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*Ecotoxicology of pharmaceuticals: making sense of the published literature
Report of the WP4 Workshop; 28 – 31 January, 2008, Harrogate, UK*

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1. Executive summary

Over the past ten years there has been increasing interest over the effects of pharmaceuticals on aquatic organisms and a large number of publications have reported effects data for pharmaceuticals in aquatic systems. This report summarises the output from a four-day expert workshop held in January 2008 that synthesised and analysed the published data in order to:

- a) identify what data is available;
- b) assess whether there is evidence for environmental risks;
- c) determine the ecological relevance of the published endpoints;
- d) evaluate and develop methods for assessing ecotoxicity of pharmaceuticals; and
- e) identify gaps in the knowledge and develop recommendations for future research work.

On the basis of the discussions, the workshop concluded the following:

1. A large body of data is now available on the ecotoxicity of pharmaceuticals; this indicates that a large proportion of pharmaceuticals are not highly toxic to aquatic organisms;
2. With a few exceptions, reported effect concentrations are higher than maximum concentrations measured in the natural environment suggesting that many pharmaceuticals probably pose a low risk to ecosystems;
3. Studies have reported non-standard effects on organisms, many of these ‘novel’ endpoints can be linked to ecologically relevant endpoints such as reproduction, growth and predator avoidance;
4. Existing predictive and extrapolation approaches such as QSARs and the use of acute:chronic ratios are inappropriate for use on pharmaceuticals;
5. Pharmaceuticals will occur in the environment as mixtures. For pharmaceuticals of the same class, it should be possible to estimate the combined risk of the mixture using concentration addition calculations;
6. Many pharmaceuticals will be metabolised or degrade in the environment. It may be possible to use information on the structure and properties of any transformation product to identify substances that pose the greatest risk to the environment;
7. A wealth of data is generated during the development of a pharmaceutical. This data could help to inform the environmental risk assessment of pharmaceuticals;
8. Risks of pharmaceuticals in the future might change due to changes in climate (which may result in increased disease pressures) and during pandemic situations.

2. Introduction and background

Over the past few years there has been a dramatic increase in the amount of research being performed into the potential effects and risks of pharmaceuticals in the natural environment. It is now timely to review this body of information in order to explore amongst other things:

- a) the implications of the published data in terms of environmental risks;
- b) the advantages and limitations of different testing approaches;
- c) relationships between different endpoint types and sensitivity of different organism classes;
- d) the application of predictive approaches;
- e) the integration of alternative endpoint into risk/hazard assessment; and
- f) major knowledge gaps and future research needs.

An EU funded workshop (funded as part of the FP6 KNAPPE project) therefore brought together scientists from Europe and N. America to discuss these issues. This report summarises the main findings of the Workshop. A more detailed manuscript of the Workshop findings is currently being produced and this will be submitted by the end of the year for publication in the Journal *Reviews in Environmental Contamination and Toxicology*.

3. Workshop structure

The workshop ran over four days and involved a series of plenary sessions and working group discussions involving two groups. The workshop participants are listed below:

Group 1

Name	Affiliation
Thomas Backhaus	Göteborg University, Sweden
Alistair Boxall (Chair)	University of York, UK
Jason Weeks	Cefas, UK
Silke Hickman	UBA, Germany
Chris Metcalfe	Trent University, Canada
Oliver Straub	Roche, Switzerland

Group 2

Name	Affiliation
Bryan Brooks	Baylor University, USA
Daniel Gouy	Sanofi-Aventis, France
Karen Kidd	University of New Brunswick, Canada
Melanie Netherton	University of York, UK
Joanne Parrot	Environment Canada, Canada

Each Working Group was tasked with addressing a series of questions:

Group 1: Risk Issues

1. What are the impacts of recently studied pharmaceuticals on environmental health and how do effects concentrations compare to exposure data?
2. What are the potential risks of substances that have yet to be studied? - Due to resource limitations, only a small proportion of pharmaceuticals in use today have been investigated. So using existing data along with models and information on mode of action, can we identify those substances that are likely to be of most concern and hence should be a priority for future work?
3. What are the risks of mixtures? – It has been demonstrated that pharmaceuticals are unlikely to appear in the environment on their own and so the current ‘single substance’ approach to environment risk assessment could be underestimating impacts.
4. Should we worry about the transformation products? – Most work to date has focused on the parent compounds yet; we know that transformation products will be produced in animals, in the environment and in treatment processes. It is important that we begin to understand the potential impacts of these metabolites.

