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Endocrine Disrupting Chemicals in the Environment

Summary

Xenoestrogens, or endocrine disrupting chemicals (EDCs) are defined as substances that disturb the hormonal balance and can thus have deleterious effects in humans or animals, or their offspring. Foremost here are the sex hormones and substances with analogous effects, since these are closely linked to reproductive and developmental abnormalities observed in humans and animals.

Most recently, the question has been intensively discussed as to what level of EDCs can interfere with the endocrine homeostasis of humans and animals, and what effects on individuals or populations may result. Switzerland is not alone in being confronted with these questions. Other countries and the EU have also identified considerable research gaps which need to be filled through specific research efforts. The chemical industry has also begun larger-scale research programmes.

The present report describes the situation in Switzerland and contains a review of current knowledge, our assessment of current pollution levels as far as available data allow, and an indication of gaps in knowledge which need to be filled in order to make a more precise assessment of the situation.

State of knowledge

Hormones, as the body's own chemical messengers between tissues and cells, regulate a multiplicity of processes. Female and male sex hormones (estrogens and androgens) play decisive roles in reproduction and the development of an organism. If a foreign chemical has the same effects as the body's own estrogens or androgens, it is defined as having estrogenic or androgenic activity. If a chemical inhibits the effects of the body's own sex hormones, however, its effect is described as anti-estrogenic or anti-androgenic.

The timing of the effect of a hormone or an endocrine disrupter can be decisive. During early development, the development of the sex organs and specific centres in the brain are controlled by sex hormones. Disruption by EDCs can then lead to permanent defects or dysfunction of organs. In a mature organism, the hormones stimulate or inhibit organ functions. They are also involved in the regulation of sperm formation and the female cycle. These effects are reversible, and cease as soon as the disrupter is no longer present.

In general, an organism is exposed not to single environmental chemicals but to many. In the case of endocrine disturbances, it is still not clear how these mixtures work. It is

assumed that chemical disturbances that occur through the same mechanisms are additive. Intensifying (synergistic) or inhibiting (antagonistic) effects however cannot be ruled out.

Methods of determining the endocrine effects of a chemical

In vivo methods allow the endocrine effects of substances in the intact organism to be determined. In vitro methods are used to analyse effects at the level of cells and molecules. They are suitable for a first rough evaluation of chemicals (screening, see chapter 2.2).

Most **in vivo assays** are carried out on mammals. For the determination of estrogenic or androgenic action, the effects on the genital tract of rats or mice are frequently used. Mostly, the increase in weight of the womb (uterus) caused by estrogens is measured. Anti-estrogenic and anti-androgenic effects are detected through the inhibition of effects normally induced by estrogens or androgens. In addition to these tests aimed specifically at sex hormones, various (standardised) tests, such as reproductive or developmental studies, are carried out. The latter can indicate the presence of endocrine disruption, since hormones (above all, sex hormones) are particularly significant for reproduction and development, and thus any disturbance caused by chemicals that mimic sex hormones may be recognised. With conventional techniques, it is however difficult to distinguish between an endocrine disturbance and other toxic effects (e.g. toxins that disrupt the hormonal balance secondarily).

In egg-laying vertebrates (birds, fish, reptiles and amphibians) an estrogenic effect can be determined by the presence of the egg yolk protein precursor vitellogenin. Vitellogenin is normally produced only by sexually mature (i.e. adult) female animals under the control of estradiol. It is produced by the liver, secreted into the bloodstream, and taken up by the egg cells. Chemicals with estrogenic effects therefore induce vitellogenin in sexually immature females or in males. An anti-estrogenic effect is characterised by the inhibition of estradiol-induced vitellogenin formation. The induction of vitellogenin synthesis is currently the most commonly used assay in fish and amphibians. Hormonal status, influence of endocrine homeostasis, and gonad histology are additional parameters.

