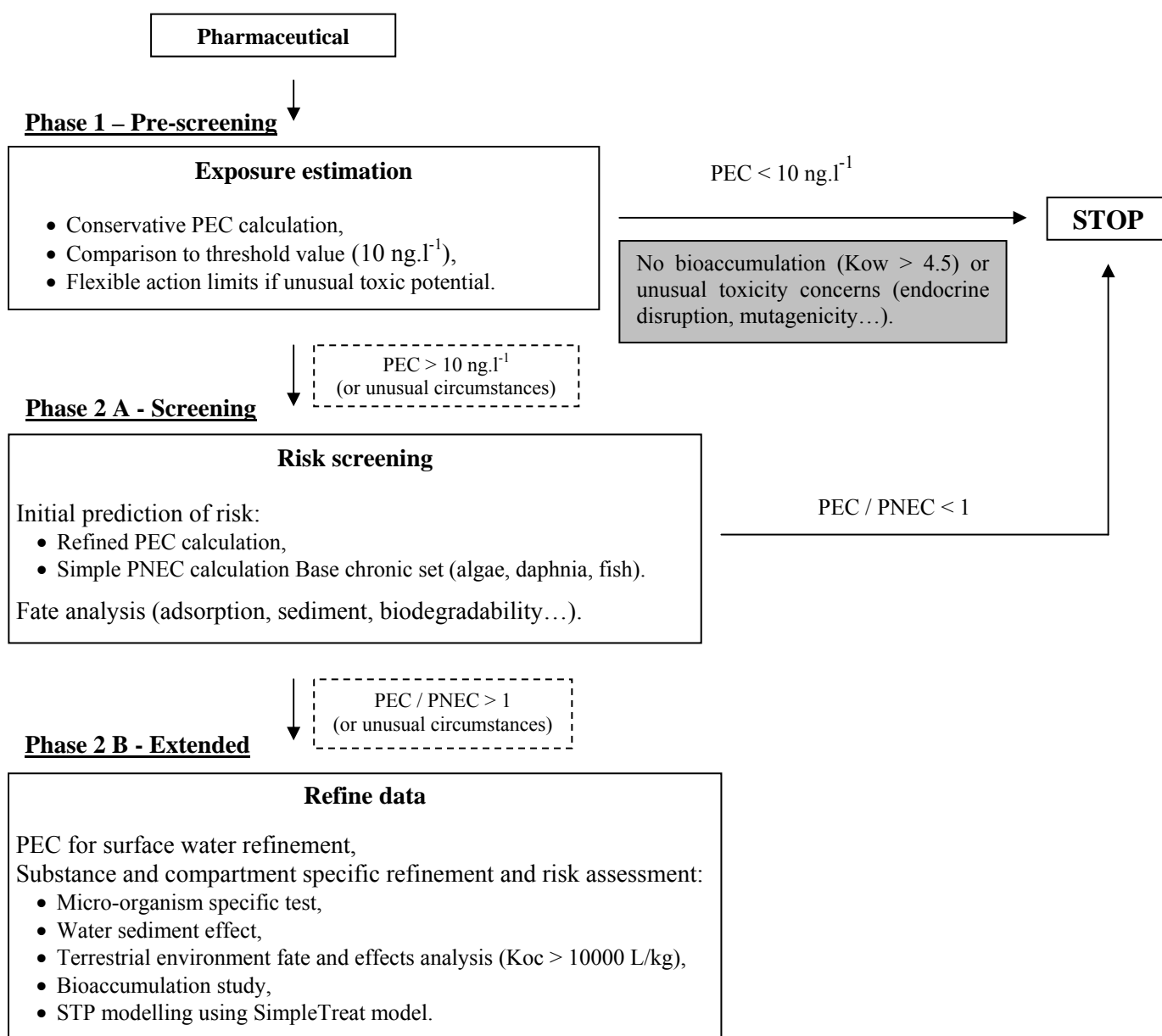


Figure 1 : 2006 EMEA guideline representation (revised from Bound and Voulvoulis 2004).

I.2 FDA risk assessment methodology for human pharmaceuticals (Figure 2):

The Food and Drug Administration (FDA) methodology (FDA 1998) is roughly similar to that of the EMEA. However, prior to any exposure assessment, it assumes to examine the depletion mechanisms of the considered molecule in the environment of potential concern (water, terrestrial...). If depletion is rapid and complete, then only a microbial inhibition test is required to assess the impact of the molecule on STP processes. If not, an EIC (Expected Introductory Concentration, equivalent to the EMEA PEC) is calculated using the following equation (Equation 3).

$$EIC = \frac{\text{production} \times D}{365 \times Q_{\text{effluent}}}$$

EIC : Expected Introductory Concentration ($\mu\text{g/l}$).

Production : amount of active compound produced for direct use (kg/year)

Qeffluent : quantity entering publicly owned treatment works (l/day)

D : conversion factor ($10^6 \mu\text{g/kg}$)

Equation 3: Expected Introductory Concentration (FDA, 1998).

IF EIC is lower than $1 \mu\text{g.L}^{-1}$ in STP effluents or lower than 100ng.L^{-1} in surface waters, if microbial inhibition tests results do not suggest a potential risk for STP processes and if log K_{ow} is lower than 3.5, therefore, the risk is considered negligible. In other cases, risk ratio based assessment is done (as in the EMEA methodology). However, to limit the number of assays needed, assessment is tiered and chronic assays are used only if acute tests have failed to show the harmlessness of the substance.

In this methodology, $EC(LC)_{50}$ / MEEC ratios stand for PEC/PNEC ratios, however, these two ways of calculation are equivalent. The threshold value of 100ng.L^{-1} assumed by the FDA guideline is based on acute toxicity data gathered for 60 pharmaceuticals (FDA, 1998) whereas the threshold value 10ng.L^{-1} of assumed by the EMEA has been criticized (CSTEE 2001; Straub 2002) because it was not selected on a scientific basis.

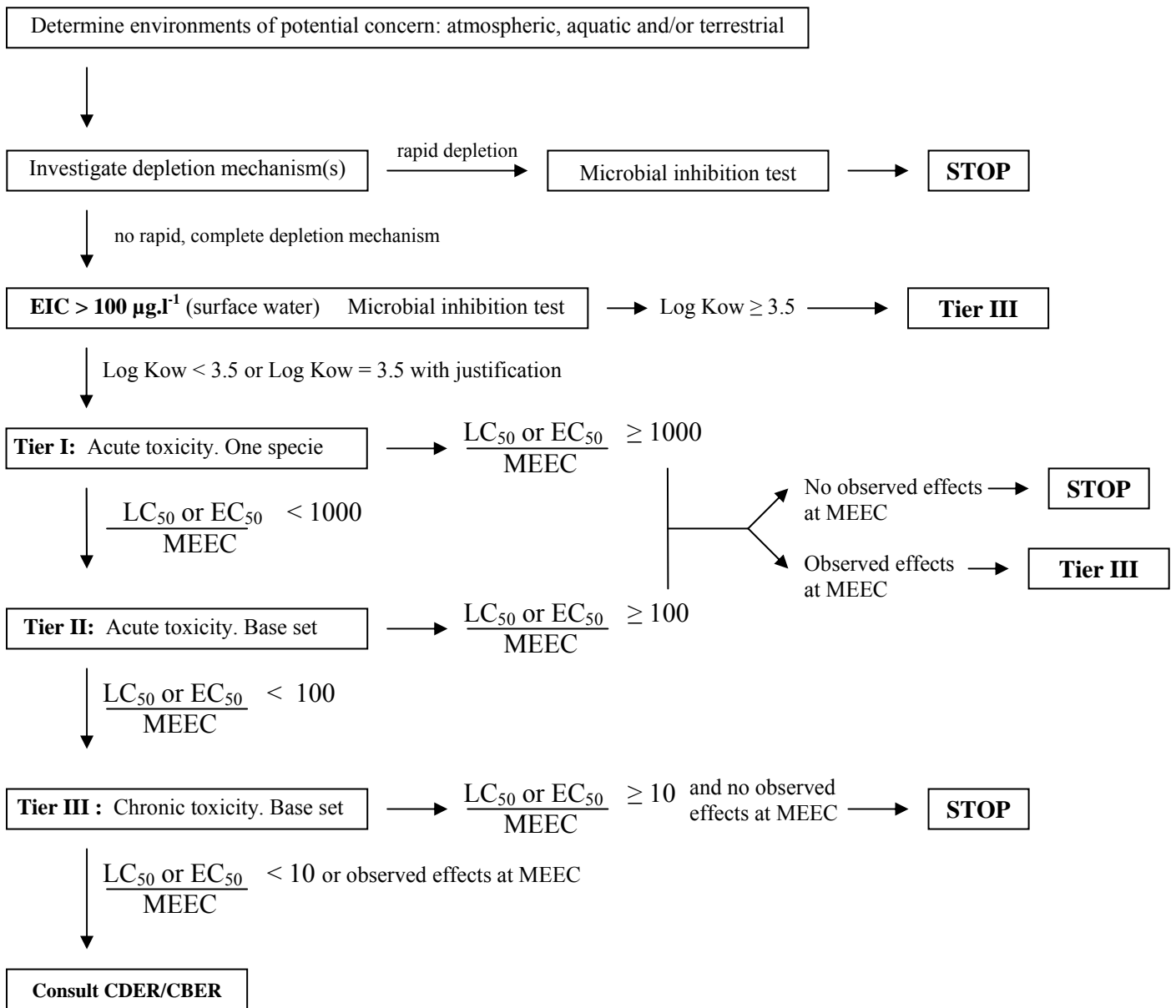


Figure 2 : FDA tiered approach to fate and effects testing (Adapted from Bound and Voulvoulis, 2004).

MEEC: Maximum Expected Environmental Concentration.
 CDER: Center for Drug Evaluation and Research.
 CBER: Center for Biologics Evaluation and Research.

II. Review of prioritization strategies and risk assessment for human pharmaceuticals in different European countries:

For the last ten years, a number of risk assessment and/or prioritization strategies have been implemented in Europe and United-States. Most of them were conducted according to the EMEA guideline.

Denmark

One of the first environmental risk assessment for human pharmaceuticals has been conducted in 2000 (Stuer-Lauridsen et al., 2000). The methodology was based on the use of risk ratios (PEC/PNEC). PEC values were calculated using the EMEA guideline (version of 1994 year). Risk ratios and PEC for surface water were calculated using the following equations (4 and 5):

$$RCR = \frac{PEC_w}{PNEC}$$

Equation 4: Risk Characterisation (EU, 1994).

$$PEC_w = \frac{A \times (100 - R)}{365 \times P \times V \times D \times 100}$$

A : amount used per year (kg)
R : removal in STP, (in percent, set to 0 as a worst case hypothesis)
P : number of inhabitants
V : volume of wastewater per day per capita
D : dilution factor in the environment (a default factor of 10 was used)

Equation 5: Concentration of active substance of pharmaceutical in surface water (EU, 1994).

The selection of molecules to assess was based on the information of the sale of human pharmaceuticals in Denmark, published by the Danish Medicine Agency. The 25 most used pharmaceuticals used in Denmark in 1997 were selected. The consumption data were given in Defined Daily Dose (DDD). DDD is the *assumed average maintenance dose per day for a drug used for its main indication in adults* (www.whocc.no).

The annual consumption for pharmaceuticals in weight units was then calculated by multiplying the number of DDD with the respective DDD value for each pharmaceutical. Finally, a list of 20 molecules was chosen for the purpose of the risk assessment.

PECs for surface water were then calculated using the EMEA guideline of year 1994 and compared to the threshold value which was set at 1 ng.L⁻¹. All molecules exceeded this cut-off value and had to be submitted to the phase II assessment. All PEC values were between 1 and 100 ng.L⁻¹ and were within a factor of 2-5 of the measured concentrations. Risk ratios were calculated to assess the environmental risk but the authors noted that there was a lack of ecotoxicity data for pharmaceuticals, which precluded the calculation of risk ratios based on measured ecotoxicological data. PEC/PNEC exceeded one for ibuprofen, paracetamol and acetylsalicylic acid.

$$K_d = f_{oc} \times 0.41 K_{ow} \quad (6)$$

$$D_{ow} = K_{ow} / (1 + 10^{pH - pK_a}) \quad (7)$$

$$C_{sludge} = M_{act} / (V_w / K_d + M_{sludge}) \quad (8)$$

K_d : sorption coefficient (L.kg⁻¹)

f_{oc} : fraction of organic carbon in sludge (default value = 0.35)

D_{ow} : K_{ow} corrected for ionisable compounds

M_{act} : annual consumption of active compound

V_w : total annual wastewater in the considered country

M_{sludge} : annual sludge production

Equation 6: Determination of sorption coefficient K_d.

