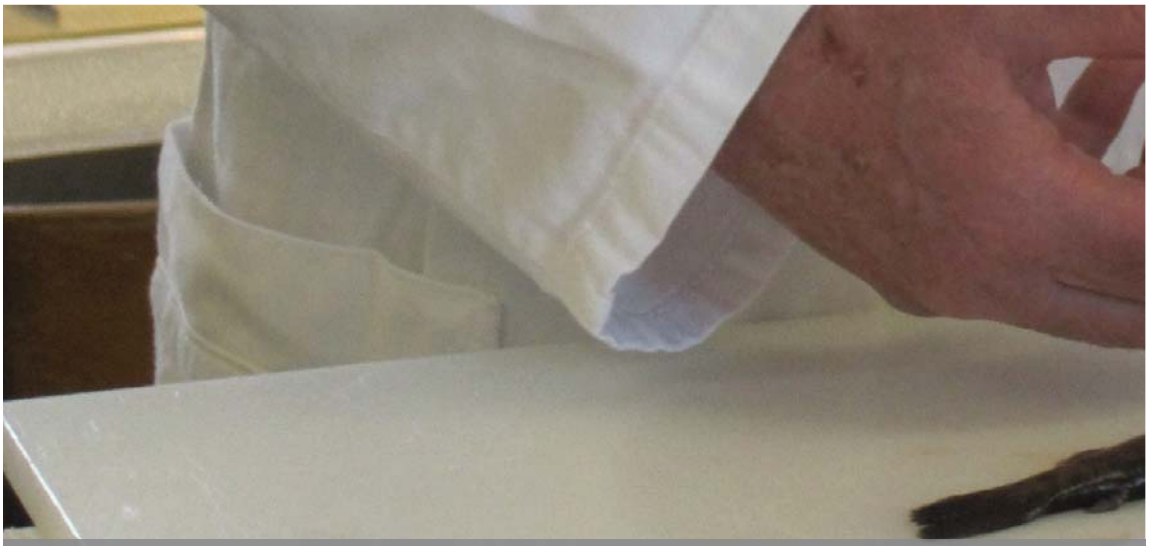

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Bioconcentration of Pharmaceuticals

