First publ. in: Toxicology Letters 131 (2002), 1-2, pp. 97-104

Environmental risk assessment of pharmaceutical drug substances—conceptual considerations

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Abstract

Drugs, i.e. active ingredients of human medicinal products, may be introduced into the environment after use in patients by sewage effluent pathways and consequently are detected at low concentrations in sewage effluents and in surface waters. Legal requirements in a number of geographical regions (Europe, US, and intended in Canada) demand environmental risk assessments (ERA) for new drug substances. Existing regulatory concepts of ERA are based initially on a set of short-term ecotoxicological studies in three to four different species, environmental behavior and the application of assessment factors to correct for the ERA inherent uncertainty. Based on theoretical considerations and the experience with a very limited, but well investigated, number of examples while considering that drugs are highly biologically active compounds, the appropriateness of this risk assessment procedure for all drug substances might be questioned. Indeed, e.g. long-term effects may occur at much lower concentrations and follow different toxicodynamic mechanism than extrapolated from short-term studies., In such cases, the application of assessment factors for deriving chronic no-observed effect concentration (NOECs) appears to be problematic. Although long-term tests with a variety of organisms would provide a complete database for the evaluation of the environmental risks, this is unachievable for all drugs due to time, money and animal welfare constraints. In order to avoid unnecessary testing, a concept is presented, which makes use of pharmacological and toxicological, as well as pharmaco- and toxicokinetic information derived from mammals during drug substance development. Useful data for adoption in a case-by-case testing strategy can be obtained by evaluating (a) the pharmacological activity, which indicates specific targets in mammalian species and may allow for an analysis, whether a similar target is available in aquatic species; (b) the mammalian toxicity, which may indicate, which targets are most susceptible to adverse effects; (c) the difference between acute and chronic effects in mammals, since the magnitude of this difference may indicate, whether long-term effects are expected at significantly lower levels than acute effects; (d) the (pharmacologically and toxicologically) effective plasma levels in mammalian test organisms, which may be compared with the relevant exposure scenario for the environment. Additionally, activity classes of compounds may be established based on experience with specific substances, in order to develop an appropriate test strategy. The above preliminary considerations should support decisions on the selection of candidate substances for chronic effects studies and for the

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appropriate selection of test species and endpoints to monitor. Generally, ecologically relevant endpoints such as impairment of growth, development and reproduction should be used to assess the ecotoxicologic effects.

Keywords: Concepts; Tiered testing; Pharmaceuticals; Tailored approaches; Risk assessment

1. Introduction

Following the European Union (EU) directive 93/39/EEC (European-Union, 1993) in context with the registration of new medicinal products in the EU, the environmental risk by use and disposal of these compounds have to be evaluated, in order to provide appropriate information to the users of these products. Similar regulations are in effect or planned in other countries such as the US and Canada (FDA, 1998; Department-of-Health-Canada, 2001). In the draft note for guidance (CPMP, 1994) and in a recently published discussion paper by the CPMP (EMEA, 2001), a procedure for such a risk assessment is described.

In essence, this procedure follows the general principle of environmental risk assessments (ERA) as applied to chemicals in the EU under the chemicals legislation, in which the intrinsic properties of the compounds are analyzed by certain experimental studies, and by application of an assessment factor the so called predicted no effect concentration (PNEC) is estimated. Additionally, the environmental exposure is calculated based on the predicted market volume, the water consumption of the target population and a dilution factor accounting for dilution of effluent when reaching the surface waters. This estimate is considered to be the predicted environmental concentration (PEC). The ratio of PEC and PNEC should provide a first estimate of the potential risk of the medicinal compound.

Given the nature of active compounds in medicinal products, it was questioned, whether the assessment of the intrinsic ecotoxicological properties of such compounds by using simplified, short-term aquatic tests, are an adequate basis for an environmental risk assessment. Indeed, the limitations of the small short-term aquatic test battery were revealed by a study of Henschel et al. (Henschel et al., 1997), whereby four pharmaceuticals and their respective metabolites were tested with different standard and non-standard ecotoxicity tests. The most sensitive tests were then non-standard tests, e.g. the BF-2 fish cell line (cytotoxicity and proliferation inhibition of fish cells) for three of the four substances. Sensitive was also the non-standard ciliate test for one, the fish embryo test for another and the Daphnia acut test for a third compound. The algal growth inhibition test was relatively insensitive for all four compounds. This demonstrates that the classical short-term aquatic tests may underestimated the toxicity of the four compounds tested. Although the CPMP discussion paper (EMEA, 2001) stated that in principle the use of short-term tests with three different aquatic species (algae, *Daphnia* sp. and fish) is recommended, however, for certain groups of compounds it is recognized that a different approach may be needed. No further guidance is given in the discussion paper or in the earlier draft guideline, what criteria should be used to determine, whether additional species, endpoints and test durations should be envisaged for a specific active compounds.