Group 2: Ecotoxicity Issues

1. How can we better assess ecotoxicity? It is clear that current standard ecotoxicity tests are probably inappropriate for assessing the impacts of PP's. The use of more subtle endpoints such as impacts on behaviour, physiology and biochemistry appear to show some merit; we may also be able to use ADME data to steer testing. A review of these methods will therefore be developed.
2. What is the relevance of the ecotoxicity data? – A number of subtle effects have been demonstrated following exposure to PP's at environmentally realistic concentrations. What is the significance of these data in terms of ecological functioning?
3. What are the indirect effects?

The main conclusions from the workshop are discussed in the following sections.

4. Workshop findings

4.1. Data availability and distribution

Over the past ten years a large body of data has been generated on the effects of pharmaceuticals on organisms within the environment. Around 20,000 study endpoints for both human and veterinary pharmaceuticals are available (see Table 1). The data include both acute and chronic endpoints and the top three study organisms are fish, water flea and other small crustaceans. The number of studies performed per class of pharmaceutical compound is shown in the Table 2. The top three drug classes studied were cholinesterase inhibitors (used in veterinary medicine), lysozyme inhibitors and antiscabies agents (e.g lindane). When all data are considered, the vast majority of studies indicate that in general pharmaceuticals are not highly toxic to aquatic organisms (Figure 1). A small proportion of compounds are however highly toxic to some organisms and endpoints (Figure 1).

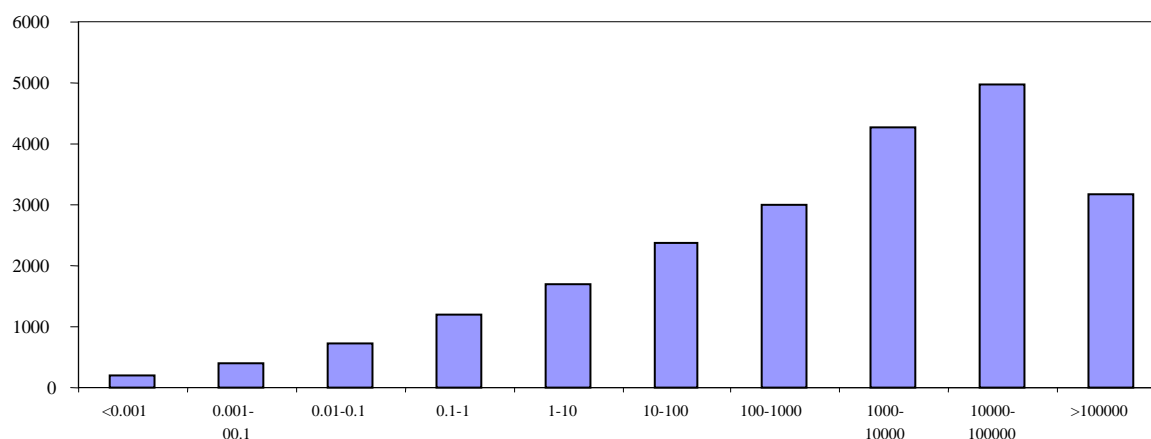
Table 1. Top ten most studied organism types covered in the database

Test Class	Number of studies
Fish	12137
Water Flea	2374
Small Crustacea	2140
Insect	1647
Algae	1403
Mollusc	1173
Protozoa	455
Amphibian	368
Worm	361
Large Crustacea	275
Rotifer	258

Table 2. Top ten most studied drug classes

Drug class	Number of studies
Cholinesterase inhibitors	2943
Lysozyme inhibitor	2435
Antiscabies agent	2102
Surface-active agents	1201
Estrogens	1186
Anti-infective agents	1109
Pentaerythritol tetranitrate reductase inhibitor	752
Anti-menopausal agents	678
D-alanyl-D-alanine carboxypeptidase inhibitor	628
Alpha-amylase	575
Anti-bacterial agents	432

Figure 1. Frequency of different effect concentration ($\mu\text{g/l}$) ranges in published ecotoxicity data for pharmaceuticals.



4.2. Comparison of effects data with measured concentrations

Workshop participants attempted to interpret the published ecotoxicological data in terms of its environmental significance. Reported effects data were compared with occurrence data (expressed as maximum measured concentrations (MECs)) to determine the ecotoxicological significance of environmental concentrations. None of the reported acute standard endpoints was higher than the MEC for any pharmaceutical (Figure 2). When standard chronic tests data are considered, effect concentrations were higher than MECs only for 17 α -ethinylestradiol and 17 α /beta estradiol (Figure 3). When non standard endpoints were considered effect

concentrations for 17alpha-ethinylestradiol, acetaminophen, 17alpha/beta estradiol, and salicylic acid were found to be above the MEC.

