Distribution of radioactivity from ^{14}C-formaldehyde in pregnant mice and their fetuses

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Abstract

The distribution of ^{14}C after the administration of ^{14}C-formaldehyde was studied in pregnant mice by a whole body low temperature autoradiographic technique. The concentrations of formaldehyde and its metabolites in maternal and fetal blood and tissues were determined in unsectioned tissues by liquid scintillation spectrophotometry. The binding of ^{14}C from ^{14}C-formaldehyde to cells and DNA in maternal and fetal mouse liver was also measured. Radioactivity of ^{14}C deriving from ^{14}C-formaldehyde was found immediately after injection, and showed strong accumulation and retention three hours after injection. The organs that had high concentrations at all studied survival intervals were maternal liver, intestinal mucosa, bone marrow, kidneys, and salivary glands. Considerable amounts of radioactivity were found in the fetuses at six hours after injection, and the concentrations were almost the same as in the maternal tissues. The elimination of ^{14}C-formaldehyde and metabolites from the placenta and fetus occurred more slowly than from maternal tissue.

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Formaldehyde is a chemical that is widely used, primarily in the production of specific resins. It is also used for various other purposes including disinfection in hospitals and dentistry.[1–3] Well known toxic effects of exposure to formaldehyde are irritation of the mucus membranes, eyes, and throat, and allergic contact sensitisation of the skin.[4–6] After long term exposure mutagenic effects have been shown in vitro[7 8] and it had carcinogenic effects in experimental animals.[9–11]

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Human exposure to formaldehyde gas at concentrations up to 2 ppm occurs in industries in Japan.[12] Important sources of indoor exposure to formaldehyde outside the workplace are tobacco smoking, and formaldehyde resins in wood products such as plywood panelling, particle board underlays, and fibreboard furniture. Atmospheric formaldehyde concentrations in the living environment frequently exceed 0·1 ppm in new homes, which use urea formaldehyde foam insulation, in vehicle exhaust emissions, and in mobile homes.[13–16] By contrast with exposure in the workplace, residential exposure may affect different subgroups of the population and frequently involves longer daily exposure periods for the young, the old, and pregnant women.

Despite its widespread use, few data are available on the chemical reactivity and potential effects of formaldehyde on pregnancy. We have studied the distribution of ^{14}C from ^{14}C-formaldehyde by a whole body low temperature autoradiographic technique to assess fetal uptake and distribution after injection into pregnant mice.

As well as this, the concentrations of formaldehyde and its metabolites in the various organs of mothers and fetuses were studied with a liquid scintillation technique.

Materials and methods

^{14}C-formaldehyde with a specific activity of 370 MBq/mmol was purchased from New England and Nuclear (Boston, MA, USA) and dissolved in 1% formaldehyde solution to obtain a concentration of 7·4 kBq/μl. Mice of the ICR strain (about 2–2·5 months old) were used. Females were paired with males overnight and the next morning (=day 0 of gestation) they were checked for vaginal plugs. On the 16th day of pregnancy, 0·05 ml of 1% formaldehyde solution containing 3·6 mg ^{14}C-formaldehyde (374 kBq)/kg body weight was injected into the tail vein. Animals were killed with gaseous carbon dioxide at five minutes, 30 minutes, one hour, two hours, four hours, six hours, 24 hours, and 48 hours after injection of ^{14}C-formaldehyde and frozen in n-hexane cooled by carbon dioxide (−78°C). The frozen animals were embedded on a microtome stage