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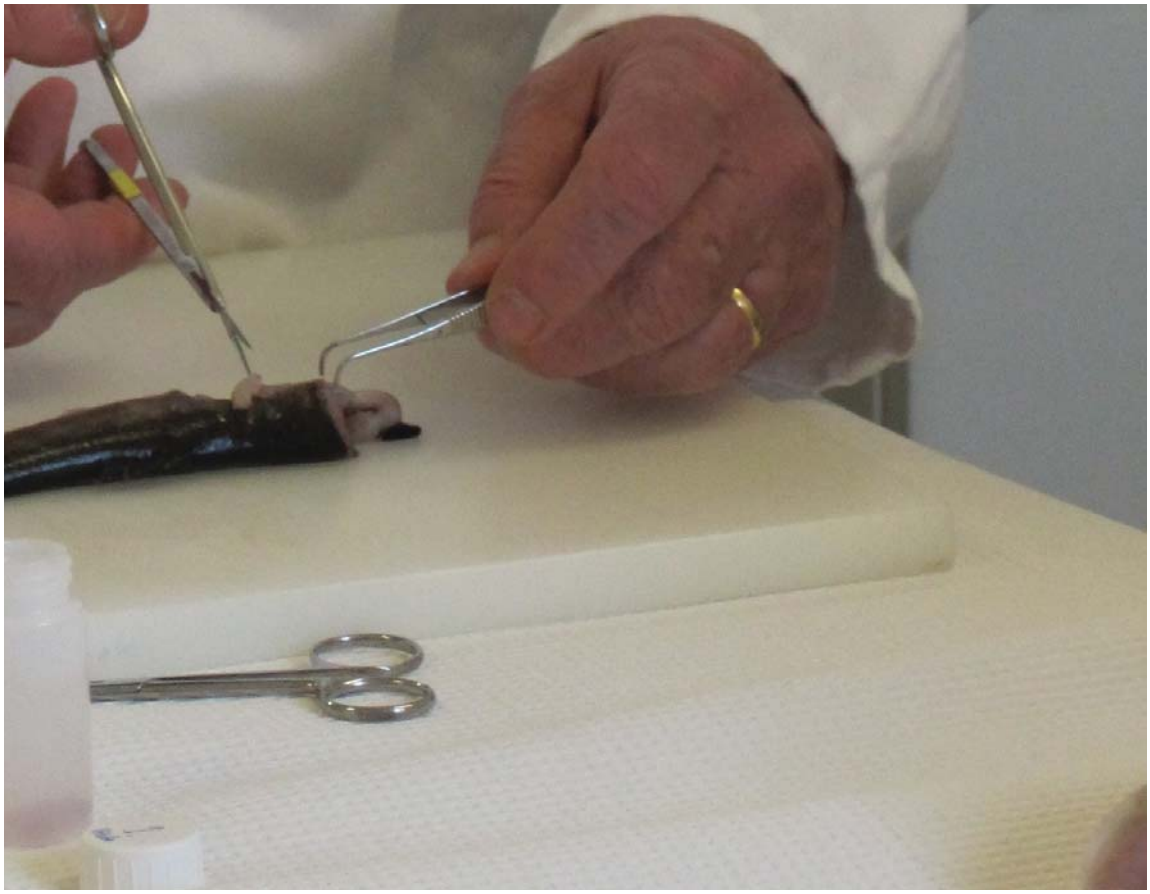
Residues of human pharmaceuticals have been widely detected in various parts of the environment and trace concentrations are often found in sewage effluent and surface waters, typically ranging from low ng L^{-1} to low µg L^{-1} levels (Lindberg *et al.*, 2005; Nikolaou *et al.*, 2007; Loos *et al.*, 2009). These concentrations, however, are orders of magnitude below the therapeutic concentrations reached in human blood plasma. Thus, the potential for a physiological impact of pharmaceuticals on water-living organisms (such as fish) have been questioned. On the other hand, the levels measured in surface waters do not simply mirror the levels encountered by the receptors or enzymes present inside the fish living in these waters. Indeed, levels of pharmaceutical in for example fish blood plasma is sometimes much higher than the levels in the surrounding water. This can be explained by the concepts of bioconcentration and bioaccumulation.



What is bioconcentration?

Bioconcentration is a process where the level of a chemical in an aquatic organism increases by uptake from the water, eventually reaching a stable concentration higher than that of the surrounding water. Bioconcentration is often presented as a bioconcentration factor (BCF), which is the concentration of the studied chemical in the entire body or in a tissue per concentration of the chemical in water (reported as L/kg). This physical property characterizes the accumulation of pollutants through chemical partitioning from the aqueous phase into an organic phase, such as the gill of a fish. Bioconcentration values are typically derived from controlled laboratory conditions, where the chemical is absorbed from the water via the gills and/or the skin.

Bioaccumulation is a similar term which is defined as the process where the

chemical concentrations build up inside an organism regardless of exposure route, i.e. dietary absorption, transport across the respiratory surface, dermal absorption etc. Thus, bioconcentration differs from bioaccumulation because the former refers to the uptake of substances into the organism from water alone; bioaccumulation is therefore the more general term because it includes all means of uptake into the organism.

What are the processes behind bioconcentration?

Bioconcentration is often described as a physico-chemical process that is more or less correlated to the octanol-water partition coefficient (K_{OW}) of the substance. Several equations have been published that describe this relation, most often with the general formula, $\log BCF = A \times \log K_{OW} - B$ (Mackay 1982; Fitzsimmons *et al*., 2001). This formula describes how chemical compounds, especially those with a hydrophobic component, partition into the lipids and lipid membranes of organisms. The concept assumes steady-state conditions, i.e. a situation when the organism is exposed for a sufficient length of time to allow uptake and excretion/metabolism to approach equilibrium. Thus, at this steady-state condition, the levels in the organisms do not change substantially. The models also more or less assumes that the chemicals are neutral, as charged molecules would have a much more restricted access to the lipid membranes of organisms. However, for some chemicals, uptake rates have been shown to remain high even after substantial ionization. Studies of water pH impact on chemical uptake for weak acids showed that the uptake rates varied little from pH 6.3 to 8.4, despite the fact that the ionization of the acids ranged from less than 1 to greater than 99.9% (Erickson *et al*., 2006). This could be explained mainly by two mechanisms, viz. reduced pH at the gill surface and that the ionized molecules contribute to the uptake by maintaining high gradients of neutral molecules across membranes (Erickson *et al*., 2006).

