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Variations in bioconcentration of human pharmaceuticals from sewage effluents into fish blood plasma

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Abstract

The "Fish Plasma Model" has been proposed for prioritizing pharmaceuticals for in-depth environmental risk assessment efforts. The model compares estimated drug concentrations in fish plasma with human therapeutic plasma concentrations in order to assess the risk for a pharmacological interaction in the fish. In this study the equation used to estimate bioconcentration from water to fish blood plasma was field-tested by exposing rainbow trout in situ to sewage effluents from three treatment plants. Measured plasma levels of diclofenac, naproxen, ketoprofen and gemfibrozil were similar or lower than those modelled, which is acceptable for an early tier. However, measured levels of ibuprofen were >200 times higher than modelled for the largest plant (Gryaab Göteborg). Comparing measured fish plasma concentrations to the human therapeutic concentrations ranked the relative risks from the pharmaceuticals. Diclofenac and gemfibrozil, followed by ibuprofen, presented the highest risk for target interactions, whereas naproxen and ketoprofen presented little risk. Remarkably, measured bioconcentration factors varied considerably between sites. This variation could not be attributed to differences in water concentrations, temperatures, pH or exposure times, thereby suggesting that chemical characteristics of effluents and/or recipient waters strongly affected the uptake/bioconcentration of the pharmaceuticals.

Keywords: Non-steroidal anti-inflammatory drugs (NSAIDs); Gemfibrozil; Rainbow trout; Plasma; Risk assessment; GC–MS

1. Introduction

Residues of human pharmaceuticals from treated sewage discharges have been widely detected in surface waters (Heberer, 2002; Kummerer, 2004), raising concerns about their potential for environmental effects (Fent et al., 2006). While only present in surface waters at trace concentrations, typically 0.001–1 μg/l (Heberer, 2002; Fent et al., 2006), continuous discharges may lead to life-long exposures of aquatic organisms in affected areas. Due to the conservative nature of physiological processes, many aquatic species at higher trophic levels, e.g. fish and amphibians, also possess similar target molecules that the drugs were designed to interact with in humans (Huggett et al., 2003; Fent et al., 2006). Thus pharmacological interactions in non-target species are possible, potentially leading to adverse effects. This has been demonstrated for synthetic oral contraceptives, which are capable of affecting the reproduction of fish downstream from sewage treatment plants (STPs), based on different lines of evidence (Purdom et al., 1994; Desbrow et al., 1998; Larsson et al., 1999; Jobling et al., 2002; Kidd et al., 2007) and perhaps more dramatically, the widespread death of vultures in Pakistan from analgesics fed to livestock (Oaks et al., 2004).

The need to assess the chronic effects of pharmaceuticals in the environment has been recognised (Williams, 2005; EMEA, 2006) and is now required for registration of new medicinal products in the EU. However, there is still a need to prioritise research efforts to assess risks posed by the multitude of products already in use (1200 active pharmaceutical ingredients in Sweden alone (Carlsson et al., 2006)). Unfortunately, relevant chronic effects data for pharmaceuticals in aquatic organisms are sorely lacking, and before these data are available it is necessary to estimate chronic effects endpoints based on existing data. This may be done by applying scaling factors to acute tox-

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icity data (Hutchinson et al., 2003; Fent et al., 2006), however, this approach can be problematic and underestimate potential effects (Sumpter and Johnson, 2005; Fent et al., 2006). Fortunately, for pharmaceuticals we already have a considerable knowledge about their potency through the efficacy and safety testing of the drugs on humans and other mammals. The Fish Plasma Model (FPM) was proposed to make use of these data for the environmental risk assessment (ERA) of pharmaceuticals to fish (Huggett et al., 2003, 2004). The model's principle is that a certain plasma concentration of a pharmaceutical is required to affect the target (receptor, enzyme etc.) in humans, therefore approximately the same level would be required to affect another species sharing the same target. Using the surface water concentration and chemical properties of a drug, the model calculates the fish steady-state plasma concentration ($F_{SS}PC$). The concentration known to give therapeutic effects in humans (H_TPC) is then compared to $F_{SS}PC$ to give the Effect Ratio (ER), i.e. $ER = H_TPC/F_{SS}PC$, which provides an estimate of the probability of pharmacological effects occurring in fish. At low ER values, the drug concentration in the fish plasma is close to the level in human plasma required to give a therapeutic effect, thus receptor mediated responses in fish are likely provided the target is conserved. At high ER values, the risk of target interactions are smaller.