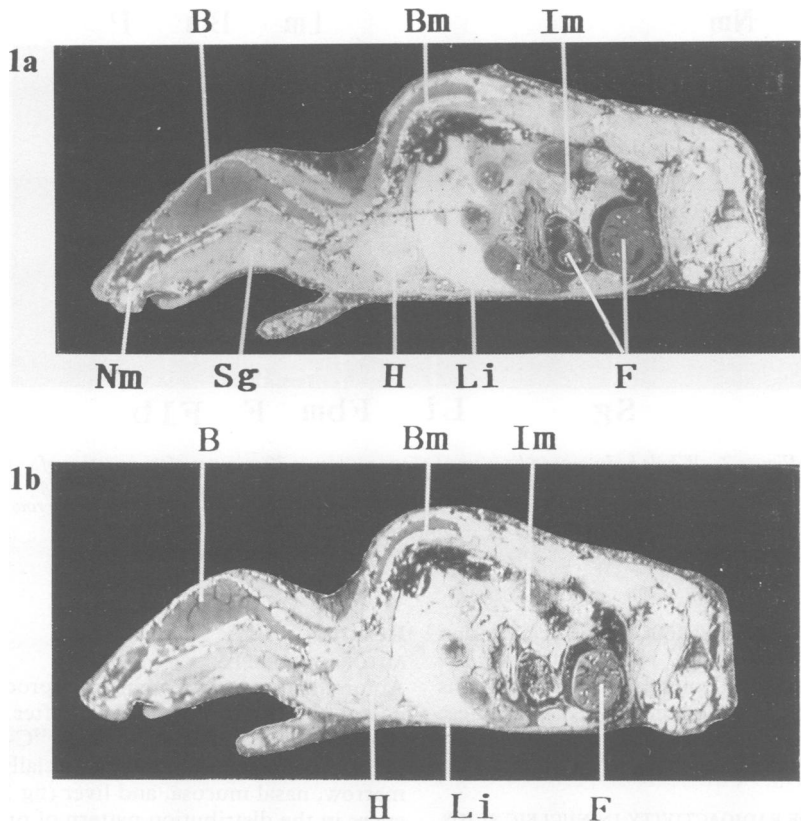

Figure 1 Whole body autoradiograms of pregnant mice five minutes after injection of ^{14}C-formaldehyde on day 16 of gestation; Hemisection exposed at −70°C (1a); freeze dried and heated section (1b). There are no obvious differences between non-volatile and volatile activity from ^{14}C-formaldehyde. B = Brain, Bm = bone marrow, Im = intestinal mucosa, F = fetus, Li = liver, H = heart, Sg = salivary gland, Nm = nasal mucosa.

with 5% aqueous sodium carboxymethyl cellulose gel.[17 18] The autoradiographic technique of Ullberg[17] was used with modification to prevent evaporation of volatile radioactivity. The frozen and embedded mice were cut into 60 μm thick sections, pressed against industrial *x* ray film (Fuji Film Co Ltd), and kept at −80°C for eight weeks. Other sections from the same parts were freeze dried overnight and heated at 50°C for 24 hours before film exposure. This allowed the volatile radioactivity to evaporate before exposure. Also, parts of the sections were then extracted stepwise with water, 10% trichloroacetic acid, 50% methanol, butanol, and heptane as described by Bergman.[19] This technique removes the metabolites that are not firmly bound. All freeze dried sections and extracted sections were exposed for eight weeks at −10°C.

MEASUREMENT OF RADIOACTIVITY IN TISSUES AND EXCRETA

Formaldehyde and its metabolites were estimated in unsectioned tissues by measurement of ^{14}C by liquid scintillation spectrophotometry. On day 16 of gestation, groups of five mice were injected with 0·025 ml of 1% formaldehyde solution containing 1·8 mg ^{14}C-formaldehyde (185 kBq)/kg body weight. They were killed five minutes, 30 minutes, one hour, three hours, six hours, 24 hours, and 48 hours after treatment in the manner described for the autoradiographic method. The radioactivity of all tissue samples (fetuses, placentas, some maternal organs, and blood) and excreta was collected by an automatic sample combustion system (ASC 113 Alloka Co Ltd). Samples collected in a plastic vial with scintillation fluid (DPO 5 mg and 0·1 mg POPOP/1 toluene) were