Which pharmaceuticals are found in fish exposed to sewage effluents?

So far, there are very few peer-reviewed reports on the levels of pharmaceuticals in fish exposed to effluent-dominated surface water. From the limited number of available studies it has been shown that more than twenty pharmaceuticals from a wide range of therapeutic classes are present in fish, including non steroidal anti-inflammatory drugs, drugs targeting the central nervous system including selective serotonin reuptake inhibitors (SSRIs), as well as steroids. *Table 1* summarizes some reported levels in fish plasma and fish tissue.

Table 1. Pharmaceuticals detected in fish exposed to sewage effluent or effluent dominated surface water.

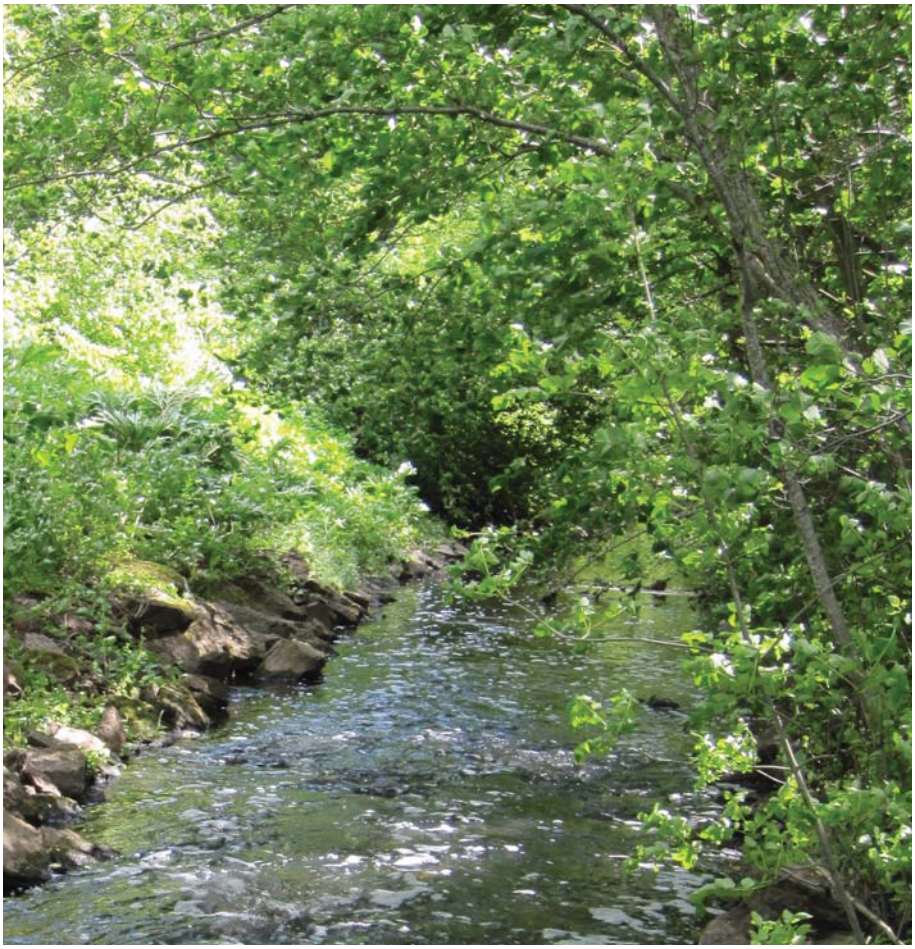
Pharmaceutical	Detected levels ng/g	Detected levels ng/ml	Sample type	Env	References
carbamazepine		0.3-1.0	blood plasma	effluent	(Fick *et al*., 2010)
carbamazepine	2.3-8.0		muscle, liver	river	(Ramirez *et al*., 2009)
carbamazepine	0.83-1.4		muscle	river	(Ramirez *et al*., 2009)
carbamazepine	0.69-16		muscle, brain	river	(Brooks *et al*., 2005)
cilazapril		0.1-0.7	blood plasma	effluent	(Fick *et al*., 2010)
diclofenac		2.2-20	blood plasma	effluent	(Fick *et al*., 2010)
diclofenac		12	blood plasma	effluent	(Brown *et al*., 2007)
diltiazem		0.9	blood plasma	effluent	(Fick *et al*. 2010)
diltiazem	0.13-0.9		muscle, liver	river	(Ramirez *et al*., 2009)
diltiazem	0.11-0.27		muscle	river	(Ramirez *et al*., 2009)
diphenhydramine	1.2-11		muscle, liver	river	(Ramirez *et al*., 2009)
diphenhydramine	0.66-1.3		muscle	river	(Ramirez *et al*., 2009)
ethinylestradiol		1200	bile	river	(Larsson *et al*., 1999)
fluoxetine	19-80		muscle, liver	river	(Ramirez *et al*., 2009)
fluoxetine	0.11-1.6		muscle, brain	river	(Brooks *et al*., 2005)
gemfibrozil	27-90		muscle, liver	river	(Ramirez *et al*., 2009)
gemfibrozil		210	blood plasma	effluent	(Brown *et al*., 2007)
haloperidol		1.2	blood plasma	effluent	(Fick *et al*., 2010)
ibuprofen		5.5-102	blood plasma	effluent	(Fick *et al*., 2010)
ibuprofen		84	blood plasma	effluent	(Brown *et al*., 2007)
ketoprofen		15-107	blood plasma	effluent	(Fick *et al*., 2010)
levonorgestrel		8.5-12	blood plasma	effluent	(Fick *et al*., 2010)
meclozine		0.1-0.7	blood plasma	effluent	(Fick *et al*., 2010)
memantine		2.3	blood plasma	effluent	(Fick *et al*., 2010)
naproxen		33-46	blood plasma	effluent	(Fick *et al*., 2010)
naproxen		14	blood plasma	effluent	(Brown *et al*., 2007)
norfluoxetine	3.2-130		muscle, liver	river	(Ramirez *et al*., 2009)
norfluoxetine	3.5-5.1		muscle	river	(Ramirez *et al*., 2007)
norfluoxetine	1.1-10.3		muscle, brain	river	(Brooks *et al*., 2005)
orphenadrine		0.9	blood plasma	effluent	(Fick *et al*., 2010)
oxazepam		0.2-0.7	blood plasma	effluent	(Fick *et al*., 2010)
risperidone		0.2-2.4	blood plasma	effluent	(Fick *et al*., 2010)
sertraline		1.1-1.2	blood plasma	effluent	(Fick *et al*., 2010)
sertraline	5.0-545		muscle, liver	river	(Ramirez *et al*., 2009)
sertraline	0.34 - 4.3		muscle, brain	river	(Brooks *et al*., 2005)
tramadol		1.1-1.9	blood plasma	effluent	(Fick *et al*., 2010)
verapamil		0.7	blood plasma	effluent	(Fick *et al*., 2010)

