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Indicative content of mercury in insectivores (Central Bohemia, Czech Republic)

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Abstract. The aim of this study was to optimize the determination of mercury content in small mammals from the Soricidae family and compare it with the mercury content in larger mammals with slower metabolism, which are also linked to the aquatic foog chain with dangerous forms of mercury. Mercury content was measured in the fur, which is the main mechanism for eliminating toxins, and in the liver. The ratio between the liver and fur served to verify the intensity of metabolism. This intensity was also monitored in small rodents with lower metabolic output. In rodents, the change in mercury content in tissues due to mercury accumulation in the fetus during pregnancy was also measured. The degree of environmental load, the degree of dependence on the aquatic food chain and the degree of mercury demethylation in the liver within a specific detection are discussed. The optimal method of biomonitoring is also discussed.

Key words. Soricidae, mercury, metabolism, fur, aquatic food sources.

INTRODUCTION

The task of this pilot study is to optimize the methodology for further research on mercury content in small insectivores (*Neomys* Kaup, 1829; *Crocidura* Wagler, 1832; *Sorex* Linnaeus, 1758) and to evaluate their data by comparing it with data on mercury content in mammals that have contact with the aquatic food chain (*Lutra* Brisson, 1762; *Neovison* Baryshnikov et Abramov, 1997). The insectivores used in this study were assumed to belong to populations that also depend on aquatic food sources. In the genus *Neomys*, this connection is close and evolutionarily determined. In the genera *Sorex* and *Crocidura*, the connection is occasional or indirect. Recaptures prove here and population or individuals, which are captured on the banks of streams, have their permanent areas here, and are therefore in constant contact with the aquatic environment. However, it is difficult for these insectivores to establish clear habitat preferences, as they are opportunists. *Sorex minutus* Linnaeus, 1766 and *Crocidura suaveolens* (Pallas, 1811) mainly collect surface-moving prey such as insects and spiders; *Sorex araneus* Linnaeus, 1758 focuses mainly on earthworms. Due to their smaller body size, shrews have increased energy requirements, faster metabolism and relatively longer fur. We already observe these tendentious metabolic differences within the family between the genera *Sorex* and *Neomys* (Gebczynka & Gebczynski 1965; Table 6).

The toxic burden of mercury is conditioned by its methylation. In contrast to the inorganic form of Hg, the methylated organic form of Hg (MeHg) is more easily absorbed by the gastrointestinal system and more easily passes through the haemoencephalic membrane into the brain, where it acts as a neurotoxin with an impact on the immune and reproductive systems (Scheuhammer et al. 2007). Methylation is usually tied to the aquatic food chain, where it is catalyzed by aquatic bacteria. Part of bivalent Hg is methylated by sulfur and iron bacteria (Selin 2009). MeHg enters the terrestrial environment through the predation of aquatic organisms, which accumulate MeHg (Gerrard & St. Louis 2001). MeHg is the predominant form of total Hg (THg) in all tissues (Evans et al. 2000). A comparison of mercury content in captured and bibliographic mammals is intended to point out several aspects:

(1) According to Evans et al. (2000), fur or feathers serve as an active mechanism for getting rid of toxins. (The thiols in keratin form strong bonds with Hg and with other metals). The concentration of both THg and MeHg is higher in the coat than in the liver. The different content of Hg in the fur must therefore depend on the contamination of the environment and on the time course of the replacement of the body cover. Fur and liver samples will be compared to determine cumulative dynamics in insectivores:

(1a) For *Sorex minutus* in the autumn set between localities No. 1 and No. 2. The comparison should indicate the environmental burden at location No. 1.

(1b) For all captured individuals of all species. In the monitored insectivores, especially *Neomys fodiens*, we can count on a closer connection to the aquatic food chain, and therefore a greater content of Hg in tissues and fur. This content may continue to increase with the specificity of fast metabolism in insectivores. The comparison should indicate the significance of the link to the aquatic environment.

(1c) For *Sorex minutus*, depending on the molting of the fur in the summer and autumn sets at location No. 1.

