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Effects of the Antiparasitic Drug Moxidectin in Cattle Dung on Zooplankton and Benthic Invertebrates and its Accumulation in a Water–Sediment System

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Abstract

Two anthelmintic macrocyclic lactones—ivermectin and moxidectin—have revolutionized parasite control in cattle. These drugs are only partly metabolized by livestock, and the main route of excretion is via feces. In seasonally inundated floodplains, cattle feces come into direct contact with surface water. Important differences in pharmacokinetics and pharmacodynamics between these drugs may bear on their ecotoxicology in aquatic ecosystems. Moxidectin strongly binds to organic matter and thereby may be consumed in aquatic food webs, but there is a scarcity of data on toxicity to freshwater invertebrates. The objectives of this work were to determine the effect of moxidectin spiked in cattle dung on survival and growth of three representative aquatic invertebrates: the zooplankton *Ceriodaphnia dubia*, the amphipod *Hyalella curvispina*, and the snail *Pomacea canaliculata*. Moxidectin-laced dung was added in microcosms and concentrations were measured in water, sediment + dung, roots of the aquatic plant *Salvinia biloba*, and the aforementioned invertebrates. The influence of moxidectin on nutrient concentrations was also evaluated. Dung was spiked with moxidectin to attain concentrations of 750, 375 and 250 µg kg^{-1} dung fresh weight, approximating those found in cattle dung at days 2, 3, and 5 following subcutaneous injection. Concentrations of moxidectin in dung during the first week of excretion were lethally toxic for the tested invertebrate taxa. The persistence of moxidectin in the sediment + dung and the uptake of the drug in roots of *S. biloba* increase its potential exposure to aquatic food webs. Moxidectin also reduced the rate of release of soluble reactive phosphorus to the water.

Veterinary pharmaceuticals are used in large amounts to treat cattle around the world (Tolls 2001). The anthelmintic macrocyclic lactones (MLs) are endectocides that provide the basis of modern parasite control. Since their development in the early 1980s, the success of the MLs has relied on their remarkable broad-spectrum activity, safety profile, and ease of administration (McKellar and Benchaoui 1996; Kita et al. 2007). Two widely used MLs are ivermectin (IVM) and moxidectin (MOX). These drugs have a common macrocyclic lactone ring that binds selectively and with high affinity on the ligand-gate chloride ion channel receptors of invertebrate nerve and muscle cells, causing irreversible opening of these channels (Rohrer and Arena 1995; Omura 2002).

Despite the similar modes of action of IVM and MOX, distinct physicochemical properties may account for differences in formulation flexibility, kinetic behaviour, pharmacodynamics, and toxicity to target and nontarget invertebrates (Prichard et al. 2012). Both MOX and IVM have high adsorption coefficients (Koc > 1000), indicating that they are not likely to be found in high concentrations in the water column (Lumaret et al. 2012), and are likely to become tightly bound to organic matter in soil and sediments, immobile in the environment (Cunningham et al. 2010).

In cattle, major differences exist between IVM and MOX with a larger volume of distribution and a faster (plasma)

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clearance for MOX (Lanusse et al. 1997; Bousquet-Mélou et al. 2004), presumably due to a more rapid partition into adipose tissue. The higher lipophilicity of MOX compared with IVM ($\log_{10}$ of the octanol/water partitioning coefficient for MOX = 6; IVM = 4.8: HyperChem 7.0, HyperCube Inc., Vellarkad et al. 1989) determines its higher proclivity to accumulate in the body fat of cattle, resulting in a longer elimination half-life (Lifschitz et al. 1999; Prichard et al. 2012).

Residues of MOX and IVM in feces following cattle injection show insecticidal activity, with effects on survival, reproduction, and development of nontarget terrestrial arthropods (Steel and Wardhaugh 2002). From many studies comparing the effects of both drugs on dung arthropods, MOX clearly appears ecologically safer than IVM (Herd 1995; Butters et al. 2012). In cattle, a dose rate of 0.2 mg kg^{-1} IVM is toxic to dung beetles feeding on the feces, whereas MOX had no adverse effects on adult dung beetles at the same dose (Fincher and Wang 1992). Fecal residues of both drugs presented a similar pattern of chemical degradation after environmental exposure of the dung, revealing that the differences in nontarget toxicity were not due to distinct drug degradation kinetics (Suarez 2002). The mechanism underlying these differences remains to be clarified. It may be linked to a lower affinity of MOX for the insect ligand-gated ion channel receptors or to IVM and MOX not targeting the same subset of receptors (Strong and Wall 1994).

Studies of the ecotoxicology of MOX and IVM in aquatic ecosystems have led to concerns that make them a high priority for further environmental study and monitoring (Lumaret et al. 2012). Due to the drugs' tendencies to absorb strongly to soil and sediment organic matter, erosion of particulate matter containing these drugs and direct excretion by treated livestock into water bodies or into land that is subsequently flooded represents the most important routes of entry into the freshwater environment (Kövecses and Marcogliese 2005). Although aquatic invertebrates are more likely to be exposed to MOX and IVM by consumption of particulate matter rather than directly from water, only a few studies have addressed exposure of aquatic benthic invertebrates to IVM via sediment (Thain et al. 1997; Davies et al. 1998; Allen et al. 2007; Egeler et al. 2010) and only two of IVM via feces (Schweitzer et al. 2010; Mesa et al. 2017). Mesa et al. (2017) studied the toxicity of IVM spiked in fresh dung on invertebrates representative of the Paraná River floodplain system. Concentrations between 458 and 1150 μg kg^{-1} were lethal for *Ceriodaphnia dubia* and *Hyalella* sp., and high concentrations of this drug were detected in the apple snail *Pomacea* sp., the aquatic plant *Salvinia* sp. and in the sediment + dung. An apparent suppression of bacterial nitrification by IVM also was observed. In contrast to this work on IVM, no studies have assessed the toxicity of MOX spiked in dung to aquatic invertebrates and the uptake of this drug in biotic compartments such as plants and invertebrates.

