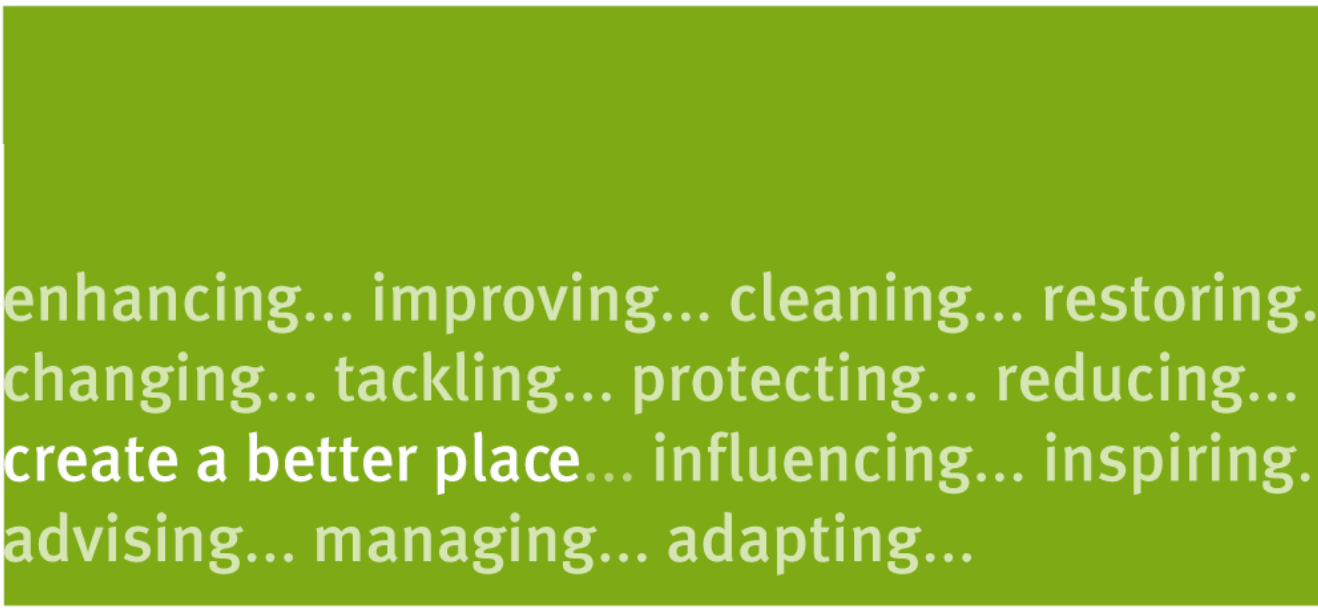

Environment Agency workshop on chronic aquatic ecotoxicity testing of human pharmaceuticals

SCHO0506BKTJ-E-P

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Executive summary

This report presents the outcomes of a workshop organised by the Environment Agency in May 2005 in London, UK.

Monitoring work has shown that many pharmaceutical substances can enter the aquatic environment at low but continuously present concentrations. These concentrations are too low to cause acute effects. However, our understanding of whether these levels can harm aquatic life over time is still limited.

In this context, the workshop aimed to:

- create a productive dialogue and encourage collaborative work by gathering together pharmacology, ecotoxicology and chemistry experts from the industrial, research and regulatory sectors; and

- establish a consensus on how and when to assess chronic impact through low-level exposure of pharmaceuticals in the aquatic environment.

In the first session, attendees discussed how chronic aquatic effects should be measured and, more specifically, the adequacy of standard laboratory tests and the usefulness of extrapolation approaches. The second session focused on when chronic aquatic effects should be measured. Areas discussed included the basis for substance prioritisation and the issue of mixtures in effluents.

There was considerable agreement at the workshop on many of the topics under discussion. We present here the main discussion points for each session, a summary of the workshop conclusions and the overall research needs and recommendations identified.

Contents

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1. Introduction and workshop objectives

This document summarises the discussions and outcome of a workshop on chronic aquatic ecotoxicity testing of human pharmaceuticals held in London on 19 May 2005.

1.1 Background

Over the last decade the potential impacts of pharmaceuticals in the environment have attracted increasing interest from the scientific community and the media. Several pharmaceuticals and their breakdown products have been found in watercourses in Europe and the USA. Recent survey work by the Environment Agency supports the findings in other countries and suggests that low concentrations of many pharmaceuticals are likely to enter the aquatic environment via sewage discharges as well as from agricultural use (e.g. spreading of sludge) (Environment Agency 2000 & 2003, Ashton *et al.* 2004). Although these substances are released at concentrations too low to cause acute effects such as rapid death, there are concerns about whether these low levels have the potential to harm aquatic life over time.

In October 2003 the Environment Agency published a Position Statement[1] on pharmaceuticals, which identified a number of areas where further work is necessary to help quantify risks to the aquatic environment. One of the main issues was the need to understand better the impact, if any, of low but continuously present concentrations of pharmaceuticals in watercourses across England and Wales.

1.2 Objectives

The workshop was part of the Environment Agency's current work programme on human pharmaceuticals. It aimed to:

- create a productive dialogue and encourage collaborative work by gathering together pharmacology, ecotoxicology and chemistry experts from the industrial, research and regulatory sectors; and

- establish a consensus on how and when to assess chronic impact through low-level exposure of pharmaceuticals in the aquatic environment.

The focus of the workshop was on scientific issues and research needs, and it was not intended to address any specific regulatory or testing regime. Its

[1] Available on the Environment Agency's website www.environment-agency.gov.uk (> About us > Policy > Position Statements > Human Pharmaceuticals)

scope therefore included both ‘old’ and ‘new’ substances. However, it did not include veterinary medicines.

2. Issues addressed

The workshop consisted of a short introductory session, followed by two discussion sessions with three parallel working groups addressing the selected issues. Group feedback and plenary sessions followed each discussion.

Annex A contains the workshop agenda and Annex B lists the members of the three working groups. The workshop presentations are given in Annex C.

A short review on the chronic risks of human pharmaceuticals in the aquatic environment was commissioned prior to the workshop in order to inform discussions (see Annex D).

2.1 Session questions and tasks

Session 1: How should we measure chronic aquatic effects for pharmaceuticals?

- Under what circumstances are standard laboratory tests adequate (algae, plant, invertebrate, and fish)?[2]
- Can extrapolation approaches help?

Session 2: When should we measure chronic aquatic effects for pharmaceuticals?

- Can classes of, or individual, pharmaceuticals be prioritised for chronic assessment and if so, on what basis, e.g. exposure or mode of action?
- When and how should we consider mixtures in effluents e.g. additivity and synergism, Direct Toxicity Assessment (DTA) and biomarkers for specific receptors?

Working group tasks

To identify:

1. Areas where existing knowledge and evidence is sufficient
2. Areas where ongoing research and development (R&D) will fill knowledge gaps
3. R&D needs

[2] Although sediments were not considered, they may represent an important sink for some substances and are therefore an important issue in this area.

3. Summary of discussions

Session 1: How should we measure chronic aquatic effects for pharmaceuticals?

1. Are standard laboratory tests adequate (algae, plants, invertebrates and fish)?

Use of a suite of standard chronic tests[3] was discussed:

- aquatic plants, e.g. *Lemna* sp. OECD 221;
- algae, e.g. OECD 201;
- invertebrates, e.g. *Daphnia* OECD 211;
- fish, e.g. fish early life stage (FELS) OECD 210.

The group agreed that such a suite is a useful basis for standard chronic toxicity evaluation. However, pharmaceuticals may act via mode(s) or mechanism(s) of action,[4] which impact specific receptors either by intention or coincidentally. Standard tests may tend to underestimate these specific effects if they do not address relevant receptor-mediated effects, contain the appropriate sensitive species, or consider exposure at sensitive life stages.

Knowledge of these modes of action is therefore important and should be used to target data collection, including:

- **Testing for specific effects**. For instance, standard tests do not consider the potential for the development of bacterial resistance from the release of resistant bacteria or the direct effects of pharmaceuticals on ecological communities. Some workshop participants questioned whether this effect was of direct environmental significance if microorganism populations continued to perform their ecological function.

- **Testing additional species**. For algae, there is the potential to test at community level (e.g. studies by Hans Blanck and co-workers on algal communities). In addition, invertebrate species absent from the standard OECD test suite have been identified as potentially sensitive, e.g. recent work highlighting the effects of selective serotonin re-uptake inhibitors (SSRIs) on molluscs (Fong *et al.* 1998, Honkoop *et al.* 1999, Cunha and Machado 2001).

- **Selecting the life-cycle stage tested**. The standard *Daphnia* test does not cover sexual reproduction. In addition, specific concerns were raised

[3] Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals (www.oecd.org/document/22/0,2340,en_2649_201185_1916054_1_1_1_1,00.html)

[4] Mode of action is defined here as the general description of key events leading to the pharmacological endpoints. Mechanisms are descriptions of specific biochemical or biophysical processes. A mode of action may therefore comprise several mechanisms.

over the FELS tests,[5] as their coverage of the fish life cycle is limited. The OECD Validation Management Group for Ecotoxicity Tests (VMG-eco) is currently validating a three-week fish screening test that will be able to detect oestrogens and androgens (and their antagonists), and aromatase inhibitors. However, the results of the screening by themselves cannot be used for risk assessment and are not applicable to non-endocrine modes of action. Future work by VMG-eco may include the development of:

- partial fish life-cycle tests that cover the two critical life stages for many endocrine disrupters (i.e. development to sexual maturity and reproduction);
- a fish full life-cycle test (one or two generations), which would cover all possible modes of action.

These tests are likely to have some applicability to pharmaceuticals with non-endocrine activity. VMG-eco is also developing tests for endocrine disrupters using amphibians, and invertebrates such as mysids, which may also have some applicability to pharmaceuticals. The group concluded that the FELS test may need to be supplemented by further testing in some cases. Simply increasing the duration of the test was considered inappropriate; instead, all available information should be used to target the most appropriate life-cycle stage.

It should be noted that environmental mutagenic load is not currently considered.

Overall, the group concluded that results from standard chronic tests:

- could not be considered in isolation; and
- will be insufficient in some cases.

Lack of response in such tests cannot always be taken to imply that a pharmaceutical poses no environmental risk. All available data should be reviewed and a process put in place in order to identify when and how to select additional tests. The Environment Agency is collaborating with pharmaceutical industry representatives to attempt to develop such a process by creating a decision tree.

In addition to endocrine disrupters, the group identified two groups of pharmaceuticals for which standard tests are most likely to be inadequate:

- **Antibiotics**. Tests using blue-green algae have been shown to be 20–500 times more sensitive to antibiotics than green algae (Holten Lützhoft *et al.* 1999, Ferrari *et al.* 2003). Higher plants could also be used more effectively as test species for ecotoxicity, as the chloroplast may be directly affected (Krajcovic *et al*. 1989).

[5] Currently two FELS tests are available: OECD 210 (until feeding larvae stage); and OECD 212 (short-term toxicity test on embryo and sac-fry stages completed prior to total yolk sac resorption).

- **Cytotoxic anticancer drug**s. These compounds, which are designed to kill cancer cells, are highly potent and thus used in very small quantities. They have always been assumed to be highly toxic. Genotoxic activity can be assumed to be relevant to all taxa and ecologically relevant effects can only be analysed under sub-lethal concentrations. In contrast to human risk assessment, scientific concern over genotoxicity in wildlife populations addresses a broader horizon termed 'genotoxic syndrome', which encompasses endpoints such as growth, disease resistance and reproductive health (Kurelec 1993). The standard suite of ecotoxicological tests, while perhaps theoretically adequate as a risk assessment tool, presents logistical difficulties and intrinsic risks. Sample availability is limited because, in general, very little compound is ever prepared. The handling, shipment and use of samples for testing may also present unnecessary exposure risks.

In addition to identifying the above groups of substances where standard chronic tests were unlikely to provide all the information needed to make an adequate assessment of risks, the workshop also identified several groups of substances where they thought chronic toxicity testing should be prioritised. These were:

- endocrine disrupters
- β-adrenergic receptor blockers
- cytotoxics
- fungicides
- antiparasitics (avermectins).

Endocrine disrupters and β-adrenergic receptor blockers have been shown to have unusually high acute/chronic ratios. Cytotoxics have special issues as discussed above, while fungicides and anti-parasiticides such as avermectins are designed to be acute toxins.