Several studies have been conducted in the US where pharmaceutical residues have been measured in tissues of fish from effluent-dominated rivers (Brooks *et al*., 2005; Ramirez *et al*., 2007, Ramirez *et al*., 2009). So far these investigations have indicated that the fish is safe for human consumption but the ecological implications for the fish remains to be studied further.

In a study performed within the MistraPharma research programme, the bioconcentration of 25 pharmaceuticals were investigated in rainbow trout exposed to treated effluent from three Swedish sewage treatment plants (Fick *et al.,* 2010). Out of the 25 selected pharmaceuticals, 17 were detected in fish plasma. One of the pharmaceuticals, the synthetic progestin levonorgestrel, was detected in fish plasma at levels that even exceeded the human therapeutic plasma concentration. Zeilinger *et al.* (2009) recently showed that exposure to as little as 0.8 ng / L levonorgestrel, the lowest concentration tested, resulted in strongly impaired reproduction of fish. In accordance, the study by Fick *et al*. showed that an effluent concentration of 1 ng/L resulted in a highly potent plasma concentrations in exposed rainbow trout. The MistraPharma study is the first study showing that fish exposed to sewage effluents can bioconcentrate pharmaceuticals to plasma levels equal to, or even exceeding, the human therapeutic plasma concentration. This suggests that certain pharmaceuticals could cause pharmacological effects on fish living in effluent-dominated surface waters. This study also shows that several pharmaceuticals can bioconcentrate quite significantly, as the levels found in fish plasma were up to 12000 times that of the water concentration (Fick *et al.,* 2010).