Land use on the Middle Paraná River floodplain in Argentina has changed significantly in recent decades, as the expansion of upland soybean production has forced the relocation of cattle to marginal floodplain sites, where the stocking density has recently increased greatly (PROSAP 2009; Quintana et al. 2014). Injection of cattle herds with MOX and IVM has been a practical and accessible tool for parasite control in this region (Mesa et al. 2017). The availability of these drugs without a veterinarian prescription and the presence of several generic formulations in the pharmaceutical market have led to a high frequency of treatment of herds without requiring a parasitological diagnosis. In the Paraná River floodplain, cattle enter wetlands for grazing and drinking immediately following injection, depositing feces that may represent a threat to these aquatic ecosystems (Mesa et al. 2017).
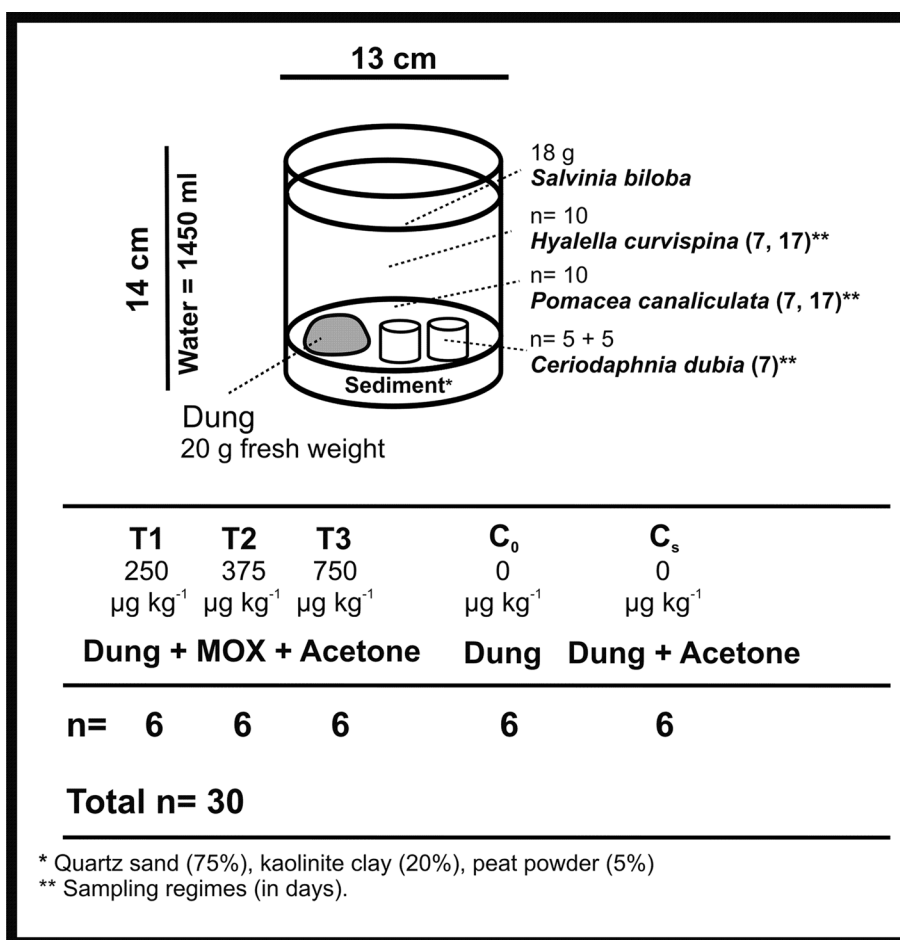
The present study examined the uptake and effects of MOX in dung on three aquatic invertebrates and a macrophyte typical of the floodplain system. The purposes of this work were (1) to determine the effect of MOX spiked in dung on survival and growth (indicated by length) of the planktonic cladoceran *Ceriodaphnia dubia*, the pleustonic amphipod *Hyalella curvispina*, and the benthic snail *Pomacea canaliculata*; (2) to analyze the concentration of MOX in water, sediment + dung, roots of *Salvinia biloba*, and the aforementioned invertebrates; and (3) to evaluate the influence of MOX nutrient release to water. We hypothesized that MOX is less toxic to aquatic invertebrates than IVM and that uptake of this drug in aquatic organisms would be greater than we previously observed for IVM.

