Environment and Pharmaceuticals

A publication containing facts and reflections about how pharmaceutical products and pharmaceutical residues can affect our environment and, as a result, our health. Published in collaboration between Apoteket AB (The National Corporation of Swedish Pharmacies), Stockholm County Council and Stockholm University

Pharmaceuticals, environment and health

In the national and international activities that are carried out to protect the environment, limiting the spread of substances that do not occur in nature is central. This must be weighed against the fact that many of these substances are of considerable importance for our economy, our peace of mind, our health and our quality of life. Unnatural substances are inevitable in our society. Environmental protection activities must, therefore, concentrate on investigating and revealing their properties and on ensuring that they have a minimal impact on the environment and that their use and disposal take place in a manner that as far as possible prevents them from spreading.

For some 150 years mankind has learnt to produce substances never before seen in nature. Consequently, no special processes have evolved in fauna and flora to deal with and break down these unnatural substances.

Unfortunately, not all the properties of substances are immediately apparent. Their effects on our health or in our environment may only occur after a long period, perhaps after centuries, and these effects may not appear until soil sediment and water concentrations have reached a certain level.

It is important to observe maximum caution when unnatural substances are introduced and to show the utmost care when it comes to early signs of unwanted effects.

Pharmaceuticals form a group of unnatural substances which are of considerable importance, both for the quality of our lives and our health and for our economy and our peace of mind. They are made, of course, with the express aim of exerting a biological effect. In the task of achieving *A Nontoxic Environment*, the national environmental quality target, drugs are thus an important area to take into account. Having control over their environmental aspect is essential if drugs, which are perhaps the best instrument of healthcare, are to be developed and utilised in the best possible way. No-one should need for environmental reasons to refrain from medical treatment based on drugs.

There are too many examples of early warning signs, where lessons have been drawn too late and where the result has been fatal for both people and other creatures on our planet. We must be aware of the risks, and all the forces for good must now lend a hand so that in the future we can avoid the problems described in the chapter entitled “Late Lessons from Early Warnings”, which rounds off this publication. Application of the precautionary principle is fundamental if we are to achieve this.

Jan Bergqvist | *Chairman of the Board of Apoteket AB (The National Corporation of Swedish Pharmacies)*

Contents

Published by: Apoteket AB, 2006
Original title: Läkemedel och miljö
ISBN: 91-85574-55-4

This publication is produced according to Apoteket's environmental regulation.
More information (in Swedish) at www.apoteket.se

Preface by the Editorial Committee

Drugs form a globally distributed group of products which are handled by many interested parties. Knowledge of the environmental effects of drugs is often inadequate. If environmental activities surrounding drugs and their use are to be successful, the collaboration of a large number of parties is needed: those engaged in academic research, drug manufacturers, the health services, pharmacies and authorities.

In August 2004 the Swedish Medical Products Agency presented the results of its enquiry "Environmental effects of pharmaceuticals, cosmetics and hygiene products". This report is an excellent source of knowledge, which gives structure to this area, besides pointing out the many gaps in our existing knowledge.

The concepts *health risk* and *environmental risk* are often used in the debate. They may be regarded as two different perspectives of the same concept. Health risk is a term we usually reserve for our own species – mankind, while the other term, environmental risk, encompasses all species. Health risk is often used in connection with our most important food (water), which is a finite resource. There have been reports of pharmaceutical residues in both European and American watercourses. The use of drugs is increasing as a result of an increasing population, the rise in consumption of developing countries, a greater average duration of life and, not least, the introduction of new and valuable drugs. Health risks associated with our being exposed for long periods via our environment to sub-therapeutic concentrations of drugs, concentrations which are also expected to increase, must be surveyed more effectively.

Drugs, health and the environment interact with one another in a complex way. In this publication Apoteket (The National Corporation of Swedish Pharmacies), Stockholm County Council and Stockholm University have brought together various interested parties to give an overview of these interactions, each of them contributing their specific knowledge. The publication also seeks to draw attention to the important balance between the desirable properties of drugs and their adverse environmental properties.

This publication is addressed to employees and decision-makers in the pharmaceutical sector, authorities, the health services, the wastewater treatment industry and academic research.

Each author/group of authors is responsible for the contents of their chapters, which are written so that they can be read independently. This means that the same or similar information may be found in more than one chapter.

Bengt-Erik Bengtsson *Bo Gunnarsson* *Tom Wall* *Åke Wennmalm*

1 Drugs and the flow of the substances they contain

Staffan Castensson and Bo Gunnarsson, Apoteket AB (The National Corporation of Swedish Pharmacies)

Drugs are products that are designed to diagnose, prevent, cure or mitigate disease. For thousands of years mankind has sought and found in nature substances which have these properties. From the animal and plant kingdoms, components have been obtained (and sometimes concentrated) to be later made into preparations used as a drug for internal or external use. Minerals, too, were sometimes concentrated and used for these purposes. Although these products potentially posed an acute risk to health, they hardly amounted to an environmental problem, with the possible exception of concentrated minerals containing substances such as mercury, lead or arsenic.

During the 19th century, when chemists learned to synthesise organic substances, the arsenal of drug substances was enlarged. Natural substances came to be made on a large scale at low cost and substances never before seen in nature were introduced. The development and manufacture of drugs became an industry. Classification, effect evaluation and quality assurance evolved and drugs became one of the most important tools of healthcare. The manufacture and use of drugs took off in earnest in the second half of the last century. From an environmental perspective, their manufacture came to be regarded and regulated in the same way as chemical manufacture generally.

The use of drugs came to enhance people's quality of life, prolong the lives of individuals and, in many cases, provide security for those who suffered disease. Animal drugs also took on considerable importance for livestock farming. Environmental aspects in connection with the use of drugs were obliged to take a back seat in favour of the therapeutic properties of drugs. As products, drugs came to occupy a unique position.

Drug design and function

To enable a drug to exert its effect, it is administered in a number of ways. In principle, it can be administered via all body openings, applied to various body surfaces or inserted directly into the tissues. The route of administration in turn affects the extent to which a drug is absorbed by the body, in comparison to the amount which directly leaves the body and also what remains behind in the product after use.

A finished drug consists of both the basic formulation that makes up its dosage form and the packaging enclosing it. In addition, there are usually one or more outer layers of packaging and sometimes various aids/accessories. The dosage form and its packaging form a unit, namely the pharmaceutical product.

In exceptional circumstances, a pharmacologically active substance may serve directly as a suitable dosage form, which after packaging is ready for use. Normally a more comprehensive formulation is made, where the aim is to attain the desired properties through processing and the addition of excipients. A dosage form should, for example:

- Enable a desired method of administration
- Facilitate accurate dosing
- Simplify the use of the drug
- Satisfy stability aspects
- Control the release and uptake of the drug

The choice of packaging is influenced by a large number of functional requirements, such as:

- Providing protection during use, storage and transportation against light, oxygen, carbon dioxide, humidity, microorganisms and evaporation
- Facilitate handling and use
- Identify and informing

Use of drugs

Measuring the use of drugs from an environmental perspective runs into considerable difficulties. In Sweden we make use of more than 1,000 active substances in drugs, in addition to which there are approximately the same number of excipients and also packaging materials. We know a lot about the effect that these active substances have on humans, but very little about their concentrations, breakdown and effects on plants and animals in the soil and water. These substances are present in about 7,000 different pharmaceutical products and new substances come along each year. Most new drugs that receive marketing authorisation, however, are made from previously known and used substances.

Quantities

Monitoring the use of drugs by monetary standards is not meaningful from an environmental perspective; the price or cost of a drug does not reflect its environmental impact. Monitoring drug use by measuring the quantities of substances may be of interest for an individual substance, but adding together quantities for classes of drugs is of limited value, since different substances have different environmental effects and different substances can have an environmental impact at very different concentrations.

Keeping track of the number of drug packs is one way of measuring drug use and can give some idea of the distribution of drugs nationwide, although pack sizes can vary and be intended for drug treatment of varying duration. In 2004 Swedish pharmacies sold more than 147 million packs of drugs. A Swedish citizen buys about 15 drug packages a year.

The existing measure of drug use best suited in an environmental context is the number of drug doses. Although this measure has been created on the basis of treating diseases in people (or animals) and not on the basis of the effects that can be brought about in nature, it is the only measure of effect generally employed today.

For most drugs, there is a defined daily dose (DDD), which is the normal dose per day for the main indication of the drug. Drugs belonging to certain classes, such as skin preparations, infusion solutions and animal drugs, may lack a DDD.

In Sweden about 5.4 billion DDDs were sold in 2004, which implies an average of about 550 DDDs per inhabitant a year. Their distribution among the pharmacological classes in recent years is shown in the diagram alongside. The drugs have been divided into pharmacological groups according to the ATC (Anatomical Therapeutic Chemical Classification) system.

The two groups of drugs which show the strongest growth and at the same time account for a substantial proportion of drug use are group C (Cardiovascular system) and class N (Nervous system). In both these groups new drugs are being introduced with highly specific mechanisms of action. The statins, which belong to group C, have shown the largest growth, with an increase of 35 million DDDs in 2003. Statins lower human cholesterol levels, although their effects may be entirely different in other organisms.

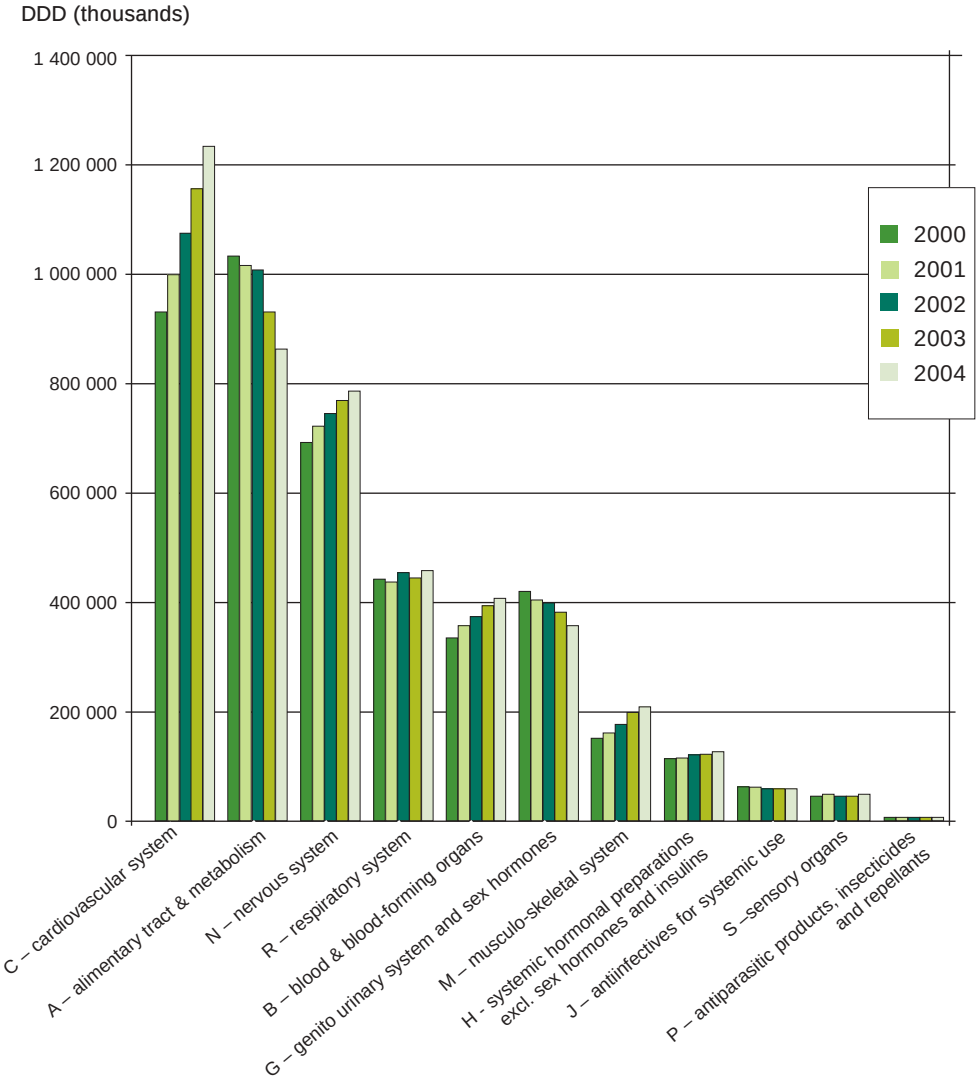
One class of drugs in group N that is increasing consists of the selective serotonin re-uptake inhibitors or SSRIs, which are used for depressive illnesses. Little is known about how these substances affect the nervous system or what effects they have generally on organisms other than humans.

Among the sex hormones in group G there are many substances that are of importance from an environmental perspective. There has been discussion of contraceptives and hormone replacement agents and attention has been drawn to the risks of oestrogen treatment. There has been an overall decrease in this group in recent years.

In group D (dermatologicals) it is difficult or impossible to establish meaningful DDDs.

Another group, which is not quite as large, but which may be important in the context of the environment, consists of antiinfectives (J). The amounts used of these drugs are relatively stable over the years.

About two per cent of all drug packs are intended for use in animals. While this may appear to be a small amount, it may be of considerable importance from an environmental perspective. A single pack may contain a relatively large amount of a drug substance and these drugs are used in small geographical areas. They can also directly impact on the environment, e.g. antiparasitic agents, and, in addition, are used in a food-production environment.



Sales by pharmacies of drugs between 2000 and 2004 measured in defined daily doses (DDDs) per ATC group. Groups D (dermatologicals) and L (antineoplastic and immunomodulating agents) have been excluded from the diagram because of the difficulty of establishing relevant DDDs. Group V (various) has been excluded owing to the low level of DDDs.

Source: Sales statistics, Statistical Dept., Apoteket AB.

Environmental aspects of drugs

The environmental consequences of the manufacture and use of pharmaceutical products are entirely dominated by the risks associated with various active substances, together with a number of excipients, when they are released into the environment. The table below gives examples from the drugs distributed in Sweden by Apoteket AB of substances that pose a hazard for the environment, together with their quantities.

Environmental aspect assessment (Sweden) according to the report of the Swedish Medical Products Agency, August 2004.

Substance	Function	Annual amount in 2002 (kg)	Initial assessment
Atenolol	Beta-blocker	4 500	Risk to aquatic environment cannot be ruled out
Dextropropoxifen	Analgesic	1 800	Risk to soil environment cannot be ruled out
Docusate sodium	Excipient	1 570	Local risk to sediment-living organisms in freshwater environments
Diazepam	Anxiolytic	183	Risk to soil environment cannot be ruled out
Diclofenac	Anti-inflammatory	3 960	Potentially bioaccumulating
Ethinylestradiol	Sex hormone	6.4	Risk to aquatic environment
Ibuprofen	Anti-inflammatory	68 200	Potentially bioaccumulating
Ketoprofen	Anti-inflammatory	62 700	Risk to aquatic environment cannot be ruled out
Norethisterone	Sex hormone	50	Risk to aquatic environment cannot be ruled out
Oxazepam	Anxiolytic	642	Risk to aquatic environment cannot be ruled out
Oxytetracycline	Antibiotic	293	Risk to microorganisms in sewage treatment plants cannot be ruled out
Paracetamol	Analgesic	418 000	Risk to aquatic environment
Ranitidine	Antiulcer	8 360	Risk to soil environment cannot be ruled out
Simvastatin	Lipid-lowering	1 430	Risk to aquatic and soil environments cannot be ruled out; also potentially bioaccumulating
Tetracycline	Antibiotic	2 400	Risk to microorganisms in sewage treatment plants cannot be ruled out
Estradiol	Sex hormone	153	Risk to aquatic environment
Estriol	Sex hormone	38	Risk to aquatic environment

Active substance and excipients

The hazard posed by the active substance and certain excipients is bound up with the extent to which they inherently bioaccumulate in plants and animals, break down with difficulty (persist) and are poisonous (ecotoxic). The risk of an adverse environmental impact depends on the extent to which the environment is exposed to high concentrations of a substance. Apart from the fact that the amount released into the environment is crucial, bioaccumulation and persistence will reinforce the degree of exposure. The real harm is ultimately determined by the ecotoxic properties of a substance.

Packaging

Packaging materials, like aids and accessories, consume a small, though not insignificant, proportion of natural resources. They can impact on the environment through pollution caused by incineration and landfill. No pharmaceutical packaging is re-used, although some recycling takes place when the user is able to sort parts of a pharmaceutical product into recyclable fractions, such as cardboard or glass. Other packaging waste is best disposed of by incineration and energy recovery and, as far as possible, landfill should be avoided. One special problem arises from pharmaceutical packaging that retains residues of the active substance after use, owing to the impossibility of using up the entire contents intended for administration, e.g. inhalers, ointments and eye preparations. Such packaging should be disposed of in such a way that leakage to the environment is by and large completely avoided. The same applies to dosage forms where large residues of active substance remain enclosed after use, e.g. in transdermal patches.

Pharmaceutical waste

For both the health services and the public, systems exist in Sweden for disposing of surplus drugs. In both cases incineration is used as a method of getting rid of these products. In two studies carried out by the Swedish polling organisation SIFO (Sifo Research & Consulting AB, 2001, and Sifo Research & Consulting, 2004) about 40 per cent of those who had recently used drugs claimed to have returned any that were left over to a pharmacy. A substantial proportion of those questioned replied that they let the drugs “sit in the cupboard” until further notice, for possible use at a later

date, many of them saying that they intended to return what was left over to a pharmacy later on. The total proportion of the population who return leftover drugs to a pharmacy can in this way be put at about 65 per cent (Discarded drugs, National Board of Health and Welfare, 2004).

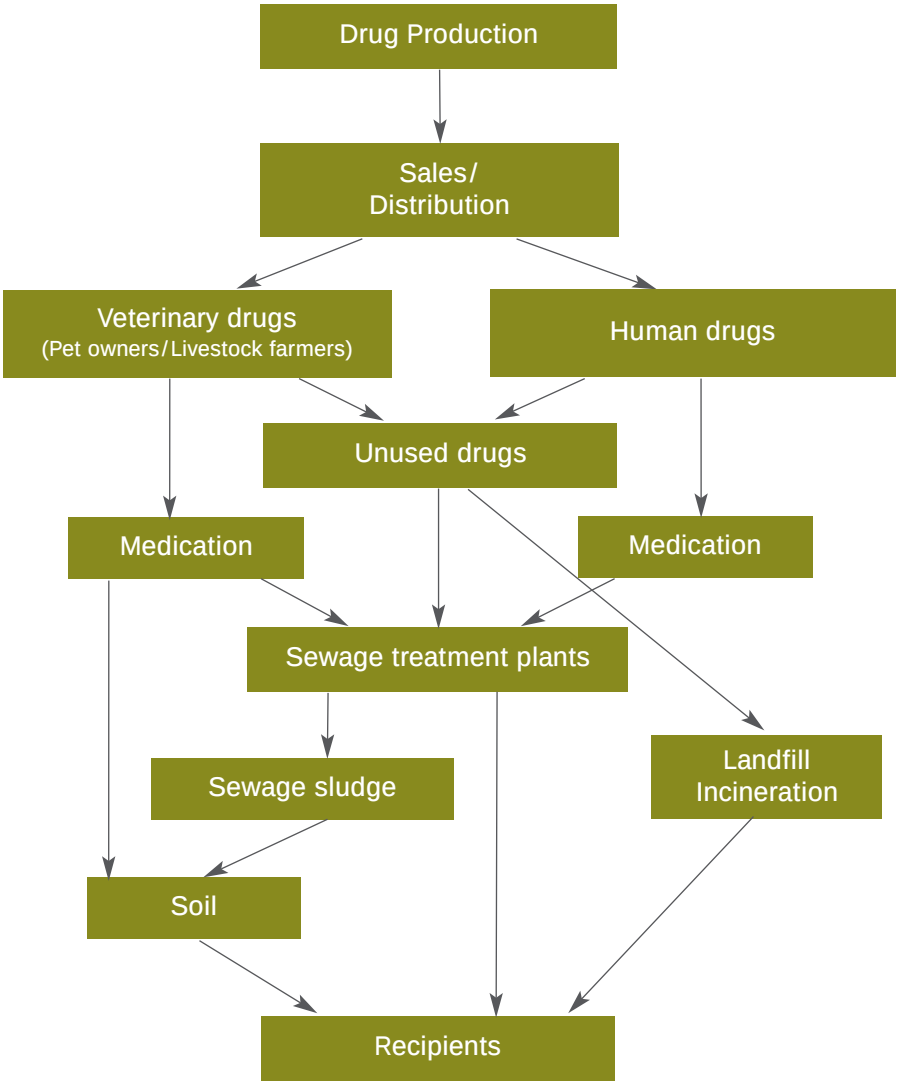
The proportion of drugs which never get used may be put at about 5 per cent. This estimate is based on studies of what is handed in at pharmacies and on the SIFO studies mentioned above. The drugs which predominate (in terms of the numbers of packs) among the ones that are left over are drugs for cardiovasvular disease, asthma, the nervous system and the gastro-intestinal tract. Of the leftover drugs, therefore, about 65 per cent are returned to pharmacies for environmentally acceptable disposal, while the rest may spread in an uncontrolled manner.

Environmental aspects and design

The design of a pharmaceutical product has so far focused on giving the product good therapeutic, technical and user-friendly properties, with any environmental aspects less commonly being taken into account. At present halogenated plastics (PVC or PVdC) are predominantly found in the extremely common blister packs. They have an adverse impact on the environment during manufacture, may contain harmful additives and, when incinerated, give rise to the acidic hydrogen chloride, which also contributes to the formation of dioxins. Alternative plastic materials such as polypropene or polyester produce carbon dioxide and water when incinerated, and efforts are therefore being made to phase out the halogenated plastics. There are also examples today of designs of active substances with better environmental properties in the form of a smaller risk of bioaccumulation and persistence than similar active substances (Kümmerer et al., 2000). In the development of an active substance, therefore, good environmental properties can be added as an additional design goal.

Where do drugs end up?

To understand the environmental consequences that drugs have when they reach the user, we must follow the route they take through society and see in what way and in what amounts they can reach different parts of the environment.



The flow of drug substances and metabolites to the environment.

Use

The pharmaceutical products that are used will be absorbed by the body to a widely differing extent, depending on the route of administration, their dosage form and the physicochemical properties of the active substance. Drugs that are not absorbed either remain in the actual pharmaceutical product or are released from it and pass the body without being taken up. In the first case typical examples are various dosage forms designed to be applied to the skin to give a local effect or to penetrate the skin to produce what is known as a systemic effect. In these dosage forms (e.g. ointments, powders, transdermal patches) the drug substance is dissolved or embedded in a much larger amount than the body is able to absorb. The rest is either washed off the skin or remains inside the patch. In the second case a drug passes through the gastro-intestinal tract, for instance, without being absorbed. The drug which is taken up by the body will in turn leave the body in unchanged form or after it has been metabolised to more water-soluble forms (conjugates, breakdown products or conjugates of breakdown products).

After use

Drugs that are administered to humans and animals leave the body intact or metabolised via urine and faeces. Any drug remaining after being applied to skin and mucosal surfaces is washed off or removed by other means. Drugs that are volatile, such as inhalation gas, leave the body in the same way as they are absorbed.

Human drugs, once used, nearly always end up in the sewage system, which in turn usually leads to a sewage treatment plant and eventually the receiving waters. The latter can also acquire drug residues as a result of leakage from landfill sites and agricultural land on which sludge from treatment plants has been spread. Drugs directly enter the soil layers (in the case of animals in the open) or are added to farmland via the spread of manure. These residues can have a direct effect on farmland or eventually, through leaching processes, reach the receiving waters. Emissions can be measured for a number of drug substances, and various studies add to our knowledge of the spread of drug residues in soil and water (Holm et al., 1995; Ternes, 1998; Halling-Sørensen et al., 1998; Heberer, 2002; Reddersen et al., 2002).

The environmental impact of pharmaceutical products in the atmosphere may be regarded as slight owing to the small amounts and the large degree of dilution involved. Nitrous oxide for anaesthesia and CFCs in aerosol propellants have a high greenhouse gas factor and they also lead to thinning of the ozone layer. Drugs were excluded from the 1987 Montreal protocol on the use of Freons; nevertheless, virtually all drug aerosols with these propellants have been phased out (Replacement of Chlorofluorocarbons in Metered Dose Inhalation Products; Matters Relating to the Replacement of CFCs in Medicinal Products).

The damage that is known to have affected the environment has come about from localised effects, where the concentration of a drug substance and/or its metabolites has for various reasons become too high. Such situations may occur in direct conjunction with a drug leaving the body, such as the antiparasitic agent ivermectin and its effect on dung-living insects (Bloom and Matheson, 1993), or where the total amount of the diffuse flows entering the sewage system is so extensive that an environmental impact occurs in sewage treatment plants or downstream of them.

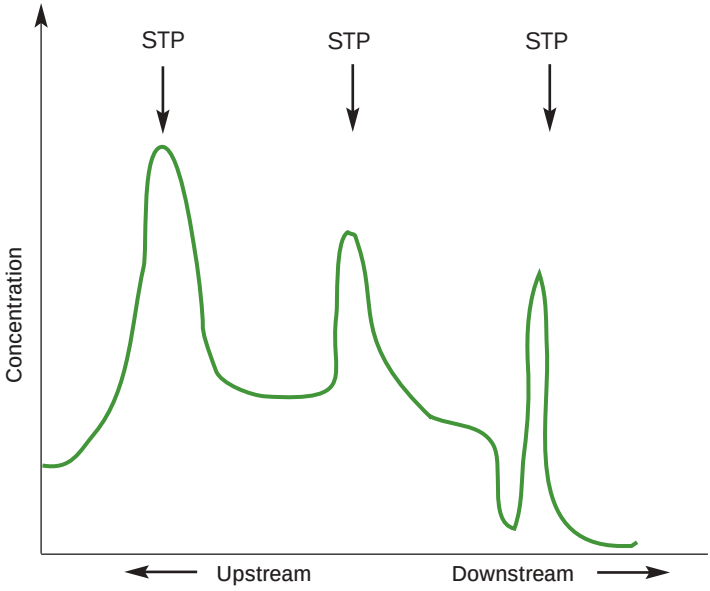
When it comes to understanding and estimating the effect of the total diffuse flows, one can today make a risk assessment based on the estimated concentrations of an active substance and/or its metabolites in various defined compartments of the environment (the surface water, groundwater, sludge etc.). The estimated concentration is arrived at by first estimating the inflow to the sewage systems due to the consumption of a particular drug within a geographical area minus the deactivation that arises through metabolism. In a treatment plant further breakdown may occur due to a biological or chemical effect, although there are also examples of reactivation of an active substance through conjugate degradation (Panter et al., 1999). At the treatment plant significant separation of the substance may take place through adsorption to sludge. The degree of breakdown and adsorption is extremely varied (0–100%) and depends on the properties of the substance. Consequently, by and large the same concentrations can usually be observed during both the inflow to and outflow from a sewage treatment plant.

Spread in nature via sewage

Sewage treatment plants (STP) release drug residues into the natural environment in concentrations usually measured in the range of ng/L – microgram/L. If the spread of these residues is followed in a runoff area,

the concentrations will fall due to dilution and breakdown (e.g. hydrolysis, photolysis or biodegradation) and as a result of the substances being distributed to other parts of the environment (water, air, soil, sediment, the living flora and fauna). This distribution can be assessed with the help of the known physicochemical properties of the substances. The concentrations may also increase as a result of the water system receiving more drug residues along the runoff area.

With the aid of simulation models, the flow of drug substances can be followed along a water system, giving a rough estimate of the concentrations that may be expected to occur (Schowanek and Webb, 2002; Anderson et al., 2004). As the diagram below shows, the concentration varies due to various reducing effects or additions to the flow. A test involving field sampling has mainly confirmed the usability of the model as a tool for assessing the risk to humans, based on the limit of the daily intake (Anderson et al., 2004).



Changes in the concentration of a drug substance along, for example, a river system vary with additions from a sewage treatment plant (STP) and reducing effects such as breakdown and binding to sediment.

References

Anderson P D, D'Aco V J, Shanahan P, Chapra S C, Buzby M E, Cunningham V L, DuPlessie B, Hayes E P, Mastrocco F J, Parke N J, Rader J C, Samuelian J H, Schwab B W (2004). Screening Analysis of Human Pharmaceutical Compounds in U.S. Surface Waters. Environ. Sci. Technol. 38(3): 838-849.

Bloom R, Matheson J (1993). Environmental assessment of ivermectins by the US Food and Drug Administration. Vet. Parasitol. 48: 281-294.

Halling-Sørensen B, Nors Nielsen S, Lanzky P F, Ingerslev F, Holten-Lützhoft H C, Jørgensen S E (1998). Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere 36: 357-393.

Heberer T (2002). Occurrence, fate and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology Letters 131: 5-17.

Holm J V, Rugge K, Bjerg P L, Christensen T H (1995). Occurrence and distribution of pharmaceutical compounds in the groundwater downgradient of a landfill. Environ. Sci. Technol. 29: 1415-1420.

