

All three solvents were rapidly biotransformed with metabolites appearing mainly in the liver and kidney immediately after inhalation. Both urinary and biliary excretion of metabolites of the solvents took place, the time course of which was found to be roughly correlated to their respective retention times in fatty tissues. Excretion of methylene chloride and chloroform metabolites appeared to be largely complete within 8 hours after inhalation whereas non-volatile radioactivity from ^{14}C -carbon tetrachloride was registered in the intestinal contents still 48 hours after inhalation. Metabolites of the three solvents accumulated both in the kidney and in the liver after inhalation as shown by autoradiography of dried, evaporated sections as compared to low-temperature autoradiography of hemisections. In neither case were the liver and kidney cleared of radioactivity within the time of the autoradiographic study. The distribution picture of methylene chloride metabolites 24 hours after inhalation (Fig. IV:2) strongly resembles that of formaldehyde (Fig. III:4), with the notable exception of the relatively high concentration of radioactivity in the liver, indicating that the radioactivity present in several organs such as the kidney, bone marrow, gastro-intestinal mucosa, thymus and salivary gland is due to the incorporation of radioactivity via normal metabolic pathways. Methylene chloride has been shown to be metabolized *in vitro* to formaldehyde (6, 234), which probably explains the incorporation of radioactivity observed *in vivo* in this study into tissues with a rapid cell turnover or high rate of protein synthesis. The remarkably high amount of radioactivity in the liver, as compared to e.g. the gastro-intestinal

mucosa, is surprising in view of the results of Reynolds and Yee (345), who reported similar patterns of incorporation of radioactivity into rat liver microsomal lipids and proteins after administration of ^{14}C -methylene chloride or ^{14}C -formaldehyde. The autoradiographic results indicate that the liver radioactivity after administration of ^{14}C -methylene chloride is due not only to the incorporation of radioactivity from the one-carbon pool via formaldehyde but also via other biotransformation products. The nature of this metabolite is obscure but it seems unlikely that it would cause severe liver injury in view of several studies reporting a very low acute hepatotoxicity of methylene chloride in mice (143, 222, 316). However, continuous exposure of mice to 5000 ppm methylene chloride for only 6-7 days produced slight fatty degeneration of the liver and swelling and necrosis of scattered hepatocytes (470). A comparison of the autoradiographic distribution patterns of non-volatile and non-extractable radioactivity within the liver after inhalation of ^{14}C -labeled methylene chloride, chloroform and carbon tetrachloride shows that the liver radioactivity after administration of ^{14}C -methylene chloride is evenly distributed whereas that after inhalation of ^{14}C -chloroform or ^{14}C -carbon tetrachloride has an uneven, mottled appearance, which may indicate formation of a methylene chloride metabolite of a different nature than the free radicals assumed to arise during the biotransformation of chloroform or carbon tetrachloride. The distribution of radioactivity within the liver observed after inhalation of ^{14}C -chloroform or ^{14}C -carbon tetrachloride at the whole-body autoradiographic level observed in this study is assumed to represent a centrilobular

localization of their metabolites, which has been observed by micro-autoradiography both in the case of chloroform (193) and carbon tetrachloride (351). Non-volatile and non-extractable radioactivity in the kidney cortex was noted after administration of all three ^{14}C -labeled chlorinated methanes but a strikingly higher level was observed after inhalation of ^{14}C -chloroform. This finding supports the observation that chloroform is a potent nephrotoxin in mice whereas methylene chloride and carbon tetrachloride produce functional and morphological injury to the mouse kidney only at near lethal dose levels (316, 317) and, since the study was performed with male mice, the results of Ilett *et al.* (193) showing a high amount of covalent binding of radioactivity from ^{14}C -chloroform in the male mouse kidney, indicating a relationship between covalent binding of a reactive chloroform metabolite and kidney injury in male mice. They also showed an accumulation of firmly bound radioactivity in the necrotic renal tubules, which, at the whole-body autoradiographic level, is seen as a specific localization of radioactivity to the kidney cortex as observed by Taylor *et al.* (439) up to 24 hours after administration and in this study up to 48 hours after administration.