These groups of compounds require special consideration with regard to environmental testing strategies and risk assessments.

2. Can extrapolation approaches help?

2.1 Can we use a representative range of model drugs to predict the toxicity of a wide range of substances?

It was agreed that grouping substances by therapeutic classes for this purpose is meaningless. Modes of action could be used to define groupings; 34 reference compounds were proposed at a recent Society of Environmental Toxicology and Chemistry (SETAC) Pellston Workshop (Ankley *et al.* 2005). This list was based on mode of action covering a variety of therapeutic classes and aimed to help devise targeted chronic ecotoxicity testing in order to inform which pharmaceutical modes of action are of potential concern. Criteria for choosing reference compounds also included the availability of some published aquatic ecotoxicological data.

The group identified three main issues:

- the lack of data on chronic ecotoxicity in general and on reference compounds;
- the lack of understanding of the mode and precise mechanism of action of some substances, e.g. anti-epileptics;
- the continuous development of pharmaceuticals with new modes of action by industry.

2.2 Do you agree with the application of an assessment factor to aquatic acute toxicity data?
There was general agreement that a safety factor of 10,000 could be used on acute toxicity data (experimental and QSAR-derived (quantitative structure activity relationships)) only if no other data (e.g. mode of action, mammalian data, information on reference compounds) were available. This is a factor of 10 greater than the assessment factor routinely applied for risk assessment purposes to non-biologically active, industrial chemicals[6] and was suggested as a pragmatic and precautionary approach to help rank substances and trigger data collection.

Acute–chronic toxicity ratios (ACRs) for some substances with specific modes of action may be over 10,000, (e.g. 150,000 for ethinyloestradiol and ~50,000 for propanolol, a β-adrenergic receptor blocker). However, we currently do not know the extent to which other substances and mode(s) of action may exhibit the same characteristics, as ACRs are lacking for many, if not most substances (Ankley *et al.* 2005).

Pragmatically, given the data currently available (see Review Table 1 in Annex D), it is believed that a safety factor of 10,000 on acute toxicity data would be protective for most substances in the absence of other relevant information. This assumes that the PNEC (predicted no effect concentration) thus derived would be used only in the context of prioritising substances for further investigation, as it may overestimate chronic toxicity in many cases (particularly for substances with general narcotic modes of action). In addition, more ACR data are needed across a range of substances with specific modes of action to enable a more robust use of safety factors.

Through its trade association, Pharmaceutical Research and Manufacturers of America (P*h*RMA), the research-based pharmaceutical industry in the USA is currently collating a database for pharmaceutical substances containing all available ecotoxicological data from peer-reviewed scientific literature in English.

2.3 Mammalian dataset extrapolation?
The use of mammalian data to inform ecotoxicologal testing strategies (e.g. Huggett *et al.* 2003, 2004) is continually being developed. Knowledge of receptors and a wide range of data are used to screen compounds. Steps include identifying and locating receptors likely to be affected by the pharmaceutical substance, based on mammalian mode of action; isolating the

[6] An assessment factor of 1,000 is used on short-term toxicity data if at least one $L(E)C_{50}$ is available from each of the three trophic levels of the base set (algae, *Daphnia*, fish) (ECB 2003).

organ system; studying the drug/receptor mechanism *in vitro* using standard and non-standard tests and data on substance properties and receptor binding/affinity. Some felt that the approach remained too focused on the therapeutic mode of action, thereby neglecting potentially significant side effects.

Current knowledge tends to indicate that metabolism in fish is very similar to that in mammals; fish have many of the same P450 and Phase II metabolic enzymes contained in species used in pre-clinical studies (see work by Buhler and Stegeman). Some attendees, however, cautioned that while the above approach may help identify potentially hazardous substances, differences in exposure routes and in subsequent metabolism between mammals and fish may change the target site concentrations and hence confound the extrapolation of potential effects from mammals.

With regard to extrapolation of responses in other non-target species, two strands emerged:

- **Current use of the extrapolation approach**. If an affected mammalian receptor is known to occur in, for instance, invertebrate organisms, the approach can be (and is) used as an indicator of potential effects and a trigger for further testing. The latter is essential: although receptors may be conserved in these species, their specific function and hence the effects of pharmaceuticals may differ from those in mammals. For example, the potential action of SSRIs in molluscs can be predicted from the presence of serotonin receptors in these organisms. Further investigation is then necessary to decipher specific effects; in this case, the induction of spawning (Fong 2001, Fong *et al.* 1998 & 2003).[7]

- **Limitations and data gaps**. The group agreed that the approach is a good starting point but is limited by lack of knowledge relating to the physiology of non-target organisms. Data are needed to:
  - understand the function of conserved receptors in non-target species;
  - understand the relative sensitivity of organisms containing these receptors;
  - target future ecotoxicological testing;
  - identify additional effects, which cannot be predicted from the current approach (mediated via receptors or pathways not occurring or not affected in mammals).

In addition, ecotoxicity is not solely receptor-mediated; other effects such as DNA damage leading to mutagens may occur. However, DNA damage may be predicted from mammalian safety studies; typically, pre-clinical evaluations are available for the substance. Again, these studies can be used to trigger further testing on non-target organisms, if required.

[7] This occurred at concentrations exceeding those detected in the environment.

2.4 Can QSARs be used? If not, what is required to enable their use in the future?

At present, QSARs can be used to estimate acute ecotoxicity where the predominant mode or mechanism of toxicity is known. QSARs may be specific to either a mode or mechanism of action, or cross several modes/mechanisms. It is commonly assumed that a QSAR based on a single mechanism of action will predict toxicity more accurately than one based on a broader selection.

QSARs may predict the toxicity of narcotic chemicals (e.g. polar and non-polar narcosis), as well as some 'bioreactive' mechanisms. However, the group agreed that QSARs could not currently be used to predict chronic effects or more specific mechanisms of toxicity, e.g. potent electrophiles. Nevertheless, all expressed an interest in seeing such models developed and evaluated according to the OECD principles for validation.

In order to generate the necessary data, researchers should concentrate on:

- identifying the pharmaceuticals' mode(s) of action; and
- prioritising those of potential environmental significance.

Session 2: When should we measure chronic aquatic effects for pharmaceuticals?

1. **Can classes of, or individual, drugs be prioritised for chronic assessment? On what basis, e.g. exposure, mode of action?**

The group first discussed prioritisation on the basis of exposure. Identified criteria for prioritisation included:

- **Exposure predictions and environmental monitoring**.

- **Mammalian toxicology indicating carcinogenic, mutagenic or reprotoxic properties**. This criterion alone is not sufficient to identify substances of potential environmental concern because these effects are generally studied at concentrations above those detected in the environment. However, it adds to the weight of evidence collected.

- **Persistence, bioaccumulation and toxicity (PBT)**. PBT criteria as per the European Union *Technical Guidance Document on Risk Assessment Part II* (TGD) (ECB 2003) can be used as a prioritisation tool. However, concern was expressed that many environmental models do not accurately predict the environmental behaviour of pharmaceutical compounds, which are chemically different from many of the datasets used to develop the models. For example, some of the models available (e.g. EPIWIN) may over-predict bioconcentration if based solely on the *n*-octanol:water coefficient ($\log K_{ow}$) because pharmaceutical substances are generally designed to metabolise rapidly. In addition, they often use the neutral form of the compound; most pharmaceuticals will exist as ions in water at environmental pH levels.

Thus, other factors such as metabolism, adsorption, hydrolysis, degradation and structural features should be considered in addition to modelled data as advised, for instance, by the TGD. If PBT properties are assessed using modelled data, efforts should be made to verify them.

In the absence of experimental toxicity data in non-target organisms, the group considered that potency in mammals can be used as a surrogate, although the approach is not ideal and should be used in the short term only. It was also noted that potency is irrelevant in the absence of the target receptor. However, the use of potency is currently more appropriate than QSAR predictions provided it is used in conjunction with other mammalian data.

- **Large ACRs for other reference substances with the same mode of action**. When ACRs exist for one substance, these can be used to assess potential chronic impacts across a group of substances with the same mode of action, e.g. β-adrenergic receptor blockers may be prioritised on the basis of the ACR (~50,000) available for propanolol.

The group then discussed prioritisation on a mode of action basis. Grouping and prioritising substances on this basis was seen as a promising approach for organisms such as fish that are phylogenetically close to mammals. The group agreed that only a relatively small subset of the modes of action of existing drugs are likely to be of environmental significance (i.e. combining environmental exposure and potential effects).

The approach suggested was to:

- Focus on the most important (environmentally relevant) modes of action, e.g. the list of 34 reference compounds based on mode-of-action that were identified at a recent SETAC Pellston Workshop (Ankley *et al.* 2005). The data obtained can then be used, together with relevant mammalian data on individual compounds, to make a more informed prioritisation of pharmaceuticals of potential concern. Using the propanolol ACR (~50,000) to help prioritise atenolol for further testing is a good example of how such reference data can be used.

- Subsequently, prioritise substances within these groups according to:
  - occurrence in the environment (predicted or measured), e.g. surveys by the Environment Agency, US Environmental Protection Agency (USEPA) and other regulatory authorities;
  - tonnage;
  - PBT.

A trigger of 1 tonne was proposed, with all substances (or sum of substances acting on the basis of the same mode of action) produced above this trigger qualifying for further investigation. Another suggestion was the adoption of a sliding scale of tonnage triggers driven by known potency at the mammalian receptor. This stems from the realisation that highly potent pharmaceuticals with potential environmental effects may only be produced in kilogram

quantities but be present in sewage effluents at environmentally active concentrations (e.g. ethinylestradiol; Routledge *et al.* 1998).

Special consideration would be given to those substances administered in a way that creates point sources from which subsequent environmental hotspots are likely, e.g. substances used in old peoples' homes and psychiatric units. Products likely to have highly seasonal use patterns may also require special consideration, as the assumption that use will be evenly distributed throughout the year will not be valid.

All available information should be used to assess each substance; in particular, all data obtained from pre-clinical and clinical trials, e.g. persistence in humans may indicate slow metabolism in the environment.

Care should be taken not to always assume that pharmacological potency is the sole determinant for identifying environmental risk. Although lack of toxicity at the therapeutic dose is a criterion for selection and development as a medicine for the majority of pharmaceuticals, there are some classes of pharmaceutical for which potential for toxicity at therapeutic doses is assumed. An example is cytotoxic anticancer medicines, which are highly toxic and likely to provoke adverse reactions even at the therapeutic doses. Identification of such circumstances could be used to identify priority substances.

Additionally, high priority may be given to modes of action eliciting an effect across a wide range of species. Some members of the group felt that this approach was not yet possible due to the uncertainties and lack of data associated with effects on non-target species. Once specific modes of action have been identified, further research is needed to identify the presence and role of receptors across species in order to define suitable endpoints for testing. The 'omics' fields (genomics, proteomics, and metabolomics) are likely to play an important role in this area.

2. When and how should we consider mixtures in effluents e.g. additivity and synergism, DTA, biomarkers for specific receptors?

Pragmatically, it is not possible to consider mixtures – even for substances with the same mode of action – during the approval process for pharmaceutical substances because it does not seem possible to predict what environmental mixtures may occur within a reasonable degree of accuracy and precision. However, some attendees felt that a task force should be set up to:

- aid the exchange of information between regulators and industry; and
- encourage groups of companies manufacturing substances with similar or identical modes of action to submit co-ordinated environmental data packages.

Post-authorisation, the group agreed that an approach using concentration additivity adjusted for potency (based on acute or chronic toxicity tests in

appropriate species; e.g. Cleuvers 2003, 2004) for substances with the same mode of action is worth considering. The group agreed that, although concentrations in sewage treatment plant effluents are often very low, the probability of co-occurrence is high. Just as mixtures of similarly acting endocrine disrupters at individually negligible concentrations can act additively to produce effects, there is no reason to suppose that other substances with specific receptor-mediated actions could not act similarly. Predictions based on drug interactions are not currently possible as knowledge is too limited. Moreover, the group agreed that the probability of synergism was low.

Overall, the issue of pharmaceutical mixtures in the environment cannot be isolated and should be studied within the wider issue of chemical mixture toxicity. Investigating chemical mixtures from sewage treatment plant effluent should not be prospective, but impact-driven. This has been achieved using chronic or sub-chronic toxicity tests, e.g. in the case of pulp mill effluents in Canada (e.g. Bailey and Young 1997, Dubé *et al*. 2000, Hewitt *et al.* 2003).