Equation 7: Determination of D_{ow} (K_{ow} corrected for ionisable substances).

Equation 8: Determination of C_{sludge}, concentration of active substances sorbed on sludge.

For a few compounds, estimated and experimental K_d values were available, and predicted concentrations in sewage sludge were calculated using equations 6, 7 and 8. Results showed large differences in the predicted concentrations, depending on the mode estimation of K_d (based on K_{ow} or D_{ow}) and also depending on the use of estimated or experimental values.

United Kingdom

A report on the environmental risk assessment was commissioned from the UK environment agency, in order to assess the environmental risk for human pharmaceuticals (Hilton et al., 2003). Assessment is tiered in two main phases: selection of molecules and risk assessment. The selection approach is somewhat different from all the other approaches reviewed and was determined by crossing different approaches: EMEA guideline (2001 version), OSPAR dynamec system and literature review. This approach is displayed in figure 3 and summarized below:

1) Calculation of risk ratios using EMEA guideline (PEC/PNEC ratios). Two lists of priority compounds were built using two different ways of calculation for PNEC values:

- determination of PNEC using therapeutic dose of the active substance ($PNEC_D = \text{therapeutic dose} / 1000$)
- determination of PNEC using a QSAR approach ($PNEC_T = \text{QSAR value} / 100$).

2) Effect assessment using the OSPAR PBT criterion (OSPAR dynamec, OSPAR 2006; 2002). In this system, each single substance is defined by three criteria: persistence, bioaccumulation and toxicity (PBT), defined by physicochemical data. However, the data were available only for a few of the compounds.

3) Review of literature data. Existing data on occurrence of pharmaceutical substance and existence of analytical method or analytical feasibility (if there is no existing methodology) are reviewed. Reviewing the work from Hilton et al. (2003), it seems that these two last criteria have been determining in the choice of the final priority list: the final list (11 compounds) only contains 2 of the 19 pharmaceuticals selected on the basis of PEC/PNEC ratios and OSPAR criterion.

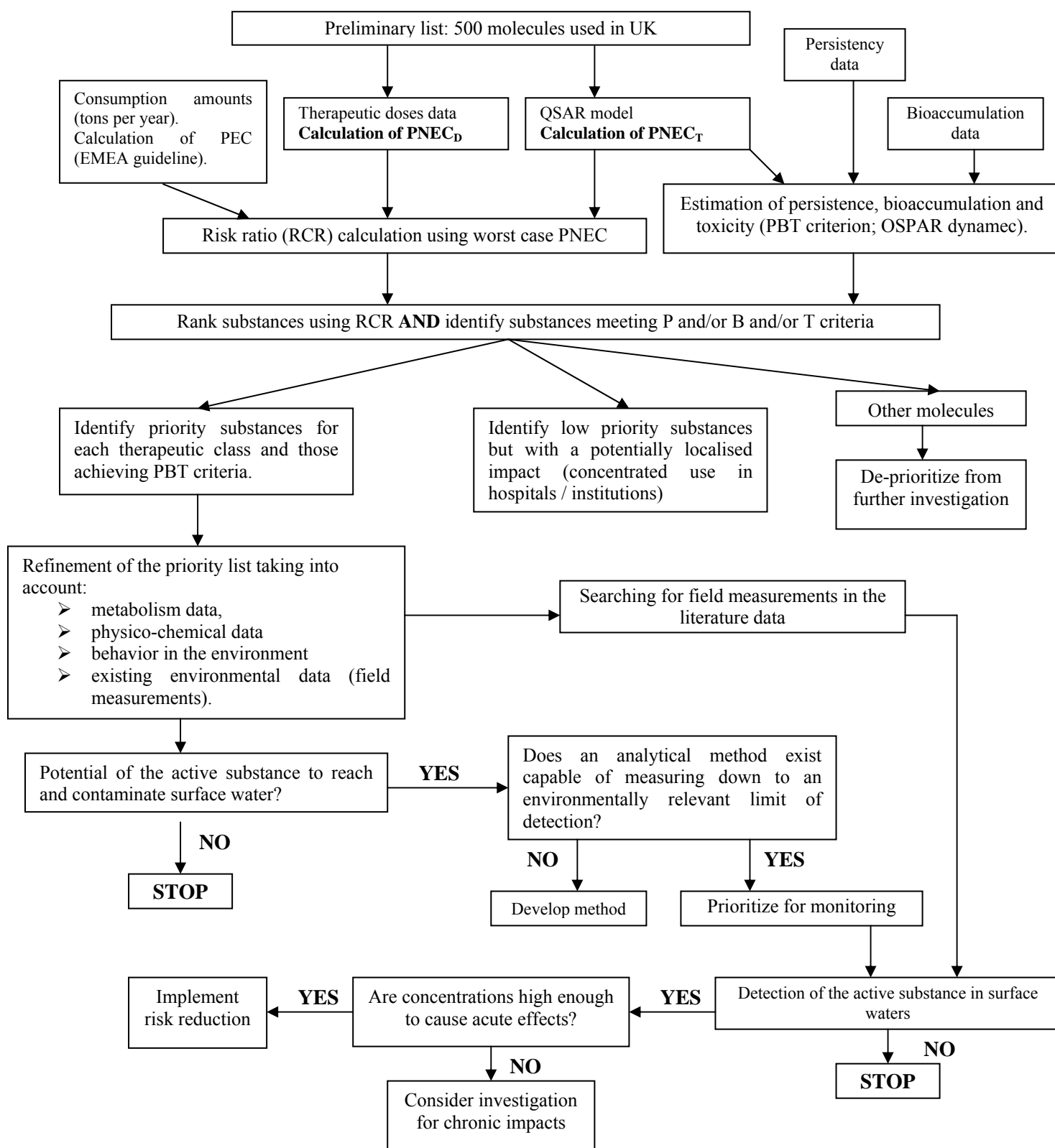


Figure 3: Tiered approach for selection of pharmaceuticals (Hilton et al., 2003).

Another aquatic environmental assessment of pharmaceuticals has been realised in England by Jones et al. (2002). This assessment was performed on the same basis as the one done by Stuer-Lauridsen et al. (2000) and used PEC/PNEC ratios. PEC values for the 25 most consumed pharmaceuticals, (in terms of DDD) were calculated using the former EMEA guideline (2001 version). PNEC values were calculated using available ecotoxicological data. In addition to risk assessment, basic environmental modelling was performed to determine bioconcentration factor, soil adsorption coefficient and behaviour in WWTP. All these parameters were modelled using different software from the EPIWIN suite (from the USEPA).

Germany

An environmental risk assessment for the 111 highest-selling human drug substances (annual sales > 5000 kg) has been conducted in Germany (Huschek et al., 2004). Risk assessment was conducted using EMEA guideline (2003 version). PECs were calculated using Equation 1. Phase 2 PEC were calculated using actual annual sales of pharmaceuticals (years 1996-2001).

2003 version of the EMEA guideline did not take into account the fraction of pharmaceuticals excreted, therefore, Huschek et al. determined a PT_{index} (which is equivalent to the Fexcreta from EMEA guideline 2006) to establish refined PEC values. Refined PECs were calculated by correcting Phase I PEC by the PT_{index} . Results showed that PEC values were comparable to STP concentrations. The study of Huschek et al. was the first to introduce the excreted fraction of pharmaceuticals in the calculation of PECs; the authors quoted the fact that the excretion rates are important to consider for PEC refinement. The authors noticed that between 2001 version of the EMEA guideline and 2003 version, the calculation of PECs have changed. The 2003 version introduces the use of DDD in the calculation (see Equation 2). This led to differences in PEC values and therefore in the PEC/PNEC risk quotients.

PEC /PNEC ratios were calculated and ratios above 1 were only achieved for the antibiotics ciprofloxacin and clarithromycin and also for acetylsalicylic acid, paracetamol, povidone iodine and ethinylestradiol.

Sweden

In December 2002, as part of the work of achieving national environmental goals, a report was commissioned from the Swedish Medical Products Agency. The report was to include a risk assessment of environmental effects of pharmaceuticals based on their occurrence in the environment in relation to their current sales volumes (Carlsson et al., 2006). The risk assessment was based on the EMEA guideline (2006 version). The selection of pharmaceuticals was based on the list of the 100 most sold pharmaceuticals, in terms of DDD, for the year 2002. Twenty-seven pharmaceuticals were finally selected using the following criteria:

- molecule occurring on the list of the 100 most sold pharmaceuticals,
- molecule reported as observed in the environment,
- molecule otherwise highlighted in the literature.

The risk assessment was then conducted as described in the EMEA guideline (2004 version). However, for the calculation of phase I PEC, the sold amount of pharmaceuticals was used as a measure of the factor “DOSE_{Ei} x F_{pen}/100” (see Equations 1 and 2).

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, as in Jones et al. (2002). Refined PEC were calculated using STP removal rates calculated with the Simple Treat 3.1 modelling software (Struijs et al., 1991). Metabolism data were also taken into account, the fraction of parent compound excreted after metabolism was included in the calculation. Metabolites lacking environmental and/or ecotoxicity data were regarded as parent compound and added to this fraction. PNEC values were calculated in accordance with EMEA guideline and the European TGD and finally risk ratios were calculated.

All risk quotients were below one except for ethinylestradiol (18), estradiol (180), estriol (1.6) and paracetamol (1.41). It is worth noting that except paracetamol, all the molecules displaying a risk quotient higher than one are steroid estrogens.

The main conclusions of this work are:

- The acute risk linked to pharmaceuticals is negligible,
- The chronic risk cannot be rule out,
- Ecotoxicological data were scarce,
- Risk assessment based on acute toxicity test do not adequately reflect the potential for long term exposure to sub-acute levels,
- The ecotoxic potency of a compound is to be taken into account at an early stage of the risk assessment, irrespective of the quantity released into the environment
- Studies are to be directed toward bioaccumulation potential, indirect effects, and mixture effects.

A risk assessment has been implemented at a national scale in Sweden (www.janusinfo.se/environment). It aims to provide information for healthcare practitioners in order to reduce the residues of medicinal products in the ground, water and air, which is one of Stockholm County Council's five most important environmental issues. Pharmaceuticals are environmentally classified according two criteria: environmental risk on one hand and environmental hazard, estimated by the PBT index, on the other hand. Environmental risk refers to acute toxicity for the aquatic environment. Environmental risk is based on the ratio between predicted environmental concentration of the substance (PEC) and the highest concentration of the substance that does not have a harmful effect in the environment. Environmental hazard expresses the inherent environmentally damaging characteristics of the substance using the PBT (Persistence, Bioaccumulation, Toxicity) index. The PBT index is part of the OSPAR dynamec system to assess the hazard of single substances (OSPAR 2002) in the following terms:

- Persistence - ability to resist degradation in the aquatic environment,
- Bioaccumulation - accumulation in adipose tissue of aquatic organisms,
- Toxicity – the potential to poison aquatic organisms.

Italy

In Italy, an assessment of the contamination of the aquatic compartment by human pharmaceuticals was conducted in 2005 (Zuccato et al., 2005). The assessment was tiered into three phases:

- Pre-selection of pharmaceuticals (prioritization),
- Samplings in STP and surface waters,
- Comparison of results from sampling campaign with theoretical prioritization.

The pre-selection was based on two main parameters: sales figures for pharmaceuticals (consumption amounts) and metabolism rate of the different compounds in the human body. Pharmaceuticals were ranked according to predicted environmental loads, calculated by multiplying sales figures by the rate of metabolism in man, according to Equation 9. The authors only considered the excretion rates of pharmaceuticals as unchanged molecule and did not take into account the metabolites. According to the authors, this approach gave a good estimation of the quantities of active molecules reaching the environment.

$$\text{Load} = \text{prescription amount} \times \text{excretion rate}$$

Load: predicted environmental load (tonnes)

Prescription amount: sale figures (tonnes for year 2001).

Excretion rate: excretion rate of pharmaceutical as unchanged molecule (%)

Equation 9: Calculation of predicted environmental loads for pharmaceuticals (Zuccato et al., 2005).

To this first set of molecules, selected on predicted environmental loads, the authors added molecules known to be persistent and molecules with high activity and potential toxicity but with low environmental load such estrogens and anti-cancer drugs. Preliminary list of selected molecules (obtained with calculation of environmental loads) and final list of pharmaceuticals selected for analysis are displayed in table 4. Field measurements in STP effluents revealed ofloxacin, furosemide, l'atenolol, hydrochlorothiazide, carbamazepine, ranitidine, ciprofloxacin, sulfamethoxazole and ibuprofen were found in the range of the hundred ng.L⁻¹.

