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Review Paper

VETERINARY PARASITICIDES – ARE THEY POSING AN ENVIRONMENTAL RISK?

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Summary: The environmental risks of pharmaceuticals have been studied less frequently in comparison to other chemicals such as pesticides and biocides. Nevertheless, during the last few years, veterinary and human medicinal products gained increasingly more attention.

Medicinal products for use in veterinary medicine include various groups of chemicals, used for a wide range of purposes for companion and farm animals. The parasiticides and antibiotics are two of the most important groups and as such used fairly often in animal treatment.

There are different entry routes of veterinary drugs into the environment. Manure of treated farm animals may contain significant amounts of the active ingredients or metabolites. They can be excreted from treated animals in agricultural soils directly (pasture) or with the application of manure as a fertiliser. The aquatic environment can also be one of exposure compartments.

In this review we will focus on certain veterinary parasiticides and give a few examples how they can be excreted into environment and what is their environmental persistency and toxicity to some aquatic and terrestrial organisms.

Key words: Environmental pollutants – toxicity; antiparasitic agents – pharmacokinetics; biodegradation; feces – chemistry; animals, domestic

Introduction

A general term, parasiticide, is used to describe a medicinal product that is effective in killing of different forms of parasites. It does not mean that the drug kills all parasite species, merely that it will kill at least one species of parasites. Most antiparasitic drugs are usually effective in killing several related species of parasites. Others, on the other hand, may have broad spectrum properties and they are effective against a wider range of parasites. In the early years of drug development, the compounds discovered were usually effective only against some of the parasites' species in one of the major groups such as the helminths. In this case, the drugs were collectively called antihelmintics. Similarly, those compounds active only against insects were called insecticides and those, effective only against ticks and mites (the acarina), were called acaricides.

More recently the avermectins and the milbemycins (collectively called the macrocyclic lactones) have been marketed as broad spectrum parasiticides with most of them having activity against nematodes, insects, mites and ticks. Therefore they can be classified as anthelmintics, insecticides and acaricides.

Veterinary parasiticides can be divided into a number of main classes, namely the ectoparasiticides, the endectocides, and the endoparasiticides (including anthelmintics and antiprotozoals). Generally speaking the ectoparasticides are antiparasitic agents used to control external parasites; endectocides are antiparasitic agents used to treat both internal and external parasites, whereas endoparasiticides are used to control internal parasites including gastrointestinal nematodes and lungworms. In human medicine prevalence of

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parasitic invasions is not as high as in veterinary medicine. It is known, that classes like the two major groups within the anthelmintics – avermectins and benzimidazoles were developed initially for veterinary use only, and since they are the most frequently used parsiticides they will be the main focus of this review.

The discovery of macrolide endectocides (avermectins e.g. ivermectin, abamectin, doramectin, milbemxcin, eprinomectin, selamectin) revolutionized the treatment and prevention of parasitic diseases. They are widely used because of their broad spectrum of activity against ecto- and endo-parasites, high efficiency and high safety margin. The most frequently used avermectins are ivermectin (introduced in mid-1980s as probably the most broad-spectrum anti-parasite medication ever), abamectin and doramectin.

Benzimidazoles constitute one of the main groups of antihelmintics used clinically and they are the largest chemical family used to treat endoparasitic diseases in domestic animals (1). Those of current interest are mebendazole, fenbendazole, oxfendazole, oxibendazole, albendazole and triclabendazole (2). All compounds in this group have also a broad spectrum of activity, a wide safety margin and are often effective against adults, larvae, and eggs.

Pharmacological properties

Avermectins

Avermectins are insecticidal or anthelmintic compounds derived from the soil microorganism *Streptomyces avermitilis*. They belong to a group of chemicals called macrolactones (3). Chemical structure of avermectins is presented in Figure 1. Their mode of action includes strong chloride influx into nerve cells, which results in disruption of nerve impulses, blocks the channel causing nerve hyperexcitation and decreases nerve transmission. They are potent agonists at the $GABA_A$ (gama amino butyric acid) receptor but they also interact with GluCl (glutamate-gated chloride) channels in the nervous system of a parasite (e.g. arthropod, nematode). Visible activity, such as feeding and egg laying in parasites, stops shortly after exposure, though death may not occur for several days (4).