If an impact is measured, diagnostic tests such as biomarkers of effects are then needed to identify causation. However, it is acknowledged that linking cause(s) and effects is complex. Moreover, although chronic DTA tests are available, the UK approach is currently based on acute toxicity testing and is therefore inappropriate for assessing chronic impact.

Alternative approaches include *in situ* studies and the creation of artificial effluents to define thresholds of adverse effects. However, using synthetic effluents to test real-world hypotheses may not be valid, as the test system may not contain all the materials that would be present in a 'real' situation. In all cases, the difficulties in extrapolating from laboratory studies to environmental impact were highlighted.

If a concern is raised regarding the presence and potential impact of a substance or group of substances, the group suggested that the next step should be the investigation of more efficient sewage treatment, e.g. the current demonstration programme for the removal of endocrine disrupters developed by the Environment Agency and the water companies.[8]

[8] See www.environment-agency.gov.uk (> Business and industry> Business sectors > Chemicals > I am interested in chemicals> Substances we are working on> Chemical groups> Endocrine Disrupters)

4. Conclusions

There was considerable agreement at the workshop on many of the topics under discussion.

Measurement of chronic effects
Standard chronic tests may sometimes be insufficient when assessing specific effects resulting from a particular mode of action. Where available, knowledge of modes of action is important in order to target data collection. The development of a decision tree would assist in identifying the need for further investigation and in selecting additional tests.

Extrapolation of the mammalian dataset to fish responses may be useful. The approach can also be used as an indicator of potential effects and a trigger for further testing in other non-target organisms known to contain the same or similar receptors.

QSARs are, at present, inadequate for the prediction of chronic or specific effects for pharmaceuticals but should be developed and evaluated.

Overall, all available data including the results of pre-clinical and clinical tests should be used to:

- assess and prioritise substances; and
- develop models.

Prioritisation for further investigation
Prioritisation of substances for further investigation should be done on the basis of environmental exposure (estimated or measured) and all other available data (fate and behaviour, mammalian toxicology, ecotoxicity, etc.).

Prioritising by mode of action is at present difficult due to lack of data for non-target species that are phylogenetically distant from mammals. Further work is therefore needed to derive data, particularly for reference compounds.

If no chronic ecotoxicological data are available, a safety factor of 10,000 might be used on acute toxicity data (experimental and QSAR-derived) as a default aid to prioritisation in the absence of knowledge of specific environmental concerns.

Assessment of mixtures
Predicting and assessing the potential impact of groups of substances with the same mode of action (e.g. following a monitoring survey) may be possible on the basis of concentration additivity, after adjusting for relative potency.

In effluents, the impact of pharmaceutical mixtures cannot be considered in isolation. The whole effluent and its constituents should be assessed on an impact-driven basis, using diagnostic tools to identify causation.

Research needs and recommendations
A chronic impact decision tree should be created to assist in the development of an effective and efficient testing strategy.

The primary focus should be on generating more data, particularly for:

- reference compounds (e.g. the SETAC Pellston list); and
- compounds for which there currently are no available ecotoxicity data.

This will also inform ACRs, the use of safety factors and the development of QSARs. However, the use of animals in aquatic testing should be minimised wherever possible.

More data are needed on species identified as potentially sensitive to certain classes of compounds, e.g. molluscs and SSRIs. Overall, understanding of receptors in non-target species should be developed.

The screening tools used to develop new drugs should be evaluated to see if they could be adapted to screen drugs of potential concern in the environment.

Increased collaboration and data-sharing between both the research-based and the generic pharmaceutical industries and regulators should be encouraged. The formation of a joint task force should be considered.

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List of acronyms and abbreviations

ACR	Acute-to-Chronic toxicity ratio
DTA	Direct Toxicity Assessment
EC_{50}	Concentration effective against 50 per cent of the organisms or animals tested
FELS	Fish Early Life Stage
LC_{50}	Concentration lethal to 50 per cent of the organisms or animals tested
OECD	Organisation for Economic Co-operation and Development
PBT	Persistence, Bioaccumulation and Toxicity
QSAR	Quantitative Structure Activity Relationship
R&D	Research and Development
SSRIs	Selective Serotonin Re-uptake Inhibitors
TGD	Technical Guidance Document
VMG-eco	OECD Validation Management Group for Ecotoxicity Tests

Annex A: Workshop agenda

9.15 Arrival and coffee

9.45 Introduction from chairman (Andy Croxford)

9.50 Background to project (Jo Kennedy)

10.00 Review of chronic testing of pharmaceuticals (Mark Crane)

10.10 Overview of questions to be addressed in the workshop and introduction to breakout groups – questions from delegates (Andy Croxford)

10.15 Breakout 1: Delegates will be divided into three groups, with coffee available at 11.00. Questions to address during this session:

- How should we measure chronic aquatic effects for pharmaceuticals?
  1. Are standard laboratory tests adequate (algae, plant, invertebrate, fish)? Under what circumstances?
  2. Can extrapolation approaches help?
     (i) Can we use a representative range of model drugs to predict the toxicity of a wide range of substances?
     (ii) Do you agree with the application of an assessment factor to aquatic acute toxicity data?
     (iii) Mammalian dataset extrapolation?
     (iv) Can QSARs be used? If not, what is required to enable their use in the future?

12.15 Plenary feedback from Breakout 1 (10 minutes per group)

12.45 Lunch

13.30 Plenary discussion

14.00 Breakout 2 (three groups as above):

- When should we measure chronic aquatic effects for pharmaceuticals?
  1. Can classes of or individual drugs be prioritised for chronic assessment? On what basis, e.g. exposure, mode of action?
  2. When and how should we consider mixtures in effluents, e.g. additivity and synergism, DTA, biomarkers for specific receptors?

15.30 Plenary feedback from Breakout 2 (10 minutes per group; coffee available at 15.30)

16.00 General discussion and identification of next steps

Annex B: Groups

Group 1
Jo Kennedy (chair)
Virginia Cunningham
Steve Dungey
Andreas Kortenkamp
Frank Mastrocco
Peter Matthiessen
Richard Murray-Smith
Chris Watts

Group 2
Paul Whitehouse (chair)
Keith Solomon
Andy Stubbings
John Sumpter
Helen Thompson
Roy Thompson
Jean-Marc Vidal

Group 3
Tatiana Boucard (chair)
Katie Barrett
Steve Binks
Mark Crane
Mark Cronin
Jeanne Garric
Pascal Michoux
Henry Stemplewski

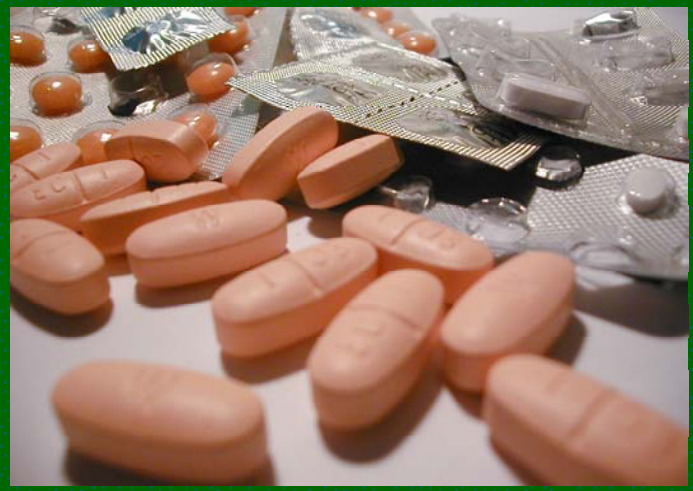
Annex C: Presentations

Andy Croxford and Jo Kennedy – The Environment Agency


Environment Agency
Workshop on chronic aquatic toxicity testing for human pharmaceuticals
19th May 2005
creating a better place

Aims of the day

- How and when to measure chronic impact?
- Future direction of research



creating a better place

Today's approach

- Scene setting presentations
- Breakout groups on the "how" and the "when"
- Looking to identify:
  - where existing knowledge is sufficient
  - where on-going R&D will fill gaps
  - further R&D needs

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Outputs

- Workshop report
  - Presentations
  - Breakout session discussions
  - Key recommendations
  - Will be circulated in draft for comment

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Background

- Environment Agency work on pharmaceuticals
  - Background on regulatory role
  - Present work programme on pharmaceuticals

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Our regulatory role

Manufacturing

Marketing and Use

Disposal

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Our regulatory role

Manufacturing

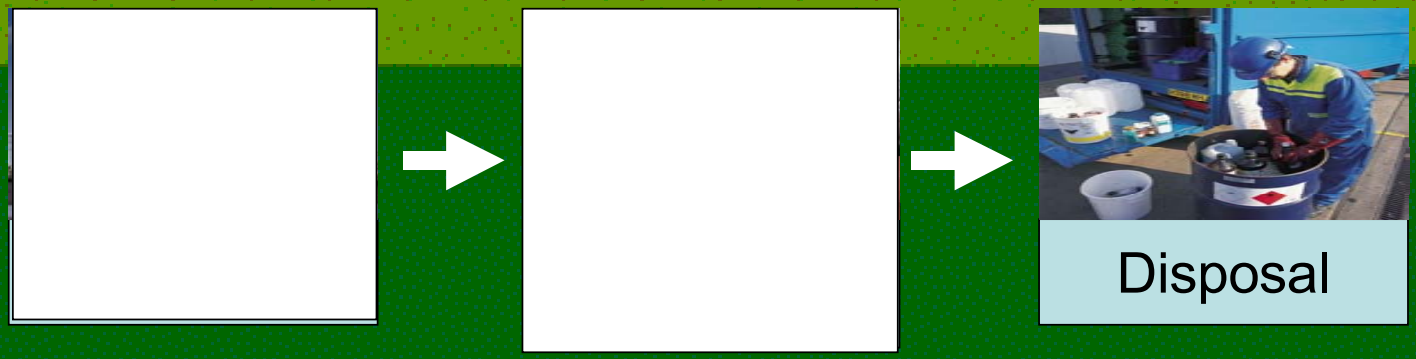
- IPPC sites
- Some formulators?

Our regulatory role

Marketing and Use

- No regulatory role in the risk assessment and authorisation of new products
- Competent Authority is the MHRA, working to EMEA guidance

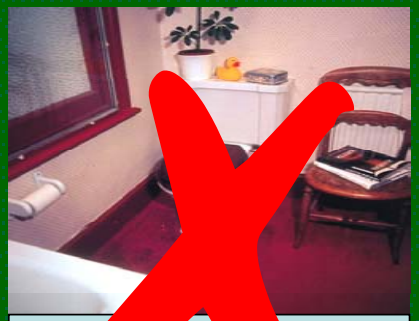
Our regulatory role



- Emissions to land, air or water from IPPC sites
- Trade and hospital effluents direct to watercourse
- Sewage work effluents
- Movements of Special Waste
- Waste sites (incinerators, landfills)

Our regulatory role

Drinking Water



Use & disposal in the home

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Existing Work Programme

- Two pieces of R&D
  - Review of environmental information (2000)
  - Ranking and targeted monitoring exercise (2003)
- Publication of Position Statement (Oct 2003)
- Copies downloadable from our chemical strategy web pages

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Existing Work Programme

Environment Agency

Are pharmaceuticals having Chronic Impacts?

- Small quantities routinely discharged from sewage treatment works
- Continuous exposure
- Biologically active molecules
- Concentrations too low for acute impacts
- Chronic impacts as individual substances or groups of substances with the same mode of action?

Existing Work Programme
Agency Position Statement

Environment Agency

"We call on on the pharmaceutical industry to..

take steps to reassure the Environment Agency that the low levels of pharmaceuticals being measured in rivers are unlikely to cause significant harm to the aquatic environment."

