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The impact of coccidiostats monensin and lasalocid on Cd and Pb uptake in the isopod *Porcellio scaber*

Primož Zidar[a,*], Suzana Žižek[b]

[a] Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia
[b] Veterinary Faculty, University of Ljubljana, Gerbičeva 60, SI-1000 Ljubljana, Slovenia

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ABSTRACT

Monensin and lasalocid are carboxylic ionophore antibiotics used as coccidiostats in the poultry industry. They enter the environment with the use of broiler excrements for manure. As ionophores, they affect the transport of cations across membranes. We studied the bioaccumulation of cadmium and lead in woodlice when concurrently exposed to either monensin or lasalocid. At monensin concentrations (1.8 mg/kg food) comparable to those that could be expected in nature, there was no effect on cadmium accumulation. At 100 times higher ionophore concentrations the presence of monensin or lasalocid resulted in significantly lower cadmium or lead content in woodlice. However, under natural conditions such high levels of ionophores are unlikely. The use of ionophore-contaminated manure in areas where metal pollution is probably does not result in any increased risk to soil invertebrates.

1. Introduction

Monensin and lasalocid are carboxylic ionophore antibiotics used in the poultry industry for prevention and treatment of coccidiosis, a parasitic disease of the intestinal tract caused by coccidian protozoa. In the treated broiler chickens ionophores are only partially metabolised and excreted primarily via faeces. Of the lasalocid in the chicken excreta, 74.3–76.9% is in the active form (EMA, 2004) and more than 50% of monensin is excreted as the parent compound (Davison, 1984) when administered by gelatine capsules in a single dose. When administered in the feed, the concentrations of the parent compound are probably lower, as reported by EFSA (2010) for turkeys. In the faeces from poultry farms which are used as manure on arable land, Žižek et al. (2011) measured monensin concentrations of 0.72 mg/kg wet manure. No measured concentrations in broiler faeces could be obtained for lasalocid. In this way coccidiostats enter terrestrial ecosystems. Environmental risk assessment of ionophores from broiler production, including monensin and lasalocid (Hansen et al., 2009; Žižek et al., 2011), confirmed that both substances could represent a potential risk to the terrestrial environment.

When in the environment, the coccidiostats undergo both biotic and abiotic degradation, with microbial degradation being the prevalent (Sassman and Lee, 2007). The rate of decay depends on the organic content of the soil, soil moisture, temperature and pH (Sassman and Lee, 2007; Yoshida et al., 2010). The reported monensin half-lives in soil are between 2 days (Sassman and Lee, 2007) and 22.7 days (Yoshida et al., 2010). EFSA (2004) reports lasalocid half-life values of 0.6–14.2 days. The reports on the predicted environmental concentrations (PEC) of monensin vary greatly and are between 0.05 mg/kg soil (Žižek et al., 2011) and 1.12 mg/kg soil (EFSA, 2005). The PEC for lasalocid is estimated at 0.58 mg/kg soil (EFSA, 2004). If the manure is used on grasslands, the coccidiostats might contaminate the organic layer of soil that serves as food for many soil organisms.

In the present study, we were interested in the effects of ionophores on metal accumulation in terrestrial invertebrates following concurrent administration of ionophores and metals. The presence of ionophores in the environment simultaneously with Cd or Pb may alter metal assimilation in non-target organisms. Ionophores bind numerous mono- and divalent cations, primarily in dimer complexes that facilitate the passage of ions through hydrophobic lipid membranes (Celis et al., 1974; Elsasser, 1984) in either direction. As reported in Elsasser (1984) and Kirk et al. (1994), feeding monensin and lasalocid to chickens and sheep alters cation availability, distribution and bioactivity. The complexation affinity and thus the transmembrane transport vary among ionophores and cations. Lasalocid is usually classified as a divalent ionophore that effectively combines with divalent cations like Ca^{2+} and Mg^{2+} besides monovalent Na^{+} and K^{+} (Dowling, 1992). Studies on supported liquid membranes demonstrated that lasalocid also facilitates Pb and Cd transport (Aouad et al., 1998; Canet and Seta, 2001; Tayeb et al., 2005). The mediation for Pb is more

* Corresponding author. Tel.: +386 1 320 33 76; fax: +386 1 257 33 90.
E-mail addresses: Primoz.Zidar@bf.uni-lj.si (P. Zidar), Suzana.Zizek@vf.uni-lj.si (S. Žižek).

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successful than for Cd as the complexation rate is generally higher when the metal cation is smaller (Canet et al., 2002; Canet and Seta, 2001). Monensin is usually classified as a monovalent ionophore that combines more readily with Na^+ and K^+ ions (Dowling, 1992) although it also facilitates the transport of divalent cations like Ca^{2+} (Ambroz et al., 1990). Monensin also mediates the transmembrane transport of Pb in rats (Hamidinia et al., 2002, 2006), lowering its intracellular concentrations, which might help to treat lead intoxication (Hamidinia et al., 2006). On the other hand, Khan et al. (1993) found that concurrent oral administration of monensin and lead to broiler chicks increased Pb levels in the liver. No data on the influence of monensin or lasalocid on metal transport and accumulation in non-target invertebrates could be found.