Kasserade läkemedel. Förslag på åtgärder för att minska mängden överblivna läkemedel. Socialstyrelsen, 2004.
[Discarded drugs. Suggested measures for reducing the amount of leftover drugs. Swedish National Board of Health and Welfare, 2004.]

Kümmerer K, Al-Ahmad A, Bertram B, Wießler M (2000). Biodegradability of antineoplastic compounds in screening tests: influence of glucosidation and of stereochemistry. Chemosphere 40: 767-773.

Miljöpåverkan från läkemedel samt kosmetiska och hygieniska produkter. Rapport från Läkemedelsverket. Augusti 2004.
[Environmental impact of drugs and of cosmetic and hygienic products. Report from Swedish Medical Products Agency. August 2004.]

Matters Relating to the Replacement of CFCs in Medicinal Products (CPMP/III/5462/93).

Panter G H, Thompson R S, Beresford N, Sumpter J P (1999). Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. Chemosphere 38: 3579-3596.

Reddersen K, Heberer T, Dünnbier U (2002). Identification and significance of phenazone drugs and their metabolites in ground- and drinking water. Chemosphere 49: 539-544.

Replacement of Chlorofluorocarbons (CFC) in Metered Dose Inhalation Products (CPMP/III/5378/93).

Schowanek D, Webb S (2002). Exposure simulation for pharmaceuticals in European surface waters with GREAT-ER. Toxicology Letters 131: 39-50.

Sifo Research & Consulting AB, 6815000, Stockholm, Sweden, 2001.

Sifo Research & Consulting AB, 1511677, Stockholm, Sweden, 2004.

Ternes T (1998). Occurrence of Drugs in German Sewage Treatment Plants and Rivers. Water Research 32: 3245-3260.


HEAD

2 The sewage treatment plant –how does it work?

Berndt Björlenius and Cajsa Wahlberg, Stockholm Water Company

History and development

Right up to the end of the 19th century sewage and domestic waste were emptied directly into our lakes via gutters and open ditches. The first public sewer in Stockholm was constructed in 1864 on Krukmakargatan in the district of Södermalm, although no mention was made of treating the sewage. As the population grew, the burden on the surrounding waters became greater and greater, and in 1932 bathing in the Strömbadet open-air swimming baths in Stockholm was banned. Even before then it had been realised that it did not do to discharge raw sewage indiscriminately, and work began on building the first sewage works. The Åkeshov sewage treatment plant in Bromma was one of the earliest and started operation in 1934. It actually contained only a coarse screen and a sedimentation step (Cronström, 1986).

Treatment of organic material

Since then environmental and health considerations have placed increasingly strict requirements on the quality of the treated sewage, and sewage treatment plants have undergone continual development. To start with, screens and sludge separation were introduced and in return for a fairly low investment the degree of sewage treatment was relatively high. The focus of reduction was on the visible pollutants, floating objects and sedimenting sludge. It was gradually realised that the oxygen demand of the treated sewage was still high, while at the same time suspended solids and bacteria were present in large quantities. In the 1950s and 1960s the treatment plants introduced biological treatment in order to break down dissolved organic material, the active sludge process becoming the stable process adopted in many places. The water was now “doubly” treated.

Need to decrease nutrients

Around a decade later it was realised that nutrients also needed to be removed. The first of these was phosphate, an important limiting substance

for the primary production of algae. In Sweden chemical precipitation with iron sulphate or other metal salts was introduced. The measures that were adopted were extensive: through state grants many treatment plants were equipped with the “third step” – secondary precipitation – and discharges of phosphorus fell markedly.

Fifteen years later there were alarming reports of widening bottom areas without higher life in nearby coastal areas. Emissions of nitrogen from farming, road traffic and the energy sector, and to a lesser extent from the sewage treatment plants, were assumed to be the culprits. Nitrogen reduction was introduced at treatment plants close to the coast. In general, biological nitrogen reduction was chosen, which involved enlarging the biological treatment step. In the active sludge step the effluent then came to have a retention time of up to four times as long, and this, besides giving a significant reduction in nitrogen, also enabled better degradation of organic substances.

In connection with the enlargement to enable nitrogen reduction in the 1990s, official requirements governing the phosphorus content of the effluent from the largest treatment plants were tightened up. A final filter step was introduced for the separation of particles, which further reduced the remaining phosphorus.

Need to stabilise the sludge

Besides treating the sewage, treatment plants also have some form of sludge processing. During the treatment process sludge is separated from the incoming water and also as a residual product of biological and chemical treatment. In order to reduce the amount of sludge and break down organic material, the sludge is often stabilised by means of digestion – the anaerobic degradation of organic material. In the course of digestion high-energy digester gas is produced, which contains primarily methane and carbon dioxide. The digested sludge is dewatered in order to increase its dry solids content. The dewatered sludge contains large amounts of nutrients, mainly phosphorous, and is used in a number of different ways, from the fertilisation of agricultural land and soil manufacture to covering landfills.

Thanks to continuous development, Swedish sewage treatment plants are now efficient when it comes to the far-reaching reduction of organic and suspended material, phosphorus and nitrogen. Viewed internationally, Sweden has come a long way, with good and robust technology for treat-

ing sewage from 90 per cent of its inhabitants. The remaining 10 per cent have some form of individual solution to the problem of sewage, such as a three-compartment septic tank, infiltration or a closed tank. All the 7.7 million people living in densely populated areas are connected to a sewage treatment plant. 94 per cent of them are connected to a plant with mechanical-biological-chemical treatment, while the remaining 6 per cent are served by plants with either biological or chemical treatment.

The sewerage network

The raw sewage produced by society is usually collected with gravity sewers connected to pumping stations in the sewerage network. The pumping stations are situated at the lowest point of the network and from them the sewage is pumped to larger pipes, which eventually empty into the treatment plant, possibly via more pumping stations. There are striking similarities between the shape of the network and the branches of a tree.

The sewers come in a number of different materials and dimensions and, depending on their location, groundwater and surface water (known as drainage water) can leak into them. This means that the hydrological situation in the ground can affect the sewerage network to varying degrees, depending on its design and condition.

Some of the water that runs into the sewers is storm water, i.e. rainwater from a hard surfaces, roads, roofs etc. A distinction is made between separated and combined sewers. The sewers in modern residential areas are separated, which means that the storm water is discharged directly into the receiving water body, while the wastewater (and drainage water that forces its way in) is led to a sewage treatment plant. Nowadays storm water from busy traffic routes, for example, is also treated in simple treatment installations before being discharged into the receiving water body. In combined systems, which are found mainly in older areas and town centres, storm water goes with other wastewater to the treatment plant.

Consequently the proportion of storm water and drainage water compared to the total flow reaching the treatment plant varies considerably between different sewerage networks. A separate network means that the inflow is less, with less risk of overflow discharges of raw sewage, both in the network and at the treatment plant. The drawback of separate systems is their cost. The great majority of population centres have a mixture of combined and separated sewers.

Processes at the treatment plants

A treatment plant can be divided into two main parts – one for sewage and one for sludge. In Sweden the sewage in nearly all cases is treated mechanically, biologically and chemically. The sewage sludge is in larger treatment plants normally stabilised by means of anaerobic digestion. The flows of sewage are large. In Stockholm's biggest treatment plant, Henriksdal, an average of 2,500 litres a second are treated from a connected population of 650 000 (see the illustration overleaf).

Mechanical treatment

When the incoming water reaches the treatment plant, it is first treated mechanically. In order to remove the solid pollutants, three main treatment steps are used in sequence:

1. Screens (alternatively strainers)
2. Sand traps (alternatively strainers)
3. Primary sedimentation

The different steps remove particles of successively smaller size. Without the mechanical pretreatment, objects and particles would enter the plant with the water and cause operating problems such as wear and tear and blockages. The total retention time for the pretreatment step is around 2–3 hours. Pretreatment is the process at the treatment plant which has the largest treatment effect for the money invested; however, the degree of treatment is not sufficient for today's high discharge standards. Following pretreatment, the water is usually piped to the biological step.

Screens can have a large number of designs. They usually consist of closely spaced narrow metal bars. The distance between the bars or "column width" varies. The traditional coarse screens have a column width of 20–30 mm, while the newer fine screens have a column width of 3–10 mm. Objects and particles with a diameter exceeding the column width get caught in the screen. The trapped screenings are removed with automated rake arms and transported on a conveyor belt to dewatering equipment. The compressed screenings are transported in containers to incineration for district heating. The screenings consist mainly of plastic, paper, sanitary towels, swabs and other refuse which does not dissolve in water. In Stockholm the amount involved is 32 tonnes a week.

The function of the sand trap (or grit chamber) is to separate particles with a diameter exceeding about 0.15 mm, which means the separation of sand, seeds and coffee grounds. The traps may be aerated or unaerated. In an aerated sand trap several processes take place at the same time. The correct speed of water is achieved by means of a stream of air, and precipitation chemicals added are oxidised and form chemical flocs or clumps of particles for the separation of phosphorus, and fat is removed on the surface of the sand trap. The sand that has been removed is pumped up to a sand dewaterer and also, in some cases, to a sand cleaner. After dewatering, the sand is collected in containers for transportation to landfill.

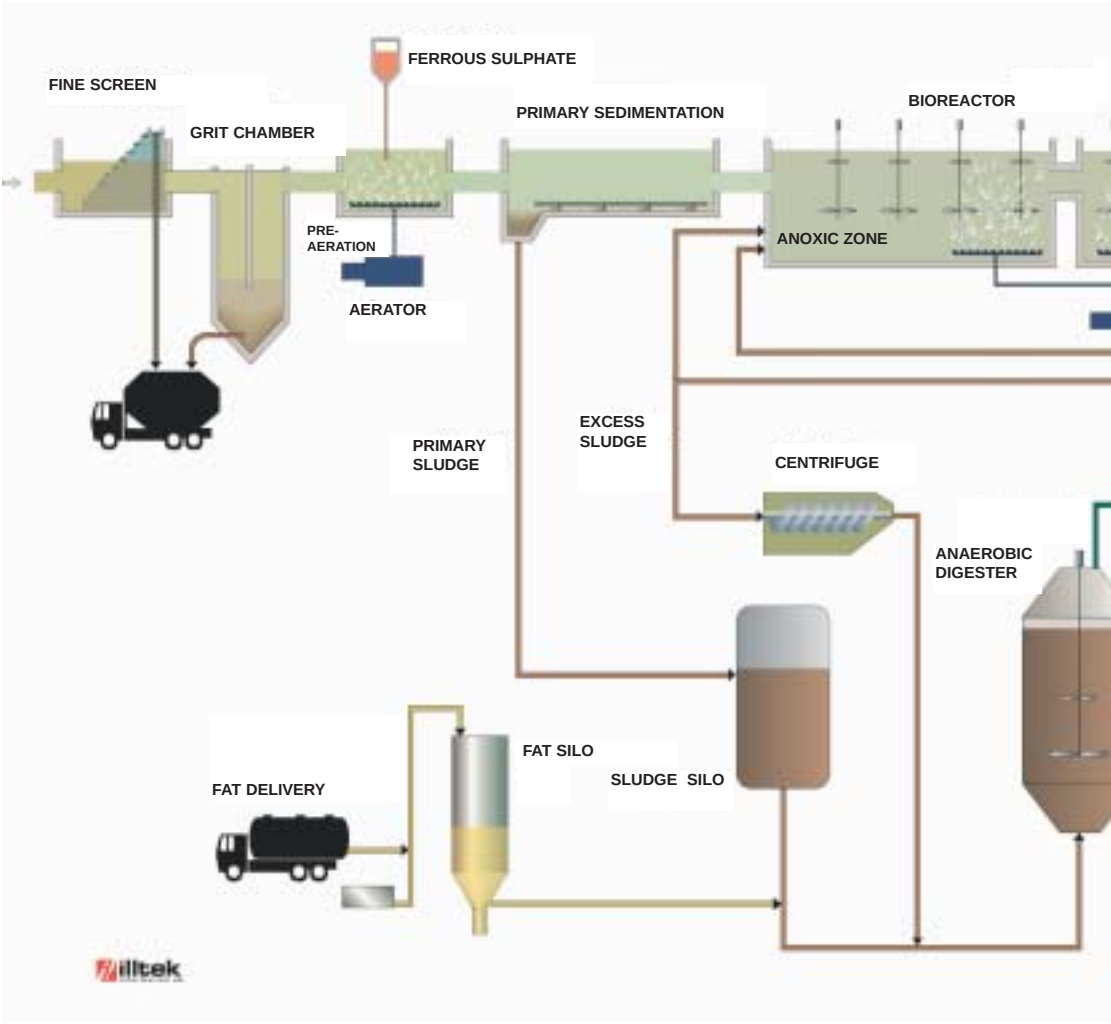
During pre-aeration floating sludge sometimes forms, consisting mostly of fat. Another phenomenon is that volatile components can evaporate during pre-aeration. Solvents that have been discharged into the sewers are often first detected in the aerated sand trap at the treatment plant.

Chemical precipitation

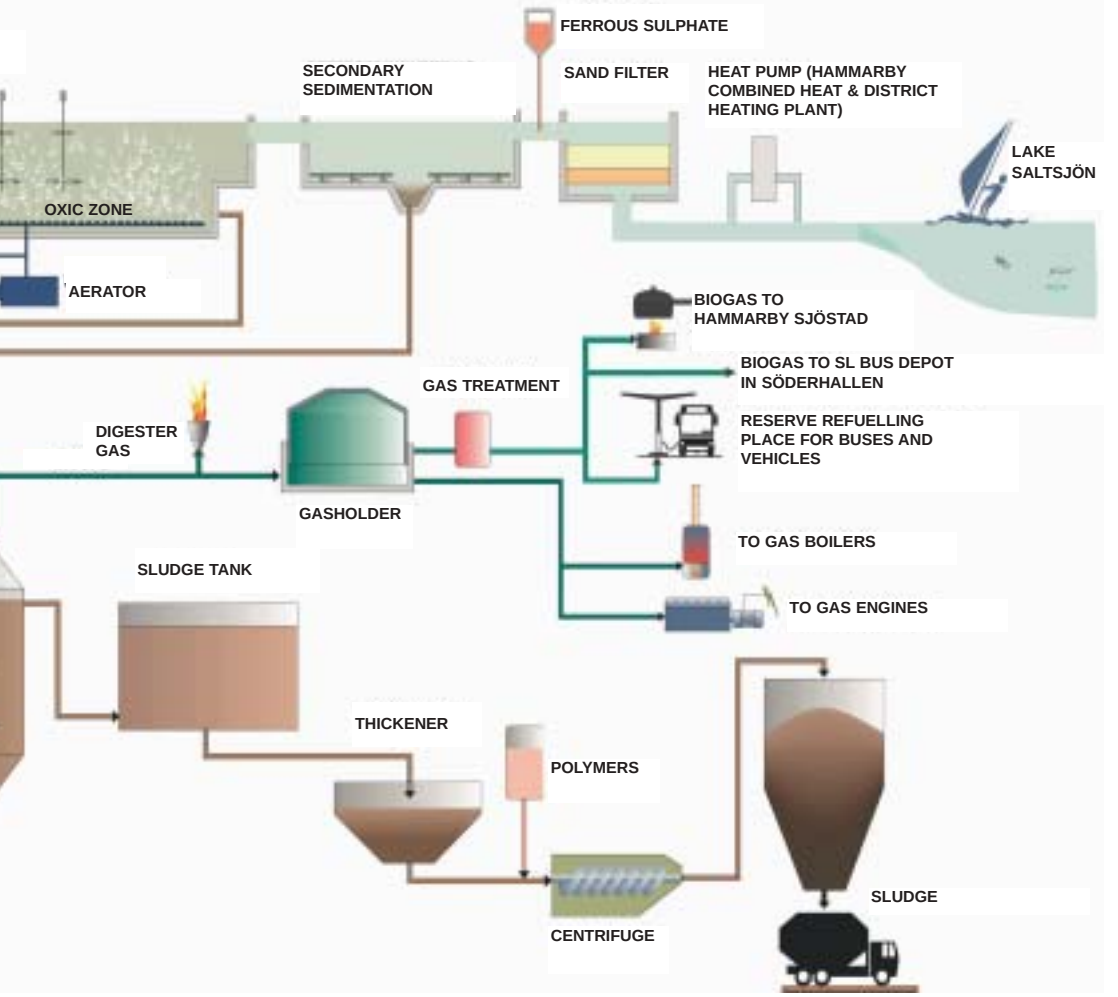
The sewage contains large amounts of phosphorus, which will cause eutrophication of lakes and watercourses unless it is removed. The reduction of phosphorus is achieved by means of chemical treatment. Through the addition of a metal salt, e.g. iron sulphate, the phosphorus is precipitated and forms iron phosphates and ferric hydroxide flocs. In order to get the iron sulphate to mix properly, air is blown into the water in a pre-aeration tank. Instead of iron sulphate, salts of aluminium, for example, can be used. After the pre-aeration tank the water flows into pre-sedimentation tanks, where the flocs sink to the bottom and form sludge. The sludge goes to sludge treatment.

Chemical precipitation carried out before biological treatment is called preprecipitation. In this method precipitation chemicals are added, generally at the plant inlet, and floc formation takes place in the sand trap or during pre-aeration and floc separation in the primary sedimentation tank. A variety of metal salts are used as precipitants. The salt precipitates the dissolved phosphorus in the form of a sparingly soluble metal phosphate. At the same time metal hydroxide is precipitated, forming gelatinous flocs which bind to the precipitating metal phosphates and other suspended solids present in the water. This means that not only dissolved phosphorus, but also organically bound phosphorus and suspended material are reduced. Occasionally synthetic polymers are added during preprecipitation to cause the particles to clump together better.

Process diagram for Henriksdal, the largest treatment plant in Stockholm.



2,500 litres of sewage per second (average) are treated here.



During presedimentation with chemical precipitation over 80 per cent of suspended solids can be reduced, and over 60 per cent of organic material (measured as COD, chemical oxygen demand) and the same amount of phosphorus are removed from the sewage. Effective preprecipitation can have a positive effect on the process throughout the treatment plant, from the standpoint of both water and sludge. Of great importance is the use of precipitation chemicals containing low amounts of heavy metals to prevent the sludge content of these from increasing.

Besides preprecipitation, simultaneous precipitation and postprecipitation and, in some cases, direct precipitation may also occur. During simultaneous precipitation the precipitants are added directly in the biological step. During postprecipitation a precipitant is added to the sewage in a subsequent sedimentation step or at a filter. In the case of direct precipitation, precipitation occurs in a single step directly after pretreatment of the sewage. There is no biological treatment step in a direct precipitation plant.

Biological treatment

Raw sewage contains large amounts of organic material, resulting in a lack of oxygen in lakes and watercourses, which kills the organisms living on the bottoms. Raw sewage also contains nitrogen, which causes eutrophication of the water. These substances must therefore be removed from the effluent before it is discharged into the receiving water body.

In the biological step at the treatment plant, use is made of microorganisms, primarily various bacteria, for the biological treatment of the pretreated water. The most common treatment today is the active sludge process, which was developed in the UK at the beginning of the last century in order to prevent further contamination of watercourses. The process that was introduced mimicked the self-treatment of the watercourses, though in a much more compact form. The oxygen-consuming sewage now had its oxygen requirements satisfied in the treatment plants' biological step.

The active sludge process is based on flocs (which mostly consist of bacteria) being kept floating with air and sometimes also with mechanical agitation. The bacteria degrade the organic material, whereupon carbon dioxide and water is formed at the same time as new biomass is created. After passing through the active sludge tank, the sludge is carried along in the effluent. It is allowed to sediment in the subsequent sedimentation process.

A large part of the sludge is pumped by the return sludge pumps to the start of the aeration tank, where it is seeded into "new" incoming, pretreated water. Recirculation of the sludge is necessary in order to maintain an adequate sludge content in the aeration tank. A small substream containing excess sludge, i.e. the net amount of sludge produced, is removed from the system.

The sludge consists of newly created cell mass, precipitated metal salts (mainly iron and aluminium) to which phosphorus (and also calcium) is bound, and degradation residues.

The extent of treatment is in general very high and uniform, with a reduction in organic material, measured as BOD_7 (biochemical oxygen demand), of between 90 and 95 percent.

Of the organic material that enters the treatment plant, 30–40 per cent is oxidised to carbon dioxide and water, 40–45 per cent forms new biomass through sludge growth and 10–25 per cent is inert material which passes unchanged through the plant, ending up in the sludge phase. In the digestion tank the excess sludge that has formed is broken down further.

In the biological step also suspended material, nitrogen, phosphorus and inorganic dissolved and solid substances, including metals, are separated.

Biological nitrogen reduction with predenitrification has been introduced at many large treatment plants in Sweden that are close to the coast. The nitrogen is transformed in the biological nitrogen reduction to nitrogen gas, which is returned to the atmosphere, 80 per cent of which already consists of nitrogen. This process is based on active sludge tanks, connected in series, which contain different amounts of oxygen in the water. In those parts of the tanks which are aerated and contain free oxygen (oxic zones), one type of microorganism thrives that oxidises ammonium nitrogen to nitrite and nitrate nitrogen. In the anoxic zones the growth takes place of other kinds of bacteria, which reduce nitrite and nitrate nitrogen to free nitrogen. These processes are called nitrification and denitrification respectively.

In the biological step a number of reactions take place: physical/chemical adsorption and absorption, chemical precipitation and, not least, biochemical reactions and microbial processes such as growth and breakdown. These processes occur at the same time and it is sometimes difficult to determine which process is doing what in the complex system.

The biochemical and microbiological processes are sensitive to disturbances, inhibition and toxic substances. High flows at the biological step can even flush out the active biomass in what is known as sludge escape. The biological step must therefore be protected against abnormal effects such as high sludge and hydraulic loads and inhibiting and toxic substances.

The sensitive properties of the biological step mean that this step is often the bottleneck of the treatment plant, particularly when biological nitrogen reduction takes place. The nitrification bacteria are particularly sensitive to toxicants and if they are inactivated, weeks can pass before the nitrogen treatment is fully operative once again.

Sand filters

Sand filters are becoming increasingly common at treatment plants, the main reason for this being the stricter requirements relating to phosphorus. The filters make the operation of the treatment plant more reliable. Sludge escape can be stopped and presedimented water, which the biological step is not able to receive, can undergo additional treatment in the filters before the effluent is released into the receiving water body. The phosphorus reduction can be further strengthened by dosing precipitation chemicals directly at the filters.

The separation of suspended solids and phosphorus in sand filters is highly efficient. Normally the amount of the outgoing suspended solids is around 1 mg/l and that of total phosphorus around 0.1 mg/l.

The usual type of downstream filter separates particles which accumulate in the filter bed, which is usually 1.5 m deep and may consist of several different sand and shale materials of varying density. The pressure drop over the filter increases gradually and after several hours the filter must be backwashed. Effluent water (and sometimes air) is forced contrary to the normal direction of the current. Particles that are caught in the filter bed are flushed out and accumulate in the water above it. This backwash water is piped either to the treatment plant intake or to the biological step. The flushing is usually carried out once a day, generally two to three times in succession, to get the filters really clean.

The total retention time in a treatment plant varies from four to five hours for a simple plant to up to one day for plants with enlarged nitrogen treatment, which requires a long retention time in the biological step. When the effluent finally leaves the treatment plant, the number of particles (measured as suspended solids) has decreased by up to 99 percent.

Organic material has also decreased by as much as 99 per cent, measured as BOD_7 (somewhat smaller if COD is measured instead). Phosphorus reduction may amount to 98 per cent and more than 80 per cent of the nitrogen may have disappeared if the plant is run with nitrogen treatment.

The treated sewage, if possible, is discharged a long way out from the shore in deep water. In this way the dilution is large and the risk of infection being spread is minimal. In Sweden treated sewage is no longer chlorinated (Lind, 2004).

Sludge treatment

The aim of sludge treatment is to deal with the sludge that is separated in the water treatment line. Primary sludge and biological excess sludge must be stabilised and the water content of the sludge must be reduced. Finally, it has to be possible to transport the sludge away from the treatment plant, in a handy way.

The sludge treatment generally starts with thickening of the primary and excess sludge so that the dry solids content increases. The primary sludge is pumped from the presedimentation to the thickener or directly to sludge stabilisation. The biological excess sludge is often taken from the return sludge flow from the active sludge step and is pumped to the thickening step.

The sludge can be thickened by three methods: gravity or flotation thickening or centrifugation. Synthetic polymers are sometimes added for better flocculation of the particles. The most common type of thickening is gravity thickening. The sludge is allowed to settle in the thickener, and overflow water is piped back to the plant inlet.

Thickening centrifuges for biological excess sludge are becoming more and more common. With centrifugation the dry solids content can be raised considerably in the best case, from 0.5 per cent to 8 per cent without the addition of polymers. Common thickeners produce a 2–3 per cent increase in the dry solids content.

Sludge stabilisation – digestion

A common stabilisation method is digestion in a digester. During digestion some of the organic material in the sludge is degraded in an anaerobic environment to form methane and carbon dioxide. In a stable sludge the troublesome odours have also been eliminated and a large proportion of the pathogenic bacteria and viruses have been killed.

The cold sludge from the thickeners is heat-exchanged with the hot sludge from the digestion tank that has been digested. The temperature during digestion is normally kept at around 35°C (mesofilic digestion). A less common alternative is to digest the sludge at a higher temperature, 50–55°C (thermofilic digestion). The digestion tank has a retention time of at least 15 days. Digestion consists of three main processes:

1. Hydrolysis of organic material
2. Acidic fermentation
3. Methane formation

The third process is the most sensitive and requires a high temperature.

The digester gas formed (known as biogas) is very high in energy. It consists of 65–70 per cent methane, the rest being mainly carbon dioxide. The gas is collected in a gasholder and is used as heating fuel, for the production of electricity and for heating in an internal combined power and district heating plant. Following treatment, in which the carbon dioxide is removed, the biogas is also used as vehicle fuel. The gas enables treatment plants to satisfy their own heating requirements, part of their electricity needs and some transportation using their own vehicles fuelled by biogas. The gas yield is about 35 litres per person (connected to the sewerage system) per day. From each kilo of organic substance added, 500–700 litres of biogas are formed. In several of the country's major treatment plants digestible materials are added directly to the digestion tank in order to increase the production of biogas. Fat from fat separators in restaurants provides a good supply of biogas.

After digestion the digested sludge is dewatered in centrifuges, filter belt presses or other equipment. Polymers are added to keep the sludge particles together during the process, facilitating dewatering and increasing the dry solids content. In this way it is possible to achieve more then 30 per cent of dry matter. The sludge becomes easier to handle and takes on the consistency of moist earth. The remaining water is firmly bound to the particles and can be separated by drying.

For economic reasons the centrifuges are operated to produce the maximum dry solids content. A higher dry solids content gives smaller volumes and thereby lower transportation costs.

The reject water from the sludge dewatering is piped back into the treatment plant at the inlet. Besides particles, it contains a high amount of ammonium- nitrogen and amounts to 15–20 per cent of the total amount of incoming ammonium.

Separation of substances with different properties

Substances with different properties reach the treatment plant: dissolved, particle-bound, water-soluble and fat-soluble substances. The various stages of the treatment plant deal with the substances in different ways. Some are separated only from the water phase, while others are broken down, wholly or partially. Volatile substances escape into the atmosphere. Generally speaking an attempt is made to separate as much as possible in particle form. Separation mainly takes place by gravity, by means of sedimentation. Presedimentation, intermediate sedimentation and final sedimentation are the large separation steps in a treatment plant and the separated material makes up what we refer to as sludge.

One important and inescapable fact is that by and large no persistent material actually disappears in a treatment plant. Substances which do not degrade finish up in either the atmosphere, the sludge or the receiving waters. Most heavy metals and classical organic micropollutants, such as PCB, are adsorbed to particles and thus distributed to the sludge phase. Only a small percentage are discharged with the effluent into the receiving waters.

Dissolved substances are mainly reduced in the biological step if they are readily degradable. The exception is phosphorus, which is precipitated chemically with a metal salt. Dissolved substances also bind to particles, when they can accompany the sludge to digestion.

At really large treatment plants the treated sewage is filtered through a sand bed before being discharged into the receiving water body. The most important function of the filter is to separate additional particles and thus particle-bound substances.

The amount of floating materials, such as oils and fat, is relatively small after their passage through the screens at the start of the treatment plant, if the screens have a small column width. Hydrophobic substances, mainly fat, are separated in some plants in a fat trap. Otherwise the small

amount of fat accumulates in a small part of the surface of the water during presedimentation and is removed via floating sludge gullies. The floating sludge is often piped to sludge treatment. Fats are best broken down in the digester, when they produce a relatively large amount of biogas.

