Accumulation and distribution of lead in the archiacanthocephalan *Moniliformis moniliformis* from experimentally infected rats

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SUMMARY

It recently became clear that adult eo- and palaeacanthocephalans parasitizing fish can bioconcentrate several heavy metals to significantly higher concentrations than the tissues of their definitive hosts. Following this discovery the lead accumulation of the archiacanthocephalan *Moniliformis moniliformis* was investigated using experimentally infected male Wistar rats of the CD-M-strain. The worms were allowed to grow up for 4 weeks post-infection followed by a 3 weeks oral lead exposure of the rats. After the exposure period the rats were killed and the metal levels were determined in muscle, liver, intestine and kidney of the rats as well as in different organs of female and male acanthocephalans. Lead concentrations were found to be highest in female *M. moniliformis* followed by the kidneys of the rats. Male worms contained approximately the same lead concentration as the hosts' kidneys. Lead analysis of the worms' organs revealed the highest lead concentration in the eggs of female acanthocephalans, followed by the cement gland of male Worms. Whilst the lead burden of the presoma was higher than that detected in the kidneys of the rats, the lead content of the metasoma was even lower than in the kidneys. A lead uptake of *M. moniliformis* from the intestinal lumen of the host became apparent as the faeces of infected rats contained significantly less lead compared to the uninfected conspecifics. Thus, this study reveals that lead accumulation also occurs in archiacanthocephalans parasitizing mammals. But the degree of metal bioconcentration is considerably lower compared to eo- and palaeacanthocephalans in fish. Anyway, due to a lack of adequate sentinel species in terrestrial biotopes the host–parasite system rat– *M. moniliformis* appears to be a useful and promising bioindication system especially in urban ecosystems in temperate regions.

Key words: *Moniliformis moniliformis*, acanthocephalans, lead accumulation, rats, bioindication.

INTRODUCTION

Environmental associated aspects of parasitology as well as the recognition of parasitic diseases in environmental impact studies are of growing interest (see e.g. reviews by MacKenzie *et al*. 1995; Lafferty, 1997; Sures, Siddall & Taraschewski, 1999*a*). In contrast to a still increasing number of papers dealing with effects of pollution on fish parasites (Lafferty, 1997) and the accumulation of xenobiotics in helminths of fish (Sures, Taraschewski & Siddall, 1997*a*; Sures *et al*. 1999*a*) less information is available on environmental impact studies of mammalian helminths (see e.g. Sures, Jürges & Taraschewski, 1998). The highest accumulation of heavy metals described so far was found for adult acanthocephalans parasitizing fish (Sures *et al*. 1999*a*; Sures & Taraschewski, 1999) with levels being up to 2700 times higher in the parasites compared to the muscle of the host (Sures, Taraschewski & Jackwerth, 1994).

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Concerning mammalian parasites a number of reports on metal concentrations in nematodes were published mainly as part of investigations dealing with the chemical composition of the hog roundworm *Ascaris suum* (see Enigk, Feder & Dey-Hazra, 1973; Ince, 1976; Greichus & Greichus, 1980; Sures *et al*. 1998). Greichus & Greichus (1980) found the significantly highest concentrations of different elements (Cd, Cu, Fe, Mn, Pb, Se, Zn) in hog kidneys compared to *A. suum*. The levels of most of these elements in the parasites were on average only as high as in the muscle of the pigs (Greichus & Greichus, 1980; Sures *et al*. 1998). An investigation of cattle naturally infected with the liver fluke, *Fasciola hepatica*, revealed interesting differences. Although the cadmium content of *F. hepatica* was considerably lower than that in the tissues of cattle, the concentration of lead in the digenean was significantly higher compared to muscle, kidney and liver of the host (Sures *et al*. 1998). But until now, there has only been 1 study on metal concentrations in acanthocephalans compared to their mammalian definitive hosts (Scheef, Sures & Taraschewski, 2000). After oral cadmium exposure *Moniliformis moniliformis* was found to contain 20, 23 and 119

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times more cadmium than host's kidney, liver and intestine, respectively. Obviously nothing is known about the distribution of metals within the body of acanthocephalans as fish acanthocephalans are too small to dissect them prior to metal analysis. But this information would be very interesting as there are no obvious signs of metal toxicity within the worms. Even female *Pomphorhynchus laevis* sampled from the field with lead concentrations of 193 μg/g (wet weight) were found to release eggs containing acanthors (Sures *et al*. 1994) which indicates an unimpaired healthy state of the worm.