In vitro methods involve cells, cell extracts and reconstituted cell systems. The affinity of chemicals to hormone receptors is determined via receptor binding studies. Binding to the appropriate receptor is required for the receptor-mediated hormonal effect of a substance. If a substance binds to hormone receptors, the receptor can also be blocked, the body's own hormone can be prevented from binding to it, leading to an anti-estrogenic or anti-androgenic effect. Non receptor-mediated chemical interactions cannot be detected in this way.

Some estrogen-sensitive cell lines react to estrogenically effective chemicals with accelerated cell proliferation. This phenomenon can be exploited, for example in the E-screen assay using human breast cancer cells MCF-7. Furthermore, the synthesis of particular proteins under the influence of hormones is being studied in hormone-sensitive cells. Various reporter gene systems are being developed using new molecularbiological techniques. Reporter genes are inserted into human, animal or yeast cells. They are then 'switched on' by chemicals that bind to hormone receptors. The products of the reporter genes can easily be detected. To determine estrogenic chemicals in fish, primary liver cells are usually employed. Here, as with in vivo tests in fish, the formation of the egg yolk protein precursor vitellogenin is determined.

Endocrine disrupters

Although no intensive screening for endocrine disrupters has taken place to date, some of these chemicals have already been identified (see chapter 2.3):

- Natural estrogens, such as 17β-estradiol, estrone and estriol, are effective even at very low concentrations.
- Synthetic estrogens, such as 17α-ethinylestradiol (EE_2, the estrogenic compound used in oral contraceptives) and diethylstilbestrol (DES), are very potent hormones developed for medical use.
- Phyto-and myco-estrogens, such as isoflavone and other compounds, are synthesised by the plants themselves, or by fungi that infest the plants. Their effect is estrogenic, although some anti-estrogenic effects have also been described.
- Metabolites of alkylphenolpolyethoxylates (APEO: nonylphenol, octylphenol and further breakdown products) are currently used primarily as the active substances in industrial detergents, and are also to be found as additives in paints, pesticides and other products. They act estrogenically and are of particular significance in sewage contaminated rivers.
- Various organochloropesticides, such as DDT and its breakdown products DDE, DDD, Lindane (γ-hexachlorcyclohexane, γ-HCH) and kepone (chlordecone) are also endocrine disrupters. While the isomer o,p'-DDT acts estrogenically, p,p'-DDE is anti-androgenic. Methoxychlor is metabolised by organisms into estrogenic metabolites. Lindane (γ-HCH) has effects on mammalian reproduction, although the mechanisms are unclear. Chlordecone demonstrates an estrogenic effect. The pesticides endosulfan, toxaphen and dieldrin are also possibly estrogenic.
- Certain industrial chemicals used in plastics (bisphenols, especially bisphenol A) show estrogenic effects. Phthalates have so far been shown to have estrogenic effects in vitro, but their effect on whole organisms is not clear.
- Various chlorinated and hydroxylated biphenyls (PCB, OH-PCB) that are widespread due to their minimal breakdown, show estrogenic and anti-estrogenic effects.
- Various polychlorinated dibenzo-p-dioxins and furanes show anti-estrogenic effects.
- The fungicide vinclozoline has an anti-androgenic effect.
- Organotin compounds such as tributyltin (TBT) and triphenyltin (TPT), both of which are used in antifouling paints for ships, show an androgenic effect on various marine snail species, even at the very low concentrations of a few nanogrammes per litre. The use of these antifouling paints on small boats is now usually prohibited. However,

organotin compounds are used today in antifoulings for large vessels, in agriculture (TPT, vegetable cultivation), in wood preservatives and in textile finishing.

Wildlife studies

In the following cases, endocrine disturbances have either been proved, or a connection between the observed effects and certain hormonally active chemicals is suspected (see chapter 2.4):

Studies in England and the USA show that male rainbow trout, flounder and carp in polluted areas synthesise vitellogenin. These findings are associated with alkylphenol pollution as well as contamination by natural and synthetic estrogens. Ovarian tissue has been formed in the gonads of male fish (intersex). Furthermore, in Canada, retarded development of the sex organs of male fish, and the masculinisation of female fish, have been detected downstream of pulp factories and paper mills. The phytoestrogens sitosterol and stigmasterol, found in the waste water from paper mills, are under suspicion as the possible cause.

