ACTA THERIOLOGICA
Vol. 27, 35: 499—507, 1982

Contamination of Roe Deer by Mercury Compounds[1]

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Kryński A., Kałuziński J., Wlazełko M. & Adamowski A., 1982: Contamination of roe deer by mercury compounds. Acta theriol., 27, 35: 499—507 [With 2 Tables]

The purpose of the present study was to determine differences in mercury contamination of tissues in roe deer originating from two habitats, forest and agricultural, differing in respect of the amount of mercury fungicides introduced into them. Mercury content was determined in liver, kidney and muscular tissues. Samples were taken from 46 individuals obtained from the forest population and 53 from the field population. Identification was carried out by the sedimentation method after Krylova, as modified by Smirnova (1977). It was found that mercury concentration in all the roebuck tissues examined from the field population is significantly higher than in males from the forest population. In the group of does, however, whether from forest or field, there were no significant differences in the mercury concentration in muscles, liver or kidneys. Roebucks thus differ from does in respect of the difference in the dynamics of mercury accumulation and elimination processes. Mercury concentration in roe deer tissues, with the exception of the kidneys in roebucks from the field population, does not exceed the level accepted under FAO/WHO regulations for food substances.

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1. INTRODUCTION

The increasing use of chemicals in all natural habitats has an important influence on contamination of animal organisms. Populations of game animals continuously living in biotopes where there is a given intensity of the action of chemical compounds may form an objective index of the contamination of the habitat.

The source of contamination of animal organisms by mercury compounds, *e.g.* in a habitat with intensive agricultural management, may be fungicides, and also the smoke and dust accompanying the burning of coal and its derivatives. The majority of Polish and foreign studies have been concentrated chiefly on contamination of the biological habitat by mercury of industrial origin. Such studies have dealt, *inter alia*

[1] Praca wykonana w ramach problemu MR-II/15 koordynowanego przez Instytut Ekologii PAN.

with mercury concentration in the tissues of domestic animals (Juszkiewicz *et al.*, 1977; Juraskova *et al.*, 1976; Kabata-Pendias *et al.*, 1979; Maggi *et al.*, 1979; Szprengier, 1975) and in the tissues of wild animals (Büring *et al.*, 1978; Cumbie, 1976; Frank *et al.*, 1979; Hoffman *et al.*, 1979; Holt *et al.*, 1979; Tataruch *et al.*, 1979, 1980). Little is known about the effect of the preparations from the group of mercury fungicides used in plant protection on the degree to which the tissues of game animals are contaminated. It is, however, known that mercury compounds produce lasting contamination of soil and via plant food pass into the tissues of herbivorous animals (Magas, 1975; Prior, 1976). Thus mercury fungicides, used in Poland up to recent years and seed dressing, may form a serious source of contamination of animal organisms. The degree to which game animals are exposed to contamination by mercury compounds should be greater, where their trophic links with an agricultural habitat are closer.

The purpose of the present study is to determine the differences in contamination by mercury compounds of tissues in roe deer originating from two habitats: typically agricultural, and forest, which may *inter alia* provide evidence of the close ecological tie of these animals with one, and not another, habitat.

2. MATERIAL AND METHODS

Material for these studies was obtained from roe deer shot in the experimental area of the Polish Hunting Association Research Station at Czempiń, and also in the woods of the "Zielonka" Hunting Experimental Centre, Institute of Forest Protection, Agricultural Academy in Poznań. The experimental area of the Polish Hunting Association Research Station at Czempin, 15,000 ha in extent, of which 14,000 ha consists of fields, is a typical agricultural habitat, in which large-scale fields predominate, forming 70% of the whole area of the range (Kałuziński & Pielowski, 1976). Agriculture in this area is characterized by intensive use of chemical and machines, and by the development of accompanying small industry. The experimental area of the Station is inhabitated by a numerous roe deer population, average density from 1977—79 being 12 individuals per 100 ha (Kałuziński, 1982). The forest of the "Zielonka" Hunting Experimental Centre form a dense stretch of wooded land 8,000 ha in extent. Average density of roe deer is 15 individuals per 100 ha (Fruziński *et al.*, 1982).