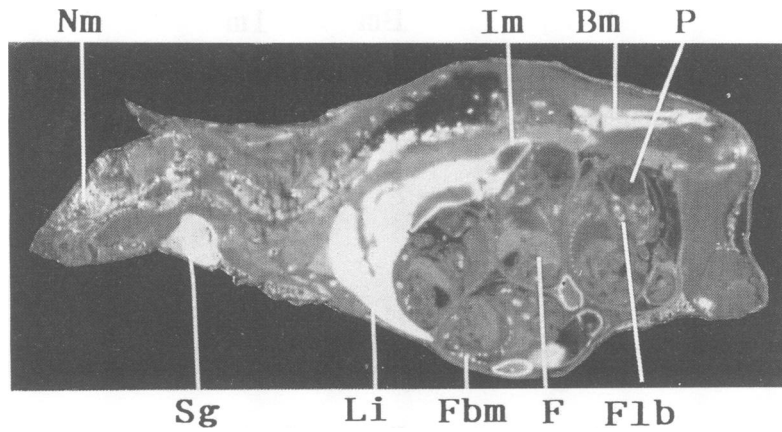

Figure 2 Whole body autoradiogram of pregnant mouse 30 minutes after injection of ^{14}C-formaldehyde on day 16 of gestation; hemisection exposed at −70°C: note the high uptake of radioactivity (white area) in liver (Li), intestinal mucosa (Im), bone marrow (Bm), and salivary gland (Sg). P = placenta, Flb = fetal limb bud, Fbm = fetal bone marrow.

counted in a Packard Model 460C liquid scintillation spectrophotometer.

The mean fetal and placental concentrations within each mother were determined and these values were used for statistical comparison by Student's *t* test.

DETERMINATION OF RADIOACTIVITY IN NUCLEIC ACIDS

Samples of maternal and fetal livers were treated by standard and routine techniques involving solution of DNA in trichloroacetic acid and assessment of ^{14}C activity in the DNA fraction and the acid insoluble protein fraction by liquid scintillator spectrophotometry.

Results

AUTORADIOGRAPHY

Autoradiograms of pregnant mice produced by using a low temperature technique after five minutes showed a high uptake of injected ^{14}C-formaldehyde or metabolite radioactivity, especially in the bone marrow, nasal mucosa, and liver (fig 1a). No differences in the distribution pattern of maternal tissues were seen between frozen and freeze dried heated sections. Non-volatile radioactivity derived from ^{14}C-formaldehyde was most prominent in the bone marrow, intestinal mucosa, and liver (fig 1b).

Figure 2 shows the distribution of radioactivity 30 minutes after injection. Low temperature

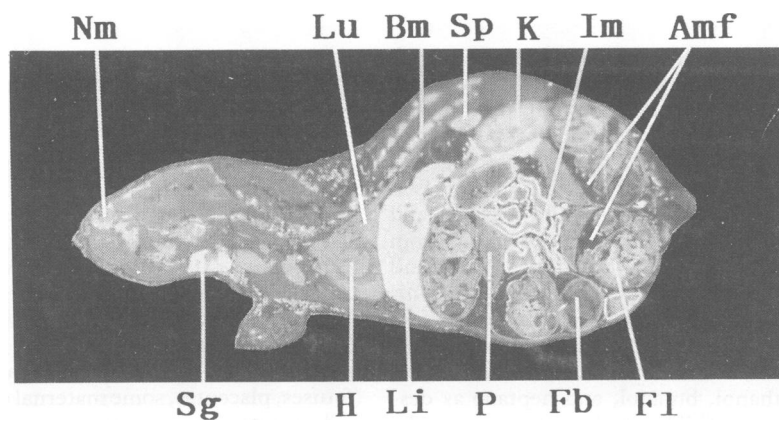

Figure 3 Whole body autoradiogram of pregnant mouse one hour after injection of ^{14}C-formaldehyde on day 16 of gestation; hemisection exposed at −70°C: detail of an autoradiogram showing fetal organs: fetal liver (Fl), fetal brain (Fb) or conceptus organs such as placenta (Pl) or amniotic fluid. K = kidney, S = spleen.