The incorporation of radioactivity into tissues with a rapid cell turnover or high rate of protein synthesis evident after administration of ^{14}C -methylene chloride to mice was also observed to a slight extent after administration of ^{14}C -chloroform and ^{14}C -carbon tetrachloride. This finding would support the possibility that chloroform is biotransformed to CO_2 via formaldehyde (354, 461) and that carbon tetrachloride is biotransformed to CO_2 via chloroform (174). However, in the case

of carbon tetrachloride, the localization of non-volatile radioactivity in the gastro-intestinal mucosa is remarkable in several aspects. The appearance of non-volatile radioactivity in the gastro-intestinal mucosa is extremely rapid and the radioactivity is not evenly distributed between the various parts of the gastro-intestinal tract but shows an accumulation in the mucosa of the glandular part of the stomach and in the mucosa of the colon and rectum. This is not observed after administration of methylene chloride, chloroform or formaldehyde. Thus, it seems as if the radioactivity registered in the gastro-intestinal mucosa is due not only to the incorporation of radioactivity via the one-carbon pool but also to the incorporation of another, presumably very reactive, carbon tetrachloride metabolite. Radioactivity from ^{14}C -carbon tetrachloride has been found to be irreversibly bound to lipids in the rat intestinal mucosa after oral administration (73). The autoradiographic results show that binding also occurs to proteins in the intestinal mucosa, since the lipids would be extracted during the last step of the autoradiographic procedure, and that binding takes place also after inhalation of carbon tetrachloride. Alpers and Isselbacher (16) noted inhibition of protein synthesis and polysome breakdown in the mucosa of the jejunum and ileum in rats fed with carbon tetrachloride. The autoradiographic results indicate that the effects of carbon tetrachloride may be even more pronounced in the mucosa of the stomach, colon and rectum. Neither lipid peroxidation nor necrosis were observed in the intestinal mucosa of rats receiving carbon tetrachloride (16, 73). The strong accumulation of non-extractable carbon tetrachloride metabolites in the

gastro-intestinal mucosa may thus seem to be relatively harmless. However, workers chronically exposed to carbon tetrachloride vapors showed several signs of gastro-intestinal distress such as hypertonicity, irregular peristalsis and spasmodic contractions of the stomach and intestines and also excessive gastric secretion, mucosal changes, hematemesis and rectal bleeding (430).

In this study, the testicular interstitial cells showed a moderate level of non-volatile radioactivity after inhalation of both ^{14}C -chloroform and ^{14}C -carbon tetrachloride. The radioactivity was extractable in the case of chloroform, which has been reported to cause increased testicular weight but no histopathological changes in the testes of chronically exposed animals (444). In the case of carbon tetrachloride the radioactivity was non-extractable, which may possibly be associated with testicular atrophy observed in chronically exposed animals (4). Acute exposure to carbon tetrachloride produced no alterations of testicular morphology (199) but a slight binding of radioactivity from a single dose of ^{14}C -carbon tetrachloride to testicular lipids has been noted (73, 466). The autoradiographic study demonstrates the firm binding of radioactivity also to testicular proteins. Non-volatile radioactivity from ^{14}C -carbon tetrachloride was also firmly bound in the vaginal and uterine mucosa. Little is known about the effects of carbon tetrachloride on the female reproductive tract. However, diminished fertility was demonstrated in rats chronically exposed to 200 or 400 ppm carbon tetrachloride although this could have been due to effects on both males and females (402).

Elimination studies

Uptake. The doses of ^{14}C -labeled methylene chloride, chloroform and carbon tetrachloride administered to mice by inhalation were calculated from the differences between the amounts of radioactivity added to the inhalation apparatus and the amounts recovered by repeated washings of the apparatus with scintillation fluid. These values were used to calculate the uptake of the three solvents. The uptake of all three solvents was high amounting to about 87% for methylene chloride and about 94% and 84% for chloroform and carbon tetrachloride respectively (Table IV:1).

Elimination in the exhaled air. About 63% of the administered dose of methylene chloride was eliminated unchanged in the exhaled air in 8 hours whereas about 29% and 70% of the administered doses of chloroform and carbon tetrachloride respectively was recovered as volatile material in the same period of time (Table IV:1). The time course of the cumulative elimination of the three solvents in the exhaled air is shown in Fig. IV:6. About 97% of the exhaled amount of methylene chloride was recovered during the first 2 hours whereas the recovery of the same proportions of the exhaled amounts of chloroform and carbon tetrachloride required 3 hours and 5 hours respectively. Radioactive CO_2 was found in the exhaled air after administration of all three solvents. Chloroform was biotransformed to a large extent to CO_2 ; about 28% of the dose was recovered as $^{14}\text{CO}_2$ in the exhaled air in 8 hours whereas about 4.5% of the doses of ^{14}C -methylene chloride and ^{14}C -carbon tetrachloride was exhaled as $^{14}\text{CO}_2$ (Table IV:1,