The FPM requires evaluation on several levels prior to any widespread use to assess the risks posed by pharmaceutical residues in the environment and/or as a means to prioritise research into their effects. The first stage involves examination of how well the pharmaceutical concentration in fish blood can be theoretically modelled for a given water concentration (the focus of this paper). The second level of evaluation involves determining if the H_TPC giving known effects in humans is a good marker for the onset of pharmacological effects in fish. Finally, an understanding of the relationship between pharmacological effects in fish versus established harmful effects, such as decreased growth, survival or reproduction, must be gained.

Obviously, it is critical that the first step, i.e. calculating pharmaceutical bioconcentration in to fish blood, be as accurate as possible for the entire FPM to function effectively. We are concerned that using a too simplistic approach, such as solely using the uptake of pharmaceuticals by fish from clean water in aquaria (Schwaiger et al., 2004; Triebskorn et al., 2004; Mimeault et al., 2005), may inaccurately assess the risks to aquatic organisms posed by pharmaceuticals in real-world environmental exposures. Environmental exposures are notably more complex, involving variations in exposure concentrations with time, mixtures of pharmaceuticals and the presence of suspended solids, colloids and surfactants from the sewage effluents. Thus field exposure studies are necessary to fully assess the magnitude of pharmaceutical bioconcentration in fish, particularly as bioconcentration may be higher at the low concentrations found in the field and decrease as the higher levels typically used in aquarium studies are approached (Schwaiger et al., 2004; Mimeault et al., 2005). Additionally, the bioavailability of a drug may be significantly less than 100% due to adsorption to particles and colloidal dissolved organic matter present in environmental waters. Many of the more water insoluble pharmaceuticals, e.g. gemfibrozil and 17α-ethinylestradiol, adsorb to particulate matter in sewage (Ternes et al., 2004), a factor determining the efficacy of their removal in sewage treatment plants (STPs). Once discharged to surface waters it is highly likely that the residual drugs will also undergo partitioning to some extent. Partitioning between the particulate, colloidal and truly dissolved fractions influences the uptake of other contaminant groups and moderates their toxicity to aquatic organisms (Haitzer et al., 1998). For fish, the binding of chemicals to colloids may restrict their transport across the gills to just the truly dissolved species (Erickson and McKim, 1990). Estrogens can partition to river colloids (Liu et al., 2005; Zhou et al., 2007), however data for non-hormonal pharmaceuticals and effects on bioconcentration are lacking.

A prerequisite for evaluating the model with actual sewage is that the pharmaceuticals chosen are readily measurable in the effluent and that they bioconcentrate sufficiently to be quantified in the fish blood plasma. Pharmaceuticals with $\log K_{ow}$ 3–5 should be sufficiently water soluble to be present in the dissolved phase of the effluent (rather than being removed with the sewage sludge) while being lipophilic enough to bioconcentrate. The family of non-steroidal anti-inflammatory drugs (NSAIDs) fits these criteria and may be useful in testing the usefulness of the FPM approach. They are used in large volumes worldwide and are present in treated sewage effluents at significant concentrations (typically 0.1–2 μg/l) (Fent et al., 2006). Additionally, their uptake into laboratory fish has been demonstrated and some chronic toxicity data collected. For example, at environmentally relevant concentrations (lowest observable effect concentration of 1 μg/l), diclofenac accumulation caused histopathological and cytopathological alterations in kidneys, liver and gills in rainbow trout (*Oncorhynchus mykiss*) (Schwaiger et al., 2004; Triebskorn et al., 2004) and brown trout (*Salmo trutta*) (Hoeger et al., 2005). Ibuprofen was shown to be able to disrupt the heat shock response in rainbow trout at concentrations as low as 1 μg/l (Gravel and Vijayan, 2007). Gemfibrozil, a blood lipid-regulating agent, may also be useful in testing the model. In aquaria, uptake across the gills of goldfish (*Carassius auratus*) resulted in significant reductions in plasma testosterone at a concentration of 1.5 μg/l (Mimeault et al., 2005).

In this paper we demonstrate the uptake of NSAIDs and gemfibrozil from treated sewage effluents into fish blood plasma. We also investigate how well a previously proposed model for bioconcentration can predict the plasma concentrations of these pharmaceuticals in fish exposed to three different sewage effluents. The risks of a target interaction within the fish are then evaluated based on a comparison with human therapeutic concentrations.

2. Materials and methods

2.1. Fish

Juvenile rainbow trout of both sexes (weighing approximately 70 g) were obtained from Antens fiskodling AB, Sweden. As per standard practice, and to allow the collection of bile for other studies (Pettersson et al., 2006; Samuelsson et al., 2006), the fish were not fed throughout the experiments. Experiments were carried out according to animal ethics permit no. 295-2004 to D.G.J. Larsson. At the end of the exposure the fish were stunned by a blow to the head and