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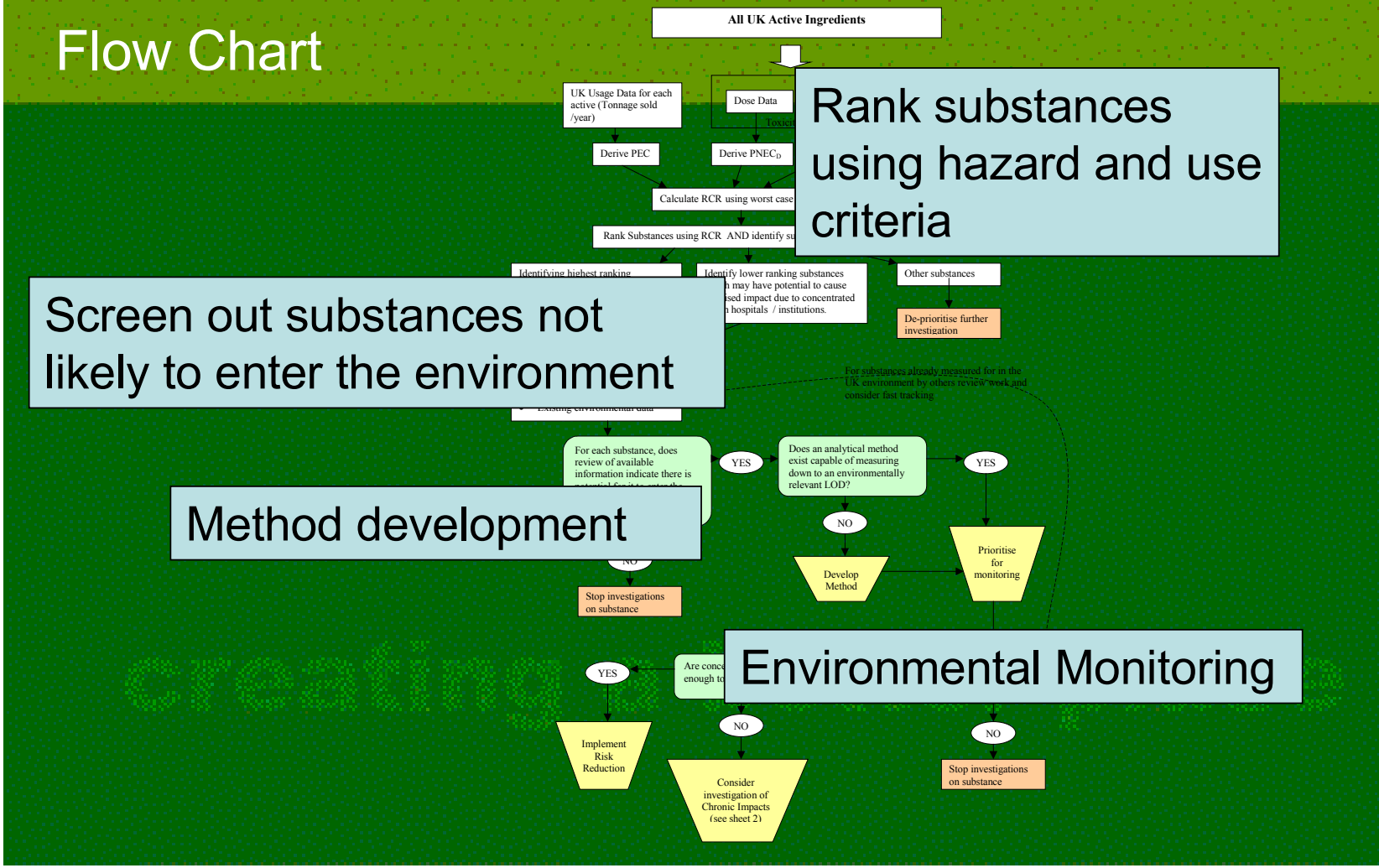
Existing work programme

Flow Chart



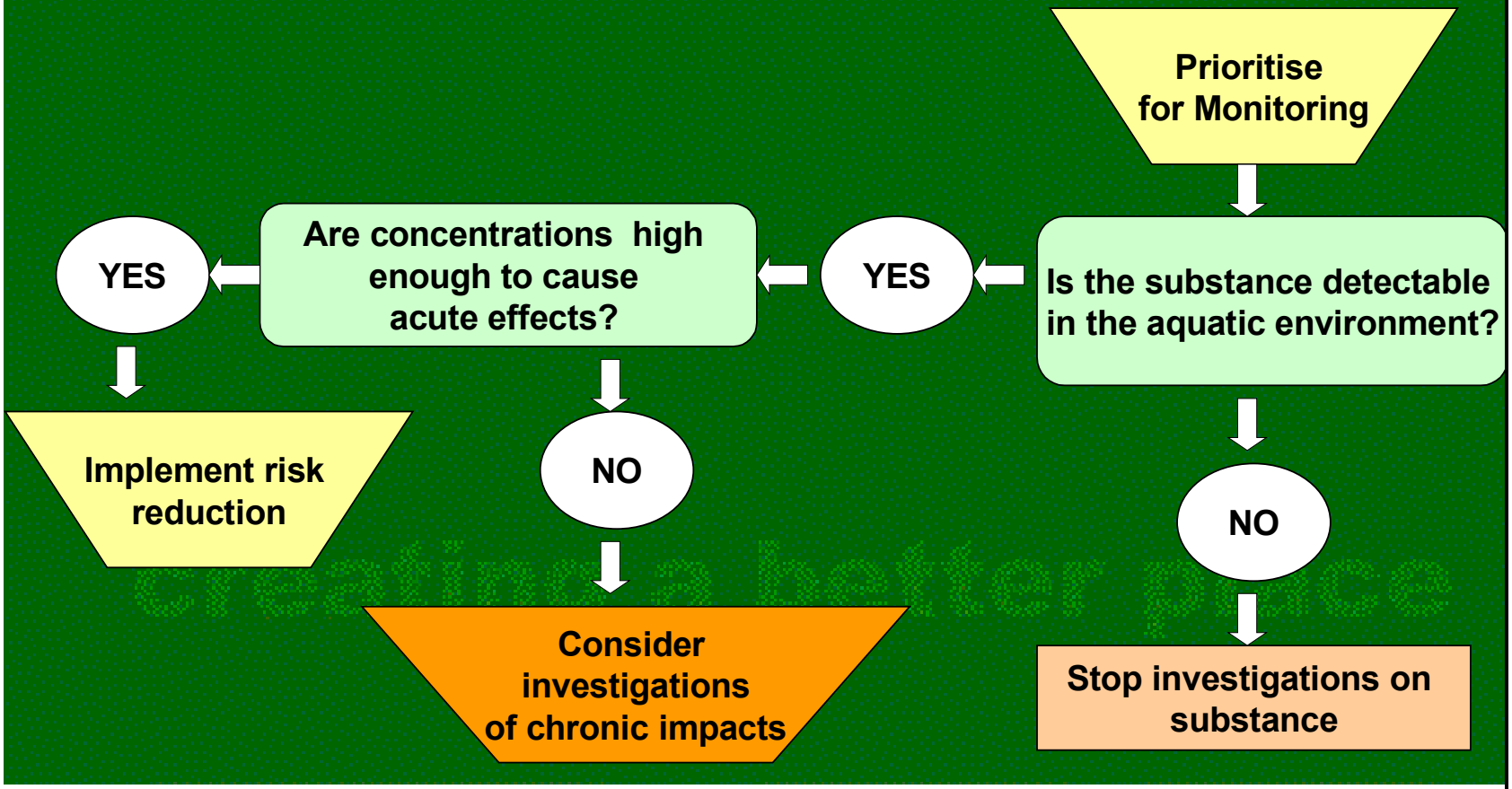
Rank substances using hazard and use criteria

Screen out substances not likely to enter the environment

Method development

Environmental Monitoring

Existing work programme



Existing work programme

Environment Agency

Consider investigations of chronic impacts

- How?
- When?

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Existing work programme

Environment Agency

Consider investigations of chronic impacts

- How?
- When?
- Completion of the flow chart

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Mark Crane – Watts & Crane Associates

Chronic Risks of Human Pharmaceuticals in the Aquatic Environment

Review of Current Knowledge

Mark Crane and Chris Watts

Watts & Crane Associates

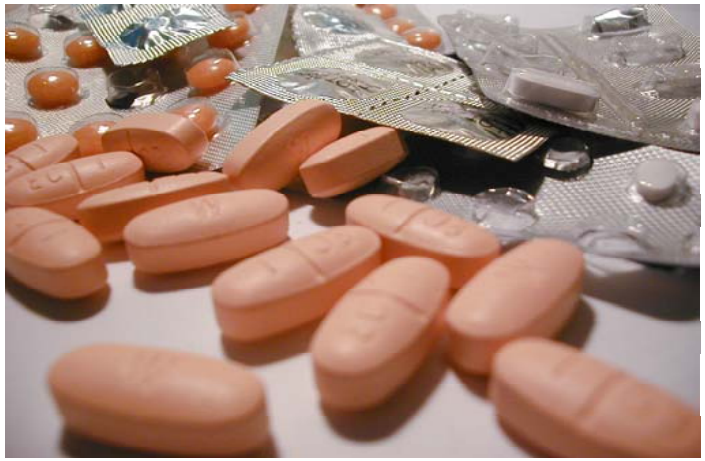
Introduction

- Environmental risks of pharms increasingly important issue for regulators and industry.
- Driven by widespread detection of pharms in environmental samples.
- Exposure of aquatic wildlife to human pharms most likely from sewage treatment works discharges.
- Exposure therefore at continuous, low concentrations.

Watts & Crane Associates

Chronic testing requirements

- Calls for chronic aquatic testing of human pharmaceuticals.
- E.g., Environment Agency *Position on Pharmaceuticals in the Aquatic Environment.*
- Chronic tests adopted in recent draft ERA guidance by EMEA.



What is a chronic aquatic toxicity test?

- Study in which organisms are exposed to different concentrations of a chemical and observed over a long period, or a substantial part of their lifespan.
- Chronic tests usually include additional measures of effect such as growth or reproduction.
- Contrast with acute toxicity tests, which often use mortality as the only measured effect.

When are chronic tests necessary?

- When assessment factors applied to acute results suggest potential risks that could be refined by chronic tests.
  - ➢ Applies to some human medicines (but not many?).
- When modes of toxic action differ from acute to chronic tests.
  - ➢ Applies to steroid hormones & ß-blockers (and others?).
- When the database for establishing acute-to-chronic ratios is insufficient.
  - ➢ Applies to most classes of human medicines .

Watts & Crane Associates

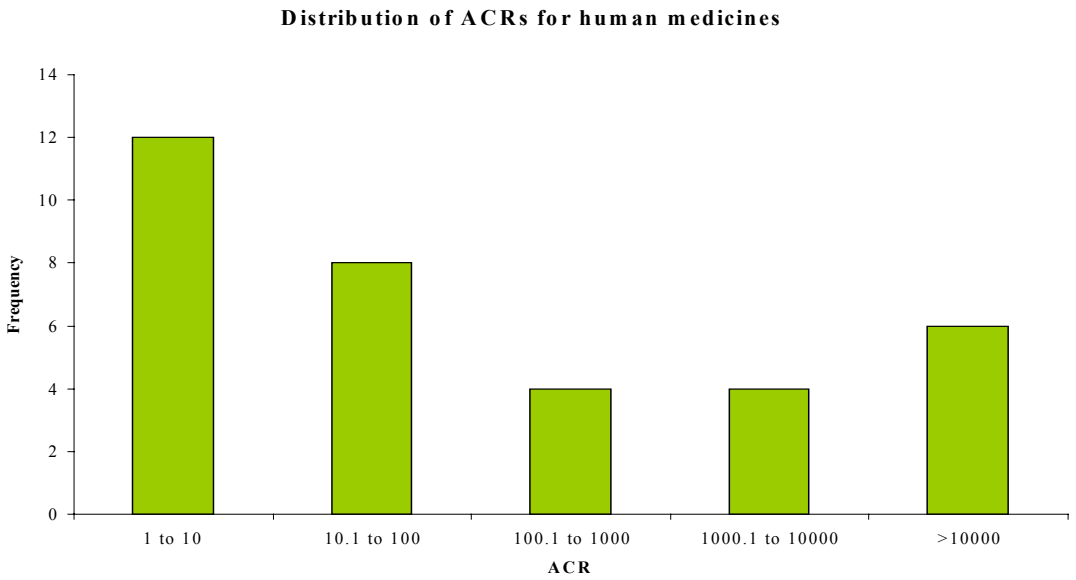
A reliable acute-to-chronic toxicity database is the key to establishing appropriate assessment factors

Available ACRs (fish and invertebrates, n=34)

Class	n	ACR range
Androgen	1	1000000
Anti-androgen	1	3.6
Anti-depressant (SSRI)	5	2.3 - 13.3
Anti-epileptic	1	3108
Anti-hyperlipoproteinemic	2	>312 – 1428
Bone resorption inhibitor	1	43.9
Cholinergic agonist	1	42.9
NSAID	8	1.68 – 1258
Oestrogen	9	<10 – 390000
Topical keratolytic	1	5.9
X-ray contrast medium	1	1
? -adrenergic receptor blocker	3	6.8 - >48600

Available ACRs

- Mean = 51967
- Median = 32.8
- Mode = 10.0.