In veterinary medicine avermectins are frequently used as anthelmintics against internal and external parasites of cattle, pigs, and horses, sheep and goats as well as cats and dogs. The recommended

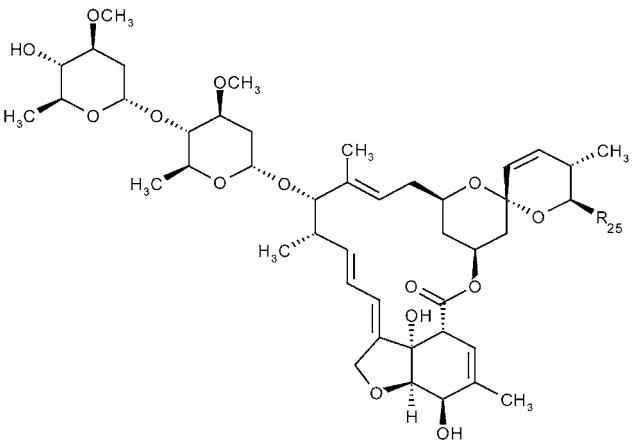

Figure 1: Chemical structure of avermectins

dose of avermectins for all domestic animals is 200 μg/kg b.w. applied in injectable or oral form and 500 μg/kg b.w. in topical form of the drug (5-7). They are excreted mainly through faeces, with up to 98 % being excreted as the non-metabolised drug (8,9). Although, drug formulation, dosage and route of administration are the most important factors in determination of the elimination profile and persistence of faecal residues of avermectins, the majority of the administrated dose is usually excreted in first 10 days after application (10,11). Avermectins are highly insoluble in water and have a strong tendency to bind to faeces and soil particles. Faecal residues or metabolites of avermectin drugs might be highly toxic for non-target organisms living in soil (9). The disturbances that macrocylic lactones can produce on non-targeted invertebrates and on their associated participation in dung degradation and soil element recycling are unpredictable and can negatively affect biodiversity and the agricultural ecosystem sustainability (12). The combination of their physical/chemical properties (non-volatile, low water solubility, strong affinity for lipids and strong sorption to organic matter, soil and sediment) with the high excretion rate of the parent compound from treated animals has raised concerns that toxic levels of avermectins are entering and persisting in various environmental compartments. Consequently they may pose an ecotoxicological risk, especially during periods of their frequent use when large number of animals is treated on a limited area.

Benzimidazoles

The benzimidazoles bind to free β-tubulin, inhibiting its polymerisation and thus interfering with microtubule-dependent glucose uptake (13-15). Binding of benzimidazoles to β-tubulin is reversible and saturable. The depolymerization of microtubules damages the integrity and transport functions of cells within the parasite and thereby disturbs the parasitic energy metabolism. The antiparasitic effect is a lethal, but relatively tardy process. Because benzimidazoles progressively deplete energy reserves and inhibit excretion of waste products and protective factors from parasite cells, an important factor in efficacy of the benzimidazoles is prolongation of contact time between drug and parasite (1).

All benzimidazoles share the same central structure with 1, 2-diaminobenzene – e.g. thiabendazole. Other members of this group – albendazole, fenbenfazole, oxfendazole - have a substitution on carbon 5 of the benzene ring. They are slightly soluble in water (from to 40 10 ng/g). Chemical structure of benzimidazoles (thiabendazole) is presented in Figure 2.

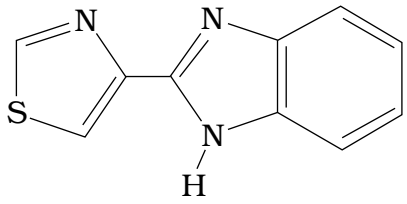

Figure 2: Chemical structure of benzimidazoles (thiabendazole)

The most effective benzimidazoles are less readily metabolized to inactive soluble products than earlier compounds, i.e., the kinetics of elimination is slower. After administration, anthelmintics are usually absorbed into the bloodstream and transported to different parts of the body, including the liver, where they are metabolized and eventually excreted in the faeces and urine. Following single oral administration benzimidazoles have relatively short time of elimination. The very low maximal concentrations in faeces were detected 36 h (thiabendazole), 96 h (albendazole) or 168 h (oxfendazole, febendazole) after treatment.

