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Toxicology

STUDIES ON METHYLMERCURY COMPOUNDS IN ICTALURUS NEBULOSUS (Le SUEUR, 1819) EXPOSED TO MERCURY CHLORIDE

BADANIA NAD WYSTĘPOWANIEM ZWIĄZKÓW METYLORTĘCIOWYCH W TKANKACH SUMIKA KARŁOWATEGO (ICTALURUS NEBULOSUS (Le SUEUR, 1819) PODDANEGO DZIAŁANIU CHLORKU RTĘCI

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Individuals of *Ictalurus nebulosus* (Le Sueur, 1819) were bathed in solutions of HgCl₂ and CH₃HgCl. Methylmercury compounds were detected in muscles, liver, and kidneys of those individuals exposed to HgCl₂, which indicates mercury methylation to have taken place in tissues of the fishes studied.

INTRODUCTION

The problem of mercury compounds methylation in animal organisms including fishes, has not been adequately explained so far. Results of the few studies reported tend to diverge. The course of methylation occurring in an organism, provided it does take place, seems to be affected by both the metabolic properties of the species concerned and environmental factors. The few experiments carried out on fishes failed to definitely detect the mercury methylation. Rainbow trout (Salmo gairdneri) kept in mercuric chloride solutions showed no organic mercury compounds, while traces of methylmercury were found after exposing the fishes to phenylmercury acetate (Matida et al., 1971). No methylmercury compounds were determined in rainbow trout exposed to labelled inorganic mercury (Olson et al., 1973; Uthe et al., 1973).

The objective of the present paper was to find out whether methylmercury compounds are detectable in fishes exposed to inorganic mercury (HgCl₂).

MATERIAL AND METHODS

The experiment was run on 100 60-90 g individuals of *Ictalurus nebulosus* caught in the Miejskie Lake near Ostrów Lubelski. The fishes were divided into 3 experimental groups (A,B, and C) and a control, and acclimated for 2 weeks in aquaria.

Total mercury and methylmercury compounds in muscles, liver, and kidneys were determined in each group, the control included.

The control

The control consisted of 20 individuals kept in clear running water at 11-13°C. Mercury compounds were assayed prior to the experiment and when the experimental fishes were tested.

Group A

Group A consisted of 40 individuals bathed 1 h a day on 6 consecutive days in a mercury chloride solution of 0.15 mg Hg/l at 20° C. After each bathing, the fishes were transferred to clean water of the same temperature; after the last bath they were gradually adapted to the $11-13^{\circ}$ C water in which they stayed until samples were taken. Mercury compound determinations in 20 individuals were made after 1 week, the remaining individuals being examined 4 weeks after the last bathing.

Group B

The 20 individuals making up the group were bathed in a methylmercury chloride solution of 0.15 mg Hg/l, the temperature and conditions in the aquaria being the same as in Group A. Mercury compounds assays were performed 1 week after the last bath.

Group C

The 20 individuals making up the group were bathed for 1 h a day on 6 consecutive days in a CH₃HgCl solution of 0.15 mg Hg/l at 11-13°C; then the fishes were transferred to clean water of the same temperature in which they stayed for 4 weeks awaiting the assays.

A prolonged period of keeping the Group C fishes in aquaria was introduced in order to detect changes, if any, occurring in the methylmercury compounds/total mercury ratio in the liver and kidneys, the total mercury level being assumed to remain unaltered. To maintain the Group C total mercury content in muscles on the level identical to that in Group B, the temperature range of $11-13^{\circ}$ C was found necessary to be used.

Total mercury was determined by means of atomic absorption spectrophotometry performed in a Coleman MAS-50 analyser following mineralisation of a sample with nitric and sulphuric acids (Szprengier, 1972; Żmudzki and Szprengier, 1973).