In the following, considerations are presented as guidance, what criteria could be useful tools to pre-evaluate the potential for the ecotoxicological effects, based on the large database, which is typically developed during the development of a drug substance. These criteria should help to determine an adequate testing strategy for the ecotoxicological effects of medicinal compounds.

2. Available relevant pharmacological, pharmacodynamic and toxicological information

Common procedures in the research and development of medicinal compounds provide a huge database, which should be used for a rationale of an adequate testing strategy for the ecotoxicological properties.

The first information on the type of activity of an active substance is gained in research, where a certain pharmacodynamic property is evaluated for treatment of human diseases. This desired property in a patient might provide for an unwanted adverse effect in e.g. a non-target aquatic species. Thus the specific mode of action is a very relevant basis for further considerations of a test strategy in environmental organisms. It must be emphasized, however, that although the intrinsic activity properties of a pharmaceutical compound and thus the mode of action may be similar across species, the actual affected endpoints, e.g. downstream of a receptor interaction, may differ dramatically amongst classes of organisms and species within the same classes.

For example serotonin (5-hydroxy-tryptamine, 5-HT) is a neurotransmitter not only found in humans and mammalians but also in all other phyla thus far examined including invertebrates. However, the 5-HT induced effects, downstream of the 5-HT receptor, differs among the various species (*humans*: regulation of appetite, sleep, sexual arousal, and depression; *bivalves*: regulation of reproductive processes e.g. spawning, oocyte maturation, germinal vesicle breakdown, sperm reactivation and parturition; *freshwater gastropods* (*Lymnaea stagnalis* and *Biomphalaria glabrata*): regulation of egg laying and induction of penile erection; *protozoans*: cilia regeneration; nudibranches: ciliary reaction; *Aplysia*: regulation of muscle contraction; *crustaceans*: regulation of ovarian growth) (Fong, 2001; Fong et al., 1998; Muschamp and Fong, 2001). This also implies that 5-HT analogues (e.g. Methiothepin) or 5-HT re-uptake inhibitors [SSRI: fluoxetine (Prozac®), fluvoxamine (Luvox®) or paroxetine (Praxil®)] may adversely influence the normal function of various species at concentrations magnitudes lower than those normally applied to humans i.e. in patients treated for depression or obsessive-compulsive behaviors associated with Tourette's Syndrome. However, whether or not aquatic organisms can be adversely affected at the concentrations of antidepressants released into the environment is at present difficult to determine. The mere fact that similar if not identical pharmacological targets are present in species other than the human should drive the environmental testing and risk assessment of tailored pharmaceuticals with highly specific activities. Consequently, the pharmaceutical database i.e. compound class, type of pharmacological activity, efficacy, treatment doses, and physico-chemical parameters, could serve as a primary source of information of preliminary risk estimation. The combined information on the pharmacodynamical properties of such a compound and the distribution of identical or similar physiological targets in environmental organisms, should lead to a directed and tailored environmental testing.

In contrast to the wanted pharmacodynamical properties, discussed above, unwanted (adverse) reactions during pharmacological and toxicological testing of a pharmaceutical are a completely different entity. Important pieces of information on the adverse effects of a compound, which may be useful for targeting an ecotoxicological test strategy, can be obtained from the mammalian toxicology database as well as from patient information. This database should provide an indication of the ratio of acute and chronic toxicity, target organs, specific effects regarding genotoxicity, reproduction and developmental toxicity or immunotoxicity.

Particularly relevant for further ecotoxicological testing considerations are characteristic parameters the relationship of pharmacological and toxicological responses to the plasma levels, and information regarding the metabolism and excretion of the compound in question. Especially the potential metabolites formed may indeed form a complete ecotoxicological relevant entity of their own. For example while the lipid regulator, clofibrate, will not be detected in the environment, its major metabolite clofibric acid appears in significant amounts in the environment (Ternes, 1998). The question thus arises whether clofibric acid can adversely affect cholesterol synthesis, thus steroid genesis and consequently could influence endocrine regulation in aquatic species (Pfluger and Dietrich, 2001).