These findings indicate that a) acute effects of pharmaceuticals on the environment are unlikely; b) standard chronic effects are possible for some substances at environmentally realistic concentrations; and c) subtle effects (not covered in standard toxicity investigations) are also possible for some pharmaceuticals at environmentally realistic concentrations. The ecological relevance of these subtle effects is however not known (this is discussed later).

Figure 2. MEC vs acute effect concentrations for a range of pharmaceuticals

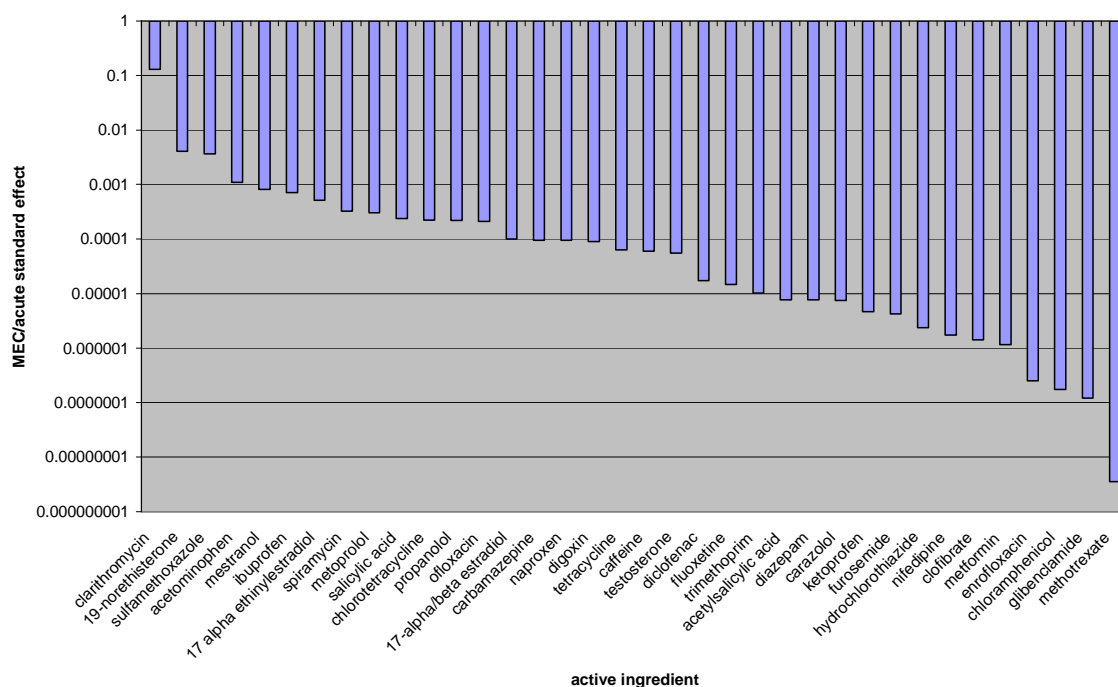


Figure 3 . MEC vs standard chronic effect concentrations for a range of pharmaceuticals

