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Problematika veterinarskih zdravil v okolju

Izvleček

V enaindvajsetem stoletju naj bi v okolju obstajalo več kot 100.000 različnih kemikalij, od tega pa jih danes 30 % predstavlja snovi farmacevtskega izvora. Z večanjem ozaveščenosti glede varovanja okolja se je vzporedno začela razvijati tudi ekotoksikologija – veda, ki še vedno velja za sorazmerno novo znanstveno disciplino. Zdravila za uporabo v veterinarski medicini z ekotoksikološkega vidika še do nedavnega niso predstavljala večjega problema, vendar pa so njihove značilne lastnosti in pogosta uporaba na velikem številu živali pripomogle k temu, da so upravni organi in tudi znanstveniki pristopili k tej problematiki bolj načrtno.
Raziskave so pokazale, da so ena izmed bolj uporabljenih skupin zdravil avermektini, ki se uporabljajo v veterinarski medicini kot antiparazitiki. Znano je, da se ti v nespremenjeni obliki z živalskimi iztrebki izločajo v okolje. V študijo smo vključili avtohtono slovensko pasmo ovc – istrsko pramenko, ki smo jo tretirali z avermektini. Po uspešno razviti metodi določanja časovnega profila njihovih zaostankov v iztrebkih in njihove razgradnje pod različnimi pogoji smo določili koncentracije, ki bi lahko škodljivo delovale na koristne organizme v okolju, in na podlagi tega pripravili oceno tveganja.
Ekotoksikološke raziskave tako omogočajo razširjen vpogled v možne posledice, ki zaradi nekontrolirane uporabe potencialnih onesnaževal okolja tam nastanejo.

Ključne besede: veterinarska zdravila, istrska pramenka, ekotoksikologija, ocena tveganja, odpadki

Veterinary medicines as an environmental problem

Summary

There are over 100.000 chemicals of different origin present in the environment in the 21. Century, among which30 % of them belongs to pharmaceuticals. Widespread concern about the environmental impacts of chemicals led to the development of a new scientific discipline – ecotoxicology. Veterinary medicines posed no threat until recently. As global livestock industry and also number of companion animals is (still) growing, the usage of mentioned substances is intensively expanding. Due to the increased use and their special chemical character, scientists and governmental institutions started with a more focused approach and in-depth studies regarding the problem.
Avermectins are one of most frequently used veterinary medicines. After their use, they eliminate from the body in their active form, end up in the environment and as such interfere with a diverse range of biological systems. Indigenous sheep breed IstrianPramenka treated with therapeutic dosages were used in the study. Time profiles of excretion and concentration of avermectins in faeces were established, using a chemical procedure. Degradation of avermectins under different conditions was also followed. We performed also ecotoxicological studies looking at the effects on soil dwelling organisms with a final conclusion in an environmental risk assessment scheme.
Performed ecotoxicological study enables an in-depth view into the possible consequences of such large-scale pharmaceuticals use.

Key words: veterinary medicines, IstrianPramenka, ecotoxicology, risk assessment, pharmaceutical waste

1. Introduction

Widespread concern about the environmental impacts of chemicals in general started in late 1960's, after Carson published her book "Silent spring" about negative effects of pesticides. Afterwards there was a growing need for extensive studies related to environmental consequences especially to organisms living in the environment, which led to the development of a new scientific discipline - ecotoxicology. The original definition of ecotoxicology was given by Truhaut in 1977 (Walker et al., 2001). Ecotoxicology isan interdisciplinary environmental science, dealing with the interactions between environmental chemicals and biota, studying the adverse effects at different levels of biological organisation.

In general, environmentally occurring chemicals can be divided into two groups: naturally occurring and "artificially developed" substances. The first group includes heavy metals, polycyclic aromatic carbons, nitrogen oxides etc. whilst the latter refers to pesticides, industrial chemicals, consumer products like dyestuffs and cosmetics, and pharmaceuticals (human and veterinary). Veterinary medicines are used in various therapeutic means but also in terms of prophylaxis, e.g. as growth promoters. In agricultural livestock production especially antibiotics, antiparasitics and growth promoters are significantly used (Halling-Sørensen et al., 1998; Jorgensen and Halling-Sørensen, 2000).