Table IV:1. The uptake of ^{14}C -labeled methylene chloride, chloroform and carbon tetrachloride by mice and the elimination of radioactivity in the exhaled air and urine during 8 hours after inhalation. Mean values of four mice $\pm$ S.E.M.

	inhaled			eliminated in the exhaled air			eliminated
	amount		dose	unchanged	as CO ₂	as CO	in urine
	μCi	uptake	μCi	dpm·10 ³	dpm·10 ³	dpm	dpm
	(μl)	%	(mg/kg)	(% of dose)	(% of dose)	(% of dose)	(% of dose)
methylene chloride	1.44 (10)	86.9±2.2	1.25±0.03 (461.3±11.7)	1749.8±109.2 (63.4±4.0)	129.7±24.0 (4.6±0.9)	16322±816 (0.59±0.03)	29516±1572 (1.07±0.06)
chloroform	1.33 (5)	93.9±0.4	1.24±0.005 (278.5±1.1)	802.6±35.8 (29.3±1.3)	765.3±46.5 (28.4±1.7)	-	248737±7019 (9.1±0.3)
carbon tetra-chloride	1.30 (5)	84.2±2.7	1.10±0.04 (268.6±8.6)	1699.6±79.4 (70.4±3.3)	109.3±7.0 (4.5±0.3)	-	11538±664 (0.48±0.03)