Spiramycine, bezafibrate, erythromycine, lincomycine and clarithromycin were found in the ten ng.L⁻¹ range. Despite very high predicted environmental loads, amoxicillin had low concentrations in STP effluents, suggesting that this molecule was rapidly degraded in the environment. In river waters (Po and Lambro rivers), concentrations were lower than in STPs effluents with the exception of spiramycin, bezafibrate and lincomycin for which concentrations were roughly the same. Table 1 displays the results for the molecules found in higher concentrations. Salbutamol, cyclophosphamide, omeprazole, methotrexate, ethinylestradiol, enalapril and diazepam were only detected at very low concentrations (in the ng.L⁻¹ range) or were below detection limits.

Concentrations of pharmaceuticals detected in STP effluents and surface water (expressed in ng.L⁻¹, median value)					
STP effluents		Lambro river		Po river	
ofloxacin	600	ofloxacin	306	ofloxacin	33
furosemide	585	furosemide	254	lincomycin	32.5
atenolol	466	atenolol	241	carbamazepine	23
hydrochlorothiazide	439	hydrochlorothiazide	255	aténolol	17
carbamazepine	291	carbamazepine	175	ibuprofen	13
ranitidine	288	lincomycin	74	spiramycin	10
ciprofloxacin	251	spiramycin	57	hydrochlorothiazide	4.5
sulfamethoxazole	127	bezafibrate	24	furosemide	3.5
ibuprofen	121	ibuprofen	20	erythromycine	3.2

Table 1: Comparison of concentrations of pharmaceuticals, detected in STP effluents and surface water (Zuccato et al., 2005).

United States

Two recent studies have been conducted in USA, directed to effect assessment of human pharmaceuticals. The first one, by Cooper et al. (2008) is based on a compilation of available information (CAS number, molecular weight, melting point, pKa, Kow, water solubility, measured environmental concentration, environmental half-life and persistence, quantities used and toxicity) in a free access database (PEIAR database). In this study pharmaceuticals were ranked using five different combinations of physico-chemical data and toxicological data, to emphasize different risks. One of these ranks used QSAR modelled toxicity data using the ECOSAR (USEPA) software.

Another study (Kostich and Lazorchak, 2008) studied the environmental exposure and potential biological effects for the 50 most dispensed pharmaceuticals. The consumption masses for pharmaceuticals were indirectly calculated from dollar sales data. Predicted concentrations took into account metabolic inactivation and also disposal of unused medicines. Concentrations reaching wastewater and in biosolids were calculated using the two following equations (10 to 12).

$$(\text{API activity}) = (M \cdot (1 - F_i) \cdot (1 - F_w)) + (M \cdot F_w) \quad (10)$$

M: mass dispensed

F_i: fraction inactivated (by metabolism)

F_w: fraction wasted

$$\text{PEC}_{\text{wastewater}} = (\text{API activity introduced annually}) / (\text{annual wastewater volume}) \quad (11)$$

$$\text{PEC}_{\text{biosolids}} = (\text{API activity introduced annually}) / (\text{annual biosolids volume}) \quad (12)$$

Equation 10: Predicted API activity released in the environment

Equation 11: Predicted wastewater concentrations

Equation 12: Predicted biosolids concentrations

To assess the potential biological effects of pharmaceuticals, authors used several methodologies summarized here:

1) Non-human exposure rates: Assuming exposure to non-target organisms *via* equilibration with wastewater, the concentration of pharmaceutical dissolved in the modelled organism's extracellular fluid would approach the concentration dissolved in wastewater (this assumption is close to the one assumed by Huggett et al., 2003). Therefore, an "exposure multiple" for each pharmaceutical was expressed as the ratio between wastewater PEC and human peak freely dissolved plasma concentration of the pharmaceutical. An "exposure multiple" was also expressed for microbial inhibition as the ratio of wastewater PEC and the lowest minimum inhibitory found for a specific pharmaceutical.

2) One of the main questions addressed in the ERA of pharmaceuticals is the sensitivity of non-target taxa to human pharmaceuticals, due to high differences in biology and physiology. Authors reviewed the molecular targets of the selected studied pharmaceuticals and search for potential similar targets in non-human species.

This analysis, along with the information about the physiological roles of these molecular pathways in different organisms can provide guidance on the range of species and the type of endpoints that should be considered in chronic toxicity studies.

France:

Two prioritization strategies were recently conducted in France. One with regard to the use of pharmacological data to assess the biological effects of pharmaceuticals and one with a special emphasis on bioaccumulation. The first one (Besse and Garric, 2008) is fully described in the second part of the document as we give it as an example of prioritization approach.

The second one is summarized here: this strategy was conducted for hospital effluents (Jean, 2008). A priority list was implemented with regard on consumption amounts in hospitals and bioaccumulation potential. Bioaccumulation potential was determined by reviewing modelled bioconcentration factor (BCF) from CAS database® values for a high number of pharmaceuticals. To take into account the ionization of pharmaceuticals in the environment, BCF were reviewed for four environmental relevant pH values ranging from 6 to 9. A compound was considered bioaccumulating if at least one of the estimated BCF value was higher than the threshold value 1000. The recommended value of TGD is set at 2000, however, the more conservative value of 1000 was chosen to stand for uncertainties of estimated values (which could differ from real ones).

One of the main assumption of this work is that, considering environmental concentrations for pharmaceuticals which are low (ng.l⁻¹ to µg.l⁻¹ range), the main risk is linked to a chronic exposition and to a bioaccumulation of pharmaceuticals in aquatic organisms which could lead to critical internal concentration in the organisms and toxicity. Moreover bioaccumulation is the main way, with drinking water, which can lead to human contamination. As pharmaceutical compounds are continuously released in the aquatic environment (“pseudo-persistence”), the risk of bioaccumulation is to consider.

This review allowed to highlight the following facts:

- most of the studies implemented in Europe use the European Medicines Agency (EMA) guideline for ERA as a basis of their work,
- calculated PEC values are in general in good agreement with measured field values,
- chronic ecotoxicological data are lacking, which makes hard to implement accurate ERAs.
- some methodologies have been implemented in order to bypass the lack of ecotoxicological data.

All these points are discussed in the third part of this report.

Example of prioritization strategy

A prioritization strategy, with special emphasis on metabolism data and pharmacological data has been conducted in France in 2008 (Besse and Garric 2008). This work was addressed in the framework of the French Plan National Santé Environnement (PNSE 2004) and in Europe, within the European Water Framework Directive. However, although it is possible to estimate the PEC for a number of compounds, ecotoxicological data are too scarce to calculate PNEC values for most of the pharmaceuticals currently in use. In a first step (Besse et al., 2008), all ecotoxicological data (acute and chronic) were compiled in a database and a first risk assessment using PEC/PNEC ratios was implemented following the EMEA recommendations. However the review of available ecotoxicity data showed that only six compounds bring together the conditions required by the EMEA guideline. Even when referring to the European TGD (2003) for pharmaceuticals with limited chronic data (1 or 2 NOECs from different trophic levels), it was only possible to calculate PNEC values and PEC/PNEC quotients for a further 16 compounds.

Consequently, a pragmatic prioritization approach was implemented, which aimed to identify priority human pharmaceuticals and metabolites to monitor in surface water. Moreover, this approach allowed to identify some data gaps that should be filled to allow a better assessment of these compounds. The approach used was based both on the calculation of PEC values and on a decision scheme based on selected quantitative and qualitative biological data: ecotoxicological, pharmacological and physicochemical data.

The prioritization scheme (fully detailed and available in Besse and Garric, 2008) consisted of three tiers:

- First, a preliminary exposure-based classification was established using PEC values.
- Second, this preliminary classification was reviewed using a case-by-case expert approach considering the potential environmental effect, using ecotoxicological data, pharmacological, mammalian toxicological and physicochemical data as well as available data on the environmental behaviour of pharmaceuticals.

- Finally, an additional selection was made in order to select priority compounds belonging to the same pharmacological and chemical class (e.g., compounds with the same chemical structure and the same mechanism of action (MoA)).

The candidate pharmaceuticals list was based on the data provided by the French Health Product Agency (Agence Française de Sécurité Sanitaire des Produits de Santé, AFSSAPS, Paris), which covered exhaustive sales data for drugs delivered in France for hospitals and pharmacies, including over-the-counter drugs, for the year 2004. The candidate list consisted in 120 parent pharmaceuticals and 30 metabolites, selected metabolites were targeted in a previous work (Besse et al., 2008).

I. Exposure assessment:

The first step of the prioritization strategy classified pharmaceuticals compounds according to their probable exposure concentration in the aquatic environment. This was based upon the premise that the pharmaceuticals used in higher amounts have a potential to reach the aquatic environment in greater quantities and therefore may present a higher risk for the aquatic environment. The exposure-based classification implemented was derived from the EMEA guideline (EMEA, 2006) and used the following equations (13 and 14).

$$PECa = \frac{\textit{consumption}}{WWinhab \times hab \times Dilution \times 365} \quad (13)$$

$$PECb = \frac{\textit{consumption} \times \textit{Fexcreta}}{WWinhab \times hab \times Dilution \times 365} \quad (14)$$

Consumption (mg/year): quantity of an active molecule consumed in France (AFSSAPS data)

WWinhab: volume of wastewater per person per day (default of 200 l.inhab⁻¹.day⁻¹)

Fexcreta: excretion fraction of the unchanged active molecule (parent drug or metabolite).

Dilution: dilution factor from WWTP effluents to surface water (default value of 10)

hab: number of inhabitants in the defined zone (set at 60 million for France).

Equation 13 and Equation 14: Predicted environmental concentrations (Besse and Garric 2008).

Pharmaceuticals were subsequently ranked accordingly and classified in 6 different classes summarized in Table 2.

Priority class	Priority rank according to the exposure criteria	Comments
I A	highest risk compounds	PECa and PECb higher than 100 ng.l ⁻¹ . High consumption and limited metabolism.
I B	potentially hazardous compounds but limited data	PECa higher than 100 ng.l ⁻¹ . High consumption. No data on metabolism
II A	potentially hazardous compounds	PECa higher than 100 ng.l ⁻¹ and PECb higher than 10 ng.l ⁻¹ . High consumption and intermediate metabolism.
II B	unclassified priority risk	PECa lower than 100 ng.l ⁻¹ but higher than 10 ng.l ⁻¹ . No data on metabolism. No definitive conclusion, need further investigation
III	very low risk for the environment (extensive metabolism)	PECa higher than 100 ng.l ⁻¹ but PECb lower than 10 ng.l ⁻¹ . High consumption but extensive metabolism.
IV	very low risk for the environment (low consumption amount)	PECa lower than 10 ng.l ⁻¹ . Low consumption amount .

Table 2: Ranking of pharmaceuticals according to their exposure assessment (Besse and Garric, 2008).

II. Effect assessment:

Second and third phase were dedicated to the effect assessment. In the second phase, pharmaceuticals were reviewed according to ecotoxicological, physicochemical and pharmacological data with emphasis on human toxicity (mechanism of action, side effects, specific organ toxicity, cytochrome and para-glycoprotein P modulation). The implemented strategy is summarized in figure 4.

This effect assessment was based on the following assumptions:

- For non-mammalian animals with targets similar to those of mammals, biological effects may occur.
- Known adverse human side effects of pharmaceuticals may also be valuable to indicate potential harmful effects on non-target organisms.
- Several APIs are known to interact with Cytochrome P-450, therefore there is a potential risk of disruption in the homeostasis of non-target organisms.

- Several pharmaceuticals are known to interact with Glycoprotein-P (P-gp), a multidrug transporter that actively transports xenobiotics out of the cell, preventing the accumulation of toxic compounds; therefore an inhibition of its expression by a specific drug could result in enhancing the sensitivity of organisms to environmental pollutants.