(2) Part of MeHg demethylates in the liver. It seems that the higher the proportion of Hg in the liver, the lower its methylated fraction tends to be (Scheuhammer et al. 2007). However, our determination does not distinguish between free Hg and methylated MeHg element and only determines the total THg content. To verify the influence of metabolic intensity on Hg deposition in individual species, we will therefore use the THg ratio between fur and liver. For insectivores with an accelerated metabolism, these values have not yet been determined. The ratio of increasing heat production to decreasing body weight in mammals is the most striking in insectivores. Heat production is associated with basal metabolic rate (BMR) (Geiser & Baudinette 1990), which is higher in insectivores than, for example, rodents of the same size. BMR represents the amount of energy released at rest and in a neutral environment. In insectivores, the intake of food volume per unit of weight is therefore greater, and the assumption for the accumulation of heavy metals also increases with it. Soricinae have extremely high metabolic output (Taylor 1998). A comparison of fur and liver will serve:

(2a) To compare the cumulative dynamics in captured insectivores. Measurements will also be used in the wood mouse rodent (*Apodemus flavicollis* (Melchior, 1834)) and other mammals linked to the aquatic food chain. These comparisons are also intended to enable the estimation of the MeHg content in relation to THg and, together with point 1a), enable the determination of the environmental burden at the Roudný location.

(2b) for determining the relative share of THg in the liver and fur.

(3) Considering that Hg also leaves the tissues through accumulation in the fetus during pregnancy, the values in the fur and in the liver of a male and a pregnant female of the species *Apodemus flavicollis* will be compared.

MATERIAL AND METHODS

The capture of the studied individuals (*Sorex minutus*, *Sorex araneus*, *Crocidura suaveolens* and *Apodemus flavicollis*) took place at the Roudný locality in the region of Podblanicko (Central Bohemia, the district Benešov) with the predicted occurrence of an increased mercury content. Geopark Roudný is located on a former gold mine, where mercury was used to amalgamate gold. The last historical stage of mining took place here from roughly 1882 to 1950. The studied individuals were caught in live traps at night and in the morning on the banks of the Bořkovický stream below the sludge lagoon pond (49°37'N 14°49'E). The stream is protected by a forest and belongs to the Blanice basin. In the upper part of the stream, growths of sulfur bacteria were found, which are supposed to catalyze the methylation of mercury. The only species of insectivore that was repeatedly captured (two pieces each) in all seasons and also at another reference site was *Sorex minutus* due to its constant presence at the Roudný site of interest.

The capture of the first set of monitored individuals took place at the beginning of August 2020, when the animals should be in summer fur. At the beginning of the second half of May, according to Borowski (1973), in the *Sorex minutus* species, the double spring molting to the summer coat (2.5–3.7 mm) is completed. We find increased summer fur similar to the *Sorex araneus* species (3.5 mm) from the beginning of June. In *Neomys fodiens* (Pennant, 1771), the second spring molt is completed in mid-August, but 90% of the population completes molting into the summer coat (3.6–6.8 mm) in May (Borowski 1973).

The capture of the second set of monitored individuals took place at the beginning of October. In the *Sorex minutus* species, autumn molting into winter fur (4.5–5.9 mm) starts from mid-September and ends at the beginning of November. We therefore find increased winter fur similar to *Sorex araneus* (6.6 mm) from the beginning of November.

In *Neomys fodiens*, autumn molting begins in late August, and 75% of the population acquires its winter coat (6.9–9.5 mm) by mid-October (Borowski 1973). In *Crocidura suaveolens*, the autumn molt begins in September and ends in mid-October (Huminski & Wojcik-Migala 1967). From the point of view of autumn molting and the attempt to capture fresh winter fur in the samples, it would therefore be more optimal to postpone the second capture to the beginning of November, but the food links at this time would probably be less connected to the aquatic food chain, which in insectivores is mainly insects. The liver, whose mercury content can indicate the degree of demethylation, is able to reflect short-term dietary information (Braune et al. 2006), which, in case of a change in diet, disrupts the continuous trend in metal accumulation. This information is evident when the content of Hg in the liver is higher than in the coat.