Materials and Methods

Test Organisms

A four-species, water–sediment microcosm experiment was performed to simulate exposure of freshwater invertebrates to direct deposition of fresh cattle dung spiked with MOX into water bodies. The design of this microcosm was similar to that performed in a previous study with IVM (Mesa et al. 2017) for comparative purposes. Each microcosm consisted of a glass vessel containing water, sediment, a small floating aquatic fern (*Salvinia biloba*), and three invertebrates—the zooplanktonic microcrustacean *Ceriodaphnia dubia* (Crustacea: Branchiopoda), the benthic amphipod *Hyalella curvispina* (Crustacea: Amphipoda) and the apple snail *Pomacea canaliculata* (Mollusca: Gastropoda) (Fig. 1). These invertebrates were selected as representative of planktonic and benthic taxa of floodplain water bodies along the

Fig. 1 Experimental design of the water–sediment test system. Nominal concentrations of moxidectin spiked in fresh cattle dung relative to the three treatments (T1–T3). MOX-free controls contained either dung alone (C_0) or dung + solvent (C_s)

T1	**T2**	**T3**	**C$_0$**	**C$_s$**
250 μg kg⁻¹	375 μg kg⁻¹	750 μg kg⁻¹	0 μg kg⁻¹	0 μg kg⁻¹
Dung + MOX + Acetone			**Dung**	**Dung + Acetone**
n= 6	6	6	6	6
Total n= 30				

* Quartz sand (75%), kaolinite clay (20%), peat powder (5%)
** Sampling regimes (in days).

Middle Paraná River and of South American floodplains in general. *S. biloba* was included as a widely distributed macrophyte in wetlands of this floodplain system. All of these taxa were taken from our own stock cultures. Water temperatures averaged 25 ± 1 °C in these cultures. *C. dubia* were fed with *Chlorella* sp. ad libitum, *H. curvispina* with Tetramin® fish food, and snails with romaine lettuce every day before the initiation of the experiments.

Spiking Dung with MOX

Fresh cattle dung used in the experiments was collected near wetlands of the Middle Paraná River system where cattle congregate to sleep. Dung was collected from cattle that had not been injected with MLs (MOX and IVM) for at least 7 months before to ensure the minimum concentration or inexistence of these drugs in their bodies. Dung was kept refrigerated at 6 °C for 24–48 h until the initiation of the experiment. Moxidectin pure standard (Sigma-Aldrich, 97%) was used to prepare the stock solutions. Stock solutions and dilutions in cattle dung were prepared with acetone as solvent (Fig. 1). Twenty grams of fresh cattle dung were spiked with different concentrations of MOX. The applied

nominal MOX concentrations were 750 (T3), 375 (T2), and 250 (T1) μg kg⁻¹ dung fresh weight, corresponding to those found in dung at days 2, 3, and 5, respectively, in studies conducted following subcutaneous injection (200 μg kg⁻¹) (Lifschitz et al. 1999; Suarez et al. 2003). The percentage of the administered dose excreted in dung is 44% (Lifschitz et al. 1999, 2000).

The MOX solutions were sprayed on the surface of the 20 g of fresh dung to attain the different nominal concentrations. A sufficient volume of acetone was used to ensure homogeneous application of MOX solution on all of the surface of the fresh dung. Special care was taken to allow a complete absorption of the solution into the dung. An identical methodology was used in Mesa et al. (2017) and confirmed analytically by high recovery rates compared with the nominal concentrations. Samples were left for 90 min after adding MOX solutions to allow evaporation of the acetone.

Microcosm Set-up

Six test vessels (glass vessels, 13-cm diameter, 14-cm height, 1.45-L volume) were prepared for each treatment (T1, T2, and T3), six for the control (C_0), and six for the

solvent control (C$_s$) (total = 30 test vessels) (Fig. 1). The artificial sediment based on OECD guideline 218 (OECD 2004) consisted of quartz sand (75%, 291 grams) kaolinite clay (20%, 29 g), and peat powder (5%, 0.83 grams). This sediment had a water content of 32% (1.25 L) and a pH of 6.8, which had been adjusted using CaCO$_3$. Unchlorinated water was included in each vessel.

Individuals of *C. dubia* were placed inside two small vessels (6-cm height, 2-cm diameter, 30-mL volume, covered with a 50-μm mesh) to enable the rapid visual inspection of the individuals (Fig. 1). The experiment was carried out under a light regime of 12 h light/12 h dark at constant environmental conditions (continuously gently aerated water, water temperature 25 ± 1 °C), without food addition. During the course of the experiment, evaporated water was replaced by unchlorinated tap water.

Thirty randomly selected individuals of each studied taxon were taken from cultures for length measurements before the initiation of the experiment (day 0). The exposure started when the 20 g of fresh cattle dung with the different nominal concentrations of MOX was placed into each vessel. To simulate direct dung deposition by cattle into water bodies, solid fresh dung was introduced into each vessel from the surface of the water, falling through the water column, and remaining on the sediment until the end of the experiment. Some of the added dung broke apart and became mixed with the sediment.

One day after addition of dung, ten *C. dubia* (5 in each small vessel), ten *H. curvispina*, and ten *P. canaliculata* were introduced to the vessels (Fig. 1). The number of individuals used in this experiment was the same that in Mesa et al. (2017) for comparative purposes. Juvenile individuals of each taxon were selected according to approximate length criteria: *C. dubia* 0.7 ± 0.1 mm, *H. curvispina* 3 ± 1 mm, and *P. canaliculata* 10 ± 1 mm (shell length). Eighteen grams (wet weight) of *S. biloba* was included in each test vessel, covering 70% of its surface (Fig. 1).