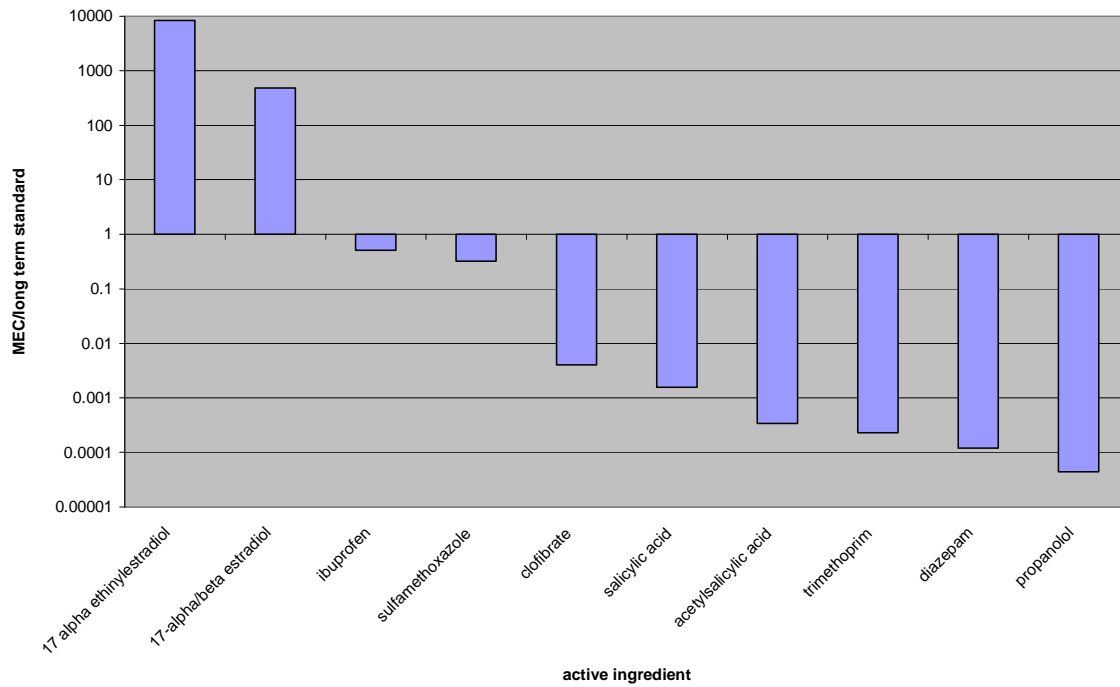
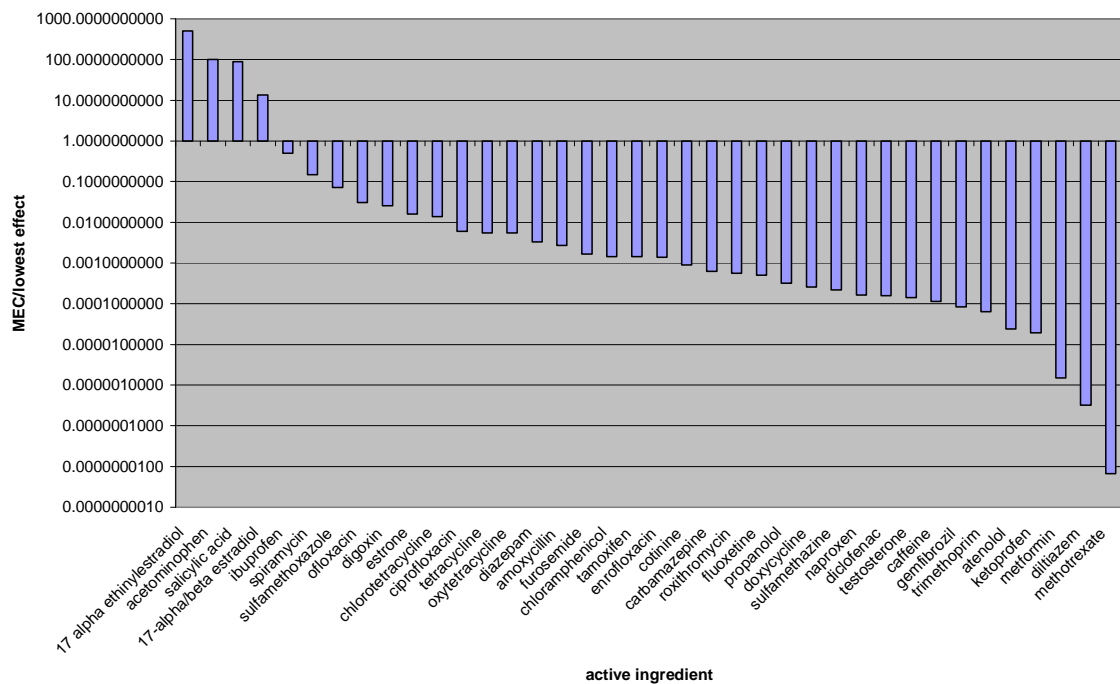


Figure 4 MEC vs lowest reported effect concentrations (including standard tests and ‘novel’ tests for a range of pharmaceuticals



4.3. Ecological relevance of reported 'novel' effect concentrations

A number of non-standard ecotoxicity studies have been performed on pharmaceuticals. These studies have involved the use of invertebrates and fish and have looked at a range of endpoints including effects on nest holding, egg production, heart rate, activity and feeding rate and behaviour. As many of these 'novel' effects can occur at environmentally realistic concentration levels, workshop participants discussed whether or not the results should be of concern to the scientific community. Participants therefore attempted to link published novel endpoints to important ecological functions.

The results of these discussions are summarised in Table 3. Participants agreed that a number of the reported endpoints observed for different drug classes can be linked to either reproduction, growth and predator avoidance. Depending on the exposure levels, further study of these classes may therefore be warranted.