Methylmercury compounds were assayed by means of gas chromatography according to Westöö (1968) in a Pye-Unicam 104 analyser with electron capture detector. 230×0.6 cm columns filled with 5% Carbowax 20 M on Chromosorb G 60/80 mesh

were used. Nitrogen was the carrying gas (60 ml/min. flow); the column and detector temperatures were 155 and 250°C, respectively*.

Statistical treatment of the results involved testing the significance, at 95% confidence level, of differences between the muscles, liver and kidneys mercury contents in the experimental and control fishes with the use of Student's t test.

RESULTS

Table 1 presents data on the contents of total mercury and methylmercury compounds in muscles, liver, and kidneys, of the Experimental and control *Ictalurus nebulosus*,

The control

The muscles showed the presence of methylmercury compounds only, while the liver and kidneys contained none of these compounds in concentrations exceeding the sensitivity treshold of the technique (0.02 mg/kg).

Total mercury and methylmercury compounds contents in muscles, liver, and kidneys of all the experimental fishes were significantly higher than those in the control (p < 0.05).

Group A

Methylmercury compounds were found to occur in every tissue examined (muscles, liver, kidneys). The muscle methylmercury content was almost 10 times higher than in the control. The muscles showed a much higher percentage of methylmercury compounds relative to total mercury than did the liver and kidneys. Four weeks after the last bath the methylmercury/total mercury ratios in muscles and kidneys were similar to those after 1 week, the ratio markedly decreasing in the liver.

Group B

The liver and kidneys total mercury contents were much higher than those in muscles, while the methylmercury/total mercury ratio was much higher in muscles.

Group C

Similarly to Group B, the methylmercury/total mercury ratio was higher in muscles than in the liver and kidneys,

Higher, by 3-10%, muscle contents of methylmercury in the control and Groups B and C (Table 1) may have resulted from the different weights of samples taken for the total mercury and methylmercury determinations, 5 and 10 g, respectively, and from the fact that total mercury was determined on fresh tissues while methylmercury compounds were assayed on frozen (-20°C) material.

*All the mercury assays were performed in the Pharmacology and Toxicology Department, Veterinary Institute, Puławy.

Table 1

Mean contents of total mercury and methylmercury compounds in muscles, liver, and kidneys of *Ictalurus nebulosus* exposed to six 1-h baths in HgCl₂ and CH₃HgCl 0.15 mg Hg/l solutions (the values reported are given in mg/kg)

Group	Solution and temperature	Time lapse after last bath	Muscles			Liver			Kidneys		
			Total Hg	Methyl Hg	Methyl Hg %	Total Hg	Methyl Hg	Methyl Hg % total Hg	Total Hg	Methyl · Hg	Methyl Hg <u>%</u> total Hg
Control	_	-	0.045	0.046	102	0.068	_	_	0.055	-	_
Group A	HgCl ₂ 20°C	1 week	0.590 0.736	0.431 0.548	73 74.5	1.610	0.504	35 11.3	3.920	0.266 0.288	6.8 5.4
Group B	CH₃HgCl 20.°C	1 week	5.193	5.469	105	17.830	12.686	71.1	12.720	6.601	52
Group C	CH ₃ HgCl	4 weeks	5.897	6.540	110	8.471	5.691	67.2	5.495	3.531	64.3

DISCUSSION

The studies show that in *I. nebulosus* exposed to mercury chloride, methylmercury compounds are produced in muscles, liver, and kidneys. Since the compounds occurred in muscles (Group A) in concentrations 10 time those in the control, mercury chloride was presumably transformed within the fish organisms. No methylmercury compounds were detected in the liver and kidneys of the control. Their presence in Group A, although the percentage was lower than in muscles, further supports the contention of the endogenous formation of these compounds after a mercury chloride treatment (Table 1).

The results suggest mercury methylation to occur in fishes, at least in *I. nebulosus*.

