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DERMAL ABSORPTION AND DISTRIBUTION OF ^{14}C CARBARYL IN WISTAR RATS

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Abstract: The level of ^{14}C carbaryl was determined in blood (leukocytes, erythrocytes, all blood cells, plasma) and organs (brain, heart, lungs, liver, spleen, skin at the site of exposure) of male Wistar rats after dermal administration. The application liquid was ^{14}C carbaryl solution in 96% ethyl alcohol. This preparation, possessing an activity of 670 kBq/ml, containing 1.67 mg of carbaryl, was applied to the skin of the tail according to Massmann's method in own modification. The amount of the preparation per 1 cm^2 of the tail skin was 0.19 mg of carbaryl (74.4 kBq). The tails of experimental rats were exposed to ^{14}C carbaryl by soaking for 4 h daily: once, twice or three times. Beta radiation from ^{14}C was measured in homogenized organs (brain, heart, lungs, liver, skin) and in blood by computer controlled Wallac scintillation counter Model 1409, using Multi Calc software. The dermal absorption of carbaryl at the site of exposure and in the surrounding area of about 2 cm was observed already during 4 hour exposure. Carbaryl reached plasma within 4 h of a single dermal exposure and penetrated into leukocytes, erythrocytes, heart, liver, lung, kidney and brain. The largest amount of ^{14}C carbaryl, about 2% of absorbed dose, was detected in liver.

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INTRODUCTION

Carbamate insecticides, including carbaryl, are esters of carbaminian acid. Carbamates are absorbed through the alimentary tract, airways and intact skin. When absorbed, they undergo rapid transformations at the site of exposure (nervous system, plasma) and in other tissues. The metabolites produced show various toxicity, sometimes equal to or greater than the administered substance [13].

Carbamates are metabolised by the hydrolysis of carbamoyl bond, as well as by oxidation mechanisms. The hydrolysis of carbamates takes place with the contribution of various enzymes, including cholinesterase, while the hydrolysis of the carbamoyl bond is a detoxication process. In the living organism, apart from dioxide, methylamine and 1-naphthol are subject to further decomposition. The main detoxication changes of carbamates, including carbaryl, take place in the liver under the influence of microsome oxydases [5].

The studies conducted by McCracken [11] showed that carbaryl was hydrolyzed by liver, lung, and skin at a lower rate by microsomal fractions (Vmax nmol/min/g 2.1 ± 0.25; 1.6 ± 0.25; 0.2 ± 0.035 respectively) compared to cytosolic fractions (Vmax nmol/min/g 6.7 ± 0.75; 1.4 ± 0.36; 0.5 ± 0.12 respectively) and plasma (Vmax nmol/ min/ml 3.0 ± 0.25). Hydrolysis involved carboxylesterases.

In the organism of mammals, carbaryl is decomposed to three main metabolites of hydroxyl structure, which are excreted with urine in the form of glucuronides or sulphates. Studies by Chin and Sullivan conducted *in vitro* showed that foetal liver performed the metabolic processes of demethylation, hydrolysis, hydroxylation and oxydation. The authors confirmed that ^{14}C carbaryl applied in their study produced naphtyl glucuronide and naphtyl sulphate in the kidney, and naphtyl sulphate in the lung [2, 13].

The mechanism of the toxic effect of carbamates consists in the inhibition of esterases, especially acetylocholinesterase. Intraperitoneal administration of a

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dose of 70 mg/kg to rats caused inhibition of brain cholinesterase activity by 47% after 60 min [6]. Animals exposed orally to 25 mg/kg carbaryl had a 34% decrease in WBC counts. A 34% decrease in WBC and a 13% increase in RBC counts were observed at the 50 mg/kg oral dose [9]. Carbaryl appeared to exert a teratogenic effect on guinea pigs, puppies, and mice, and had an embryotoxic effect on hamsters [3, 12].

Studies performed with the use of ^{14}C marked pesticides enabled us to select the organs which most strongly absorb the preparation and to recognize its metabolism, ways of excretion and secretion of metabolites. The studies conducted with the use of ^{14}C carbaryl dermally applied on female Fischer-344 rats (young 33-day-old and adult 82-day-old rats) showed that the absorption was stronger in the young rats [14]. In the studies on rabbits, the dose of 20 μCi ^{14}C carbaryl was applied on the neck skin. The measurements of radioactivity in different organs showed that carbaryl is a pesticide rapidly absorbed by the tissues and 95% of total dose is metabolized within 24 hours after application. A high level of radioactivity was observed also in excreta, urine and faeces [15]. In the studies conducted by Declume and Bernard [4], the distribution of 1-Naphtyl-N-methyl-^{14}C carbamate was determined after the administration of a single oral dose to mice at the 18th day of pregnancy. A quick transport of ^{14}C carbaryl to the placenta was observed 5 h after the administration. Radioactivity of the kidney, spleen, and heart of the foetus was higher than in the analogous organs of the mother, and was maintained after birth. Similar to mice, in pregnant rats the transition of ^{14}C carbaryl was also noted. Eight hours after the intraperitoneal administration of the dose of 2.8 μCi/kg the presence of carbaryl was observed in the brain, heart, and lungs of the foetus, the values being considerably higher than in the organs of the mother [16].