For determining the winter and summer coat, the length of the coat, or the change in color to a darker one, which mosaic-likely accompanies the course of the autumn molt, was decisive. The summer coat at the time of the first capture should therefore accumulate metabolic products for approximately 2.5 months in *Sorex minutus*. Now of the second capture, the summer coat should accumulate metabolites for approximately 4.5 months, and the winter coat for less than a month at most. In case of incomplete molting, the percentage of winter and summer fur must be considered in order to assess the cumulative period of fur.

The capture of the studied individuals of *Sorex minutus* at the reference location Prague-Zbraslav took place on the banks of the Břežanský stream (49°58'N, 14°25'E) belonging to the Vltava basin at the beginning of November. The stream bank was 3–6 m away from the parallel, busy road. The one-month delay in this capture was due to the absence of the observed species at the previously selected reference locations.

Table 1. Overview of captured individuals at particular locations

ID	species	date	location / No.	weight [g]	note
summer 2020					
1	*Sorex minutus*	6 August 2020	Roudný / 1	3.0	male
2	*Sorex minutus*	12 August 2020	Roudný/1	4.0	male
3	*Apodemus flavicollis*	12 August 2020	Roudný/1	46.0	pregnant female
autumn 2020					
4	*Sorex minutus*	7 Octobur 2020	Roudný/1	3.0	male
5	*Sorex minutus*	7 Octobur 2020	Roudný/1	3.0	female
6	*Apodemus flavicollis*	7 Octobur 2020	Roudný/1	26.0	male
7	*Sorex araneus*	7 Octobur 2020	Roudný/1	7.0	–
8	*Crocidura suaveolens*	7 Octobur 2020	Roudný/1	9.5	–
9	*Sorex minutus*	16 November 2020	Zbraslav/2	2.5	–
10	*Sorex minutus*	16 November 2020	Zbraslav/2	2.4	–
11	*Neomys fodiens*	24 October 2020	Chřášťany/3	9.6	–

Table 2. Concentration of the mercury (Hg µg/g) in the organs of *Sorex minutus* in autumn at the locations Nos. 1 and 2

location	ID	liver	fur	coat length mm/%
Roudný No. 1	4	0.044	0.375	5/7
	5	0.062	0.350	5/7
Zbraslav No. 2	9	0.436	2.116	5/100
	10	0.171	1.002	5/100

The capture of *Neomys fodiens* took place on the banks of the Tloskovský stream near Chřášťany (49°47'N, 14°34'E) in the region of Podblanicko, belonging to the Sázava basin in October 2020 due to its absence at the Roudný locality. The stream was about 100 m away from a busy road. At the Zbraslav and Chřášťany localities, live hunting traps were also used in the night and morning hours. The rodent *Apodemus flavicollis* with more or less continuous and irregular molting was also caught in the Roudný locality in summer and autumn (Fullagar 1967). *Apodemus flavicollis* has defended permanent ranges but also explores outside the home range. Out of four visits to the site, the species was captured only two times, each time by one piece, so it is unlikely that their home range was on the banks of a stream where they would regularly receive food. On the other hand, visits to the stream bank were most probably also related to food intake and thus to occasional contact with the aquatic food chain. The ratio of the possible content of Hg in the liver and fur can indicate the regularity of food contacts, if THg also contains a representative proportion of MeHg. The capture took place in accordance with the applicable legal regulations of the Czech Republic.

Fur and liver samples were taken in a volume of 0.05 g, stored in –15 °C and dried. Two measurements were averaged to determine one sample. Each analyzed a different loading of sample that was not modified in any way before analysis. The samples were analyzed on an AMA 254 analyzer; the determination principle is atomic absorption spectrometry. The device uses the technique of generating vapors of metallic mercury with subsequent capture and enrichment on a gold amalgamator. The detection limit of the instrument is 0.01 µg Hg.