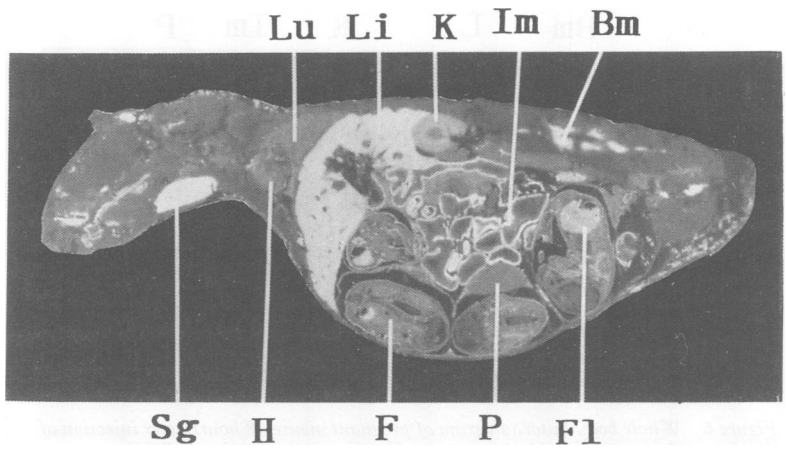

Figure 4 Whole body autoradiogram of pregnant mouse six hours after injection of ^{14}C-formaldehyde on day 16 of gestation; Hemisection exposed −70°C: Radioactivity is obvious in maternal liver (Li), intestinal mucosa (Im), salivary gland (Sg), and fetal liver (Fl).

autoradiography showed especially high concentrations of radioactivity in the liver, intestinal mucosa, bone marrow, and salivary glands. Nasal mucosa also had high labelling compared with other tissues. Radioactivity in the fetal tissues was less than in maternal tissues. It was seen mainly in the bone marrow and limb buds.

Figure 3 shows autoradiograms obtained at one hour after injection. Radioactivity in maternal organs had accumulated mainly in the liver, spleen, intestinal mucosa, lung, heart, and salivary glands. In the fetal tissues the radioactivity was mainly in the liver. At one hour after injection amniotic fluids were essentially devoid of ^{14}C in comparison with the fetal liver. The skeleton and skin showed clear labelling.

The distribution pattern in the maternal and fetal tissue obtained at two to four hours after injection was similar to the pattern at one hour. In autoradiograms prepared at six hours after injection (fig 4), the maternal liver, intestinal mucosa, kidney, and salivary gland remained highly radioactive. Also, fetal liver regions of these autoradiograms showed ^{14}C activities comparable with those in the maternal kidney. Although the distribution patterns of animals killed 24 hours after injection seemed unchanged from those of animals killed at six hours after injection (fig 4), the amounts of radioactivity present in the maternal bone marrow and intestinal

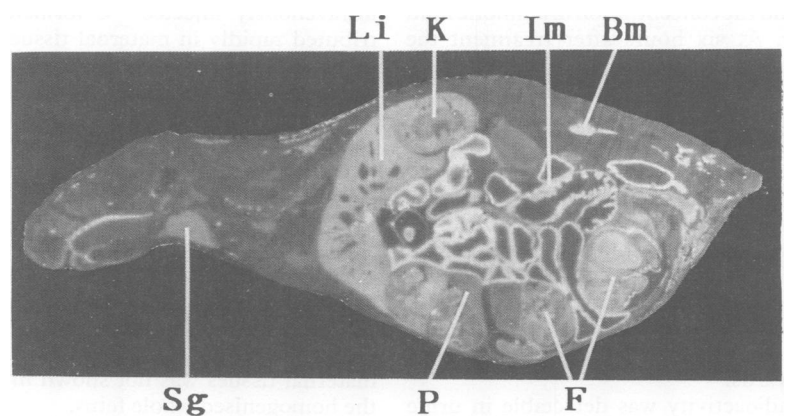

Figure 5 Whole body autoradiogram of pregnant mouse 24 hours after injection of ^{14}C-formaldehyde on day 16 of gestation; hemisection exposed at −70°C: intestinal mucosa (Im) shows intense radioactivity.