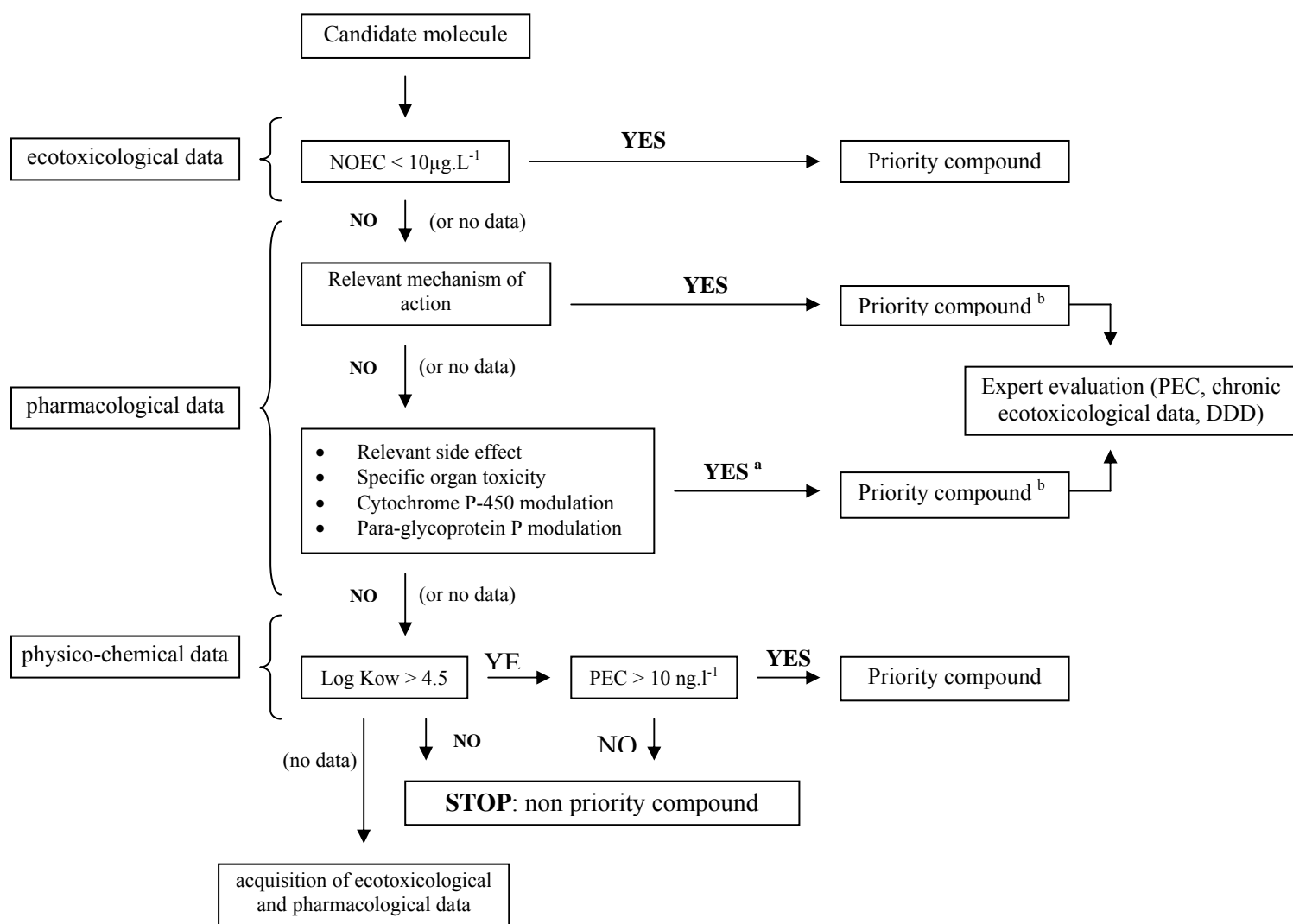


Figure 4: Prioritization of pharmaceuticals based on ecotoxicological, pharmacological and physicochemical data. (a) Yes, if the compound meets at least two of the selected criteria (e.g., relevant side effect and cytochrome inhibition). (b) For priority compounds selected based on pharmacological data, the final inclusion on the priority list is to be discussed case-by-case by taking into account PEC values and biological data.

Finally, to select priority pharmaceuticals displaying the same MoA, an additional step was implemented using available data: NOEC, DDD and PEC values for pharmaceuticals.

III. Results and discussion:

A final priority list of 40 compounds and 14 metabolites was obtained with several therapeutic and chemical classes represented. Results are displayed in annexes. 21 parent compounds have been already detected in surface waters (mainly β -blockers, anti-inflammatories and antibiotics). 5 metabolites have also been measured in surface waters, other metabolites have not been searched yet. PEC values refined by STPs removal rates are in good agreement with field measurements. Even if the strategy is widely applicable in different countries, the priority list obtained may not be relevant for others countries than France, mainly due to differences in the consumption profile of pharmaceuticals.

The prioritization strategy was successfully applied to the French situation; however, this methodology can be improved:

- STP removal rates should be included in the PEC calculation, when available,
- fate data (biodegradation, photodegradation and hydrolysis time) should be included,
- degradation by-products of pharmaceuticals should be taken into account,
- there is a need to investigate the relevance of use of pharmacological data to assess the risk of pharmaceuticals for non mammal organisms.

Finally it is important to consider that:

- selection of molecules for the candidate list was based on consumption in terms of tonnage, therefore, some highly active molecules but with a low tonnage of consumption may have been ignored,
- cytotoxic compounds and sexual steroids (estrogens, progestagens and androgens) were excluded from this strategy. As these molecules display specific MoAs, authors consider that they have to be assessed in a specific procedure,
- biocides and X-ray contrast media compounds were excluded from this work,
- analytical feasibility was not taken into account.

Discussion

I Exposure assessment:

A number of risk assessment and prioritization strategies have been conducted for the last ten years, especially in the last two years. Here are discussed the different results and the perspectives given by these different works with regard to exposure assessment. Figure 5 summarizes the different factors that can modify the quantities of pharmaceuticals reaching wastewaters and surface waters.

I.1. Accuracy of PEC calculation with field measurements:

Most of the conducted studies were based, for the exposure assessment, on the EMEA guideline. The exposure assessment for surface water seems to be quite well estimated. Comparison of PECs with field measurements is in good agreement, especially when considering the simplicity of the models and the remaining uncertainties. A number of authors agree with this conclusion (Bound and Voulvoulis 2006, Besse et al., 2008, Castiglioni et al., 2004, Huschek et al., 2004; Carlsson et al., 2006). Nevertheless, some uncertainties are to be taken into consideration in the assessment of the exposure, and are discussed below.

I.2. Consumption amounts:

In every study, the basis of the exposure assessment is the actual amounts of pharmaceuticals dispensed over a year. These actual amounts can be found even in tonnage (kg) or number of DDDs for every single active molecule, or even in terms of total dollar value of product sold (Kostich and Lazorchak, 2008); depending on the availability of the data. About the consumption data, there is a need to carefully consider whether or not, the consumption amounts are based on prescription data only, or also include over-the-counter (OTC) drugs (drugs which are delivered without prescription) as this can lead to large differences in the estimation of concentrations entering the environment.

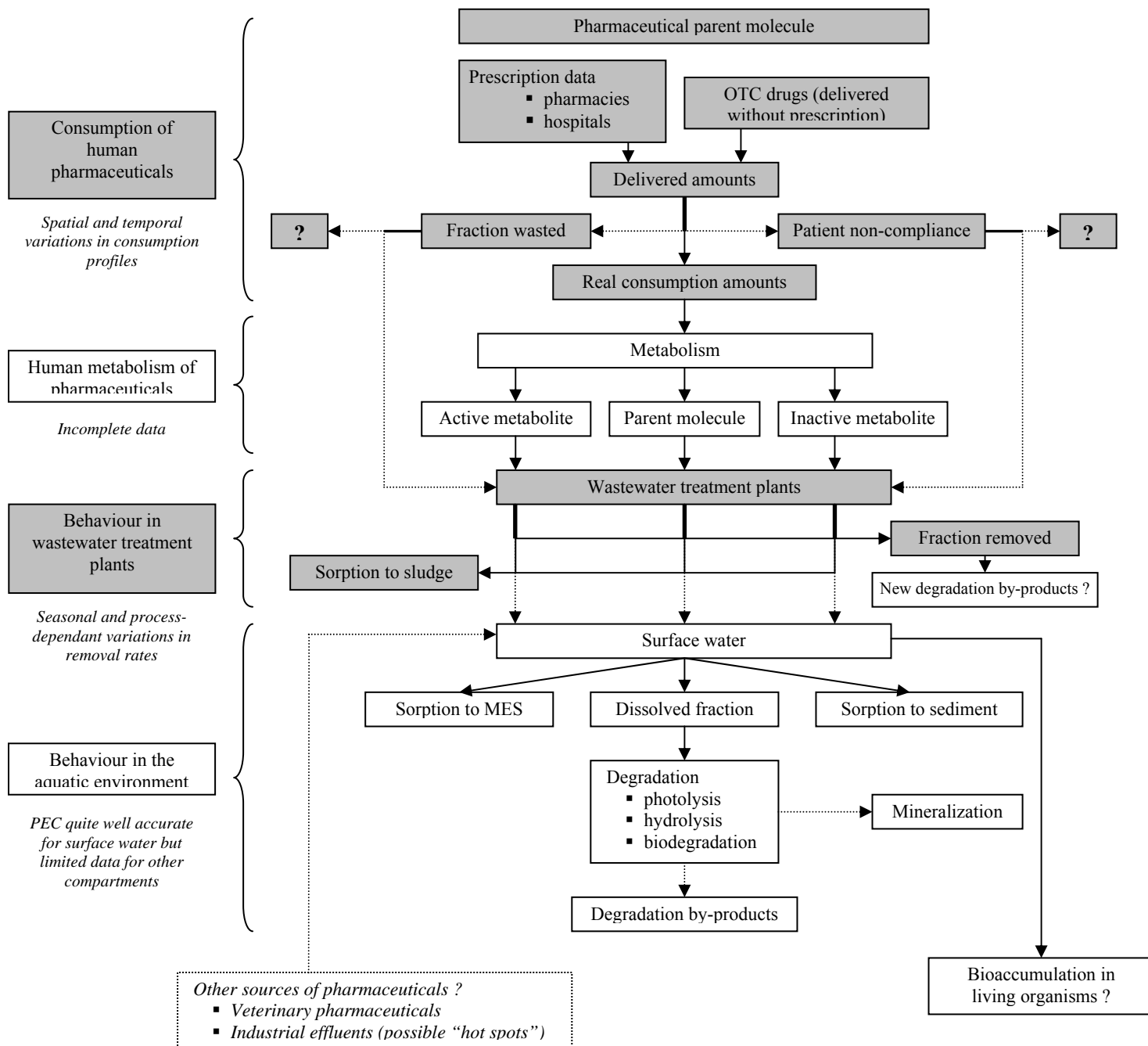


Figure 5 : Factors that can affect the quantities of pharmaceuticals reaching the aquatic environment.

Actual amounts in terms of tonnage are the most simple and direct to use, however, the availability of such data depends on the existence of regulatory agencies in a country and the possibility to share such data. It is to be noted that an assessment of pharmaceuticals based on the most consumed pharmaceuticals in terms of tonnage can ignore molecules used in low tonnages but with a high activity and concern for the environment (as ethinylestradiol). Consumption data can also be found in number of DDD. As it exist standardized DDD values (WHO, www.whooc.no/atcddd/indexdatabase/) for each active molecule, it is possible to simply convert number of DDD in tonnage of active molecule. However, more than one DDD can be found for the same active substance, depending on the indication and way of administration, therefore, there is a need to carefully choose the DDD value.

One advantage of DDD is that it can be considered as representative of the activity of the molecule (Besse and Garric, 2008). Therefore, number of DDDs can be considered as a normalization of the quantity by the potency for an active molecule and therefore gives the number of active doses that can reach the aquatic environment. As an example, ethinylestradiol which is consumed in low amounts due to its very low DDD rises in the most consumed pharmaceuticals when numbers of DDDs are considered.

I.3. Uncertainties and limitations in the exposure assessment:

Patient non-compliance / Wasted fractions of pharmaceuticals: Another major uncertainty remains on the quantity actually consumed by patients. Data provided by regulatory agencies give information on the quantities delivered in hospitals and pharmacies per year but cannot give any information on the patient compliance. Patient non-compliance can be quite large, and quantities actually consumed by people may be lower than the quantities delivered, especially for drugs which do not require medical prescription as NSAIDs (Bound and Voulvoulis, 2006; Besse et al., 2008). The study of Kostich and Lazorchak (2008) is the only one which takes into account the disposal of unused medicines. Authors quote the fact that accounting for disposal is important, especially for pharmaceuticals where metabolic inactivation is nearly complete, as it can avoid the underestimation of PEC vales for such molecules.

The study assumes three different values for wasted fraction, for short-term therapy, long-term therapy and topical medicines, considered as conservative by the authors. All wasted fractions are assumed to reach wastewaters which is a worst-case hypothesis and may not reflect the reality.

Temporal and spatial variations in consumption: PEC values are generally calculated using human consumption data over 1 year, which gives an average consumption for the year. Therefore neither spatial variations nor local variations can be taken into account: local variations can include specific local consumption of pharmaceuticals, which are likely to differ from one region to another; temporal variations especially concern pharmaceuticals used in acute treatments, such as antibiotics, for which quantities consumed and subsequently quantities reaching the aquatic environment can vary over the year (Besse et al., 2008).

Metabolism of pharmaceuticals: Human metabolism is an important step which must be taken into account for several reasons. First, metabolism is the primary transformation and inactivation process that undergo pharmaceuticals; therefore, metabolism can lead to drastically reduced amounts of parent pharmaceuticals reaching wastewaters. On the other hand, metabolism can give rise to new molecules (pharmacologically active or not) that can be of environmental concern if excreted in significant amounts. Metabolism data is generally available for pharmaceuticals; however, data are often scarce and incomplete, which limits the calculation of reliable excretion rates values (Besse et al., 2008; Huschek et al., 2004).