RESULTS AND DISCUSSION

Given that the Hg content in tissues is at site No. 2, which was the only one exposed near the road (source category: air pollution from combustion engines), greater than at location No. 1, we do not need to take into account the effect of environmental contamination at location No. 1.

The content of Hg is higher in *Neomys fodiens* than in *Apodemus flavicollis* with a minimal connection to the aquatic food chain, even than in other insectivores from locality No. 1, except individual No. 2 *Sorex minutus* and individuals No. 8 *Crocidura suaveolens*. These individuals probably specialized in collecting aquatic insects. Because the intensity of metabolism is comparable in the genera *Neomys* and *Crocidura* (see Table 6), but the accumulation of metabolites

Table 3. Mercury content (Hg µg/g) in the captured mammals with different links to the aquatic food chain

species	location / ID	season	liver	fur
Neomys fodiens	3 / 11	autumn	0.160	0.575
Sorex minutus	2 / 9	autumn	0.436	2.116
Sorex minutus	2 / 10	autumn	0.171	1.002
Sorex minutus	1 / 4	autumn	0.044	0.375
Sorex minutus	1 / 5	autumn	0.062	0.350
Sorex minutus	1 / 1	summer	0.131	0.137
Sorex minutus	1 / 2	summer	0.167	1.192
Sorex araneus	1 / 7	autumn	0.198	0.189
Crocidura suaveolens	1 / 8	autumn	0.547	1.028
Apodemus flavicollis	1 / 3	summer	0.029	0.084
Apodemus flavicollis	1 / 6	autumn	0.027	0.068

Table 4. Concentration of the mercury (Hg µg/g) in the organs of *Sorex minutus* at the location No. 1

month	ID	liver	fur	coat length mm/%
August	1	0.131	0.137	3/100
	2	0.167	1.192	3/100
October	4	0.044	0.375	5/10
	5	0.062	0.350	5/10

in the coat is twice as much in *Crocidura* as in individual No. 2 *Sorex minutus*, it cannot be ruled out that the double content is an indication of the environmental burden at the Roudný site, which, however, does not reach the level at site No. 2. This would also be confirmed by the significantly higher content of Hg in the liver of *C. suaveolens*, which could be identified with the food specialization of *Neomys fodiens* in the short term. A higher liver content of Hg was also recorded in *Sorex araneus*. Its liver value was higher than the Hg content in fur and could indicate short-term contact with aquatic food sources. (The lower content of Hg in the fur of *Neomys fodiens* could also be due to the presence of fresh winter fur, which we only assume due to the ambiguity of the measurement: approx. 7 mm).

If significant values in the coat of individual No. 2 will be understood as unrepresentative specialization on aquatic food sources, the remaining samples will represent an increase of Hg in the fur over two months in the fur by more than 150% (i.e. 0.2 µg/g). However, it was not possible to capture the effect of coat replacement due to the fact that the summer coat (90%) prevailed even during the second capture in October.

A higher proportion of THg in the liver of insectivores, expressed by a lower ratio number, suggests the possibility of more intenze demethylation. From the comparison with *A. flavicollis*, it is evident that the increased BMR in insectivores is not reflected in the liver share of Hg. In the mink, which has a lower proportion of Hg in the liver and a more intensive metabolism than the otter, Evans et al. (2000) reports a more active use of fur in Hg elimination. (In other tissues it has values comparable to otter). He assumes that the higher content of Hg in the fur of the mink compared to the otter in tab no. 5 is related to more active elimination of toxins, which would correspond to a higher value of basal metabolism in mink. (The accumulation of Hg in fur is 4.1% more effective in mink at maximum values and 4.3% at minimum values). In otters, which are about 10 times heavier, we find a larger proportion of Hg in the liver than in mink. However, from Wolfe & Norman's (1998) data, we can observe a decrease in the proportion and amount of THg in the liver in most samples, along with the increase in mink weight (n=6). Only two