Can BCF be used in prioritization?

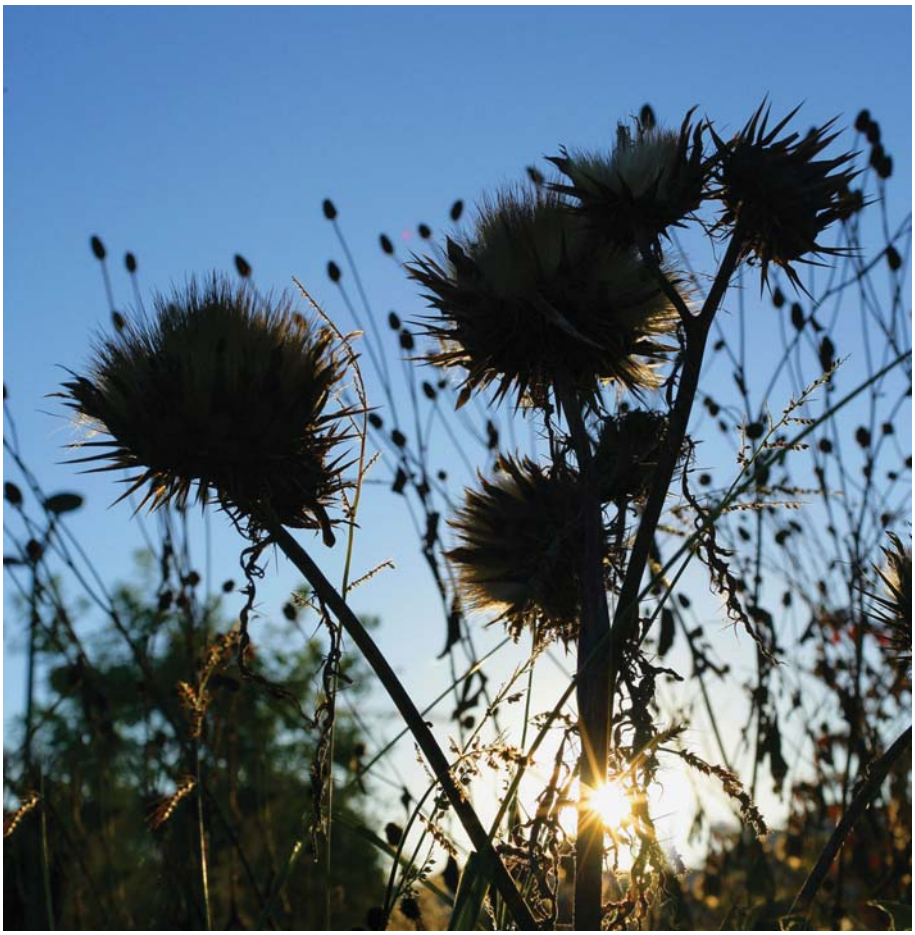
As there is a very large number of pharmaceuticals, a major challenge is how to prioritize research efforts to assess the potential risks associated with their usage. There is a need to develop novel test strategies, which has been recognized both by industry, authorities and academia (Huggett *et al*., 2003; Besse and Garric 2008; Gunnarsson *et al*., 2008; Brooks *et al*., 2009). To what extent chemicals bioconcentrate and bioaccumulate can be used directly as a tool to prioritize chemicals and this is e.g. one of the criteria used for the environmental risk assessment within REACH, the European chemical legislation (ECHA 2008). Bioconcentration studies or estimates present information on the dose that aquatic species are exposed to, which is very useful since we already have a considerable knowledge about the potency of pharma-ceuticals, at least in mammals, through their efficacy and safety testing. One option would therefore be to use existing mammalian data to assess the likelihood for a pharmacological effect in other species. It may sound



strange to compare fish and humans but due to the conservative nature of physiological processes, many aquatic species and particularly fish and amphibians, possess similar target molecules to those the drugs were intended to interact with in humans (Gunnarsson *et al*. 2008). This similarity implies that if the plasma level in fish is high enough, a similar pharmacological response could occur as in the intended target species, i.e. humans. The best available example of this are the effects of ethinylestradiol, a synthetic estrogen present in many birth control pills, on sexual differentiation and fertility of fish living downstream from sewage treatment plants (Larsson *et al*., 1999; Lange *et al*., 2009).