Other sources of pharmaceuticals: As some pharmaceutical compounds are used both in human and veterinary medicine, there are still uncertainties about the actual amounts of pharmaceuticals reaching surface waters. This is particularly the case for antibiotic and antiprotozoal compounds. Theoretically, including veterinary consumption is likely to ensure a more comprehensive PEC. However, routes of administration and ways of reaching the aquatic environment differ between veterinary and human pharmaceuticals (Besse et al., 2008). Other sources of contamination such as industrial effluents (production facilities and conditioning facilities) should be considered, such sources could result in hot spots of contamination, however no data are available on this possible source at the moment.

Removal in STPs: Uncertainties also lie in the removal rates of pharmaceuticals in STPs. Review of available data shows that existing values are scarce and that there is a high heterogeneity in removal rates. STP efficiency toward pharmaceuticals can be influenced by the season (Castiglioni et al. 2006), therefore leading to varying surface water concentrations among the year. Results of a study (Yu et al., 2006) on 12 pharmaceuticals belonging to different therapeutical classes showed that the extents of removals were highly variable and could not be correlated to drug classification or structure. Existing measured removal rates are still scarce and only concern a limited number of pharmaceuticals, therefore, a number of studies have used modelling software such as STPWIN (Carlsson et al., 2006; Jones et al., 2002) or Simple treat (recommended by EMEA guideline) to predict removal rates in STP for pharmaceuticals.

Molecule	Calculated rates (%)		Measured rates (%)		
	degradation	adsorption	Stumpf et al., 1999	Castiglioni et al., 2006	Paffoni et al., 2006
Propranolol	0,18	12,58	34	35 (24)	22
Propranolol HCL	0,09	1,78	NA	NA	NA
Metoprolol	0,09	2,15	10	25 (24)	10
Erythromycin	0,13	6,23	-	0	42
Ofloxacin	0,09	1,76	-	43 (md) – 57 (md)	40
Fluoxetine	0,34	32,06	-	-	-
Diazepam	0,11	4,31	-	-	-
Ibuprofen	0,31	28,4	75	77 (28)	96
Diclofenac	0,53	56,55	75	41 (29)	27
Diclofenac potassium	0,09	1,77	NA	NA	NA
Diclofenac sodium	0,09	1,77	NA	NA	NA

Table 3 : Comparison of measured and calculated STP removal rates (Cemagref 2008).

As STP removal rates are lacking, it is logical to rely on modelling, however, results from table 3 show that there are large differences in measured and calculated values. There is a need to better study the accuracy of existing models for the estimation of removal rates for pharmaceuticals.

Degradation of pharmaceuticals in the environment: PEC calculation cannot take into account abiotic and biotic degradation processes that can occur in surface waters. Abiotic processes are reported to be most important ones (Fent et al. 2006). Photolysis and hydrolysis were suggested to be rapid ways of removal of amoxicillin in the environment (Andreozzi et al. 2004).

This statement was supported by the fact that amoxicillin was only detected in surface waters at low levels (Zuccato et al. 2005; Paffoni et al. 2006). The β -blocker propranolol was reported to be rapidly photodegraded and therefore is not expected to be persistent in surface waters (Qin-Tao and Williams 2007). On the other hand, most of the pharmaceuticals are continuously released in the environment. This fact could balance the degradation processes in the environment, therefore some authors have suggested that pharmaceuticals should be considered as “pseudo-persistent” contaminants, due to this continuous release (Daughton and Ternes 1999).

Results from a study assessing the biodegradability of pharmaceuticals (Yu et al., 2006) showed that if abiotic degradation was low, biotic degradation for some pharmaceuticals were important (For aerobic biodegradation experiments of selected pharmaceuticals, 8 of the 12 drugs tested exhibited biotransformations greater than 80% after 50 days of incubation). This study also tests the BIOWIN software to predict biodegradability and concluded that the submodels within BIOWIN were not able to match consistently the experimental determinations of PPCP biodegradation; two submodels tended to overestimate biodegradability while the remaining two submodels tended to underestimate biodegradability.

Finally, environmental degradation of pharmaceuticals can give rise to degradation by-products that may be of environmental relevance. As an example, it has been shown that Photodegradation metabolites could be more toxic than the parent compound (Isidori et al. 2006; 2005; DellaGreca et al., 2004).

Default values used in PEC calculation: Others great uncertainties lie in the default values proposed by the model used to calculate PECs, such as volume of wastewater and dilution factor used in the EMEA guideline. As an example for France, the default value for quantities of effluent is set to $200 \text{ L.inhab}^{-1}.\text{day}^{-1}$ which is a mean value that can be accepted at the national. However, for some specific French regions, this value may drop to $150 \text{ L.inhab}^{-1}.\text{day}^{-1}$; using this last value in our calculation significantly increases PEC values by 25%. To this extent, PEC values calculated for the STP effluents are more reliable than surface water PECs (Besse et al., 2008). Moreover, dilution factor from STP to surface waters is often set at a default value which is not representative for local specifications.

I.4. Calculation of PEC for other compartments:

PECs are often calculated for surface waters but more rarely for suspended matter sewage sludge or sediment. (Stuer lauridsen et al., 2000 ; Jones et al., 2002).

Partition to sludge: In their risk assessment for pharmaceuticals, Stuer-lauridsen et al. (2000) calculated predicted concentrations for sludge using estimated and calculated Kd (partition coefficient). Results showed that there were large differences (several orders of magnitude) in the values of PEC depending on the way of calculation and the Kd value. Another study (Jones et al., 2002) calculated fraction sorbed to sludge using the modelling software STPWIN. However, the calculated values could be inaccurate: Kd (or Koc) modelling is mainly based on the Kow. However, as quoted by several authors (Fent et al., 2006; Wells, 2006; Tolls, 2001), Kow may not be an accurate descriptor of the environmental behaviour (sorption, bioaccumulation) of pharmaceuticals in the environment. Moreover, the majority of pharmaceuticals are polar ionisable compounds, and it would be more accurate to use the logDow, Kow corrected by the pKa (Stuer-Lauridsen et al., 2000; Wells 2006).

Partition to sediment: To our knowledge, no PEC calculation has been conducted for water sediment. This is a gap as some pharmaceuticals can be found in sediment. (Petrovic et al., 2002; Daughton and Ternes, 1999). Partition to sediment is generally assessed by the Koc coefficient, however, measured Koc are scarce at the moment and estimated Koc values may not reflect the reality for pharmaceuticals (see above).

As the pharmacokinetic behavior of pharmaceuticals is influenced by the same parameters that can modify environmental behavior such as pH and pKa, it makes sense to draw a parallel between pharmacokinetic and environmental criteria. Williams et al. (2006) recently studied the correlation between the environmental partitioning coefficient Kd and the distribution volume Vd, which measures the distribution of a pharmaceutical within the body. These results suggest that pharmacokinetic parameters should be helpful to estimate environmental behavior for pharmaceuticals.

Bioaccumulation in living organisms: For some authors (Jean 2008), the main risk for aquatic organisms is the continuous exposure to low concentrations of pharmaceuticals and therefore a possible bioaccumulation in living organisms which could result in toxic effects.

Bioaccumulation is estimated by the bioaccumulation factor (BCF) which takes into account the hydrophobicity of the molecule and the living organism considered. For pharmaceuticals, there is a lack of experimental values for BCF. Therefore, the bioaccumulation can only be afforded with calculated values. In his study, Jean (2008) used calculated values from the CAS database®, mainly based on log Dow (log Kow value corrected by pH). Environmental relevance of such calculated values must be investigated. A very recent study (Paterson and Metcalfe, 2008) investigated the uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (*Oryzias latipes*). BCF values of 74 and 117 were determined for fluoxetine and norfluoxetine respectively. The results indicate longer persistence and greater potential for the bioaccumulation of fluoxetine and norfluoxetine in fish tissues than would be predicted from prior half life estimates derived using mammalian species.

II. Effect assessment:

The environmental effects for pharmaceuticals are much more difficult to assess than the environmental exposure. The different approaches attempting to assess the environmental effects of pharmaceuticals are depicted below. Table 4 summarizes the different parameters considered in the different assessment strategies.

II.1. Ecotoxicological data:

PEC/PNEC risk quotients: To assess the risk for the aquatic environment, the most classical approach is to calculate PEC/PNEC risk quotients. The EMEA guideline describes the way to calculate PNEC values; this approach is similar to that proposed by the European TGD (TGD 2003). Unlike TGD, the EMEA guideline enforces the use of chronic toxicity data and requires long-term NOEC for the base set (i.e. three NOEC values from three different trophic levels, applying an assessment factor of 10 to the lowest value). The review of available ecotoxicity data showed that only very few compounds bring together the conditions required by the EMEA guideline. Indeed the lack of chronic ecotoxicological data has been clearly highlighted by the several risk assessment and prioritization strategies implemented for the last ten years (Carlsson et al., 2006, Jones et al., 2002, Stuer-Lauridsen et al., 2000, Besse et al., 2008, Jjemba 2006).

Data	Approach	Parameters	Commentary	Reference
Ecotoxicological data	Experimental data	Acute data	Some data are available, but the acute risk for pharmaceuticals is negligible; acute data can be used to derive chronic PNEC values but relevance is questionable.	Besse et al., 2008; Carlsson et al., 2006; Jones et al., 2002 ; Stuer-lauridsen et al., 2000
		Chronic data	the more representative of aquatic hazard for pharmaceuticals but data are lacking	
	QSAR modelling	ECOSAR	Do not accurately estimate toxicity for pharmaceuticals	Sanderson et al., 2004a,b; Cooper et al., 2008
		Lienert et al. QSAR model	Allow to take into account and add the toxic potential of parent drug and metabolites in the PNEC calculation	Lienert et al., 2007
	OSPAR dynamec System for hazard assessment	Persistence	Based on experimental data or BIOWIN model	www.janusinfo.se; van wezel and Jager (2002)
		Bioaccumulation	Based on Kow, may not accurately assess the environmental behaviour for pharmaceuticals, threshold values not adapted to pharmaceuticals	
		Toxicity	Based on acute data or chronic ecotoxicological data	
Pharmacological data	Human and mammalian pharmacological and toxicological data	Mechanism of action (MoA)	Some similar MoA have been identified in human and aquatic species	Besse and Garric, 2008
		CYP 450 modulation	CYP 450 modulation can interfere with homeostatis in no-target species	
		P-gp modulation	P-gp modulation can result in enhance sensitivity of organism to environmental pollutants	
		human side effects	Can explain some observed toxic MoA in non-target species	
		Therapeutic plasma concentration	Can be used to estimate response in fish	
	For antimicrobials, suggested to estimate the response in aquatic organisms (other than microbes)		Kostich and Lazorchak, 2008	
	Antimicrobial sensitivity	Inhibitory concentration for antibiotics	Can provide information on environmental effects of antibacterial pharmaceuticals (antimicrobial resistance and growth inhibition of beneficial microbes).	Kostich and Lazorchak, 2008
	Evolutionary approach	Conservation of human drug targets in aquatic organisms	Can provide indication on pharmaceuticals molecular targets and taxa of concern, can be used to assess relevance of ecotoxicological data and to select relevant species for bioassays	Kostich and Lazorchak, 2008 Gunnarsson et al., 2008

Table 4: Review of existing strategies for assessing the biological effects of pharmaceuticals on aquatic species.

Use of intrinsic properties of a compound for environmental hazard assessment: The OSPAR dynamec (OSPAR 2006, 2002) approach include a ranking classification for single substances based on three criterions (PBT criterion). This approach has been used to describe the hazard of pharmaceuticals in the environment (www.janusinfo.se/). Persistence in the environment (based on degradation time DT_{50}), Bioaccumulation potential (based on BCF or log Kow) and Toxicity according to the following recommendations:

- Persistency (P): Half-life ($T_{1/2}$) of 50 days,
- Liability to Bioaccumulate (B): $\log Kow \geq 4$ or $BCF \geq 500$,
- Toxicity (T): acute $L(E)C_{50} < 1$ mg/l, long-term $NOEC < 0,1$ mg/l.

The estimation of the toxicity is based on available ecotoxicological data, therefore this approach meets the same limitation (data gaps) as the risk quotients approach. Estimation of persistency is based on experimental data, but in the absence of such data, the dynamec guidance document recommends the use of the BIOWIN model to estimate the persistency. A study conducted in 2002 (van Wezel and Jager, 2002) compared the OSPAR dynamec system and the EMEA guideline for risk assessment and prioritisation of pharmaceuticals. Results from this study suggest that the OSPAR dynamec is not adapted for pharmaceuticals. Log Kow for pharmaceuticals are often lower than 3, which prevent an efficient discrimination between molecules according to the bioaccumulation potential.