Therefore the aims of this study were (1) to investigate whether the enormous metal accumulation capacity of eo- and palaeacanthocephalans from aquatic definitive hosts also occurs in the archiacanthocephalan *M. moniliformis* parasitizing rats for metals other than cadmium, and (2) to gain a greater understanding of metal distribution in acanthocephalans. Due to their size some *M. moniliformis* were dissected prior to analysis and different parts of the body were analysed in respect of their metal burdens.

MATERIALS AND METHODS

Maintenance and infection of rats

Male Wistar CD rats, *Rattus norvegicus*, (3 months old) were obtained from a commercial supplier (Charles River, Sulzfeld, Germany). Individual rats were maintained at 20 °C in laboratory cages, fed on commercial pellet food (Atromin, Lage, Germany) and were allowed to drink *ad libitum*. The animals were free of intestinal helminths as proven by spot checks of the faeces. Twice a week they were additionally fed with biscuits to guarantee a sufficient supply of saccharides for *Moniliformis moniliformis* (Nesheim *et al*. 1977, 1978; Crompton, 1991). To obtain cystacanths of the acanthocephalan, cockroaches (*Periplaneta americana*) were fed with eggs of *M. moniliformis* containing infective acanthors. The eggs were sampled from a gravid female worm and suspensions of eggs were pipetted on slices of potatoes which were fed to the cockroaches. At 60 days post-infection (p.i.) rats were infected with the cystacanths of *M. moniliformis*. Two *P. americana* were given to each rat (Exp. 1) which were allowed to feed on the cockroaches (infection intensities of *P. americana* with *M. moniliformis* are summarized in Table 1). In the second experiment (Exp. 2) cystacanths were dissected from cockroaches and 10 larvae were given to each rat in a sugar solution, after the rats were trained to drink sugar solution from an Eppendorf pipette.

Experimental design

Rats were randomly divided into different groups and treated according to Table 2. After infection the acanthocephalans were allowed to grow for 4 weeks before exposing the rats to lead. Metal exposure was performed using 2 different methods. In the first experiment rats were orally dosed by contaminated biscuits with lead every 2 days for a period of 3 weeks. As the rats of Exp. 2 were trained to drink sugar solution from an Eppendorf pipette, lead was added to the sugar solution and administered orally to the animals every 2 days for a period of 3 weeks. The rats were weighed each week to determine the appropriate amount of lead which had to be administered (2 μg/g in Exp. 1 and 5 μg/g in Exp. 2, respectively). These lead concentrations were equivalent to an estimated daily lead uptake by humans (Sabbioni *et al*. 1981). Lead solutions were prepared by dissolving solid lead nitrate ($Pb(NO_3)_2$ (Merck, Darmstadt, Germany) in distilled water.

Sampling and analytical procedure

Following exposure the rats were killed, dissected and samples of muscle, liver, kidney and intestine as well as the parasites were taken with the aid of stainless steel scissors and forceps which had been previously cleaned with 1% ammonium–EDTA solution and double-distilled water (Table 2). Additionally, samples of faeces from 1 exposed only and from 1 exposed and infected rat (Exp. 1) were collected every 2 days (the days without lead exposure) at the same time to evaluate possible differences of the lead excretion between infected and uninfected rats.

The infection intensity of *M. moniliformis* was determined according to the method of Bush *et al*. (1997). The wet weight of the worms was noted for both sexes to determine if any deleterious effects of the metal on the growth of the parasites occurred. For a more detailed picture of the distribution of lead within the acanthocephalan all female and male worms from Exp. 1 were dissected prior to digestion to enable analysis of metal concentrations in different tissues of the parasites. All samples of host organs and the parasites were frozen at −26 °C until processing for lead analysis. Prior to the digestion process all samples were homogenized with a dispersing tool (Ultra-Turrax T 25, Janke & Kunkel, Staufen, Germany). Sample portions of about 200 mg (wet weight) were digested with 1·8 ml of nitric acid (Suprapur, Merck, Darmstadt, Germany) using a microwave digestion system as described earlier (Sures, Taraschewski & Haug, 1995). To determine the detection limit analytical blanks were prepared in a similar manner without insertion of a sample. Lead analysis was performed using a Perkin Elmer Model 4100ZL atomic absorption spectrometer equipped with a Zeeman effect background correction system. The metal concentration in each sample was calculated from the corresponding regression line (correlation factor $r \geqslant 0{\cdot}99$) using the

