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DISTRIBUTION AND BINDING OF 1, 3, 5 [U14C]-TRIOXANE IN MATERNAL AND FETAL RATS

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Abstract. The tissue distribution and binding of ^{14}C activity were studied at different time intervals following a single oral administration of 1,3,5[U^{14}C]-trioxane (^{14}C-TOX) (40 mg/kg : 1.6 MBq/kg) to pregnant rats. Animals were killed on the 21st day of gestation 3, 24 or 48 hours after administration of TOX.

In maternal rats, 3 hours after administration, the highest levels of total radioactivity were found in the liver and plasma, followed by a slow, gradual decline with time. The level of ^{14}C-activity in the whole fetus was comparable to that of the maternal kidney through the study. The radioactivity in the fetal kidney and liver at the end of 48 hours after single administration was higher than at 3 hours after administration. Slow decline in radioactivity was observed with time in the fetal brain, skin and carcass. However, after 48 hours the level of total radioactivity in the fetal kidney and brain was more than twice as high as in the corresponding maternal organs. Three hours following ^{14}C-TOX administration 35—41% of the respective total ^{14}C radioactivity in maternal liver and kidney was firmly bound to the macromolecules, while the fetal liver and kidney showed 100—72% binding with respect to their total radioactivity.

INTRODUCTION

Trioxane (1, 3, 5-trioxane, $C_3H_6O_3$), a formaldehyde polymer, has a cyclic structure, contrary to chain paraformaldehyde. It is used as a half-product in the production of tarnform, a plastic material. Trioxane is also planned to be used as an insect-proofing agent, which may result in a greater number of occupationally and non-occupationally exposed people. Trioxane belongs to the substances with slight systemic toxicity under condidions of acute exposure. In fact, it does not pose a risk of acute occupational poisoning, while LC_{50} of this compound is above 26 g/m^3 for the rats, and LD_{50} per os for the rats is over 5 g/kg b.w. (3, 4).

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During chronic inhalatory exposure trioxane produces an irritant effect on mucous membranes of the respiratory tract as well as CNS impairment (4, 7). Based on animal studies on chronic inhalation toxicity, exposure limits of trioxane in the workplace atmosphere have been determined in Poland: 15 mg/m^3 as a time weighted average and 75 mg/m^3 as a short-term exposure limit (5). Trioxane is not mutagenous for the Salmonella typhimurium strains used in Ames test (8). It does not produce an increased number of polychromatic erythrocytes with micronuclei in mouse bone marrow (13). Nor does it induce dominant lethal mutations in the germ cells of male rats (1). Trioxane administered *per os* at doses of 0.2 and 0.5 LD_{50} has been associated with significant maternal toxicity, embryonal and fetal lethality, congenital malformations and retarded fetal development. Administration of trioxane at a dose of 0.1 LD_{50} still induced fetotoxic and teratogenic effects without apparent toxicity to the dams (14).

The purpose of the present study was to determine the transplacental transport and the distribution of trioxane and/or its metabolites in pregnant female rats and their embryos.

MATERIALS AND METHODS

1,3,5[U^{14}C]-trioxane (1,3,5-trioxane, ^{14}C-TOX), chromatographically pure, was obtained from the Institute of Radiation, Faculty of Chemistry, Technical University of Lodz. Specific activity of ^{14}C-TOX was 3.6 MBq/mmol. The experiments were performed on 3.5-month-old female Wistar rats and their embryos. The tissue distribution and binding of ^{14}C activity were studied at different time intervals following a single administration, by gavage of 1,3,5-[U^{14}C]-trioxane (40 mg/kg, 1.6 MBq/kg) to pregnant rats. Two experiments were performed. In the first experimental series tissue distribution of ^{14}C was determined, in the second one total and protein-bound ^{14}C-activity was measured in tissue homogenates. Trioxane was dissolved in 0.9% NaCl (0.86 MBq/ml). Venous blood samples (30 µl) were drawn from mothers 1, 3, 5, 24 and 48 h after administration of ^{14}C-TOX, centrifuged in heparinized capillary. The radioactivity was measured only in the plasma because, as it was proved in another study (9), erythrocytes did not bind ^{14}C after ^{14}C-TOX administration. Assuming 7 ml of blood per 100 g of rat body weight, and taking into account a hematocryte value, the content of trioxane and/or its metabolites in plasma was calculated.