Responses of Invertebrate Taxa

Survival and length of *C. dubia* were recorded at day 7. Survival of *H. curvispina* and *P. canaliculata* were observed at day 17, whereas length was recorded at days 7 and 17. These days were the same as in the experiments of Mesa et al. (2017) to facilitate comparison. Each surviving *C. dubia* individual was observed under a microscope, and length was determined from the head just above the compound eye to the base of the tail spine. After 7 days of exposure, individuals of *H. curvispina* were collected from three replicates of the controls and treatments by passing the sediment and water through a strainer. Length was measured from high-resolution photographs of each individual under a stereoscopic microscope, digitized using the TPSdig2

program (Rohlf 2006). On the same day, five individuals of *P. canaliculata* were removed from each replicate (*n* = 30 for each treatment and control) and shell length was measured according to Boulding and Hay (1993). Mortality was determined when snails failed to maintain the operculum closed. At the end of the experiment (day 17), all individuals of *H. curvispina* and *P. canaliculata* were sieved from water and sediment + dung using a 200-μm mesh. Surviving individuals of *P. canaliculata* were placed in dechlorinated tap water for one day to flush undigested sediment out of the mantle cavity and gut, which would otherwise create an inaccurate assessment of the body burden (King and Davies 1987; Van Roon 2000). The snails were anesthetized using 1 g L^{-1} of benzocaine–methanol solution (Garr et al. 2012), and later the visceral mass was separated above the edge of the mantle for MOX analysis (Gomot-de Vaufleury and Pihan 2002).

Analysis of MOX Concentrations

Samples of each treatment were analyzed to determine MOX concentrations, and additional samples of fresh dung were prepared to corroborate the absence of MOX in control samples. *Salvinia* roots, sediment + dung, and water were taken from three test vessels of controls and treatments at the middle (day 7) and at the end of the experiment (day 17). Entire *Salvinia* plants were removed, washed, and their roots separated and homogenized for further analysis. Samples of sediment were collected with a spoon (4 g wet weight approximately). Particulates of dung with MOX that were incorporated within the sediment were included in these samples. Ten milliliters of water were obtained with a graduate pipette. For each control and treatment, surviving individuals of *C. dubia* and *H. curvispina* and visceral mass of *P. canaliculata* were separately pooled and preserved at −20 °C for analysis of MOX concentrations.

The extraction from samples and analysis of MOX were carried out following Lifschitz et al. (1999). Concentrations were determined by high-pressure liquid chromatography (HPLC) with a Shimadzu 10 A fluorescence detector (Lifschitz et al. 1999; Mesa et al. 2017). The extraction of MOX from spiked and experimental samples was performed following the technique described by Lifschitz et al. (1999) and adapted by Mesa et al. (2017) for these matrixes. Samples were weighed, homogenized, and combined with the internal standard compound (abamectin). Briefly, 0.5 g of water, 0.5 g of sediment + dung, and 0.1 g of *Pomacea* and roots of *Salvinia* were combined with abamectin (Sigma-Aldrich) as the internal standard (10 ng). For *Ceriodaphnia* and *Hyalella*, the total amount of sample collected was used. One milliliter of acetonitrile was added, and the mixture was shaken (Multi Tube Vortexer, VWR Scientific Products, USA) for 20 min. After mixing the dung, visceral mass of *Pomacea* and roots of *Salvinia* samples were sonicated

during 10 min (Transsonic 570/H, Lab Line Instruments Inc., USA). The solvent-sample mixture was centrifuged at 2000g for 15 min. The supernatant was manually transferred into a tube and the procedure repeated once for sediment + dung, snails, and root samples.

The pooled supernatants were then placed on an Aspec XL autosampler (Gilson, Villiers Le Bell, France) for the automatic solid-phase extraction process. The methanol elution was collected and concentrated to dryness under a stream of nitrogen. The derivatization of MOX was done with 100 µl of a solution of N-methylimidazole (Sigma Chemical, St. Louis, MO) in acetonitrile (1:1) and 150 µl of trifluoroacetic anhydride (Sigma Chemical) solution in acetonitrile (1:2). After completion of the reaction (< 30 s), an aliquot (100 µl) of this solution was injected directly into the HPLC system. MOX concentrations were determined by HPLC using a Shimadzu 10 A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C18 column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 µm, 4.6 mm × 250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (1.6/60/38.4) mobile phase at a flow rate of 1.5 mL/min at 30 °C. MOX was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). Calibration curves were established using least squares linear regression over a range between 0.5 and 150 ng g^{-1}; correlation coefficients were > 0.99. The limit of quantification was established by HPLC analysis of blanks spiked with the internal standard and by measurement of the baseline noise at the time of retention of the MOX peak. The mean baseline noise plus six (10) standard deviations was defined as the theoretical quantification limit. The quantification limits of the analytical technique were 0.5 ng g^{-1} (dung + sediment, *Pomacea* and *Salvinia*) and 0.2 ng g^{-1} (water, *Ceriodaphnia* and *Hyalella*).

Physicochemical Variables

Conductivity, pH (corrected to 25 °C), dissolved oxygen (all measured with a Hanna meter), and water temperature (standard thermometer) were determined daily during the experiment in all test vessels at the same time of the day. Ten milliliters of subsurface water were obtained with a pipette from the controls and treatments for nutrient analyses. Water samples were taken before the beginning of the experiment (day 0) and at days 1, 4, 7, 11, 13, and 16. Water was immediately filtered through Whatman GF/F glass-fiber filters and kept frozen until determination of dissolved components. Total ammonia ($NH_4^+ + NH_3$) was determined by the indophenol blue method (Koroleff 1970), whereas unionized ammonia (NH_3) was estimated from pH and water temperature according to Emerson et al. (1975). Nitrate + nitrite

($NO_3^- + NO_2^-$) was measured by reduction of NO_3^- with hydrazine sulfate and subsequent colorimetric determination of NO_2^- (Hilton and Rigg 1983) and soluble reactive phosphorus (SRP) by the ascorbic acid method (Murphy and Riley 1962).