- Female marine gastropod species from around the world display the characteristics of masculinisation (imposex) in coastal areas considerably contaminated with organotin compounds.
- In Lake Apopka (Florida), severe contamination with pesticides (Dicofol, DDT, DDE, DDD) is held responsible for the marked decrease in the alligator population, changes in the ovaries of female alligators, and abnormalities of the testes, vas deferens and penis of male animals.
- Feminisation of male seagulls has been observed in the USA, and linked with DDT or DDE contamination. Furthermore, increased eggshell breakage among birds of prey in the 1950s to 1970s has been traced back to the effects of DDT and DDE.
- Female common seals from the Waddenzee demonstrated reduced fertility if fed with local fish that were severely contaminated with DDE and PCB. A link between PCB pollution and reproductive disorders has also been shown in mink. Furthermore, the contamination of fish through PCB is thought to be an important factor in the decline of the European otter population.
- Of the few remaining Florida panthers, most show developmental and reproductive disorders. This is thought to be largely due to the high levels of contamination of the females by DDE and PCB.
- Disturbances of reproduction and damage to the ovaries and uterus in sheep have been attributed to phytoestrogens (clover species rich in isoflavonoids). Isoflavone-rich fodder additives have also led to infertility in the cheetah.

Epidemiological studies in humans

The synthetic estrogen diethylstilbestrol (DES) has produced foetal malformations and other effects. Prescribing DES to pregnant women has resulted in malformations of the genital tract of male offspring and a higher incidence of a specific kind of vaginal cancer in female offspring.

Furthermore, a drop in sperm density and quality has been observed over the last few decades in men of several different countries, with some regional variation (including countries in which there was no reduction). Various studies have showed a trend towards an increased incidence of testicular cancer. An increase in cases of undescended testicles and urethral fissures (hypospadia) has also been detected. These effects may therefore be linked to the action of endocrine disrupters on the foetus; a causal relationship, however, has not yet been proven.

The situation in Switzerland

To gather the data existing in Switzerland, a survey of 246 selected cantonal agencies, research and university institutions and organisations was carried out. The results of the survey and other information available in the literature has been summarised (see chapter 3).

Contamination of the environment with endocrine disrupters

Natural and synthetic estrogens: There are no systematic studies of the occurrence of natural or synthetic estrogens in Switzerland. Such studies are however now being initiated. The few data previously published (on sewage plant effluent, surface waters, drinking water) stem from England, Germany and Israel. Natural estrogens such as 17β-estradiol and estrone, and synthetic estrogens such as 17α-ethinylestradiol have been detected at concentrations of a few nanogrammes per litre in the effluent from waste water treatment plants. 17β-estradiol and estrone (Israel) and 17α-ethinylestradiol (England, Germany) have been found in surface waters and drinking water.

Phyto- and myco-estrogens: With the exception of a few plant steroids found in water from the Rhine, no data from Switzerland are available. In Germany, β-sitosterol has been detected in sewage plant effluent and in some rivers, streams and drinking water at low concentrations of a few nanogrammes per litre.

Alkylphenolpolyethoxylates (APEO) and their breakdown products: Since the limitation placed on the use of APEO in 1987 (prohibited in washing powders), concentrations of metabolites (especially nonylphenol) in waste water have decreased markedly. In 1997 however the compounds were still detected at concentrations of a few microgrammes per litre in effluent from water treatment plants and also in sewage sludge. Elevated values were found in samples of drainage water and flowing water receiving waste water from the textile industry.