Based on the available information on rats (Hamidinia et al., 2002, 2006) and broiler chickens (Khan et al., 1993), monensin was expected to alter Pb availability to terrestrial invertebrates. Lead emissions have decreased after the phase-out of leaded petrol, but lead can still enter terrestrial ecosystems by atmospheric deposition mostly from mining and smelting sources (Reuer and Weiss, 2002). Furthermore, cadmium bioaccumulation is also of potential interest in agricultural areas. Cadmium enters terrestrial environments with the use of pesticides, phosphate fertilizers, manure, sewage sludge, and with atmospheric deposition (Thornton, 1992). It is therefore very likely to be present in the environments where coccidiostat-contaminated broiler waste is used for manure, but no data exists on the effects of coccidiostats on its bioaccumulation.

As a model organism to study the bioaccumulation of lead and cadmium in the presence of ionophores, the woodlouse species *Porcellio scaber* was selected for two main reasons: first, our previous work (Žižek et al., 2011) has demonstrated that carboxylic ionophores have no detrimental effects on woodlice; and second, woodlice are well known metal bioaccumulators (Hopkin, 1989).

Besides the harmful effects that monensin and lasalocid can have on some terrestrial organisms such as springtails and enchytraeids (Jensen et al., 2009), earthworms (Žižek et al., 2011) or face flies (Broce et al., 1988), their presence in the environment could also have some collateral effects.

2. Materials and methods

2.1. Test animals

The test animals (*P. scaber*) originated from the laboratory culture at the Department of Biology, University of Ljubljana. They were kept in a climate chamber at $20 \pm 1\,^{\circ}C$ with a 16/8 h photo period and caged in glass containers with moist sand and peat on the bottom. They were fed fallen leaves from various trees, with periodical additions of potatoes, fresh vegetables, apples and commercial rabbit food (Hobby Vit, HP Hobby program, Hoče, Slovenia). All tests were performed with adult animals of both sexes weighing between 30 and 45 mg. Pregnant females were excluded.

2.2. Food preparation

Monensin in the form of monensin A sodium salt (90% purity) was obtained from Sigma–Aldrich (Germany) and lasalocid A sodium salt (99.3% purity) was purchased from Alpharma (Belgium). Pb (99.9% purity) and Cd (99.9% purity) atomic spectroscopy standards were purchased from Baker (Holland) and Perkin-Elmer (USA), respectively.

Animals were exposed to test concentrations of ionophores and metals via food. The food pellets consisted of ground maple leaves (42%), ground commercial rabbit food (25%) and potato powder (33%). Demineralised water (36 mL; approx. 5–6 mL g^{-1} dry food) was heated on a magnet mixer to 65 °C, and 2.5 g of maple leaves and 1.5 g of rabbit food were added. Test substances were added at this point. Ionophores were dissolved in acetone and metals were dissolved in water (stock solution: 1 g/L in 2% nitric acid). Next, 2 g of potato powder were mixed in and the mixture was left to thicken (approx. 30 s). Pellets were formed using plastic blisters. They were left to solidify and dry for 24 h at room temperature and 4–5 h at 60 °C. Dry food pellets were weighed and stored in a freezer until use.

The effects of monensin were studied in two experimental sets. The nominal monensin concentrations were 5 and 50 mg/kg dry food in the first experimental set and 150 and 300 mg/kg dry food in the second. The lower monensin concentrations were combined with cadmium (at 10 and 100 mg/kg dry food) and the higher with cadmium and lead (both at 100 mg/kg dry food). Lasalocid was tested with cadmium at 100 and 300 mg/kg dry food. For negative controls, food was prepared without any additions. Solvent control was prepared by adding acetone.

The metal concentrations were selected on the basis of literature data on values that can be expected in polluted environments (Adriano, 2001). The lowest used monensin concentration is close to levels that can occur in nature in a worst case scenario (Hansen et al., 2009), while the higher monensin and lasalocid concentrations are 2–200 times higher than PEC values for soil (Hansen et al., 2009; Žižek et al., 2011).

2.3. Test design

Tests were performed using Lufa 2.2, a standardized natural soil with 3.7% organic matter, 6.8% clay and pH of approx. 6.0 (1 M KCl). Glass jars (100 mL) were filled with approx. 30 g moist soil (60% WHC), and four (for the experiments with monensin) or five (for the experiment with lasalocid) isopods were introduced. There were six replicate jars for each treatment, including the controls. Isopods received food pellets in a small plastic blister (diameter approx. 1 cm) on the soil surface. Food was replaced once a week. The uneaten food was dried and weighed to calculate food consumption.

Test jars were covered with perforated lids and placed on trays in a climate chamber at 21 °C, with 85% RH and a 16/8 h light/dark cycle. Animals were checked twice per week for mortality and moulting. Any dead animals found at that time were removed from the jar. Moisture content was adjusted weekly by weighing the containers and replenishing the water loss with deionised water. Animals from two replicates from each treatment were removed after 14 days and the remaining four replicates were exposed for another two weeks in order to study temporal changes in metal accumulation.

2.4. Chemical analyses

After exposure, the animals were fed clean food for 24 h to purge their gut. Then they were lyophilised, weighed and completely digested in a concentrated nitric acid/perchloric acid mixture (7:1, v/v). After evaporation of the acid, the residue was dissolved in 0.1% HNO_3. Cd and Pb concentrations in whole animals were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100). Metal analyses in the food pellets (4–5 samples/concentration) were performed in the same way as animal samples. Verification of the analytical procedure was performed using the certified reference material for trace metals, TORT-2 (National Research Council of Canada), made of lobster hepatopancreas. The reference material was dried in a vacuum desiccator, weighted (10–20 mg per sample) and digested and analysed in the same way as the rest of the samples. The measured Cd and Pb concentrations in the reference material did not differ by more than 10% from the certified concentrations.