Environmental fate

Chemicals that come in contact with natural ecosystems will be distributed into different environmental compartments. To understand their potential environmental fate, it is first necessary to assess their probable concentration in these compartments. For pharmaceuticals, environmental concentrations depend initially on the route of drug administration, drug formulation and pharmacokinetics, the dose applied and the frequency of treatment as well as on the number and category of treated animals. Furthermore, the environmental risk assessment requires reliable information on their physical/chemical properties e.g. solubility, adsorption as well as information on their behaviour and persistence in the environment and ecotoxicology. Knowledge of all above mentioned points could enable us to evaluate environmental risk of pharmaceuticals use as well as to predict possible danger for animal and human health. In case of veterinary pharmaceuticals, pasture ecosystems have been of greatest concern.

While talking about the fate of pharmaceuticals in the environment a few very important terms have to be introduced. The K_{ow} or n-octanol - water partition coefficient of chemicals, is simply a measure of the hydrophobicity of an organic compound and it is commonly used as a good estimate of the potential bioaccumulation. Water solubility itself is of great importance for understanding the soil mobility of organic chemicals. On the other hand, the lipophilicity may be of great importance for potential accumulation of chemicals in living organisms (16).

A compound with a high K_{ow} is therefore considered relatively hydrophobic and would tend to have low water solubility, a large soil/sediment adsorption coefficient and a large bioconcentration factor. The K_{ow} of a compound can also be used to find the distribution coefficient (K_d) of a particular contaminant. It is a ratio between contaminant concentration in the solid phase (soil or sediment) and contaminant concentration in the liquid phase (pore water). Distribution coefficient is therefore a direct expression of the partitioning of substance between the aqueous and solid (soil or sediment) phase. For many soils and chemicals, the distribution or partition coefficient can be estimated using as the ratio between soil organic matter (mass of organic carbon per mass of soil). It is called organic carbon normalized sorption coefficient (K_{oc}). It is an indicator of mobility of pharmaceuticals in the environment. Substances with K_{oc}'s >1000 likely have low mobility. Values for macrocyclic lactones range from 3231 (eprinomectin) to 86900 (doramectin) and from 31500 to 50000 for fenbendazole (a ben-

zimidazole). There is no data available for imidazothiazoles, tetrahydropyrimidines, silicylanilides, or other benzimidazoles (17). The fact that the soil organic carbon content seems to be mainly responsible for the adsorption of at least non-polar organic chemicals led to the assumption that soil sorption processes are partitioning processes between water and lipophilic soil phase (16).

Physicochemical properties of some avermectins and some benzimidazoles are presented in Table 1.

Table 1: Physicochemical properties of some avermectins and some benzimidazoles

Pharmaceutical	Solubility in water	Koc	Kd	Kow	Ref
Abamectin	7.8 µg/l	5300-15700		9900	ARS Pesticide Properties Database (18,19,20)
Doramectin	25 µg/l	7520 silty loam 13330 clay loam 86900 silty clay loam	70.8 silty loam 23.4 clay loam 562 silty clay loam	9700	(21)
Ivermectin	4 mg/l	12600-15700		1651	(22)
Albendazole	0.53-0.59 mg/l	1862		1380	SRC PhysProp database, (23)
Mebendazole	71.3 mg/l	n.a.		239	SRC PhysProp database, (24)
Fenbendazole	10-30 ng/l	12022	630-1000	7079	SRC PhysProp database, (24)