Organochlorine pesticides: DDT and its breakdown products DDD and DDE have been found in sediments from the Rhine (at levels of a very few microgrammes per kg dry weight) and at higher concentrations in lake sediments. In Switzerland there have been numerous studies on the contamination of fish with organochlorine compounds. However, due to the use of different methodologies the results can be compared only with difficulty. High levels of pollution are found in Lago Maggiore. Fillets of the most heavily contaminated fish species show values of several mg/kg fresh weight. In Lake Geneva, the Hochrhein and southern Oberrhein, contamination of fish is decreasing. In addition to fish, birds (cormorant, great crested grebe) in Lago Maggiore also show high levels of DDT and its metabolites (in some cases over 10 mg/kg fresh weight).

γ-hexachlorcyclohexane (lindane) has been shown in individual samples of sewage sludge, in the water and sediment from the Rhine and in soil samples from forest floors. Fish and birds show lindane contamination levels of a few microgrammes per kg fresh weight. Fat-rich eels from the Rhine, however, have levels of up to 40 microgrammes/kg.

Very little is known about environmental pollution in Switzerland by other organochlorine pesticides such methoxychlor, chlordecone, endosulfan, toxaphene and dieldrin.

Bisphenol A: No Swiss studies on environmental bisphenols are known. In drinking water, as well as in the Rhine delta and in surface waters in Japan and the USA, bisphenol A has been found at concentrations of several ng/l.

Polychlorinated biphenyls (PCB): Environmental pollution through PCBs has been investigated in numerous Swiss studies. PCB has been demonstrated in suspended solids and sediments from surface waters. The PCB content of burbot and perch from Lake Geneva has been decreasing since the end of the 1970s. In the Rhine (southern Oberrhein, Hochrhein) a decrease in levels of low-chlorine PCB contamination in fish (eel, roach, barbel) was shown between 1990 and 1995. The PCB content of fish from rivers and streams varies widely. More recent studies from various Swiss waterways have given levels of up to several hundred microgrammes per kg fresh weight for river trout fillets. In addition to fish, other organisms, soils and sewage sludge have been examined for PCB. The sewage data point to a decrease in PCB input.

Phthalates: Only very few studies exist of environmental pollution by phthalates in Switzerland. Various phthalates have been studied in the course of Rhine monitoring. Most samples show traces of these compounds.

Vinclozoline: Environmental concentrations of vinclozoline have barely been researched in Switzerland at all. Within the Rhine Monitoring Programme the compound has been demonstrated in individual samples of Rhine water at low ng/l concentrations.

Organotin compounds: The sale of antifouling paints containing the organotin compounds tributyltin (TBT) and triphenyltin (TPT) has been prohibited since 1990. The results of the ban have been investigated. A reduction of concentrations in harbour water has been established. However, no clear reduction in pollution was observed in sediments or biota (mussels) in harbours and sewage sludge continue to be contaminated by organotin compounds.

Observation of wildlife

The European otter has become extinct in Switzerland. According to in-depth studies in Great Britain, a principal reason for this may be contamination of prey fish with PCB, and the associated reproductive problems.

Since the beginning of the 1980s the catch made by anglers in Swiss rivers has decreased markedly. It is assumed that catch size is associated with population size, at least for trout. At the moment, it is not possible to demonstrate a clear correlation between decrease in fish numbers and causative factors (e.g. EDCs). A study in various rivers and streams of eastern Switzerland shows organ damage in brown trout and grayling. The liver and kidneys are particularly affected. The causes of the damage remain unknown.

A drop in population has been observed in a number of bird species (peregrine falcon, hawk, sparrowhawk) since the 1950s. This has been attributed to DDT. In the 1970s the populations reached their lowest point, but have since then been increasing.

Reproductive disorders and other health problems in humans

Cancer statistics for the cantons Neuchâtel and Vaud show an increased incidence of testicular cancer. However, in the German-speaking part of Switzerland, no such change has been detected. The available data on the incidence of undescended testicles and hypospadia do not permit any conclusions to be drawn about changes over time.