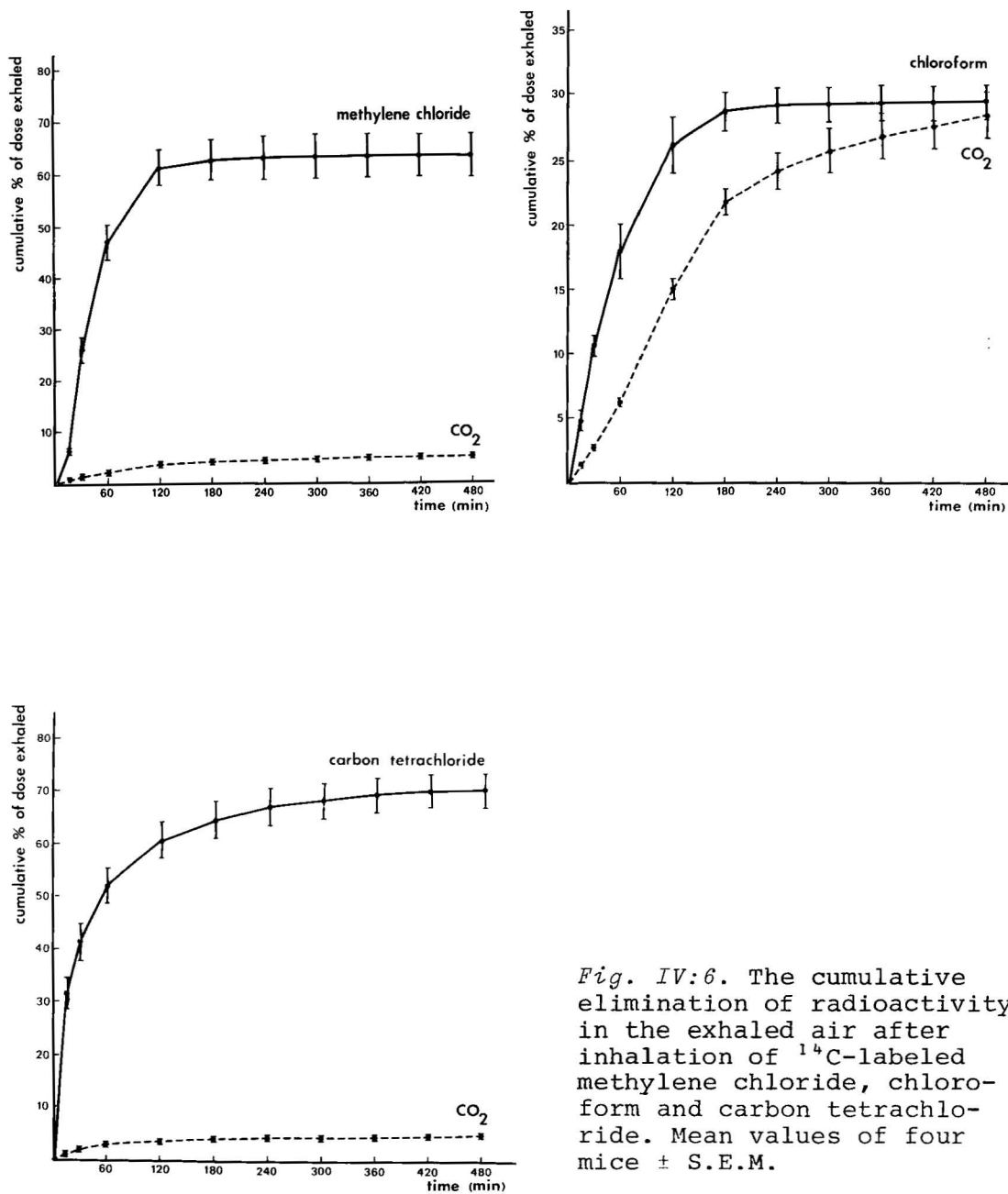


Fig. IV:6. The cumulative elimination of radioactivity in the exhaled air after inhalation of ^{14}C -labeled methylene chloride, chloroform and carbon tetrachloride. Mean values of four mice $\pm$ S.E.M.

Fig. IV:6). In addition, a small part, about 0.6%, of the administered dose of methylene chloride was exhaled as ^{14}CO (Table IV:1). No radioactivity from ^{14}C -chloroform or ^{14}C -carbon tetrachloride was recovered as ^{14}CO . Calculation of the elimination rates of methylene chloride and chloroform in the exhaled air gave unexpectedly low values for the initial 15 minute period as illustrated by the semilogarithmic plots of elimination rates against time in Fig. IV:7. These values were excluded from the experimental data during the subsequent computerized non-linear regression analysis. The elimination rate curve for methylene chloride in the exhaled air showed an initial rapid decrease (15-270 min) followed by a slower decrease (270-450 min) with a very good fit to equation:

$$Y = 3.092 \cdot e^{-0.0330 \cdot t} + 0.0060 \cdot e^{-0.0057 \cdot t}$$

with apparent half-lives of:

$$t_1 = 21.0 \text{ min}, \quad t_2 = 120.7 \text{ min}$$

Similarly, a very good fit was obtained between the experimental data and a computer calculated two-exponential curve for the elimination rate of chloroform in the exhaled air characterized by an initial rapid decrease (15-330 min) followed by a slower decrease (330-450 min).

$$Y = 0.799 \cdot e^{-0.0241 \cdot t} + 0.0036 \cdot e^{-0.0048 \cdot t}$$

with apparent half-lives of:

$$t_1 = 28.8 \text{ min}, \quad t_2 = 144.9 \text{ min}$$

In contrast, computerized non-linear regression analysis of the experimental data for the elimination rate of carbon tetrachloride in the exhaled air yielded a three-exponential decay