Finally, the pharmacological and toxicological investigations in conjunction with a more broadened biological/phylogenetic understanding will demonstrate, how species specific certain re-

sponses are, thus providing additional information regarding the requirement of further ecotoxicological considerations and testing. For example if a response is relatively unspecific, in functional systems which are preserved over a large variety of species as discussed for serotonin above, additional and extensive testing should be discussed. On the other hand, if the compound is tailored for such specific conditions that it is unlikely that this response can occur in environmental organisms, no additional testing should be envisioned.

3. Use of pharmacodynamic information from mammalian species in ecotoxicological test strategies

Typically, information of the pharmacodynamic activity can contain observations made in in vitro studies and in vivo studies. In vitro studies could demonstrate the activity of a drug substance on a variety of systems, such as receptors, specific tissues and organs. In vivo studies, on the other hand, will demonstrate in which way specific activities cause responses in the whole organism. This information can be a useful tool in selecting test organisms in an ecotoxicological test strategy. For instance, if the pharmacological effect is based on a specific receptor mediated reaction (see for example serotonin above), it has to be considered, whether this receptor is likely to exist in the suite of test organisms available for standard laboratory testing and what endpoints may be suitable to demonstrate adverse effects causal to exposure by this pharmacological entity.

For example juvenile fathead minnows were exposed to the potential model androgen methyl testosterone (Zerulla et al., 2002), however, instead of an androgenisation as expected, a feminization response of the fish was observed. This feminization response, as determined via vitellogenin protein and mRNA, was clearly coupled to the presence of an aromatase, enabling the aromatization of testosterone to an estrogen. Thus, in addition to the absolute requirement of understanding the physiology, underlying kinetics and reliability of the endpoints e.g. vitellogenin employed (Schmid et al., 2002), the mechanism of compound interaction and in this case specifically the metabolism must be investigated in order to allow proper risk assessment. Indeed, generalizations can prove to provide for false-positive answers as recently shown by Hutchinson et al. (Hutchinson, 2002), whereby the classic synthetic non-steroidal estrogen or 'endocrine disrupter' diethylstilbestrol (DES) demonstrated interaction with the ecdysteroid receptor of the marine copepod *Tisbe battagliai*, however, many known endocrine disrupters and related active pharmaceuticals did not, thus severely questioning the presence of an endocrine mediated effect in this species.

In most cases, however, it is unlikely that scientific knowledge on environmental organisms is so detailed that the existence of certain receptors can be confirmed. The pharmacological response, though, may be an indicator for an adequate test strategy. For example, it may be worthwhile to consider the use of an early life stage test in fish, when a compound is known to affect the development and growth of blood vessels, an endocrine controlled mechanism in higher organisms, in developing organs or tissues. In contrast, *Daphnia magna* is unlikely to show any response to this particular pharmacological effect, since these organisms have no vascular system.

On the other hand, compounds such as antibiotics, known to interfere particularly with the metabolic system in the microbial cell, may show distinct effects in microbial test systems, while other test organisms may be less sensitive under acute exposure situations. Thus, the antimycotic compound metronidazole was tested in fish, crustaceans, and algae. EC_{50} values for in the algal tests (*Selenastrum capricornutum*, *Chlorella* sp.) were between 12 and 45 mg/l, in fish (*Brachydanio rerio*) and crustacean (*Acatia tonsa*) there were no effects at 500 and 100 mg/l, respectively, which were the highest concentrations tested (Lanzky and Halling-Soerensen, 1997). The antibiotic ciprofloxacin had an EC_{50} of 0.005 mg/l in the cyanobacterium *Microcystis aeruginosa*, 0.6 mg/l in activated sludge, 2.97 mg/l in the alga *S. capricornutum*, while in fish and *D. magna* there was no effect up to the highest test concentration of 100

and 60 mg/l (Halling-Sorensen et al., 2000). These examples indicate the relative sensitivity of microorganisms to antibiotics in comparison to higher organisms. However, these observations should not come as a surprise as e.g. the cyanobacterium *M. aeruginosa* and the green algae *S. capricornutum* are phylogenetically much closer to the pathogenic bacteria, i.e. their specific type of gyrases inhibited by fluorquinolone antibiotics (Backhaus et al., 2000), than to organisms of higher phylogenetic class (crustaceans and fish).