The cantons of Neuchâtel and Vaud also show an increase in the incidence of breast cancer. The cause of this increase is given as improved diagnosis. The cantonal cancer registers show substantial regional variations in the incidence of breast cancer.

There are no known systematic studies of male or female reproductive disorders with respect to contamination by environmental chemicals.

Assessment of pollution

An initial assessment of pollution has been carried out, as far as the data allow, for the natural estrogens 17β-estradiol and estrone, the synthetic estrogen 17α-ethinylestradiol, breakdown products of alkylphenolpolyethoxylates, PCB and tributyltin. The data for other endocrine disrupting chemicals are insufficient (see chapter 4).

In the effluent from water treatment plants in Germany, England and Israel, the levels of natural estrogens have reached concentrations that trigger the formation of vitellogenin (an egg yolk protein precursor) in male rainbow trout. From this, we cannot rule out estrogenic effects on fish in Switzerland, at least in areas near water treatment plants.

Synthetic estrogens in the environment have not yet been investigated in Switzerland. Data from Germany and England demonstrate that the concentrations of 17α-ethinylestradiol in drains from sewage plants and in rivers can reach levels at which fish show an estrogenic effect. Analogously, estrogenic effects on fish in Switzerland cannot be excluded.

The concentrations of breakdown products of alkylphenolpolyethoxylates (nonylphenol, nonylphenolmono- and diethoxylates, nonylphenoxy acetic acid derivatives) in sewage plant effluent and rivers in Switzerland lie below the concentrations which induce vitellogenin synthesis in male rainbow trout. In the vicinity of sewage plants that are severely contaminated with alkylphenols, estrogenic effects on fish cannot be ruled out. The nonylphenol concentrations that are considered to have no effect on aquatic systems (Predicted No Effect Concentration, PNEC) are sometimes exceeded in sewage plant effluent.

The PCB contamination of prey fish in lakes is at a level at which otter populations can survive. Sublethal effects such as lowered vitamin A contents cannot be excluded. In rivers and streams, however, the contamination of fish is highly variable, and the threshold value of 50-74 microgrammes PCB/kg fresh weight is usually exceeded. Whether this contamination is damaging to the establishment of a stable otter population cannot be stated with certainty, and is most probably dependent on other factors affecting the quality of the habitat (e.g. available food).

Aquatic concentrations of the organotin compound tributyltin in harbours are above the level at which sensitive types of marine snail show induction of male sex organs in females. However, no effects on molluscs are known in Switzerland.

Virtually no data exist on the contamination of amphibians, reptiles, birds and wild mammals. This is also true for humans, with the exception of data on a few selected chemicals. The extent of pollution for these groups of vertebrates and for humans cannot therefore be assessed.

Research need

Inadequate knowledge about pollution in Switzerland, and the possible disturbances associated with it, point to a requirement for research. There is a need for research into identifying EDCs, characterisation of their effects and risk assessment.

No extensively validated assay system for identifying chemicals with hormonal effects is currently available. It is therefore imperative to develop new in vivo and in vitro assays, to validate existing ones, and to supplement standardised test guidelines. This will make further research necessary into parameters that are influenced by hormones, and include little-researched hormonal disturbances (e.g. androgenic effects, progestins, effects on the thyroid). Furthermore, the studies should be extended to other species (amphibians, invertebrates).

Environmental samples (e.g. of waste water, drinking water) can be screened for known substances by analytical chemistry, and their endocrinological effects detected by biological assay systems. The chemicals responsible for these disturbances can be identified with a combination of both methods (Toxicity Identification and Evaluation, TIE).

For the assessment of risk to humans and animals it is necessary to elucidate the overall pollution situation, the environmental behaviour of the chemicals and their effects on humans and various animal species. Further, the mechanisms by which the endocrine disrupters work, and the interactions of various chemicals, must be studied.

The models available today were set up for the assessment of risks due to individual chemicals. It is therefore necessary to develop new models and assessment concepts that include combined effects of various different chemicals.