In order to determine contamination of tissues by mercury compounds tissue samples were taken from 53 individuals, 19 bucks and 34 does, orginating from the field population. In the case of the forest population samples were taken from 46 individuals, 31 bucks and 15 does. Roe deer from both habitats were obtained during the shooting season of 1977/78, bucks — during the spring-summer months, and does during the autumn-winter months.

Roe deer from the Zielonka area were obtained from the central part of the forest, situated in every case over 1000 m to fields. This distance to a great extent

eliminated the possibility of obtaining individuals which might have fed on cultivated fields.

Identification of total mercury contents in sections of muscles, liver and kidneys was carried out by the quantitative sedimentation method after Krylova, as modified by Smirnova (Žulienko *et al.*, 1974; Smirnova, 1977). Tissue samples were taken about 2 hours after the animals were shot. For objective reasons it did not prove possible to obtain a complete set of samples from each individual. The tissues were kept frozen in polyethylene packing at a temperature of —20°C. Burning of the thawed sections, weighing about 40 g, was made by the wet mineralization method, using sulphuric and nitric acids. Losses of mercury vapour were eliminated by using the precautions given in the method. Proceeding in accordance with the method, the colour of the sediment from the mercury-iodic-copper complex was compared with the colour of the standard scale. The above method revealed 0.025 microgrammes in the 40 g sample tested, which corresponded to the level of 0.006 ppm of mercury.

Table 1

Quantity of seed dressings used at the Research Station of the Polish Hunting Association at Czempiń.

Culture	Area, ha	The kind of fungicides	Per 1 ha, gms	Total kgs
Rye	1672	Zaprawa R	400	66.8
Other cereals	4493	Zaprawa T	200	89.8
Maize	2478	Funaben T	200	49.5
Rape	645	Zaprawa GT	300	19.3
Pea	249	Zaprawa GT	300	7.4
Sugar-beet	1338	Oksafon T	300	40.1

Data on the amount of fungicides applied per area in both the experimental areas were obtained by means of records and questioning. In the experimental field area it became necessary, in order to define the amount of fungicides introduced into the habitat, to collect exact data on the area of crops on which mercury compounds had been introduced in the form of seed dressing (Table 1). In the experimental forest area questioning established the fact that no mercury compounds had been used for the last 10 years.

Statistical calculations in this study were made by the method of variance analysis in the two-direction system (Oktaba, 1976). The value of the Fischer-Snedecor F test was found for the significance of the following differences: (1) between sexes, (2) between areas, (3) co-action of the effects of sex and areas. When test F showed significant differences in the effects examined, the smallest proved difference NIR was calculated, using the equation $t \times Sr$, where t=tabular value of the Student t distribution with level of significance of 0.01, and Sr=error in mean difference.

3. RESULTS

The seeds of the majority of cultivated plants, and particularly of cereals, are treated with seed dressings, which introduce, *inter alia*, mercury compounds into the biological habitat. In 1979 a considerable

percentage of seed grain of rye cultivated in the field area had been treated with R seed dressing containing mercuric phenyl acetate (Table 1). According to the estimated data obtained, despite a tendency to gradual withdrawal of mercury dressings from use in the field area, forming about 11% of the study area, 66.8% kg of seed dressing R, containing 2.4% of mercury, had been introduced into the soil. It must be emphasised that the data obtained relate only to crops in large fields, and do not include vegetable production, since the area occupied by vegetables is only 0.5% of the whole study area, although the concentration of seed dressings used on them is far higher.

Table 2

Average concentration of mercury in ppm in tissues of field- and forest-living roe deer.
NIR — smallest proved difference ($t \times Sr$).
NS — $p < 0.01$ non-significant difference.
Forest — forest-living roe deer, Field — field living roe deer.
n — number of animals

Items	Muscles		Liver		Kidneys	
	Forest	Field	Forest	Field	Forest	Field
Males	0.013	0.047	0.015	0.036	0.027	0.053
n	28	14	25	19	31	19
Females	0.021	0.020	0.027	0.022	0.042	0.042
n	10	32	13	34	15	34
NIR for sex	NS		NS		NS	
NIR for territory	0.003		NS		NS	
NIR for cooperation of sex and territory	0.005		0.004		0.009	

As roe deer were obtained only from central part of the forst in the Zielonka wooded area, and that this to a great extent eliminated the possibility of their feeding in cultivated fields, it was assumed that there was no contamination of this habitat by the mercury compounds used in agriculture in the form of seed dressings, and that no significant amounts of mercury compounds should be found in the tissues of these individuals.