The data on the occurrence of this process in fishes, known so far, are scanty and have been obtained from rainbow trout only: Those data differ from the results presented in theat no mercury methylation was found after a mercury chloride treatment (Matida et al., 1971; Olson et al., 1973; Uthe et al., 1971), methylmercury traces were found only after treating rainbow trout with phenylmercury acetate (Matida et al., 1971), no methylmercury compounds were detected in experimental rabbits fed with a diet containing mercury chloride and phenylmercury acetate (Szprengier, 1976).

The present results for Group B and C showed the methylmercury total mercury ratios to be lower in the liver and kidneys than in muscles in the fishes treated with mercury chloride, which is consistent with the results obtained for certain Pacific fishes (Rivers et al., 1972). The latter author's opinion on the transformation in fish organs of toxic organic compounds into less toxic and better excreted inorganic ones seems plausible. A similar process was also observed in mammals (Buhler et al., 1975; Norseth et Clarkson 1970).

Translated: mgr Teresa Radziejewska

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Streszczenie

Sumiki karłowate (*Ictalurus nebulosus*) poddano kąpielom w roztworze HgCl₂ oraz CH₃HgCl o stężeniu 0,15 mgHg/l jedną godzinę dziennie przez sześć kolejnych dni. Oznaczenia związków rtęci w mięśniach, wątrobie i nerkach ryb wykonano po upływie jednego i czterech tygodni od ostatniej kąpieli. Rtęć całkowitą oznaczano metodą spektrofotometrii atomowo-absorbcyjnej, a związki metylortęciowe metodą chromatografii gazowej.

Stężenia rtęci całkowitej i związków metylortęciowych w mięśniach, wątrobie i nerkach ryb poddanych działaniu rtęci nieorganicznej i organicznej były znacznie wyższe w porównaniu do grupy kontrolnej ($P \le 0.05$).

U ryb kąpanych w roztworze HgCl₂ stwierdzono związki metylortęciowe we wszystkich badanych tkankach. W wątrobie i nerkach ryb kontrolnych nie stwierdzono związków metylortęciowych, obecność ich u ryb kąpanych w HgCl₂ świadczy o tym, że powstały w organizmie po zadziałaniu chlorkiem rtęci. Związki metylortęciowe w mięśniach sumików kąpanych w HgCl₂ występowały w ilości 10-krotnie wyższej niż u ryb w grupie kontrolnej, w związku z tym należy przypuszczać, że chlorek rtęci został przekształcony w ich organizmie.

М. Студницка

ИССЛЕДОВАНИЕ СОДЕРЖАНИЯ МЕТИЛРТУТЬЕВЫХ СОЕДИНЕНИЙ В ТКАНИЯХ СОМИКА-КОШКИ (ICTALURUS NEBULOSUS) ПОДВЕРГНУТОГО ДЕЙСТВИЮ СУЛЕМЫ

Резюме

Сомики-кошки (Ictalurus nebulosus) помещали в раствор HgCl2, CH_2HgCl при концентрации 0,15 мг Hg/π на 1 час в течение дней. Определение соединении ртути в мышцах, печени и почках рыб проводили спустья 1 и 4 недели от последнего помещения в растворе. Общую определяли методом атомно-абсорбционной спектрофотометрии, вые соединения методом газовой хроматографии. Концентрация общей метилртутьевых соединений в мышцах, печени и почках рыб подвергнутых действию неорганической и органической ртути, была эначительно выше концентрации этих соединений у контрольных рыб (Р 0.05). У рыб выдерживаемых в растворе HgCl2 обнаружено метилртутельне соединения во всех исследованных тканиях. В печени и почках контрольных рыб не нашли метилртутьевых соединений. Их присутствие у рыб помещенных в раствор ${\rm HgCl}_2$ свидетельствует о том, что они возникли в организме после действия сулемы. Метилртутьевые соедниения в мышцах сомика-кошки выдерживаемого в HgCl, наблюдались в количестве в 10 раз выше, чем у рыб контрольных. В связи с этим надо полагать, что HgCl2 был подвергнут переобразованию в их организме.

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