Modelling ecotoxicological data with ECOSAR software: As ecotoxicological data are lacking, some authors have investigated the use of the ECOSAR software to predict acute toxicity for pharmaceuticals and to rank pharmaceuticals accordingly (Sanderson et al., 2004a; 2004b; Cooper et al., 2008). However, there are two limits in the use of ECOSAR software applied to pharmaceuticals. First, QSARs only allow the modelling of acute toxicity data, which are of limited relevance as it is established that the risk linked to pharmaceuticals is chronic (Carlsson et al., 2006, Fent et al., 2006). Second, ECOSAR model has been implemented for compounds with high hydrophobicity and specific MoA. Therefore, the assessment of toxicity is mainly base on Log Kow value and non-specific narcosis endpoints which may be not relevant for pharmaceuticals, which are compounds with Kow generally lower than 3 and design to exert specific MoAs. Table 5 displays comparative results between ECOSAR modelled toxicity and experimental values. Even if only a few pharmaceuticals are tested, results show that large differences .

Molecule	ECOSAR algae LC ₅₀ 96 h (mg/l)	algae experimental toxicity	ECOSAR daphnids LC ₅₀ 48 h (mg/l)	daphnids experimental toxicity	ECOSAR fish LC ₅₀ 96 h (mg/l)	fish experimental toxicity
Propranolol	5.464	0.7 – 5.8	2.352	1.4 – 7.7	29.504	
Propranolol HCL	1914		3293		3352	
Metoprolol	13.985	7.3	8.244	63.9 - 438	116.283	
Erythromycin	17.432	0.0366	7.822	211	99.647	410 to > 1000
Ofloxacin	1249	12.1	1415	17.41	25465	> 1000
Fluoxetine	0.84	0.031	0.178	0.82	1.716	
Diazepam	5.428		2.258	4.3 – 14.1	27.955	12,7
Ibuprofen	7.51	315	38.51	132.6	31.759	173
Diclofenac	23.978	16.3	34.144	80.1	27.715	> 2
Diclofenac potassium	3059		5313		5473	
Diclofenac sodium	2911		5057		5209	

Table 5: Comparison of modelled and experimental toxicity for pharmaceuticals on aquatic organisms (Cemagref 2008).

Lienert et al. QSAR approach: Lienert et al. (2007) have proposed a screening tool to identify pharmaceuticals, including their human metabolites, with high environmental risk, mainly based on the use of modelling toxicity from quantitative structure activity relationships (QSAR). Their methodology does not consider chronic toxicity (due to lack of chronic data and appropriate QSAR) or specific toxic mode of action of parent drug or metabolite, but mainly baseline toxicity (i.e. narcosis). Nevertheless, in a more complex approach, the inclusion of known specific toxicity effects is possible and was shown for algal toxicity of β -Blockers. (Escher et al. 2006).

The approach is based on literature data of human metabolism and excretion, pharmaceuticals sales data and physicochemical properties of parent drugs and metabolites, and ecotoxicological data when exist. It allows to estimate a risk quotient of parent drug and metabolite mixture ($R_{q_{mix}} = PEC_{wastewater}/PNEC_{mix}$). When ecotoxicity data are missing, baseline acute toxicity is modelled with lipophilicity ($D_{lipw, pH7}$) estimates using QSAR. The mixture effect of each parent drug and its metabolites is treated with the model of concentration addition, assuming a similar mode of toxic action of all components. From literature information, the main phase of their procedure consists in a tiered estimation of the effect concentration (EC_{50}) of the mixture of the parent drug and metabolites, based on the assessment of the baseline toxicity of parent drug and next the relative potency of metabolites.

The relative toxicity potential of each metabolite is assessed in using the ratio between baseline toxicity of parent drug and modelled baseline toxicity of metabolite. Next it becomes possible to calculate the toxic potential of the mixture (when no toxicophore is present in metabolites). The procedure gives help to screen the risk of pharmaceutical including their metabolites. Nevertheless some uncertainties exist due to limitation of QSAR modelling when considering very hydrophilic drug and due to possible specific toxicity (by example sulfamethoxazole showed specific toxicity in algae) which is no accounted for when one models the baseline toxicity.

Ecotoxic potential: Jjemba (2006) conducted a study on the excretion and ecotoxicity of pharmaceuticals in the aquatic environment. The author investigated the correlation between several pharmacokinetic parameters and the behaviour of pharmaceuticals in the environment. He proposed a simple equation (Equation 15) to rank pharmaceuticals according to an ecotoxicity potential:

$$EP = \frac{T}{V \times NOEC}$$

EP: ecotoxic potential
T: overall residence time of the compound in the environment
V: concentration of the compound in the environment
NOEC: No Observable Effect Concentration

Equation 15: Determination of the ecotoxic potential of a compound.

Author suggests that the quantification of ecotoxicity should reflect an element of the overall residence time of the compound in question, its bioavailability, to the susceptible organisms, and its concentration in the environment. Time of residence is a main parameter of this model but no data are available at the moment for pharmaceuticals, and the author assumes a default value of 1 year (i.e. all year continuous discharge). Preliminary results of this study indicate a high EP for ethinylestradiol and carbamazepine, and a low EP for diclofenac and ibuprofen for daphnids.

II.2. Pharmacological data:

Several authors consider that the use of existing pharmacological, toxicological and pharmacokinetic data is likely to be helpful in assessing the risk of pharmaceuticals, as they could provide a better understanding of the fate and effect of pharmaceuticals in the aquatic environment (Fent et al. 2006; Jjemba 2006; Lange and Dietrich 2002; Seiler 2002; Besse and Garric, 2008). Pharmacological data alone are not sufficient enough to assess the risk for the aquatic environment, however, such data can give information on the mechanism of action (MoA) and the toxicity of the pharmaceuticals.

Mammalian pharmacological and toxicological data: The use of mammalian pharmacological and toxicological data was proposed to help to prioritize the potential impacts of pharmaceuticals to fish (Huggett *et al.* 2003). This model is based on the conservation of many enzyme/receptor systems between mammalian and teleost fish. In this model, a measured human therapeutic plasma concentration (H_TPC) is compared to a predicted steady-state plasma concentration (F_{SSPC}) in fish. The major assumption for F_{SSPC} derivation is that the pharmaceutical absorption from water to fish is driven by its hydrophobicity, therefore, only passive mechanism are taken into account in this model. (i.e. Log Kow). Finally an effect ratio ($ER = H_TPC / F_{SSPC}$) is computed. The lower the ER, the greater the potential for a response in fish.

Use of free plasma concentrations of antimicrobials in human has recently been suggested to assess the critical concentrations in aquatic organisms, other than microbes (Kostich and Lazorchak, 2008). This is based of the assumption that therapeutic plasma concentrations for antibacterials are an indicator of concentrations inducing effects in organisms. This assumption remains to be confirmed.

Mechanism of action (MoA): Mechanism of action of pharmaceuticals may provide useful information regarding the potential toxic effects on environmental targets. Pharmaceuticals, unlike other pollutants such as polycyclic aromatic hydrocarbons or pesticides, are molecules designed to exert a specific mode of action with a limited toxicity. Extensive metabolic and toxicological studies are central to the development of drugs and can provide valuable information to guide ecotoxicological studies (Owen et al., 2007).

For non-mammalian animals with receptors similar to those of mammals, similar biological effects or adverse reactions may occur; it was recently suggested that cardiovascular dysfunction could be one of the consequence of the waterborne exposure of fish to β -blockers (Owen et al., 2007). On the other hand, unexpected chronic effects may occur in lower organisms due to biological differences in pharmacodynamics and physiology (Fent et al., 2006; Seiler, 2002; Lange and Dietrich, 2002).

Recently, the evolutionary conservation of drug targets has been investigated (Gunnarsson et al., 2008; Kostich and Lazorchak, 2008).

The conservation of drug targets (by ortholog prediction) have been investigated for 1318 human drug targets and were predicted for 16 species, including species used in risk assessment (Gunnarsson et al., 2008). Prediction results estimated to 86% the homology of drug targets for the zebrafish, to 35% for *Daphnia pulex* and to 35% for green alga. For certain drugs, individual targets such as enzymes are well conserved, suggesting that tests on evolutionary distant organisms would be highly relevant for these drugs. Authors suggest that such result could be use to i) guide ERA by improving the possibilities to identify species sensitive to certain types of pharmaceuticals and ii) to interpret the relevance of existing ecotoxicological data.

The same general conclusions were drawn in the study of Kostich and Lazorchak (2008), in which the sequence conservation in drug target was used as a guidance on the range of species and type of endpoints that should be considered in chronic toxicity studies.

Side effects: Known side effects of pharmaceuticals may also be valuable to indicate potential harmful effects on non-target organisms as it as already been shown for diclofenac in vultures (Oaks et al. 2004), and in fish (Schwaiger et al. 2004; Triebkorn et al. 2004). Taking into account such effects could make it possible to target the harmful impacts of these compounds, at least on non-target vertebrates. Side effects have been used as an effect criteria in the prioritization strategy from Besse and Garric (2008).

Cytochrome P-450 modulation: Several drugs are known enzymatic inductors or inhibitors of the cytochrome P-450. P-450 isoforms are involved in a number of physiological reactions: transformation of both endogenous compounds and xenobiotics, synthesis and degradation of several steroids, prostaglandins, fatty acids, and other endogenous molecules (Stegeman et al., 1992). Therefore, modulation of the enzymatic response may lead to disruption in the homeostasis of non-target organisms. Interference between pharmaceuticals and the metabolizing enzyme have been recently shown *in vitro* (Thibaut et al., 2006).

Para glycoprotein-P (P-gp) modulation: Several pharmaceuticals are known to interact with P-glycoprotein (P-gp). P-gp is a protein acting as a multidrug transporter that actively transports xenobiotics out of the cell, preventing the accumulation of toxic compounds (Endicoot and Ling, 1989; Tutundjian and Minier, 2002). Glycoprotein P is involved in the multi-xenobiotic resistance (MXR) system. Increases in P-gp expression have been reported for aquatic organisms from polluted areas (Toomey and Epel, 1993; Britvic and Kurelec, 1999).

As P-gp could play an important role in the protection of the organism from toxic effects caused by xenobiotics, a modulation in the expression of the P-gp and particularly an inhibition of its expression by a specific drug could result in enhancing the sensitivity of organisms. P-gp modulation by pharmaceuticals in aquatic species remains to be studied.

Comparative pharmacology: Comparative pharmacology could also be useful to understand toxicity pathways of pharmaceuticals. At the moment, studies have only considered the major MoA of pharmaceuticals in ecotoxicity assays. However, evidence shows that compounds belonging to same pharmacological and chemical classes (e.g. compounds with same mechanisms of action), can display a high variability in toxic values on same species and endpoints (Garric et al. 2006; Dzialowski et al. 2006; Henry et al. 2004). Indeed, pharmaceuticals are not only characterized by their principal MoA but also by some additional pharmacological characteristics that should be taken into account. In the case of β -blockers, several authors (Fraysse and Garric 2005; Fent et al. 2006) have suggested that differences in toxicity should be partially explained by pharmacological properties specific to these compounds such as receptor selectivity or membrane-stabilizing activity.

For SSRIs, results from Henry et al. (2004) show that NOEC values on the reproduction of *C. dubia* range over two orders of magnitude. Pharmacological data indicate that even if SSRIs have a greater selectivity for blocking serotonin reuptake (their principal MoA), they also have affinities to some other receptors and reuptake inhibitor activities on other systems such as the noradrenergic or dopaminergic systems, (Hyttel 1993; Dulin et al. 2002). Such “secondary” MoAs could help to understand toxic responses (Besse et al., 2008).

Antimicrobial potency of antibiotic agents: The potency of an antibacterial agent is generally assessed by its Minimum Inhibitory Concentration (MIC). Kostich and Lazorchak (2008) suggested that MIC of such compounds could be used as an estimation of the critical concentrations inducing microbial effects in the environment, in particular antimicrobial resistance and growth inhibition of beneficial microbes. Using this approach, authors suggest that wastewater concentrations for pharmaceuticals are unlikely to select for clinically resistant microbes, in the absence of strong synergies between antibacterials. This conclusion remains to be confirmed as mechanism of selection of resistant bacteria in the environment remains unclear.

Conclusion

Prioritization of human pharmaceuticals is necessary due to the high number of pharmaceuticals used, which hinders the possibility to assess the ecotoxicity of every compound. To implement a relevant prioritization strategy, there is a need to accurately assess the environmental exposure and the environmental effects. From the review of ERAs and prioritization strategies conducted in the last ten years, it is possible to highlight the following parameters and to propose some concluding remarks:

For the exposure assessment:

- The use of simple models, as EMEA model, to calculate PECs for surface water is in general in good agreement with field measurements.
- Accurate, consumption amounts are essential, but data are sometimes unavailable, depending on the country.
- Metabolism data and excretion rates are essential but data are often incomplete or unavailable.
- STP removal rates are lacking which is a major limitation of the accuracy of ERA.
- PEC for other compartments than water column is not well assessed.
- Bioaccumulation of pharmaceuticals remains to be more studied.