Data Analyses

Survival and length data were transformed with arcsine and log ($x + 1$), respectively, and then were analyzed by one-way ANOVA followed by Dunnett's test or, in case of variance heterogeneity, Dunnett's T3 test. If no significant differences between control and solvent control were detected using the Mann–Whitney U test, treatments were compared with the pooled controls. In case of significant differences, they were compared with the solvent control. Data normality was tested with the Kolmogorov–Smirnov goodness-of-fit test, and homogeneity of variance was assessed with Cochran's test. To determine if there was a significant effect of MOX on nutrient release to the water, the relationship between the concentration of each nutrient in water at day of peak concentration (dependent variable) and the nominal concentration of MOX in each treatment (independent variable) was assessed by Linear Regression. Only the value of each nutrient at its peak observed concentration was considered, because it corresponded to the maximal net release (accumulated over time) from dung (Hou et al. 2013). Statistical analyses were conducted with PAST software (version 2.17, Hammer et al. 2001).

Comparison of IVM and MOX Uptake

Uptake of MOX at day 17 in sediment + dung, roots of *Salvinia*, and visceral mass of *Pomacea*, expressed as the percent ratio between measured concentrations in each item and the nominal target concentration of MOX in the dung added to each treatment, were compared with the concentration based on data reported for IVM in Mesa et al. (2017) at the same day. Identical microcosm design, methods, and analytical methodologies between the IVM and MOX experiments facilitated this comparison. Concentrations of IVM reported in Mesa et al. (2017) in sediment + dung, roots of *Salvinia*, and visceral mass of *Pomacea* were transformed to uptake values as described earlier. The nominal concentrations of MOX and IVM in fresh dung in this study and in the Mesa et al. (2107) study were based on observed values during first week of excretion after cattle injection (MOX days 2 and 5, 750 and 250 µg kg^{-1}; IVM days 3 and 7, 1150–457 µg kg^{-1}). All tests were performed at the 5% level of significance using PAST software (version 2.17, Hammer et al. 2001).

Results

Effect of MOX on Invertebrates

There were no significant differences in survival and growth (indicated by length) of invertebrates between control and solvent control treatments (Mann–Whitney U test, $P > 0.05$). Survival of *H. curvispina* and *P. canaliculata* was significantly reduced at MOX concentrations between 250 and 750 μg kg^{-1} compared with the pooled control value (Table 1). MOX was highly toxic to *H. curvispina*, producing complete mortality of individuals by day 7 of the experiment in all treatments (250-750 μg kg^{-1} MOX). By day 17, survival of *P. canaliculata* was 63 and 80% at concentrations of MOX in dung of 375 and 250 μg kg^{-1}, respectively, whereas 80% mortality was observed at a concentration of 750 μg kg^{-1}. No significant effects on growth of the studied taxa were detected at the different nominal concentrations of MOX (ANOVA and Dunnett's test, $P > 0.05$; Table 1).

Concentration of MOX in Water, Sediment + Dung, and Invertebrates

No MOX was detected in the additional control samples of fresh dung. For all treatment levels, measured concentrations in freshly prepared dung samples spiked with MOX were in good agreement with nominal concentrations. Recovery (measured concentrations of MOX as % of the nominal concentrations) ranged from 77 to 100%. Table 2 shows the concentrations of MOX measured in water, sediment, roots of *S. biloba*, and invertebrates at days 7 and 17 of the experiment. No MOX was detected in any of the water samples. Among invertebrates, MOX was only detected in *P. canaliculata* at all studied concentrations (250–750 μg kg^{-1}). Concentrations of MOX in sediment + dung, roots of *S. biloba*, and *P. canaliculata* did not show an increase with time, being similar at days 7 and 17 (ANOVA and Dunnett's test, $P > 0.05$; Table 2).

Effects of MOX on Nutrient Dynamics

Water temperature, pH, conductivity, and dissolved oxygen remained relatively constant throughout the study across all treatments, with values of 24 ± 1 °C, 7.5 ± 0.5, 540 ± 10 μS cm^{-1}, and 7.8 ± 0.5 mg L^{-1} respectively. At the beginning of the experiment, values of pH were lower in treatments 2 and 3 (T2, T3) than in treatment 1 (T1) and controls (C_s and C_0) (Fig. 2a). Concentrations of $NH_4^+ + NH_3$ showed similar trends in the controls and the MOX treatments and were significantly higher at day 1 compared with the later days of the experiment (ANOVA and Dunnett's test, $P < 0.001$;

Table 1 Effect of moxidectin (MOX) in dung on survival[a] and growth in length[b] of *Ceriodaphnia dubia*, *Hyalella curvispina* and *Pomacea canaliculata* at days 7 and 17 of the experiment