Table 3. Predicted ecological relevance of some of the novel endpoints that have been observed for pharmaceuticals

Ecological relevance	Novel endpoints	Substance class showing endpoint
Reproduction	nest holding, egg production, ovo-testis, heart rate, nest defence, activity, spawning	androgens, antifungals, estrogens, lipid regulators, NSAIDs, SSRIs, diuretics
Growth	heart rate, histopathology, feeding rate, feeding behaviour	B-blockers, NSAID, SSRI, antibiotic, antiepileptic, diuretic, lipid regulator
Predator avoidance	activity, heart rate	B-blockers, antibiotic, diuretic

4.4. Can the effects of pharmaceuticals be predicted using QSARs or acute:chronic ratios?

For other classes of chemicals, extrapolation approaches (e.g. use of acute:chronic ratios) and QSARs have been proposed for estimating ecotoxicity values. In order to test whether these approaches might be suitable for use on pharmaceuticals, the workshop evaluated a range of different approaches for ecotoxicity estimation. Example outputs from these analyses are

shown in Figures 5 and 6. The workshop concluded that currently available QSAR approaches and the application of typical acute: chronic ratios for estimating chronic toxicity from acute toxicity are probably inappropriate for use on pharmaceuticals. Further development work is therefore required in this area.

Figure 5 QSAR Predicted versus mean observed acute toxicity values for *Pimephales promelas*

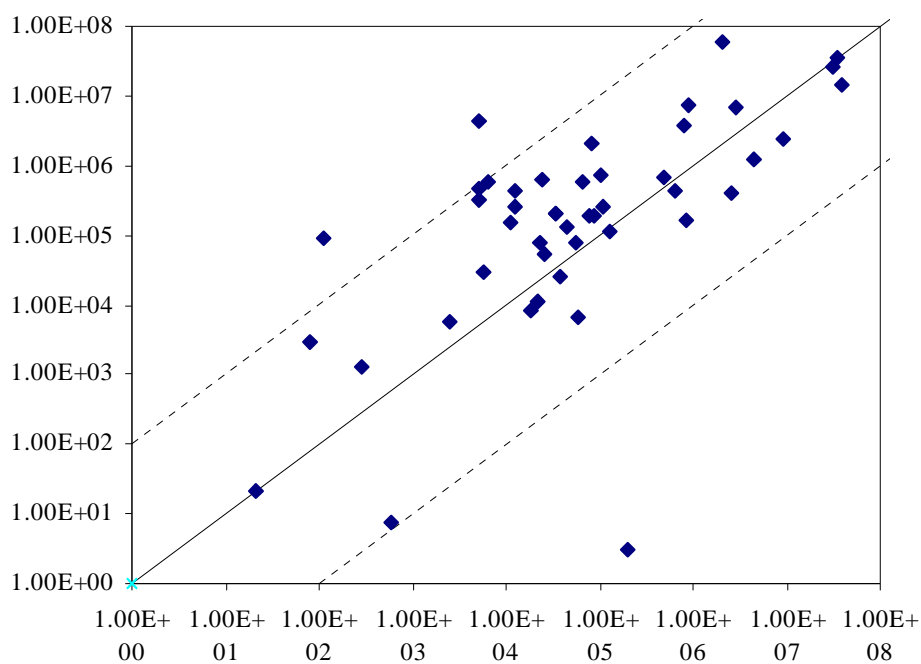
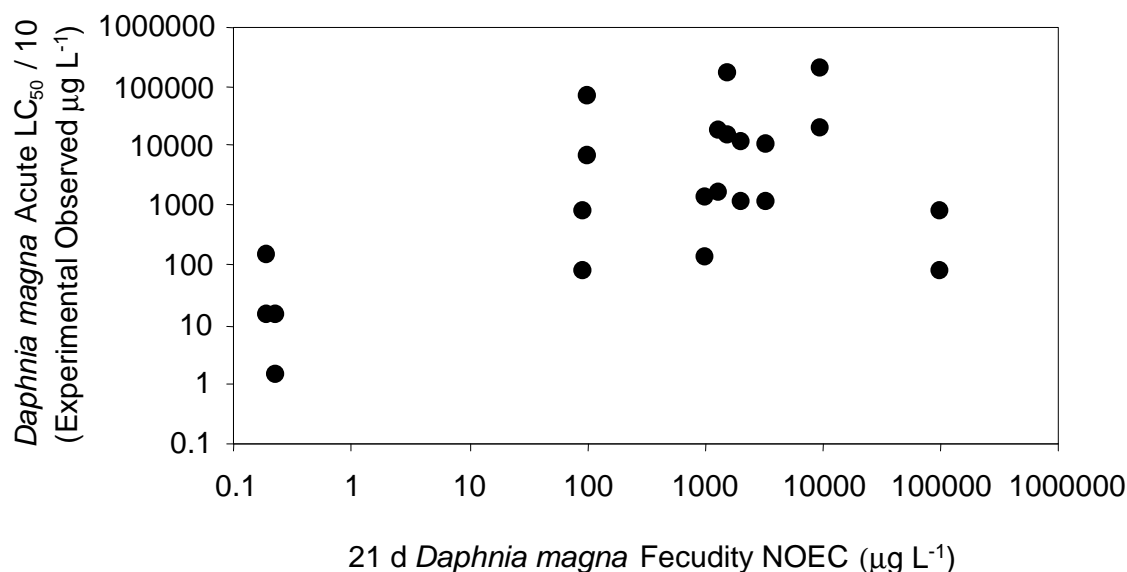