Table 1 summarizes studies done investigating the sorption behaviour of some avermectins and benzimidazoles in soils. The available data indicate that compounds are highly sorbed to soils with organic carbon normalised sorption coefficients (K_{oc}) from 1862 to 86900. The fact that all values are greater than 1000 indicates that the substances are not particularly mobile in the environment. High K_{ow} values cause limited aqueous solubility of presented compounds. Therefore, when drug residues reach the environment they tend to be adsorbed on soil or sediment particles. Their degradation to less toxic and more water soluble degradation products are known. Degradation products of avermectins are more likely to leach from dung and, therefore, pose less risk to dung-dwelling organisms. But on the other hand, degradation products leached from dung and soil into surface and ground waters may pose greater risk to aquatic organisms (17). Avermectins e.g. ivermectin undergo rapid photodegradation as a thin, dry film on a glass with half-life (DT_{50}) of 3 h (25). The half-life for ivermectin photodegradation in the surface water is 12 h in summer and 39 h in winter (25). Halley et al. reported also that the degradation half-life of ivermectin in soil or faeces-soil mixture was in the range of 91 to 217 days in the winter and 7 to 14 days in the summer and that the degradation products are less toxic than ivermectin. A soil column leaching experiment performed by Halley et al. was proved that ivermectin is rather immobile in soil and not readily leached through soil into ground water (25). The consequence of that could be its accumulation in soil. McKellar also reported (26) that no apparent degradation of ivermectin residues in faeces of cattle treated with pour-on or subcutaneous preparations over a 45-day time period was observed. Similar results were obtained by Sommer and Steffansen in Danish and Tanzanian weather conditions where photodegradation had minimal effect (27). Fisher and Mrozik (28) also reported that the half-life of abamectin degradation in sandy loam, clay and sand soil ranged from 20 - 47 days and 13 degradates were identified. No degradation was observed in sterile soil which indicates that soil organisms are responsible for degradation. Taylor (29) reported the same behaviour in soil for doramectin as it is known for ivermectin and abamectin. Sorption properties have been examined in three natural soil types with a variety of proportions of sand and clay. Depending on soil type, the aerobic degradation of doramectin was observed. The half-life was 61 - 79 days in the dark at 22 °C (29). Kolar et al. established DT_{50} value of 23 and 22 days for dissipation of abamectin and doramectin from sheep faeces under the field conditions in the pasture, respectively (11).

Benzimodazoles are slightly soluble in water and as avermectins they have high tendency to bind soil

and organic matter. There are limited data on the degradation of benzimidazoles. Nevertheless, some investigations on the biodegradability of febendazole indicate that it is degraded slowly (30). Persistence in soil or faeces is not known.

Ecotoxicology

Ecotoxicology is the study of harmful effects of chemicals on ecosystems (31). The main theme of (eco)toxicology is the relationship between the quantity of chemical to which an organism is exposed and the nature and degree of consequent harmful and toxic effects. The toxicity is usually evaluated using dose-response relationships and also enables basis for assessment of hazards and risks posed by environmental contaminants. The usual parameters used while assessing ecotoxicity of certain chemical are lethal concentration (LC_{50}) and effective concentration (EC_{50}). Lethal toxicity testing represents the median lethal concentration, whereas effective concentration investigates adverse response other than death. Both measurements are carried out on 50% of the population. Ecotoxicological studies are very important and essential also for the assessment of environmental effects of veterinary drugs. There are several organisms involved in toxicity testing: terrestrial organisms (invertebrates; soil dwelling organisms), vertebrates (birds, mammals, reptiles, amphibians), aquatic organisms (water fleas; fish), plants (algae).

Also for avermectins, it is very important to predict the environmental risk of their possible non-controlled and irregular use. Accordingly, an increase in knowledge about the elimination profile from treated animals, the rate of degradation and the distribution of avermectins in the environment, especially in pastures, is needed. Their residues in faeces of treated animals and in soil have toxic effects on some dung-associated insects, especially their larval forms (32-34), beetles (34-36), faeces- and soil-invertebrates (37) and some other decomposer organisms in temperate climate pasture (12, 18, 38, 39). They show effects on reproduction, biological function and survival of non-target aquatic and terrestrial organisms which have an important role in the food web (40, 41). Interruption of the food web may affect the diversity of a system or may influence the relative importance of one species assemblage over another (40). In this case, avermectins might influence the food web, due to their known effects on the species involved in faeces decomposition which are also a part of the food chain (41). Avermectins are toxic also to avians e.g. abamectin dietary LC_{50} values for bobwhite quail and mallard duck of 3102 mg/kg and 383 mg/kg was established, respectively (18, 20). The most sensitive organisms to avermectins are some freshwater organisms, such as *Daphnia magna* and fish (e.g. rainbow trout) (18).