The animals were killed on the 21st day of gestation, 3, 24 or 48 h after the administration of ^{14}C-TOX. The liver, kidneys, lungs, brain, spleen, heart, a piece of fat of pregnant females and liver, kidneys,

brain, a fragment of skin and carcass of fetuses, placenta and amniotic liquid were removed and homogenized, when necessary, before determination of the radioactivity. The maternal tissue samples, placentas and three whole fetuses were taken from each of three pregnant rats at respective time points for radioactivity determination. From each pregnant rat 2—3 pooled samples of amniotic liquid, fetal livers, brains, carcasses and skin fragments and one pooled sample of fetal kidneys were prepared. In order to determine the bound radioactivity, tissues homogenates were precipitated with cold 10% (w/v) trichloricacetic acid (TCA). After centrifugation, the precipitates were washed twice with 70% ethanol to remove the unbound or loosely bound radioactivity. All the tissue homogenates and final dried precipitates were digested according to Mahin and Lofberg (10). All radioactivity measurements were carried out using LKB-Wallace LSC Model 81000 and tritosol (6) as the scintillation mixture. The counting was corrected with the use of the channel ratio method. The radioactivity determined in tissue samples was converted to μg-equivalent of ^{14}C-TOX per g of tissue on the basis of the specific activity of the administered dose.

RESULTS

The radioactivity of the plasma of pregnant female rats which were administered ^{14}C-TOX in a single, intragastric dose was highest in the 5th hour following the administration (Table 1). The half-life of ^{14}C in plasma, determined according to the graphic method, was approximately 14.5 hours.

Three hours after ^{14}C-TOX administration a high content of ^{14}C was detected in the liver and plasma of the female rats. In other tissues the content of ^{14}C was several times lower during the whole time (Table 2) of the experiment. The concentration of ^{14}C in the whole fetus 3, 24 and 48 hours following the administration, was higher than in maternal tissues with the exception of liver and plasma (Table 1). In all the examined tissues and organs of the pregnant rats and their fetuses about 9%, 7.4% and 4.2% of the administered dose were detected 3, 24 and 48 hours after the administration, respectively.

The highest ^{14}C concentration, 3 hours after its administration, was detected in the liver, plasma and kidneys of the pregnant rat, and in the fetal skin and carcass (Table 3). After 24 hours an increase in ^{14}C level could be observed in the kidneys, lungs, spleen and heart of maternal pregnant female as well as in the placenta and fetal liver and kidneys. In all the remaining tissues, a decrease in radioactivity was

TABLE 1. ^{14}C-activity in plasma following a single oral dose of 1,3,5[U-^{14}C]-trioxane to pregnant rats

Time	^{14}C activity in plasma (kBg/ml)	µg-equivalent ^{14}C-trioxane	percentage of dose administered
		in total plasma volume of rat	
1 h	0.39±0.07	132.6±23.2	1.03±0.18
2 h	0.58±0.09	194.3±30.9	1.51±0.24
3 h	0.87±0.10	299.9±34.7	2.33±0.27
5 h	1.76±0.22	601.0±75.9	4.67±0.59
24 h	0.58±0.06	198.2±20.6	1.54±0.16
27 h	0.48±0.05	160.9±16.7	1.25±0.13
48 h	0.25±0.03	77.2± 9.0	0.60±0.07

a — Values are means ± SD of three animals

TABLE 2. Contents of [^{14}C]-activity derived from 1,3,5[C^{14}C]-trioxane following a single oral administration [40 mg (1,6 MBq/kg][a]