The sludge – a controversial soil improver

After the sludge has been dewatered it can be used for soil amendment. It contains nutrients, such as nitrogen and phosphorus, as well as large amounts of humus-forming organic material and makes excellent fertilizer for fields, energy forests and soil manufacture. The sludge is also used on landfills, being mixed into the intermediate layer and used as a final covering. The sludge from the Henriksdal treatment plant in Stockholm is used to restore large areas of former mining land in Aitik outside Gällivare in the north of Sweden. Some municipalities incinerate the sludge with domestic waste and in this way are able to utilise the inherent energy.

If the nutrients in the sludge are to be used in the ecocycle, the content of heavy metals and ecologically harmful organic substances needs to be very low. The quality of the sludge is carefully controlled and in many municipalities sludge must be approved for spreading on arable land by a local consultation body, with representatives of environmental authorities, consumers, the Swedish Society for Nature Conservation and the Federation of Swedish Farmers. In recent years the content of harmful substances in the sludge has been the object of hot debate and the spreading of sludge on fields has virtually ceased.

One opening for the spreading of sludge can be found in the ReVAQ research project (which stands for pure plant nutrients from wastewater), in which eight municipalities are currently taking part, with several other stakeholders (see www.revaq.se). The project is a joint venture between municipal wastewater treatment plants, food industry, farming organisations, the environmental movement and also consumers and food retailers, the aim being to establish whether the use of the waterborne sewerage systems can be developed so that sludge from them can be used on cultivated land from a sustainable perspective in accordance with national environmental goals.

For the treatment plants taking part, the project entails a number of undertakings, apart from the need, of course, to comply with existing legislation. Among other things, work must be actively carried out on

limiting pollutants at their source, the sludge must be made hygienic so that there is no risk of the spread of infection, the heavy metal content of the agricultural soil must be checked before the sludge is spread on it, the ratio of cadmium to phosphorus in the sludge must be reported and the sludge must be certified by an independent party. If the requirements of the ReVAQ agreement are satisfied, the sludge may be spread on fields. In 2003 ReVAQ sludge was spread over an area of approximately 1,200 hectares in Sweden (Hansson, 2004).

Regular analyses have been made of the heavy metal content of sludge for just over thirty years, and in the last twelve years it has been analysed for some organic micropollutants. During this period substantial improvements have taken place. The amount of cadmium, for example, has fallen by about 96–97 per cent since the beginning of the 1970s, while there has been a 92–93 per cent fall in mercury. The organic indicator substances (PCB, PAH and nonylphenol) have also decreased.

New techniques of utilising nutrients in sludge are currently being tested in several places. Through supercritical oxidation or wet chemistry methods, phosphorus in particular and also other substances can be recovered from the sludge. Other methods aim to extract the phosphorus from the ash which is a product of sludge incineration.

Treatment plants and drug substances – a complex relationship

The municipal treatment plants have been designed to deal with domestic sewage. The sewage produced by other activities in society is required to be of a "domestic nature" if it is to go to the municipal treatment plant. Industries linked to the sewerage network therefore often have internal treatment plants and equalisation tanks before discharging their sewage into the network.

Research is being carried out into the importance and function of treatment plants in regard to the reduction of drug substances. In general, reduction varies considerably between different substances. It is quite clear that treatment plants are not designed to degrade all types of drug substances. Alternatively, and with greater objectivity, it may be said that drugs are not formulated in order to be degradable in treatment plants!

Active drug substances are generally excreted from the body in the form of water-soluble metabolites or conjugates. If these are not biodegradable, they will end up in the effluent, unless the conjugates are

split in one of the processes at the treatment plant and the fat-soluble parent substance is reformed. There are some antibiotics, e.g. fluoroquinolines, which have turned out to be more particle-bound than one might expect on the basis of their physicochemical properties, which means that they are also present to a greater extent in sludge (Johansson et al., 2003). The alarm is sometimes sounded about an increase in resistance to antibiotics. In the biological treatment process there are a large number of bacteria of various strains and it is likely that resistance to antibiotics could be passed on from one bacterium to another. Here, too, more research is needed to establish whether resistance is transferred and, if so, whether the bacteria are capable of surviving in the natural environment. The reduction of bacteria of all types, however, is large across the processes of the treatment plants (>99.9 per cent, or from 10^{11} to 10^{5} /100 ml).

In general, it may be said that the development of treatment plants with increasingly advanced processes has produced better treatment results. It is likely that drug substances are mainly reduced in the biological treatment step. The introduction of biological nitrogen reduction has increased the opportunities of breaking down organic substances, since a longer retention time in the plant leads to better treatment. This is also true where drug substances are concerned. For example, studies of the effects of oestrogen in effluent have shown that better reduction occurs, the longer the period of contact in the biological treatment step (Svensson et al., 2003). Treatment plants take different forms in different parts of the country and the reduction of drug substances may also vary as a result.

Urine separation is being discussed as a future solution for returning nutrients from people to agriculture. It is of especial importance here to draw attention to the way in which drug substances risk being spread to the environment, as they are generally excreted in urine.

Pollution must be stopped at source

The best way of ridding sludge and effluent of heavy metals and organic micropollutants is by mitigation at source, i.e. ensuring by various means that they never reach the sewers. Once they have been mixed with sewage, it is much more difficult to remove them even if the treatment processes were to be enlarged with additional steps. Chemicals are highly diluted in the enormous amounts of sewage processed by the treatment plants.

There are several important reasons for removing unwanted substances, preferably at the production stage. Apart from protecting the receiving

waters and enabling the sludge to form part of the ecocycle by using it as soil improvement material, this is presumably a successful strategy for achieving the quality objective of a Non-Toxic Environment set by the Swedish Parliament. Substances from chemicals, drugs and products are spread to the environment not only from treatment plants. Chemicals evaporate from construction materials, textiles, plastics etc. and are spread directly into the surrounding air, while storm water and surface run-off carries with it dissolved exhaust fumes from vehicles and particles eroded from tyres and road surfaces.

The personnel and processes of the treatment plants also need to be protected against toxic substances. The heart of the treatment process is the biotreatment, which consists of naturally occurring bacteria and microorganisms, which can easily be destroyed by toxic chemicals. The nitrogen removal is particularly sensitive and can remain out of action for weeks.

As a result of exercising supervision over industries and other activities connected to the sewerage network and laying down requirements for the pretreatment or local treatment of sewage and for the choice of chemicals made by companies, there has been a radical decrease in the accumulation of many heavy metals and organic substances. It is more difficult to limit the flow of substances whose spread is diffuse, such as plasticisers and flame retardants, which leach from electronic products and construction materials, or of chemicals that are destined to end up in the sewerage system, such as detergents and personal care products. One way of doing this is to inform the public about choosing ecolabelled products (such as the Nordic Swan or Good Environmental Choice) and about never pouring chemicals down the drain.

The Water and Sewerage Act can be used in the task of limiting the flow of unwanted substances. It lays down that treatment plants do not need to accept water with a different composition from ordinary domestic wastewater. Other important tools are the substitution principle and the precautionary principle, both of which are enshrined in the Environmental Code.

In the case of drugs, it is not always so easy to make use of these tools. We are obliged to accept the use of certain preparations and the fact that they or their metabolites reach the sewerage system, although we must at least prevent unused drugs from ending up in the toilet or the sink. Here Apoteket has an important role to play in collecting leftover drugs.

New treatment processes

Research is being carried out on the additional processing of already treated effluent. It may be possible in the future to add an extra treatment step to remove some of the substances from the effluent that the existing treatment process is unable to get rid of. Drugs form an important group, although there are also other chemicals whose amounts would decrease in the event of such additional treatment. However, an entire battery of various separation or oxidation techniques will probably be needed in order to free sewage completely from unwanted substances, and so far we know too little to justify investing in such relatively expensive technology. Moreover, it is important to point out that substances that accumulate in the sludge are not reduced by this means.

The best road to go down for the environment as a whole is to stop producing substances that are not suited to the ecocycle and to adapt the use of chemicals in our society to what treatment plants and receiving waters can cope with.

References

Cronström, A (1986). Vattenförsörjning och avlopp, Stockholms tekniska historia. [Cronström, A (1986). Water supplies and sewage, The technical history of Stockholm]

Hansson B, Envisys AB. Personal communication (2004).

Johansson M, Lindberg R, Wennberg P, Tysklind M (2003). Screening av antibiotika i avloppsvatten, slam och fisk under 2002/2003. Rapport, Umeå universitet.
[Johansson M, Lindberg R, Wennberg P, Tysklind M (2003). Screening of antibiotics in sewage, sludge and fish in 2002/2003. Report, University of Umeå.]

Lind A, Swedish Water. Personal communication (2004).

Svenson A, Allard A-S, Ek M (2003). Removal of estrogenicity in Swedish municipal sewage treatment plants, Water Research 37, 4433-4443.

http://www.revaq.se

3 The spread of drugs in soil and water

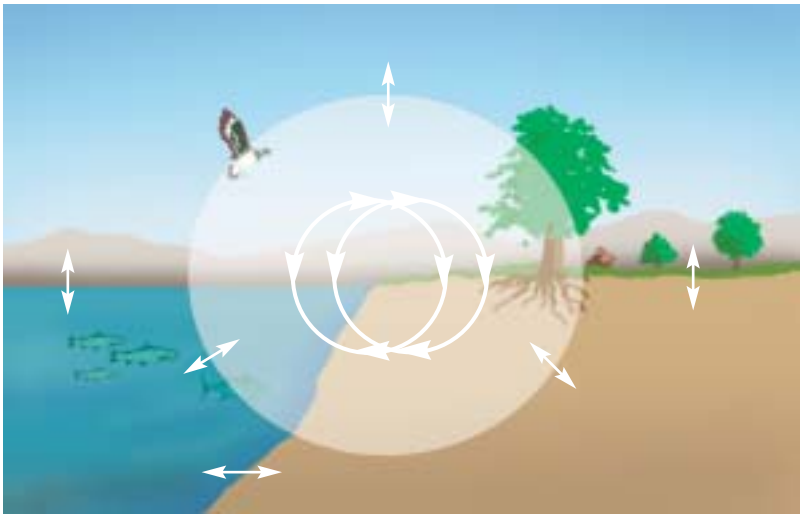
Mats Tysklind, Jerker Fick, University of Umeå, and Roland Kallenborn, University Centre in Svalbard

The natural interplay in the environment

In the last ten years improved analytical instrumentation and refined methods have made it possible to detect a number of new types of chemicals in the environment (Kümmerer, 2004). Among other substances, traces of pharmaceutical and personal care products have been discovered in various environmental tests. The detection of drugs in the environment has given rise to discussions about the term environmental pollutants. Generally speaking, *environmental pollutants* may be described as unwanted chemical substances which break down slowly and have a negative impact on various organisms. On the basis of a wide definition such as this, drug residues and various personal care articles in environmental tests would also be included in the term environmental pollutants.

When discussing the spread of environmental pollutants, it is a good idea to start from the natural interplay in the environment. In general, one can describe processes in the environment as interplay between five "spheres", namely the atmosphere, the geosphere, the hydrosphere, the biosphere and the anthroposphere, which is a part of the biosphere, (see the illustration on the next page).

The atmosphere is the thin layer of gas that surrounds our planet, and it is here that the most effective breakdown of chemical substances takes places, i.e. photochemical degradation. The atmosphere is also extremely efficient in dispersing chemical substances over large areas via air currents. The geosphere is the ground on which we stand, while the hydrosphere is made up of the seas and lakes, which cover 70 per cent of the earth's surface. The biosphere is a collective term for everything living on the earth – plants as well as animals and the anthroposphere is the result of human influence.



A continual interplay between five spheres: the atmosphere, the geosphere, the hydrosphere, the biosphere and the anthroposphere.

These different spheres are interconnected and are entirely dependent on one another. Their composition is also changing constantly and various organic and inorganic substances are continually undergoing transportation and transfer via a number of linked reactions. We can illustrate all this with an example: the exhaust pipe on a car (part of the anthroposphere) emits quantities of carbon-based chemical pollutants into the atmosphere. These chemical pollutants are eventually degraded by sunlight and reactive compounds. Many of them break down completely and form carbon dioxide and water. The newly formed carbon dioxide can either be taken up by plants (part of the biosphere) or be dissolved in the sea (part of the hydrosphere), where carbonates (which become part of the geosphere) can be formed.

The way in which a chemical substance is distributed among the different spheres is governed entirely by its chemical properties. The three properties that have the largest influence are:

Degradability. The speed with which a substance is degraded. If the substance degrades rapidly, it does not have time to travel a long way from the source of the emission. If metabolites or transformation products are formed, these can also affect the environment, even if their properties are entirely different from the original substance.

Volatility. How volatile a substance is, i.e. the ease with which it passes into the gas phase, determines how much of it can be transported and react in the atmosphere.

Water solubility. The ease with which a substance can be dissolved in water. Water-soluble substances are found in watercourses and fat-soluble substances in sediment and soil. Fat-soluble substances have properties that enable them to accumulate in various food chains and to have a large effect on the biosphere.

Anthropogenic environmental pollutants (i.e. pollutants arising from human activity) may be described in this context as chemical compounds which come from the anthroposphere and are distributed among and affect ecosystems and reactions in the various spheres. Examples of their influence are the effect of chlorofluorocarbons (CFCs) on the ozone layer or the impact of trace metals on vegetation etc. Traditional environmental pollutants such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) are spread in the environment through intentional or unintentional use. These substances are very persistent and take a long time to break down. Their levels in the environment can therefore increase, even if the rate at which they are added is very low. Their environmental stability, combined with the fact that they are to some extent volatile, means that they also can travel for long distances in the atmosphere and/or in ocean currents and so be found a long way from their original sources. In actual fact, many of the most common environmental pollutants have been encountered in samples from very remote locations (e.g. Antarctica and the Pacific Ocean), which is a clear indication that they present a global problem (see, for example, Lohmann et al., 2001).

The spread of pharmaceuticals

When describing the spread of an environmental pollutant, there are a number of factors that must be considered, including the nature of the sources of emission, distribution properties, and environmental stability. In these respect pharmaceuticals differ from the more traditional environmental pollutants in that their emissions are directly connected to humans. The largest flow of drugs into the environment comes from people who are under medical treatment. Drugs are eliminated from the body either in unchanged form or as metabolites in faeces and urine and find their way to sewage treatment plants (STP) (see the diagram in Chapter 1 on the flow of drugs in society). Point sources are of limited importance globally, but can effect the environment locally. One of the point sources is hospitals. In the case of certain drugs hospitals are the only source, since some medication and therapeutical treatment only take place there. Usage of iodised X-ray contrast agents is such an example. Other point sources include livestock rearing and fish farming, although all the point sources play a limited role in the overall global context, even if severe local impact has been demonstrated. On this basis we may conclude that a feature of a "hot spot" is a large population of drug consumers occupying a small area.

How drugs later behave in the environment and what happens in the long term will depend on their chemical and physical properties. Pharmaceuticals present a very diverse group of chemicals, which include a large number of compounds with a wide range of properties. In general however, we can conclude that drugs are much more water-soluble than traditional environmental pollutants. Moreover, drugs are, as a rule, not volatile, i.e. they will not be present in the atmosphere and so will not travel long distances. Geographical distribution of most drugs will thus depend on transport via water. This combination of discharge sources and chemical properties means that sewage treatment plants play a central role, since the majority of drugs will pass such a plant on their way into the environment. This route is different compared to most traditional environmental pollutants.

The distribution and behaviour of different drugs in the aqueous phase will differ, both between different pharmaceutical classes and between drugs belonging to the same class. Drugs and their metabolites may be divided roughly into three groups:

- those that break down rapidly
- those that are water-soluble and stable
- those that are fat-soluble and stable

The drugs in these three general categories will be encountered in different places since they vary in their chemical and physical properties.

Drugs that break down rapidly will be found close to their sources and then only if they have been released in large amounts. Acetylsalicylic acid is an example of such a drug.

Drugs that are both water-soluble and stable will pass through a treatment plant more or less unchanged and be dispersed in the surrounding watercourse. Bezafibrate and other lipid-lowering drugs are examples of drugs of this kind.

Drugs that are fat-soluble and stable will adsorb to a considerable extent to sludge particles through the sewage treatment process. Fluoroquinolones (a group of antibiotics) are examples of such drugs.

Many research groups have started to study in detail how the distribution of the various drugs is affected by various factors and properties. The basic problem is that drugs, as a group, also differ here markedly from the more traditional environmental pollutants. The knowledge we have of the way in which PCBs and dioxins, for example, distribute can only be transferred to a limited extent to drugs. As matters stand at present, there are many substantial question marks in this area.

Where have pharmaceutical residues actually been found?

Levels of pharmaceuticals in environmental samples can vary considerably depending on the distance to primary sources and matrix, etc. The concentrations typically measured are in the order of 1 ng to 1 mg per litre, and they are generally found in complex environmental samples, such as sewage. This combination of complex matrixes and low concentrations places considerable demands on the analytical methods used. Technological improvements in recent times, such as refined methods of detection and separation, have made it possible to search for smaller amounts and greater numbers of drugs in the environment. However, there are still no analysis methods for quite a number of the drugs that may theoretically be present

in the environment, which makes their detection impossible at present. Currently, various research activities are contributing to this field and a steady stream of new and improved methods are appearing. At present, there are excellent analytical methods for detecting and quantifying about one-hundred different drugs in the environment. The major surveys that have been conducted have shown that about 50–75 per cent of the drugs for which a search is being made are found (Daughton and Ternes, 1999, Kolpin et al., 2002, Dębska et al., 2004). Geographically, most studies have been carried out in the USA, Germany, Switzerland, Denmark, the Netherlands and France, and for the most part in densely populated areas. However, drugs have also been found in the middle of the North Sea (Buser et al., 1998) and antibiotic-resistant bacteria have been found in Antarctica (Alam and Singh, 2002), which suggests that some drugs already have a global environmental dimension.

Groundwater

Up until now there have been few reports of drugs in the groundwater and most of these surveys show that the detected drugs originate from nearby landfills or other point sources and are therefore geographically limited. However, diclofenac, an analgesic/anti-inflammatory agent, and clofibric acid, a metabolite of the lipid-lowering agent clofibrate, have been found in groundwater without it being possible to identify a separate source. Clofibric acid is regarded as one of the most persistent drug residues and can still be found in different environmental samples, despite the fact that clofibrate has been withdrawn from the market (Buser et al., 1998; Zuccato et al., 2000).

Drinking water

The first pharmaceutical substance detected in drinking water was clofibric acid, found by a German research group in Berlin ten years ago (Stan, 1994). Since then several drugs have been found in drinking water in Germany (Ternes, 2001). They include two other lipid-lowering agents: bezafibrate and a metabolite of fenofibrate. Also found were phenazone (an analgesic) and carbamazepine (an anti-epileptic). These results awakened a large debate, resulting in an investigation by the research group into whether the drug residues that were found could affect people's health.

They showed that drinking water from taps in Germany is safe to drink, the amounts they found being at least one-thousand times (150,000 times in more than 90 per cent of cases) below the therapeutic doses and also below the threshold values for an acceptable daily drug intake (Webb et al., 2003). Drugs have also been found in drinking water outside Germany. In Spain, for example, estradiol and estriol, two hormone preparations, were found (McLachan et al., 2001). Various types of antibiotic have also been detected in drinking water in the USA (Ye et al., 2004). Schwab et al. made a similar health risk assessment in the US which also stated that the present measured levels are very low and that they pose no appreciable human health risk (Schwab et al. 2005). Some concerns about the lack of investigations regarding the environmental or human health hazards that might be posed by chronic, sub-therapeutic exposure to pharmaceuticals have been raised however (e.g. Jones et al. 2005).

Watercourses

A number of drugs have been found in investigations of watercourses. The majority of studies have been carried out in Europe and the amounts in the watercourses are about one-tenth of those found in treated sewage, i.e. 1–100 ng/L (Daughton and Ternes, 1999, Dębska et al., 2004).

A search has been successfully conducted for drugs used for a large number of indications, although the majority of investigations have concentrated on the following classes of drugs:

- analgesic/anti-inflammatory agents
- antibiotics
- hormone preparations
- antihypertensive agents

The analgesic/anti-inflammatory agents that have been found are acetylsalicylic acid (and its metabolites), diclofenac, ibuprofen (and its metabolites), indometacin, ketoprofen, naproxen and phenazone.

The antibiotics found are chloramphenicol, erythromycin, lincomycin and roxithromycin and the two fluoroquinolones ciprofloxacin and norfloxacin. Another group often found is the tetracyclines (e.g. tetracycline, doxycycline and oxytetracycline) as well as the sulphonamides and trimethoprim.

The hormone preparations found are estradiol-17α, estradiol-17ß, ethinylestradiol, mestranol, 19-noretisterone, progesterone, testosterone and estriol.

The antihypertensive agents are exclusively beta-blockers: atenolol, betaxolol, bisoprolol, carazolol, metoprolol, propanolol and timolol.

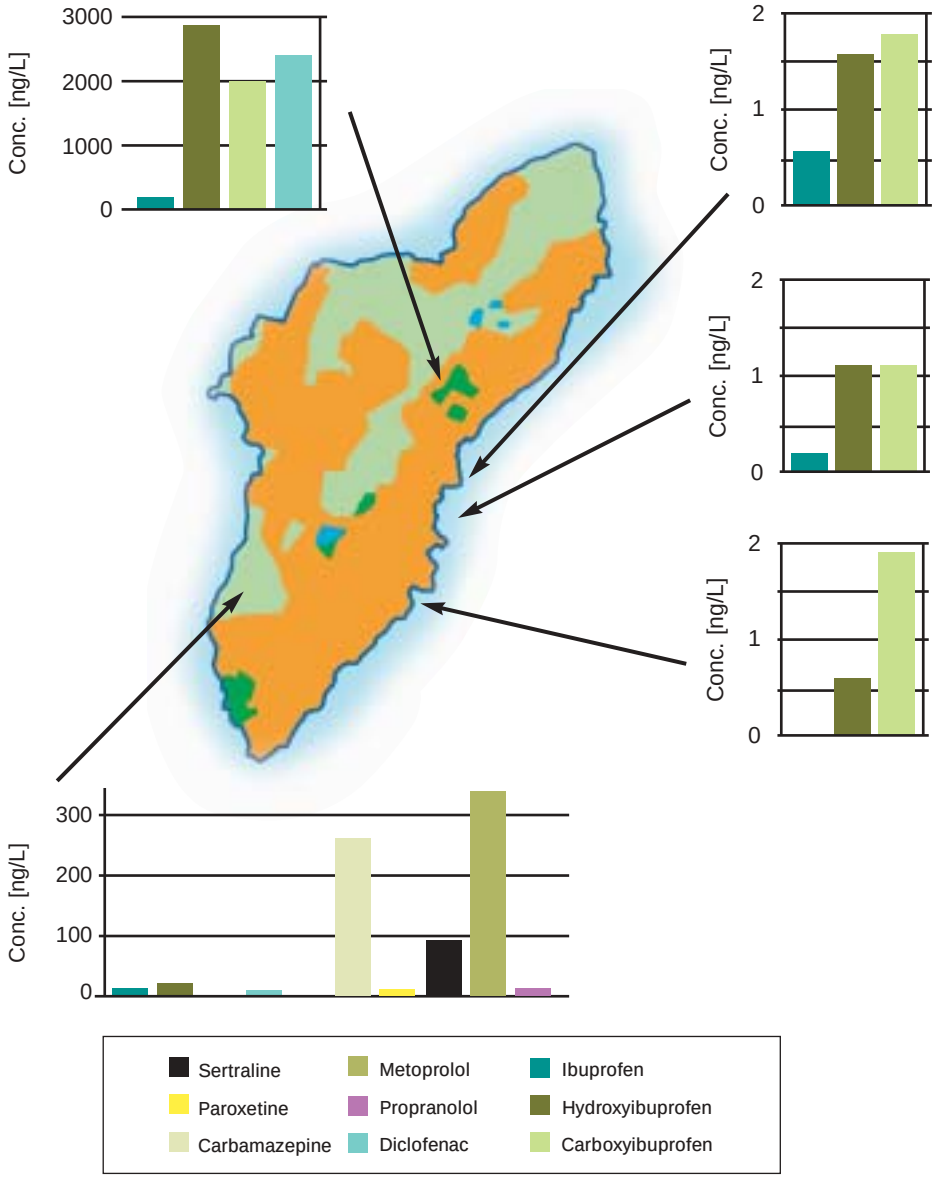
The US geological survey recently presented results from one of the larger surveys that have been made (Kolpin et al., 2002). Samples were taken from 139 rivers in thirty states and 37 different drugs were found. Of these, there were 12 antibiotics and 9 hormone preparations. The levels found were not very high and rarely exceeded the guidelines for drinking or ecological thresholds (Kolpin et al., 2002). One problem, however, was that guidelines and limits are lacking for several of the substances found. In this study five different analytical methods were used to measure the various substances, which may perhaps be an indication of the difficulties one faces when measuring amounts of drugs in the environment.

Soil and sediment

The largest sources of drugs in the soil and sediment are considered to be antibiotics and growth hormones which are used in farming and not in human medicine (Thiele-Bruhn, 2003). The drugs directly enter arable land via grazing animals and/or fertilisers. The presence has been demonstrated in fertilisers of several different preparations for use in veterinary medicine, e.g. sulphonamides and trimethoprim (Haller et al., 2002), as well as other antibiotics such as tetracyclines, aminoglucosides, ß-lactams and macrolides (Thiele-Bruhn, 2003). The fact that this is not just a localised problem was clearly shown by an Italian investigation, which found drugs dispersed over arable land in a number of Italian watercourses (Zuccato et al., 2000). The usage of digested sludge from sewage treatment plants in agriculture has raised the question of whether this can contaminate agriculture areas with drug residues that are bound to sludge (Lindberg et al., 2005).

Surveys in the Nordic countries

Some recent surveys have shown that the presence of pharmaceutical residues may pose an environmental problem in this region (Swedish Medical Products Agency, 2004). The Norwegian Institute for Air Research (NILU) found several pharmaceutical residues present in sewage, sewage effluent



Amounts (ng / L) of a number of drug substances in sewage from a psychiatric ward (upper left diagram) and the university hospital (lower left diagram) and in seawater around Tromsø (diagrams on the right).

and seawater around Tromsø in the north of Norway (Weigel et al., 2004), see the illustration containing the map.

The survey detected the presence in sewage of anti depressive agents (sertraline, paroxetine), analgesics and their degradation products (diclofenac, ibuprofen, carboxy ibuprofen, and hydroxy ibuprofen), beta-blockers used for the treatment of cardiovascular diseases (meto-prolol, propranolol) and anti-epileptics (carbamazepine). The presence of these drugs was shown to be influenced mainly by discharges from the University Hospital and a psychiatric department (see illustration on the next page). Of these substances, ibuprofen and its degradation products were also found in the seawater around Tromsø (Weigel et al., 2004).

In Sweden surveys of drug residues in the environment have begun and in the last few years the results of several such surveys have been published (Swedish Medical Products Agency, 2004). Stockholm County Council carried out a survey of sewage and effluent material, investigating levels of antibiotics in three large sewage treatment plants in Bromma, Henriksdal and Käppala in Stockholm (Wennmalm, 2003). At the same time samples from Lake Mälaren and Lake Saltsjö were examined. Only low levels of most antibiotics were found in the effluent (about 10 ng/L), compared with slightly higher amounts of sulfamethoxazole (max 130 ng/L), trimethoprim (max 470 ng/L) and metronidazole (max 80 ng/L). In Mälaren and Saltsjö only norfloxacin, ofloxacin and trimethoprim were found, and then in very small amounts (max 10 ng/L).

A survey has also been carried out at the Rheumatic Hospital in Spenshult in the south of Sweden, where drug residues at the hospital's own sewage treatment plant were studied. It was shown that 11 of the 14 drugs looked for could be detected in the incoming sewage. The drugs found in the largest concentrations were ibuprofen (77–116 mg/L). The survey also showed that the levels of drugs leaving the treatment plant were below 1 mg/L. The degree of treatment, i.e. the proportion of drug that the treatment plant was able to remove, was shown to vary between 0 and 99 per cent for the various drugs. The content of 26 drugs was measured at the Ryaverken plant in Gothenburg as part of a large EU project under the name REMPHARMAWATER (Andreozzi et al., 2003). The survey showed that 14 of the 26 drugs could be detected in amounts between ng and mg per litre. Ibuprofen was the drug that was detected in the highest amount (7 mg/L).

In another survey of the presence of antibiotic residues in the effluent of five treatment plants, eleven types of antibiotics were detected in amounts between ng and mg per litre. Fluoroquinolones, trimethoprim and doxycycline were measured in all the samples. The results showed that penicillins and cephalosporin's have short half-lives, only a small number of substances from these classes of drugs being detectable in less than half the samples (Lindberg et al., 2005).

References

Alam S I, Singh L (2002). Proteolytic heterotrophic bacteria of cyanobacterial assemblage from Schirmacher oasis, Antarctica, capable of growing under extreme conditions. Current Science, 83/8, 1000-1004.

Andreozzi R, Raffaele M, Paxéus N (2003). Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere, 50, 1319-1330.