Figure 6 Comparison of measured chronic ecotoxicity values with estimated values obtained using acute data and an acute: chronic ratio of ten.



4.5. Effects of mixtures

Pharmaceuticals usually do not occur as individual, isolated compounds in the environment. Instead, the typical exposure is towards pharmaceutical mixtures. According to our current knowledge the toxicity of mixtures of similarly acting compounds can be described by the concept of Concentration Addition (CA), which implies that the toxic unit of a mixture – the ratio between the total mixture concentration and a pre-defined effect concentration of the mixture, which is most often its EC50 – equals the total sum of the toxic units of its components:

$$\sum_{i=1}^n \frac{c_i}{EC50_i} = \frac{c_{Mix}}{EC50_{Mix}} \text{ Equation 1}$$

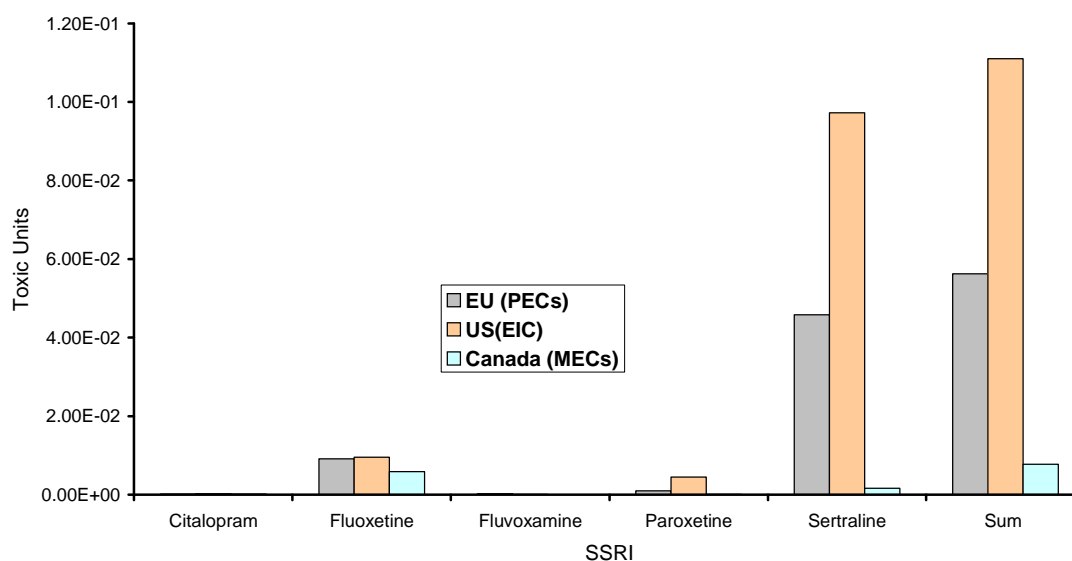
where c_i gives the concentration (or dose) of the i th component in a n -compound mixture and $EC50_i$ denotes the concentration of that substance which provokes 50 % effect if applied singly. c_{Mix} is the total concentration of the mixture and $EC50_{Mix}$ gives the mixture concentration that provokes 50% total effect. It should be noted, that CA implicitly assumes that all EC50 values have been recording in the same assay. The validity of CA has been demonstrated in a range of different assays and pharmaceuticals, e.g. for antibiotics,

estrogens, anti-inflammatory drugs, β -blockers or selective serotonin re-uptake inhibitors (SSRIs), a group of commonly used antidepressants.

During the workshop the method was applied to SSRIs. Data were obtained on acute ecotoxicity to algae, fish and daphnids and predicted concentrations were obtained for Europe and the US and measured concentrations were obtained for Canada. The analysis (Figure 7) indicated that the co-occurrence of SSRIs is unlikely to cause acute effects in aquatic systems.

Due to a lack of data, the workshop was unable to apply the approach to chronic endpoints. We would therefore advocate that in the future these combined effects are modelled as are other mixtures of substances from the same class. Consideration should also be given to the effects of mixtures of different chemical classes (i.e. mixtures of different pharmaceutical classes as well as mixtures of pharmaceuticals with other types of chemical (e.g. pesticides).