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Table 1. Mean intensity of *Moniliformis moniliformis* in cockroaches and rats after experimental infection

	Experiment 1			Experiment 2		
		R. norvegicus ($n = 20$)			*R. norvegicus* ($n = 13$)	
n *M. moniliformis*	*P. americana* ($n = 20$)	Exposed	Unexposed	*P. americana* ($n = 41$)	Exposed	Unexposed
$\bar{x}$	6·7	20·2	14·4	7·7	8·0	7·7
S.D.	5·2	16·7	8·9	8·1	2·8	2·3
X_{min}	0	0	5	0	3	4
X_{max}	17	38	35	32	10	10

Table 2. Experimental design

Experiment	Group	*n* rats	Lead exposure $C_{Pb^{2+}}$ (μg/g)	*M. moniliformis*	Analysed tissues
1	Exposure	10	2	−	Host: muscle, liver, intestine, kidney, dissected *M. moniliformis*
	Infection	10	−	+	
	Exposure and infection	10	2	+	
2	Control	10	−	−	Host: muscle, liver, intestine, renal cortex and medulla, individual male and female *M. moniliformis*
	Exposure	9	5	−	
	Infection	6	−	+	
	Exposure and infection	7	5	+	

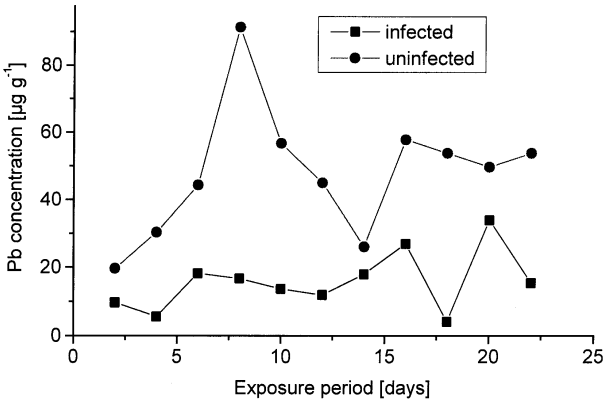

Fig. 1. Lead concentration in the faeces of the rats of Exp. 1.

standard addition method. Regression lines were determined for each sample type (blank, parasites, each of host tissues and faeces) using concentrations (ng/ml) and peak areas (Ext s) for the corresponding *xy* values. Lead concentrations in the parasites and host tissues were determined as μg/g wet weight.

Statistical analysis

For statistical analysis the Mann–Whitney U-Test, Friedman-Test and the Wilcoxon-Test were applied with a significance level of $P \leqslant 0{\cdot}05$. Additionally, the ratio of the metal concentration in the parasites to that in different host tissues ($C_{[parasite]}/C_{[host\ tissue]}$) was determined as described by Sures *et al*. (1999*a*).

RESULTS

Infection of intermediate and final hosts

The mean infection intensities of *M. moniliformis* in the intermediate host were similar in both experiments (Table 1). Due to different modes of infection of the final host, rats of the first experiment were found to contain approximately twice as many adult worms inside their intestine as rats of Exp. 2. In contrast to Exp. 1, each rat in Exp. 2 contained at least 1 adult worm and no rat was uninfected. Considering mean and standard deviation of the infection intensities the application of isolated cystacanths in a sugar solution resulted in less varying values than an inoculation procedure using whole cockroaches (Table 1). Infection intensities of lead exposed and unexposed rats showed no significant difference (Mann–Whitney U-Test, $P > 0{\cdot}05$, in both cases).