However, parasitic invasions are still a common issue in domestic animals. Parasite infections, even at sub-clinical levels, can decrease the growth, maturation and productivity of livestock (DesCôteaux et al., 2001) and hence animals have to be treated

Avermectins as antiparasitics present a group of veterinary medicines that are still of high scientific interest due to their specific metabolism. They are poorly metabolized; measured concentrations of the parent compound in faeces reach up to 80–98 % of the initial administered dose and less than 2 % is excreted in urine (Chiu et al., 1990; Herd et al., 1996).

Recently, Danaher et al. (2011) published a major review on methods for the determination of macrocyclic lactones – ML's (avermectins and milbemycines) in various biological matrices and environmental samples. As environmental samples present a very specific type of analyte, present at low concentrations and along with potentially interfering compounds, effective extraction and purification are essential prior to the analysis.

When avermectins are used for treatment of a herd and according to the properties they have, they will end up either in soil or in the aquatic environment. There is an on-going debate about detrimental effects of avermectin usage especially on grazed pastures. Reports on the toxicity of avermectins to soil invertebrates are rather scarce, except for the earthworms (Svendsen, 2005).

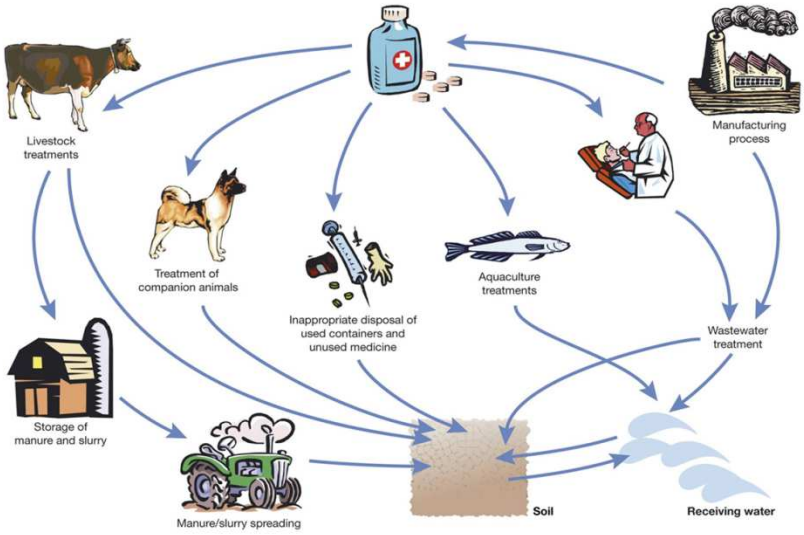

Figure 1: Routes of pharmaceuticals entering the environment
Source: reprinted from Boxall A., 2004

2. Materials and methods

We used several different materials and methods. Our model animals were sheep – which were treated (subcutaneously) by two different avermectins: abamectin and doramectin. We collected their faeces samples for chemical and ecotoxicological analysis. We also sampled soil from the sheep pasture.
Analysis of samples: according to the paper published by Kolar et al., 2005.

Figure 2. Solid phase extraction
Source: Personal archive, 2005

Ecotoxicological methods: according to the paper published by Kolar et al., 2008.

Figure 3. Standard soil-dwelling organism (earthworm) exposed in Lufa 2.2 soil
Source: Personal archive, 2008

We decided to select four species of soil invertebrates for performing toxicity studies in soil and in faeces of treated sheep: the springtail *Folsomia candida*, the enchytraeid*Enchytraeuscrypticus*, the isopod *Porcellioscaber* and the earthworm *Eiseniaandrei*. They were exposed according to proposed guidelines with some modification when the test matrix used differed from the standard Lufa 2.2. soil. It means that besides natural, standard Lufa 2.2 soil, we also used faeces samples from treated sheep with the purpose to mimic the real field situation and compare the outcome with validated laboratory tests.

3. Results

As faecal elimination is environmentally of most concern, we first studied the behaviour of abamectin and doramectin residues in sheep faeces. Abamectin and doramectin showed different characteristics. Doramectin showed efficient excretion pattern whilst abamectin on the contrary, excreted less efficiently -leading to higher residual concentrations in faeces. The time profile of excretion of doramectin and abamectin via sheep faeces after a single subcutaneous dose of 0.2 mg/kg body weight (b.w.).is presented in Figure 4.