Figure 7. Toxic unites for individual SSRIs and SSRI mixtures in the EU, US and Canada



4.6. Do metabolites pose a risk?

Following administration, a pharmaceutical may be metabolised and consequently, a mixture of the metabolite(s) and the parent compound may be released to the environment. Once in the environment, both the metabolites and the parent compound may be further degraded by abiotic and biotic processes. The fate and effects of all the transformation products (i.e.

metabolites and degradation products) may differ from that of the parent compound which means that the environmental risks could be very different from those of the parent compound. With the exception of a few drugs, limited information is available in the public domain on the ecotoxicity of metabolites and transformation products.

The workshop therefore explored ways in which transformation products of most concern could be identified without the need for extensive testing. Data is available on the ecotoxicity of pesticide transformation products that provides useful information on which factors make a transformation product more ecotoxic than the parent compound. The majority of pesticides transformation products is less toxic or has similar toxicity to their associated parent compound. However some are more than 3 times more toxic than the parent compound. These observed increases can be due to a number of reasons:

- 1) the uptake of the transformation product into organisms is greater than for the parent compound, due to either an increase in lipophilicity or a change in dissociation or both;
- 2) the transformation product contains the active moiety of the parent compound; and
- 3) the transformation reaction results in the introduction of a toxic moiety which is not present in the parent compound.

Using the above knowledge, if you know the structure of a metabolite or transformation product of a pharmaceutical, it is possible to identify which substances are likely to be more toxic than the associated parent compound. The lipophilicity and dissociation behaviour of a molecule can be estimated using quantitative structure-property relationships. If you know the active moiety (or pharmacophore) of the parent compound, then a quick visual assessment of the transformation product can tell you whether this moiety is present in the transformation product molecule. A number of approaches are available to identify whether a new toxic moiety has been introduced during the transformation reaction. These approaches generally use structural alerts that are associated with a range of environmental models of action. If the transformation product contains one of these structural alerts but this alert is not present in the parent compound, then the transformation product may be more toxic than the parent compound.

4.7. Use of information on properties, safety and efficacy in humans

The registration dossier for a new drug is composed of 3 sections containing information that may be used for environmental risk assessment these include:

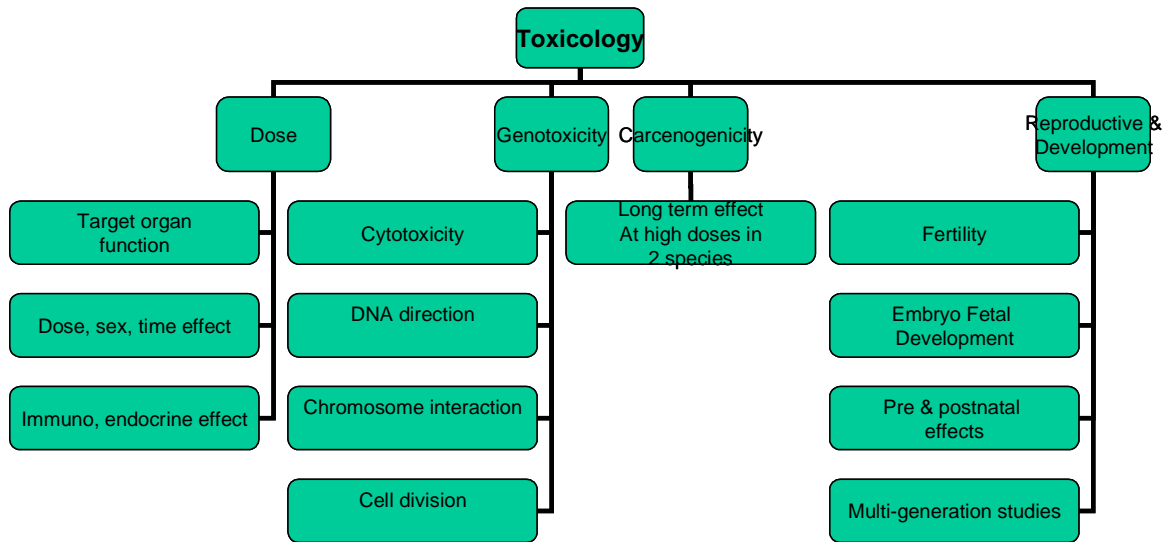
1) the pharmaceutical section the contains information on the physical and chemical properties of the active substance and excipients;

2) the nonclinical section contains information on the biological properties of the compound; and

3) The clinical section which includes information on the activity and tolerance as well as metabolism and pharmacokinetic parameters to justify the therapeutic benefit of the drug.