Buser H-R, Müller M D, Theobald N (1998). Occurrence of the pharmaceutical drug Clofibric acid and the herbacide Mecoprop in various Swiss lakes and in the North Sea. Environmental Science & Technology, 32, 188-192.

Daughton C S, Ternes T A (1999). Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives, 107, 907-938.

Dębska J, Kot-Wasik A, Namieśnik (2004). Fate and analysis of pharmaceutical residues in the aquatic environment. Critical Reviews in Analytical Chemistry, 34, 51-67.

Haller M Y, Müller S R, McArdell C S, Alder A C, Suter M J-F (2002). Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. Journal of Chromatography A, 952, 111-120.

Halling-Sørensen B, Nors Nielsen S, Lanzky P F, Ingerslev F, Holten Lützhøft H C, Jørgensen S E (1998). Occurrence, fate and effects of pharmaceutical substances in the environment. A review. Chemosphere, 36, 357-393.

Jones O A, Lester J N, Voulvoulis N (2005). Pharmaceuticals: a threat to drinking water. TRENDS in Biotechnology 23, 163-167.

Kolpin D W, Furlong E T, Meyer M T, Thurman E M, Zaugg S D, Barber L B, Buxton H T (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science & Technology, 36, 1202-1211.

Kümmerer K [editor] (2004). Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks. Springer-Verlag.

Lindberg R, Wennberg P, Johansson M, Tysklind M, Anderson B A V (2005). Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environmental Science & Technology 39, 3421-3429.

Lohmann R, Ockenden W A, Shears J, Jones K (2001). Atmospheric distribution of polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs), and nonortho biphenyls (PCBs) along a North-South Atlantic transect. Environmental Science & Technology, 35, 4046-4053.

Läkemedelsverket (2004). Miljöpåverkan från läkemedel samt kosmetiska och hygieniska produkter, Rapport från Läkemedelsverket. Augusti 2004. [Swedish Medical Products Agency (2004). Environmental effects of pharmaceuticals, cosmetics and hygiene products, Official report from the Medical Products Agency. August 2004.]

McLachan J A, Guillette L J, Iguchi T Jr., Toscano W A Jr. (2001). Environmental hormones: The scientific basis of endocrine disruption. Annals of the New York Academy of Sciences, 1-142.

Ternes T A (1998). Occurrence of drugs in German sewage treatment plants and rivers; Water Research, 32, 3245-3260.

Thiele-Bruhn S (2003). Pharmaceutical antibiotic compounds in soil – a review. Journal of Plant Nutrition and Soil Science. 166, 145-167.

Stan H-J, Linkerhägner M (1992). Identification of 2-(4-Chlorophenoxy)-2-methyl-propionic acid in groundwater with GC-AED and GC-MS. Vom Wasser 79, 75-88.

Schwab B W, Hayes E P, Fiori J M, Mastrocco F J, Roden N M, Cragin D, Meyerhoff R D, D'Aco V J, Anderson P D (2005). Human pharmaceuticals in US surface waters: A human health risk assessment. Regulatory Toxicology and Pharmacology 42, 296-312.

Webb S, Ternes T, Gibert M, Olejniczak K (2003). Indirect human exposure to pharmaceuticals via drinking water. Toxicology Letters, 142, 157-167.

Weigel S, Jensen E, Kallenborn R, Kuhlmann J, Thoresen H, Hühnerfuss H (2004). Pharmaceuticals and caffeine in sewage and seawater from Tromsø/ Norway with emphasis on ibuprofen and its metabolites. Chemosphere 56/6, 583-592.

Wennmalm Å (2003). Miljöredovisning 2003 Stockholm läns landsting, rapport. [Environmental report 2003 Stockholm County Council.]

Ye Z, Weinberg H S, Meyer M T (2004). Occurrence of antibiotics in drinking water. 4th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Minneapolis, USA. 138-142.

Zuccato E, Calamari D, Natangelo M, Fanelli R (2000). Presence of therapeutic drugs in the environment. Lancet, 355, 1789-1790.

4 Ecotoxicology

Magnus Breitholtz and Bengt-Erik Bengtsson, Stockholm University

Ecosystems

To protect our environment against the unwanted effects of chemicals, we carry out environmental risk assessments, which entails assessing the risk posed by the presence of chemicals in the environment. A rather vague concept, one might think. After all,

- Which environment is the one we wish to protect?
- For what individual(s) does the presence of a chemical in the environment pose a risk?
- What is the inherent margin of error (or risk) with which the assessment is carried out?

Indeed the questions which surround environmental risk assessments are often numerous and difficult to answer for the uninitiated. Our aim, therefore, in what follows is to try and answer these questions and to clarify and simplify terms, definitions and associations that are relevant for environmental risk assessments.

In today's society the term *environment* is used in various contexts. The environment we shall be discussing here is primarily a collective term for the animals and plants that surround us. A secondary meaning of environment also encompasses the interaction between these animals and plants, i.e. what we learnt at school to call *ecology*, which includes the vital flow of energy that originates in those plants (producers) which by means of photosynthesis convert sunlight to glucose, which in turn is utilised by animals (consumers) higher up in the food web (SFM, 1992). However, the environment also refers to the natural environment in which animals and plants live, together with the oxygen, carbon and nitrogen cycles which enable them to grow and multiply. In order to make clear what we wish to protect, we should perhaps replace the word environment with *ecosystem*, which is simply a summary definition of biological communities and the

non-biological environments in which they live. The biological communities are made up of populations of various species, which in turn consist of individuals from each species.

Each individual belonging to a species competes with the others for food and living space in order to continue living and to reproduce. Populations within and between species do likewise. In other words, in nature there is constant competition, in which strong individuals survive and pass on their genes to future generations. This natural selection results in the population adapting to existing conditions, migrating or dying out (Ulfstrand, 1996). Evolution or nature takes its course, as we say in everyday speech. The environment we wish to protect then is a diversity of ecosystems, which among themselves or under the influence of one another are continually evolving in order to adapt to changed environmental conditions. In the last few centuries, however, many populations, communities and ecosystems have found it difficult to adapt to the rapid and often turbulent changes brought with it by our industrialised society. Evolution, as Darwin once said, is a slow process in which natural selection works silently generation after generation, century after century, millennium after millennium (Uddenberg, 2004). Evolution, as we view it today, has also come to include "technical selection", which operates in parallel with natural selection, but over a shorter time perspective than before.

As everyone will appreciate, we have taken up a large challenge when we say that we seek to protect the environment, since the number of ecosystems is virtually endless and each system has its own character, function and flow of energy. What is a rule on land can function entirely differently in the sea and in yet another way in a freshwater system, such as a lake or a stream. It goes without saying that this diversity makes it practically and economically impossible to protect all species, populations, communities and ecosystems, despite our having set environmental targets for the preservation of biological diversity, which includes both the wealth of species and the number of individuals within each species (Naturvårdsverket, 1994). What then should we focus on with a view to protecting it? It is this question, perhaps, that is most discussed, and the difficult part is to answer it within the framework of an environmental risk assessment. Historically, we have focused firmly on preserving species high up in the food web. One rule of thumb has been (and still is, to a large extent) that the more closely a species resembles people in physiological terms, the greater the reason for protecting it. This is despite the fact that we know

that ecosystems consist of different organisational levels, where different organisms have specific roles and where those that have key functions in the system are not necessarily the largest or the most best-looking. Bear in mind, for example, that without algae and plants we would be without life-sustaining energy from the sun. Without insects, crustaceans and fish, this energy could not be transported further up the food web and eventually be at our disposal. Our wish, in other words, is to preserve today's diversity of species and to protect our ecosystems, although for practical and economic reasons we are unable to do this fully. This is where the risk we take through simplifications and generalisations lies, which we make when we select a very limited number of representatives of the inhabitants of ecosystems, which we then use in order to study how they are affected by our emissions.

Toxicity

To describe a chemical as toxic means that it has adverse biological effects on cells, organs, tissues or physiological systems, which may result in diseases, chronic injuries or, at worst, death. Even if it is sometimes said that it is only the dose which determines whether or not a chemical is toxic, there are today refined explanations of what is and is not toxic (SFM, 1992). For example, with today's biochemical and genetic methods, it is possible to focus with greater precision on the mechanisms of action that lie behind a toxic response. Other important factors which govern the toxicity of a chemical are its absorption and distribution in the body and how efficiently it is neutralised by the body. Sometimes this "detoxification process" may give rise to metabolites that are more toxic than the parent substance. Broadly speaking, these processes unfold in similar ways in different species, although even small evolutionary differences can result in two related species responding in opposite ways to chemical exposure. This fact is exploited, for example, in the control of insects in agriculture and silviculture, where toxiicants are designed to have an effect on target organisms, but not on organisms that may be of positive significance to the environment in which the activity in question takes place. A distinction is also made between acute and chronic toxicity. Acute toxicity normally occurs after a short period of exposure to a high dose or concentration of a chemical and its outcome is nearly always fatal, whereas chronic toxicity occurs after a long period of exposure to a low dose or concentra-

tion. Even here death may result, although sublethal effects, such as impaired reproduction or development, are generally studied in tests involving chronic exposure.

There are a number of (direct or indirect) mechanisms of action that make chemicals toxic (SFM, 1992). Chemicals can bind to molecules such as nucleic acids (DNA and RNA), lipid membranes, hormones and proteins and either destroy the molecule or modify its structure, which in turn leads to changes in physiological functions. In other cases a chemical may bind to receptors or transport proteins designed for hormones, for example, thereby blocking or reinforcing a natural function. Enzymes often work through different substances binding to an "active region" on them. We usually say that the substance must fit this active region like a key in a lock. Certain chemicals can form a bond and thus block the function of the enzyme, while others change the shape of the "lock", preventing the natural substance from binding correctly. The nervous system is an example of a target organ for chemicals that bind to enzymes. At the synapses of the nerve endings a neurotransmitter is released, which triggers a neural response. This response is often short-lived and decreases naturally when the neurotransmitter is broken down by specific enzymes. Chemicals which prevent the breakdown of a neurotransmitter by binding to and blocking the enzymes will in this way prevent the neural response from coming to an end. This may cause tremors, convulsions and, in the worst case, death. Classical nerve gases and certain insecticides (organophosphates) exert their effect through this mechanism. The list of toxic mechanisms is endless – some arise as a consequence of human inventiveness, while others occur unintentionally. It is important to bear in mind, however, that the enzyme and hormone systems present in mammals have arisen through evolution and are in all probability present in other lower organisms, where they control identical, similar or entirely different physiological processes. This is especially important when we study the toxicity of drugs, which perhaps have an enzyme or hormone system as a target organ in many groups of mammals.

What is ecotoxicology?

If we are to describe how toxic a chemical is in nature, we must really take into account the overall adverse impact on the various ecosystems that may come into contact with it. The branch of science which studies the effects

of chemicals on ecosystems is known as ecotoxicology. In ecotoxicology the aim is also to find out in part how chemicals are dispersed, absorbed and possibly accumulate in organisms and also how they break down and/or are transformed in the environment. In the latter case we speak of how persistent a chemical is. Persistence broadly provides us with a measure of how difficult it is for an ecosystem to break down a chemical. This may involve a single emission giving rise to relatively constant levels in the environment over a long period of time. In reality, however, the continual release (e.g. from industry) of a chemical that is readily biodegradable may affect an ecosystem in a similar way. It might be said that the rate of release is then fairly constant in relation to the ability of the ecosystem to break down the chemical, in which process the amounts in the environment remain fairly constant over time. Bioaccumulation in turn describes whether a chemical exists in higher concentrations in an organism than in its surrounding medium or its food (SFM, 1992). Bioconcentration is another term that is used, mainly in regard to organisms in the aquatic environment, and describes the concentration of a substance in an organism in relation to the concentration in the surrounding water. In order to describe in simple terms how chemicals are absorbed and possibly become bioconcentrated in organisms, we usually make use of the fat solubility of chemicals. Empirical data have shown that bioconcentration is correlated with fat solubility, at least up to a very high degree of fat solubility. In reality, however, the metabolism of an organism also influences the effectiveness with which a chemical is absorbed and neutralised, thereby playing an important part in bioconcentration. The connection between bioconcentration and fat solubility, in other words, should be regarded as a rough ecotoxicological tool.

Ecotoxicology then is an interdisciplinary subject which draws on environmental chemistry, toxicology and ecology. We are thus dependent on competence and capacity in analytical chemistry and on analytical sensitivity to the substances to be studied, within the concentration ranges that can produce biological effects even at very low amounts, albeit during a long period of exposure. The ability to detect and test metabolites is also something we cannot do without. Relatively free access to the active substances/classes of substances is a condition for the intercalibration of methods and also for testing by independent research groups/test laboratories. Greater openness in regard to toxicological test results (including negative results) increases the possibility for the research community to take note of existing information and reduces expensive duplication of

work. It is also this type of information that should in the future constitute the basis for a more selective choice of which test methods/test organisms should come into question in the first place, on the basis of known or probable biological effects. Finally, ecotoxicology makes great demands on ecological competence, which facilitates our understanding of what environmental consequences we can attribute to the test results we obtain in the laboratory. As we learn more in this area, it may also become relevant to supplement or replace existing ecotoxicological methods with ones that have a better predictive value for the environmental risk assessment than the methods in use today.

It is there then that the great difference between health and environmental risk assessments lies. Whereas a health risk assessment assesses the risk (including toxicity) to a species (man), an environmental risk assessment seeks to take account of millions of species that differ in regard to physiology, sensitivity, living strategies, habitats, niches, positions in food webs and roles in the energy flow.

This evolutionary tree seeks to remind us of the common origin of living things. It signifies, for example, that the enzyme and hormone systems found in humans and other mammals are also in all probability found in other “lower-order” organisms, in which they may control identical, similar or entirely different physiological processes. This is something we must take into account when we carry out an environmental risk assessment of a drug.

Why the aquatic environment?

Why is the aquatic environment so important when we seek to assess the environmental effects of drugs? If we look at the diagram on page 15 of Chapter 1, we see that a drug or its breakdown products sooner or later risk ending up the aquatic environment, generally after passing through our municipal sewage treatment plants for used or discarded preparations or via industrial processes for drug manufacture and treatment plants connected to them. In other words, drug residues are more likely to end up in the aquatic environment than in any other part of the environment. In the aquatic environment they could theoretically be metabolised via food chains and possibly find their way back to humans, e.g. via fish or other food from the aquatic environment. The main concern perhaps is not people and their health in this context, but whether organisms that make up a significant part of the aquatic ecosystem can be affected. One important exception/special case, however, may exist in the form of the large-scale use of antibiotics in marine aquaculture, e.g. cultivated giant shrimps which are imported mainly from Asia (Holmström et al., 2003). There is a clear risk here of resistant pathogens, including human pathogens, developing locally and then spreading on a global scale with the product. This may impact on everything from bacteria, microalgae, single-celled organisms, invertebrates (e.g. crustaceans, worms and molluscs), higher plants and fish to various animals that in turn feed on them, e.g. fish-eating birds and mammals. These organisms can show both similarities and dissimilarities to people, which determines what effects may develop and should therefore be studied. We may perhaps share one and the same enzyme system with relatively primitive organisms, but where evolution has successively homed in on another mechanism in man than the one which perhaps occurs in a mollusc (such as a snail or mussel). This may mean that a desired medical effect in humans may have an effective but fatal outcome in another groups of organisms. If we take into account the fact that the aquatic environment includes hundreds of thousands of species that have specialised in different ways through adaptations to different environments, we can easily understand that the risk is great that a particularly weak link may exist and may therefore be affected as well.

For us, water is relatively seldom an important source for the intake of foreign bodies, since we only take in waterborne substances via the water we drink and the water that is used in the preparation of our meals. In the case of aquatic organisms, on the other hand, water is a much more impor-

tant source of exposure, since they are also exposed via their gills during oxygen replenishment. In the case of smaller organisms and organisms at young evolutionary stages, with thin walls separating them from the environment, osmotic transport in or out of the organism is also an important route of exposure. Aquatic organisms may thus, in principle, be expected to be more sensitive to substances present in water than we are. The aquatic environment in turn offers a wide spectrum of life forms, with far-reaching specialisation and sometimes extreme adaptations and mutual dependencies (compare, for example, the complexity of the coral reef). Many organisms feed by filtering particles that are suspended in the water or by "eating their way through" and/or living in the sediment particles that make up the soft sea floor. In the case of a substance which is sparingly soluble in the aquatic phase and so tends to bind to small particles, exposure via the aquatic phase (i.e. the concentration in the water) is of secondary importance for how these organisms are exposed. It follows from this that tests carried out on organisms that are exposed to/consume particle-bound substances should be considered for an environmental risk assessment of sparingly soluble substances.

Ecotoxicological test methods

In laboratory tests of various organisms one can investigate whether a substance causes visible or microscopic changes in different organs, physiological changes, reduced capacity for reproduction, fetal damage, cancer, behavioural changes etc. One can also find out the mechanisms of action of a substance through various experiments often carried out in vitro in test tubes, using, for example, tissue homogenate, cell or organ cultures. Unfortunately, the majority of methods are not specific or particularly suitable in general for use in an environmental risk assessment.

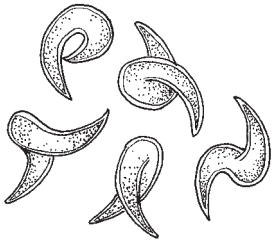
Given what we really wish to protect (i.e. ecosystems) through environmental risk assessments, there are many who think that even the ecotoxicological methods of testing and tools in use today are not suitable for predicting the effects of chemicals in the environment, particularly where drugs are concerned. It is clear that from a strictly ecological point of view we should study the effects of chemicals directly in the environment. It is difficult, however, to attribute an environmental effect in nature to a specific substance, since simultaneous exposure to a mixture of several different substances with overlapping effects often occurs, and

also in organisms that even under natural conditions show considerable variation in number and condition. Impaired reproductive capacity, fetal damage, inhibited growth and developing, a skewed gender balance, changes in mating behaviour or behavioural changes regarding the care of offspring are all effects which may occur and are particularly serious, since in the long term they can change the composition of an ecosystem and impoverish its diversity. Despite this, they can be difficult to substantiate in the environment before the extent of the damage has become unacceptable. Our aim must therefore be to focus as far as possible on prevention, i.e. we should seek to predict the potential effects of a chemical in the environment and then by various means prevent them from taking place.

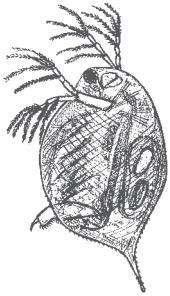
An environmental risk assessment (see Chapters 7 and 8) is therefore broadly based on a strategy whose aim is to estimate, on the one hand, what concentrations or amounts will reach the environment and, on the other hand, whether these estimated amounts or concentrations will have any effect on a small selection of organisms, which will then represent the whole ecosystem.

The methods of testing required by present-day legislation have come into existence in order to illustrate in a simple manner acute effects on representatives of three stages of a notional food chain. A primary producer (a plant) utilises solar energy and serves as food for a crustacean, which is in turn eaten by a fish. Although this is a good principle, it is, as mentioned, simple as applied in present-day practice. In a microalga (generally *Selenastrum capricornutum*) a study is made of growth inhibition in a number of new individuals through asexual multiplication for 72 hours. In the water flea (usually of the *Daphnia* family) mortality/immobility is measured after 48 hours, the result being expressed as EC_{50}, i.e. the concentration which after 72 and 48 hours respectively reduces growth and the number of mobile individuals by 50 per cent. In fish (generally an adult zebra fish) the number of individuals that have died after 96 hours is measured. Use is then made of the concept LC_{50}, i.e. the concentration which after 96 hours kills 50 per cent of the fish (96-h LC_{50}). Of these tests, the alga test is probably the most relevant, since what is measured is a sublethal (nonfatal) effect and several generations of the alga are exposed. In order for comparable sensitivity to be attained for crustaceans, at least reproduction should be included. In the case of *Daphnia* and other water fleas, this test usually includes only parthenogenesis (non-sexual reproduction) and also part of the early larval development. Parthenogenesis is,

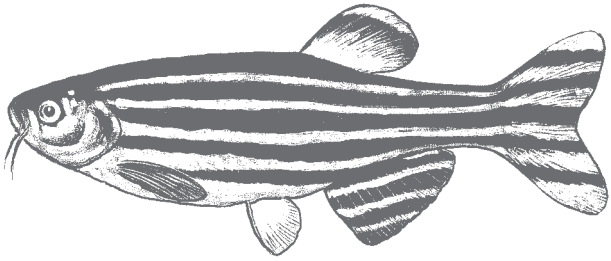
however, not especially typical for crustaceans and does not include, for example, mating behaviour and any effects on males and their role in reproduction. For this reason there are OECD proposals for alternative test methods for crustaceans, which give much more information about the possible toxic effect than the established acute test with *Daphnia*.



Selenastrum capricornutum (microalga)



Daphnia magna (water flea)



Danio rerio (zebra fish)

There are several levels of ambition/methods for testing the toxicity of chemicals in aquatic organisms. One of the simplest involves the use of a combination of three short-period tests (2–4 days) on representatives of a simple food chain consisting of a microalga, a crustacean and a fish. These are represented here by *Selenastrum capricornutum* (microalga), *Daphnia magna* (water flea) and *Danio rerio* (zebra fish).

In traditional drug testing use is made of experimental animals that *show large similarities with man*. With regard to environmental risk assessments, it is also necessary to focus on what is very *different from man*, but where an adverse effect may, nevertheless, occur. If a substance severely affects, for example, the ability of chlorophyll to convert solar energy, this certainly has no counterpart in man, although it may have very serious consequences for the entire ecosystem on which we are dependent. One must also understand the dilemma present in selecting a reasonable number of representative organisms and effect criteria in order to carry out a necessary environmental risk assessment at a reasonable cost. We can then ask ourselves whether we can accept, as is the case today, that a single microalga, a single crustacean and a single fish, whose growth and survival we measure in acute tests, are sufficient to enable us to assess potentially acute and chronic effects in the environment (see Chapters 7 and 8). We can easily see that this is an enormous simplification and a concession aimed at keeping the costs of producing new chemicals and drugs at a low level. This "haggling" naturally involves some uncertainty and thus a greater "risk" of our missing adverse environmental effects. In the case of chemicals in general, where there is not necessarily a desired biological effect, one may perhaps allow a slightly more basic analysis. In the case of drugs, on the other hand, there may be reasons to adopt a somewhat more ambitious strategy. One should be able, for example, to make use of our knowledge of the mechanisms of action to select which environment-related tests are most necessary, instead of treating all drugs as if they were potentially dangerous or harmless to an equal degree. We could then carry out much better risk assessments founded on reasoned knowledge and on this basis a more effective test strategy (e.g. a stepwise test procedure) in order to detect environmental toxicity. In this way it might perhaps be possible in some cases to dispense with the microalga test in favour of a test of some higher plant, or to replace acute tests of water fleas with tests that cover both the sensitive period of moulting or sexual reproduction of some other species of crustacean or a mollusc. Instead of acute fatality tests on zebra fish, one could also, for example, employ fish embryo tests with many physiological effect variables that measure sublethal effects (e.g. heart rate, organ development and deformities). There is considerable scope, therefore, for developing and using test methods that have higher sensitivity and relevance after a more intellectual prior examination of what effects could be expected, even if this initially takes place at the cost of standardisation.

Experimental animals – ethical aspects

Considerations of animal ethics have meant that tests of vertebrates (and hence fish) have become fenced with restrictions. Acute tests on fish where nothing other than mortality is measured have therefore been the object of severe criticism. As an alternative, the development has taken place, especially in Germany, of embryo tests on zebra fish that not only reduce the number of experimental animals, but also include measurements of much subtler features than mortality (Schulte & Nagel, 1994). It is very likely that tests of this kind will be increasingly employed in the future as a replacement for acute tests on adult fish. For many years attempts have also been made to replace fish tests with cell cultures of various fish species; however, this has so far not produced sufficiently practicable results to allow the replacement of tests on living fish.

To a greater and greater extent we may expect the number of animal experiments to decrease and be replaced by alternative test methods. This approach has been actively promoted mainly by animal rights activities and other pressure groups both inside and outside Sweden. Society has also taken on board some of the criticism of animal experiments, and all licences for experiments on vertebrates in Sweden now go through animal ethics committees. This has in turn increased the need for alternative test methods, e.g. using cell cultures, in order to bring down further the number of classical animal experiments.

In Sweden a new authority, the Animal Protection Agency, has recently been established, whose tasks include controlling and developing this activity. In the EU we also have ECVAM (the European Centre for the Validation of Alternative Methods), which has even more specialised skills for moving developments in the same direction and in accordance with existing EU policy, without thereby endangering its scientific basis. Today the risk assessment of chemicals, including the effects of drugs in the environment, is based on relatively simple tests on microalgae and small crustaceans (usually *Daphnia*) and acute tests (which measure fatal doses) on fish. Proposals have recently been made for the simplification of this procedure in order to reduce the use of experimental fish. A simplified pilot test is therefore being proposed, involving a very small number of fish, in order to see whether higher sensitivity can be achieved than by using microalgae and *Daphnia*. Only if the pilot test points to greater toxicity than in relation to the other two species will a full acute test on fish be carried out. Under this proposal, the number of fish used when

testing drugs will fall by more than 70 per cent (Hutchinson et al., 2003). In the future we can expect to see further departures from conventional LC_{50} tests on fish. Only about ten years ago alternative proposals were made for fish tests using fish embryos instead of adult fish. Recently an American pharmaceutical company, Phylonix Pharmaceuticals Inc., acquired a US patent on the actual principle of testing drugs with zebra fish embryos. The conclusion has thus been drawn that this methodology should have a wide application and significance for the future test of drugs.

Environmental risk assessments of drugs in the 21st century?

Drugs, unlike general chemicals used in industrial processes or products, are designed to have a biological effect in man. There is nothing strange about the possibility of other species also being affected. All life has the same origin and the substances that form part of our systems, such as hormones, are found in everything from fish to crustaceans. Sometimes they have the same function as in ourselves, and sometimes a completely different one. This fact, in particular, is something that the future environmental risk assessment of drugs should lean towards. We only have to find out which organisms in the ecosystems we wish to protect are most exposed and most sensitive and whether these particular organisms have the same, similar or entirely different enzyme, hormone or nervous systems, but where the specific mechanism we wish to control in people is in some way involved. Other particularly important questions to be considered in an environmental risk assessment include:

- How and where is the substance emitted? How is it distributed in the environment?
- What is the situation regarding bioavailability and which classes of organisms (e.g. sediment filterers) and which organisms, depending on physicochemical properties of the substance, are primarily in the risk zone?

In 2004 the Swedish Medical Products Agency presented a report which dealt with the environmental impact of drugs (Läkemedelsverket, 2004). According to the report, there is a considerable shortage of facts to enable either environmental toxicity assessments or environmental risk

assessments to be carried out. One particular shortcoming mentioned is that the existing test methods do not take account of the biological activity that characterises the pharmaceutical substance. In an examination made by Stockholm County Council of 159 drug substances, only two were found to be readily degradable; the others were persistent or lacked data about their degradability (Miljöklassificerade läkemedel, 2005). There was a potential for bioaccumulation in 54 of the 159 substances. Low or moderate ecotoxicity applied to 62 substances, compared with high or very high ecotoxicity in 97 substances. These include many modern drugs which have specific mechanisms of action and which are used by a large proportion of the population, e.g. drugs for cardiovascular disease such as beta-blockers, calcium antagonists, ACE inhibitors and modern anti-cholesterol drugs . We have considerable knowledge of the effect of these substances in man, but rather inadequate knowledge of their effect on fauna and flora generally. No clear difference in regard to environmental impact could be shown between drugs for different therapeutic areas. On the other hand, in some classes of drugs with the same mechanism of action it appears that the degree of environmental impact can vary. Examples are the fluoroquinolones, which are used, for example, in urinary tract infections, and beta-blockers, used in cardiovascular disease.

Another class of interest with a specific mechanism of action is modern antidepressants, the selective serotonin reuptake inhibitors (SSRIs), whose use is increasing. The report of the Swedish Medical Products Agency here includes substances which are provisionally classified as toxic/highly toxic and persistent from an ecological point of view, where our knowledge of their effects in man is relatively good, but rather thin where other species are concerned. SSRI drugs all appear to be persistent and ecotoxic, though not bioaccumulating (Läkemedelsverket, 2004).

Other classes of drugs which may include toxic/highly toxic and persistent substances are drugs to combat tumours and disorders of the immune system, cytostatic/cytotoxic agents, antibiotics (see Chapter 5) and hormones (see Chapter 6).

The pharmaceutical industry, the research community and the authorities are agreed that it is not the acute toxic effects that cause us concern in the case of drugs. The ecotoxicological tests carried out on drugs today are insufficient to allow us to predict what may happen in nature. Instead we should focus on the chronic effects that may occur during a long period of exposure to low doses. This requires a greater number of both analytical

and ecotoxicological methods and tests. In addition, it is necessary for the environmental risk assessment to become closer to the health risk assessment in order to take note of the biological effects and mechanisms of which we are already aware. In this connection ecotoxicologists whose work involves environmental risk assessment should have access to valuable pharmacological registers.