Growth of the worms

Comparing the wet weight of female and male worms between exposed and unexposed rats 51 days p.i., no significant difference (Mann–Whitney U-Test, $P > 0{\cdot}05$) was found, but it was observed that the mass of female *M. moniliformis* was approximately 3 times that of male worms. The weight also corresponded very well with the length of the worms. While male helminths were found to be $5{\cdot}1 \pm 0{\cdot}6$ cm (exposed rats) and $5{\cdot}0 \pm 0{\cdot}5$ cm (unexposed rats) in

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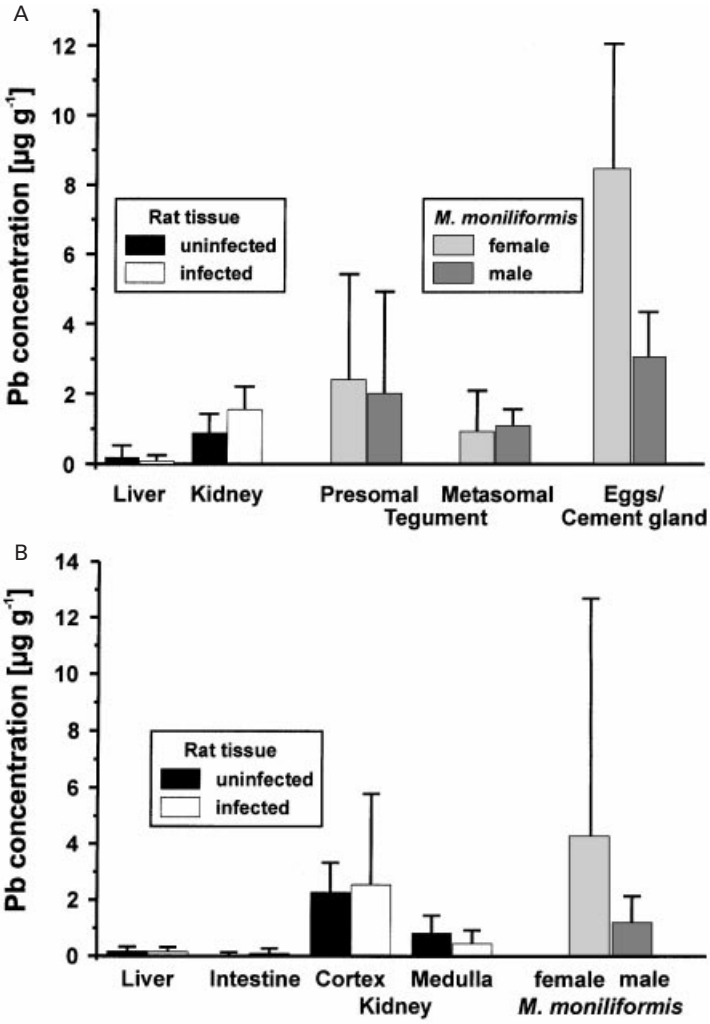

Fig. 2. Lead concentrations in different organs of rats and *Moniliformis moniliformis* orally exposed to lead (lead levels in the unexposed animals as well as in muscle of all rats and intestine of the rats from Exp. 1 ranged below the detection limit). (A) Results from Exp. 1; (B) results from Exp. 2.

length their female conspecifics were twice as long with a length of $10{\cdot}6 \pm 1{\cdot}2$ cm and $10{\cdot}9 \pm 1{\cdot}0$ cm, respectively.

Lead analysis

The detection limit for lead was found to be 11 ng/ml. Lead was continuously excreted with the faeces of the rats (Fig. 1). While there was a straight increase in the lead excretion with the faeces of the uninfected rats until day 8, the lead levels found in the faeces of the infected rats were lower and less fluctuating (Exp. 1). Comparing the mean lead concentration (mean $\pm$ S.D.) over the exposure period, the faeces of the infected rats ($16{\cdot}4 \pm 8{\cdot}9$ μg/g) contained significantly less lead (Mann–Whitney U-Test, $P \leqslant 0{\cdot}001$) than the faeces of their uninfected conspecifics ($50{\cdot}8 \pm 17{\cdot}8$ μg/g).

Regarding the organs of the definitive host only, the highest metal levels were found for the kidney and the liver of the rats (Fig. 2) with the significantly highest lead concentrations (Wilcoxon-Test, $P \leqslant 0{\cdot}01$ in Exp. 1 and $P \leqslant 0{\cdot}05$ in Exp. 2) in the kidneys of the rats with mean Pb concentrations of 1·57 μg/g (Exp. 1) and 2·28 μg/g respectively, for the renal cortex (Exp. 2). No clear tendency emerged between the lead levels in organs of infected and uninfected hosts. While the kidneys of the infected rats in Exp. 1 were found to contain significantly higher concentrations (Mann–Whitney U-Test, $P \leqslant 0{\cdot}001$) than those of the uninfected ones, no difference was found for renal medulla and cortex of the rats from Exp. 2. The lead exposure obviously did not affect the lead levels in the muscle of all rats and the intestine of the rats from the first experiment as the analysed lead burdens in these organs ranged below the detection limit. Unexposed rats and worms from both experiments did not contain lead.

