Pharmacologically active substances in the aquatic environment

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Abstract

In recent decades, there has been an enormous increase in drug usage in human and veterinary medicine around the world. Drugs can enter the environment in parent form or as metabolic by-products; the primary source of contamination of the aquatic ecosystem is wastewater from hospitals and households. The purification technologies used in wastewater treatment plants are in most cases able to eliminate the given drugs only partially, for which reason surface and ground water can easily be contaminated with these substances. This paper describes the effects of pharmacologically active substances, the most frequent contaminants of surface waters in the Czech Republic, on fish and other aquatic organisms.

Drugs, fish, negative impact, state of health

Introduction

Monitoring the negative impact of xenobiotics on organisms in the aquatic environment is an extremely complicated business. The causes of lower growth rates, reduced fertility and high mortality are extremely variable; the state of health of a fish may be affected by a single etiological factor (e.g. a specific toxic substance) or, in the majority of cases, the given situation is the result of a combination of a number of factors such as the poor quality of feed, an unsuitable temperature regime and the simultaneous action of various toxic substances, all frequently in combination with anthropogenic factors (Leatherland and Woo 1998).

The increase in drug use is the result of the increasing human population and the ageing of this population. The "western lifestyle" is leading to a growth in the use of drugs such as antidepressants, sleeping pills, and drugs treating blood pressure and the cardiovascular system. Moreover, many drugs are cheap and readily available, which often leads to their excessive use or abuse. In human medicine, a significant growth has been recorded in non-steroidal antiphlogistic drugs, antibiotics, contraceptives and other types of pharmaceutical drugs (Fent et al. 2006).

The primary source of contamination of the aquatic ecosystem is wastewater from hospitals and households. Following the application of drugs, their pharmacologically active substances or their metabolites are excreted from the organism, usually in the faeces or urine, and pass through the sewer system to end up at wastewater treatment plants. The purification processes used are, in the majority of cases, only capable of eliminating these drugs in part, for which reason the subsequent contamination of surface water, and in rare cases groundwater as well, with these substances may occur. Seepage from inadequately secured municipal waste dumps and excrement produced by livestock treated with drugs and applied to the soil may also be additional sources of contamination of the aquatic environment with pharmacologically active substances. The contamination of water may also occur during the use of stabilised sewage sludge as secondary fertiliser in agriculture (Fent et al. 2006; Kotyza et al. 2009 and Kožíšek et al. 2012).

Drugs can be detected primarily in surface waters, though they can also be found to a lesser extent in groundwater and, in isolated cases, in drinking water (Reemtsma and

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Jekel 2006). The occurrence of individual substances in surface waters is extremely variable and is dependent on drug use in the given region, the physicochemical properties of the water and the specific drug in question (Fent et al. 2006 and Kotyza et al. 2009). Average concentrations of drugs in surface waters range from tens to hundreds of ng per litre.

According to the Czech Hydrometeorological Institute, the most frequently detected drugs include stimulants of the central nervous system, non-steroidal antiphlogistic drugs, antimicrobials, anti-epileptic drugs, nephrotropic x-ray contrast substances and peripheral vasodilators (Prášková et al. 2012).

Of the many drugs that have been intensively studied in recent times, the significant contaminants include substances capable of interfering with the organism's hormonal balance (known as endocrine disruptors). This involves, first and foremost, extremely widespread contraceptives and other drugs used in hormonal treatments (Morteani et al. 2006).

The National Institute of Public Health performed the first systematic screening of selected human drugs in drinking water in the Czech Republic in 2011 in response to the alarming situation regarding the occurrence of these pollutants in surface waters and the rather distorted information presented by some of the media. The results of the first stage of blanket sampling of drinking water from the main distribution networks were extremely good. Sporadic findings (3%), mainly of ibuprofen and carbamazepine, were recorded during analysis of samples from water mains that use raw water from middle and lower reaches. The content of these drugs was in single digits of $ng \cdot l^{-1}$ (Kožíšek et al. 2012).

In 2014, according to the ranking of Top 10 Drugs from the State Institute for Drug Control, the drugs issued in the largest number of packages were those containing metformin (anti-diabetic, 3.30 million packages), atorvastatin (antihyperlipidemic drug, 2.88 million packages), metoprolol (antihypertensive drug, 2.27 million packages) and ramipril (antihypertensive drug, 2.25 million packages). In terms of indication groups, the largest number of packages of human drugs issued in 2014 were drugs indicated for the cardiovascular, nervous and GIT systems.