	percent of dose administered		
	3 h	24 h	48 h
Plasma	2.3 ±0.27	1.54 ±0.16	0.60 ±0.07
Liver	3.3 ±0.42	2.90 ±0.35	1.80 ±0.16
Kidneys	0.12 ±0.03	0.15 ±0.01	0.12 ±0.009
Lungs	0.09 ±0.01	0.09 ±0.01	0.05 ±0.008
Brain	0.09 ±0.005	0.02 ±0.003	0.01 ±0.008
Spleen	0.02 ±0.001	0.07 ±0.02	0.04 ±0.003
Heart	0.03 ±0.002	0.03 ±0.003	0.03 ±0.003
Placentas	0.02 ±0.004	0.04 ±0.002	0.003±0.005
All fetuses	3.04 ±0.42	2.60 ±0.60	1.50 ±0.74
Whole fetus	0.38 ±0.03	0.29 ±0.03	0.16 ±0.04
Fetal liver	0.02 ±0.008	0.03 ±0.003	0.016±0.0002
Fetal kidneys	0.001±0.003	0.001±0.0002	0.002±0.0002
Fetal brain	0.008±0.001	0.005±0.0006	0.006±0.001
Fetal carcass	0.33 ±0.03	0.22 ±0.02	0.11 ±0.02

a — Values are means ± SD of three pregnant animals

TABLE 3. Radioactivity levels in tissues following a single oral administration of 1,3,5 [^{14}C]-trioxane[a]

	µg-equivalent 1,3,5 [U^{14}C]-trioxane/g tissue		
	3 h	24 h	48 h
Plasma	21.9±2.4 (0.17±0.019)	14.2±1.5 (0.11±0.012)	6.2±0.75 (0.0048±0.006)
Liver	36.0±5.1 (0.28±0.04)	29.6±3.9 (0.23±0.03)	16.7±1.3 (0.13±0.01)
Kidneys	11.9±2.6 (0.09±0.02)	14.2±0.9 (0.11±0.007)	9.0±0.8 (0.07±0.006)
Lungs	7.7±1.3 (0.06±0.01)	9.0±1.2 (0.07±0.009)	6.4±1.0 (0.05±0.008)
Brain	7.7±0.51 (0.06±0.004)	1.3±0.3 (0.01±0.002)	1.3±1.8 (0.01±0.006)
Spleen	5.1±0.26 (0.04±0.002)	14.2±3.9 (0.11±0.03)	7.7±2.6 (0.06±0.02)
Heart	3.9±0.26 (0.03±0.002)	5.1±0.5 (0.04±0.004)	3.9±0.5 (0.03±0.004)
Fat	3.9±0.77 (0.03±0.006)	1.3±0.3 (0.01±0.002)	2.6±0.5 (0.02±0.004)
Placenta	7.7±1.3 (0.06±0.01)	9.0±0.5 (0.07±0.004)	7.7±1.3 (0.06±0.01)
Fetus	12.9±1.0 (0.10±0.008)	10.3±1.2 (0.08±0.009)	9.0±2.6 (0.007±0.02)
Fetal liver	9.0±3.9 (0.07±0.03)	16.7±1.3 (0.13±0.01)	12.9±1.3 (0.10±0.01)
Fetal kidneys	9.0±3.9 (0.07±0.03)	15.4±2.6 (0.12±0.02)	20.6±2.6 (0.16±0.02)
Fetal brain	10.3±1.3 (0.08±0.01)	6.4±0.8 (0.05±0.006)	7.7±1.3 (0.06±0.01)
Fetal skin	20.6±2.6 (0.16±0.02)	11.6±0.8 (0.09±0.006)	11.6±1.3 (0.09±0.01)
Fetal carcass	15.4±1.3 (0.12±0.01)	10.3±0.8 (0.08±0.006)	9.0±1.3 (0.07±0.01)
Amniotic liquid	10.9±3.5 (0.15±0.047)	2.1±0.39 (0.028±0.005)	0.8±0.09 (0.011±0.001)

a — Values are means ± SD of three pregnant animals. Values reported in parentheses are percent of dose administered/g tissue.

noticed, and ^{14}C was eliminated fastest from the maternal brain and amniotic liquid. The half-lives of ^{14}C, estimated according to the graphic technique amounted to appr. 8 and 9 hours for the brain and amniotic liquid, respectively. Fourty eight hours after the administrations a further decrease in ^{14}C concentration in the tissue was detected. In the maternal spleen and heart, in the placenta, in the liver and kidneys of the fetus, however, the concentration of ^{14}C was the same or higher than 3 hours after the administration. The level of ^{14}C was considerably higher in the whole fetus than in the placenta and approximated the ^{14}C concentration in maternal kidneys (Table 3).