The workshop participants discussed how this information could be used to inform the environmental risk assessment. It was concluded that the development phases for a new drug provide scientific information (e.g. Figure 8) which should be used in a scientific comprehensive integrated evaluation of the environmental risk. This however is an area which requires significantly more research. The use of proteomics and metabolomics may also provide useful information.

Figure 8 Types of information generated in the mammalian toxicology assessment of a pharmaceutical



4.8. Conclusions

On the basis of the discussions, the workshop concluded the following:

1. A large body of data is now available on the ecotoxicity of pharmaceuticals. This data covers a range of species and endpoints. Data on effect distributions indicates that many pharmaceuticals are not highly toxic to aquatic organisms;
2. Generally, reported effect concentrations are higher than maximum concentrations measured in the natural environment suggesting that many pharmaceuticals probably pose a low risk to ecosystems. There are however some exceptions where effects on reproduction and growth and novel impacts have been seen in the laboratory at concentrations close (or lower) than those seen in surface waters;
3. A number of 'novel' endpoints have been observed in the laboratory. For many of these, it is possible to identify a potential link with ecologically relevant endpoints such as reproduction, growth and predator avoidance. In instances where these novel endpoints are observed at environmentally realistic concentrations, further experimental work is warranted to understand the implications on ecosystem health;
4. Existing predictive and extrapolation approaches such as QSARs and the use of acute:chronic ratios are inappropriate for use on pharmaceuticals;
5. Pharmaceuticals will occur in the environment as mixtures. For pharmaceuticals of the same class, it should be possible to estimate the combined risk of the mixture using concentration addition calculations. Other approaches may be required for mixtures of pharmaceuticals from different classes;
6. Many pharmaceuticals will be metabolised or degrade in the environment. The potential impacts of the resulting transformation products should be assessed. It may be possible to use information on the structure and properties of any transformation product to identify substances that pose the greatest risk to the environment;
7. A wealth of data is generated during the development of a pharmaceutical. This data could help to inform the environmental risk assessment of pharmaceuticals.
8. Risks of pharmaceuticals in the future might change due to changes in climate (which may result in increased disease pressures) and during pandemic situations.

4.9. Recommendations

The workshop identified a number of recommendations for further research:

1. Further work is required to understand the ecotoxicity of metabolites and environmental degradation products and approaches need to be developed for identifying transformation products that are likely to pose the greatest risk to the environment. Data from the mammalian ADME and toxicity packages could provide valuable information on transformation product exposure and activity;
2. Further work is required to understand the significance of novel endpoints (including results from studies employing proteomics and metabolomics) in terms of their ecological relevance. These studies will help to establish whether or not the standard chronic tests appropriate.
3. Consideration should be given to effects and chemical-based, post-authorisation monitoring of relevant endpoints in the natural environment in order to attempt to understand the impacts, if any, of pharmaceuticals on 'real' systems;
4. Work is required to understand how risks of pharmaceuticals may change in the future as a result of climate change and pandemics;
5. Our current understanding of behaviour of pharmaceuticals in natural systems is limited. It would be beneficial to develop a better understanding of the effects of environmental variables (DOC, pH, nutrients, multiple stressors etc.) on fate and behaviour, uptake and effects to allow better extrapolation from lab to field.
6. More synthesis of published and unpublished data would be worthwhile. Ecotoxicity studies should report background conditions (measured exposure concentrations, pH, how NOEC, EC50 derived, include all information not just summary stats, confidence limits etc.) to facilitate this synthesis. Reported effects data should be compared to monitoring data in order to identify substances of most concern. Scientists and industry should be encouraged to share data (table of parameters). Studies yielding 'surprising' results should be repeated.
7. Further work is required to understand whether and how we can extrapolate from mammalian data to environmental effects. The use of contra-indications to indicate potential for ecological risks should be considered and the utility of 'omics' based approaches should be explored for risk assessment purposes. It would be helpful if case studies could be developed for read across from mammalian data to environmental risk for a range of substances.

8. Data are available for only a few substances so methods for assessment of old substances should be explored.
9. The impacts of mixtures of a) pharmaceuticals of the same class; b) pharmaceuticals of different classes; and 3) pharmaceuticals and other substance types should be assessed. Modelling approaches are available from other sectors that could be used for this purpose.
10. Long-term studies at realistic exposure concentrations and under realistic environmental conditions might be useful for some pharmaceuticals although strong consideration would need to be given on how best to interpret these studies.
11. Most work has been performed on the aquatic environment. Further work into impacts on terrestrial systems is warranted.