For the effect assessment:

- Chronic ecotoxicological data are lacking.
- Due to gaps in ecotoxicological data for pharmaceuticals (due to the high number of pharmaceuticals), other ways of assessing the effects of pharmaceuticals have to be investigated and validated.
- Pharmacological data can be useful to estimate the biological effects on aquatic organisms however, i) the access to such data is sometimes not possible, ii) the relevance of such data for environmental considerations remains to be confirmed.

- Investigation of the evolutionary conservation of drug targets is important information that can help for a relevant use of pharmacological data, and for targeting sensitive species in bioassays.
- QSAR models should be investigated with regard to pharmaceuticals, which are compounds with specific mechanisms of action.

To conclude, it becomes necessary to harmonize the different prioritisation strategies and models now available in European countries. As pharmacological and toxicological data are not easily available, the ERA of old pharmaceuticals at least, could greatly benefit from the development of a database on existing pharmacological and ecotoxicological data

References

- Andreozzi R., Caprio V., Ciniglia C., De Champdore M., Lo Giudice R., Marotta R., Zuccato E. (2004). Antibiotics in the environment: Occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin. *Environmental Science and Technology* 38(24):6832-6838
- Ashton, D., Hilton, M., Thomas, K.V., (2004). Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. *Science of the Total Environment* 333:167–184.
- Bendz, D., Paxeus, N.A., Ginn, T.R., Loge, F.J. (2005). Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Høje River in Sweden. *Journal of Hazardous Materials* 122:195–204.
- Besse, J.-P., Kausch-Barreto, C., Garric, J. (2008). Exposure assessment of pharmaceuticals and their metabolites in the aquatic environment: Application to the French situation and preliminary prioritization. *Human and Ecological Risk Assessment* 14 (4): 665-695.
- Besse, J.-P., Garric, J. (2008) Human pharmaceuticals in surface waters. Implementation of a prioritization methodology and application to the French situation. *Toxicology Letters* 176 (2): 104-123
- Bound J.P., Voulvoulis N. (2004). Pharmaceuticals in the aquatic environment--a comparison of risk assessment strategies. *Chemosphere* 56(11):1143-1155.
- Bound J.P., Voulvoulis N. (2006). Predicted and measured concentrations for selected pharmaceuticals in UK rivers: Implications for risk assessment. *Water research* 40:2885-2892.
- Britvic S., Kurelec B. (1999). The effect of inhibitor of multixenobiotic resistance mechanism on the production of mutagens by *Dreissena polymorpha* in waters spiked with premutagens. *Aquatic Toxicology* 47:107-116.
- Budzinski, H., Togola, A. (2006). Présence des résidus de médicaments dans les différents compartiments du milieu aquatique. *Environnement Risques et Santé* 5:248–252.
- Carlsson C., Johansson A.K., Alvan G., Bergman K., Kuhler T. (2006) Are pharmaceuticals potent environmental pollutants?: Part I: Environmental risk assessments of selected active pharmaceutical ingredients. *Science of The Total Environment* 364-(1-3):67-87.
- Castiglioni, S., Fanelli, R., Calamari, D., Bagnati, R., Zuccato, E. (2004). Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy. *Regulatory Toxicology and Pharmacology* 39(1):25-32.
- Castiglioni S., Bagnati R., Fanelli R., Pomati F., Calamari D., Zuccato E. (2006). Removal of pharmaceuticals in sewage treatment plants in Italy. *Environmental Science and Technology* 40(1):357-363.

Cemagref 2008. Unpublished data.

Chang, H., Hu, J., Shao, B. (2007). Occurrence of natural and synthetic glucocorticoids in sewage treatment plants and receiving river waters. *Environmental Science and Technology* 41: 3462–3468.

Cooper, E.R., Siewicki, T.C., Phillips, K. (2008). Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment. *Science of the Total Environment* 398 (1-3): 26-33.

Crane, M., Watts, C., Boucard, T. (2006). Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Science of the Total Environment* 367 (1): 23-41.

CSTEE. (2001). Opinion on draft CPMP Discussion paper on the environmental risk assessment of medicinal products for human use, EMEA London.

Daughton C.G., Ternes T.A. (1999). Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environmental Health Perspectives* 107 Supplement 6:907-938.

DellaGreca, M., Fiorentino, A., Isidori, M., Lavorgna, M., Previtiera, L., Rubino, M., Temussi, F. (2004). Toxicity of prednisolone, dexamethasone and their photochemical derivatives on aquatic organisms. *Chemosphere* 54(5):629-637.

Dulin R., Silberstein N., Bonnin M., Saux MC. (2002). Comparison and practical guidelines of selective serotonin reuptake inhibitors [Comparaison et critères de choix des inhibiteurs sélectifs de la recapture de la sérotonine]. *Journal de Pharmacie Clinique* 21:39-46.

Dzialowski EM, Turner PK, Brooks BW. (2006). Physiological and reproductive effects of beta adrenergic receptor antagonists in *Daphnia magna*. *Archives of Environmental Contamination and Toxicology* 50(4):503-510.

EMEA 2006. Note for guidance on environmental risk assessment of medicinal products for human use. Doc. Ref. EMEA/CHMP/SWP/4447/00. Committee for proprietary medicinal products. European Agency for the Evaluation of Medicinal Products, London, UK. <http://www.emea.eu.int/pdfs/human/swp/444700en.pdf>.

Endicoot J.A., Ling V. (1989). The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annual review of biochemistry* 58:137-171.

Escher, B.I., Bramaz, N., Richter, M., Lienert, J. (2006). Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites using a mode-of-action-based test battery and a QSAR approach. *Environmental Science and Technology* 40 (23): 7402-7408.

EU 1994. Assessment of potential risks to the environment posed by medicinal products for human use, excluding products containing live genetically modified organisms. EU. Ad Hoc Working Party, III/5504/94 Draft 4.

EU TGD Technical Guidance Document. (2003). Technical Guidance Document in support of Council Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) 1488/94 on risk assessment for existing substances. Office for Official Publications of the European Communities, Luxembourg.

FDA. 1998. Guidance for Industry-Environmental Assessment of Human Drugs and Biologics Applications, Revision 1. FDA Center for Drug Evaluation and Research, Rockville.

Fent K, Weston AA, Caminada D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* 76(2):122-159.

Ferrari B, Mons R, Vollat B, Fraysse B, Paxeus N, Lo Giudice R, Pollio A, Garric J. (2004). Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? *Environmental Toxicology and Chemistry* 23(5):1344-1354.

Fraysse B, Garric J. (2005). Prediction and experimental validation of acute toxicity of β -blockers in *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 24(10):2470-2476.

Garric J., Ferrari B., Fraysse B., Mons R., Vollat B. (2006). Effects of some human pharmaceutical on freshwater organisms | [Impact de médicaments à usage humain sur les organismes aquatiques d'eau douce]. *Environnement, Risques et Sante* 5 (4):290-295.

Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Larsson, D.G.J. (2008). Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. *Environmental Science and Technology* 42 (15):5807-5813

Henry TB, Kwon JW, Armbrust KL, Black MC. (2004). Acute and chronic toxicity of five selective serotonin reuptake inhibitors in *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 23(9):2229-2233.

Hilton M.J., Thomas K.V., Ashton D. (2003). Targeted monitoring programme for pharmaceuticals in the aquatic environment. R&D Technical report P6-012/06/TR UK Environment Agency.

Huggett D.B., Cook J.C., Ericson J.F., Williams R.T. (2003). A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. *Human and Ecological Risk Assessment* 9(7):1789-1799.

Huschek, G., Hansen, P.D., Maurer, H.H., Kregel, D., Kayser, A.(2004). Environmental risk assessment of medicinal products for human use according to European Commission recommendations. *Environmental Toxicology* 19:226-240.

Hyttel J. (1993). Comparative pharmacology of selective serotonin re-uptake inhibitors (SSRIs). *Nordisk Journal of Psychiatry* 47(30):5-12.

Isidori M., Lavorgna M., Nardelli A., Parrella A., Previtiera L., Rubino M. (2005). Ecotoxicity of naproxen and its phototransformation products. *Science of the Total Environment* 348(1-3):93-101.

Isidori M., Nardelli A., Parrella A., Pascarella L., Previtiera L. (2006). A multispecies study to assess the toxic and genotoxic effect of pharmaceuticals: Furosemide and its photoproduct. *Chemosphere* 63(5):785-793.

Jean J. (2008). Identification et hiérarchisation des substances médicamenteuses bioaccumulables rejetées dans les effluents hospitaliers. Thèse pour le diplôme d'état de Docteur en Pharmacie. Thèse n° 48-2008. Université Claude Bernard-Lyon 1. Faculté de pharmacie. LYON.

Jjemba P.K. (2006). Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. *Ecotoxicology and Environmental Safety* 63(1):113-130.

Jones O.A.H, Voulvoulis N., Lester J.N. (2001). Human pharmaceuticals in the aquatic environment: a review. *Environmental Science and Technology* 22:1383-1394.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environmental Science and Technology* 36 :1202–1211.

Kostich, M.S., Lazorchak, J.M. (2008). Risks to aquatic organisms posed by human pharmaceutical use. *Science of the Total Environment* 38:329-339.

Lange R., Dietrich D. (2002). Environmental risk assessment of pharmaceutical drug substances--conceptual considerations. *Toxicology Letters* 131(1-2):97-104.

Lienert, J., Güdel, K., Escher, B.I. (2007). Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. *Environmental Science and Technology* 41 (12):4471-4478.

Oaks J.L., Gilbert M., Virani M.Z., Watson R.T., Meteyer C.U., Rideout B.A., Shivaprasad H.L., Ahmed S, Chaudhry M.J.I., Arshad M. and others. (2004). Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature* 427(6975):630-633.

OSPAR 2002. Dynamic Selection and Prioritisation Mechanism for Hazardous Substances (DYNAMEC).http://www.ospar.org/documents/dbase/publications/p00146_DYNAMEC%20Manual.pdf.

OSPAR 2006 Dynamic Selection and Prioritisation Mechanism for Hazardous Substances (New DYNAMEC Manual).
http://www.ospar.org/documents%5Cdbase%5Cpublications%5Cp00256_New%20DYNAMEC%20Manual.pdf

Owen S.F., Giltrow E., Huggett D.B., Hutchinson T.H., Saye J., Winter M.J., Sumpter J.P.(2007). Comparative physiology, pharmacology and toxicology of β -blockers: mammals versus fish. *Aquatic Toxicology* 82:145–162.

Paffoni C., Welte B., Gousailles M., Montiel A. (2006). Nouvelles molécules mises en cause par les directives Européennes : de la station d'épuration à l'usine de traitement d'eau potable. *Journal Européen d'Hydrologie*. 37(1):21-38

Paterson, G., Metcalfe, C.D.(2008). Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (*Oryzias latipes*). *Chemosphere in press*.

Petrovic M., Solé M., Lopez de Alda M.J., Barcelo D. (2002). Endocrine disruptors in sewage treatment plants, receiving water and sediments, integration of chemical analysis and biological effects on feral carp. *Environmental Toxicology and Chemistry* 21:2146-2156.

PNSE (2004). Plan National Santé Environnement. Ministère de la Santé et de la Protection sociale. Ministère de l'Ecologie et du Développement durable. Ministère de l'Emploi, du Travail et de la Cohésion sociale. Ministère délégué à la Recherche. Available at <http://www.sante.gouv.fr/htm/dossiers/pnse/rapport.pdf>.

Qin-Tao L, Williams HE. (2007). Kinetics and degradation products for direct photolysis of β - blockers in water. *Environmental Science and Technology* 41:803-810.

Sanderson H., Brain R.A., Johnson D.J., Wilson C.J., Solomon K.R. (2004a). Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular, and sex hormones. *Toxicology* 203(1-3):27-40.

Sanderson H., Johnson D.J., Reitsma T., Brain R.A., Wilson C.J., Solomon K.R. (2004b). Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. *Regulatory Toxicology and Pharmacology* 39(2):158-183.

Schwaiger J., Ferling H., Mallow U., Wintermayr H., Negele R.D. (2004). Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquatic Toxicology* 68(2):141-150.

Seiler J.P. (2002). Pharmacodynamic activity of drugs and ecotoxicology--can the two be connected? *Toxicology Letters* 131(1-2):105-115.