The estimation of ^{14}C firmly bound in the tissues proved that its proportion of the respective total ^{14}C-activity increased with time after ^{14}C-TOX administration. Only in the liver of the fetus, where the proportion of ^{14}C firmly bound was very high 3 hours after the administration (about 100%), a gradual decrease of binding could be observed (Table 4). The percentage of firmly bound ^{14}C was the lowest in the brain of pregnant rats and their fetuses 3 hours after the administration. However, 48 hours after the intoxication it was similar to the level detected in the liver and kidneys. The concentration of ^{14}C firmly bound in the tissues was higher in the brain and kidneys of fetuses than in the respective organs of the pregnant rats (Table 4).

In order to assess the absorption and distribution of ^{14}C-TOX, the distribution tissue/plasma coefficients were established for the major tissues (ratio between the µg-equivalents of ^{14}C-TOX in tissue and in maternal plasma). Three hours after the administration only in the liver of the pregnant rat the concentration of ^{14}C was higher than in the plasma. Twenty four hours after the administration, ^{14}C concentration exceeding the level in the plasma, was noticed in the maternal liver and in fetal liver and kidneys. Two days after administration, only in the brain, heart muscle and fat tissue of the pregnant rat as well as in amniotic liquid, the level of ^{14}C-activity was still lower than in the maternal plasma (Table 5).

DISCUSSION

The toxicokinetics and metabolism of trioxane in animals and humans has not as yet been clarified. The small (ca. 9%) percentage of the administered dose, found in the tissues of the pregnant rats and their fetuses 3 hours after ^{14}C-TOX administration, suggests that the compound administered orally is quickly eliminated during the first few hours following the administration. This hypothesis is supported by the results of Ligocka and Sapota et al. (9), who observed that 78.4%

TABLE 4. Recovery of total and protein bound radioactivity in tissue homogenates following a single oral administration of 1,3,5[$U^{14}C$]-trioxane[a]

	μg-equivalent 1,3,5[U^{14}]-trioxane/g tissue					
	3 h		24 h		48 h	
	Total	Bound	Total	Bound	Total	Bound
Liver	21.2±9.5	8.8±3.7 (41.5)	23.4±5.1	11.1±3.9 (47.4)	12.5±0.5	8.1±3.7 (64.8)
Kidneys	8.1±0.2	2.9±0.5 (35.8)	13.2±2.2	7.3±2.2 (55.3)	7.3±0.7	4.8±4.7 (65.8)
Lungs	6.5±0.7	2.3±0.22 (35.4)	8.1±2.9	5.5±1.5 (67.9)	4.8±0.22	3.0±0 (62.5)
Brain	3.7±0.5	0.4±0.15 (10.0)	1.5±0.2	0.7±0 (48.7)	0.7±0.15	0.4±0.02 (60.3)
Heart	3.0±0.7	1.3±0.15 (43.3)	4.8±2.0	3.0±1.0 (62.5)	2.8±0.22	2.0±0.15 (71.4)
Placenta	6.5±2.4	4.1±0.17 (63.1)	10.2±1.8	6.2±0.2 (60.8)	5.7±0.4	4.3±0 (75.4)
Fetal liver	4.3±1.1	4.3±1.8 (100)	16.1±2.1	12.5±1.1 (77.6)	9.5±1.8	6.2±1.2 (65.3)
Fetal kidneys	6.7±1.6	4.8±0.8 (71.6)	9.8±0.9	7.3±2.1 (73.5)	11.3±2.1	8.7±1.6 (77,0)
Fetal brain	6.2±2.0	0.7±0.22 (11.8)	8.1±1.3	4.0±0.9 (49.4)	4.5±0.6	2.2±0.4 (48.9)

a — Values in parentheses represent percent binding of the total radioactivity in homogenates. Values are means ± SD of three pregnant animals used in the second experiment.