Public data sets are extremely inconsistent as far as the use of veterinary drugs is concerned. According to information from the Institute for State Control of Veterinary Biologicals and Medicines, there have been increasing efforts at the EU level since 2009 to monitor consumption of veterinary antimicrobials. Changes in the consumption of these drugs are analysed in the EU in connection with the analysis of livestock populations. The current system does not, however, allow the monitoring of use by target species of animal, as a number of these drugs can be used on more than one target species. The data obtained are not, therefore, a completely relevant parameter for assessing the exposure of animals to veterinary drugs containing antibiotics, chemotherapeutics and anti-parasitics. According to data from the Institute for State Control of Veterinary Biologicals and Medicines from 2011, consumption of antimicrobial substances reached a climax in 2006, and there has been a systematic reduction in their use since. The groups of antimicrobial substances used most frequently (accounting for as much as 80% of total consumption of veterinary antimicrobials) are tetracyclines (of the total amount of TTC, chlortetracycline and oxytetracycline account for more than 70% and 10%, respectively), penicillins (80% amoxicillin), sulphonamides (40% sulphadimidine) and boosted sulphonamides. Considerable emphasis is also placed on monitoring the occurrence of limited-indication drugs, in particular third and fourth-generation cephalosporins (a moderate decline in use) and quinolones and fluoroquinolones (a slight increase in consumption). Other veterinary antimicrobials in the group limited-indication drugs (i.e. aminoglycosides – gentamicin and kanamycin, ansamycins – rifaximin) make up just a small percentage of overall consumption of antimicrobial substances and their use is stable over the long term.

The results of long-term monitoring of the occurrence of foreign substances in foods of animal origin performed by the State Veterinary Institute of the Czech Republic indicate a continuing problem of positive findings of residues of malachite green and its metabolic form leucomalachite green (a drug prohibited for use on fish reared for consumption) in samples of fish, specifically carp, trout and other species of fish from rearing facilities. In a positive finding, the sum of the measured concentrations of these two contaminants exceeds the "decisive limit" (i.e. 2 $\mu g \cdot kg^{-1}$ of muscle). The situation in carp rearing is relatively favourable. In contrast, the occurrence of malachite green and its leuco-form is a long-term problem in trout farming. In 2014, residues of these drugs were found in one sample of carp (a sample taken for this purpose) and on seven trout farms, with concentrations exceeding the limit for a ruling of their edibility (the decisive limit) being recorded in one case. The indiscipline of Czech trout farmers, in addition to misconduct on the part of the foreign breeders from whom trout are imported in their early stages, was defined as the clear cause of this finding (State Veterinary Administration 2015).

Non-steroidal antiphlogistic drugs

Non-steroidal anti-inflammatory drugs (NSAID) are used to treat inflammation and pain, injuries and arthritis, to relieve fever and for long-term treatment of rheumatic illnesses (Fent et al. 2006). The cause of frequent detection of this group of drugs is their growing use in human medicine and their imperfect elimination during wastewater treatment (Nakada et al. 2006).

The most familiar non-steroidal antiphlogistic is ibuprofen, consumption of which amounts to around 200 tons a year in the Czech Republic. Diclofenac (consumption of around 20 tons a year in the Czech Republic), ketoprofen and a number of other drugs also belong to the NSAID group (Prášková et al. 2012). These drugs can subsequently be detected in surface waters as a result of the insufficient effectiveness of purification at wastewater treatment plants (Kotyza et al. 2009).

The mechanism of action of non-steroidal antiphlogistic drugs is (reversible or irreversible) inhibition of the enzyme cyclooxygenase which is the enzyme responsible for synthetizing prostaglandins derived from arachidonic acid (Vane and Botting 1998).