Concentration of MOX (μg kg^{-1})[c]	C. dubia			H. curvispina			P. canaliculata			
	Survival[a] (%)	Length[b] (mm)		Survival (%)	Length (mm)		Survival (%)	Length (mm)		
		Day 0	Day 7		Day 0	Day 7		Day 0	Day 7	Day 17
0 (C_0)	97.0 (6.3)	0.8 (0.1)	0.7 (0.1)	93.0 (6.0)	4.7 (0.5)	5.9 (1.1)	100.0 (0)	13.5 (0.3)	13.8 (21)	12.0 (0.4)
0 (C_s)	93.0 (5.2)	0.7 (0.1)	0.8 (0.1)	94.0 (5.6)	5.1 (1.1)	5.4 (1.3)	100.0 (0)	13.3 (0.4)	13.3 (2.0)	12.8 (1.0)
250 (T1)	76.0 (5.4)	0.6 (0.1)	0.8 (0.1)	0**	4.8 (0.6)	–	73.3 (5.6)*	13.2 (0.3)	12.9 (2.2)	12.5 (1.0)
375 (T2)	73.3 (3.1)	0.7 (0.1)	0.8 (0.1)	0**	4.7 (0.3)	–	63.3 (8.6)**	13.9 (0.9)	13.8 (1.8)	12.5 (1.1)
750 (T3)	66.6 (5.5)	0.7 (0.1)	0.8 (0.2)	0**	4.9 (0.6)	–	20.4 (10.7)***	15.5 (0.7)	14.5 (1.2)	14.4 (0.8)

*Significantly different from the pooled control values (ANOVA, Dunnett's test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

[a]Observed at day 7 for *C. dubia* and *H. curvispina*, and day 17 for *P. canaliculata*

[b]Observed at day 0 and 7 *C. dubia* and *H. curvispina*, and day 0, 7 and 17 for *P. canaliculata*. Mean values with standard deviation in parenthesis (n = 6)

[c]Nominal concentration of MOX in cattle dung (μg kg^{-1} fresh weight). Treatments = T1, T2, T3

Table 2 Mean concentrations (standard errors in parentheses) of moxidectin (MOX) in water, sediment + dung, roots of *Salvinia biloba*, and invertebrates ($n = 3$) on days 7 and 17 of the experiment in each treatment

	Nominal MOX concentration (fresh weight)					
	Day 7			Day 17		
	250 µg kg^{-1} (T1)	375 µg kg^{-1} (T2)	750 µg kg^{-1} (T3)	250 µg kg^{-1} (T1)	375 µg kg^{-1} (T2)	750 µg kg^{-1} (T3)
Water (µg kg^{-1})	ND	ND	ND	ND	ND	ND
Sediment + dung (µg kg^{-1})	0.5 (0.1)	0.6 (0.1)	60.0 (85.1)	1.9 (2.3)	0.9 (0.3)	50.0 (34.6)
Roots of *S. biloba* (µg kg^{-1})	11.3 (5.6)	16.2 (9.3)	59.1	10.1 (1.6)	29.2 (4.3)	36.0 (11.0)
C. dubia (µg kg^{-1})	ND	ND	ND	ND	ND	ND
H. curvispina (µg kg^{-1})	ND	ND	ND	ND	ND	ND
P. canaliculata (µg kg^{-1})	43.0 (7.4)	95.0 (25.4)	71.0 (1.4)	64.0 (1.7)	89.8 (11.8)	105.0 (2.7)

No MOX was detected in the control (C_0) and solvent control (C_s) samples. *ND* not detected. The quantification limits of the analytical technique were 0.5 ng g^{-1} for dung + sediment, *Pomacea canaliculata* and *Salvinia biloba* and 0.2 ng g^{-1} for water, *Ceriodaphnia dubia* and *Hyalella curvispina*

Fig. 2b). Concentrations of unionized ammonia in controls (C_s and C_0) and T1 peaked at day 1, whereas in T2 and T3, the highest concentrations occurred at days 7 and 11 of the experiment, respectively (ANOVA and Dunnett's post hoc comparison, $P < 0.001$; Fig. 2c). Concentrations of $NO_3^- + NO_2^-$ were significantly higher on the first day of the experiment in the solvent control and all treatments (ANOVA, $P < 0.01$; Fig. 2d). Concentrations of SRP progressively increased from the beginning of the experiment, reaching the highest values at days 11 and 13 (ANOVA, $P < 0.05$; Fig. 2e).

Peak concentrations of SRP showed a significant negative relationship with nominal concentrations of MOX in dung ($r^2 = 0.55$, $P < 0.05$), whereas the inorganic nitrogen forms ($NH_4^+ + NH_3$, NH_3, $NO_3^- + NO_2^-$) did not show a significant relationship with nominal concentrations ($r^2 < 0.15$, $P > 0.05$).

Comparison of MOX and IVM Uptake

Uptake of MOX and IVM in sediment + dung was not proportional to the nominal concentrations of these drugs in dung, with considerably higher ratios at the higher dung concentrations (Table 3). In contrast, uptake of MOX and IVM for *S. biloba* and *P. canaliculata* were lower than expected at the higher nominal concentration of these drugs in dung.

Discussion

The results of this work showed that MOX in dung is accumulated by and toxic to aquatic invertebrates and that this drug accumulates and persists in sediments containing dung as well as in a representative aquatic macrophytes (Tables 1, 3). Concentrations of MOX commonly found in dung during the first week of excretion of cattle following injection

reduced the survival of *C. dubia*, *H. curvispina*, and *P. canaliculata* and significantly altered nutrient release from dung to the water column (Fig. 2).