It must, however, be pointed out that the basic source of contamination of the biological habitat by mercury compounds consists in the wastes of large-scale industry, and also from burning petroleum products and coal. The two experimental areas are situated at a similar distance from the Poznań urban and industrial centre, and are therefore exposed to a uniform degree to contamination of the atmosphere, water, soil and indirectly vegetation, by mercury compounds of industrial origin.

The data obtained justify the statement that the concentration of total mercury in muscles, liver and kidneys in roebucks from the forest

population is significantly lower than in the analogical group of roebucks from fields, whereas similar mercury levels were found in all the tissues examined from field-living and forest-living does. It must also be emphasised that mercury concentration in the organs of the field-living roebucks reaches the highest level, contrary to what is found for the same group of forest-living roebucks (Table 2).

4. DISCUSSION

The natural mercury compounds content in cultivated soil in Poland is on an average 0.06 ppm, but soil on which mercuric fungicides have been used are characterized by considerable accumulation of mercury, up to as much as 53 ppm. Cultivated plants contain on an average not more than 0.02 ppm of mercury, the greatest amount of mercury being contained in the green parts of plants. With increased mercury compounds content in the soil the concentration of mercury in cultivated plants increased markedly (even to 10 ppm), but the greatest amount of this element accumulates in the ligneous parts of plants, including the husks of seeds, according to Kabata-Pendias & Pendias (1979).

In the light of the above data it is clear that seed dressing R is a source of contamination of both soil and cereals, and in this way mercury compounds are able to penetrate into roe deer organisms. The mercury compound ingested together with food is absorbed into the blood and is partly fixed with the proteins of the systemic fluids and tissues, owing to their particular affinity with the group of sulphahydryl, carboxyl and amine amino-acids. Mercury is deposited in organs, especially in the liver and kidneys. Mercury compounds penetrate most rapidly into the blood, liver and pancreas, and are cumulated longest in muscles and brain cells (Bidstrup, 1964; Kabata-Pendias & Pendias, 1979). The kidneys possess the special property fixing metal ions, particularly mercury. As a result of the mechanism of condensation of primary urine, multiple concentration of this metal takes place in the sinuous tubules. It is not completely excreted from the kidneys, since it is permanently fixed with proteins of the epithelial morphotic cells of nephron elements.

Roebucks from the field population are characterized by significantly higher level of mercury in tissues as a consequence of mercury penetrating through seed dressings in general use in the fields during sowing time, and reaching plant shoots, chiefly shoots of cereal plants, and then the animal organism. Similar observations were made by Büring *et al.* (1978) when examining mercury concentration in muscles and liver of cock pheasants. A far higher concentration of mercury (average

164 ppb in the liver, and 16 ppb in muscles) was found in tissues of pheasants from an agricultural habitat than in similar tissues obtained from pheasants living in a habitat with predominantly green areas (12 in the liver, and 8 ppb of mercury in muscles).

There are no data on the time of elimination from the animal organism of mercury compounds ingested over a long period of time together with natural food, but it is probable that during autumn and winter a certain amount of mercury is eliminated from the organism and therefore balanced levels of this element are found in the tissues of field-living and forest-living roe deer. Other factors which determine the concentration of mercury compounds are the sex of the animals examined and the time at which they were obtained. On account of limited seasons it was impossible in our experiment to segregate these indexes simultaneously, by parallel shooting of males and females. Differences in contamination of the tissues by mercury compounds in male and female roe deer could be explained by physiological differences between the sexes. It is a known fact that 29% of organic combinations of mercury administered once only to goats is excreted with faeces within 21 days, 2.3% with urine and 0.4% with milk (Kitagawa *et al.*, 1977). According to Bohosiewicz (1979), mercury is eliminated from the organism primarily together with urine, and to a lesser degree with faeces, milk and sweat. It is also known that mercury compounds rapidly penetrate through the blood-placenta barrier, cumulating in foetal tissues (Nikonorow, 1980). It would, however, appear that these are slight quantitative differences between the ways of eliminating mercury by females and males. The physiological differences between bucks and does cannot therefore provide a satisfactory explanation of differences in mercury concentration in their organs, especially as the reverse proportion in contamination of tissues in males and females is found in the forest population of roe deer. In this habitat it remains on a significantly lower level. Considerably more mercury is found in the tissues of females obtained in autumn and winter.

