How Does the Pill Affect Fish?

Chemicals with estrogenic effects – so-called environmental hormones – are believed to be responsible for the promotion of female traits in male fish. As part of the EU program COMPREHEND, EAWAG has investigated the effects of wastewater treatment plant effluents on male rainbow trout and found increased levels of vitellogenin. This egg yolk protein precursor is normally found in high concentrations in female fish only. Effluent samples from wastewater treatment plants, which were taken during the fish exposure, underwent chemical ultra-trace analysis and *in vitro* testing for estrogenic activity. In some samples, elevated hormone concentrations could be detected and estrogenic activity could be confirmed.

Environmental chemicals that interfere with hormonal systems of humans or animals are called environmental hormones. Of primary interest are the so-called estrogens that mimic female sex hormones. These include:

- the natural estrogen estradiol and its transformation products, estron and estriol;
- synthetic estrogens, such as ethinylestradiol, the active ingredient commonly found in oral contraceptives;
- high production volume manufactured chemicals that are for instance used in industrial detergents (alkylphenol polyethoxylates and their degradation products) and in plastics (e.g., bisphenol A).

Many of these hormonally active chemicals can be detected in aquatic systems. Natural and synthetic estrogens, for example, are excreted by humans and transported to wastewater treatment plants where they are eliminated to some extent but also partially washed out into surface waters.

Mechanism of Action of Environmental Hormones

In fish, indigenous production of estradiol induces synthesis of the protein vitellogenin in the liver, the precursor of egg yolk proteins that is transported via the blood stream to the oocytes in the ovaries. It is normally only found in large quantities in the blood of sexually mature females. This is why high vitellogenin concentrations discovered in male fish in the UK in the mid-1990s caused some alarm. Fish exhibiting elevated vitellogenin concentrations were from stream sections below wastewater treatment plants; vitellogenin production was attributed to the presence of estrogen-like compounds in the treatment plant effluent. In addition, it was shown that male fish in contaminated waters showed a significant higher incidence of testicles containing female egg cells, a phenomenon called "intersex". This has recently also been observed to occur in whitefish in Lake Thun.

We can only speculate on the effect of estrogen-like chemicals in humans. It has not yet been demonstrated that postulated reductions in sperm count and sperm quality and increases in testicular cancer are linked to the increased occurrence of hormonally active chemicals in the environment.

Over 500 Potential Hormonally Active Chemicals

Over the last few years, high priority programs have been initiated on an international level to identify potential hormonally active chemicals from among the roughly 80,000 chemicals that are currently in use. The EU has recently published a list of 553 chemicals, plus 9 natural and synthetic steroid hormones, that are "suspected to interfere with hormonal systems in humans and animals living in the wild" [1]. Simultaneously, national and international projects, in which EAWAG is a participant, are examining the occurrence of environmental hormones in surface waters and determining their effect on aquatic organisms. National research programs include the National Research Program "NRP 50 – Endocrine Disruptors", started in 2001, and the network project "Fish Decline in Switzerland", which among others examines whether or not hormonally active chemicals are responsible for the observed fish decline in Swiss surface waters. Results presented in this article were obtained under the EU project COMPREHEND (**COM**munity **P**rogram of **R**esearch on **E**nvironmental **H**ormones and **EN**docrine **D**isrupters) that was completed by the end of 2001. The primary goals of COMPREHEND were to examine the presence of hormonally active chemicals in effluents of industrial and communal wastewater treatment plants all over Europe and to develop new detection methods.

Elevated Vitellogenin Concentrations also in Swiss Fish

For a period of two weeks, male rainbow trout were exposed to the effluent of the wastewater treatment plant (WWTP) Rontal in Canton Lucerne. Control fish were held for the same period of time in the stream above the point where the effluent is discharged into the river and in the laboratory. Fish were not fed during their exposure. Figure 1 shows that in control fish, vitellogenin concentrations decreased over the duration of the experiment. This is possibly due to the fish having been fed fish food containing hormonally active chemicals during the acclimatization phase. Fish exposed to the effluent of the WWTP, on the other hand, showed increased levels of vitellogenin, indicating the presence of hormonally active compounds.

Combined Chemical and Biological Analysis

Concurrent with the fish exposure experiments, water samples were taken from the effluent of the WWTP Rontal and examined for the presence of hormonally active chemicals, combining two different analytical tools. Chemical ultra-trace analysis was used to determine concentrations of known environmental hormones in the water samples. Estrogenic activity in the water samples was examined using a biological test system, in this case, a yeast estrogen screen containing a human estrogen receptor and a reporter gene. If hormonally active chemicals are present, they bind to the receptor, activate the reporter gene and can subsequently be detected by a biochemical color reaction. The intensity of the color is a