There are not that much data available for benzimidazoles, a few are mentioned in the Table 2, which gives a brief overview of the data listed in the literature. They are presented systematically, including results of toxicity testing of dung-dwelling organisms, which are one of the major concerns especially in regard of using avermectins.

The toxicity data obtained by the testing procedures are eventually used to make assessments of hazard (the potential to cause harm) and risk (the probability that harm will occur). To asses risk, you must know the toxicity of the compound in question (expressed as LC_{50}, EC_{50}, or NOEC (non observed effective concentration) values) and the anticipated exposure of the organism to the toxic compound (31). The predicted environmental concentration (PEC) and the predicted environmental no-effect concentration (PNEC) can be calculated and the risk is expressed as a risk quotient: PEC/PNEC = risk quotient. In the case of PEC, calculations are based on known rates of release and dilution factors in the environment. For the environmental release scenarios the important measures in regard of PEC are – the use and consumption, interval of medicinal treatment, the metabolic rate, the agricultural practise when collecting, storing and applying manure/slurry on the field as well as exposed area. The PEC in manure for instance, is ratio between total dose administered (mg/animal/day) multiplied by number of treatment days and divided by the total amount of manure produced during manure production period. Such examples serve mainly for studies of environmental fate and are especially important for pharmaceuticals which are excreted in urine or manure (57).

For example reported predicted environmental concentration (PEC) for doramectin at worst-case scenario (one treatment of a feedlot bovine animal, all dose excreted in first 14 days via faeces, no degradation, runoff is one-third of rainfall) ranging from 0.011 μg/L in surface runoff to 18 μg/L in wet feedlot waste (45). Reported concentrations could not pose high harmful effects on terrestrial organisms comparing to toxicity data presented in Table 2. On the other hand the risk of avermectins is higher for aquatic and dung-dwelling organisms responsible

Table 2: Toxicity data of some avermectins and some bezimidazoles to different non-target organisms (only some examples are shown)

Test organism	Species	Pharmaceutical	Toxicity data		Ref.
Aquatic organisms					
Fish	*Salmo gairdneri* (rainbow trout)	Ivermectin	LC_{50} = 3.0 mg/l	96 hours	(28)
	Oncorhynchus mykiss (rainbow trout)	Abamectin	LC_{50} = 3.2 µg/l LC_{50} = 1.5 µg/l	96 hours 96 hours	(20) (42)
	Salmo gairdneri	Abamectin	LC_{50} = 3.2 µg/l	48 hours	(18)
	Salmo gairdneri	Fenbendazole	LC_{50} = 40 µg/l	96 hours	(30)
	Lepomis macrochines (bluegill sunfish)	Ivermectin	LC_{50} = 4.8 mg/l NOEC = 0.9 mg/l	96 hours	(26)
	Lepomis macrochines (bluegill sunfish)	Abamectin	LC_{50} = 9.6 µg/l	96 hours	(20)
	Lepomis macrochines	Abamectin	LC_{50} = 9.6 µg/l	48 hours	(18)
	Cyprinodon variegatus (sheepshead minnow)	Abamectin	LC_{50} = 15 µg/l	96 hours	(20)
	Ictalurus punctatus (channel catfish)	Abamectin	LC_{50} = 24 µg/l	96 hours	(20)
	Cyprinus carpio (carp)	Abamectin	LC_{50} = 24 µg/l	96 hours	(20)
	Cyprinus sp. (carp)	Abamectin	LC_{50} = 42 µg/l	96 hours	(43)
	Lepomis macrochirus	Oxfendazole	LC_{50} > 2.7 mg/l	96 hours, 2.7 mg/l was the highest tested conc.	(44)
Crustaceans					
	Daphnia magna (water flea)	Ivermectin	EC_{50} = 0.025 ng/g NOEL = 0.01 ng/g	48 hours	(18)
	Daphnia magna	Abamectin	EC_{50} = 0.34 µg/l	48 hours	(18)
	Daphnia magna	Doramectin	EC_{50} = 0.001 mg/l	48 hours	(45)
	Gemmarus duebeni and G. zaddachi (amphipoda)	Ivermectin	LC_{50} = 0.033 µg/l	96 hours	(43)
	Daphnia magna	Fenbendazole	LC_{50} = 12 µg/l	48 hours	(30)
	Daphnia magna	Oxfendazole	LC_{50} = 52 µg/l	48 hours	(46)
Others					
	Panaeus duorarum (pink shrimp)	Abamectin	LC_{50} = 1.6 µg/l	96 hours	(20)
	Msyidopsis bahia (mysid shrimp)	Abamectin	LC_{50} = 0.022 µg/l	96 hours	(20)
	Crassostrea virginica (eastern oysters)	Abamectin	LC_{50} = 430 µg/l	96 hours was observed at the embryo-larval stage of the life	(20)
	Callinectes sapidus (blue carb)	Abamectin	LC_{50} = 153 µg/l	96 hours	(20)
Soil-dwelling organisms					
Bacteria	8 different genera of Eubacteria	Oxfendazole	No effect found on replication or growth at maximum solubility, 9 ng/g		(46)