4. Use of pharmacological and toxicological information from mammalian species in ecotoxicological test strategies

Pharmacological and toxicological information becoming available during the development process of a drug substance, comprise a large number of data on adverse effects. Information on the acute and repeated-dose including the target organs, disturbance of fertility in the male or female (or both) gender, embryotoxicity or teratogenicity, and genotoxicity are typically available. For the selection of an ecotoxicological test strategy the types of effects displayed in mammalian toxicity tests should be considered. Vertebrates have many physiological functions in common and few examples are reported, where endpoints studied in fish were similarly affected as those in mammalian species. Thus, the natural estrogen estradiol as well as the synthetic steroid ethinylestradiol (EE2) have a strong feminization effect in fish, i.e. affected reproduction and normal development in gold fish (Bjerselius et al., 2001) and fathead minnows (Laenge et al., 2001), respectively.

In addition to the direct effects elicited by a drug in a given species, secondary adverse effects as a consequence of a primary drug interaction must also be considered. For example, cyclosporine and staurosporin selectively inhibit P-glycoprotein (Pgp), a member of the ABC family of transport proteins, in fresh water mussels (*D. polymorpha* and *C. fluminea*) and thus reduce the active excretion of reactive metabolic products from these organisms potentially leading to a progressive self-intoxication or to an accumulation of other toxic xenobiotics. Other compounds with Pgp modulating or inhibiting activity or compounds that could serve as substrates for Pgp are calcium channel blockers, calmodulin antagonists, anti-hypertensives, vinca alkaloids, steroids, anti-arrhythmics, anti-parasitics and anti-estrogens (Epel and Smital, 2001). Indeed, the presence and degree of expression of Pgp's in aquatic organisms may directly influence uptake and retention of active compounds e.g. steroids, anti-steroids and possibly quinolone antibiotics (Backhaus et al., 2000), and, therefore, modulate the physiologically relevant concentrations within the exposed organisms. This example, therefore, suggests that knowledge of the environmentally present and biologically available concentration of a pharmaceutical compound may not suffice for a risk assessment (see also below).

5. Use of pharmacokinetic information in ecotoxicological test strategies

Pharmacokinetic investigations in mammalian species demonstrate the availability of the administered substance, distribution, metabolism and excretion. It further shows in combination with toxicological or pharmacological studies, at which endogenous level a drug substance exerts it activity.

In ecotoxicological studies, the exposure is typically determined on the basis of the dissolved fraction of the test compound. Although the bioavailability of the substance in the organism is not further assessed by blood plasma analysis, it might be useful for preliminary evaluation of the ecotoxicological potency of a substance to compare mammalian blood plasma levels with exposure levels in ambient waters of aquatic organisms. Further, the simplified assumption is made that similar (e.g. same order of magnitude) levels in plasma of mammalians and in the ambient environment of aquatic organisms are comparably effective. Under this assumption, effective plasma levels or plasma levels at the recommended human dose in comparison to the predicted environmental concentration may indicate whether chronic exposure of wildlife organisms is needed to be assessed in specific long-term tests.

It has to be stated, however, that there are not many examples supporting this hypothesis yet. In case of the compound EE2 it was found that the concentration of 4 pg/ml in water caused developmental and reproductive disturbances in fish (Laenge et al., 2001). In comparison, pharmacological effects (contraceptive) are found at plasma levels of 25–100 pg/ml (Hümpel et al., 1990) given a recommended daily dose of 30 μg EE2 per person. This example indicates that fish seemed to be even more sensitive to EE2 than man and a factor of at least 10 had to be applied, if the ratio of the effective environmental concentration is compared with effective plasma levels.

Future studies should confirm, whether there is a general similarity between mammalian and other vertebrate species, e.g. fish, in pharmacologically effective levels.

6. Criteria for the development of an ecotoxicological test strategy

The aforementioned considerations may be used to select a science based test strategy for the environmental hazard and risk assessment of drug substances. Apparently, the information on the pharmacodynamics and toxicology of a drug substance mainly support ecotoxicity testing specifically in fish, since common functions between mammalian species and invertebrates are not very well understood and cannot a priori be assumed (Hutchinson, 2002).

Therefore, the following example of a testing strategy is mainly focused on the aspect, whether ecotoxicity testing for a candidate substance should go beyond the standard acute tests in algae, *daphnia* and fish by performing longer-term test in fish, employing relevant endpoints.

6.1. Two tiered strategy

6.1.1. Tier 1: algae, daphnia, fish

6.1.1.1. Information.

- General level of acute toxicity and pharmacological activity (underlying mechanism) of the compound in question in the above wildlife species.
- Availability of mammalian pharmacology and toxicology as well as ecotoxicology datasets for benchmark compound(s) with comparable pharmaco/toxicokinetic and—dynamic properties as the compound in question.