Toxicity tests performed on aquatic organisms show that even trace amounts of residues of non-steroidal antiphlogistic drugs in water can have a negative impact. Zooplankton, in which the chronic action of these substances leads to a fall in reproduction, is the most sensitive. Tests of chronic (reproductive) toxicity with acetylsalicylic acid on *Daphnia magna* have demonstrated a reduction in reproductive parameters; the LOEC value of acetylsalicylic acid is 1.8 $mg \cdot l^{-1}$ (Marques et al. 2004). Ibuprofen also leads to a reduction in reproduction in *Daphnia magna* in chronic tests (EC50 13.4 $mg \cdot l^{-1}$), while a concentration higher than 20 $mg \cdot l^{-1}$ inhibits reproduction altogether (Heckmann et al. 2007). The action of NSAID on fish has not, as yet, been fully investigated. Their acute toxicity is practically excluded in view of the low concentrations of non-steroidal antiphlogistics in water. The studies performed do, however, indicate that exposing early developmental stages of fish to non-steroidal antiphlogistics leads to serious defects of development, with the kidneys and gills, in particular, being negatively affected in adults (Prášková et al. 2011). A 28-day toxicity test on a rainbow trout exposed to sub-lethal concentrations of diclofenac revealed kidney damage (degeneration of the tubular epithelium, interstitial nephritis, i.e. inflammation of the intercellular space in the kidneys) and alterations (changes) to the gills (Schwaiger et al. 2004).

Antibiotics and antibacterial chemotherapeutic agents

Antibiotics and antibacterial chemotherapeutic agents are substances with an antimicrobial action. The unsuitable and excessive use of antimicrobial preparations on humans and animals, and their release into the environment, is the principal cause of antimicrobial resistance (Kümmerer 2003). Consumption of antimicrobial agents (antibiotics and chemotherapeutic agents) amounts, according to data from the Institute for State Control of Veterinary Biologicals and Medicines from 2011, to 61 644.5 kg of active substances, the largest proportions being accounted for by tetracyclines (28 006.5 kg), broad-spectrum penicillins (10 240.5 kg), pleuromutilins (2 806.9 kg), macrolides (2 475.3 kg) and aminoglycosides (2 325.5 kg).

The main sources of antimicrobial agents in water are the urine and faeces of treated humans and animals (Kümmerer and Henninger 2003). The effectiveness of wastewater treatment plants in eliminating antibiotics ranges from single-digit percent values to a hundred percent depending on the particular substance, the season of the year and the quality of purification processes. Another significant source of drugs in water are unused and expired drugs that get into the aquatic environment by means of, for example, seepage from waste dumps or from municipal wastewater. Soil contamination may occur during the use of sewage sludge from wastewater treatment plants as agricultural fertiliser (Kim and Carlson 2007). Companies manufacturing pharmaceuticals may also be another source of contamination (Kotyza et al. 2009).

Antibiotics and chemotherapeutic agents can also get into the aquatic environment through their targeted application to fish. Currently, the preparations Flumiquil 50% plv. ad us. vet. containing fluoroquinolone flumequine and Rupin Special gran. ad us. vet. containing the tetracycline antibiotic oxytetracycline are registered for use on fish in the Czech Republic (Svobodová et al. 2007). The medicated premix Aquaflor 500 $mg \cdot g^{-1}$ containing florfenicol has been approved in the EU, and its registration in the Czech Republic is expected in the near future.

Negative effects of antimicrobial substances on free-living fish are rare; the toxic action of antibiotics is seen when concentrations of these drugs are high. Embryos represent a particularly sensitive developmental stage in fish (Madureira et al. 2011).

Anti-parasitic drugs and anaesthetics

Anti-parasitic drugs are drugs that act on external and internal parasites. Anaesthetics are substances that cause anaesthesia and are used in both human and veterinary medicine. They are used in fish, for example, for the purpose of performing veterinary and breeding operations (Svobodová et al. 2007). As is the case with other drugs, people and animals treated with these substances are a source of anti-parasitic drugs and anaesthetics in water. Data from the Institute for State Control of Veterinary Biologicals and Medicines from 2011 indicates a trend of continual reduction in the use of anti-parasitic preparations; 247.44 kg of anti-parasitic drugs were used in 2005, 105.2 kg in 2009 and 80.1 kg in 2011.

Kitchen salt, formaldehyde, preparations containing copper, organic phosphorus compounds, ammonia, acriflavine, potassium permanganate, cypermethrin, praziquantel, etc. can be used for anti-parasitic baths for fish (Svobodová et al. 2007). The only anaesthetic currently registered in the Czech Republic for fish is MS 222 (tricaine). Other anaesthetics used on fish are 2-phenoxyethanol and clove oil. Changes to the haematological profile (an increase in haematocrit and haemoglobin) have been found in the common carp following exposure to 2-phenoxyethanol (Witeska et al. 2015). Changes to the haematological and biochemical profile occurred in koi carp exposed to the anaesthetic MS-222 and clove oil (Zhang et al. 2015).