Test organism	Species	Pharmaceutical	Toxicity data		Ref.
Fungi	5 different genera of Fungi	Oxfendazole	No effect found on growth at maximum solubility, 9 ng/g		(46)
Springtails	F*olsomia fimetaria*	Ivermectin	NOEC = 0.3 mg/kg EC_{10} = 0.26 mg/kg EC_{50} = 1,7 mg/kg LC_{50} = 8.4 mg/kg		(47)
Worms	*Enchytraeus crypticus* (potworm)	Ivermectin	NOEC = 3mg/g EC_{10} = 14 mg/kg EC_{50} = 36 mg/kg		(47)
	Esienia foetida	Ivermectin	LC_{50} = 315 ng/g	28 days	(43)
	Lumbricus terrestris	Ivermectin	No effect on survival and growth	24 weeks	(43)
	Earthworm	Ivermectin	LC_{50} = 15.7 mg/kg NOEC (repro) = 4.7 mg/kg LC_{50} = 18-100 mg/kg	 28 days	(47) (28)
	Earthworm	Fenbendazole	NOEC = 56 mg/kg LOEC = 120 mg/kg LC_{50} = 180 mg/kg	28 days	(30)
	Earthworm	Oxfendazole	No effect found at highest experiment concentration, 971 mg/kg soil	28 days	(46)
Dung-dwelling organisms					
Dung beetles	*Onthophagus binodis*	Abamectin	Not affected in dung of treated cattle		(48)
	Onthophagus binodis	Fenbendazole	NOEC = 770 ng/g LC_{50} >770 ng/g	7 days	(30)
	Onthophagus gazella (immature)	Doramectin	LC_{50} =0.0125 mg/kg LC_{90} = 0.0382 mg/kg NOEC =>0.25 mg/kg	Effect endpoint used: number of brood balls	(45)
	O*nthophagus gazella* (immature)	Fenbendazole	LC_{50}= > 770 µg/g NOEC = > 770 µg/g	Amounts in spiked dung used as diet, 7 d study	(30)
	Onthophagus gazella	Ivermectin	17 days 21 days	Sensitivity of coleopteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	(49) (50)
	Onthophagus taurus	Ivermectin	15 days	% dung pat dispersal, number of beetles/pat; reductions on days 7 and 10 after treatment	(43)
Flies	*Musca vetuistissima* (bushfly)	Avermectin B1	No bush flies survived from eggs to adult following cattle injection of 200 µg/kg		(33)
	Musca domestica (house fly)	Ivermectin	30 days	Increased mortality for 20 days	(43)