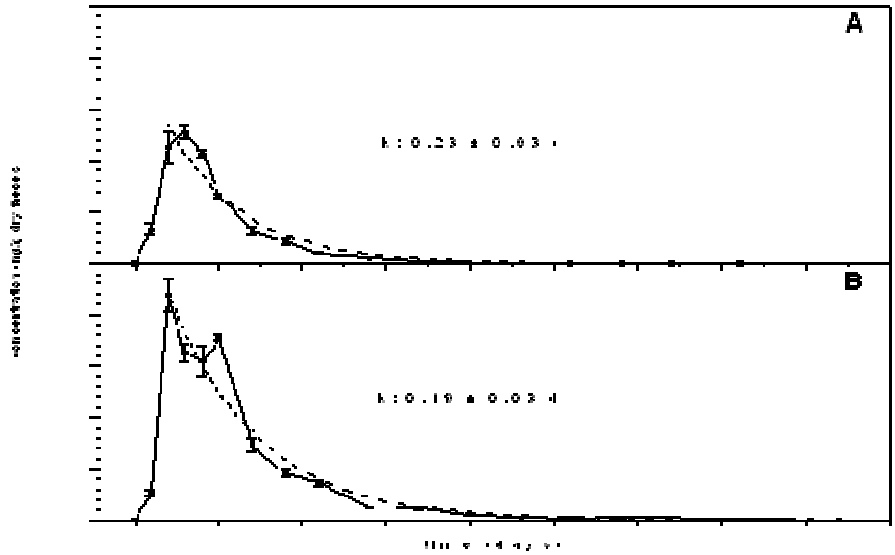

Figure 4.Excretion time profile of abamectin (A) and doramectin (B) via sheep faeces after single subcutaneous administration of 0.2 mg/kg b.w. (collective sample of six sheep with their lambs; mean ± SD; 4 parallel determinations). Dashed lines show the fit obtained applying first order kinetics; the resulting excretion rates (k ± SE) are also shown in the Figure 4

Source: reprinted from Kolar etal., 2006

According to the Figure 4, doramectin, although showing similar excretion kinetics, is excreted more efficiently from sheep than abamectin, leading to higher residual concentrations in faeces.

We also followed degradation of both substances under environmental conditions where establishedhalf-lives for dissipation(DT50) values were of approximately 23 and 22 days for abamectin and doramectin, respectively.

Furthermore we exposed non-target soil dwelling organisms to abamectin and doramectin via contaminated Lufa 2.2 soil and in faeces of treated animals. Abamectin was more toxic to soil dwelling organisms than doramectin. Soil dwelling organisms showed different sensitivity when exposed to avermectins via Lufa 2.2 soil or in faeces from recently treated sheep. No observed effect concentrations values NOECs for the most sensitive organism exposed in soil were 1.5 mg/kg in case of abamectin and 8.4 mg/kg for doramectin, respectively.NOECsthe most sensitive organism exposed in faeces samples varied from 0.811 - > 1.4 mg/kg dry faeces for abamectin and > 2.5 and < 1.4 mg/kg for doramectin (Kolar et al., 2008).

Performed calculation using tools of risk assessment gave predicted no effect concentration PNEC of 150 µg/kg dry soil for the most sensitive tested organism (based on the ecotoxicity results), while measured concentration of avermectins in soil samples was predicted effect concentration in soilPEC_{soil} 2.4µg/kg dry soil. Estimated risk quotient (RQ) was below 1.

4. Discussion

For registration, new veterinary medicinal products and risk studies have to pass the requirements stated in EU Directive 2001/82/EC (European Commission, 2001), amended by Directive 2004/28/EC (European Commission, 2004). Directive 2001/82/EC describes the assessment process in two phases: Phase I assesses the potential of exposure of the environment to the product, while Phase II is needed only if certain trigger values are exceeded and if effects of the product on particular ecosystems are observed. There are also some supporting guidance documents on environmental risk assessment like EMEA (1997), and VICH (2000, 2003), which are regularly used. With obtained data we managed to fill the gap about actual toxicity of avermectins and their effect on the non-target organisms.