Finally, in order to get as much as possible out of the environmental risk assessment of drugs in the future, we set out here an approach which is capable of combining economy with scientific content in an effective manner:

- focus on the aquatic environment, since this is where drugs end up sooner or later,
- use the same classes of test animals as for general chemicals (REACH, **r**egistration, **e**valuation and **a**uthorisation of **ch**emicals), but increase the number selected so as to have access to representatives of a greater number of and more representative ecosystems,
- increase knowledge of the basic physiology of the test animal organisms selected to represent the inhabitants of the ecosystems,
- incorporate people into the ecosystems we wish to protect or reduce the gap between the health risk to people and the environ mental risk assessment,
- use pharmacological data and in vitro (e.g. cell culture) methods to discover mechanisms of action and general biological activity,
- combine the above points in order to select in an effective manner possible target organs of the classes of test animals (i.e. representatives of the ecosystems) and in order to provide guidance as to whether their entire or perhaps part of their life cycle needs to be studied. Every simplification that is not made at the cost of scientific content and relevance is of considerable ecotoxicological benefit.

References

Holmström K, Gräslund S, Wahlström A, Poungshompoo S, Bengtsson B-E & Kautsky N. (2003). Antibiotic use in shrimp farming and implications for environmental impacts and human health. Int. J. Food. Sci. Tech., 38 (3), 255-266.

Hutchinson T H, Barrett S, Buzby M, Constable D, Hartmann A, Hayes E, Huggett D, Länge R, Lillicrap A D, Straub J O & Thompson R S (2003). A strategy to reduce the numbers of fish used in acute ecotoxicity testing of pharmaceuticals. Environmental Toxicology and Chemistry 22: 3031-3036.

Läkemedelsverket (2004). Miljöpåverkan från läkemedel samt kosmetiska och hygieniska produkter. Rapport från Läkemedelsverket. Augusti 2004. 286 sidor. [Swedish Medical Products Agency (2004). Environmental effects of pharmaceuticals, cosmetics and hygiene products. Official report from the Medical Products Agency. Aug 2004]

Miljöklassificerade läkemedel 2005, Stockholms läns landsting. [Environmentally classified drugs 2005, Stockholm County Council.]

Naturvårdsverket (1994). BiologiskMångfald i Sverige – En Landstudie. Monitor 14. Naturvårdsverkets Förlag. 280 sidor. [Swedish Environmental Protection Agency. Biological Diversity in Sweden – a National Study. Monitor 24. 280 pages.]

Personligt meddelande Åke Wennmalm, Stockholms läns landsting. [Personal communication, Åke Wennmalm, Stockholm County Council.]

Schulte C & Nagel R (1994). Testing acute toxicity in the embryo of zebrafish (Brachydanio rerio) as an alternative to the acute fish test: preliminary results. ATLA 22:12-19.

SFM (1992). Miljö från A till Ö – Svenska Folkets Miljölexikon. Det Naturliga Steget, Bokförlaget Bra Böcker. 320 sidor. [SFM (1992). The Environment from A to Z – Swedish Popular Environmental Lexicon.The Natural Step, Book Publisher Bra Böcker. 320 pages.]

Uddenberg N (2004). Idéer om Livet. Band 1: En levande materia: mångfald och utveckling. Natur and Kultur. 310 sidor. [Uddenberg N (2004). Ideas about Life. Volume 1: Living matter: diversity and development. Natur och Kultur. 310 pages.]

Ulfstrand S (1996). Fågel, Fisk & Folk. Forskningens Frontlinjer, Universitetsförlaget. 147 sidor. [Ulfstrand S (1996). Birds, Fish & People. The Front Lines of Research, Universitetsförlaget. 147 pages.]

5 Antibiotics in the environment

Niklas Johansson, Swedish Environmental Protection Agency,
and Roland Möllby, Karolinska Institute

The word antibiotic is derived from the Greek anti (= against) and biotikos (=living).

Many living organisms have the ability to form substances that can affect other organisms' capacity for growth, survival, communication, reproduction and other central processes. Microorganisms, too, have a multifaceted ability to suppress the growth and function of other microorganisms by forming, and excreting at appropriate moments, biologically active substances. We refer to substances of this kind as *antibiotics*. These naturally formed antibiotics are basically an important regulator of the structure and function of different ecosystems.

The ability of organisms to form antibiotics has also been of crucial importance for the evolution of different life forms and their ability to adapt to new environments. Even today naturally formed antibiotics are an important component of the functions of various biological systems. Shifts in these interactions or in the ability of natural antibiotics to affect various microorganisms can thus potentially lead to major changes in ecosystems. Naturally, many microorganisms have also developed various methods to counter the effects of antibiotics. The mere presence of an antibiotic results in species and strains that are less sensitive to it being favoured at the cost of species that have not developed this means of defence. Possessing or acquiring and maintaining resistance does not come without cost to an organism. In a situation uninfluenced by antibiotics, therefore, resistance can be a small, though negative, selection factor.

Paul Ehrlich's discovery of Salvarsan in 1910 and Domagk's description of the antibacterial effects of sulphonamides (sulfa preparations) in 1935 were of decisive importance to people's ability to treat and their approach to treating bacterial infectious diseases. The scope for treating infectious diseases improved further with the discovery and characterisa-

tion of penicillin and streptomycin by Alexander Fleming and Henry Waxman respectively. The therapeutic significance of these preparations was enormous, although it was soon realised that they were not sufficient to cope with all situations. This led in turn to efforts aimed at obtaining access to more specific preparations, which also had fewer pronounced side effects.

The development of new, synthetic antibiotics has been successful and has given us access to a large number of substances with more or less specific areas of application.

The present-day use of synthetic antibiotics results in a significant proportion of them finding their way into the environment. Since antibiotics are bioavailable and many of them are not readily degradable, they can also exert a biological effect in the environment for a long period. In Sweden the dominant class of antibiotics is the penicillins. These are not always readily degradable, which enables some of them to have long-term effects in the environment. Tetracyclines, on the other hand, which are used widely in veterinary medicine, are broken down relatively quickly by ultraviolet light, while macrolides and quinolone derivatives, used mainly in human medicine, are often persistent. Of the total of 80 tonnes of antibiotics used in Sweden each year, perhaps 10 tonnes may be regarded as fairly persistent substances. Antibiotics are also used in various animal feeds, although their use here has fallen markedly. In 1986 40–50 tonnes were used in feed, a figure which had fallen to 14 tonnes by 1995. At the present time their use in feed has decreased to approximately 800 kg (Swedish Board of Agriculture, 2004). The total amount of antibiotics used in veterinary medicine in 2003 was 16 tonnes. There is no absolute connection, but natural antibiotics are probably more readily degradable and so have a shorter lifetime in the environment. At present we cannot quantify the potential effect on ecosystems that today's use of antibiotics may have in the short or the long term.

When antibiotics find their way into the environment, groups of microorganisms may be inhibited by their direct effects, while others may develop more or less pronounced resistance to them. Both these effects, if they are sufficiently large, may lead not only to biodiversity potentially being put at risk, but also to changes in the function of ecosystems.

Antibiotics in the environment are not just able to affect the communities of microorganisms that are sensitive to them; there is also a possibility of microorganisms which are to a large extent dependent on the pro-

duction of antibiotics for their competitiveness being affected. The latter may "benefit" to a smaller extent from their production as a result of other groups of microorganisms increasing their resistance to antibiotics because of the pressure from synthetic antibiotics. In the long run a change in the composition of the microflora for these reasons could prove to be just as important as the change caused by the (short-lived) direct effects of antibiotics. In man, such an increase in resistance in naturally occurring pathogenic organisms could represent a potential risk factor.

The resistance that can develop may be of two kinds. It may be linked to the central genetic material of the organism, i.e. the chromosomes, and will then mainly follow the species/strain in future generations. It may also be linked to genes present in the plasmids of cells, small extra chromosomal elements (i.e. the free DNA fragments inside the cell), which contain different genes. These plasmids can be transferred between different strains of bacteria, both within the same species and between different species. When the resistance gene sits on a plasmid it can be transferred between different species of bacteria in a very short time if there is a selective pressure for this. The location of the resistance gene in the organism's genome is also important for the risk of transfer.

Bacteria lack sexual multiplication, which means that the transfer of genes present on small, transferable DNA elements (plasmids) in bacteria occurs to a much greater extent than the exchange of DNA elements from the bacterium's chromosome. The risk of gene transfer is thus greater between bacteria than, for example, between transgenic plants and bacteria.

The fact that resistance to antibiotics can be transferred from one microorganism to another, irrespective of their affinities, can in itself affect the composition, spread and function of the natural microflora.

Antibiotic resistance is also incorporated into genetically modified organisms (GMOs) as part of their design. As mentioned above, gene transfer between bacteria can occur frequently, even in environments such as soil and water (biofilms). If a gene does not confer increased survival value, it is likely to disappear relatively quickly from a bacterial population. If, however, the bacteria are exposed to selection pressure (in this case antibiotics), the antibiotic resistance gene remains in the population. Genes can also spread between plants and bacteria, albeit at a low rate of occurrence, which means that GMOs that contain resistance genes are a source of the further spread of these genes.

Antibiotics as plant protection agents

The use of plant protection agents is the subject of a National Chemicals Inspectorate regulation, which prohibits the use of such agents in Sweden if they contain antibiotics. On the other hand, use is made of four agents that contain microorganisms, which can form antibiotic substances. These agents are used for treating seed or spraying cultivation substrates in greenhouses. The reason why plant protection agents containing microorganisms are used at all is that (correctly used) they are relatively harmless to the user and nontoxic to the environment, compared with conventional pesticides,. The risk of diseases spreading when bacteria are used as active substances in plant protection agents is minimal, since the bacteria used are not pathogenic. On the other hand, their use may increase the risk of resistance to antibiotics spreading, including to strains of bacteria which may in the long term conceivably pose a threat to the health of animals or people. There are good reasons, therefore, for being very restrictive in the use of microorganisms that are capable of producing antibiotic substances used in human and veterinary medicine, particularly those which do not break down sufficiently rapidly, but risk being spread with feed and water. In the EU Sweden has taken up the question of antibiotics as plant protection agents, arguing:

- that work should start immediately on describing their current use, identifying risks and assessing relevant measures at national and at EU level,
- that advantage should also be taken of existing experience and recommendations for antimicrobial food additives in the use of plant protection,
- that attention should also be paid to the use of plant projection agents containing microorganisms capable of producing substances used in human and veterinary medicine.

It has been decided by the EU that the use of antibiotic resistance for the purpose of plant improvement should be phased out over the next few years. The antibiotic resistance markers that are, nevertheless, used in various types of research and development activities should not be of interest or of potential interest for use in human or veterinary medicine. Other markers which may be used for selection purposes include herbicide resistance. If the latter is still there when the genetically modified plant comes onto the market, there is a clear risk of herbicides being used on the "wrong" crop.

In 2000 a consultation paper entitled "National plan of action against antibiotic resistance" was circulated in Sweden by the Ministry of Health and Social Affairs. In the plan it was proposed that Sweden should "… *work towards a development whereby no use is made of antibiotic resistance genes* …". The Swedish Environmental Protection Agency emphasised in this context the importance of the need for alternative techniques to be ecologically and environmentally sound. Alternatively, they should either be non-functional after the selection process or be able to be removed from the genome before planting of the modified varieties takes place.

Any measures in this area should be concentrated on the removal of antibiotic resistance in GM microorganisms intended for open use in environments where they risk transferring resistance to human pathogenic bacteria. A discussion is currently taking place within the EU about which antibiotics this should apply to, and there are differences of opinion here between GMO producers and healthcare professionals, agricultural experts and medical microbiologists, and not least between different countries with different views concerning the use of antibiotics and the significance of the problem of resistance. GMOs which are considered to be of especial importance are those that are designed for human consumption (e.g. maize), in other words those whose genes will be present in individuals who may be taking antibiotics at the same time. The risk here of resistance genes being transferred to human microogranisms and their release into nature via sewage is obvious, even though there is as yet no agreement about the size of the risk.

From a broad environmental perspective, the goal for the use of antibiotics in society should be to prevent risks of long-term adverse effects in man and animals as a consequence of exposure to microorganisms with increased antibiotic resistance or to biodiversity or the conditions

of sustainable cultivation being put at peril. In order to achieve this goal, it is necessary to acquire additional knowledge of the extent to which the current use of antibiotics results in a biological effect in various systems in the natural environment and ultimately in man and to increase our knowledge of how much, by what routes and to what "final destinations" different types of antibiotics are added each year to the environment. We also need to acquire information about the (chemical and biological) degradation of the various antibiotics in various relevant environments. For this the development of standardised analytical methods for antibiotics and their degradation products is necessary. Finally, when choosing between various preparations, one should, wherever possible, select those with as short as possible a half-life in the environment (a "short eco-shadow").

Resistant microorganisms (bacteria)

The impact of antibiotics released into the environment consists mainly in the risk that the growth of resistant microorganisms will be favoured and also, as a consequence, the existence of resistance genes, which may spread further. Two problems can be distinguished: (a) ecological disruption caused by the growth of certain microorganisms at the expense of others, and (b) selection and maintenance of the occurrence of human pathogenic bacteria in effluent and receiving bodies of water.

Human bacteria which contain resistance genes for common antibiotics occur freely in nature, since they are created both by both human and veterinary medicine and are released without restrictions. However, it has also been found that the environment may also contain resistant bacteria to a higher degree than seen in the healthcare sector, i.e. rare bacteria which possess significant resistance may be much more common than expected. For example, vancomycin-resistant enterococci (bacteria which occur naturally in the human intestine) pose a threat to healthcare, although they are normally found only in a small number of patients each year. Despite this, large numbers of such bacteria could be found in various samples of sewage in the city of Stockholm, mostly in hospital sewage (half of all the samples) and even in receiving waters for effluent (Iversen et al., 2002). Following this survey, however, the incidence of patients reported to have been infected with this type of resistant bacteria has increased dramatically, so that there is reason to suspect that the bacteria are initially

present in the human population, without causing disease, and from here spread to the environment via the sewers, subsequently infecting sensitive individuals. One project investigating whether the detection of resistant bacteria in different samples of sewage can be used an early warning system is currently being funded by the Swedish Research Council for Environmental, Agricultural Sciences and Spatial Planning (Formas, 2003). See also Chapter 9.

The reason for the presence of these bacteria in sewage is unclear. Antibiotic resistance is expected to involve a strain on the organism, which means that the resistance should be removed by selection in the absence of a selective antibiotic pressure. One possibility, however, is that such pressure, however small, is actually present in human sewage, since vancomycin is used to some extent in our hospitals. Measuring the low concentrations of antibiotics in sewage come up against a number of technical problems, and it has not been possible to demonstrate the existence of high concentrations. Other explanations may be that the bacteria have undergone "compensatory mutations", i.e. have changed in some other way in their metabolism, so that the resistance no longer constitutes a load and can thus remain there for good. Finally, one can imagine pockets or "niches" in the sewerage system, e.g. biofilms, where these special strains multiply. At the moment, however, this is merely speculation.

Release of antibiotics into the environment

Since the 1950s the large quantities of antibiotics currently (over)used in human and veterinary medical treatment have in various ways found their way into the environment. The actual significance of this fact is still insufficiently known. We can identify the following main sources of release:

The health services

Most of the antibiotics consumed are excreted in active form via faeces and urine, in this way reaching the sewers and the environment. Antibiotics designed to cure, for example, urinary tract infections need, after all, to be active in urine. Their release by the healthcare sector, at least in non-institutional care, is a fact that is difficult to change. The handing in of leftover medication to pharmacies also applies, of course, to antibiotics.

The consumption of antibiotics in Sweden has fallen slightly in recent years. In 2003 it was 16.3 DDDs (defined daily doses) per 1,000 inhabitants per day, compared with 16.8 DDDs in 2000 (Swedres 2003).

Of particular interest is the widespread use of quinolones, e.g. ciprofloxacin and norfloxacin, which are excreted in active form in urine and are frequently used antibiotics for urinary tract infections as well as many other common infections caused by intestinal bacteria (Beam, 1994). These antibiotics are extremely persistent in nature and are expected to remain there for many years yet. For this reason and because of the emergence of the development of resistance, attempts are being made to reduce their use, so far with limited success. In France and Greece they are the commonest class of antibiotics after penicillin, with about 7 DDDs/1,000 inhabitants/day compared with about 1 in Sweden (Swedres, 2003). The long-term effects of the use of these persistent artificial antibiotics in nature remain to be seen.

Agriculture and aquaculture

Here, too, there has been a tightening up of use and some reduction. In 2003 16 tonnes of antibiotics were used in veterinary medicine, which was 7 per cent less than in 2002. In agriculture an antibiotic is often excreted directly into the environment without passing through sewage treatment plants, although even here low amounts are normally involved. One special problem, however, although a small one in Sweden but many times greater in Norway, for example, is the use of antibiotics in fish farming, where they are added directly to the water, from where they are able to spread more or less freely. Use in Sweden fell from 259 kg in 1994 to 40 kg in 2003 (SVARM, 2003). Off the coast of Norway, however, there are 200 tonnes of quinolones that have still not broken down in the sediment layers since their earlier (injudicious) use layers (Midtvedt, 1991). Antibiotic residues can also be found in imported foods, e.g. giant shrimps mainly from Asia, where antibiotics can also be used prophylactically. Apart from the risk of antibiotic residues, these shrimps also probably contain resistant pathogens in their intestines (see Chapter 4).

Manufacturing

It is here that the large amounts are produced, although their lease is controlled from manufacturing premises, for which reason they are not considered to pose a problem today, at least in Sweden. There is lack of clarity, however, as well as discussions about the significance of the release of antibiotic-producing microorganisms, various preliminary stages and breakdown products of antibiotics etc.

Laboratories

In research and diagnostics, antibiotics are used as a tool in analysis and in cultures of both human cells and microorganisms. They are also necessary in the production of GMOs. Releases from such activity have recently been regulated, and at present antibiotics that are not readily degradable may not be released without previously being inactivated or destroyed. One frequent method of inactivation is autoclaving, i.e. heating to a temperature of 120^{o}C over 20 minutes, although this does not inactivate all antibiotics.

Release of resistant bacteria/genes

People contribute to the release of resistant bacteria and their genes into the environment since they grow on host organisms (people and animals) and are excreted. When the release of resistant bacteria occurs along with the release of antibiotics, the problem arises of the antibiotic being selective for resistant bacteria, both those which occur spontaneously through mutations and those that are already resistant by virtue of a selective growth advantage. The biggest problem, however, is that the spread of resistance genes such as plasmids is favoured. Plasmids can also carry resistance genes for several different antibiotics, all of which are selected from a selective pressure of one of these antibiotics. In actual fact, the problem of resistance in the healthcare sector caused by mutations is not particularly large, the real problem being the spread of resistant bacteria and their genes. In this context, therefore, the cultivation of GM crops containing genes for antibiotic resistance stands out as being particularly misguided.

Summary

Large amounts of antibiotics are used today in human and veterinary medicine, while antibiotics are also produced naturally in the environment. The release of antibiotics into the environment increases the risk of the selection of resistant strains with a consequent ecological effect, increasing the risk of resistance in pathogenic bacteria for people and animals. Little is known about the real extent of the problem in the environment, although certain antibiotics are known to be especially persistent in nature, while others readily break down. Every occurrence of antibiotics selects resistant bacteria, and every occurrence of resistant bacteria and of resistance genes increases the likelihood of resistant bacteria growing. Since the problem of resistance is one of the greatest threats to today's healthcare, there is every reason to pay special attention to this problem in the future. A situation where there are no antibiotics in the environment and no release of resistant bacteria or genes into the environment is the vision of the future for a better environment, while human and veterinary medicine continues to work on behalf of the reduced and more rational use of this valuable class of drugs.

> "There is no longer room anywhere for the misuse of antibiotics. Microbes rule the world and we cannot interfere with them too much with impunity. The microbes are fighting back!"
>
> (Midtvedt and Norin, 2002)

References

Beam T R Jr. (1994). ASM News 60:350-351.

Formas (2003). Antibiotikaresistenta bakterier i avloppsvatten och "early warning" för resistensutveckling i samhället. Projektledare: Inger Kühn.
[Formas (2003). Swedish Research Council for Environmental, Agricultural Sciences and Spatial Planning. Antibiotic resistant bacteria in sewage and early warning of the development of resistance in society. Project leader: Inger Kühn.]

Iversen A, Kühn I, Franklin A, Möllby R. Prevalence of vancomycin-resistant enterococci in Swedish sewage. Appl Environ Microbiol. Vol 68(6):2838-42, 2002.

Jordbruksverket (2004). Jordbruksverkets foderkontroll 2003.
Rapport 2004:8 (se Internet: www.sjv.se).
[Swedish Board of Agriculture (2004). Feed control by the Board of Agriculture 2003. Report 2004:8 (see www.sjv.se).]

Midtvedt T (1991). Antibiotic prescribers – when will they ever learn?
Side Effects of Drugs Annual 15:245-5.

Midtvedt T och Norin E (2002). Antibiotika och ekologi. I Läkemedel och miljön sid 35-49, Apoteket AB.
[Midtvedt T and Norin E (2002). Antibiotics and ecology. In Drugs and the Environment pp 35-49, Apoteket AB.]

SWEDRES (Report on Swedish Antibiotic Utilisation and Resistance in Human Medicine) 2003, STRAMA (= the Swedish Strategic Programme for the Rational Use of Antimicrobial Agents), Swedish Institute for Infectious Disease Control, 2004.

SVARM 2003, Swedish Veterinary Antimicrobial Resistence Monitoring, National Veterinary Institute, 2004.

6 Hormones and endocrine-disrupting substances in the environment

Joakim Larsson, Göteborg University, Leif Norrgren, Swedish University of Agricultural Sciences, and Lars Förlin, Göteborg University

Endocrine-disrupting substances – what are they?

The different parts of the body communicate with each other via nerves and hormones. Hormones regulate such widely differing processes as salt balance, food absorption, reproduction and growth. The normal functioning of the hormonal systems is necessary if an organism is to maintain an "inner equilibrium", grow and adapt to changes in the physical environment. Disturbances of hormone signalling can therefore give rise to a number of serious effects.

Several definitions of endocrine-disrupting substances have been proposed, and what defines such a substance is not entirely obvious. One definition is as follows: "*an exogenic substance that adversely affects the health of an intact organism or its offspring, as a consequence of changes in hormonal function*". Endocrine (or hormonal) disruption as a mechanism of toxicity has received increasing attention ever since the publication by Theodora Colborn of *Our stolen future* in 1996. The clearest examples of endocrine disruptions do not come from studies in man, but from wild animal populations that have been exposed to various kinds of environmental toxicants.

This chapter gives a brief introduction to what is known about endocrine disruption in nature that may be associated with the effect of drugs in particular. The research community's knowledge in this area is still fragmentary, which means that only in exceptional cases can we be certain of whether or not drugs have hormonal effects in the environment; what is more, even in cases where effects have been shown to exist, it is still diffi-

cult to say how extensive and significant these effects are. For many classes of drugs, we can only hint at what effects might be expected if the drugs found their way in potent amounts into the environment. The most important route by which drug residues enter the environment is via sewage effluent to surface water (Chapters 2 and 3). Most studies have therefore been carried out on aquatic organisms, and this chapter focuses on effects on fish, where our knowledge is also greatest.

Many drugs affect hormone signalling

Many drugs act by affecting hormonal systems. Conditions caused by reduced production, release of or sensitivity to a hormone can often be treated with a hormone replacement therapy. The hormone used may be identical to the natural hormone or it may be a synthetic agonist (which stimulates receptors). Agonists can also be administered when there is no shortage of hormones, but when one wishes for various reasons to reinforce the effect of the natural hormone. A third application for agonists is when we seek to affect a negative feedback system in order to reduce the release of other hormones. Similarly, antagonists (which block receptors) or inhibitors of hormone synthesis are given in the event of overproduction of a hormone or when one otherwise wishes to reduce the effect of a hormone. The desired effect of hormonal drugs on patients is all in all very positive, and the dosage is adapted so as to minimise any side effects, while maintaining the desired effect. However, when hormone residues reach organisms in nature, we have no control over the dose. Moreover, organisms that are exposed in nature have no need for any hormone replacement or similar treatment, so any positive effect would be unlikely and purely coincidental in their case.

Preserved hormonal systems

Many hormonal systems are very old in evolutionary terms and are utilised by many different species. Some hormones are identical in a large range of species, such as many steroids, thyroid hormones and tyrosine derivatives, while other hormones, particularly large proteins, may differ more from each other even between closely related species. The hormone receptors, which are proteins, also differ more frequently, although as long as the hormone is shared, synthetic agonists and antagonists also usually function across species boundaries. Generally speaking, both receptors and hormo-

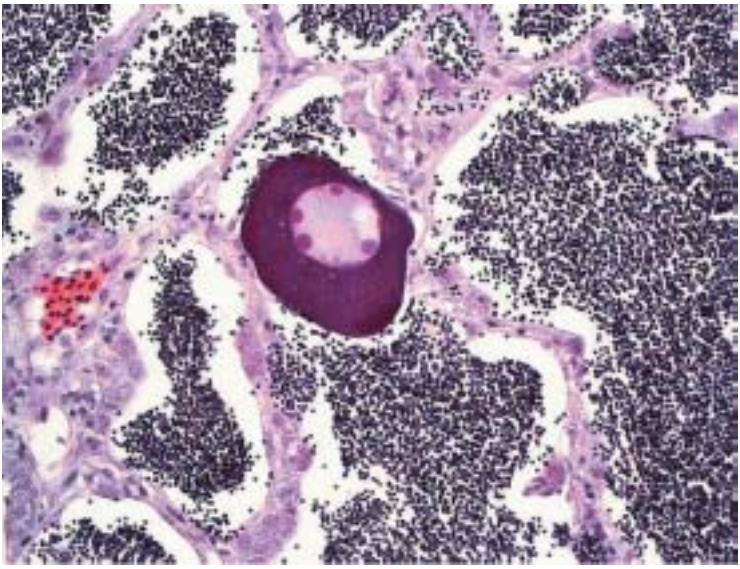
nes are more similar, the more related the organisms are. It is therefore more likely that a hormone drug designed to affect man will also be potent in other vertebrates (e.g. fish) than in crustaceans, say, even though such examples can also be found in laboratory experiments.

Effects of hormone-disrupting substances

Despite the fact that hormones and their receptors are often preserved in different species, they may have different functions and regulate different physiological processes. In egg-laying vertebrates such as birds, fish and amphibians, oestrogen controls the production of vitellogenin (an egg yolk precursor protein) in the liver. The eggs of placental mammals, such as humans, have no yolk, which means that in these species oestrogen lack this role; some other functions of oestrogen, however, are shared. Inadequate knowledge of the functional differences of hormones between species can make it difficult to assess the consequences of an exposure.

The effects that cause us concern, above all, are not acute toxic effects, but problems related to a prolonged effect on the receptor or hormonal system that the drug in question has been designed to interact with. Embryonal development in this connection is a particularly sensitive stage, where even moderate exposure can sometimes have serious consequences. Effects on the hormonal systems in adult organisms suggest that effects on the early development stages of the organism also are very likely. The effects on the health of an exposed adult individual may be relatively small, while the situation for early development stages may be much more serious, with disturbed organ development, for example.

Whenever endocrine-disrupting substances affect reproduction in wild animals the situation is particularly serious, leading in the worst case to the disappearance of populations. Reproductive disturbances observed in wild animals in parts of Sweden have especially affected top predators in the Baltic, one of the most environmentally stressed seas on the planet. An impaired reproduction may be more difficult to detect than when animals suffer clear bodily injuries, which may be more easily apparent to a lay person. As many hormonal systems are regulated by negative feedback, the body itself can sometimes compensate for an external influence, without the exposure giving rise to any serious effects for the individual. This may make it even more difficult to detect an effect when it is moderate.



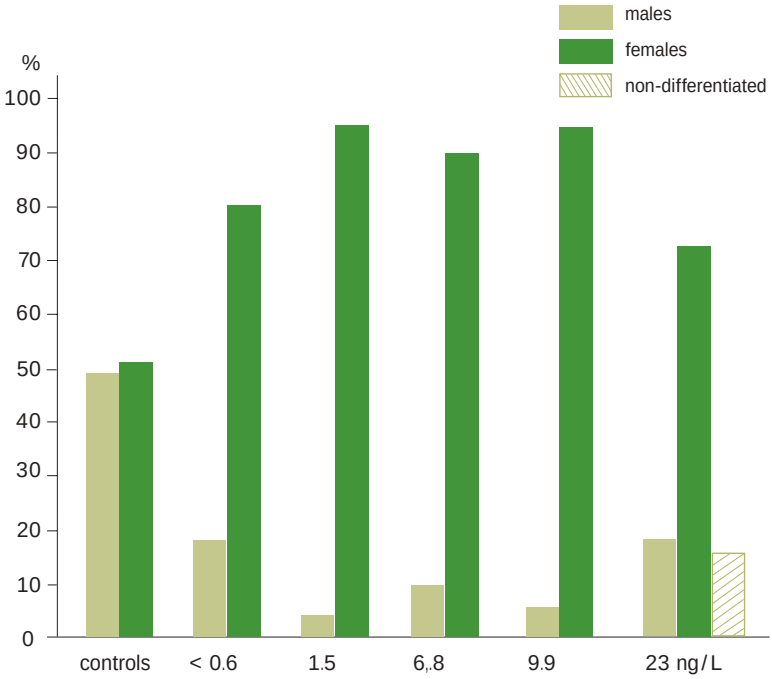
Light microscope image of an intersex roach (*Rutilus rutilus*).
The diagram shows an egg cell interspersed in the testicular tissue.