Test organism	Species	Pharmaceutical	Toxicity data		Ref.
	Haematobia irritans (horn fly)	Ivermectin	LC_{50} = 0.032 – 0.061 µg/g LC_{50} = 0.0032 – 0.0066 µg/g	Amounts in blood, 48h mortality 88 h mortality	(51) (52)
	Haematobia irritans	Doramectin	LC90(larvae) = 0.003 mg/kg NOEC = 0.0024 mg/kg	Amounts in spiked cattle dung, effects on larvae development/emergence	(45)
	Musca autumnalis (autumn house-fly)	Ivermectin	14 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	(53)
	Neomyia cornicina (dung fly)	Ivermectin	32 days 17 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	(54) (55)
	Stomoxys calcitrans (stable fly)	Ivermectin	14 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	(56)
	Scatophaga scercoraria (yellow dung fly)	Ivermectin	EC_{50} = 0.051 µg/g EC_{50} = 0.036 µg/g EC_{50} = 0.015 µg/g EC_{50} = 0.001 µg/g	24 h mortality 48 h mortality Pupariation prevented Emergence prevented (cm) all amounts in spiked cattle dung	(33)
	Scatophaga stercoraria	Ivermectin	EC_{50} = 0.051 µg/g	24 hours mortality	(43)

for dung degradation. Nevertheless, they could still pose a risk to aquatic as well as terrestrial environment, especially during periods of their frequent use in large number of animals. Climate conditions and type of soil have to be considered also.

Studies on benzimidazoles are limited, but suggest that these class of compounds are generally not toxic even to dung-dwelling organisms (17).

Results from our studies on avermectins

Although there are several reports on the environmental effects and fate of avermectins, disagreement between scientists still exists about their possible environmental impact (58, 59, 60). McKellar (26) summarized that the contributory factors to the environmental impacts of avermectin residues are the activity of excreted avermectins or their metabolites on non-target fauna, the amount and temporal nature of excretion and the stability and persistence of avermectin residues in the environment as well as environmental influences on the processes of physical degradation of excreta (e.g. sunlight, temperature, rainfall and mechanical disruption).

The aim of our work was to evaluate the possible risk of avermectin (abamectin, doramectin) use in pastured sheep. First we developed a sensitive and selective analytical tool for determination of

avermectins in sheep faeces and in soil (61), that enabled us to determine time profile of elimination of both avermectins via faeces after sheep treatment with a single subcutaneous dose of 200 μg/kg b.w. The maximal abamectin concentration in sheep faeces (1277 ng/g dry faeces) was detected on day 3 after treatment, while maximal concentration of doramectin was detected on days 2 and 5 after treatment (2186 and 1780 ng/g dry faeces, respectively). Both avermectins were excreted approximately at the same rate (k was 0.23 d^{-1} for abamectin and 0.19 d^{-1} for doramectin). The majority of both avermectins was excreted in 10 days after treatment (11).

In addition, some experiments were also performed on sheep pasture. We studied degradation time profile of both avermectins in sheep faeces and in soil under environmental conditions. Environmentally important parameters – e.g. samples moisture, temperature and weather conditions were recorded during the experiments. A rapid loss of abamectin and doramectin from sheep faeces was observed during the first 32 days. After that, concentrations of abamectin and doramectin remained constant at approximately 77 ng/g and 300 ng/g, respectively. The DT_{50} for abamectin and doramectin dissipation from sheep faeces were 23 and 22 days, respectively (16). Dissipation of both avermectins was strongly correlated with moisture content in faeces. Due to low contamination of soils, dissipation of avermectins in soil was not significant (62).

We have studied dissipation also under laboratory conditions, where results showed that abamectin and doramectin in homogenized, contaminated sheep faeces were evidently degraded under the UV light at the wavelength of 370 nm; DT_{50} of less than one day was established for abamectin and 4 days for doramectin (63).

For evaluation of possible toxicity of both avermectins to aquatic and soil-dwelling organisms some experiments were also performed. Namely, Halley et al. (18) reported high toxicity of avermectins to some freshwater organisms, such as *Daphnia magna* and fish (e.g. rainbow trout). Our investigations on toxicity of avermectins to the same and some other water organisms (*Daphnia magna*, rainbow trout, zebrafish, green unicellular algae and bacteria) and some soil-dwelling organisms (*Folsomia candida*, *Enchytraeus crypticus* and *Porcelio scaber*) confirmed high toxicity of both avermectins. Toxic effect was observed in all investigated water organisms (concentration ranges in ng/kg and μg/kg) (unpublished data) and in soil-dwelling organisms (concentration ranges in mg/kg) (unpublished data). Results show extremely high toxicity of abamectin to daphnids since the concentration 0.0094 μg/l still caused mortality and inhibited the reproduction of daphnids. The NOEC was detected at 0.0047 μg/l of abamectin and the LOEC was 0.0094 μg/L (unpublished data).