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Conclusions on ACRs

- Available ACR database insufficient to draw reliable conclusions about ACRs and appropriate assessment factors for different therapeutic classes.

- Further chronic aquatic data are required.

Chronic tests currently recommended

- Microbes and algae – all tests are chronic?
- Invertebrates – 21-d reproduction of *D. magna*.
- Fish – 14-d early life stage test

Some remaining issues

- Strengths and limitations for testing human medicines of:
  - *Daphnia magna* reproduction test?
  - Fish ELS?
- Can chronic tests be focussed better by use of:
  - Biomarker/MOA information?
  - (Q)SAR?
  - Extrapolation from mammalian data?
- Mixture toxicity
  - Should pharmaceuticals be treated differently?
  - How should environmental mixtures be tested?

Annex D: Review - Chronic Risks of Human Pharmaceuticals in the Aquatic Environment

Mark Crane and Chris Watts
Watts & Crane Associates, 23 London Street, Faringdon, Oxfordshire, SN7 7AG, UK

Abstract

The Environment Agency published its *Position on Pharmaceuticals in the Aquatic Environment* in October 2003. One of the key issues identified for regulators and industry was the need to understand better the impact, if any, of low but continuously present concentrations of pharmaceuticals in watercourses across England and Wales. This paper reviews current information on the chronic aquatic toxicity of human pharmaceuticals. It concludes that chronic tests with blue-green algae are likely to be sensitive surrogates for both algae and other unicellular organisms, although possibly not for higher plants. In contrast, there is little evidence of a general need to perform chronic aquatic invertebrate tests for all human pharmaceuticals, although further acute-to-chronic ratio data are required for the main therapeutic classes of pharmaceuticals before this issue can be fully resolved. Chronic fish tests may be necessary for some substances, but it is likely that these can be focused more accurately through use of mammalian toxicity datasets. For some substances and modes of action, life-cycle or partial life-cycle fish tests may be more relevant than reliance on early life-stage (ELS) tests, because the ELS test is unlikely to respond adequately to all pharmaceutical modes of action. Biomarkers may be useful in focusing research and testing efforts by identifying active substances and receptors of interest in aquatic species, and they may also be useful in field surveys for helping to establish possible cause and effect relationships. QSARs have been used by several authors to predict acute toxic effects, but predictions of chronic effects are currently hampered by the paucity of available chronic data to build predictive models.

Introduction

Potential risks associated with releases of pharmaceuticals into the environment have become an increasingly important issue for environmental regulators and the pharmaceuticals industry (Jørgensen and Halling-Sørensen 2000, Kümmerer 2004a, Stuer-Lauridsen 2000). This concern has been driven by widespread detection of pharmaceuticals in environmental samples as a result of improved analytical capabilities and the commissioning of focused field surveys (Daughton 2001, Focazio et al. 2004, Webb 2004b). Surface water sampling programmes in the UK (Ashton et al. 2004, Thomas and Hilton 2003), continental Europe (e.g., Alder et al. 2004, Buser et al. 1998, Calamari et al. 2003, Ternes 1998, Zuccato et al. 2004a), North America (e.g., Anderson et al. 2004, Focazio et al. 2004, Kolpin et al. 2002, Metcalfe et al. 2003, 2004) and elsewhere (Heberer 2002) have all shown the presence of many different classes of pharmaceuticals, some of which are known to be environmentally persistent (Zuccato et al. 2004a). The high polarity and low volatility of most pharmaceuticals means that they are likely to be transported to the water compartment (Breton and Boxall 2003). Although some pharmaceuticals are unlikely to be a risk to the aquatic environment because of low concentrations combined with low toxicity (e.g., iopromide, Steger-Hartmann et al. 1999, 2002), other pharmaceuticals such as natural and synthetic sex hormones are now known to pose considerable risks (Nash et al. 2004).

Pharmaceuticals used in both veterinary and human medicine have been a focus of attention, but environmental exposure scenarios differ substantially between the two. Exposure of aquatic wildlife to human pharmaceuticals is most likely to occur from sewage treatment works (STW) discharges (Focazio et al. 2004) and this exposure may therefore be at continuous, low concentrations (Breton and Boxall 2003, Daughton and Ternes 1999). Despite this, most published aquatic toxicity data and risk assessments for pharmaceuticals are based on short-term acute studies (Cunningham et al. 2004, Ayscough et al. 2002, Halling-Sørensen et al. 1998, Webb 2004a). Concerns over the possible environmental effects of low level continuous aquatic exposure to human pharmaceuticals have led to a call by the Environment Agency of England and Wales in its *Position on Pharmaceuticals in the Aquatic Environment* (Environment Agency 2003) to develop and implement chronic toxicity testing procedures using aquatic organisms. This position is in agreement with others (e.g., Bound and Voulvoulis 2004, Daughton 2003, Emmanuel et al. 2005, Ferrari et al. 2004, Zuccato et al. 2004b). As a result of such calls, chronic aquatic toxicity tests have been adopted in the most recent draft environmental risk assessment guidance document for human pharmaceuticals produced by the European Medicines Agency (EMEA 2005) in support of Directive 2001/83/EC (EC 2001).

Chronic toxicity tests are studies in which organisms are exposed to different concentrations of a chemical and observed over a long period, or a substantial part of their lifespan. In contrast to acute toxicity tests, which often use mortality as the only measured effect, chronic tests usually include additional measures of effect such as growth or reproduction. This paper

reviews available information on chronic toxicity to aquatic organisms from exposure to human pharmaceuticals, and poses a series of questions that require answers before an appropriate strategy can be proposed for assessing chronic risks to aquatic organisms from exposure to human pharmaceuticals.

Aquatic Ecotoxicity from Chronic Exposure to Human Pharmaceuticals

In this section we review evidence for the chronic effects of human pharmaceuticals on aquatic organisms. The clearest evidence for potential adverse effects from the release of human pharmaceuticals into the aquatic environment is for natural and synthetic steroids, particularly the oral contraceptive 17α-ethinyloestradiol (Young et al. 2002). Little is known about the chronic effects of most other pharmaceuticals (Schulte-Oehlmann et al. 2004), although an increasing amount of information is becoming available on the effects of antimicrobial substances (Alexy et al. 2004, Kümmerer 2004b), and new data for other classes of pharmaceuticals are regularly published in this active research area. Chronic toxicity data for algal, invertebrate and fish species are summarised in Table 1, ordered by therapeutic class, substance and taxonomic group.

Micro-organisms

Thomulka and McGee (1993) found substantial effects of several antibiotics on reproduction of the microbe *Vibrio harveyi*, but little evidence for effects on short-term bioluminescence. Similar time-dependent effects have been found for *Vibrio fischeri* exposed to antibiotics (Backhaus and Grimme 1999, Froehner et al. 2000).

Several authors have measured alterations in microbial assemblages after exposure to antibiotics at concentrations similar to those found in hospital wastewaters (Al-Ahmad et al. 1999, Kümmerer et al. 2000, Stanislawska 1979). Ash et al. (2002) found evidence of resistance to imipenem and the beta-lactams ampicillin, cefotaxime and ceftazidime in bacteria cultured from water samples taken from US streams. Sediments below fish farm cages may also contain reduced microbial numbers and activity as a result of the veterinary application of antimicrobials (Hansen et al. 1992).

The major concern over the effects of human pharmaceuticals on microbial assemblages is the development of antimicrobial resistance. Resistance has been found in natural aquatic environments, although it seems likely that this has been caused by the release of resistant bacteria into the environment and possible gene transfer, rather than through direct exposure of naturally occurring microbes to antibacterial substances and subsequent natural selection (Kümmerer 2004c, Ohlsen et al. 1998, 2003, Wiethan et al. 2002). The implications of antimicrobial resistance for aquatic ecosystem structure and function remain unknown, but the human health implications of widespread resistance are of clear concern.

Algae and higher plants

Most chronic aquatic toxicity data for human pharmaceuticals, other than synthetic steroids, are available for algae (Webb 2004a), probably because these are the briefest and therefore least expensive chronic toxicity tests to run.

In Table 1, algal species were sensitive (NOEC <1mg l^{-1}) to several different therapeutic classes, including fluoroquinolone and sulfonomide antibacterials, selective serotonin reuptake inhibitors (SSRIs), β-adrenergic receptor blockers, and oestrogens.
There is evidence from Table 1 and elsewhere (Boxall et al. 2003, Halling-Sørensen 2000, Holten-Lützhøft et al. 1999) that microalgae and blue-green algae, such as *Microcystis aeruginosa*, are considerably more sensitive to antibiotics than standard algal toxicity test species such as *P. subcapitata*. However, sensitivity does not seem to differ greatly between these algal groups for non-antimicrobial substances, with a 21-d EC50 of 13.3 mg l^{-1} for *M. aeruginosa* exposed to tiludronate (Sanofi 1996), which is similar to that reported for *P.subcapitata*.

There is also a lack of consistency in the response to pharmaceuticals of green algae and higher aquatic plants such as duckweeds, although higher plants have often been more sensitive in the limited number of comparisons to date (Pro et al. 2003, Cleuvers 2003).

Invertebrates

Table 1 shows that exposure of some invertebrate taxa to ethinyloestradiol causes adverse effects at very low concentrations of ~1 ng l^{-1} or less (Belfroid and Leonards 1996, Schulte-Oehlmann et al. 2004). Maximum concentrations of 3.4 ng l^{-1} ethinyloestradiol in UK waters have been measured (Williams et al. 2003), so biological effects on invertebrates at low ng l^{-1} levels are environmentally relevant. Other oestrogens and the androgen methyltestosterone also cause effects at very low concentrations, and a wide range of other therapeutic classes cause effects at concentrations <1 mg l^{-1}. In contrast to this, invertebrates tend not to be affected to as great an extent as algae by exposure to antimicrobials (Holten-Lützhøft et al. 1999).

Sediments may act as a sink for contaminants, including pharmaceuticals, and provide a continuous chronic source of these to sediment-dwelling organisms, including invertebrates (Drewes et al. 2002, Heberer et al. 2002, Holthaus et al. 2002). However, rather few studies have been performed with pharmaceuticals on sediment-dwelling organisms.

Fish and amphibians

It is now widely accepted that aquatic vertebrates are highly sensitive to endocrine modulation, especially through exposure to steroid oestrogens excreted by women either naturally or as a result of oral contraception

(Desbrow et al. 1998, Vos et al. 2000). Fish are also sensitive to other sex hormones such as methyltestosterone (Zerulla et al. 2002), and to β-adrenergic receptor blockers, as shown in Table 1. Sensitivity can manifest itself through reduced fecundity, which means that partial life-cycle studies such as the fish early life stage (ELS) test may not measure important effects.

There is little evidence for any direct adverse effects of antibiotics on vertebrates such as fish at environmentally realistic concentrations (Canton and van Esch 1976, Lanzky and Halling-Sørensen 1997, Marking et al. 1988). However, some poorly soluble pharmaceuticals do have the potential to bioaccumulate (Delépée et al. 2004, Luneestad 1992, Migliore et al. 1993), so exposure to higher organisms through the food chain is possible. Brown et al. (2004) suggest that known thyroid-active pharmaceuticals that are not retained by STWs, so may be present in surface waters (e.g., indomethacin, naproxen, salicylates, clofibric acid, carbamazepine, and iodocontrast agents) should be investigated for their long-term ability to alter fish thyroidal status under realistic environmental conditions.

Finally, it is important to recognise that there is considerable pressure from some regulatory authorities and animal welfare pressure groups to reduce the amount of vertebrate testing. Methods for either reducing the number of fish that are tested, or eliminating the need for fish tests altogether, would therefore be of considerable use during the environmental risk assessment process (Hutchinson et al. 2003a).

Methods and strategies available for assessing chronic effects

Acute versus chronic toxicity testing

Webb (2004a) shows that most human pharmaceuticals have low acute aquatic toxicity. This is by design, because pharmaceuticals should not be acutely toxic to the target species, although they do need to have targeted, chronic pharmacological activity to be truly effective medicines. It is this distinction between low acute toxicity and high chronic activity, by design, that has led to calls for a focus on chronic rather than acute toxicity tests by the Environment Agency and others. Debate over the value of acute versus chronic tests applies only to aquatic animal testing with invertebrates and fish, as standard 96-h algal and 7-d higher plant test methods are generally considered to be chronic.