Hormones

Humans and animals excrete hormonal substances into the environment that were either produced by the endocrine glands in their own organism (endogenous) or those administered to them in hormonal preparations (exogenous). Hormones and their synthetic analogues are excreted from the organism in urine and faeces, and are found in low concentrations ($ng \cdot l^{-1}$) as wastewater contaminants (Aga 2008). A significant group of hormonal substances is made up of the female sex hormones oestrogens (estrone E1, estradiol E2, estriol E3) and gestagens (progesterone) and the male sex hormones androgens (testosterone). Synthetic oestrogens, such as 17α-ethinyl estradiol (EE2) and mestranol (MeEE2), the main source of which in water is human hormonal contraception, are also hormones that have a significant environmental impact (Aga 2008). MeEE2 was used primarily in older human contraceptive preparations, while norethisterone and levonorgestrel continue to be frequently used human gestagens (Hynie 1998).

The main sources of hormonal preparations in the aquatic environment are municipal wastewater and sewage and wastewater from livestock farms containing the urine and faeces of treated humans and animals containing residues of pharmacologically active substances and their metabolites. Natural steroid hormones contained in waste from intensive livestock (e.g. cattle, pigs and poultry) farms and drugs used as growth stimulators on livestock farms and in aquaculture are another source (Schiffer et al. 2001 and Ying et al. 2002).

The complete elimination of hormonal substances and their metabolites from water often does not occur at wastewater treatment plants, for which reason hormones may also occur in trace concentrations in water flowing from wastewater treatment plants and, subsequently, in surface waters. A large quantity of hormones is also found in sewage sludge (Aga 2008).

During monitoring of the occurrence of lipophilic pollutants in the aquatic environment, their detection in various tissues of aquatic organisms is often employed. In the case of monitoring the occurrence of steroid hormones in the aquatic environment, determination of selected biochemical markers that indicate the presence of substances with endocrine effects in the aquatic environment is used because steroid hormones are rapidly metabolised in the organism and in view of the natural occurrence of steroids in fish. The most important markers include vitellogenin in the plasma or whole-body homogenates (Blahová et al. 2010 and Mikula et al. 2009). Certain studies have used the determination of male sex hormones, e.g. testosterone and 11-ketotestosterone, for the detection of anti-androgenic pollutants (Randák et al. 2006 and Blahová et al. 2010).

Progestins (gestagens) in the aquatic environment are of human and veterinary origin. Synthetic progestins are used in contraceptive preparations. Extremely little study has, however, been conducted as regards their ecotoxicological potential. A negative effect on reproductive parameters has, however, been proven (these substances work as "endocrine disruptors") at already environmental concentrations ($ng \cdot l^{-1}$) (Fent 2015).

References

Aga DS 2008: Fate of pharmaceuticals in the environment and in water treatment systems. CRC Press, Boca Raton. 391 p

Blahová J, Havelková M, Kružíková K, Hilscherová K, Halouzka R, Modrá H, Grabic R, Halířová J, Jurčíková J, Ocelka T, Haruštiaková D, Svobodová Z 2010: Assessment of contamination of the Svitava and Svratka rivers in the Czech Republic using elected biochemical markers. Environ Toxicol Chem **29**: 541-549

Fent K 2015: Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment. Environ Int **84**: 115-130

Fent K, Weston AA, Caminada D 2006: Ecotoxicology of human pharmaceuticals. Aquat Toxicol **76**: 122-159

Heckmann LH, Callaghan A, Hooper HL, Connon R, Hutchinson TH, Maund SJ, Sibly RM 2007: Chronic toxicity of ibuprofen to *Daphnia magna*: Effects on life history traits and population dynamics. Toxicol Letters **172**: 137-145

Hynie S 1998: Farmakologie v kostce. Triton, Praha. 461 p (In Czech)

Kim SC, Carlson K 2007: Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environ Sci Technol **41**: 50-57

Kotyza J, Soudek P, Kafka Z, Vaněk T 2009: Léčiva - „nový" environmentální polutant. Chemické Listy **103**: 540-547 (In Czech)