Based on gained experimental results we partially assessed possible environmental risk related to the use of avermectins in sheep grazing in Slovenian Karst. We estimated PEC of abamectin and doramectin according to the experimental data obtained in our experiments with time profile of excretion and degradation of avermectins in sheep faeces after single subcutaneous administration of 200 μg/kg body weight for both substances (unpublished data). The worst case scenario was used for calculations (30 sheep kept at limited area of 800 m^2 for 9 days; excretion of the total dose given during that time; no degradation of avermectins in faeces; entire average monthly rainfall occurring on day 9; runoff is half of the rainfall). Calculated PECs as well as experimental data were compared to toxic concentrations of both substances for tested aquatic and soil-dwelling organisms. From results we may conclude that the detrimental environmental effect of tested avermectins for soil-dwelling and aquatic organisms after their single administration to sheep is unlikely to occur (unpublished data). But additional experiments are needed for environmental assessment after repeated applications in sheep grazing in karst region.

Conclusions

The fate and behaviour of pharmaceuticals in the environment have been studied for several decades (64, 65, 66). More recently several reviews on use, emission, fate, occurrences and effects of pharmaceuticals have been published (16, 67-72). The environmental risk of the use of medicinal products is currently assessed at their registration procedure, but the methodology has not been finalised yet (73-75). In the thesis of Montforts from 2005 (76), an in-depth study was made about European legislation and guidance documents for the risk assessment and this work is a very good starting point for more precise insights in the field of pharmaceuticals in the environment.

There are still a lot of opened issues related manly on frequent and repeated applications of veterinary drugs. In addition different environmental conditions (e.g. climate conditions, soil type) should

be considered as well. Large-scale, long-term and multidisciplinary field studies are needed to monitor the effects of fecal residues on dung degradation and pasture productivity. Systematic studies would enable us to develop modelling approach, mainly focused on prediction.

In future we will continue with our studies which in order to understand how certain veterinary medicinal products reach the environment – determination of time profile of elimination and process afterwards – degradation pathways, persistence and toxicity to non-target species in the environment.

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PROTIZAJEDAVSKA ZDRAVILA ZA UPORABO V VETERINARSKI MEDICINI – ALI PREDSTAVLJAJO TVEGANJE ZA OKOLJE?

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Povzetek: Študije, ki bi preučevale, ali zdravila predstavljajo tveganje za okolje, so v primerjavi s tovrstnimi študijami pesticidov in biocidov redke. Počasi pa se to razmerje popravlja, tako da so v zadnjih nekaj letih začeli aktivno raziskovati tudi uporabo humanih in veterinarskih zdravil in njihovih možnih vplivov na okolje.

Zdravila za uporabo v veterinarski medicini predstavljajo zelo raznoliko skupino, ki se uporablja za različne namene, tako pri ljubiteljskih kot farmskih vrstah živalih. Protizajedavska zdravila in antibiotiki spadajo v najpomembnejši skupini in so tudi najpogosteje uporabljani pri zdravljenju živali.

Zdravila za uporabo v veterinarski medicini pridejo v okolje na različne načine. Eden od možnih načinov je z blatom, ki ga izločijo zdravljene živali neposredno na pašne površine ali posredno s sredstvi za gnojenje, kot je gnojevka. Tudi vodni ekosistemi so pogosto izpostavljeni tovrstnim vplivom in so pomemben pokazatelj kontaminacije.

V prispevku bomo predstavili nekatera protizajedavska zdravila in podali nekaj primerov, kako le-ta vstopajo v okolje, kako so v njem obstojna in kakšna je njihova toksičnost za nekatere vodne in zemeljske organizme.

Ključne besede: okolje, onesnaževalci – toksičnost; protiparazitarna sredstva – farmakokinetika; biodegradacija; zdravila, ostanki – analize; feces – analize; živali, domače