Table 5. Concentration of THg (in µg/g) in the liver and fur in various mammals

species	weight [g]	liver min–max	fur min–max	author
Apodemus flavicollis	26.0	0.027–0.029	0.068–0.084	own results
Sorex minutus	3.0–4.0	0.044–0.436	0.137–2.116	own results
Sorex araneus	7.0	0.198	0.189	own results
Neomys fodiens	9.6	0.160	0.575	own results
Crocidura suaveolens	9.5	0.547	1.028	own results
Neovison vison	500–1,700	1.50–5.14	17.26–54.05	Evans et al. 2000
Procyon lotor	3,500–9,000	1.02–3.29	6.93–21.97	Wolfe & Norman 1998
Lontra canadensis	5,000–14,500	1.61–3.35	10.56–22.23	Evans et al. 2000

Table 6. BMR (basal metabolic rate) values; BMR (%) – % increase in BMR output compared to predicted BMR output in non-soricidal mammals of similar size

species	weight [g]	BMR [ml O_2/g h] / weight	BMR [%]	author BMR
Sorex minutus	3.3	8.6	366	Taylor 1998
Sorex araneus	7.7	6.08	328	Taylor 1998
Crocidura suaveolens	6.5	2.90	149	Taylor 1998
Neomys fodiens	13.5	2.90	183	Taylor 1998
Apodemus flavicollis	28	1.65[1]	–	Taylor 1998
Neovison vison	660	0.74	–	McNab 1988
Lontra canadensis	10,000	0.45[2]	–	McNab 1988

[1] the BMR value was read in the rodent *Peromyscus sitkensis* which is comparable in size to *Apodemus flavicollis*. According to Corp et al. (1999), the values of both species are interchangeable.
[2] the BMR value was read from *Lutra lutra* which is comparable in size to *Lutra canadensis*.

samples have the opposite trend, which may be a response to the current consumption of contaminated food, whereas before the animals could have used food sources outside of water. From the data of Evans et al. (2000), it follows that of the total amount of Hg, MeHg in the liver of otters makes up 60%, i.e. 13% less than mink and 83% in fur, i.e. 17% more than mink. It seems that the higher the proportion of Hg in the liver, the lower its methylated fraction could be. The ability to demethylate may be related to this. Faster metabolism eliminates toxins more effectively and demethylation is apparently more effective here. It is assumed that during demethylation, Hg binds to organically bound selenium (selenomethionine), which forms an insoluble complex with Hg in the liver. An exponential decrease in Hg concentration with increasing Se was found in fish muscle (Chen et al. 2001). In association with selenium, the liver can accumulate higher doses of Hg without symptoms of intoxication (Scheuhammer 1987). If the intenzity of metabolism also plays a role, demethylation can also be affected by a change in energy expenditure. The decrease in energy consumption is related, for example, to rigidity in Crocidur or to the consequences of social thermoregulation, when it can be a decrease of up to 49% (Fedyk 1971).

In terrestrial species outside the aquatic food chain, the value in the liver is <0.5 μg/g, but in predators a high exposure >20 μg/g (Scheuhammer et al. 2007). For free-living mammals, a content of 1.1 μg/g is given as a problematic content of Hg in the liver and a content above 30 μg/g as an intoxication threshold (Eisler 1987). The lowest dose of MeHg in food for observable intoxication effects in mink is 0.18 μg/g. A lethal dose of MeHg in food is given as ≥1 μg/g (Scheuhammer et al. 2007). The lethal value recorded in the free environment in mink was: liver/58.2 ppm, fur 34.9 ppm and in experimental mink with a diet containing 1.8 ppm MeHg after 59 days was:

Table 7. Proportional share of THg in the liver of various mammals based on minimum and maximum values in Table 5

species	THg min–max
Sorex minutus	3.11–4.85
Sorex araneus	0.95
Crocidura suaveolens	1.87
Neomys fodiens	3.59
Neovison vison	11.28–10.51
Lontra canadensis	6.55–6.63
Apodemus flavicollis	2.51–2.89

liver 24.3 ppm; fur 1.5 ppm (Wobeser & Swift 1976). Thus, the experimental mink was probably contaminated with a larger proportion of MeHg. The proportion of Hg between their fur and liver was 1.66 for natural mink and 16.2 for experimental mink. We assume that a lower ratio number indicates more intense demethylation. In case of occasional contact with the aquatic food chain, an inverse ratio of Hg content can also occur, when the liver, which reacts quickly to short-term contact, has a higher Hg content than the fur. As an example, we present the raccoon *Procyon lotor* (Linnaeus, 1758) at a distance of 15 km from the lake: liver 7.02 ppm; coat: 4.05 ppm. An individual at a distance of 0.5 km already had the opposite values: liver 3.29 ppm; fur: 27.97 ppm (Wolfe & Norman 1998).