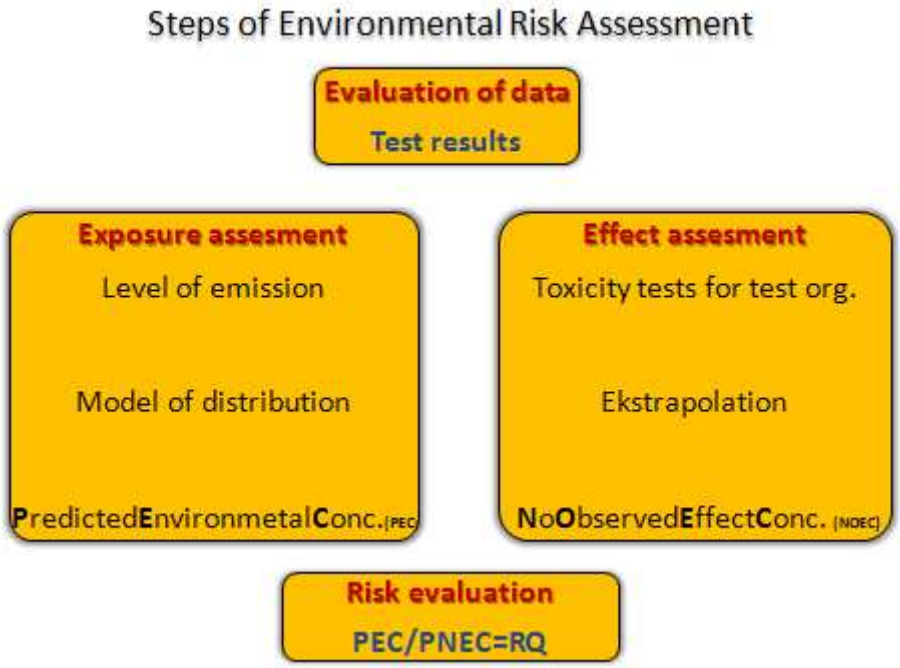

Figure 5.Schematic steps of Environmental RiskAssessment (RAS)
Source: Personal archive, 2011

Within our research we did not observe RQ to be above value 1, but we have to consider severalfacts under real-life conditions: residues in faeces mainly degrade in time after exposure on pasture and may as well be mixed with the dung from untreated animals; repeated treatments; faeces bioassays performedclearly indicated toxicity. This speaks for the complexity of RAS. Another important factor is that current standard ecotoxicity tests are probably not sufficient for assessing the impacts of many pharmaceuticals. The use of more subtle endpoints, such as changed behaviour, physiology and biochemistry in future should be considered (Boxall, 2004).

Many studies so far showed that pharmaceuticals are present in the environment (Halling-Sørensen et al., 1998). Further concern is therefore connected to ways of their removal. In our case, avermectins degraded under environmental conditions, but that is not always the case. Pharmaceuticals can be removed when treated through physical processes, such as sorption or volatilization, biological degradation or chemical reactions, for instance, through treatment with ozone. The suitability of different options is likely to be highly specific for each substance. For example, the antibiotic ciprofloxacin is removed by strong sorption on to suspended solids of sewage sludge whereas diclofenac and 17α-ethinylestradiol undergo significant biodegradation in aged activated sludge (Boxall, 2004).

Many of the treatment methods, whilst removing the pharmaceuticals, may also produce transformation products that are more persistent and mobile than the parent compounds, some of which may also have similar or enhanced toxicity. The significance of transformation products of pharmaceuticals resulting from the parent compounds during natural and technical photolytic processes and advanced oxidation processes has only recently started to attract the interest of the scientific community, while regulatory instruments don’t pay much attention to that.

In Slovenia, pharmaceutical waste is regulated by “Uredba o ravnanju z odpadnimizdravili”, 2008, but that is (only) for human and veterinary medicines which are of direct use (tablets, suspensions etc.). So far, there is no systematic tier for

following presence of pharmaceuticals or their metabolites/transformation products in surface waters or pasture soils in Slovene legislative.
In future, more detailed studies are necessary for explaining different ecotoxicity values (sensitivity between soil and faeces avermectin tests), and test new modelling approaches (quantitative structure–activity relationships). However, the most important among all is “the public awareness” by using as little pharmaceuticals as possible.

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