Kožíšek F, Čadek V, Jeligová H, Pomykačová I, Svobodová V 2012: Výskyt humánních léčiv v pitných vodách v České republice. Zpráva o kvalitě pitné vody v ČR za rok 2011. SZÚ Praha. Available at: www.szu.cz (In Czech)

Kümmerer K 2003: Significance of antibiotics in the environment. J Antimicr Chem **52**: 5-7

Kümmerer K, Henninger A 2003: Promoting resistance by the emission of antibiotics from hospitals and households into effluents. Clin Microb Inf **9**: 1203-1214

Leatherland JF, Woo PTK 1998: Fish diseases and disorders. Non-infectious disorders – 2nd Vol. Rowe Limited, Eastbourne. 386 p

Madureira TV, Cruzeiro C, Rocha MJ, Rocha E 2011: The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal) - experimental assessment using a zebrafish embryo test. Environ Toxicol Pharm **32**: 212-217

Marques CR, Abrantes N, Goncalves F 2004: Life-history trans of standard and autochthonous cladocerans. I. Acute and chronic effects of acetylsalicylic acid. Environ Toxicol **19**: 518-526

Mikula P, Kružíková K, Dobšíková R, Haruštiaková D, Svobodová Z 2009: Influence of propylparaben on vitellogenesis and sex ratio in juvenile zebrafish (*Danio rerio*). Acta Vet Brno **78**: 319-326

Morteani G, Moller P, Fuganti A, Paces T 2006: Input and fate of anthropogenic estrogens and gadolinium in surface water and sewage plants in the hydrological basin of Prague, Czech Republic. Environ Geochem Health **28**: 257-264

Nakada N, Tanishima T, Shinohara H, Kiri K, Takada H 2006: Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res **40**: 3297-3303

Prášková E, Voslářová E, Široká Z, Plhalová L, Mácová S, Maršálek P, Pištěková V, Svobodová Z 2011: Assessment of diclofenac LC50 reference values in juvenile and embryonic stages of the zebrafish (*Danio rerio*). Polish J Vet Sci **14**: 545-549

Prášková E, Voslářová E, Široká Z, Svobodová Z 2012: Vliv antiflogistik na vodní organizmy. Veterinářství **62**: 374-375 (In Czech)

Randák T, Žlábek V, Kolářová J, Svobodová Z, Hajšlová J, Široká Z, Jánská M. Pulkrabová J, Čajka T, Jarkovský J 2006: Biomarkers detected in chub (*Leuciscus cephalus* L.) to evaluate contamination of the Elbe and Vltava Rivers, Czech Republic. Bull Environ Contam Tox **76**: 233-241

Reemtsma T, Jekel M 2006: Organic pollutants in the water cycle. Wiley-VCH Verlag GmbH and Co. KgaA, Weinheim. 368 p

Schiffer B, Daxenberger A, Meyer K, Meyer HHD 2001: The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. Environ Health Persp **109**: 1145-1151

Schwaiger J, Ferlinga H, Mallowa U, Wintermayr H, Negelea RD 2004: Effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout. Aquat Toxicol **68**: 141-150

Svobodová Z, Kolářová J, Navrátil S, Veselý T, Chloupek P, Tesarčík J, Čítek J 2007: Nemoci sladkovodních a akvarijních ryb. Nakladatelství Informatorium, Praha. 264 p (In Czech)

State Veterinary Administration 2015: Kontaminace potravinového řetězce cizorodými látkami. Situace v roce 2014. Informační bulletin č. 1/2015. 176 p. Available at: www.eagri.cz (In Czech)

Vane JR, Botting RM 1998: Anti-inflammatory drugs and their mechanism of action. Inflam Res **47**: 78-87

Witeska M, Dudyk J, Jarkiewicz N 2015: Haematological effects of 2-phenoxyethanol and etomidate in carp (*Cyprinus carpio* L.). Vet Anaest Analg **42**: 537-546

Ying GG, Kookana RS, Run YJ 2002: Occurence and fate of hormone steroids in the environment. Environ Int **28**: 545-551

Zhang Z, Liu D, Ding H, Jiang Z 2015: Effects of MS-222 and clove oil on blood biochemical parameters and immune indices of Koi Carp *Cyprinus carpio*. Fisher Sci (Liaoning) **34**: 71-76