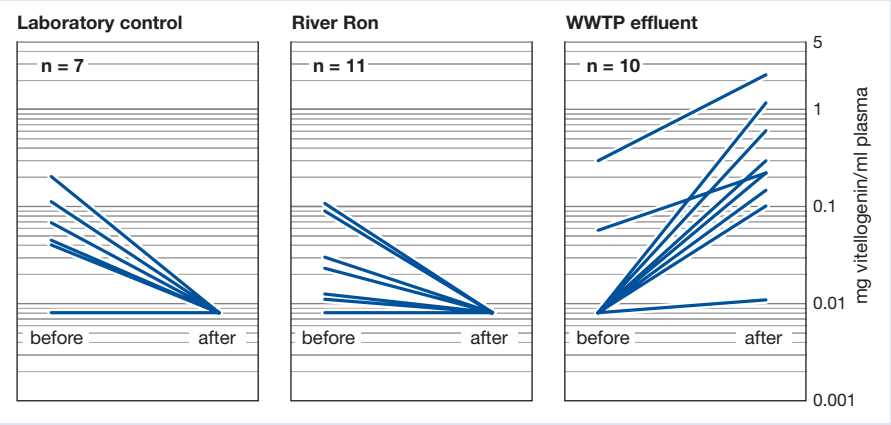

Fig. 1: Vitellogenin induction in individual male rainbow trout (n = number of male fish).

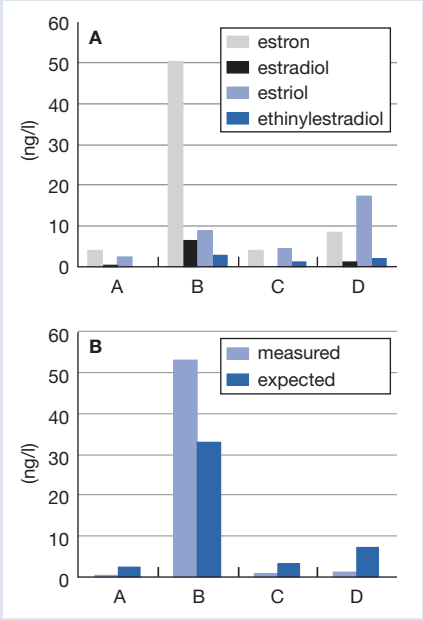

Fig. 2: Analyses of four water samples from the effluent of the WWTP Rontal
(A) Concentrations of natural and synthetic steroid hormones.
(B) Expected and measured estrogenic activities expressed as estradiol equivalents.

measure of the estrogenic activity of the sample and is expressed in estradiol equivalents. Either one of these two methods on its own is insufficient for characterizing a water sample; the combination of the two however, gives us information on actual concentrations of environmental hormones and on the estrogenic activity of a given sample, resulting in a number of advantages:

- a lower probability of false negative results, due to inhibition of the yeast screen;
- identification of water samples in which individual hormonally active components are present below the minimum effect concentration, but show estrogenic activity in combination [2];
- the possibility of identifying unknown environmental hormones in hormonally active samples.

Expected and Measured Concentrations in WWTP Effluent

Assuming a ratio of 1:1 between men and women and that 60% of the women are menstruating and 0.8% are pregnant, we can calculate an average excretion of 3.1 µg estradiol per day and person (including men) [3]. Calculating the estradiol discharge for the population served by the WWTP Rontal, and assuming that 50% of steroid hormones are eliminated in the treatment process, we would expect an average estradiol concentration of 1.6 ng/l in the WWTP effluent. This value compares well with the measured average concentration of 2.0 ng/l estradiol as determined by chemical analysis (Fig. 2A). A similar result was found for ethinylestradiol, where the measured concentration of 1.5 ng/l is in good agreement with the expected concentration of 3 ng/l (Fig. 2A). It should be noted that sample B showed exceptionally high concentrations of estradiol and estron.

Expected and Measured Hormonal Activities in WWTP Effluent

Based on chemically determined concentrations of hormonally active compounds, the overall estimated estrogenic activity of a water sample (expressed in estradiol equivalents) may be calculated using the relative hormone activities of the individual compounds, as determined with the yeast estrogen screen (Fig. 2B). Assigning estradiol a reference activity of 1, the degradation products estron and estriol show relative hormonal activities of 0.474 and 0.003, respectively, while the synthetic steroid hormone ethinylestradiol shows the same activity as estradiol. Estrogenic activities of industrial chemicals, on the other hand, are typically lower by several orders of magnitude. Since they can be present in far higher concentrations than natural and synthetic estrogens, however, they cannot be neglected. Nonylphenol, for example, has a hormonal activity roughly 40,000 times lower than estradiol, but is present in the effluent of the WWTP Rontal at concentrations that are 1000 times higher than estradiol, resulting in an estrogenic activity of 0.04 ng/l.

Estrogenic activities measured by the yeast estrogen screen generally agree well with the calculated activities. Solely in sample B, the calculated estrogenic activity is lower, accounting only for 60% of the measured activity (Fig. 2B). This is strong indication that there are other, possibly unknown, chemicals present that exhibit hormonal activity. They need to be identified using chemical analytical methods.

Additional research in this area is essential in order to gain a more complete picture of the complex problem of endocrine disruption. Beyond identifying hormonally active compounds and determining their concentrations, it will be important to investigate the effect of environmental hormones and mixtures thereof on populations, aquatic communities and entire ecosystems.

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[1] KOM (2001) 262; to be found under: http://europa.eu.int/eurlex/de/com/cnc/2001/com2001_0262de01.pdf
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[3] Johnson A.C., Williams R.J., Ulahannan T. (1999): Comment on "Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening" Environmental Science &. Technology *33,* 369–370.