Considering also the parasites, it is clear that the acanthocephalans contained the highest concentrations of lead in both experiments (Fig. 2). Analysis of the lead distribution within the worm's body revealed the highest concentration of lead in the eggs of female *M. moniliformis* followed by the cement

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glands of the male acanthocephalans (Fig. 2A). The presomal tegument of female and male worms contained approximately the same amount of lead. Lowest concentrations were found in the metasomal tegument of female and male worms. Average lead concentrations of the whole worms were found to be significantly higher (Wilcoxon-Test, $P \leqslant 0{\cdot}01$) in males as well as in females compared to liver, intestine and renal medulla of the rats (Fig. 2B). The comparison of lead concentrations in renal cortex, female and male worms revealed no significant differences (Friedman-Test, $P > 0{\cdot}05$) and although the mean lead level was higher in female acanthocephalans than in males, statistical analysis showed no significant difference (Wilcoxon-Test, $P > 0{\cdot}05$). Bioconcentration factors for lead were 25, 39, 2 and 9 for female worms and 7, 11, 0·5 and 3 for male *M. moniliformis* compared to host's liver, intestine, renal cortex and medulla, respectively (Exp. 2).

DISCUSSION

The highest concentration of lead in the host–parasite system investigated within this study was recorded for *M. moniliformis* after experimental oral lead exposure of the rats. Kidney and liver were found to contain the highest amounts of lead considering only the organs of the rats. This is in good accordance with the literature as both tissues are known as the main accumulation organs for metals in mammals (Merian, 1991). The intestine as the major uptake site for lead after oral exposure (Merian, 1991) exhibited only lead levels above the detection limit in the second experiment using a higher oral lead dose. Comparing liver lead levels of exposed rats from the two different experiments, no increase of the tissue levels after the higher oral dose was found. As rat kidney and the parasites were treated different in both experiments, a comparison of the lead levels is difficult.

Earlier studies with mammals naturally infected with helminths never included acanthocephalans but focused mainly on nematodes (Enigk *et al*. 1973; Ince, 1976; Greichus & Greichus, 1980; Sures *et al*. 1998). For example, concentrations of lead in the liver of unexposed pigs were at least twice as high as those recorded from the intestinal parasite *A. suum* (Greichus & Greichus, 1980; Sures *et al*. 1998). Only the liver fluke *F. hepatica* was found to contain 172, 53 and 115 times more lead than the muscle, kidney and liver, respectively, of its bovine host. These bioconcentration factors (ratio[$\text{metal}_{\text{parasite}}$]/[$\text{metal}_{\text{host tissue}}$]) for lead were remarkably higher than those obtained in the present study for *M. moniliformis* compared to the rat tissues. The high lead accumulation capacity of the digenean is probably related to the excretion of the respective metal by the host. It is known that in mammals lead is excreted from the liver into the intestine via bile (Lehnert & Szadkowski, 1983). As *F. hepatica* lives in the bile ducts surrounded by bile liquid, lead could be absorbed across the tegument of the flukes in a lipophilic form after complexing with bile acids. In contrast, *M. moniliformis* takes up lead from the intestinal lumen of the rats which became apparent by comparing the amounts of lead excreted with the host's faeces. The infected rats excreted significantly less lead with their faeces than the uninfected ones. Thus, the acanthocephalans are able to reduce the concentration of lead in the gut content and probably also the amount which could run through the hepatic intestinal cycle as demonstrated for *Pomphorhynchus laevis* (Sures & Siddall, 1999). But, in contrast to the results on the lead accumulation of *P. laevis* which significantly reduced the amount of lead in the intestinal wall of its host chub, no effect of the lead uptake by *M. moniliformis* on the lead burden in the rat tissues could be found in the present study.