It would appear, in connection with the above, that mercury level in the tissues of the roe deer examined continues to depend on the time the animals were obtained, and the processes of cumulation and elimi- to which crops are saturated with mercury fungicides. With less con- to which crops arre saturated with mercury fungicides. With less contamination of the roe deer organism by mercury compounds, accumulation of this elements increases gradually in a forest habitat, reaching maximum concentration in autumn and winter. With greater concentration of mercury compounds in the tissues of roe deer living in a field habitat, maximum accumulation of mercury in the liver, muscles

and particularly in the kidneys takes place during the spring-summer period, while mercury concentration decreases later on in the tissues examined. Although it is indicated in literature that mercury content in different animal organs increases proportionately with its occurrence in a natural habitat, it is not possible to rule out the possibility of there being dynamic balance with simultaneously occurring processes of absorption, cumulation and elimination of mercury compounds in an animal organism (Kabata *et al.*, 1979). This is confirmed by the data given by Żulienko *et al.*, (1975), who found that mercury concentration in the tissues of cattle killed in winter was lower than in cattle killed in summer. Seasonal fluctuations in mercury concentration were also observed in the organs of chamois and hares (Tataruch *et al.*, 1979, 1981), and followed much the same course in the case of the forest-living roe deer.

Mercury concentration in roe deer tissues does not exceed (with the exception of male kidneys from the field habitat) admissible standards for mercury content in food products of animal origin recommended by FAO/WHO. In the kidneys of field-living roebucks average mercury concentration was 0.052 ppm, and thus only slightly exceeds this level. In the remaining groups and tissues mercury concentration is far below the proposed standards. For the sake of comparison it may be stated that in Poland similar concentrations of mercury were found in the tissues of domestic animals. The following values were observed respectively for kidneys of horses, pigs and cows: 0.139, 0.050 and 0.032 ppm of mercury, whereas the following corresponding values were found in the muscles of these animals: 0.026, 0.005 and 0.007 ppm of mercury (Szprengier, 1976).

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Accepted, May 17, 1982.

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SKAŻENIE ORGANIZMU SARNY ZWIĄZKAMI RTĘCI

Streszczenie

Badania przeprowadzono na terenie doświadczalnym Stacji Badawczej Polskiego Związku Łowieckiego w Czempiniu oraz w lasach Łowieckiego Ośrodka Doświadczalnego Zielonka Akademii Rolniczej w Poznaniu. Celem pracy było ustalenie różnic skażenia rtęcią tkanek sarn pochodzących z dwóch odmiennych środowisk leśnego i rolniczego. Zasadniczym czynnikiem różniącym tereny doświadczalne pod względem skażenia ich związkami rtęci jest ilość stosowanych na terenie polnym fungicydów. Oznaczenia zawartości rtęci w tkankach sarn przeprowadzono metodą osadową według Krylovej w modyfikacji Smirnovej (1977). Metodą powyższą wykrywano 0,025 mikrograma rtęci w badanej próbie, co odpowiada poziomowi 0,006 ppm. Zawartość rtęci określano w tkankach mięśni, wątroby i nerek. Próby pobrano od 46 osobników pozyskanych z populacji leśnej i 53 z polnej. Stwierdzono, że stężenie rtęci w mięśniach, wątrobie i nerkach samców sarny polnej wynosi średnio odpowiednio 0,047, 0,036 i 0,053 ppm i jest istotnie wyższe od odpowiednich stężeń w tkankach samców sarny leśnej (0,013, 0,015 i 0,027). Nie stwierdzono istotnych różnic w stężeniu rtęci w tkankach samic sarn pochodzących z obu populacji (Tabela 2). Natomiast procesy akumulacji i wydalania rtęci charakteryzują się odmienną dynamiką uzależnioną od środowiska, w którym sarny bytują. Stężenie rtęci w tkankach badanych sarn, z wyjątkiem stężenia w nerkach samców sarny polnej nie przekracza poziomu zalecanego przyjętymi ustaleniami FAO/WHO. Wyniki badań świadczą o ścisłym powiązaniu troficznym sarn ze środowiskiem w którym bytują.