6.1.1.2. Questions.

- Is the observed ecotoxicity, in comparison to the mammalian toxicity, unexpected?
- Are the test organisms appropriate (presence/absence of enzymes, receptors, transporters etc.) for detecting effects of the compound in question? Which aquatic organism(s) are most likely affected?

6.1.1.3. Preliminary risk assessment.

- Determination of the order of magnitudes between expected environmental exposure concentrations and observed effect concentrations.

6.1.2. Tier 2: criteria for further testing

6.1.2.1. Questions.

- Is the acute/chronic ratio (i.e. the LD_{50} vs. the lowest NOEL in the most sensitive species and the most sensitive endpoint) in mammalian species > 1000?
- Is the substance genotoxic?
- What chronic effects are known (longevity (mortality), reproductive toxicity, embryo toxicity, organ toxicity, carcinogenicity)?
- Is the pharmacological/toxicological active plasma level in the mammalian test system within the same order of magnitude than the PEC?
- Are the pharmaco/toxicokinetics driven by a specific uptake/excretion mechanism (transporters; metabolism/conjugation)?

6.1.2.2. Experimentation.

- Identification of the most suitable (susceptible) species, test systems, test duration and endpoints

6.1.2.3. Risk assessment.

- Standard environmental risk assessment based on a comparison of environmental concentrations (EC) or PEC and observed no-effect concentrations in the test system(s).

7. Conclusions and recommendations

Most of the available literature on pharmaceuticals in the environment deals with the analytical detection of these compounds in the aquatic environment. For a very few of these chemicals, the environmental fate is known. Data on the effects of these compounds in environmentally relevant organisms are, with a few exceptions (Fong, 2001; Hutchinson, 2002; Pfluger and Dietrich, 2001), rare. Indeed, most currently available data on the effects of pharmaceuticals in the environment were obtained using standard test systems/procedures e.g. algal growth inhibition tests (OECD, 1984a), the *D. magna* acute and chronic toxicity test (OECD, 1984b; Wollenberger et al., 2000) or embryotoxicity tests with zebrafish (DRETA, *Danio rerio* embryo teratogenesis assay, (Dietrich and Prietz, 1999; Dietrich et al., 1998), the African clawed frog *Xenopus laevis* (FETAX, frog embryo teratogenesis assay *Xenopus*, (Kiamos et al., 1998; Neeser et al., 1996), or with the fat head minnow (*Pimephales promelas*) (Laenge et al., 2001; Schmid et al., 2002; Zerulla et al., 2002). This lack of information is not necessarily surprising in view of the tailored testing schemes and specifically defined endpoints required to detect potential adverse effects (Epel and Smital, 2001; Fong, 2001; Pfluger and Dietrich, 2001). Indeed, it is unlikely that testing for potential endocrine modulating chemicals (also known as 'endocrine disrupters') with the standard short-term acute test systems as proposed by the CPMP (EMEA, 2001) for pharmaceuticals would reveal their chronic toxicity potency. Thus in analogy to the 'endocrine disruption' problematic an approach tailored to the pharmacological function and activity, rather than a uniform approach, is of essence for the ecotoxicological testing of pharmaceuticals.

The tiered approach as suggested above demands a thorough understanding of the compound in question and an extrapolation and interpretation of the potential environmental activities in addition to the standard short term testing systems very early on in the risk assessment process. The combination of present knowledge from the base-set of pharmaceuticals registration, environmental biology and rudimentary toxicology should provide a basis to decide whether a compound presents with a potential for low, medium or high environmental risk. The second tier should provide for a testing approach specifically tailored to the type of pharmacological activity inherent to the compound in question, relevant for those compounds which have been selected preliminary as of potential medium or high environmental concern. Consequently no 'check-the-box' type of testing system should be implemented at this stage, but rather, the experimentation should be knowledge and experience driven. Although this asks for a closer interaction of industry, academia and regulatory agencies, this approach will provide for a sound and robust risk assessment.

The use of validated and standardized methods are essential in order to provide valid and reproducable results. The methods published under the OECD testing guidelines scheme are useful tools, however, specific issues of ecotoxicological relevance may require the need for adaptation and modification of those guidelines to assess additional relevant endpoints.

These considerations are thought to be a start for further research in this area, the suggestions need to be put in more concrete terms, when more data are available, which may show similarities in the toxicological and pharmacological responses in mammalian and wildlife organisms, when treated with medicinal compounds.

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