Even effects such as a change of sex or the development of egg cells within the testes may be difficult to detect. It is even more difficult to reveal if hormones act as pheromones, i.e. affect communication between individuals. Several hormones or hormone derivatives can function as pheromones at concentrations much lower than those measured in the environment (Kolodziej et al., 2003). Researchers are not primarily concerned that fish may be attracted by false pheromone signals, but instead that they become overdosed when they swim in an environment that contains altogether too many signals which swamp the natural message conveyed by pheromones.

Oestrogens

Sexual identity – sensitive to hormonal effects

Attention was drawn in the early nineties to the observations of intersex roach near municipal sewage treatment plants in the UK. This finding was the start of an area of research that focused on hormones and substances mimicking hormones, particularly oestrogen. It had been known for a long time that the sexual differentiation and development of lower vertebrates, e.g. fish, is a dynamic process which can be affected for a period after hatching. Exposure to oestrogen leads in many species to a larger proportion of females, while exposure to androgens leads to a larger proportion of males. Manipulation of sexual differentiation by means of hormones has long been a practice in fish farming in several countries. We may suspect that the physical environment, including unwanted exposure to hormone substances, to a greater extent regulates functional sex in species which lack sex chromosomes. For example, zebrafish (*Danio rerio*) exposed in the juvenile stages to ethinyloestradiol develop only into females (Örn et al., 2003), while exposure to the androgen trenbolone results exclusively in males. It has long been known that if the dosage or time of hormone treatment was not optimal, the result could be intersex fish. It was therefore a reasonable hypothesis that the intersex fish downstream of UK treatment plants had been affected by oestrogens. This was investigated by John Sumpter and his colleagues at Brunel University, and in 1994 the first comprehensive study was published, showing that carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) kept in cages downstream of sewage treatment plants were strongly affected by oestrogens (Purdom et al., 1994).



Effects of ethinyloestradiol on sexual development.
Sex ratios in zebrafish (*Danio rerio*) after exposure to ethinyloestradiol during the period of sex differentiation (redrawn from Örn et al., 2003).

Formation of yolk proteins – one way of measuring the effect of oestrogen

Effects of oestrogen in fish can be measured relatively easily. We know of several proteins that are regulated by oestrogen, including the yolk protein vitellogenin, which is normally expressed in the liver of sexually mature females under the influence of the body's own oestrogen. Vitellogenin is then transported by the blood to the growing egg follicles, where it is taken up to form the yolk. Male fish and juvenile fish normally have low or undetectable levels of both oestrogen and vitellogenin in their blood, although if they are exposed to oestrogens, the liver starts to produce vitellogenin which then can be measured in the blood plasma. Vitellogenin is often therefore used as a biomarker for the exposure to oestrogens.

Identification of oestrogens in sewage effluent

Knowledge of which oestrogens had affected the fish downstream from treatment plants was initially poor. There were suspicions that various industrial chemicals, drugs, natural hormones and household products could all be involved. Initial problems in measuring hormones present in amounts of nanograms/litre in effluents meant that it took some time before the responsible substances were identified.

One strategy for finding out which substances were affecting the fish was to separate the effluent into fractions chemically and to use biological methods to find out which fractions were oestrogenic and which were not. In order to test the fractions biologically, the researchers decided to use a simplified model to study the degree of oestrogenicity. They used yeast cells fitted with a human oestrogen receptor. The results of the UK investigations were that the greatest effects on the yeast cells came from natural oestrogen (estrone and oestradiol) and also synthetic oestrogen from contraceptive pills (ethinyloestradiol) (Desbrow et al., 1998).

Contributions from both natural and synthetic oestrogen

Once these steroids had been successfully measured, together with some other oestrogenic chemicals in sewage effluent, it was possible to compare the levels with dose-response studies of fish in which the effects on vitellogenin production had been measured (Purdom et al., 1994; Routledge et al., 1998). The comparison indicated that ethinyloestradiol and natural oestrogen, above all, were responsible for the oestrogenic effect in fish. Parallel studies from Sweden (Larsson et al., 1999), on the oestrogens content in sewage effluent, uptake of oestrogens in fish and resulting biological effects gave a similar picture, also supported by other studies in several different countries. Natural oestrogen often occurs at levels from a few ng/L to several tens of ng/L in sewage effluent, while levels of ethinyloestradiol are often between 0.5 and 3 ng/L, although levels several times higher have been measured (Heberer, 2002). Dose-response studies of vitellogenin synthesis in rainbow trout have shown effects by ethinyloestradiol at levels as low as 0.1 ng/L (Purdom et al., 1994), while levels in the range 1–10 ng/L of oestradiol and still higher for oestrone are required for a similar effect (Routledge et al., 1998).

Effective uptake of oestrogen in fish

In a study of rainbow trout exposed to sewage effluent from a small Swedish treatment plant, it was found that the concentration of oestrogens, including ethinyloestradiol, in their bile was between one-hundred thousand and one million times higher than the surrounding water and increased with time. This showed a very efficient bioconcentration of oestrogen (Larsson et al., 1999). Oestrogen is excreted from the human body in the form of conjugates with water-soluble groups such as glucuronide. Steroids in effluent, however, are mainly unconjugated (Routledge et al., 1998; Larsson et al., 1999), i.e. in the biologically active and fat-soluble form, which facilitates their uptake by organisms. Deconjugation probably takes place with the help of bacteria, e.g. *E.coli*, which are abundant in sewage treatment plants.

Synthetic oestrogen – more difficult to break down

Synthetic ethinyloestradiol is more resistant to degradation during sewage treatment and in the environment than is natural oestradiol. In degradation studies in which water from the River Thames was used, ethinyloestradiol had a half-life of 17 days and oestradiol 1.2 days. In Germany ethinyloestradiol has been found in drinking water in concentrations high enough to affect fish. There is no data indicating, however, that such small concentrations as a few ng/L can have an effect on animals or people, who drink water rather than breathe it, like fish do.

Sources of oestrogen

The principal source of natural oestrogen in effluent from sewage treatment plants is urine from women. As the production and secretion of oestrogen increase dramatically during pregnancy, the proportion of pregnant women in a community can be significant for the oestrogen load at a treatment plant. Another potential source of natural oestrogen is various preparations for oestrogen replacement. In Sweden, ethinyloestradiol is used only for contraceptives. Combined oral contraceptives, which usually contain 20–35 µg of oestrogen per tablet, today make up the largest source. New administration systems, such as patches and rings, contain much higher amounts, and most of the oestrogen remains in the patch/ring after use. It is therefore of great importance that used rings and patches are being handed in to the pharmacy for destruction (Larsson et al., 2003). The amount of gestagens (progesterone-like substances) in contraceptives is much larger than the amount of ethinyloestradiol. A fraction of certain

ethinylated gestagens, e.g. norethisterone and lynestrenol, can be aromatised to ethinyloestradiol in the body and perhaps also in the environment, although the latter possibility has not been investigated. No investigation has been made into whether oestrogen receptor antagonists, which are used in the treatment of oestrogen-dependent tumours, pass through treatment plants and reach the environment. It has been reported that certain sunscreens can be oestrogenic to fish in high concentrations, although it is not known whether such amounts occur in the environment (Inui et al., 2003).

Different effects of oestrogen

An effect on vitellogenin synthesis in fish exposed to sewage effluents has been observed in a large number of studies from different countries. COMPREHEND, a research programme initiated by the EU, started in 1999, with a view to assessing the oestrogenicity of sewage effluent in parts of Europe. The results showed that the spread of oestrogens from both municipal and industrial treatment plants was a common phenomenon in Europe. The production of large amounts of vitellogenin probably represents a cost for the fish, but there is most concern about other, perhaps more serious, disruptions caused by the effect of oestrogen, which can be difficult to measure. Examples of this are the disturbances of the development of genitals as mentioned earlier, delayed sexual development, asynchronous development of the gonads, disturbed sexual behaviour, embryolethality and various kinds of malformation in fry, which leads to abnormally high mortality. Once commonly measured parameter in fish is the size of the gonads in relation to body weight. This measure has been used for many years in environmental monitoring to allow a relatively simple assessment of sexual development. The potential effects of hormone-like substances at the level of the population have seldom been studied, one of the reasons being that a very large amount of material is necessary for an accurate assessment. In one large-scale experiment in Canada, ethinylestradiol has deliberately been added to a lake over a period of several years. This has caused the fish there to stop reproducing and the population is disappearing.

With regard to the intersexuality of roach, which prompted the investigations into the effect of oestrogen in fish, studies have been intensified to assess incidence, severity and reproduction. Investigations in recent years show that intersexuality occurs in a number of freshwater fish species such as bream (*Abramis brama*), gudgeon (*Gobio gobio*) and burbot (*Lota*

lota), and that the phenomenon also occurs in marine species such as flatfish (e.g. flounders) and eelpout (*Zoarces viviparous*). Feminised male roach downstream of UK sewage treatment plants have been shown to suffer from impaired fertility. Sperm production, sperm motility and sperm density, fertilisation ability and the proportion of fish able to release milt were all affected (Jobling et al., 2002).

There are indications that some populations of bream in the Netherlands have an overrepresentation of females. Sex ratios measured among captured fish, however, need not reflect the actual sex ratio present in nature. The collection methods, access to food, location and time of capture can affect which sex that tend to be captured, e.g. depending on natural sexual differences in growth and behaviour. In addition, the sex ratio of adult fish need not be the same as that of young fish since mortality or the tendency to migrate often varies between the sexes. Determination of the sex of embryos of species of fish that are viviparous (i.e. give birth to live young) has therefore been employed in order to circumvent these problems (Larsson and Förlin, 2002). Studies carried out in Swedish waters indicate that intersexuality in fish is not entirely connected with the degree of exposure to anthropogenic substances, but that the phenomenon also occurs in roach from lakes in area not directly influenced by human activity. Intersexuality can vary from severe, which is characterised by the presence of a large number of relatively well developed egg cells in a mature testis, to the predominant type, i.e. less developed intersexuality characterised by a few immature eggs interspersed in the testicular tissue. A number of different factors in the environment may affect sexual development in certain species, of which a hormonal exposure is one. In many cases, therefore, one should be cautious, in the absence of additional studies about drawing too hasty conclusions about the underlying causes and the consequences of intersexuality or skewed sex ratios. It should be emphasised that it is important in the future to investigate any effects over several generations and in the field at the level of the population, including the significance of genetic variations.

Studies of the effects of endocrine-disrupting substances on amphibians in the wild have also been carried out. There are signs of feminisation of leopard frogs (*Rana pipens*) in various parts of the USA where the herbicide atrazine is commonly used. Between 10 and 92 per cent of the males show different types of changes involving the gonads, such as delayed development and intersexuality. These observations have been confirmed in experimental studies with atrazine (Hayes et al., 2002).

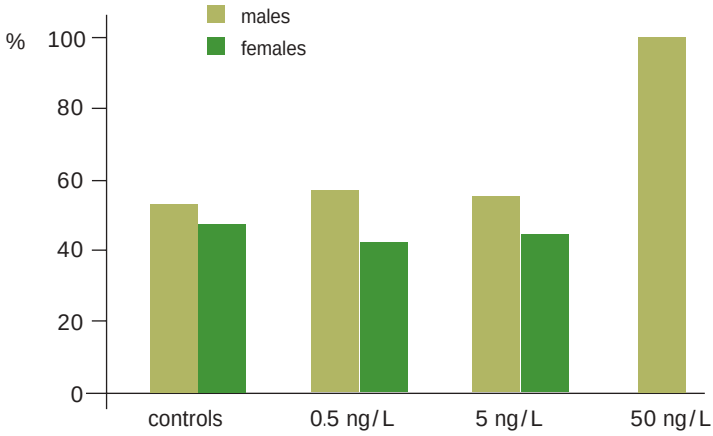
Androgens

The scientific literature on endocrine disruption in the environment is dominated by investigations into the effects of oestrogens. The effects studied are often linked to reproduction and development. Next on the list come androgens and antiandrogens. Here, too, reproduction and development are generally studied.

Androgens in sewage effluent

Androgens have been identified in water from municipal treatment plants. Studies show that the amounts involved can vary considerably, and up to several tens of ng/L of testosterone and androstenedione, for example, have been reported (Thomas et al., 2002; Kolodziej et al., 2003). The presence of androgens in municipal effluent have also been shown using *in vitro* tests with yeast cells fitted with a human androgen receptor (Svenson and Allard, 2004). In this way androgens have been detected in amounts of up to 100 ng/L of dihydrotestosterone equivalents. The majority of this is thought to have a natural origin, that is excretion by people, since men's urine contains significant amounts of androgens.

Animals such as cows and horses also excrete substantial amounts of hormones. In the vicinity of stockbreeders and stud farms, therefore, one can detect hormonal disruptions in fish which has been attributed to natural oestrogens and androgens, although it is also suspected that synthetic hormone preparations may be the cause. In a recently published American report, clear masculinisation was detected in fathead minnow (*Pimephales promelas*) in the vicinity of large breeders of beef cattle (Orlando et al., 2004). In some countries (though not in Sweden) the use of hormone supplements is a common practise to stimulate growth and increase the transformation of feed into muscle mass. However, researchers have not yet been able to decide whether natural hormones or synthetic hormone preparations are the cause of masculinisation in fish. The American study in the vicinity of cattle breeders in the USA brings to mind doping and the misuse of anabolic steroids by some bodybuilders and athletes. We are not aware, however, of any studies reporting such substances in, for example, treatment plants or in nature. Hopefully, this misuse is not large enough to have any significance for our environment. Experimental studies of zebrafish show that exposure to methyltestosterone (Örn et al., 2003) and to the synthetic anabolic steroid trenbolone results in lower concentrations of vitellogenin and a higher proportion of males.



Sex ratios in zebrafish (*Daniro rerio*) after exposure to the synthetic androgen trenbolone during the period of sex differentiation.

Ways of measuring the effect of androgens

There are several ways of measuring the effects of androgens. Androgens affect primary (the sex organs) as well as secondary sex characteristics in many vertebrates, including fish. In guppies (*Lebistes reticulata*) and related fish species, a number of androgens may cause the anal fin to develop into something which resembles the male's breeding organ or *gonopodium*. For over 20 years female mosquito fish (*Gambusia sp.*) close to pulp and paper manufacturers in the south of the USA have been known to develop a gonopodium-like structure. We are still not certain what the cause is, but there is evidence which suggests that the wastewater contains wood-derived steroids which masculinise fish. It can be difficult to establish whether sexual development is affected in organisms in the wild. In the vicinity of a large Swedish pulp mill, however, a skewed sex ratio in favour of males has been observable for several years in eelpout embryos – an effect which can be linked to the degree of exposure to effluent (Larsson and Förlin, 2002). There are many indications that this skewed effect is caused by androgens, since effluent induces a number of male sex characteristics in female fish in laboratory experiments. Examples of this are colouring in guppies and the production of spiggin, an androgen-regulated protein in the three-spined stickleback (*Gasterosteus aculeatus*). A recent study also identified and partly characterized a number of candidate androgens in untreated effluent from the mill, including progesterone (Larsson

et al, 2006). Progesterone is a steroid which is best known for it's role to maintain pregnancy in women, but it also have some androgenic activity.

One of the best-known examples of endocrine disruption in the aquatic environment is imposex in certain marine molluscs (Matthiessen and Gibbs, 1998), which is when the females develop a penis-like organ and the oviduct regresses, making the molluscs sterile. The cause is organic tin compounds (e.g. tributyl tin or TBT) present in certain antifouling paints used on boats to reduce growth on the hull. TBT appears to disrupt natural hormone signalling in exposed organisms.

It is important to remember that a decrease in the expression of female or oestrogen-regulated characteristics is sometimes (though far from always) due to androgen exposure (stimulation of androgen receptors), but may be caused, for example, by decreased stimulation of oestrogen receptors. With regard to changes of sex ratios, for example, it can often be difficult to know whether the basic cause is a change in oestrogen or androgen stimulation, or perhaps something else.

Other steroids

There are a relatively large number of investigations which describe the endocrine-disrupting effects in the environment of mainly oestrogens and also androgens. The presence of other steroids in the environment has also been reported (Kolpin et al., 2002), though to a much lesser extent. Data concerning corticosteroids and potential environmental effects are very scant, despite the very large use of these substances. Many cortisone preparations are also applied to the skin, which may result in a large proportion of them never being metabolised in the body since they are rinsed off in the course of washing and showering. Italian researchers have published a study of prednisone, a corticosteroid, showing that it has the potential to affect aquatic organisms. The transformation products formed after irradiation with ultraviolet light are particulary potent (DellaGreca et al., 2003). Norethisterone, a substance found in certain contraceptives with a progesterone-like effect, may pass sewage treatment (Kolpin et al., 2002), although little is known about its potential effects on aquatic organisms. A major problem, which applies to nearly all drugs, including steroids, apart from oestrogens and perhaps androgens, is that we do not know what specific effects to study in order to determine whether organisms in the environment are affected.

Amino acid derivatives

Thyroid hormones are amino acid derivatives with important functions in all vertebrates, including the regulation of metabolism. In frogs and toads they have a special and very pronounced role. Amphibians, unlike other vertebrates, spend the first part of their lives in water before making their way to the land. This part of their life cycle or metamorphosis is hormone-driven and can be divided into three periods: *premetamorphosis, prometamorphosis* and the final *metamorphosis*. Thyroid hormones initiate the anatomical and physiological changes that amphibians undergo during the transition from aquatic to terrestrial environments and from plant eaters to predators. Since tadpoles breathe water, they risk bioconcentrating drugs and other environmental toxicants from the water. Amphibians are regarded as an increasingly threatened group of animals. Their natural biotopes have gradually been altered as a result, for example, of changes to the agricultural landscape, drainage, the building of roads etc. Studies of chemicals that affect the development of amphibians have until now focused on general toxicological effects. Evaluation is taking place of model substances with effects resembling those of thyroid hormones. Amphibian test protocols are being coordinated by the OECD in order to obtain global acceptance in the future evaluation of chemicals that have potential thyroid gland-related effects. We are not aware of any studies of drugs, passing through sewage treatment plants, which affect the thyroid gland.

Several amino acid derivatives can function both as hormones and as neurotransmitters in nerve synapses. Such a class of drugs whose use is widespread is the beta-blockers, which inhibit the normal action of catecholamines by blocking beta-adrenergic receptors. Beta-blockers are used to a large extent to treat conditions such as high blood pressure and *angina pectoris*, and they are present in amounts of ng/L to micrograms/L in effluents from both European and American sewage treatment plants (Ternes, 1998; Hugget et al., 2003). With regard to the environmental effects of these substances, only a handful of studies have been published in the scientific literature. Recently studies were published of the effects of certain beta-blockers on three selected invertebrates and one species of fish, Japanese medaka (*Oryzias latipes*) (Hugget et al., 2002). The LC_{50}, i.e. the lethal concentration of these substances, varied between from about 1 microgram/L to tens of microgram/L. In the case of one of the beta-blockers, propranolol, the effect on reproduction was also investigated. The results of the study indicate that it is hardly likely that propranolol

affects the reproduction of the invertebrates tested in the concentrations that are found in the environment. On the other hand, this may be the case in fish, since it was found that the reproduction of medaka was affected by 0.5 microgram/L. Similar levels have been reported in surface water or effluent from sewage treatment plants.

Protein-peptide hormones

Most of the hormones in the body belong to the group of peptide and protein hormones. These hormones are involved in everything from reproduction to metabolism, the regulation of blood pressure and salt balance. Several protein hormones have been preserved among various vertebrates, although some interspecies differences in the amino acid sequence often occur. In relation to simple amino acid derivatives and steroids, protein hormones are rather fragile and probably have difficulty in withstanding breakdown at a sewage treatment plant. We know of no examples where a protein hormone has passed sewage treatment and found its way into the environment. However, there may very well be substances in effluent, perhaps from drugs, which affect the synthesis, release and action of various protein hormones in organisms in the environment.

The complicated burden of proof

The detective work of linking cause and effect

Providing proof that an organism in the environment is affected by a drug or an environmental toxicants of some kind can be rather complicated: one should be able to measure the substance in the environment, i.e. normally in water or food, and one should have observed an effect on organisms residing where the drug residues are present. It should be able to reproduce these effects in controlled laboratory experiments on the same kind of organism, where the dose that gives a particular effect must show a reasonable correspondence with the effect and the amounts of drug found in the environment. All this naturally presupposes that one knows the effect that should be studied, which is frequently not the case. Making use of knowledge of the mechanisms of action of drugs and the physiology of the organisms exposed to them can be one of several ways of finding such effects or biomarkers. From an evidential point of view, it is always an advantage if one can understand how the substance in question could give rise to the observed effect. It is often difficult to know the extent to which

wild animals are actually exposed, as they can often move between exposed and relatively unexposed areas, while at the same time the degree of exposure can vary in one and the same place over time. It is therefore important to check whether the organisms in the environment have taken up the drug in question, to what extent and preferably when. One should also know something about the specificity of the effect. Can the effect have arisen as the result of the influence of some other substance? Can it have been modified through interactions with other substances? In addition to this, there is epidemiological evidence, which may mean, for example, that the effect should only occur in places where organisms has been exposed to the drug and preferably everywhere where the drug is present in sufficient amounts. If one is able to produce data for such evidence, it is a highly convincing way to link a drug to a specific effect.

Unfortunately, such observations still tell us very little about how serious the effect of a drug is for the continued existence of the population or the ecosystem. It is sometimes possible to make qualified guesses, although in order to be able to give a really good reply to such questions, extensive epidemiological studies and controlled studies over several generations are often required, involving individual organisms as well as entire ecosystems, together with modelling. Estimating how serious expected effects may be is thus a very demanding and often impossible task.

How much evidence should we ask for?

Bearing all this in mind, it may be worth considering the circumstances in which it could be justified to make use of a precautionary principle. How strong does the evidence need to be before one should take some kind of action? Is it enough to have chemical measurements in the environment, or is it also necessary for these concentrations to have an effect of some kind in the laboratory? Does one also need to demonstrate effects in the field, or does one have to show that the exposure affects reproduction or more seriously has an effect at the level of the population or ecosystem? When we answer the question of how and when one should employ some kind of precautionary principle, we must naturally at the same time take into account the costs, in economic terms as well as in terms of the potentially adverse effects on our health. This is clearly not an easy matter.

Gaps in our knowledge and ongoing research

Large gaps exist in our knowledge of how drugs affect the development of animals. Little is known about the risks of exposure in utero, during the prepubertal period or during specially sensitive windows of development. A hitherto neglected and difficult area is the complex situation that arises from exposure to the increasing number of chemicals which together can have interacting effects, even at low doses. At the same time it is difficult to answer such questions before one even knows what effects the individual substances can have. In the case of most drugs knowledge is lacking of specific responses in wild animals that could be used to study whether they are exposed to and affected by drugs (biomarkers). A Swedish project of this kind, supported by MISTRA (the Foundation for Strategic Environmental Research) and FORMAS (the Swedish Research Council for Environmental, Agricultural Sciences and Spatial Planning), which aims at developing so-called biological fingerprints in fish, started in 2004 and is expected to last for four years. The approach, which involves large-scale analyses of gene and protein expression, is expected to play an increasingly important role in ecotoxicology in the future. Another development activity is being coordinated by the OECD, the goal being to create laboratory models that are specially adapted to enable the prediction of long-term environmental effects of hormone-disrupting substances. So far this work has resulted in three key species being suggested: zebrafish, Japanese medaka and fathead minnow. At the moment several tests are evaluated or discussed for evaluation, including a screening assay based of adult fish (21 days), a short term embryo test (48 hours), a fish sexual developmental test based on exposure from fertlization until 60 days post-hatch and a study over several generations (about 8 months).

References

Colborn T, Dumanoski D and Peterson Myers J (1996). Our stolen future. Dutton,

Penguin Books, USA.

DellaGreca M, Fiorentina A, Lesce M R, Isidori M, Nardelli A, Previtera L and Temussi F (2003). Identification of phototransformation products of prednisone by sunlight: Toxicity of the drug and its derivates on Aquatic organisms. Environmental Toxicology and Chemistry 22, 534-539.

Desbrow C, Routledge E J, Brighty G C, Sumpter J P and Waldock M (1998). Identification of estrogenic chemicals in STW effluents. 1. Chemical fractionation and in vitro biological screening. Environmental Science and Technology 32, 1549-1558.

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C and Vonk A (2002). Feminization of male frogs in the wild. Nature, 419, 895-896.

Heberer T (2002). Occurrence, fate and removal of pharmaceutical residues in the Aquatic environment: a review of recent data. Toxicology Letters 131:5-17.

Hugget D B, Brooks B W, Peterson B, Foran C M and Schlenk D (2002). Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (B-Blockers) on aquatic organisms. Archives of Environmental Contamination and Toxicology 43, 229-235.

Hugget D B, Khan I A, Foran C M and Schlenk (2003). Determination of beta-adrenergic receptor blocking pharmaceuticals in United States wastewater effluent. Environmental pollution, 121, 199-205.

Inui M, Adachi T, Takenaka S, Inui H, Nakazawa M, Ueda M, Watanabe H, Mori C, Iguchi T and Miyatake K (2003). Effect of UV screens and preservatives on vitellogenin and choriogenin production in male medaka (Oryzias latipes). Toxicology 194, 43-50.

Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty G C, Sumpter J P, Tyler C R (2002). Altered sexual maturation and gamete production in wild roach (Rutilus rutilus) living in rivers that receive treated sewage effluents. Biology of Reproduction 66, 272-281.

Kolodziej E P, Gray J L and Sedlak D L (2003). Quantification of steroid hormones with pheromonal properties in municipal wastewater effluent. Environmental Toxicology and Chemistry 22, 2622-2629.

Kolpin D W, Furlong E T, Meywe M T, Thurman E M, Zaugg S D, Barber L B and Buxton H T (2002). Pharmaceuticals, hormones and other organic waste-water contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science and Technology 36, 1202-1211.

Larsson DGJ (2003). Potentiella miljörisker med EVRA och NuvaRing. Information från Läkemedelsverket 14(1), 4-7.
[Larson D G J (2003). Potential environmental risks associated with EVRA and NuvaRing. Information from the Swedish Medical Products Agency 14 (1), 4-7.]

Larsson D G J, Adolfsson-Erici M, Parkkonen J, Pettersson M, Berg H, Olsson P-E, Förlin L (1999). Ethinyloestradiol – an undesired fish contraceptive? Aquatic Toxicology 45, 91-97.

Larsson D G J and Förlin L (2002). Male-biased sex ratios of fish embryos near a pulp mill: Temporary recovery after a short-term shutdown. Environmental HealthPerspectives. 110, 739-742.

Larsson D G J, Adolfsson-Erici M and Thomas P. (2006). Charactarization of putative ligands for a fish gonadal androgen receptor in a pulp mill effluent. Environmental Toxicology and Chemistry 25, 419-427.

Matthiessen P and Gibbs P E (1998). A critical appraisal of the evidence for tributyltin mediated disruption in molluscs. Environmental Toxicology and Chemistry 17, 37-43.

McAllister B G and Kime D E (2003). Early life exposure to environmental levels of the aromatase inhibitor tributyltin cause masculinisation and irreversible sperm-damage in zebrafish (Danio rerio). Aquat. Toxicol. 65, 309-316.

Orlando E F, Kolok A S, Binzcik G A, Gates J L, Horton M K, Lambright C S, Earl Gray L Jr., Soto A M and Guillette L J Jr. (2004). Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. Environmental Health Perspectives, 112, 353-358.

Purdom C E, Hardiman P A, Bye V J, Eno N C, Tyler C R and Sumpter J (1994). Estrogenic effects from sewage treatment works. Chem. Ecol. 8, 275-285.

Routledge E J, Sheahan D, Desbrow C, Brighty G C, Waldock M and Sumpter J P (1998). Identification of estrogenic chemicals in STW effluents. 2. In vivo responses in trout and roach. Environmental Science and Technology 32, 1559-1565.

Svenson A and Allard A-S (2004). Occurrence and some properties of the androgenic activity in municipal sewage effluents. Journal of Environmental Science and Health. Part A – Toxic/Hazardous Substances & Environmental Engineering, A39 (3), 693-701.