Stegeman J.J., Brouwer M., Richard T.D.G., Förlin L., Fowler B.A., Sanders B.M., van Veld P.A. (1992). Molecular responses to environmental contamination : enzyme and protein systems as indicators of chemical exposure and effect. in Hugget R.J., Kimerly R.A. (Eds.), *Biomarkers : biochemical, physiological and histological markers of anthropogenic stress*. Lewis publishers, Chelsea, MIn USA, pp. 235-335.

Straub J.O. (2002). Environmental risk assessment for new human pharmaceuticals in the European Union according to the draft guideline/discussion paper of January 2001. *Toxicology Letters* 135(3):231-237.

Struijs J., Stoltenkamp J., van de Meent D. (1991). A spreadsheet based box model to predict the fate of xenobiotics in a municipal wastewater treatment plant. *Water Research* 25:891-900.

Stuer-Lauridsen F, Birkved M, Hansen LP, Holten Lutzhoft HC, Halling-Sorensen B. (2000). Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere* 40(7):783-793.

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32: 3245–3260.

Thibaut R., Schnell S., Porte C., 2006. The interference of pharmaceuticals with endogenous and xenobiotic metabolizing enzymes in carp liver: an *in vitro* study. *Environmental Science and Technology* 40:5154–5460.

Togola, A., Bristeau, S., Amalric, L. (2007). Occurrence of pharmaceuticals in aquatic systems of Loire-Brittany Basin (France). Poster communication. ERAPharm International Conference on Pharmaceuticals in the Environment. Lakeside Conference Centre, York, UK.

Tolls J. (2001). Sorption of veterinary pharmaceuticals in soils: A review. *Environmental Science and Technology* 35(17):3397-3406.

Toomey B.H., Epel D. (1993). Multixenobiotic resistance in *Urechis caupo* embryos: protection from environmental toxins. *Biology Bulletin* 185:355-364.

Triebkorn R., Casper H., Heyd A., Eikemper R., Köhler H.R., Schwaiger J. (2004). Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (*Oncorhynchus mykiss*) *Aquatic Toxicology* 68(2):151-166.

Tutundjian R., Minier C. (2002). Les protéines de résistance multiple et leur exploitation pour la biosurveillance chez les organismes aquatiques. *Regard sur la biochimie* 4:37-50.

van Wezel AP, Jager T. (2002). Comparison of two screening level risk assessment approaches for six disinfectants and pharmaceuticals. *Chemosphere* 47(10):1113-1128.

Vasskog, T., Berger, U., Samuelsen, P.J., Kallenborn, R., Jensen, E. (2006). Selective serotonin reuptake inhibitors in sewage influents and effluents from Tromsø, Norway. *Journal of Chromatography A* 1115:187–195.

Wells M.J.M. (2006). Log Dow: Key to understanding and regulating waste-water-derived contaminants. *Environmental Chemistry* 3:439-449.

Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Löffler, J., Reincke, H., Schmidt, R., Stachel, B., Von Tümpling, W., Wanke, A. (2004). Pharmaceuticals in the river Elbe and its tributaries. *Chemosphere* 57:107–126.

Williams M., Saison C.L.A., Williams D.B., Kookana R.S. (2006). Can aquatic distribution of human pharmaceuticals be related to pharmacological data? *Chemosphere* (65):2253-2259.

Yu J.T., Bower E.J., Coelhan M. (2006). Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent. *Agricultural Water Management* 86:72-80.

Zuccato E, Castiglioni S, Fanelli R. (2005). Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. *Journal of Hazardous Materials* 122(3):205-209.

Annexes

Priority list of pharmaceuticals (parent compounds and metabolites) implemented with the Besse and Garric (2008) methodology for the French environment.

molecule	PEC a (ng.l ⁻¹)	PEC b (ng.l ⁻¹)	exposure priority class	therapeutic / chemical class	reason(s) for including the compound in the priority list	metabolite(s)	found in surface water (reference)	Additional data need
allopurinol		150	IA	antigout	PEC value	oxypurinol		confirm occurrence in water ; ecotoxicological data
amiodarone	555		IB	antiarrhythmic	high Kow ; adverse effects linked to iode CYP-450 and P-gp inhibitor	N-desethyl amiodarone		confirm occurrence in water or sediment ; may sorb to WWTP sludge due to high Kow ; may search for its active metabolite
amoxicillin		6,847	IA	antibiotic penicillin	PEC value ; antibiotic		Zuccato et al., 2005 Paffoni et al., 2006	confirm occurrence in water, maybe readily degradable (Zuccato et al., 2005) ; ecotoxicological data in fish
amphotericin B		415	IA	antifungal	PEC value ; kidney toxicity			confirm occurrence in water
atenolol		419	IA	ATH β-blocker	PEC value		Zuccato et al., 2005	ecotoxicological data
bezafibrate		476	IA	blood lipid lowering agent (fibrate)	PEC value ; muscular disease (rhabdomyolysis) ; PPAR agonist		Zuccato et al., 2005 Wiegel et al., 2004	ecotoxicological data
buflomedil		291	IA	anti-ischemic	PEC value			confirm occurrence in water ; ecotoxicological data
carbamazepine	765		IB	anticonvulsivant	PEC value ; may be persistent in the aquatic environment ; P450 inducer	10,11-epoxy- carbamazepine	Zuccato et al., 2005 Wiegel et al., 2004	
ceftriaxone		315	IA	antibiotic cephalosporin	PEC value ; antibiotic			confirm occurrence in water, maybe readily degradable ; ecotoxicological data
ciprofloxacin		139	IA	antibiotic fluoroquinolone	PEC value ; antibiotic ; high ecotoxicity		Zuccato et al., 2005	ecotoxicological data
clarithromycin		62	IIA	antibiotic macrolide	ATB ; high ecotoxicity on blue-green algae ; CYP-450 and P-gp inhibitor		Zuccato et al., 2005 Wiegel et al., 2004	
cyamemazine	124		IB	antipsychotic	endocrine and metabolic disorders in man due to dopaminergic receptor blockade			confirm occurrence in water ; ecotoxicological data
diclofenac		35	IIA	NSAID	high Kow ; adverse effects on kidney		Ashton et al., 2004 Budzinski & Togola 2006	ecotoxicological data
diosmin	8,528		IB	vitaminic P	flavonoid ; potent estrogenic activity	diosmetin (deglycosylated form)		confirm occurrence of diosmetin in water rather than diosmin ; evaluate diosmetin estrogenic activity
doxycycline		103	IA	antibiotic tetracycline	PEC value ; antibiotic			confirm occurrence in water ; complexing properties of cyclines and possible sorption to suspended matter (Hirsch et al., 1999)
fluoxetine		9	III	antidepressant SSRI	agonist of serotonergic receptors ; high ecotoxicity ; P-gp inhibitor	norfluoxetine	Vasskog et al., 2006 Kolpin et al., 2002	ecotoxicological data in fish
fosfomicin		155	IA	antibiotic phosphonic	PEC value ; antibiotic			confirm occurrence in water
furosemide		486	IA	diuretic	PEC value		Zuccato et al., 2005	ecotoxicological data in fish
ibuprofen		1,370	IA	NSAID	PEC value ; potential renal toxicity	2-OH-ibuprofen carboxy-ibuprofen	Zuccato et al., 2005 Budzinski & Togola 2006	ecotoxicological data

molecule	PEC a (ng.l ⁻¹)	PEC b (ng.l ⁻¹)	exposure priority class	therapeutic / chemical class	reason(s) for including the compound to the priority list	metabolite(s)	found in surface water (reference)	Additional data need
ketoprofen		421	IA	NSAID	PEC value ; potential renal toxicity		Budzinski & Togola 2006	ecotoxicological data
losartan	334		IB	ATH sartan	MoA ; decrease in aldosterone secretion	5-carboxylic acid metabolite		confirm occurrence in water ; ecotoxicological data
metformin		16,367	IA	antidiabetic	PEC value			ecotoxicological data
metronidazole		150	IA	antiprotozoal	PEC value ; antiprotozoal activity	OH-metronidazole		confirm occurrence in water
naftidrofuryl	1,039		IB	anti-ischemic	antagonist activity of serotonergic 5-HT ₂ receptors			confirm occurrence in water
naproxen		597	IA	NSAID	PEC value ; potential renal toxicity		Budzinski & Togola 2006	ecotoxicological data on fish
ofloxacin	94		IIA	antibiotic fluoroquinolone	PEC value ; ATB ; high ecotoxicity		Zuccato et al., 2005	
oxazepam		207	IA	benzodiazepine	PEC value		Togola et al., 2007	ecotoxicological data
paracetamol	64,101		IA	antipyretic / analgesic	PEC value		Budzinski & Togola 2006	ecotoxicological data
piperacillin		102	IA	antibiotic ureidopenicillin	PEC value ; ATB			confirm occurrence in water, maybe readily degradable ; ecotoxicological data
pravastatin		125	IA	blood lipid lowering agent (statin)	adverse effects on striated muscle ; evidence of endocrine disruption in insects by fluvastatin ; MoA ; carcinogenic to rodents			confirm occurrence in water ; ecotoxicological data
prednisolone	85		IIB	corticoid	immunomodulating properties ; metabolism data		Chang et al., 2007	ecotoxicological data
pristinamycin	910		IB	antibiotic streptogramine	PEC value ; ATB			confirm occurrence in water ; ecotoxicological data
propranolol	68		IIA	ATH β-blocker	high ecotoxicity ; adverse effects on thyroid	4-OH-propranolol	Ashton et al., 2004 Miège et al., 2006	
ranitidine	133		IA	antacid	PEC value		Zuccato et al., 2005	ecotoxicological data
sertraline	20		IIA	antidepressant SSRI	serotonergic activity ; high Kow ; high ecotoxicity ; P450 inhibitor		Vasskog et al., 2006	ecotoxicological data on fish and algae
sulfamethoxazole	153		IA	antibiotic sulfonamide	PEC value ; ATB synergy with trimethoprim		Ashton et al., 2004 Wiegel et al., 2004	ecotoxicological data of mixture with trimethoprim
tramadol	177		IA	analgesic	PEC value	demethyltramadol		ecotoxicological data
trimethoprim	38		IIA	antibiotic benzylpyrimidine	synergy with sulfamethoxazole		Ashton et al., 2004 Wiegel et al., 2004	ecotoxicological data of mixture with sulfamethoxazole
valproic acid	1,357		IA	anticonvulsivant	PEC value ; P450 inhibitor			confirm occurrence in water maybe extensively removed in WWTP (Yu et al., 2007)
vancomycin	21		IIA	antibiotic glycopeptide	ATB			confirm occurrence in water, could be extensively removed in WWTP (Paffoni et al., 2006)

metabolite	PEC b (ng.l ⁻¹)	parent compound	reason(s) for inclusion on priority list	pharmacological activity	found in surface water (reference)	Additional data need
salicylic acid	ND	aspirin	active metabolite of the prodrug	responsible for pharmacological activity	Ternes 1998	
fenofibric acid	1148	fenofibrate	active metabolite of the prodrug	responsible for pharmacological activity	Ternes 1998	
perindoprilat	192	perindopril	active metabolite of the prodrug	responsible for pharmacological activity		confirm occurrence in water / ecotoxicological data
ramiprilat	125	ramipril	active metabolite of the prodrug	responsible for pharmacological activity		confirm occurrence in water / ecotoxicological data
demethyltramadol	355	tramadol	active, high excretion rate (60%)	active, analgesic activity		confirm occurrence in water / ecotoxicological data
hydroxy-ibuprofen	1370	ibuprofen	high excretion rate (25%)	inactive	Benz et al., 2005	ecotoxicological data
carboxy-ibuprofen	2027	ibuprofen	high excretion rate (37%)	inactive	Benz et al., 2005	ecotoxicological data
acetylsulfamethoxazole	229	sulfamethoxazole	high excretion rate (60%)	inactive	Ashton et al., 2004	ecotoxicological data
14-OH-clarithromycin	52	clarithromycin	active, synergy with parent drug	active, synergy with parent compound on some bacterial strains		confirm occurrence in water / ecotoxicological data
norfluoxetine	24	fluoxetine	active, high excretion rate (28%)	equipotent to parent drug		confirm occurrence in water / ecotoxicological data
OH-metronidazole	234	metronidazole	active, high excretion rate (28%)	30% to 65% of the activity of metronidazole		confirm occurrence in water / ecotoxicological data
β-hydroxy-acid metabolite	87	simvastatin	active metabolite of the prodrug	responsible for pharmacological activity		confirm occurrence in water / ecotoxicological data
2-OH-atorvastatin	ND	atorvastatin	active metabolite of the prodrug	responsible for pharmacological activity		confirm occurrence in water / ecotoxicological data
4-OH-atorvastatin	ND	atorvastatin	active metabolite of the prodrug	responsible for pharmacological activity		confirm occurrence in water / ecotoxicological data

ND: not determined