The lead accumulation in *M. moniliformis* resembled results on the cadmium uptake and accumulation in the same host–parasite system published recently (Scheef *et al*. 2000). Thus, *M. moniliformis* is able to accumulate lead and cadmium to a higher degree than its final host. But it is noticeable that the bioconcentration factors for cadmium determined for *M. moniliformis* (Scheef *et al*. 2000) were higher than those determined for lead, which is in contrast to results on metal accumulation in acanthocephalans parasitizing fish (e.g. Sures, Taraschewski & Rydlo, 1997*b*). In several field studies fish acanthocephalans were found to show higher bioconcentration factors for lead than for cadmium (summarized by Sures *et al*. 1999*a*). In a recent comparison of the accumulation of 17 different elements in *Acanthocephalus lucii* and its fish host perch, bioconcentration factors regarding the intestinal wall of the host can be listed as follows: Cu = Ag > Pb > Tl > Cd > Sr > Ca > Ba > Zn > Fe > Ni > Al = Mg > Co = Ga = Mn, with a value for lead being approximately twice that of cadmium (Sures *et al*. 1999*b*). The bioconcentration of lead in *M. moniliformis* is also relatively low compared to the values obtained for fish acanthocephalans. Compared to the fish host intestine, e.g. *P. laevis* contained 280 higher lead levels (Sures *et al*. 1994) whereas in the present study *M. moniliformis* accumulated between 11 and 39 times more lead than the intestine. Reasons for these differences are not clear, but the route of host exposure to the metal and hence the chemical form of lead in the intestine may play an important role. While fish acanthocephalans are most likely exposed to bile-bound lead excreted by host liver (Sures & Siddall, 1999), the rats were orally exposed to lead salts. Thus, the metal enters the intestine without being bound to bile salts in the liver, and therefore its bioavailability could be reduced compared to bile-bound lead.

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The question of a sex-specific accumulation of metals in *M. moniliformis* is not easily answered. Although no significant differences were found in respect of the lead levels between female and male worms, the eggs of the females contained considerably higher amounts of lead than the cement glands of the males. Additionally, a previous investigation showed higher cadmium levels in female worms compared to their male conspecifics (Scheef *et al*. 2000) and it was suggested that female *M. moniliformis* are probably able to discharge metals via the shells of their eggs (for morphology of egg-shells see Taraschewski (2000)). The same conclusion may be drawn from results on element levels in the fish tapeworms *Bothriocephalus acheilognathi* (Riggs, Lemly & Esch, 1987) and *Bothriocephalus scorpii* (Sures, Taraschewski & Rokicki, 1997*c*). In both studies the highest levels of selenium (Riggs *et al*. 1987) and of cadmium and lead (Sures *et al*. 1997*c*), respectively, were found in gravid proglottids as compared to the anterior part of the cestode's strobila. Thus, a possible storage/elimination of lead in the eggs/cement gland may result in a detoxification of the metal. Comparing the infection intensities as well as the size and the weight of the helminth from lead-exposed and untreated rats no significant differences were found, indicating that at least no obvious deleterious effects of the metal exposure on the rate of establishment and survival of *M. moniliformis* occurred. Similar results were obtained from an *in vitro* lead exposure of cystacanths of *Pomphorhynchus laevis*, in which also no obvious negative effects on the growth of the worms were found (Sures & Siddall, 1999).

The results presented in this article demonstrate that species belonging to all groups of acanthocephalans (eo-, palae- and archiacanthocephalans) bioconcentrate heavy metals to a higher degree than their hosts. Due to its lead and cadmium accumulation capacity *M. moniliformis* might be used as an accumulation indicator for heavy metals in terrestrial biotopes, especially urban ecosystems, as the number of animals which might be used for these purposes is limited (Schubert, 1991). Advantages of using helminths as bioindicators are among other things their wide geographical range, their abundance and easy sampling, and the obvious tolerance against metal toxicity (Sures *et al*. 1999*a*). The mobility of the host allows us to calculate the average exposure within the natural home range of the definitive host (Sures *et al*. 1997*a*). Rats are widely distributed and abundant in all kinds of terrestrial biotopes. They are used as final hosts by *M. moniliformis* but also by different other helminths like cestodes, which have also been shown to accumulate metals (Sures *et al*. 1997*c*). Thus, rats and their helminths appear to be a very useful and promising tool in environmental monitoring. More field and experimental studies are required to evaluate the relationship between parasite bioaccumulation and environmental metal exposure and to validate the role of helminths in environmental biomonitoring.

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