It is worth briefly considering the assumptions that underpin reliance on acute tests to predict chronic toxicity in other areas of aquatic toxicity. For the purposes of determining chronic impacts, acute toxicity studies are viewed as ‘accelerated’ tests in which duration of exposure is replaced by intensity of exposure. In other words, exposure of an organism to concentrations of test substance many times greater than those likely to be encountered in the environment are assumed to mimic longer exposure to much lower, environmentally realistic concentrations. For this to be a reliable basis for

hazard and risk assessment the mode of toxic action must remain the same between acute and chronic exposure.

In the context of substance testing for environmental risk assessment, the *ratio* between acute and chronic toxicity is of considerable importance. This is because consistent acute-to-chronic ratios (ACRs) allow use of acute data, with application of an appropriate assessment factor, to be used as surrogates for chronic data. Webb (2004a) compared ACRs for all published ecotoxicity data on human pharmaceuticals up to the year 1998. ACRs for *Daphnia* spp. (n = 7) ranged from 1 to 1428, with a median of 43. As Webb (2004a) points out, this is rather similar to ACRs reported for invertebrates exposed to industrial chemicals (range 1.6 to 1030; median 22.1), suggesting that there may be no need for invertebrate exposure to human pharmaceuticals to be assessed in a manner that differs from other chemicals. Table 1 includes and updates Webb's (2004a) summary of ACRs. For invertebrates the mean ACR for all data is 1367 (n = 28; standard deviation = 5613; range = 1 - 29800) and the median is 19.3. The dataset is highly skewed by an ACR of 29800 for propranolol. When this datum is removed, the mean ACR is 314 (n = 27; standard deviation = 687; range = 1 - 3108) and the median is 17.6. The median values of ACRs for invertebrates from Table 1 are therefore very similar to those of industrial chemicals, and an assessment factor of 1000 applied to acute data would protect against effects observed in most chronic tests. However, coverage of therapeutic classes remains sparse, with sufficient results available only for SSRIs and non-steroid anti-inflammatory drugs.

There remain too few ACRs for fish, other than for sex hormones, for any conclusions to be drawn. However, for sex hormones and β-adrenergic receptor blockers there is clear evidence that ACRs for fish can be very high.

Use of sub-organismal biomarkers

Several authors have recommended the use of sub-organismal biomarkers for detecting either exposure to, or the effects of, human pharmaceuticals. This includes both the more traditional enzymatic and cellular biomarkers (e.g., Jos et al. 2003, Nunes et al. 2004) and newer biomarkers from the fields of genomics, proteomics and metabolomics (e.g., Miracle et al. 2003, Snape et al. 2004, Viant et al. 2003). One of the major values of biomarkers may be as rapid screens to help prioritise further study (e.g., Dinan et al. 2001).

The use of vitellogenin induction as a biomarker of exposure to steroid oestrogens is now widespread. However, gene expression profiles have also been used to assess the exposure of largemouth bass to 17β-oestradiol (Larkin et al. 2003), and the proteomics of similar oestrogenic exposures were reported by Shrader et al. (2003) for zebrafish. Many other biomarker techniques for use in either laboratory, in situ caged, or resident biota studies are available, such as the comet assay (Steinert 2000, Vinette et al. 2003), DNA damage assays (Black and Belin 1998), bacterial genotoxicity assays (e.g., Giuliani 1996, Hartmann et al 1998) and cDNA microarrays (Snape et

al. 2004). However, none of these approaches has been used extensively for detecting or assessing human pharmaceutical effects.

There are few examples for human pharmaceuticals in which biomarker data have been related to adverse outcomes at the whole organism level. Exceptions to this, once again, are for ethinyloestradiol and other (anti)oestrogenic or (anti)androgenic pharmaceuticals (Jensen et al. 2004, Nash et al. 2004, Segner et al. 2003b&b). Ankley et al. (2002) also report such a link between aromatase inhibition and reduced fecundity in fish exposed to fadrozole.

Biomarkers may be useful in helping efficiently to direct research and testing towards substances with biological activity and modes of action that are relevant to particular taxonomic groups (Henschel et al. 1997). They may also help to demonstrate possible cause and effect relationships in field surveys. However, they are unlikely to be useful as sole endpoints in an environmental risk assessment in which links between chronic exposure and demographic endpoints (survival, growth and reproduction) are required by decision makers.

Modelling

(Q)SAR

Kümmerer (2004d) suggests that because of the relatively recent appearance of pharmaceutical effects as a research topic of environmental interest, there are too few data available to generate QSARs. However, Breton and Boxall (2003) suggest that there are many existing QSAR models that could be used to estimate the fate and effects of pharmaceuticals, or at least to prioritise studies.

Ashton et al. (2004) used ECOSAR (Meylan and Howard 1998) to predict the acute aquatic toxicity of pharmaceuticals marketed in the UK so that they could prioritise substances for a field monitoring programme. Boxall et al. (2000), Jones et al. (2002) and Sanderson et al. (2003, 2004a&b) also used ECOSAR to fill gaps in available acute toxicity datasets for prioritisation purposes. However, no QSARs for chronic effects are yet available because of the absence of sufficient chronic toxicity data to help build these models.

Extrapolation from mammalian data

Several authors have noted that pharmaceuticals are extensively studied in mammals during product development and that information from this can be used to predict effects in other organisms, particularly fish (Länge and Dietrich 2002). For example, Huggett et al. (2003, 2004) used measured human therapeutic plasma concentrations of drugs to predict steady state plasma concentrations in fish and to compute an effects ratio. When applied to 28 pharmaceuticals, this approach identified 17β-oestradiol and 17α-oestradiol as substances of potential concern. Initial validation of the model has also

shown that this may be a promising approach for focusing and prioritising testing resources.

However, it should be noted that mammalian modes of action are not a completely reliable means of predicting effects in other organisms (Länge and Dietrich 2002, Seiler 2002). For example, fluoxetine induces spawning in molluscs (Fong 2001, Fong et al. 1998), which cannot be predicted from effects in mammals. Further research is therefore required before knowledge of modes of action of pharmaceutical classes in non-mammalian species is sufficient for reliable extrapolation (Hutchinson 2002).

Mixture toxicity

Several authors have called for mixture toxicity to form part of pharmaceutical risk assessment, as these substances are likely to be found in different combinations in the environment (Brain et al. 2004b, Daughton 2003, Emmanuel 2005).

The logistics of mixture toxicity studies are complex and could include laboratory (e.g., Cleuvers 2003) or microcosm tests (e.g., Brain et al. 2004b, Richards et al. 2004). However, despite the obvious importance of understanding the effects of chemical mixtures in the environment, there seems little justification for treating pharmaceuticals differently to other industrial and plant protection substances which may also be found in environmental mixtures. Indeed, O'Brien and Dietrich (2004) suggest that detailed investigation of highly complex issues such as the mixture toxicity of pharmaceuticals may be less cost-effective than simply investing in upgraded STWs to prevent the entry of such mixtures into the environment in the first place.

Conclusions

1. Chronic testing for algae should be with blue-green algae, as these are likely to be sensitive surrogates for both algae and other unicellular organisms. There is little information available to determine whether algal data can also be used as surrogates for higher aquatic plants.

2. There is little evidence of a general need to perform chronic aquatic invertebrate tests for all human pharmaceuticals. However, more acute-to-chronic ratio data are required for the main therapeutic pharmaceutical classes before this issue can be fully resolved.

3. Chronic fish tests may be necessary for some substances, but it is likely that these can be focused more accurately through use of mammalian toxicity datasets. For some substances and modes of action, life-cycle or partial life-cycle fish tests may be more relevant than early life-stage tests. This is because the early life-stage test is unlikely to respond adequately to all pharmaceutical modes of action.

4. There is a need for regular collation of all ACR data from the white and grey literature on human pharmaceutical effects on aquatic organisms to help build an adequate database. This exercise may need to be supplemented by additional acute and chronic testing to fill data gaps for particular taxonomic groups and therapeutic classes.

5. Sub-organismal biomarkers may be useful in focusing research and testing efforts by identifying active substances and receptors of interest in aquatic species. They may be useful in field surveys for helping to establish possible cause and effect relationships.

6. QSARs have been used by several authors to predict acute toxic effects, but predictions of chronic effects are currently unavailable because of the paucity of chronic data.

Outstanding questions

1. What level and frequency of environmental exposure, or other factor, should trigger chronic aquatic testing of a human pharmaceutical, and how should substances identified for testing be prioritised?

2. Are standard algal, waterflea and fish tests adequate for chronic toxicity testing of human pharmaceuticals?

3. Are toxicity tests with higher aquatic plants, such as duckweeds, necessary to protect natural plant populations from the chronic effects of human pharmaceuticals?

4. Is application of an assessment factor to aquatic invertebrate acute toxicity data sufficient to protect natural invertebrate populations from the chronic effects of human pharmaceuticals? If so, what should this factor be?

5. Are mammalian datasets a reliable means of focussing fish acute and chronic toxicity tests?

6. When is the fish ELS test insufficient for protecting natural fish populations from the chronic effects of human pharmaceuticals, and what could replace or supplement it?

7. Do sub-organismal biomarkers have a practical role when assessing the chronic aquatic environmental effects of human pharmaceuticals?

8. Can QSARs currently be used to predict the chronic aquatic environmental effects of human pharmaceuticals? If not, what is required to enable their use in the future?

9. Is toxicity due to endocrine disrupting substances a special case, or are there wider implications for the toxicity testing of other therapeutic classes of human pharmaceuticals?

10. Is the mixture toxicity of human pharmaceuticals a special case, or should it be treated in the same way as for other chemical mixtures?

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Table 1. Chronic toxicity data for aquatic organisms exposed to human pharmaceuticals

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
Androgen	Methyltestosterone	Fish	*Carassius carassius*	0.00001		Fujioka (2002)
Androgen	Methyltestosterone	Fish	*Oryzias latipes*	<10.0 ng/l	>1000000	Hutchinson et al. (2003b)
Androgen	Methyltestosterone	Fish	*Pimephales promelas*	0.01		Zerulla et al. (2002)
Androgen	Methyltestosterone	Invertebrate (snail)	*Lymnaea stagnalis*	1.0 ng/l		Czech et al. (2001)
Androgen	Methyltestosterone	Invertebrate (snail)	*Marisa cornuarietis*	<100 ng/l		Schulte-Oehlmann et al. (2004)
Anti-androgen (non-steroidal)	Flutamide	Fish	*Oryzias latipes*	1.0	3.6	Hutchinson et al. (2003b)
Anti-bacterial	Trimethoprim	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (aminoglycoside)	Neomycin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (aminoglycoside)	Streptomycin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (cephalosporin)	Cephalexin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (fluoroquinolone)	Ciprofloxacin	Plant (duckweed)	*Lemna gibba*	0.106 (EC10)		Brain et al. (2004b)
Anti-bacterial	Levofloxacin	Plant (duckweed)	*Lemna gibba*	0.013 (EC10)		Brain et al.

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
(fluoroquinolone)						(2004b)
Anti-bacterial (fluoroquinolone)	Lomefloxacin	Alga (green)	Unspecified	2.0		FDA-CDER (1996)
Anti-bacterial (fluoroquinolone)	Lomefloxacin	Plant (duckweed)	*Lemna gibba*	0.008 (EC10)		Brain et al. (2004b)
Anti-bacterial (fluoroquinolone)	Norfloxacin	Plant (duckweed)	*Lemna gibba*	0.206 (EC10)		Brain et al. (2004b)
Anti-bacterial (fluoroquinolone)	Ofloxacin	Alga (blue-green)	*Synechococcus leopolensis*	0.005		Ferrari et al. (2004)
Anti-bacterial (fluoroquinolone)	Ofloxacin	Alga (diatom)	*Cyclotella meneghiniana*	0.0312		Ferrari et al. (2004)
Anti-bacterial (fluoroquinolone)	Ofloxacin	Alga (green)	*Pseudokirchneriella subcapitata*	2.5		Ferrari et al. (2003, 2004)
Anti-bacterial (fluoroquinolone)	Ofloxacin	Invertebrate (rotifer)	*Brachionus calyciflorus*	12.5		Ferrari et al. (2003
Anti-bacterial (fluoroquinolone)	Ofloxacin	Invertebrate (waterflea)	*Ceriodaphnia dubia*	10.0		Ferrari et al. (2003, 2004)
Anti-bacterial (fluoroquinolone)	Ofloxacin	Plant (duckweed)	*Lemna gibba*	0.121 (EC10)		Brain et al. (2004b)
Anti-bacterial (macrolide antibiotic)	Erythromycin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (macrolide antibiotic)	Lincomycin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial	Roxithromycin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al.