OBJECTIVE

The aim of the study was the determination of the level of ^{14}C carbaryl in blood (leukocytes, erythrocytes, all blood cells, plasma) and organs (brain, heart, lungs, liver, kidney, skin at the site of exposure and the area around the site of exposure) in male Wistar rats after dermal administration.

MATERIALS AND METHODS

Radioactively marked ^{14}C carbaryl was applied for the study (1-Naphtyl-N-methyl-^{14}C carbamate). The study was conducted on male Wistar rats with the body mass of 200-300 g, fed with standard fodder LSM [8] and watered *ad libitum*.

The application liquid was ^{14}C carbaryl solution in 96% ethyl alcohol. The preparation of the activity of 670 kBq/ml, containing 1.67 mg of carbaryl, was applied to the skin of the tail according to Massmann's method in own modification [17]. The amount of the preparation per 1 cm^2 of the tail skin was 74.4 kBq (0.19 mg of carbaryl).

The animals were divided into three experimental groups and one control group, three animals per group. The tails of experimental rats were soaked in the application liquid for 4 h daily. The first group was exposed once, the second group twice, and the third group - three times. Animals from the control group were not exposed to skin penetration, neither of carbaryl nor 96% ethyl alcohol.

Animals of all groups were anaesthetized with Vetbutal administered intraperitoneally. The material for the study was taken directly after exposure, then 6 h and 20 h after exposure. Heart blood was taken into heparin captured test tubes. The presence of ^{14}C carbaryl was determined in the following organs: brain, liver, heart, lungs, kidneys, tail skin from the site of exposure, and tail skin at a distance of at least 2 cm from the exposure site, as well as in erythrocytes, leukocytes, all blood cells and plasma.

0.5 g excisions of the organs were homogenized in a manual homogenizer in 5 ml PBS (buffered solution of the physiologic salt with calcium chloride and magnesium chloride, Biomed, Lublin, Poland). 10 ml of scintillation liquid (Sigma) was added to the homogenate, and the mixture was shaken at room temperature in a laboratory shaker for a period of 4 h.

Separation of leukocytes and erythrocytes from blood was carried out according to Böyum's method [1]. 0.5 samples of erythrocytes, leukocytes, all blood cells, and plasma were diluted each in 10 ml scintillation liquid, and then shaken for 4 h at room temperature in a laboratory shaker.

Beta radiation of ^{14}C was measured by computer controlled Wallac scintillation counter, Model 1409, using MultiCalc software. Measurements performed for each sample lasted 5 min and were repeated three times.

RESULTS

At a single exposure, ^{14}C carbaryl applied to tail skin of male rats was absorbed at the rate of 0.15 μg/cm^2 during 4 h (1.4 μg/ 9 cm^2). The rates of ^{14}C carbaryl absorbed during exposures repeated twice or three times were smaller: 0.02 μg/cm^2 and 0.012 μg/cm^2 respectively (Tab.1).

^{14}C carbaryl absorbed at the rate of 1.4 μg/ 9 cm^2 penetrated the surrounding skin quite quickly. So 6 h after exposure, the amount of pesticide in the exposure zone was only 0.3 μg, and after 20 h it fell down to 0.05 μg. It suggests that only 21.4% and 3.57% of absorbed ^{14}C carbaryl respectively, persisted still in the application zone. During 6 h about 80% of the absorbed amount of pesticide left this zone and penetrated the surrounding skin, blood and internal organs.

In the skin at a distance of 2 cm from exposure zone the smallest percent of ^{14}C carbaryl was detected directly after exposure. This was 2.7% of the absorbed dose, which was observed in the application zone. Later, during

Table 1. The amount of ^{14}C carbaryl absorbed by tail skin of Wistar rats during 4 hours.

Number of exposures	Amount of ^{14}C carbaryl absorbed	
	Total amount (µg)	Amount per cm ^{2} (µg)
Single exposure	1.4	0.15
Twice repeated exposure	0.18	0.02
Three times repeated exposure	0.12	0.012

Table 2. ^{14}C carbaryl in the tail skin of Wistar rats after dermal application.

Region of skin	Amount of ^{14}C carbaryl absorbed					
	Directly after exposure		6 h later		20 h later	
	µg	%	µg	%	µg	%
Application zone	1.4	100	0.3	21.4	0.05	3.57
At a distance of 2 cm from exposure zone	0.03	2.7	0.06	4.6	0.057	4.1

Table 3. The amount of ^{14}C carbaryl in internal organs of Wistar rats after single dermal exposure.