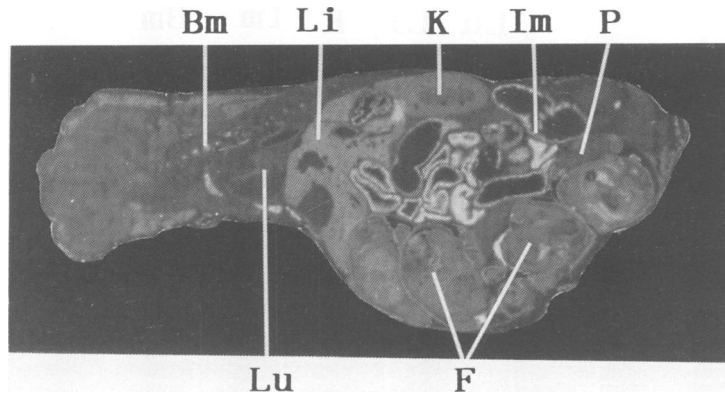

Figure 6 Whole body autoradiogram of pregnant mouse 48 hours after injection of ^{14}C-formaldehyde on day 16 of gestation; Hemisection exposed at −70°C: ^{14}C-activity from ^{14}C-formaldehyde remains in maternal organs and fetus.

mucosa were higher than in the liver and kidney. Radioactivity was seen in the placenta, uterus, and fetus at 24 and 48 hours after injection (figs 5 and 6).

LIQUID SCINTILLATION SPECTROPHOTOMETRY

From the quantitative liquid scintillation spectrophotometric data it was obvious that radioactivity was distributed rapidly throughout the body. The organs that had the highest concentration of ^{14}C-formaldehyde and its metabolites in mice killed soon after the injection were the maternal liver and salivary gland (table 1). There were significant increases in activity in these organs between 30 minutes and three hours. The radioactivity had declined in all organs, by six hours.

The concentration of ^{14}C-formaldehyde and its metabolites in the placenta and fetal body were about half of the amounts in maternal blood at five minutes after injection, and the concentration in amniotic fluid was even lower. At six hours after treatment the radioactivity in the placenta, uterus, and fetal body was higher than in maternal blood. Concentration in the homogenised whole foetus was similar to that in the placenta (table 2).

Radioactivity in the maternal salivary gland showed the highest concentration among all tissues measured at three hours after treatment. The ^{14}C-formaldehyde and metabolic concentrations in fetal liver peaked at three hours and declined afterwards. The concentrations of radioactivity in maternal and fetal brain were equivalent at five minutes, but the fetal brain had significantly more radioactivity at six hours and afterwards.

No volatile radioactivity was detectable in urine and faeces.

Urinary excretion of non-volatile ^{14}C-activity was measured at six hours, 24 hours, and 48 hours. Concentration in urine was especially high in the samples at six hours (table 1). The total elimination of the activity via the urine in the first 48 hours was about 11% and in the faeces 0·7%. Total residual activity in the mother and fetuses at 48 hours was 29·6% of the administered dose.

RADIOACTIVITY IN NUCLEIC ACIDS

Figure 7 presents results showing radioactivity in maternal and fetal liver cells and hepatic nuclei. The quantity of ^{14}C in the DNA fraction represented 20% of the total radioactivity of maternal hepatic cells and 50% of the fetal hepatic cells both at six hours and 24 hours after injection.

Discussion

Because of its high degree of water solubility, intravenously injected ^{14}C-formaldehyde was distributed rapidly in maternal tissues. The extensive distribution of radioactivity from ^{14}C-formaldehyde was consistent with other reports, regardless of the route of administration.[20-23] Autoradiograms of frozen hemisections and freeze dried hemisections obtained at all survival intervals showed no differentiation for volatile or non-volatile substances. The distribution from the autoradiographic analysis showed particular target organs in the mother, such as bone marrow, liver, intestinal mucosa, and salivary gland. The pattern of accumulation of label at each time after treatment was different in different tissues—for instance, the decline at 30 minutes seen in some maternal tissues was not shown in fetal organs and the homogenised whole fetus.