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
(macrolide antibiotic)						(2004b)
Anti-bacterial (macrolide antibiotic)	Tylosin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (penicillin)	Amoxicillin	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
Anti-bacterial (penicillin)	Amoxicillin	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (sulfonamide)	Sulfadimethoxine	Plant (duckweed)	*Lemna gibba*	0.044 (EC10)		Brain et al. (2004b)
Anti-bacterial (sulfonamide)	Sulfamethazine	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-bacterial (sulfonamide)	Sulfamethoxazole	Alga (blue-green)	*Synechococcus leopolensis*	0.0059		Ferrari et al. (2004)
Anti-bacterial (sulfonamide)	Sulfamethoxazole	Alga (diatom)	*Cyclotella meneghiniana*	1.25		Ferrari et al. (2004)
Anti-bacterial (sulfonamide)	Sulfamethoxazole	Alga (green)	*Pseudokirchneriella subcapitata*	0.09		Ferrari et al. (2003, 2004)
Anti-bacterial (sulfonamide)	Sulfamethoxazole	Invertebrate (rotifer)	*Brachionus calyciflorus*	25.0		Ferrari et al. (2003
Anti-bacterial (sulfonamide)	Sulfamethoxazole	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.25		Ferrari et al. (2003, 2004)
Anti-bacterial (sulfonamide)	Sulfamethoxazole	Plant (duckweed)	*Lemna gibba*	0.011 (EC10)		Brain et al. (2004b)
Anti-bacterial	Sulfochlorpyridazin	Plant (duckweed)	*Lemna minor*	2.33 (EC50)		Pro et al.

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
(sulfonamide)	e					(2003)
Anti-bacterial (tetracycline)	Chlortetracycline	Plant (duckweed)	*Lemna gibba*	0.036 (EC10)		Brain et al. (2004b)
Anti-bacterial (tetracycline)	Doxycycline	Plant (duckweed)	*Lemna gibba*	0.055 (EC10)		Brain et al. (2004b)
Anti-bacterial (tetracycline)	Oxytetracycline	Plant (duckweed)	*Lemna gibba*	0.788 (EC10)		Brain et al. (2004b)
Anti-bacterial (tetracycline)	Oxytetracycline	Plant (duckweed)	*Lemna minor*	4.92 (EC50)		Pro et al. (2003)
Anti-bacterial (tetracycline)	Tetracycline	Plant (duckweed)	*Lemna gibba*	0.23 (EC10)		Brain et al. (2004b)
Anti-depressant (SSRI)	Citalopram	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.8	4.9	Henry et al. (2004)
Anti-depressant (SSRI)	Fluoxetine	Alga (green)	Unspecified	0.001		FDA-CDER (1996)
Anti-depressant (SSRI)	Fluoxetine	Invertebrate (amphipod)	*Hyalella azteca*	>43 mg/kg		Brooks et al. (2003)
Anti-depressant (SSRI)	Fluoxetine	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.056		Brooks et al. (2003)
Anti-depressant (SSRI)	Fluoxetine	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.089	5.7	Henry et al. (2004)
Anti-depressant (SSRI)	Fluoxetine	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-depressant (SSRI)	Fluvoxamine	Alga (green)	Unspecified	31		FDA-CDER (1996)
Anti-depressant	Fluvoxamine	Invertebrate	*Ceriodaphnia dubia*	0.366	2.3	Henry et al.

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
(SSRI)		(waterflea)				(2004)
Anti-depressant (SSRI)	Paroxetine	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.22	2.8	Henry et al. (2004)
Anti-depressant (SSRI)	Sertraline	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.009	13.3	Henry et al. (2004)
Anti-depressant (SSRI)	Sertraline	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-diabetic (biguanide)	Metformin	Alga (green)	*Desmodesmus subspicatus*	>320.0 (EC50)		Cleuvers (2003)
Anti-diabetic (biguanide)	Metformin	Plant (duckweed)	*Lemna minor*	110.0 (EC50)		Cleuvers (2003)
Anti-epileptic	Carbamazepine	Alga (blue-green)	*Synechococcus leopolensis*	17.0		Ferrari et al. (2004)
Anti-epileptic	Carbamazepine	Alga (diatom)	*Cyclotella meneghiniana*	10.0		Ferrari et al. (2004)
Anti-epileptic	Carbamazepine	Alga (green)	*Desmodesmus subspicatus*	74.0 (EC50)		Cleuvers (2003)
Anti-epileptic	Carbamazepine	Alga (green)	*Pseudokirchneriella subcapitata*	>100.0		Ferrari et al. (2003, 2004)
Anti-epileptic	Carbamazepine	Fish	*Danio rerio*	25		Ferrari et al. (2003)
Anti-epileptic	Carbamazepine	Invertebrate (midge larva)	*Chironomus riparius*	0.625 mg/kg		Nentwig et al. (2004)
Anti-epileptic	Carbamazepine	Invertebrate (oligochaete worm)	*Lumbriculus variegatus*	>10 mg/kg		Nentwig et al. (2004)

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
Anti-epileptic	Carbamazepine	Invertebrate (rotifer)	*Brachionus calyciflorus*	0.377		Ferrari et al. (2003, 2004)
Anti-epileptic	Carbamazepine	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.025	3108	Ferrari et al. (2003, 2004)
Anti-epileptic	Carbamazepine	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Anti-epileptic	Carbamazepine	Plant (duckweed)	*Lemna minor*	25.5 (EC50)		Cleuvers (2003)
Anti-hyperlipidemic (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor)	Atorvastatin	Plant (duckweed)	*Lemna gibba*	0.085 (EC10)		Brain et al. (2004b)
Anti-hyperlipoproteinemic	Clofibric acid	Alga	Unspecified	5.4 (EC10)		Köpf (1995)
Anti-hyperlipoproteinemic	Clofibric acid	Alga (blue-green)	*Synechococcus leopolensis*	23.5		Ferrari et al. (2004)
Anti-hyperlipoproteinemic	Clofibric acid	Alga (diatom)	*Cyclotella meneghiniana*	>100.0		Ferrari et al. (2004)
Anti-hyperlipoproteinemic	Clofibric acid	Alga (green)	*Desmodesmus subspicatus*	115.0 (EC50)		Cleuvers (2003)
Anti-	Clofibric acid	Alga (green)	*Pseudokirchneriella*	75.0		Ferrari et al.

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
hyperlipoproteinemic			*subcapitata*			(2003, 2004)
Anti-hyperlipoproteinemic	Clofibric acid	Fish	*Danio rerio*	70		Ferrari et al. (2003)
Anti-hyperlipoproteinemic	Clofibric acid	Invertebrate (midge larva)	*Chironomus riparius*	>8 mg/kg		Nentwig et al. (2004)
Anti-hyperlipoproteinemic	Clofibric acid	Invertebrate (oligochaete worm)	*Lumbriculus variegatus*	>8 mg/kg		Nentwig et al. (2004)
Anti-hyperlipoproteinemic	Clofibric acid	Invertebrate (rotifer)	*Brachionus calyciflorus*	0.246		Ferrari et al. (2003
Anti-hyperlipoproteinemic	Clofibric acid	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.64	>312	Ferrari et al. (2003, 2004)
Anti-hyperlipoproteinemic	Clofibric acid	Invertebrate (waterflea)	*Daphnia magna*	0.01	1428	Köpf (1995)
Anti-hyperlipoproteinemic	Clofibric acid	Plant (duckweed)	*Lemna minor*	12.5 (EC50)		Cleuvers (2003)
Anti-hypertensive	Losartan K	Alga (blue-green)	Unspecified	556		FDA-CDER (1996)
Anti-hypertensive	Losartan K	Alga (green)	Unspecified	143		FDA-CDER

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
						(1996)
Anti-hypertensive (angiotensin-converting enzyme inhibitor)	Captopril	Alga (green)	*Desmodesmus subspicatus*	168.0 (EC50)		Cleuvers (2003)
Anti-hypertensive (angiotensin-converting enzyme inhibitor)	Captopril	Plant (duckweed)	*Lemna minor*	25.0 (EC50)		Cleuvers (2003)
Anti-infective	Lorcabef	Alga (green)	Unspecified	13		FDA-CDER (1996)
Anti-inflammatory (corticosteroid)	Budesonide	Alga (green)	Unspecified	10		FDA-CDER (1996)
Anti-protozoal	Metronidazole	Alga (green)	*Chlorella* sp.	2.03 (EC10)		Lanzky & Halling-Sørensen (1997)
Anti-protozoal	Metronidazole	Alga (green)	*Pseudokirchneriella subcapitata*	19.9 (EC10)		Lanzky & Halling-Sørensen (1997)
Anti-psychotic	Riseperidone	Alga (blue-green)	Unspecified	<100.0		FDA-CDER (1996)
Anti-psychotic	Riseperidone	Alga (green)	Unspecified	<10.0		FDA-CDER (1996)
Anxiolytic; muscle	Diazepam	Invertebrate	*Hydra vulgaris*	<0.01		Pascoe et al.

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
relaxant (benzodiazepine)		(cnidarian)				(2003)
Aromatase inhibitor	Fadrozole	Fish	*Pimephales promelas*	0.002		Ankley et al. (2002)
Benign prostatic hypertrophy drug (5α-reductase inhibitor)	Finasteride	Alga (green)	Unspecified	≥49		FDA-CDER (1996)
Bone resorption inhibitor	Alendronate	Alga (green)	Unspecified	0.5		FDA-CDER (1996)
Bone resorption inhibitor	Etidronic acid	Alga (green)	*Pseudokirchneriella subcapitata*	1.3 – 13.2		Gledhill & Feijtel (1992)
Bone resorption inhibitor	Etidronic acid	Invertebrate (waterflea)	*Daphnia magna*	>12.0	43.9	Gledhill & Feijtel (1992)
Bone resorption inhibitor	Tiludronate	Alga (blue-green)	*Microcystis aeruginosa*	13.3 (EC50)		Sanofi (1996)
Bone resorption inhibitor	Tiludronate	Alga (green)	*Pseudokirchneriella subcapitata*	36.6 (EC50)		Sanofi (1996)
Calcium channel blocker	Amlodipine	Invertebrate (cnidarian)	*Hydra vulgaris*	<0.01		Pascoe et al. (2003)
Cardiotonic (digitalis medicine)	Digoxin	Invertebrate (cnidarian)	*Hydra vulgaris*	<0.01		Pascoe et al. (2003)
Central nervous system stimulant	Caffeine	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Cholinergic agonist	Nicotine	Invertebrate (waterflea)	*Daphnia pulex*	<0.07	42.9	FDA-CER (1996)

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
Diuretic	Bendroflumethiazide	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
Diuretic (loop)	Furosemide	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
Nicotine metabolite	Cotinine	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Non-steroid anti-inflammatory drug	Acetaminophen (paracetamol)	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Non-steroid anti-inflammatory drug	Acetylsalicylic acid (aspirin)	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
Non-steroid anti-inflammatory drug	Diclofenac	Alga (blue-green)	*Synechococcus leopolensis*	10.0		Ferrari et al. (2004)
Non-steroid anti-inflammatory drug	Diclofenac	Alga (diatom)	*Cyclotella meneghiniana*	10.0		Ferrari et al. (2004)
Non-steroid anti-inflammatory drug	Diclofenac	Alga (green)	*Desmodesmus subspicatus*	72.0 (EC50)		Cleuvers (2003)
Non-steroid anti-inflammatory drug	Diclofenac	Alga (green)	*Pseudokirchneriella subcapitata*	10.0		Ferrari et al. (2003, 2004)
Non-steroid anti-inflammatory drug	Diclofenac	Fish	*Danio rerio*	4		Ferrari et al. (2003)
Non-steroid anti-inflammatory drug	Diclofenac	Invertebrate (rotifer)	*Brachionus calyciflorus*	12.5		Ferrari et al. (2003
Non-steroid anti-inflammatory drug	Diclofenac	Invertebrate (waterflea)	*Ceriodaphnia dubia*	1.0	22.7	Ferrari et al. (2003, 2004)
Non-steroid anti-inflammatory drug	Diclofenac	Plant (duckweed)	*Lemna minor*	7.5 (EC50)		Cleuvers (2003)