From the current content of the liver, it is therefore possible to read the content in the coat over a continuous time horizon. Evans et al. (2000) gives the ratio between MeHg and Hg in mink, from which it follows that 80.3% of MeHg is contained in the liver and 65% in the fur. Therefore, if we subtract the percentages of MeHg between the fur and the liver, we get, after rounding, a value of 20 ppm for both natural and experimental mink, which we can consider lethal and which roughly corresponds to the content in the muscles (natural: 15.2 ppm; experimental: 16 ppm). In the brain, apparently due to the haemoencephalic membrane, the content was reduced to (natural: 13.4 ppm; experimental: 11.9 ppm).

If we compare the liver values of Hg from the mentioned localities in mammals having contact with aquatic food sources, the environmental burden at the Roudný locality is minimal. An order of magnitude lower values of THg in *Apodemus flavicollis* at least allow us to state a links to the aquatic food in insectivores at the Roudný locality. According to Decree no. 153/2016 Coll. the preventive value of Hg in soil is 0.3 mg.kg^{-1} dry matter, which roughly corresponds to the data obtained at the location in mammals.

It is obvious that the most suitable tool for biomonitoring is feathers and fur for their easy and constant supply in marked individuals. The age of the cumulative cover and the metabolically based rate of demethylation provide more precise information. The liver is a suitable bioindication in the event of an emergency burden in a so far non-risky environment, or in the event of a change in dietary habits. The neurotoxic effect of MeHg is enhanced by organophosphate insecticides by increasing the inhibition of cholinesterase. Therefore, it is also advisable to evaluate their drift from the nearest fields when determining the burden.

From the work of Sanchez et al. (2007) it is evident that lower bone Hg values (0.12–3.09 μg/g) than old males (0.61–12.63 μg/kg) were recorded in aged *Crocidura russula* (Hermann, 1780) females in a contaminated environment. Juvenile males and females had comparable values in the range (0.27–3.73 μg/g). The likely reason is lactation and pregnancy. In an uncontaminated environment with values (0.47–1.74 μg/kg) these differences were not apparent. Measurements performed in bone have an approximate ratio of 1:4 to liver Hg content (Sanchez et al. 2007).

Table 8. Conversion of liver Hg content (range μg/g) in different environments in *Crocidura russula* (BMR (ml O_2/g h) = 2.45/9.6 g; BMR (%) = 141)

contaminated environment	juvenile	senile
females	2.24–9.2	0.48–12.36
males	1.08–14.92	2.44–50.52
uncontaminated environment		
females	1.88–3.52	3.16–6.96
males	3.88–4.32	1.88–6.44

A pregnant female *Apodemus flavicollis*, which was captured at locality No. 1 in summer, did not indicate this tendency when compared with the autumn male. The Hg content in the liver exceeded the male by 11% and in the fur by 24%. After subtracting the fetus (6 developed embryos weighing 13 g), the weight of the female (33 g) was 27% higher.

The collection of samples for the determination of Hg from fur and liver (for estimation of demethylation activity) was limited by the possible number of analyzes provided by the analytical laboratory of the University of Chemistry. The resulting values therefore have no statistical evidence and are only indicative. As well as assumptions and considerations that could only be developed by further measurement. Crucial to the significance of metabolism for demethylation intoxication would be the measurement of the proportion of organic and inorganic Hg in model species of the family Soricidae at selected localities and at the same time monitor these ratios in the fur during the progress of individual molts in marked individuals.

Statements and declarations

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