The major excretion of ^{14}C from injected formaldehyde was in urine (11% of the dose) and hardly any was excreted in faeces. Roughly 30% of the total

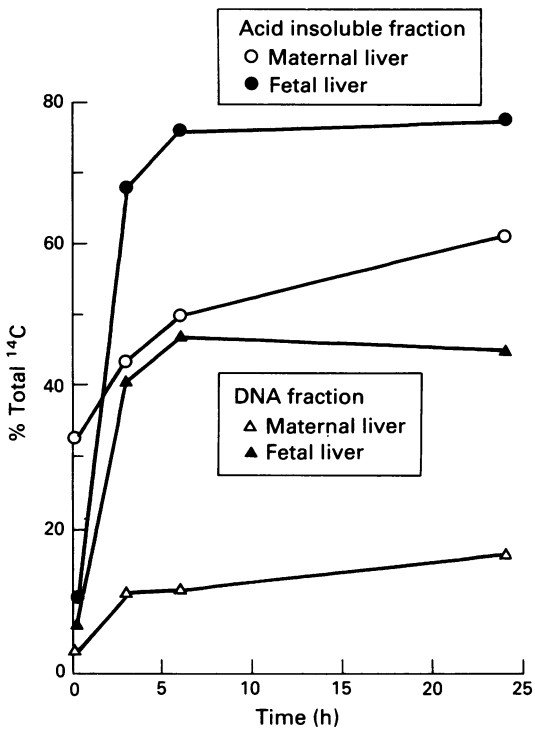

Figure 7 Binding of ^{14}C from ^{14}C-formaldehyde to cells and DNA of maternal and fetal mice livers. Upper two lines show % of ^{14}C in acid insoluble fraction (M: Mother, F: fetus), lower two lines show % of ^{14}C in DNA.

formaldehyde taken into the body still remained in the tissues after 48 hours. The distribution of ^{14}C derived from formaldehyde in pregnant female mice seems to be similar to that in male mice.[21 22] Our present study on liver tissue showed that the ^{14}C from formaldehyde was in the DNA fraction at least in the first day, uptake was maximum at six hours after treatment, and unchanged at 24 hours. This indicates incorporation of metabolites, on the basis of a rapid elimination of formaldehyde itself from the animal by excretion.[24]

Autoradiograms show the fate of radioactivity in the fetus, placenta, and amniotic fluid. The presence of radioactivity in the early phase after treatment indicates that the transplacental passage of the compound or its metabolites is rapid. By contrast with water soluble formaldehyde, we have previously shown that lipid soluble styrene does not immediately pass into fetal tissues.[18] Concentrations of styrene and their metabolites seemed to be much lower in fetuses than in the maternal organs after injection and radioactivity accumulated in the amniotic fluid and placenta.[18] Such accumulation was not found for ^{14}C-formaldehyde.

Only a few studies have been carried out on the effects of formaldehyde on fetuses.[25 26] Gofmekler *et al* reported tetratogenic effects on fertility with changes in fetal, and organ weights, and histopathology in fetuses of mothers exposed to formaldehyde. Our study shows that the elimination of formaldehyde and its metabolites from fetal tissues is slower than from maternal tissues. This is especially so in the fetal liver and brain, at least at 16 days of fetal development: the concentrations were twice as high in the fetal brain compared with the maternal brain at 24 hours after injection. Moreover, our data on the percentage of ^{14}C radioactivity accumulated in the DNA fraction of liver tissue shows it to be much higher in fetal than in maternal hepatic cells. There are no data on metabolic enzymes for formaldehyde in fetal liver and it is not known whether these are the same as those in maternal liver.

In summary, special attention should be paid to the fact that the rate of removal of radioactivity from ^{14}C-formaldehyde in the fetus was slower than that in maternal tissue after a single injection. Further study is needed to determine the effects of chronic exposure on fetal tissues.