TABLE 5. Distribution coefficient tissue/plasma of [^{14}C]-activity derived from 1,3,5[U^{14}C]-trioxane following single oral administration

	Time (h)		
	3	24	48
Liver	1.60±0.21	2.12±0.26	2.81±0.22
Kidneys	0.53±0.13	0.97±0.06	1.58±0.13
Lungs	0.35±0.06	0.64±0.08	1.04±0.17
Brain	0.34±0.02	0.11±0.02	0.25±0.07
Spleen	0.22±0.006	1.0 ±0.29	1.38±0.32
Fat	0.20±0.03	0.12±0.02	0.40±0.08
Heart	0.19±0.006	0.34±0.03	0.70±0.08
Placenta	0.32±0.05	0.67±0.03	1.35±0.28
Fetal liver of fetus	0.42±0.08	1.19±0.11	2.06±0.30
Fetal kidneys	0.38±0.16	1.13±0.22	3.35±0.54
Fetal brain	0.46±0.07	0.43±0.05	1.26±0.21
Fetal skin	0.88±0.11	0.85±0.05	1.95±0.30
Fetal carcass	0.70±0.06	0.75±0.06	1.52±0.29
Whole fetus	0.56±0.05	0.73±0.08	1.47±0.37
Amniotic liquid	0.50±0.13	0.14±0.03	0.13±0.01

Distribution coefficient is the ratio of µg-equivalent 1,3,5[U^{14}C]-trioxane/g tissue to µg-equivalent/ml plasma.
Values are means ± SD of three pregnant animals.

of intragastrically administered ^{14}C-trioxane was eliminated during 24 hours, out of it 75.8% — with the expired air and 12.6% with the urine. During the first 8 hours about 65% of the ^{14}C was excreted with the expired air. The excretion with faeces is negligible. Only 0.7% of the intragastrically administered ^{14}C was eliminated in this way during 72 hours (9). The decrease of the radioactivity in most of the organs of the pregnant rats as well as in the fetuses between 3rd and 48th hour after administration takes place very slowly and after 48 hours the level of ^{14}C exceeds 50% of the radioactivity observed 3 hours after ^{14}C-TOX administration. ^{14}C (administered as ^{14}C-TOX) is transported during the 1—3 hours after oral administration from the blood of the mother to the tissues of the fetus to a greater extent than to the most organs and tissues of the mother herself. Moreover, between 3rd and 48th hour after oral administration of ^{14}C-TOX, the level of radioactivity in the liver and kidneys of the fetus increases, whereas in the respective organs of the female rat it gradually decreases. During the same period of time the drop of radioactivity in the fetal brain is several times lower than in the brain of the mother. These data suggest that in the case of repeated administration, retention of trioxane and/or its metabolites in liver, kidneys and brain of the fetus can be higher than in the respective organs of the mother.

The absorption by ethonolamine of the ^{14}C from the expired air of the rats intoxicated with ^{14}C-trioxane and the fact that expired ^{14}C is not absorbed by water, seem to suggest that CO_2 may be the main trioxane metabolite excreted through the respiratory tract (9). It is known that most of formaldehyde administered to the animals is also excreted with the expired air as CO_2 (15). Thus, we cannot exclude a possibility that trioxane is decomposed in animal tissues into 3 formaldehyde molecules, and is subsequently metabolized to formic acid, then to CO_2 and water (11, 12).

A considerable level of radioactivity in the tissue precipitates, despite the intensive extraction with polar and non-polar solvents, may be an evidence that a significant part of ^{14}C is either permanently bound or built into the macromolecules. It is not possible, however, to state in what chemical form and to which macromolecules ^{14}C is bound in the tissues, due to the lack of sufficient data on the trioxane metabolism. Neverthless, the metabolic decomposition of trioxane to CO_2 suggests that the atom of carbon from the trioxane molecule may enter C_1-pool, which makes carbon the precursor of numerous chemical compounds. The degree of ^{14}C binding to macromolecules in different organs varied, the highest being in the organs with highest metabolic activity, including the liver of mothers, placenta and the livers and kidneys of the

fetuses. An increase of the concentration of ^{14}C firmly bound to the macromolecules was detected in all examined organs between the 3rd and 48th hour after the administration of ^{14}C-TOX. This finding may suggest that the part of ^{14}C, which was initially loosely bound in the tissues was then gradually built into the macromolecules. On the other hand, it indicates that the fragments of trioxane molecules which contain ^{14}C are not eliminated even during two days after the administration of the compound.

The results obtained in the present study prove that apart from the toxic effects exerted by trioxane and/or its metabolites in the tissues of the maternal rat and its placenta (14), the trioxane and/or its metabolites are transported to the fetus where they can directly produce disorders of the histogenesis and metabolic processes.

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