Based on many studies comparing the effects of both drugs on terrestrial arthropods (Diptera and Coleoptera), it was concluded that MOX is ecologically safer than IVM (Herd 1995; Fincher and Wang 1992; Hempel et al. 2006). In this study, MOX and IVM in dung showed different effects on survival of aquatic invertebrates. In accordance with the hypothesis of this work, toxicity of MOX for *C. dubia* was lower than that we previously observed with IVM in a similar microcosm experiment (Mesa et al. 2017); survival of this taxon was greater than 50% when it was exposed to dung with MOX at concentrations between 250 and 750 µg kg^{-1}, whereas IVM caused complete mortality of *C. dubia* at similar concentrations (458–1150 µg kg^{-1}). In addition, both MOX and IVM in dung were lethal for *H. curvispina*; a complete mortality of this taxon was observed at concentrations of both drugs found in cattle dung during the first week of excretion. *Hyalella* could be exposed to these drugs either from sediment or water or through their feeding habits (Saigo et al. 2009; Mesa et al. 2017). In contrast with our hypothesis, toxic effects of MOX on *P. canaliculata* were greater than previously observed for IVM, producing mortality > 70% at concentrations in dung of 750 µg kg^{-1}. All snails survived upon exposure to IVM in dung in a similar experiment (Mesa et al. 2017).

MOX in dung did not show a significant effect on growth of the aquatic taxa. This result agrees with Mesa et al. (2017), where IVM did not show an effect on growth of the same taxa at concentrations between 22 and 1150 µg kg^{-1}. In contrast, Schweitzer et al. (2010) found that 1314 µg kg^{-1} of IVM in dung had a significant effect on larval growth of chironomids. Differences in toxicological effects of MOX and IVM on invertebrates may be explained by the distinct pharmacodynamic and pharmacokinetic characteristics of

Fig. 2 Mean values (n = 3) of pH (**a**) and concentrations (mg N L^{-1}) ▶ of ammonium + ammonia (**b**), unionized ammonia (**c**), nitrate + nitrite (**d**), and soluble reactive phosphorus (**e**) in the control (C_0), solvent control (C_s), and treatments (T1, T2, T3: see Fig. 1) during the experiment. Vertical bars are standard errors based on three replicates. Peak concentrations are marked with arrows

these drugs. Although the mechanisms are not well known, the specificity of MOX and IVM for different glutamate-gated chloride channels, different affinities of these drugs for the invertebrate ligand-gated ion channel receptors, and distinct targeting to the same subset of receptors could explain their different effects on the survival and growth of aquatic invertebrates.

In contrast with our hypothesis, uptake of MOX and IVM was observed only for *P. canaliculata*, and uptake values were lower than expected at higher nominal concentrations of this drug in dung (Table 3). Detoxification mechanisms in snails (Mackay and Fraser 2000; Ruamthum et al. 2010; Lingpeng et al. 2011) may limit the uptake and increase elimination of these drugs. The observation that *S. biloba* took up MOX approximately in proportion to nominal values in dung (Table 3) indicates that it is suitable for monitoring of MOX pollution in freshwater ecosystems.

The lack of detectable MOX in water and its high concentration in sediment + dung are because this drug is highly hydrophobic (Prichard et al. 2012). As discussed for IVM by Mesa et al. (2017), MOX and IVM are highly lipophilic, binding tightly to soil and sediments, and therefore are classified as immobile (Park et al. 2013). Concentrations of MOX and IVM in sediment + dung were 8 and 5 times higher at the highest nominal concentrations compared with the lowest concentration at day 17 of the experiment (Table 3 and Mesa et al. 2017). Uptake and persistence of these drugs in sediment also have been observed in other studies of aquatic ecosystems (Boxall et al. 2004; Sanderson et al. 2007), including experimental additions of IVM to sediment (Davies et al. 1998; Egeler et al. 2010) and dung (Schweitzer et al. 2010; Mesa et al. 2017). Contamination of aquatic ecosystems with these drugs presents a potential risk for the aquatic biota due to strong binding to sediment and uptake by macrophytes and the persistence in these compartments over time.

Regarding nutrient cycling, MOX showed a negative effect on SRP release, suggesting that this drug could favor the retention of this nutrient in sediment + dung. Exchange of phosphorus between the sediment and the water column is generally considered as an abiotic process driven by changes in pH and redox potential (Maine et al. 1992), but microorganisms have a significant role through mineralization of organic P compounds (Gächter and Meyer 1993; Hupfer and Lewandowski 2008). Neither antibacterial nor antifungal properties of MOX have been previously observed (Lumaret
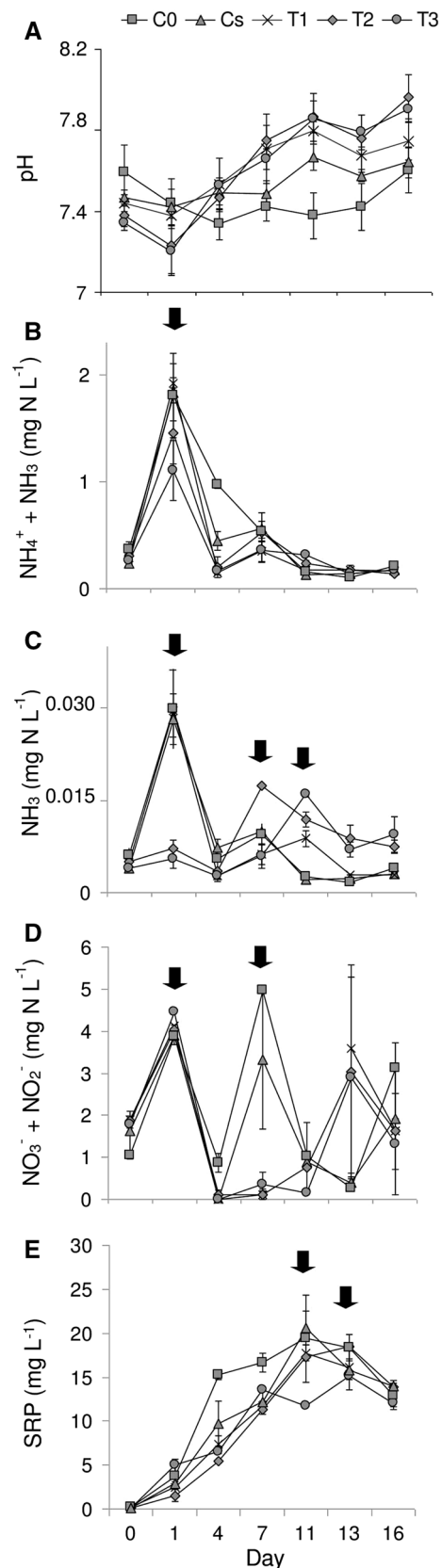