Thomas K V, Hurst M R, Matthiessen P, McHugh M, Smith A and Waldock M J (2002). An assessment of in vitro androgenic activity and the identification of environmental androgens in United Kingdom estuaries. Environmental Toxicology and Chemistry 21, 1456-1461.

Örn S, Holbech H, Madsen T H, Norrgren L and Petersen G (2003). Gonad development and vitellogenin production in zebrafish (Danio rerio) exposed to ethinylestradiol and methyltestosterone. Aquatic Toxicology 65, 397-411.

7 Assessment in the chemicals control of environmental hazards and risks

Alf Lundgren, Swedish Chemicals Inspectorate

Chemicals play an important role in modern society; they are found in most of our everyday activities and have contributed to the material prosperity we currently enjoy. Their use has risen exponentially since the Second World War, but this has also contributed to the downsides of this prosperity. The degree of inadvertent exposure of people and the environment to a very large number of chemical substances is increasing all the time. Our knowledge of exposure to and the effects of chemicals, however, is inadequate, particularly where the environment is concerned.

The classical environmental toxicants (DDT, PCBs, heavy metals etc.) were in focus for a long time in the environmental field, although recently attention has increasingly been directed at the large amounts of chemicals that are used today in society. In the European market it is estimated that about 30,000 chemical substances are in use. Everyone who uses or handles chemicals has a responsibility to prevent any harm arising from their use.

Manufacturers and importers of chemicals have a special responsibility for chemical safety in that they have a duty to provide users with relevant information about the hazards of chemical preparations/substances so that the user can devise a safe method of handling them. The Swedish Chemicals Inspectorate is the central authority in Sweden with responsibility for overseeing that manufacturers and importers take on their responsibility.

Chemicals control is by its nature international for two reasons – chemical substances do not recognise national borders but can be distributed via the air and water over large areas, and trade in chemicals is a global phenomenon. In Agenda 21 of UNCED (the United Nations Conference on Environment and Development) the whole of Chapter 19 is devoted to chemicals control.

The international trade in chemicals resulted in the OECD making early efforts to develop the assessment of the hazards and risks of chemical substances and to harmonise the assessments made by different member states. From the standpoint of free trade, it was important for technical barriers to trade not to come about as a result of unnecessary national differences. The EU is also essentially a free trade organisation and it is not surprising, therefore, that laws and regulations in the area of chemicalscontrol are totally harmonised within its borders. This means that laws and regulations are neither more nor less comprehensive in Sweden than in the other EU member states and that Sweden's efforts aimed at improving and modifying the rules are made within the scope of EU activities.

Risk assessment – a synthesis of a series of sub-analyses

Definitions

The term risk analysis is defined in the report of the Chemistry Commission (SOU 1984:77), which preceded the setting up of the Swedish Chemicals Inspection as a new state authority in 1986:

Risk analysis: an assessment of the probability that adverse effects will occur and of their possible extent.

This definition places extensive demands on the assessment, including quantitative estimates of the probability of adverse effects occurring. In practice, it is seldom possible to make such precise assessments, for which reason one speaks more about risk characterisation:

Risk characterisation: An estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartments (e.g. soil, air or water) due to actual or predicted exposure to a substance, and may include a "risk estimation", i.e. the quantification of this likelihood (TGD 2003).

The term risk assessment below will be used to cover both risk analysis and risk characterisation. One important component of risk assessment is the hazard analysis, which also serves on its own as a basis for classifying and labelling the environmental hazards of chemicals.

Hazard analysis: An assessment of the intrinsic properties of chemical substances to cause adverse effects to man or in the environment (SOU 1984:77).

Risk assessments can take different forms

Three models of risk assessment may be distinguished.

The first is a model which makes use of a generic risk assessment. This type of assessment serves as the basis for the EU's risk assessment of new and existing substances and biocides and involves starting from potential general scenarios that could apply to the whole of the EU. The scenarios have been chosen so as to represent a realistic worst case, the reason being to ensure a high level of protection. In the EU the assessments are carried out at three levels, which represent the local, regional and continental scales.

In the second type of assessment, use is made of a site-specific risk assessment. Here the starting point is an actual scenario, e.g. the area affected by a point source with its specific amounts released and environmental conditions. This model is used mainly in the USA, although it may also be relevant to more refined assessments on the local scale in the EU's assessments.

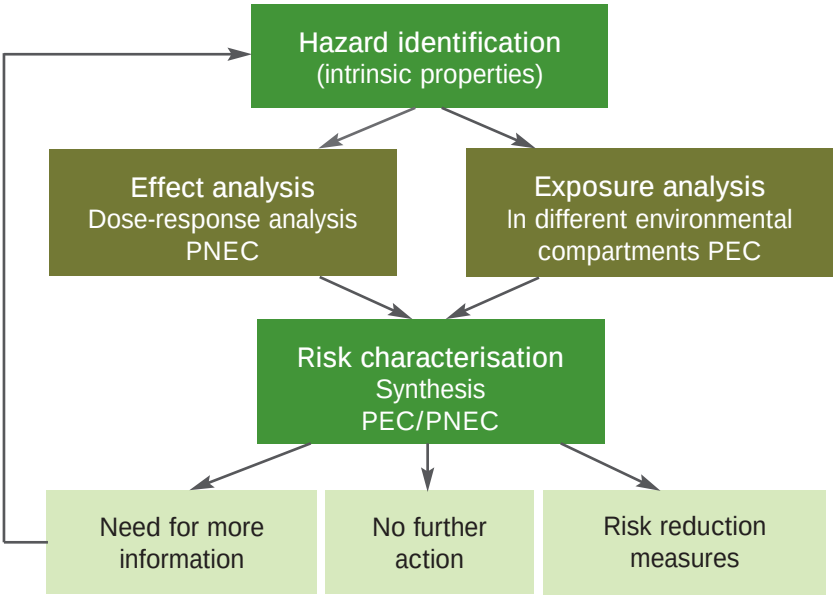
A third form is a probabilistic risk assessment, and with this one starts to come closer to the stricter definition of risk analysis. Here account is taken of the statistical distribution of the parameters involved, such as the sensitivity to the chemical substance of different species, variations in the rate of flow (and thus dilution) into the watercourses serving as a receiving body for point sources etc. This risk assessment model is uncommon as it requires access to rather extensive information, although parts of the concept may be found in the other two models.

Regardless of which model is used, information of exposure and effects is combined into a risk assessment (see the skeleton diagram on the next page). The effect analysis results in a PNEC (predicted no effect concentration) regarding long-term effects. The exposure analysis results in a PEC (predicted environmental concentration). All risk assessments serve as a basis for a decision of some kind. In the EU programme for existing substances there are three possible conclusions resulting from the risk assessment:

i) There is a need for further information and/or testing.

ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.



Skeleton diagram of the risk assessment process in the EU.

Risk assessment – a step-by-step process

Access to data for the risk assessment may vary within very wide limits as far as both quality and extent are concerned. Along with this comes a varying degree of uncertainty in the assessment. The risk assessment process then often has to be carried out in steps, where one proceeds from limited data with a high degree of uncertainty towards increasingly comprehensive and realistic material with a lower degree of uncertainty. In the risk assessment the uncertainty of the assessment is compensated for by

various application factors (see below). One of the advantages of a step-by-step procedure is that one can keep down the costs of the risk assessment and, for example, base one's decisions on relatively simple assessments for low-risk chemicals.

Hazard identification

Hazard identification is based entirely on the intrinsic properties of the chemical substances and in the risk assessment it comprises the first step in the process. The aim is to identify relevant environmental effects and their dose-response relationships. In this step the PNEC is determined, although at the same time one draws up proposals for environmental hazard classification.

Classification of substances dangerous to the environment

Specific criteria of hazard applicable to the aquatic environment and to substances that deplete the ozone layer have been part of EU regulations since 1991 (KIFS 1994:12, reprinted in KIFS 2001:3), and criteria for the land environment are under development in the OECD. In broad terms, one can say that substances that are very toxic to aquatic organisms (LC_{50} ≤ 1 microgram/L) are classified as environmentally dangerous. Substances that are toxic or harmful to aquatic organisms (1microgram/L < LC_{50} ≤ 100 microgram/L) are classified only if they are not readily degradable or bioaccumulative (BCF >100). The BCF or bioconcentration factor is the ratio between the concentration in the test organism, e.g. a fish, and the surrounding medium, e.g. water, at equilibrium.

Substances which are sparingly soluble in water, a property which may affect the interpretation of their toxicity, may also be classified if they are bioaccumulative and do not readily degradable. The specific criteria refer to the results of standardised tests in Appendix 5 of the EU Substance Directive (67/548/EEC), which are mainly copies of the OECD's Test Guidelines for Chemical Substances. In the absence of data from these standard tests, the classification may be based on other equivalent tests or, in certain cases, be calculated from quantitative structure-activity relationships (QSAR). The assessment of aquatic toxicity is based on information concerning toxicity for three groups of organisms (fish, crustaceans and algae) that represent three levels in the food chain. The most sensitive group determines the aquatic toxicity.

The classification and labelling of chemicals are extremely important carriers of information about the hazardous properties of chemicals. Insofar as they are based on intrinsic properties, they are also of general application and independent of how or where the chemical is used. Information about the hazardous properties of chemicals is essential to enable users to be able to design their use in a safe manner and prevent exposure which can lead to environmental injury.

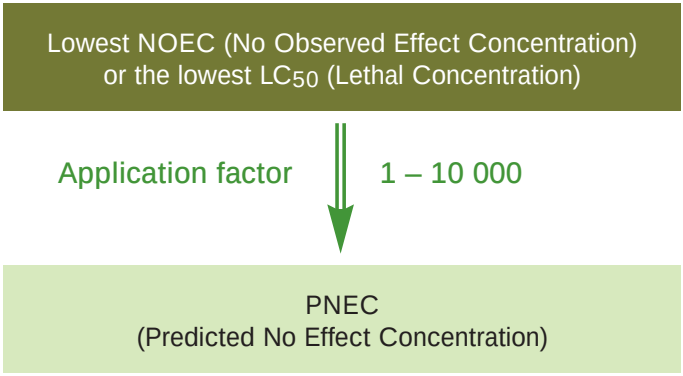
The EU classification criteria, with certain minor modifications, form part of the UN's ongoing work according to Agenda 21 for GHS (Global Harmonization of Systems for Hazard Classification and Labelling of Chemicals) (United Nations, 2003).

Effect assessment – calculation of PNEC

In the EU risk assessment the PNEC is calculated for organisms in different environmental compartments separately:

1. Sewage treatment plants (microorganisms)
2. Inland water (organisms living in water and sediment)
3. Terrestrial ecosystems (above-ground, soil and groundwater communities)
4. Top predators
5. Atmosphere (qualitative assessment only)
6. Marine waters (organisms living in water and sediment)
7. Marine top predators

When calculating the PNEC, the uncertainty of the data is taken into account in the form of application factors that are designed to compensate for variations in sensitivity between species, uncertainty in the assessment of long-term effects from short-term tests, transfer of laboratory data to the field etc. Normally the NOEC (no observed effect concentration) from the most sensitive species forms the basis of the PNEC calculation. If large amounts of relevant test data are available, however, a statistical calculation of PNEC can be carried out. The application factors for aquatic organisms, for example, vary from 10,000 (when only data from short-term tests are available from the three tropic levels) to between 1 and 5 (when comprehensive data are available from field experiments or model ecosystems). (The trophic level is one of the levels in the food chain, e.g. plant – plant eater – predator.)



Procedure for calculating the PNEC of aquatic organisms with the help of application factors.

Exposure assessment – calculation of PEC

The environment may be exposed to chemical substances throughout their life cycles, from manufacture to waste. In the EU's risk assessment one derives the concentration of the substance in all environmental compartments liable to exposure. This analysis normally includes the following steps in the life cycle:

- Production
- Transport and storage
- Formulation of chemical preparations
- Industrial/professional use and trade
- Consumer use
- Lifetime of goods
- Waste management (incl. treatment, storage, destruction and recycling)

Included in the exposure assessment are all direct emissions of the substance as well as unintentional formation of the substance, such as occurs in incineration processes or as a consequence of the degradation of other chemical substances. Consideration should also be given to whether the substance can be degraded, biotically or abiotically, in the environment to give stable degradation products. In such cases an assessment of these degradation products should also be made.

The PEC of the various environmental compartments may be arrived at through model calculations or by direct measurement (e.g. in industrial

programmes for emission control). Where relevant and representative measurements exist, these are given precedence. In view of the uncertainty in the exposure assessments, both measured data and model calculations should be included in the analysis. TGD 2003 (see references) contains guidance for interpreting the various outcomes of direct measurements and model calculations.

In the first stage an analysis takes place of the releases from each stage in the life cycle as well as of that environmental compartment which receives them. The environmental fateof the substance is then analysed, together with routes of exposure and biotic and abiotic transformation processes. Quantification of the distribution and degradation in the environment as a function of time and space results in the calculation of the PEC.

A number of exposure models may be used for these calculations and one of them, EUSES, is based directly on the TGD and is used routinely in the EU risk assessments. The exposure analysis according to the TGD and EUSES can be carried out on widely varying data and allows a step-by-step procedure, from a worst case scenario with very conservative assessments to increasingly realistic assessments with increasing and more precise knowledge of patterns of use, releases etc. The principle is that in the absence of information on any point, conservative default values are used to enable a PEC value always to be calculated. The basis for the regional scenario, for example, for surface water is that the area is 200 x 200 km with 20 million inhabitants and that 10 per cent of the production and use of the whole of Europe takes place there, i.e. 10 per cent of the calculated releases take place within this area. The TGD also contains more detailed scenarios for certain types of chemicals (so-called emission scenario documents).

Risk assessment – synthesis of the effect and exposure assessments

Once the effect and exposure assessments are completed, a direct comparison between the PEC and PNEC is made in an environmental risk assessment (cf. the skeleton diagram on page 108). If the PEC/PNEC ratio is greater than 1, a risk of harm is judged to exist. If it is less than 1, no risk is judged to exist. No extra margin of safety (MOS) is used for the environmental risk assessment since the uncertainty of the data is already built into the calculations through application factors and conservative

defaults. One possible exception may be that when authorising biocides, one should ensure that less environmentally hazardous alternatives are available if the PEC/PNEC ratio is between 0.1 and 1. If alternatives exist which are otherwise equivalent, consideration should be given to withholding authorisation of the biocide. This is an application of the substitution principle that has been written into the regulation for biocides.

PBT and vPvB substances – an important special case

While the classical risk assessment, as it has been described above, has worked satisfactorily on the local and regional scales, it has proved to be inadequate on the global scale. Experience has shown that substances which degrade very slowly and which accumulate in plants and animals, such as DDT, PCBs etc., can have toxic effects after a much longer time and at a greater distance from the sources than substances without these properties. Long-term exposure from substances of this type and the fact that many top predators have long life cycles make it difficult to detect potential effects at an early stage. Once effects start to make their appearance and measures are put in place to stop the emissions, it may take a very long time, perhaps several generations, before environments that have been affected recover and the risk of further damage has been eliminated. In the TGD these problems are discussed in the chapter on marine risk assessment, although in the proposed new set of rules (REACH) they should take into account all environmental compartments.

What are basically involved are substances which are persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) or have other properties that may give rise to similar concern.

In the TGD specific criteria are given for PBT and vPvB substances (see the table on the next page). If a substance fulfils the criteria, this results in the conclusion that there is a need for risk reduction measures. Since this assessment applies regardless of the PEC, it is a question of the phasing out of the emissions.

Harmonisation of hazard and risk assessments

A chemical substance may have several different applications, e.g. an industrial chemical may also serve as an active substances in a plant protection agent. In the EU industrial chemicals and plant protection agents

Criterion	PBT criteria	vPvB criteria
P	Half-life >60 days in marine water or >40 days in freshwater or >180 d days in marine sediment or >120 days in freshwater sediment	Half-life >60 days in marine water or freshwater or >180 days in marine or freshwater sediment
B	BCF >2,000	BCF >5,000
T	Chronic NOEC <0.02 mg/l or CMR or endocrine-disrupting effects	Not applicable

Table showing the criteria for identifying PBT and vPvB substances according to TGD (2003).

are subject to different sets of rules, although it goes without saying that the assessment of the risk of harm should conform to the same principles, regardless of the application. It would be unreasonable if the risk of environmental damage caused by a substance were to differ depending on how it has been used before it finds its way into the environment. In EU there is a clear ambition to harmonise the hazard and the risk assessments in the various sets of rules in order to avoid unnecessary anomalies, which has found clear expression in, for example, its marine strategy (EU 2002). The procedures described above are based on the TGD (2003). The TGD has been developed for and is followed in environmental risk assessments of general chemicals (existing and new substances) and of biocides. The EU's Water Framework Directive (2000/60/EC) describes how to calculate water quality standards (i.e. the threshold values for chemical substances in water). The description follows the TGD and the standards basically amount to the PNEC. The risk assessment of plant protection agents follows the same principles as the TGD, with only minor deviations. The TGD is increasingly starting to become something of a norm for the risk assessment of chemical substances, although the task of harmonising the rules continues and it is particularly important not to incorporate unnecessary anomalies into new rules and guidelines.

References

SOU (Statens offentliga utredningar) 1984:77. Kemikaliekontroll.
ISBN 91-38-08497-X.
[SOU (Swedish Government Official Reports) 1984:77. Chemical control.
ISBN 91-38-08497-X.]

TGD 2003. Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for existing substances.
(See http://ecb.jrc.it/existing-chemicals/)

EUSES 2.0 (see http://ecb.jrc.it/existing-chemicals/)

KIFS (1994:12). Kemikalieinspektionens föreskrifter (KIFS 1994:12) om klassificering och märkning av kemiska produkter.
[KIFS (1994:12). General Recommendations issued by the Swedish Chemicals Inspectorate on the Classification and Labelling of Chemical Products.]
(See http://www.kemi.se/Kemi/Kategorier/Lagar/KIFS/1026453232.html)

OECD Test Guidelines for Testing of Chemicals.
(See http://www.oecd.org/)

EU 2002. Communication from the Commission to the Council and the European Parliament. Towards a strategy to protect and conserve the marine environment. COM (2002) 539, final.

United Nations (2003). Globally Harmonized System for Classification and Labelling of Chemicals (GHS). United Nations Publication No. E.03.11.E.25.
ISBN 92-1-116840-6.

EU legislation. (See http://europa.eu.int/eur-lex/en)

8 Environmental risk assessment and environmental classification of drugs

Bo Gunnarsson, Apoteket AB (The National Corporation of Swedish Pharmacies), and Åke Wennmalm, Stockholm County Council

Drugs are chemical products designed to diagnose, cure or alleviate disease. Each pharmaceutical product is usually made up of the active substance and one or more excipients, together with the packaging. The excipients may be designed, for example, to give the product sufficient volume and shelf life, make it easy to manufacture or take, or give it colour.

Drugs (apart from some used for diagnosis) are designed to have some kind of effect on the patient's organs or tissues or on microorganisms present inside or on the patient. This means that they can also affect other organisms, such as mammals, fish and lower order species. Drugs may also affect plants.

Most drugs are designed to be swallowed. In order to survive its passage through the stomach without being broken down by the acid environment and gastric enzymes, the pharmaceutical substance must be resistant. Drugs have been deliberately given properties that can distinguish them from other chemicals: they are chemically stable and they have a biological effect on many cells and organisms.

Drugs are usually excreted in the urine via the kidneys. Many of them are present in the urine without having undergone a chemical change, when they retain their biological activity. Excretion in urine is an important reason why drugs can affect the environment, primarily the surface water and the groundwater.

There are various methods of describing the environmental impact of chemical products (see Chapter 7), and these methods can also be applied to drugs, which are, after all, chemicals (see Kümmerer, 2004). One can carry out a *hazard identification* and a *risk assessment*. In a hazard identifica-

tion one assesses the inherent chemical and physical properties of the substance and decides on the basis of these data, together with information about the substance's ecotoxicological properties, the degree of inherent hazard posed by the substance (KIFS, 1994). In the risk assessment a determination according to a model takes place of the concentration of the substance (its PEC or predicted environmental concentration) that may be expected to be found in the environment, e.g. in surface water. By calculating the ratio of the PEC and the highest concentration that can be considered harmless (the PNEC or predicted no effect concentration), a quantitative measure of the risk is obtained.

Risk assessment of drugs

The model described below is connected with the method described in the EMEA guidelines for the risk assessment of drugs (EMEA, 2005). The assessment relates to the aquatic environment and is carried out in three stages:

a. Exposure assessment – calculation of PEC

The sales of the product in a particular region are calculated as the amount in weight of the active substance (AS). In the case of drugs in Sweden, this information is available in statistics from Apoteket AB. The entire sold quantity of active substance in the product is assumed to be consumed, excreted and diluted in sewage (a standard figure of 200 L/person/day) and the effluent from the treatment plant to the receiving body of water is assumed to bring about further dilution (by a standard factor of 10). A rough estimate of the PEC can then be made as follows:

$$PEC_{AS} = \frac{AS\ (kg/yr) \times 10^{9}}{\text{number of inhabitants} \times 200 \times 10 \times 365}\ \mu g/L$$

If this concentration does not exceed 0.01 µg/L and if there are no special reasons for assuming that the substance produces an environmental impact, the environmental risk is considered to be so small that no further investigation is required.

b. Effect assessment – calculation of PNEC

The NOEC (no observed effect concentration) is the highest concentration of the active substance which in the aquatic environment has been shown not to have any adverse effect on either of three aquatic organisms: normally a fish, *Daphnia magna* and algae (see page 64). If these organisms differ in their sensitivity to the active substance, the lowest concentration is chosen in the evaluation. An assessment factor is introduced to compensate for the fact that the NOEC in acute tests is probably much higher than for chronic exposure, that there may be differences in sensitivity between different populations of the organisms tested, that higher sensitivity may be found in species not tested and that laboratory tests can give different results to those found in nature. The assessment factor, depending on the quality of the data, test conditions etc., is set at a value of between 1 and 10,000. In the effect assessment of drugs a common value for the assessment factor is 100. This means that the highest concentration of the active substance that can be assumed to have any effect on aquatic organisms is:

$$PNEC_{AS} = NOEC_{AS} / 100$$

c. Risk assessment through an overall appraisal of the exposure and effect assessments

If the exposure assessment (see a. above) shows that PEC >0.01 µg/L, or if special reasons exist, a risk assessment is made by calculating the ratio PEC/PNEC. If the ratio >1 (i.e. if PEC_{AS} is greater than $PNEC_{AS}$), adverse effects from the active substance may be expected in the aquatic environment, whereas if the ratio <1 (i.e. if PEC_{AS} is smaller than $PNEC_{AS}$), adverse effects from the active substance should theoretically not be expected in the aquatic environment.

This type of rather rough assessment can only be used to give an approximate idea of the risk of adverse environmental effects. The method contains many approximations which both over- and underestimate the risk of an adverse environmental impact.

Risk assessment and environmental classification

A risk assessment of a drug or other chemical, as can be seen from the above, is often associated with several uncertainty factors. Both the PEC and the PNEC are based on approximations and assumptions, and these quantities vary in value geographically and over time. The substance may come to be used in entirely different amounts than assumed and several manufacturers of the same substance may enter the market, which means that an overall assessment in all likelihood is required. The basis for a risk assessment is a knowledge of the substance's inherent adverse properties, i.e. how dangerous it is. It is therefore important for these properties (which determine whether it is environmentally hazardous) are determined with as much accuracy as is reasonable. The hazard assessment is independent of exposure, the number of manufacturers, water flows etc. On the basis of how dangerous they are, drugs (in common with other chemicals) are placed in a hazard class. The hazard assessment and risk assessment cannot replace one another, but complement one another well.

Hazard assessment and environmental classification of drugs

There is currently no official standard for how drugs should be classified or undergo a hazard assessment from an environmental standpoint, either internationally or nationally in Sweden. The Stockholm County Council and Apoteket AB, following consultation with the Swedish Chemicals Inspectorate and other ecototoxicology experts, have produced a working model (the "Stockholm model") for the classification of drugs, which is described below. The aim of this model is, by using it, to enable it to be tested, developed and refined to become an instrument which, on the one hand, provides prescribers, patients and other interested parties in the pharmaceutical field with valuable environmental information and, on the other hand, encourages drug manufacturers to develop drugs in the future which have the smallest possible environmental impact.

At the initiative of the Swedish Association of the Pharmaceutical Industry (LIF) and in collaboration between LIF, the Swedish Medical Products Agency, Apoteket AB, the Swedish Association of Local Authorities and Regions, and Stockholm County Council, the model has been developed in regard to a number of important points. The hope is that the developed model can be established as a Swedish standard ("the Swedish model").

Both models are described below:

I. "The Stockholm model"

a. Assessment criteria

Inherent environmental hazard is assessed on the basis of the criteria (i) biodegradability, (ii) potential bioaccumulability and (iii) toxicity to aquatic organisms, as follows.

Persistence is assessed according to the OECD's test guidelines or other equivalent degradation tests.

Potential to bioaccumulation is assessed from the n-octanol/water partition coefficient, Pow, where substances with log Pow >3 are judged to be potentially bioaccumulative. If simulation data for bioaccumulability (actual bioaccumulation in the fatty tissue of a test organism) are available, they also can be used.

Toxicity to aquatic organisms from ecotoxicological basic data comprising the three trophic levels fish, Daphnia (crustaceans) and algae. The tests used are:

Acute toxicity test of fish. A short-term test primarily aimed at determined the LC_{50} or lethal concentration, the test concentration at which 50 per cent of the fish are expected to die after 96 hours of exposure.

Acute toxicity test of Daphnia sp. A short-term test aimed at determining the EC_{50} or effect concentration, the test concentration at which 50 per cent of the test animals are expected to become immobilised after 24 or 48 hours of exposure.

Growth inhibition test of algae. A short-term test aimed at determining the IC_{50} or inhibition concentration, the test concentration which is expected to cause 50 per cent inhibition of growth or rate of growth of the algae after 72 hours of exposure.

If the three species tested differ in the sensitivity they show to the test substance, the value for the most sensitive organism is used in the assessment. The toxicity is divided into four categories:

$LC/EC/IC_{50}$	< 1mg/L	very high toxicity
$LC/EC/IC_{50}$	1-10mg/L	high toxicity
$LC/EC/IC_{50}$	10-100mg/L	moderate toxicity
$LC/EC/IC_{50}$	>100mg/L	low toxicity

b. Overall appraisal and evaluation

Weighting of the three assessment criteria mentioned above takes place as follows:

(i) for persistence	
Readily biodegradable	0
Not readily biodegradable	3
(ii) for potential to bioaccumulation:	
Yes	3
No	0
(iii) for toxicity:	
Very high toxicity	3
High toxicity	2
Moderate toxicity	1
Low toxicity	0

Overall appraisal means that the sum of the weights for a drug's biodegradability (0 or 3), its potential for bioaccumulation (0 or 3) and toxicity (0–3) is added. A drug that is readily biodegradable, lacks bioaccumulative potential and is of low toxicity thus receives the total value zero (0+0+0), while a drug that is not readily biodegradable, is potentially bioaccumulative and is of high toxicity receives the value 9 (3+3+3). The total weighted value should be regarded as an indication of the inherent environmental hazard of the active substance and can give a pointer to substances that it may be of interest to study further from the standpoint of environmental hazard.

The above classification model does not take account of the drug's metabolites, which may be more or less environmental hazardous. It is also based on acute effects, i.e. it does not take account of long-term exposure of aquatic organisms to low concentrations. For additional information about the model, reference should be made to an article in Drug Information Journal (Wennmalm and Gunnarsson, 2005). The results of the classification of 159 active drug substances can be found on the Stockholm County Council's website for pharmaceutical information (Janus).

II. "The Swedish model"

This differs in two important respects from the Stockholm model. It is based on a combination of risk and hazard assessments and risk/hazard are presented at three different target group levels, namely patients, prescribers and specialists. The option is also introduced of assessing drug metabolites, together with PBT and vPvB assessments (see Chapter 7).

a. Patient level

Environmental information is given through a single verbal risk assessment consisting of one sentence, as follows:

PEC/PNEC $\leq$ 0.1	Use of the medicine has been considered to result in insignificant environmental risk
0.1 < PEC/PNEC $\leq$ 1	Use of the medicine has been considered to result in low environmental risk
1 < PEC/PNEC $\leq$ 10	Use of the medicine has been considered to result in moderate environmental risk
PEC/PNEC > 10	Use of the medicine has been considered to result in high environmental risk

If there is not sufficient data to calculate the PEC/PNEC, the following statement will be used:

Risk of environmental impact cannot be excluded due to lack of data

In the case where the PEC:PNEC<1 but the medicine is flagged as a potential PBT or vPvB, the risk phrase will be replaced with the phrase 'Hazardous environmental properties'.

b. Prescriber level

This level will repeat the environmental risk information given in Patient level, but will also include additional information about the environmental persistence (degradation) and bioaccumulation of the active substance. The following statements will be used:

Degradation:	The medicine is degraded in the environment or The medicine is slowly degraded in the environment.
Bioaccumulation:	No significant bioaccumulation potential or Potential to bioaccumulate in aquatic organisms

If the pharmaceutical fulfills the criteria for PBT (Persistent, Bioaccumulative and Toxic) and/or vPvB (very Persistent and very Bioaccumulative), the following phrase should be added: The substance fulfills the EU criteria for PBT/vPvB classification.

c. Specialist level

This level contains detailed environmental information such as:

- Risk assessment, i.e. PEC/PNEC, calculations as well as the specific PEC and PNEC calculation, given in microgram/l, where applicable.
- Total sold amount in kilograms of the active substance on the market (including all products and enantiomers containing the same active substance) in the most recent year for which data are available.
- Results from ecotoxicity tests (given in microgram/l).
- Results from degradation tests.
- Partition coefficient, (e.g. octanol/water (log Kow or log D) or other indicator of bioaccumulation if more appropriate).
- Test guidelines used (e.g. OECD, FDA).
- Information about which forms the pharmaceutical is excreted as, parent compound as well as metabolites, and the percentages thereof.
- Results of CMR (Carcinogenic, Mutagenic, Reprotoxic) tests and statement on endocrine disrupting potential.
- Pharmacological activity of the metabolites.
- Data interpretation in the context of risk and hazard assessment.