Organs	% of ^{14}C carbaryl absorbed by skin (1.4 µg)					
	Directly after exposure		6 h later		20 h later	
	µg	%	µg	%	µg	%
Brain	0.0071	0.5	0.010	0.7	0.013	1.0
Liver	0.026	1.92	0.035	2.97	0.031	2.27
Kidney	0.0049	0.37	0.0062	0.53	0.0031	0.34
Lung	0.0033	0.24	0.0040	0.29	0.0038	0.27
Heart	0.0020	0.14	0.0025	0.18	0.0024	0.18

Table 4. Distribution of ^{14}C carbaryl in the rat blood after a single dermal exposure.

Components of blood	Amount of the absorbed dose of ^{14}C carbaryl					
	Directly after exposure		6h later		20 h later	
	µg	%	µg	%	µg	%
All blood cells	0.053	3.68	0.025	1.76	0.024	1.76
Plasma	0.035	2.56	0.042	2.88	0.027	1.92

the next 6 and 20 h this percent increased to about 4%. Thus, 20 h after exposure, the rate of ^{14}C carbaryl in the exposure zone and in the surrounding area had a similar degree, about 4% (Tab. 2).

During a single exposure, ^{14}C carbaryl penetrated into all examined organs, but in different amounts. Thus, directly after dermal application lasting 4 h, the largest amount, about 2% of the absorbed dose, was detected in liver. Later, during the next 6 h, it increased to about 3% and 20 h after exposure was nearly the same. Similar concentration of ^{14}C carbaryl, but not as high as in liver, was detected in brain 20 h after exposure. In the remaining organs: kidney, lung and heart, the amounts of absorbed ^{14}C carbaryl were very low (Tab. 3).

Directly after twice and three times repeated exposure, the amounts of ^{14}C carbaryl absorbed by tail skin were 0.18 µg/9 cm^2 and 0.12 µg/9 cm^2 respectively. The concentrations of ^{14}C carbaryl detected in examined organs were very low, but their distribution was similar to that observed after single exposure. The highest concentrations were detected in liver and brain.

Dermally applied ^{14}C carbaryl penetrated blood during exposure lasting 4 h. High amounts of pesticide were detected in plasma directly after exposure and 6 h after exposure: 2.56% and 2.88% of the absorbed dose, respectively. Later, after 20 h, the amount decreased. The amount of ^{14}C carbaryl in all blood cells was the highest directly after exposure: 3.68% of the absorbed dose. The distribution of ^{14}C carbaryl was similar in erythrocytes and in leukocytes (Tab. 4).

DISCUSSION

The results of the studies showed that ^{14}C carbaryl applied dermally, whether in a single dose, twice or three times, is absorbed by tissues. Directly after exposure, as well as 6 h and 20 h after application, ^{14}C carbaryl was detected in the skin at the sites of application and in the skin at the distance of 2 cm from exposure site. It was also detected in the brain, liver, kidney, heart, lungs, plasma, all blood cells, erythrocytes, and leukocytes.

Studies by Knaak *et al.* [7] showed that 74–94% of dermally applied ^{14}C carbaryl remained on the skin surface for as long as 72 h. However, during this period a part of the preparation penetrated into the skin and after 72 h its level increased to 29.3% of applied dose. After 144 h the amount of the preparation in the skin reached its maximum level (43.1%).

The results of our studies showed that during a single exposure lasting 4 h, ^{14}C carbaryl applied to the tail skin was absorbed by male rats at the rate of 0.15 µg/cm^2. Similar results obtained Knaak *et al.* [7], who detected that carbaryl was absorbed by male rats at the rate of 0.18 µg /cm^2/h. In the present study, ^{14}C carbaryl absorbed at the rate of 1.4 µg/ 9 cm^2 penetrated the surrounding skin and blood quite quickly. Thus, 6 h after dermal application the amount of pesticide in the exposure zone was only 0.3 µg, and after 20 h it decreased to 0.05 µg.

The results of the studies by Knaak *et al.* [7] showed that ^{14}C carbaryl was rapidly absorbed from the skin into the blood and distributed to the kidneys and liver. Similar results were obtained in our studies. About 2.56% and 3.68% of ^{14}C carbaryl absorbed by the skin were detected after exposure lasting 4 h in plasma and all blood cells, respectively.

Metzler *et al*. [10] reported that carbaryl penetrated skin and reached a steady state concentration amounting to 6% of the applied dose within 1 h after application. The penetrated dose was absorbed by plasma, rapidly distributed to kidneys, liver and heart, and eliminated by these tissues. In our studies, ^{14}C carbaryl penetrated the internal organs quickly, but its concentration was variable, the highest in liver and brain.

CONCLUSIONS

^{14}C carbaryl was absorbed by the tail skin of male rats in the rate of 0.15 µg/cm^2/4 h.

Directly after dermal exposure lasting 4 h, ^{14}C carbaryl was distributed in the internal organs: liver, brain, lung, heart, kidney and in the blood.

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