Fig. 2 (legend above)

Table 3 Concentrations and uptake (in percent) of moxidectin (MOX) and ivermectin (IVM) in sediment+dung, roots of *Salvinia biloba*, and visceral mass of *Pomacea canaliculata* at the two nom- inal concentrations during the first week of the experiments of this study (MOX) and the study of (IVM, Mesa et al. 2017)

	750 µg kg^{-1} MOX (day 2)[a]		250 µg kg^{-1} MOX (day 5)[a]		1150 µg kg^{-1} IVM (day 3)[a]		458 µg kg^{-1} IVM (day 7)[a]	
	Concentration (µg kg^{-1})	Uptake[b] (%)	Concentration (µg kg^{-1})	Uptake[b] (%)	Concentration[c] (µg kg^{-1})	Uptake[b] (%)	Concentration[c] (µg kg^{-1})	Uptake[b] (%)
Sediment+dung	50 (34.6)	6.6	1.87 (2.3)	0.7	119 (41.3)	10.3	9.2 (0.8)	2
Roots of *S. biloba*	36 (11)	4.8	10.1 (1.6)	4.4	81.7 (1.5)	7	45.2 (2.0)	9.8
P. canaliculata	105 (2.7)	14	64 (1.7)	25.6	157.8 (45)	13	61.2 (0.6)	13.3

[a]Nominal concentrations of both drugs in dung based on observations made during the first week of excretion after cattle injection (Lifschitz 1999; Lifschitz et al. 2000)

[b]Uptake is expressed as the ratio between observed concentrations and nominal concentration of the drug in dung relative to each treatment, in percent

[c]Mesa et al. (2017)

et al. 2012), but this topic has been poorly studied (Koops and Pommerening-Röser 2001).

On the other hand, the kaolinite clay present in the arti- ficial sediment could have contributed to the adsorption of the phosphate released from dung (Charfi et al. 2013). The sorption of phosphate on kaolinite is maximum around pH 4.0 and decreases with increasing pH (Chen et al. 1973). MOX may be considered a weak acid due to its pKa, which has been calculated as 12.8 ± 1.0 (Awasthi 2012). There- fore, lower values of pH may have occurred in the interstitial water of dung+sediment with higher MOX content, even though the pH of water in the vessels did not change. In addition, an initial slight decrease in pH was observed in water of treatments with higher MOX content, which also would decrease the percentage of unionized NH_3 (Emerson 1975). The effect of MOX on phosphate release from dung as well as the previous observation of a significant impact of IVM on nitrogen transformations (Mesa et al. 2017) point to the need for more study of how these drugs may affect nutrient cycling in aquatic ecosystems.

The significant increase in cattle densities in the Middle Paraná River floodplain in recent decades and the accompa- nying massive usage of antiparasitic drugs pose a potential threat to aquatic ecosystems that demands further study. This topic should be investigated under more realistic conditions using higher tier test systems, such as larger microcosm or preferably outdoor mesocosm systems, in which longer-term effects and population recovery are monitored (Campbell et al. 1983; Van den Brink et al. 2006; OECD 2006). Fur- ther investigations in the field are required to address pos- sible effects on aquatic invertebrates that may be caused by direct excretion of dung containing MOX and IVM into surface water. In the floodplain systems of Middle Paraná River, cattle are typically injected with IVM at a dose of 200 µg kg^{-1} and after 15 days they receive a second injec- tion with the same concentration. During summer-spring,

herds of approximately 150 cows enter floodplain wetlands in groups of 20 and remain approximately 2 h for drinking, grazing, and thermoregulation. During this time, cattle def- ecate, each animal eliminating between 2 and 8 kg of dung with IVM directly into water.

This study provides a better understanding of the effects of MOX on invertebrates typical of floodplains and many other freshwater ecosystems. To our knowledge, this is the first study to show persistence of MOX in freshwater sedi- ments and uptake of this drug in macrophytes and inverte- brates. The potential transfer of these compounds into higher trophic levels (including fish, birds and mammals) and their toxic effects (including possible risk for humans) merits urgent study. Risk mitigation measures may be necessary to avoid the introduction of anthelmintic compounds into aquatic environments. It may be appropriate, for example, to recommend that producers keep treated cattle away from waterbodies for at least a week following treatment to reduce the potential for contamination of the aquatic ecosystem.

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