Concluding remarks

The work that has been done in Sweden on the environmental assessment and environmental classification of drugs is unique. Releases of drug residues into the environment have not yet been shown to have any direct effects on people's health. This work may thus be said to be preventive and in accordance with the precautionary principle.

The development of a model for environmental risk and hazard assessments has indicated shortcomings in the test data that exist for the majority of drugs with regard to the possibility of a good assessment. These shortcomings must be eliminated. It would also be desirable if the risk assessment could be based only on chronic toxicity data instead of acute data, which is the most usual alternative today. In addition, the pharma-

cological effect of the drug in the test organisms should be assessed instead of its ecotoxic effects. At present non-lethal pharmacological effects of the drug in aquatic organisms are not included in the risk assessment, which is unfortunate. Greater attention to excreted metabolites of the drug is also desirable.

To make this possible, the pharmacological and ecotoxicological effects of the excretion products must be determined.

As these lines make clear, extremely thorough work is needed before adequate risk and hazard assessments can be made for the entire range of drugs. It is likely that more extensive tests will be carried out on new drugs in the future, while existing drugs will scarcely be the object of new and comprehensive evaluations. In this way the natural turnover of the range of drugs will primarily serve as the basis for better environmental assessments in the future.

References

Kümmerer K [editor] (2004). Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks, 1-527. Springer-Verlag.

Kemikalieinspektionens allmänna råd till föreskrifterna (KIFS 1994:12) om klassificering och märkning av kemiska produkter. KEMI. Allmänna råd. 2001:1. [General Recommendations (KIFS 1994:12) issued by the Swedish Chemicals Inspectorate on the Classification and Labelling of Chemical Products. KEMI. General recommendations, 2001:1].

EMEA. Committée for medicinal products for human use. Draft. Guideline on the environmental risk assessment of medicinal products for human use. London, 20 January 2005. (Se http://www.emea.eu.int.)

Wennmalm Å and Gunnarsson B (2005). Public health care management of water pollution with pharmaceuticals: environmental classification and analysis of pharmaceutical residues in sewage water. Drug Information Journal, vol 39, 291-297.

Janus, Läkemedelsinformation för sjukvården/Mijön och läkemedel. [Janus. Drug information for healthcare/the environment and drugs]. (See http://www.janusinfo.se/miljo).

9 Late Lessons from Early Warnings: the Precautionary Principle 1896-2000[1]

David Gee, European Environmental Agency (EEA)

"Precaution, whether or not described as a formal principle, has served mankind well in the past and the history of public health instructs us to keep the spirit of precaution alive and well" (John Graham, 2002).

John Snow and the London cholera outbreak

Graham might have been thinking of the Broad Street pump episode of 1854 when precaution did indeed serve well the people of London in drawing attention to polluted water as the cause of the cholera outbreaks that were plaguing such urban centres in those days. In a 10-day period from 31 August to 9 September 1854, there were about 500 deaths from cholera in the parish of St. James, Soho. John Snow, a London physician, was asked to investigate. Prior to this outbreak, Snow had been studying cholera and the water supplies from two different water companies in South London: one 'clean' and the other 'polluted' with sewage. This incomplete study was already producing data that supported his theory that cholera was caused by contaminated water when he went to investigate the Soho outbreak.

A short investigation revealed that virtually all of the 83 people who had died in the period between 31 August and 5 September had drawn water from the popular Broad Street water pump, rather than from the available, cleaner, yet less popular, (because it did not sparkle so much) piped water supplies. On 7 September, Snow recommended the removal of the Broad Street water pump on the grounds that there was "no...

[1] This presentation is based on "Late Lessons from Early Warnings: the Precautionary Principle 1896-2000" (EEA, 2001), supplemented by observations on several issues that have tended to dominate discussions on the integration and practical application of the precautionary principle since publication of the report.

Cholera… except amongst persons, who were in the habit of drinking the water of the (Broad Street) water pump". In Soho the local church authorities accepted the advice of Dr Snow and removed the handle from the Broad Street water pump on September 8, 1854. In so doing they helped not only to shift the focus of scientific attention from air to water pollution as the most likely cause of cholera but also to demonstrate the precautionary role of public authorities in using an uncertain but appropriate level of scientific evidence to justify action to reduce exposure to a serious hazard (EEA, 2001, Introduction). The cholera outbreak had already died out, partly because most of the population had fled, but the prompt precautionary action prevented further infection from that source and helped to establish Snow as one of the founders of modern epidemiology.

Snow later produced one of the first epidemiological maps of disease and possible causes at a presentation to the Epidemiological Society of London in 1854, which included the map of cholera deaths and the wells in the Broad Street area.

Snow's views on cholera causation were not shared by the majority of relevant scientists. The Royal College of Physicians inquiry into the earlier 1853-54 cholera outbreak had considered Snow's thesis and rejected it as 'untenable'. The London General Board of Health in 1854 took a similar view: 'we see no reason to adopt this belief'. They believed that cholera was caused by airborne contamination. This particular scientific "certainty" soon turned out to be certainly mistaken, with the last remaining doubt being removed when Koch in Germany identified the cholera vibrio in 1884. From the *association* between exposure to water polluted with human faeces, and cholera, observed by Snow in 1854, to Koch's discovery of the "*mechanism of action*", took 30 years of further scientific inquiry.

Association or causation?

This successful "precautionary prevention" in 1854, and many other examples from the "Late Lessons" report, illustrate the need to take precautionary actions when there is an appropriate level of scientific evidence about the *association* between potentially hazardous exposures and ill health, (or environmental damage), without waiting for the"certainty" of *"causation"*, or for the knowledge about *mechanisms of action* to appear, which can take many decades of further research. As Hegel observed, "the owl of Minerva spreads its wings only with the falling of the dusk" . Waiting for "dusk"

before taking action to reduce exposures, especially when there is a long time between such exposures and serious impacts, would often be too late to avoid costly damage. Precautionary action therefore may need to be taken while the "owl" is still sleeping.

The Broad Street example also serves to illustrate the contingent nature of knowledge. Today's scientific certainties can be tomorrow's mistakes, and today's research can both reduce and increase tomorrow's uncertainties, as the boundaries of the "known" and the unknown expand. Waiting for the results of more research may not only take decades but the new knowledge may identify previously unknown sources of both *uncertainty* and *ignorance*, as awareness of what we do not know expands.

"The more we know, the more we realise what we don't know" is not an uncommon scientific experience. Socrates observed that it is a wise man who acknowledges what he does not know. This is an early lesson that has been lately forgotten by many scientists and politicians, who often put "misplaced certainty" (EEA, 2001, Preface) in today's scientific knowledge, or assume that uncertainty can only be reduced, and not increased, by further research.The distinction between uncertainty and ignorance is important. *"Ignorance"* is the source of scientific *"surprises"* and is distinct from *"uncertainties"* which arise from the gaps in knowledge due to variances in sampling and measurement; parameter variability; model assumptions; and other relative simplicities.

Acting to prevent hazards in the context of ignorance presents particular challenges to decision-makers. At first sight it looks impossible to do anything to avoid or mitigate *"surprises"* when we have no idea that they are going to occur, or what they will be. Preventing hazards from "known" risks is relatively easy and does not require precaution. For example, banning smoking or asbestos today requires only acts of *prevention*. However, it would have needed *precaution*, (or foresight, based on a sufficiency of evidence), to have justified acts to avoid exposure to the lesser known risks of asbestos in the 1930's –50s, or to tobacco smoke in the 1960's. Such acts would have saved many more lives than acts of prevention alone have done. Both prevention and precaution are included as key, if undefined, principles in the European Treaty (Art. 174.2, Maastricht Treaty). Many international treaties have included reference to the precautionary prin-ciple, or, as the US prefers to say, to the precautionary approach. The third North Sea Ministerial Conference, 1990, called for "action to avoid potentially damaging impacts of substances,

even where there is no scientific evidence to prove a causal link between emissions and effects". This text has often (sometimes mischievously) been misinterpreted to mean that action is justified even where there is "no scientific evidence" that *associates* exposures with effects.

The North Sea Conference text on the precautionary principle clearly links "no scientific evidence" with the words that directly follow i.e. "to prove a *causal* link". We have already seen with the Broad Street pump example that there is a significant difference between evidence about an *"association"* and evidence that is robust enough to establish a *"causal"* link. The precautionary principle is used to justify actions on exposures when there is a "lack of full scientific certainty" about *causation*; but the need for some scientific evidence of an *association* between exposures and impacts is clear, if only implicitly so, in all serious definitions of the precautionary principle.

What is precaution?

There is still much heated disagreement and discussion about the interpretation and practical application of the precautionary principle, due, in part, to this lack of clarity over its definition. For example, most definitions, including the well known Rio and Wingspread definitions (Raffensberger and Tickner, 1999) use a double negative to define the precautionary principle: they identify reasons that *cannot* be used to justify *not* acting. They also fail to explicitly identify the sufficiency of evidence needed to justify the case specific action required to avoid serious hazards. The Communication from the EU on the precautionary principle, CEC 2001, does specify that "reasonable grounds for concern" are needed to justify use of the precautionary principle, but it does not make explicit that these grounds will be case specific: nor does it explicitly distinguish between risk, uncertainty and ignorance. The EU Commission considers that a precise definition of the precautionary principle is inappropriate, as it would be for other similarly broad legal and administrative principles such as "justice", or "equality". However, EEA experience over the last 5 years is that much unnecessary debate arises from the lack of a clearer definition of the precautionary principle. The EU Treaty, which identifies both "prevention" and "precaution" as legal and administrative principles necessary to achieve a "high level of protection" for Europe's citizens and environments, does not however, define them.

The working EEA definition of the precautionary principle used in the "Late Lessons" report has been improved in light of subsequent discussions and is provided below in the hope that it will facilitate constructive debate on its interpretation and application . The definition explicitly specifies both uncertainty and ignorance as contexts for applying the Precautionary Principle. It also identifies a case specific sufficiency of scientific evidence needed to justify public policy action to avoid or reduce hazards. It is explicit about the trade off between action and inaction. It also widens the conventionally narrow and quantifiable interpretation of "costs and benefits "to embrace the wider, and sometimes unquantifiable, "pros and cons", which can include a wider range of issues. Some of these issues, such as loss of the ozone layer, or of public trust in science, are unquantifiable, but they may sometimes have been more damaging to society than the quantifiable impacts.

The level of scientific evidence that would be appropriate to justify public action in each case varies with the pros and cons of action or inaction; with the availability of alternatives; and with the overall goals of public policy. Like the precautionary principle itself, the use of different levels of proof is not a new idea: societies often use different levels of proof for different purposes.

For example, a high level of proof (or strength of evidence) such as "beyond all reasonable doubt" is used to achieve good science where A is seen to cause B only when the evidence is very strong. Such a high level of proof is also used to minimise the costs of being wrong in the criminal trial of a suspected murderer, where it is usually regarded as better to let several guilty men go free than it is to wrongly convict an innocent man. However, in a different, civil trial setting where, say, a citizen seeks compensation for neglectful treatment at work which has resulted in an accident or ill health, the court often uses a lower level of proof commensurate with the costs of being wrong in this different type of situation. In compensation cases an already injured party is usually given the benefit of the doubt by the use of a medium level of proof, such as "balance of evidence or probability". It is seen as being less damaging (or less costly in the wider sense) to give compensation to someone who was not treated negligently than it is to not provide compensation to someone who was treated negligently. The "broad shoulders" of insurance companies are seen as able to bear the costs of mistaken judgements rather better than the much narrower shoulders of an injured citizen. In each of these two

illustrations it is the nature and distribution of *the costs of being wrong* that determines the level of proof (or strength of evidence) that is "appropriate" to the particular case.

Identifying an appropriate strength of evidence has been an important issue in the climate change debates. The International Panel on Climate Change (IPPC) discussed this issue of levels of proof before formulating their 1995 conclusion that "on the balance of evidence" mankind is disturbing the global climate. They further elaborated on this issue in their 2001 report where they identified 7 levels of proof (or strengths of evidence) that can be used to characterise the scientific evidence for a particular climate change hypothesis.

Choosing an appropriate level of proof for a particular case is clearly based on a value judgement about the acceptability of being wrong. This is why it is necessary to involve the public in decisions about serious hazards and their avoidance and to do so for all stages of the risk analysis process.

The contingency of knowledge; ignorance and "surprises"; and appropriate levels of scientific evidence for policy actions, are critical to the successful application of scientific knowledge and the precautionary principle to public policy-making. They are therefore also relevant to discussions about the potential hazards that are now emerging e.g. from nanotechnology or from the non-ionising radiations arising from the use of mobile phones.

The issue of *time* is also a critical component in discussions on the precautionary principle. For example, the time from the first scientifically based early warnings (1896 for medical x-rays, 1897 for benzene, and 1898 for asbestos) to the time of policy action that effectively reduced damage was often 30-100 years. Some consequences of the failures to act in good time (e.g. on CFCs or asbestos) continue to cause damage over even longer time periods. The ozone hole will cause many thousands of extra skin cancers in today's children but the cancers will only peak around the middle of this century because of the long latent period between exposure and effect. Such long-term impacts mean those questions of liability and compensation for foreseeable if remotely likely hazards are critical, if difficult, issues that need to be addressed when applying the precautionary principle.

Different levels of proof are illustrated in the table.

Different levels of Proof for Different Purposes: Some Examples and Illustrations

Probability	Quantitative descriptor (Probability bands based on IPCC 2001)[1]	Qualitative descriptor	Illustrations
100% probability	Very Likely	• "Statistical significance"	• Part of strong scientific evidence for "causation"
		• "Beyond all reasonable doubt"	• Most criminal law. And the Swedish Chemical law, 1973, for evidence of "safety" of substances under suspicion-burden of proof on manufacturers
90%	Likely (66-90%)	• "Reasonable certainty"	• Food Quality Protection Act, 1996 (US)
		• "Sufficient scientific evidence"	• To justify a trade restriction designed to protect human, animal or plant health under World Trade Organisation Sanitary and Phytosanitary (SPS) Agreement, Art. 2.2, 1995
50%	Medium Likelihood (33-66%)	• "Balance of evidence"	• Intergovernmental Panel on Climate Change 1995 & 2001
		• "Balance of probabilities"	• Much Civil and some administrative law
		• "Reasonable grounds for concern"	• European Commission Communication on the Precautionary Principle 2000
		• "Strong possibility"	• British Nuclear Fuels occupational radiation compensation scheme, 1984 (20-50% probabilities triggering different awards up to 50%+, which then triggers full compensation)
10%	Low Likelihood (10-33%)	• "Scientific suspicion of risk"	• Swedish Chemical law, 1973, for sufficient evidence to take precautionary action on potential harm from substances-burden of proof on regulators
		• "Available pertinent information"	• To justify a provisional trade restriction under WTO SPS Agreement, Art. 5.7, where "scientific information is insufficient"
0% probability	Very Unlikely (1-10%)	• "Low risk"	• Household fire insurance
		• "Negligible and insignificant"	• Food Quality Protection Act, 1996 (US)

Source: David Gee, EEA, 2003, based on IPCC, 2001.

[1] Third Assessment Report from the Intergovernmental Panel on Climate Change, "Summary for Policymakers" Final, 2001, simplified by removing the top (>99%) and bottom (<1%) levels.

The concept of "Early warning"

A good example of such an early warning from the EEA report "Late Lessons" case studies is that provided by the UK Medical Research Council in 1969. They were asked to assess the evidence for risks of resistance to antibiotics in humans following the prolonged ingestion of trace amounts of antibiotics arising from their use as growth promoters in animal feed. (Chapter 9, EEA "Late Lessons" report).

A sufficiency of evidence was identified and described which justified the need for public authorities to restrict the possibility of exposures to antibiotics from animal growth promoters, despite the gaps in knowledge, the need for more research, and ignorance about the mechanisms of action. This early warning was initially heeded, but was then progressively ignored by the pharmaceutical companies and regulatory authorities until 1985 in Sweden, and then in the EU in 1999, when the use of antibiotics as growth promoters was banned. Pfizer, the monopoly supplier of such antibiotics, appealed against the EU decision, pleading, inter alia, an insufficiency of scientific evidence. They lost this case at the European Court of Justice (Case T-13/99-Pfizer, 2002).

Another example of an early warning comes from the lead in petrol story, a warning that was largely ignored for over 50 years, resulting in much damage to the intelligence and behaviour of children in America, Europe and the rest of the motorised world. Yandell Hendersson, Chair of the Medical Research Board, US Aviation Service, who had been asked to look at the scientific evidence on the possible hazards of tetraethyl lead during the temporary ban on lead in petrol, in 1925, concluded:

"It seems likely that the development of lead poisoning will come on so insidiously that leaded gasoline will be in nearly universal use … before the public and the government awakens to the situation". (Rosner and Markowitz, 2002).

There are 14 case studies in the EEA "Late Lessons" Report, covering chemicals (TBT, Benzene, PCBs, CFCs, MTBE, SO2 and Great Lakes pollution); two other pharmaceuticals (DES/diethylstilboestrol, and beef hormones); two physical agents (asbestos and medical x-rays); one pathogen (BSE); and Fisheries.

Authors of the case studies in the report were asked to structure their chapters around four questions concerning the timing and origins of the first scientifically based early warnings; the responses of society; the costs

and benefits of the actions or inactions; and lessons that can be drawn that may help future decision-making to reduce the overall costs of economic activities.

All of the case studies environmental or health stressors are "false negatives" in the sense that they were regarded as not harmful for some time before evidence showed that they were indeed hazardous. The report tried to include a "false positive" case study in the report, but failed to find either authors or sufficiently robust examples to use. Providing evidence of "false positives" is more difficult than for "false negatives": how robust does the evidence of the absence of harm have to be? However, vol. 2 of "Late Lessons", which the EEA intends to publish in 2006, in partnership with Collegium Ramazzini, will include a chapter exploring the lessons to be learned from such, apparently, "false positives", as the EU ban on food irradiation and restrictions on saccharin. Why are there so many "false negatives" to write about? Conclusions based on the first "Late Lessons" case studies point to two main answers: the bias within the health and environmental sciences towards avoiding "false positives", thereby generating more "false negatives": and the dominance within decision-making of short term, specific, economic and political interests over the longer term, diffuse, and overall welfare interests of society as a whole. The latter point needs to be further explored, particularly within the political sciences. Researchers could examine the ways in which society's long-term interests can be more effectively located within political and institutional arrangements that have, or could have, a mandate to look after the longer term, and to resist the short term pressures of particular economic or political interests. The judiciary in democracies can play part of this role, as can long running advisory bodies.

The current and increasing dominance of the short term in markets and in parliamentary democracies makes this an important issue. The experiments we are conducting with planet earth and its systems requires, inter alia, more long term monitoring of "surprise-sensitive" parameters which could, hopefully, give us early warnings of impending harm. Such long term monitoring requires long term funding, via appropriately designed institutions; such funding and institutions are in short supply. The case studies in Vol. 1 of "Late Lessons" illustrate the value, but relative paucity, of such long term monitoring.

The art of accurate scientific evaluation

The evaluation of scientific evidence in the environmental health sciences, at least since 1965, is often implicitly or explicitly based on the nine, so-called, "Bradford Hill Criteria", which were produced in response to the smoking controversy (Bradford Hill, 1965). However, they have often been misused to show that there is little evidence of an association between exposures and harm (e.g. for endocrine disrupting substances, by the WHO in 2002 and by Ashby et al in 1999), when in fact the evidence suggests that there may be such a link.

Even the apparently more robust of the nine "criteria" (which Bradford Hill actually called "features" of evidence rather than "criteria") may not provide robust evidence against an association. For example, the criterion "consistency" of study findings, in the context of multi-causality, complexity and gene/host variability, is not always to be expected. As Professor Needleman, who provided the first of what could be called the second generation of early warnings on lead in petrol in 1979 has observed: *"Consistency in nature does not require that all or even a majority of studies find the same effect. If all studies of lead showed the same relationship between variables, one would be startled, perhaps justifiably suspicious"* (Needlemann, 1995).

The *presence* of consistency of results between studies on the same hazard can provide robust evidence *for* a causal link, but the *absence* of such consistency may not be very robust evidence for the *absence* of a real association. In other words, the "criterion" of consistency is asymmetrical, like most of the other Bradford Hill "criteria", a point which is often lost on the misusers of these "criteria".

Similarly, the criterion of "temporality", which says that the putative cause X of harm Y must come before Y appears, is robust in a simple, unicausal world. In a multi-causal, complex world of common biological end points that have several chains of causation this may not necessarily be so. For example, falling sperm counts, or rising breast cancer rates, can have multiple, co-causal factors, some of which may have been effective at increasing the biological end point in question in advance of the stressors in focus, thereby confusing the analysis of temporality. Chlorine based chemicals cannot be dismissed on temporality grounds as a possible causal factor in falling sperm counts just because sperm counts started falling in some regions before chlorine chemical production took off. This is because the other causal factors responsible for the earlier fall in sperm counts

could have been later joined by chlorinated chemicals, whose new, additional effects on sperm counts could have been combined with the impacts of the other, and differentially changing, co-causal factors.

The resulting overall sperm count trends could then be rising, falling or static, depending on the combined direction and strengths of the causal factors and their impact latencies.

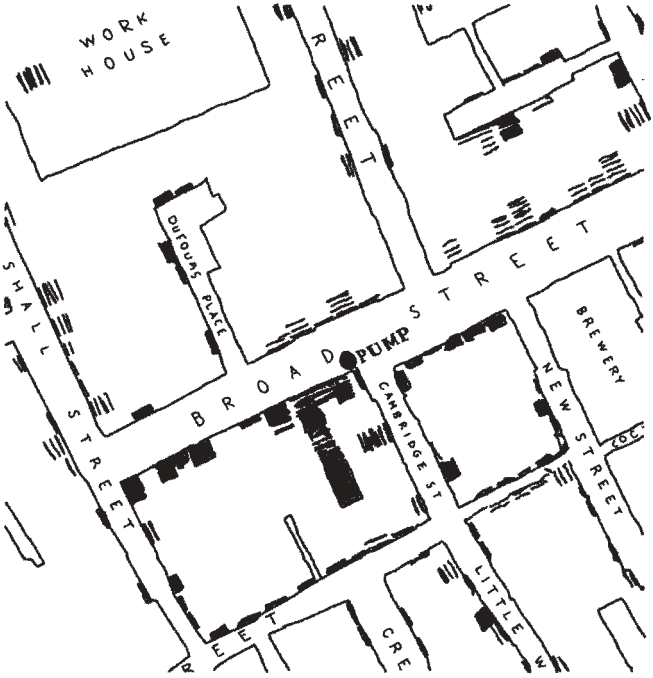
In other words, the *presence* of temporality may be robust evidence *for* an association being causal, but its *absence* may not provide robust evidence against an association. Bradford Hill was well aware of the asymmetrical nature of the "criteria": his followers have sometimes not been so aware.

Chlorine chemicals may or may not be co-causal factors in falling sperm counts: but the above use of the "temporality" argument does not provide robust evidence that they are not causally involved.

The EEA Report on the Precautionary Principle ends with "Twelve Late Lessons" which attempt to synthesise the fourteen different experiences from the very different case study chapters into generic knowledge that can inform current policy-making. The immense difficulties of doing this "in the eye of the hurricane" of current controversies, such as GMOs, nanotechnologies, and mobile phones, and without the benefit of hindsight, are recognised. The origin of each of the lessons is illustrated by several examples from the case study chapters e.g. "evaluate alternative means of providing services" (lesson 4) is illustrated with examples from the asbestos, CFC's, PCB, and antibiotics chapters. Three of the twelve lessons (Nos 3, 9 and 10) explicitly invite early involvement of the public and other stakeholders in all stages of risk analysis, a development which has been actively encouraged by many influential reports during the last decade, (e.g. the US Presidential Commission on Risk, 1996). However, this is not easy and the different ways of doing this are still at the "experimental" stage.

The capacity of "Homo sapiens" (who should perhaps be called, "Homo stupidous" as few, if any other, species consciously destroy their habitat) to foresee and forestall disasters is limited, but, unlike Albert Schweitzer, we do not think that "we will end up destroying the earth". Armed with greater humility, less hubris, and a wider and wiser application of the precautionary principle, we could use the best of systems science to foresee and forestall hazards more successfully than in the past 100 years, whilst also stimulating innovation.

We have so much to learn about the science of nature. The dramatic innovations in the way we use energy and materials to meet our needs could power an “Economic Revolution” that would put Europe at the hearth of cleaner, greener and less hazardous technologies and social systems.



This epidemiological map of the cholera outbreak in the Broad Street area in 1854 was used by John Snow to illustrate his findings. Dr Snow´s practical application of the precautionary principle was successful.

References

Ashby J et al, (1997). “The Challenge posed by endocrine disrupting Chemicals”, env Health Pers., vol 105, No 2, 164-169.

Bradford Hill A (1965). “The Environment and Disease: Association or Causation?”, Proc Royal Soc of Med., vol 58, p 295-300, London.

EEA (2001) “Late Lessons from Early Warnings. The Precautionary Principle 1896-2000”, European Environment Agency, Copenhagen.

Graham J (2002). “Europe’s Precautionary Principles: promise and pitfalls”, J of Risk Research, Vol. 5, No 4, 375.

Needleman H (1995). “Making models of real world events : the use and abuse of inference”, Neurotoxicology and Teratology, vol 17, no 3.

Raffensperger C and Tickner J A (1999). Protecting Public Health and the Environment. Implementing the Precautionary Principle, Island Press, Washington, DC.

Rosner D and Markowitz G (2002). Deceit and Denial. The Deadly Politics of Industrial Pollution, University of California Press, USA.

WHO (2002). “Global Assessment of the State of the Science of Endocrine Disruptors.”, World Health Organisation, Geneva.

Authors

Bengtsson, Bengt-Erik
Stockholm University, Stockholm
bengt-erik.bengtsson@itm.su.se

Bergqvist, Jan
National Corporation of Swedish Pharmacies, Stockholm
jan.bergqvist@hogsbo.goteborg.se

Björlenius, Berndt
Stockholm Water Company, Stockholm
berndt.bjorlenius@stockholmvatten.se

Breitholtz, Magnus
Stockholm University, Stockholm
magnus.breitholtz@itm.su.se

Castensson, Staffan
National Corporation of Swedish Pharmacies, Stockholm
staffan.castensson@apoteket.se

Fick, Jerker
University of Umeå, Umeå
jerker.fick@chem.umu.se

Förlin, Lars
Göteborg University, Göteborg
lars.forlin@zool.gu.se

Gee, David
Europen Environment Agency
Köpenhamn, Danmark
david.gee@eea.eu.int

Gunnarsson, Bo
National Corporation of Swedish Pharmacies, Stockholm
bo.gunnarsson@apoteket.se

Johansson, Niklas
Swedish Environmental Protection Agency, Stockholm
niklas.johansson@naturvardsverket.se

Kallenborn, Roland
University Centre in Svalbard
roland.kallenborn@unis.no

Larsson, Joakim
Göteborg University, Göteborg
joakim.larsson@fysiologi.gu.se

Lundgren, Alf
Swedish Chemicals Inspectorate, Sundbyberg
alf.lundgren@kemi.se

Möllby, Roland
Karolinska Institute, Solna
roland.mollby@mtc.ki.se

Norrgren, Leif
University of Agricultural Sciences, Uppsala
leif.norrgren@bvf.slu.se

Tysklind, Mats
University of Umeå, Umeå
mats.tysklind@chem.umu.se

Wahlberg, Cajsa
Stockholm Water Company, Stockholm
cajsa.wahlberg@stockholmvatten.se

Wennmalm, Åke
Stockholm County Council, Stockholm
ake.wennmalm@sll.se

Apoteket

UNIVERSITET STOCKHOLMS

Stockholms läns landsting

1590-01 • APRIL 2006 •