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Table 1 Concentration of radioactivity in maternal tissues, urine, and faeces of mice

Time after injection	*Liver*	*Lung*	*Heart*	*Salivary gland*	*Gall bladder*	*Spleen*	*Kidney*	*Urine*	*Faeces*
5 min	307·9 (58·8)	147·0 (49·4)	129·6 (57·4)	216·0 (62·9)	140·8 (86·3)	172·1 (57·3)	193·8 (44·2)	—	—
30 min	269·3 (43·4)	80·5 (16·0)	70·5 (15·7)	258·3 (67·4)	98·0 (54·3)	142·9 (80·2)	147·4 (13·3)	—	—
1 h	298·2 (66·7)	103·9 (22·2)	83·6 (20·7)	350·3 (115·8)	124·6 (49·6)	205·8 (110·0)	181·7 (47·3)	—	—
3 h	361·8 (64·5)†	166·2 (36·3)†	97·1 (7·7)	393·5 (86·7)**	142·4 (25·6)	225·4 (57·3)	230·5 (42·5)†	—	—
6 h	180·4 (42·0)	66·3 (11·2)	51·8 (8·0)	221·4 (91·3)	75·1 (13·1)	129·3 (18·1)	122·4 (31·2)	487·9 (96·6) (5·96)	120·3 (72·8) (0·32)
24 h	105·7 (37·7)	51·4 (11·6)	40·7 (7·7)	83·5 (27·2)	43·7 (13·0)	66·2 (34·3)	98·2 (29·4)	293·7 (122·8) (3·39)	156·2 (114·2) (0·30)
48 h	48·3 (10·8)	29·2 (5·1)	24·5 (4·2)	40·2 (7·3)	15·5 (4·3)	43·5 (14·8)	47·9 (8·2)	167·2 (114·6) (2·00)	36·6 (20·5) (0·12)

**$p < 0·01$, significantly different from radioactivity at five minutes; † $< 0·05$, significantly different from radioactivity at 30 minutes.
—No specimens were obtained. The results are expressed as dpm/mg (μl) tissue (mean (SD)) (n = 5).

Table 2 Maternal and fetal concentrations of radioactivity in pregnant mice

Time after injection		Blood	Brain (whole)	Liver	Placenta	Fetus (whole)	Amniotic fluid	Amnion	Uterus
5 min	Maternal	154·4 (39·9)	36·7 (4·6)	307·9 (58·8)	65·5 (27·4)		39·2 (33·8)	56·8 (23·8)	141·9 (37·3)
	Fetal		31·8 (10·4)	88·9 (39·3)		63·1 (30·6)			
30 min	Maternal	79·0 (13·4)	48·7 (3·1)	269·3 (43·4)	75·3 (22·8)		19·3 (6·9)	53·9 (9·9)	110·2 (29·9)
	Fetal		47·7 (20·1)	129·0 (42·5)		81·1 (19·7)			
1 h	Maternal	117·1 (24·1)	44·6 (0·5)	298·2 (66·7)	89·6 (39·7)		14·6 (7·9)	58·4 (29·1)	109·3 (26·5)
	Fetal		56·2 (34·6)	150·3 (4·3)		85·1 (32·0)			
3 h	Maternal	149·4 (18·9)†	53·1 (25·6)	361·8 (64·5)	120·1 (23·8)		18·8 (11·3)	80·2 (21·1)	135·5 (27·8)
	Fetal		71·0 (24·2)	263·9 (56·7)		117·7 (26·5)			
6 h	Maternal	49·8 (3·8)	22·9 (6·0)	180·4 (42·0)	62·6 (0·11)		8·9 (4·9)	51·1 (17·0)	82·4 (10·4)
	Fetal		35·0 (8·0)*	142·5 (38·1)		79·5 (25·7)			
24 h	Maternal	15·4 (5·8)	17·7 (4·2)	105·7 (37·7)	48·5 (21·3)		4·9 (1·9)	45·3 (21·1)	69·3 (20·1)
	Fetal		36·5 (12·1)**	96·2 (38·6)**		65·0 (29·0)			
48 h	Maternal	9·0 (2·5)	9·5 (2·5)	48·3 (10·8)	25·9 (4·8)			24·2 (6·0)	33·6 (7·8)
	Fetal		22·3 (5·9)**	48·2 (16·6)					

*$p < 0.05$; **$p < 0.01$, significantly different from maternal radioactivity; †$p < 0.05$ significantly different from radioactivity at 30 minutes. The results are expressed as dpm/mg (μl) tissue (mean (SD)) (n = 5).

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