What is the fish-plasma-model?

Huggett *et al.* (2003) presented a simplistic approach to predict the likelihood for pharmacological interactions in aquatic species, based on a screening-level model to predict bioconcentration followed by a comparison with human therapeutic plasma concentrations. This approach is referred to as the "the fish plasma model" (Huggett *et al.,* 2003). It assumes that two species sharing the same drug targets, i.e. receptors and enzymes etc, will require about the same plasma concentrations of a pharmaceutical to activate a pharmacological response. This approach makes it possible to generate an index of the likelihood that a fish is pharmacologically affected by a drug in the water. Huggett *et al.,* (2003) referred to this index as an "effect ratio",



whereas we have proposed the term "concentration ratio" as the index really is a ratio of two concentrations. The concentration ratio compares the blood plasma levels in humans taking a specific pharmaceutical (i.e. the human therapeutic plasma concentrations (H_TPC)), with measured or predicted steady state levels in fish blood plasma ($F_{SS}PC$; see *Equation 1*). Given that the target molecule(s) in the fish has roughly similar affinities to the drug as the human target(s) have, this concentration ratio will reflect the risk for a pharmacological response to develop in fish. If the concentration ratio is ≤ 1 then the concentration in the exposed fish is higher or equal to the known concentration that gives a pharmacological response in humans, i.e. the lower the ratio, the higher the risk for the fish.

$$CR = \frac{H_TPC}{F_{ss}PC}$$

Equation 1. CR = concentration ratio, H_TPC = Human therapeutic plasma concentration, $F_{SS}PC$ = Fish steady state plasma concentration.

One of the advantages with this approach is that the H_TPC is readily available in the literature for most pharmaceuticals. Since studies measuring plasma levels of pharmaceuticals in fish subsequent to exposure via water are scarce it is necessary, in most cases, to predict the plasma levels in fish using one of the several equations that are available to calculate the bioconcentration. Even though these equations are made for neutral compounds and describe the partition into the lipid membranes of organisms, it seems to be able to predict fish plasma levels of pharmaceuticals with relatively good accuracy (Brown *et al*., 2007; Fick *et al*., 2010). It should be stressed that even if a fish has the same plasma levels as a human using a pharmaceutical, this will only indicate the probability of a pharmacological response to develop, not whether this response is adverse or not.

We propose that one possible way forward for identifying drugs of environmental concern is to rank them based on their estimated concentration ratios. Concentration ratios could be derived in different ways. The most accurate, but also the slowest approach, is to derive concentration ratios from actual measurements of blood plasma levels in fish as in the studies by Brown *et al.* (2007) and Fick *et al.* (2010). Another alternative is to derive concentration ratios from *estimates* of blood plasma levels, which in turn are based on *measured* surface water levels of drugs. This approach will allow a more rapid screening of many drugs, but it also involves assumptions about how

well different drugs bioconcentrate (Fick *et al*., 2010). The most rapid approach, but also the approached with the most uncertainties, would be to base the calculation on *estimated* surface water levels, where for example usage, excretion and estimated removal efficiencies in sewage treatment plants could be taken into account. Although all of these strategies admittedly involve assumptions about conserved modes of action of drugs between fish and humans, it provides a possibility to apply a scientific basis to rank a large number of drugs prior to performing extensive biological tests with fish. Our strategy in MistraPharma to expose fish to effluents, screen for drugs in their blood plasma and compare measured levels with human therapeutic levels led to the identification of levonorgestrel as a drug of high environmental concern. The recent findings that similar water levels of levonorgestrel impairs reproduction in fish (Zeilinger *et al*., 2009) suggest that our strategy could be a fruitful and. new multi-residue analytical techniques (LC/LC-MS/MS) including more than 120 different pharmaceuticals have been developed and validated within the MistraPharma programme which will allow an expansion to a wider set of pharmaceuticals.

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