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
Non-steroid anti-inflammatory drug	Ibuprofen	Alga (green)	*Desmodesmus subspicatus*	315.0 (EC50)		Cleuvers (2003)
Non-steroid anti-inflammatory drug	Ibuprofen	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
Non-steroid anti-inflammatory drug	Ibuprofen	Invertebrate (snail)	*Planorbis carinatus*	1.02	1.68	Pounds et al. (2004)
Non-steroid anti-inflammatory drug	Ibuprofen	Plant (duckweed)	*Lemna gibba*	>1.0 (EC10)		Brain et al. (2004b)
Non-steroid anti-inflammatory drug	Ibuprofen	Plant (duckweed)	*Lemna minor*	22.0 (EC50)		Cleuvers (2003)
Non-steroid anti-inflammatory drug	Naproxen	Alga (green)	*Desmodesmus subspicatus*	>320.0 (EC50)		Cleuvers (2003)
Non-steroid anti-inflammatory drug	Naproxen	Plant (duckweed)	*Lemna minor*	24.2 (EC50)		Cleuvers (2003)
Non-steroid anti-inflammatory drug	Paracetamol (acetaminophen)	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
Non-steroid anti-inflammatory drug (metabolite of aspirin)	Gentisic acid	Invertebrate (waterflea)	*Daphnia longispina*	0.32	1070	Marques et al. (2004)
Non-steroid anti-inflammatory drug (metabolite of aspirin)	Gentisic acid	Invertebrate (waterflea)	*Daphnia magna*	0.32	1258	Marques et al. (2004)
Non-steroid anti-inflammatory drug	o-hydroxyhippuric acid	Invertebrate (waterflea)	*Daphnia longispina*	84.5	>21	Marques et al. (2004)

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
(metabolite of aspirin)						
Non-steroid anti-inflammatory drug (metabolite of aspirin)	o-hydroxyhippuric acid	Invertebrate (waterflea)	*Daphnia magna*	186.0	>9.7	Marques et al. (2004)
Non-steroid anti-inflammatory drug (metabolite of aspirin)	Salicylic acid	Invertebrate (waterflea)	*Daphnia longispina*	5.6	205	Marques et al. (2004)
Non-steroid anti-inflammatory drug (metabolite of aspirin)	Salicylic acid	Invertebrate (waterflea)	*Daphnia magna*	>10.0	<195	Marques et al. (2004)
Non-steroidal anti-androgen	Bicalutamide	Alga (blue-green)	Unspecified	1		FDA-CDER (1996)
Non-steroidal anti-androgen	Bicalutamide	Alga (green)	Unspecified	1		FDA-CDER (1996)
Oestrogen	17β-oestradiol	Fish	*Oryzias latipes*	10 ng/l	390000	Hutchinson et al. (2003b)
Oestrogen	Diethylstilbestrol	Fish	*Oryzias latipes*	10 ng/l	140000	Hutchinson et al. (2003b)
Oestrogen	Diethylstilbestrol	Invertebrate (copepod)	*Nitocra spinepes*	0.003	97	Breitholtz & Bengtsson (2001)
Oestrogen	Diethylstilbestrol	Invertebrate	*Tisbe battagliai*	0.01	<10	Hutchinson et

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
		(copepod)				al. (1999)
Oestrogen	Diethylstilbestrol	Invertebrate (waterflea)	*Daphnia magna*	0.062	17.6	Baldwin et al. (1995)
Oestrogen	Ethinylestradiol	Alga	Unspecified	0.054 (EC10)		Köpf (1995)
Oestrogen	Ethinyloestradiol	Fish	*Oncorhynchus mykiss*	<0.1 ng/L		Purdom et al. (1994)
				<0.3 ng/L		Sheahan et al. (1994)
Oestrogen	Ethinyloestradiol	Fish	*Oryzias latipes*	10 ng/l	150000	Hutchinson et al. (2003b)
Oestrogen	Ethinyloestradiol	Fish	*Pimephales promelas*	1 ng/L		Länge et al. (2001)
Oestrogen	Ethinyloestradiol	Invertebrate (amphipod)	*Hyalella azteca*	0.0001		Vandenburg et al. (2003)
Oestrogen	Ethinyloestradiol	Invertebrate (copepod)	*Nitocra spinepes*	0.05	10.2	Breitholtz & Bengtsson (2001)
Oestrogen	Ethinyloestradiol	Invertebrate (snail)	*Bithynia tentaculata*	<0.125 ng/l		Belfoid & Leonards (1996)
Oestrogen	Ethinyloestradiol	Invertebrate (snail)	*Lymnaea stagnalis*	<1.25 ng/l		Belfoid & Leonards (1996)
Oestrogen	Ethinyloestradiol	Invertebrate (snail)	*Marisa cornuarietis*	<1.0 ng/l		Schulte-Oehlmann et al. (2004)

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
Oestrogen	Ethinyloestradiol	Invertebrate (waterflea)	*Daphnia magna*	0.01	570	Köpf (1995)
Oestrogen	Oestradiol	Invertebrate (copepod)	*Nitocra spinepes*	0.16	10	Breitholtz & Bengtsson (2001)
Peristaltic stimulant	Cisapride	Alga (green)	Unspecified	320 ('Effects')		FDA-CDER (1996)
Peristaltic stimulant	Cisapride	Blue-green alga	Unspecified	100 ('Effects')		FDA-CDER (1996)
Topical keratolytic	Salicylic acid	Invertebrate (waterflea)	*Daphnia magna*	<20.0	5.9	Wang & Lay 1989
X-ray contrast medium	Iopromide	Alga (blue-green)	Unspecified	68		FDA-CDER (1996)
X-ray contrast medium	Iopromide	Invertebrate (waterflea)	*Daphnia magna*	>1000.0	1	Schweinfurth et al. (1996)
β-adrenergic receptor blocker	Atenolol	Invertebrate (cnidarian)	*Hydra vulgaris*	>0.01		Pascoe et al. (2003)
β-adrenergic receptor blocker	Metoprolol	Alga (green)	*Desmodesmus subspicatus*	7.3 (EC50)		Cleuvers (2003)
β-adrenergic receptor blocker	Metoprolol	Plant (duckweed)	*Lemna minor*	>320.0 (EC50)		Cleuvers (2003)
β-adrenergic receptor blocker	Propranolol	Alga (blue-green)	*Synechococcus leopolensis*	0.35		Ferrari et al. (2004)
β-adrenergic receptor blocker	Propranolol	Alga (diatom)	*Cyclotella meneghiniana*	0.094		Ferrari et al. (2004)
β-adrenergic	Propranolol	Alga (green)	*Desmodesmus*	5.8 (EC50)		Cleuvers

Therapeutic class	Substance	Taxonomic group	Species	Long-term exposure result (mg l^{-1})*	Acute to chronic ratio (if available)	Reference
receptor blocker			*subspicatus*			(2003)
B-adrenergic receptor blocker	Propranolol	Alga (green)	*Pseudokirchneriella subcapitata*	5.0		Ferrari et al. (2003, 2004)
β-adrenergic receptor blocker	Propranolol	Fish	*Oryzias latipes*	<0.0005	>48600	Huggett et al. (2002)
β-adrenergic receptor blocker	Propranolol	Invertebrate (amphipod)	*Hyalella azteca*	0.001	29800	Huggett et al. (2002)
β-adrenergic receptor blocker	Propranolol	Invertebrate (rotifer)	*Brachionus calyciflorus*	0.18		Ferrari et al. (2003
β-adrenergic receptor blocker	Propranolol	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.009		Ferrari et al. (2003, 2004)
β-adrenergic receptor blocker	Propranolol	Invertebrate (waterflea)	*Ceriodaphnia dubia*	0.125	6.8	Huggett et al. (2002)
β-adrenergic receptor blocker	Propranolol	Plant (duckweed)	*Lemna minor*	114.0 (EC50)		Cleuvers (2003)

* (NOEC in mg l^{-1} unless otherwise stated)

Glossary

Term	Definition
17α-ethinyloestradiol	Oestrogen
Acetaminophen	Paracetamol: non-steroid anti-inflammatory drug
Acetylsalicylic acid	Aspirin: non-steroid anti-inflammatory drug
ACR	Acute-to-chronic ratio
Acute toxicity	Short term, often lethal
Acute-to-chronic ratio	Ratio between acute and chronic toxic effects
Alendronate sodium	Metabolic bone disease drug
Amlodipine	Calcium channel blocker
Amoxicillin	Antibacterial
Ampicillin	Antibacterial
Atenolol	β-adrenergic receptor blocker
Atorvastatin	Statin drug
Bendroflumethiazide	Diuretic
Bicalutamide	Non-steroidal anti-androgen
Biomarker	A biomarker is a biological response to an environmental chemical, which gives a measure of exposure and sometimes also a toxic effect.
Budenoside	Anti-inflammatory
Caffeine	Central nervous system stimulant
Captopril	Antihypertensive
Carbamazepine	Anti-epileptic
Cefotaxime	Antibacterial
Ceftazidime	Antibacterial
Cephalexin	Antibacterial
Chlortetracycline	Antibacterial
Chronic toxicity	Long-term, often sublethal
Ciprofloxacin	Antibacterial
Cisapride	Peristaltic stimulant
Citalopram	Anti-depressant
Clofibrate	Antihyperlipoproteinemic
Clofibric acid	Antihyperlipoproteinemic
Cotinine	Nicotine metabolite
Diazepam	Anxiolytic; muscle relaxant
Diclofenac	Non-steroid anti-inflammatory drug
Diethylstilbestrol	Oestrogen
Digoxin	Cardiotonic
Doxycycline	Antibacterial
EC10	Concentration of substance causing a 10% effect
EC50	Concentration of substance causing a 50% effect
ECOSAR	Software for QSAR modelling

EMEA	European Medicines Agency
Erythromycin	Antibacterial
Ethinyloestradiol	Oestrogen
Etidronic acid	Metabolic bone disease drug
Fadrozole	Aromatase inhibitor
Finasteride	Benign prostatic hypertrophy drug
Flutamide	Anti-androgen
Fluoxetine	Anti-depressant
Fluvoxamine	Anti-depressant
Fluvoxamine maleate	Anti-depressant
Furosemide	Diuretic
Ibuprofen	Non-steroid anti-inflammatory drug
Imipenem	Antibacterial
Indomethacin	Non-steroid anti-inflammatory drug
Iodocontrast agents	X-ray contrast media
Iopromide	X-ray contrast medium
Levofloxacin	Antibacterial
Lincomycin	Antibacterial
LOEC	Lowest Observed Effect Concentration. The lowest tested concentration that does produce a statistically significant effect when compared with controls
Lomefloxacin	Antibacterial
Loracarbef	Anti-infective
Losartan K	Antihypertensive
Metformin	Antidiabetic
Methyltestosterone	Androgen
Metoprolol	β-adrenergic receptor blocker
Metronidazole	Antiprotozoal
Naproxen	Non-steroid anti-inflammatory drug
Neomycin	Antibacterial
Nicotine	Cholinergic agonist
NOEC	No Observed Effect Concentration. The highest tested concentration that does not produce a statistically significant effect when compared with controls
Norfloxacin	Antibacterial
Oestradiol	Oestrogen
Ofloxacin	Antibacterial
Oxytetracycline	Antibacterial
Paracetamol	see Acetaminophen
Paroxetine	Anti-depressant
Propranolol	β-adrenergic receptor blocker
QSAR	Quantitative Structure-Activity Relationship
Risperidone	Anti-psychotic

Roxithromycin	Antibacterial
Salicylic acid	Topical keratolytic
Sertraline	Anti-depressant
SSRI	Selective Serotonin Reuptake Inhibitors
Streptomycin	Antibacterial
STW	Sewage treatment works
Sulfadimethoxine	Antibacterial
Sulfamethazine	Antibacterial
Sulfamethoxazole	Antibacterial
Sulfochlorpyridazine	Antibacterial
Tetracycline	Antibacterial
Tiludronate disodium	Metabolic bone disease drug
Triclosan	Antibacterial
Trimethoprim	Antibacterial
Tylosin	Antibacterial

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