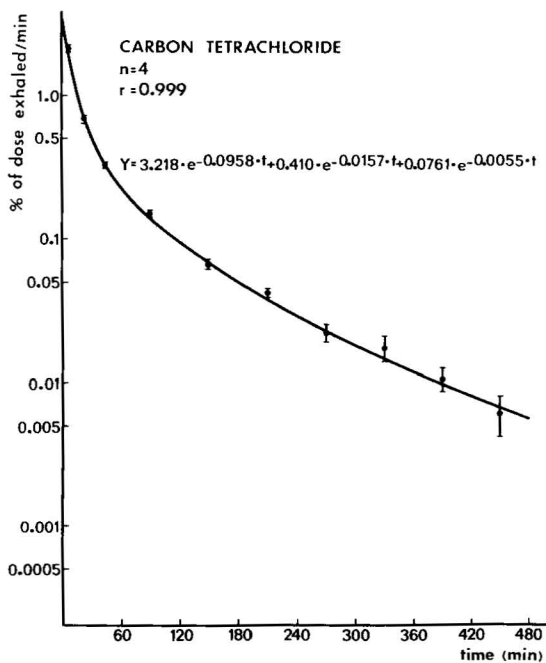
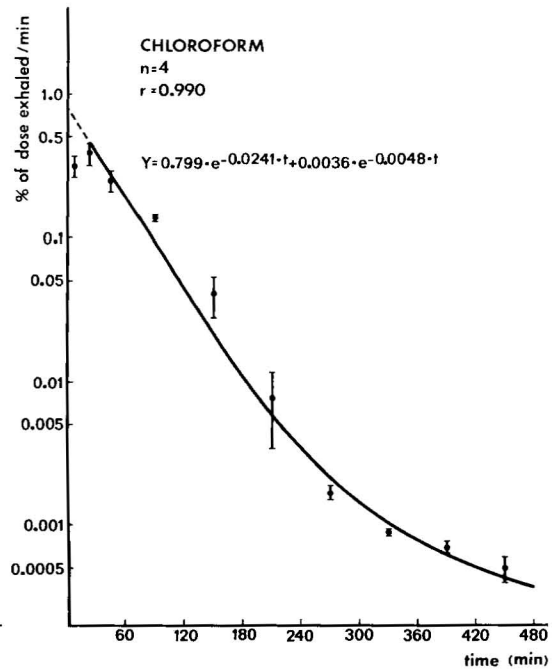
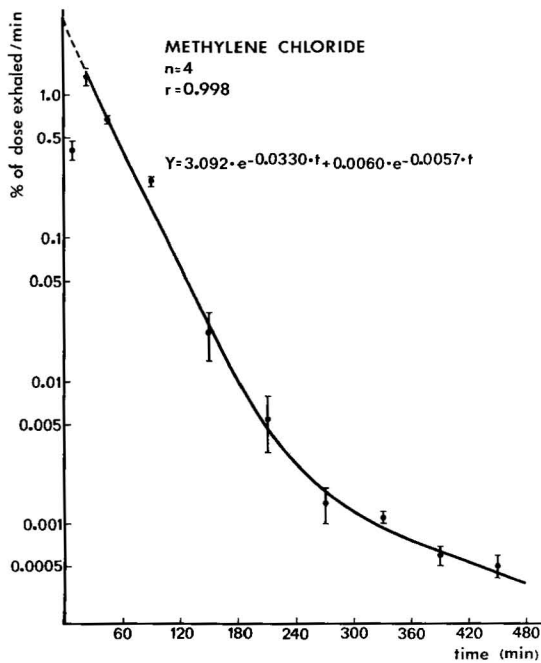


Fig. IV:7. Elimination rate curves for methylene chloride, chloroform and carbon tetrachloride in the exhaled air. Mean values $\pm$ S.E.M.

curve, characterized by an initial very rapid decrease (0-45 min) followed by a slow decrease (45-210 min) and an even slower decrease (210-450 min). The computer derived equation for the rate of excretion of carbon tetrachloride in the exhaled air was:

$$Y = 3.218 \cdot e^{-0.0958 \cdot t} + 0.410 \cdot e^{-0.0157 \cdot t} + 0.0761 \cdot e^{-0.0055 \cdot t}$$

with apparent half-lives of:

$$t_1 = 7.2 \text{ min}, \quad t_2 = 44.1 \text{ min}, \quad t_3 = 126.4 \text{ min}$$

Elimination in urine. About 9% of the dose of ^{14}C -chloroform was eliminated in the urine in 8 hours. The radioactivity from ^{14}C -methylene chloride and ^{14}C -carbon tetrachloride was recovered in the urine in amounts corresponding to about 1% and 0.5% of the respective doses (Table IV:1).

Tissue radioactivity levels. The remaining radioactivity in various tissues 8 hours after inhalation is shown in Table IV:2. The amounts of radioactivity in the brain and fatty tissues both increase in the order methylene chloride, chloroform, carbon tetrachloride. However, there is a considerable difference between the radioactivity levels in the cerebrum and in the cerebellum after inhalation of ^{14}C -chloroform. Relatively high amounts of radioactivity are also retained in the liver and kidney 8 hours after inhalation of all three radiolabeled solvents. In the case of chloroform the kidney radioactivity level is higher than that of the liver whereas the liver radioactivity levels are higher than those of the kidney after inhalation of ^{14}C -methylene chloride or ^{14}C -carbon tetra-

Table IV:2. The retention of radioactivity in selected tissues of mice 8 hours after inhalation of ^{14}C -methylene chloride (dose: $8.69 \pm 0.22 \mu\text{l} = 2759.4 \pm 69.8 \cdot 10^3 \text{ dpm}$), chloroform (dose: $4.70 \pm 0.02 \mu\text{l} = 2734 \pm 10.6 \cdot 10^3 \text{ dpm}$) and carbon tetrachloride (dose: $4.21 \pm 0.14 \mu\text{l} = 2415 \pm 77.0 \cdot 10^3 \text{ dpm}$). Mean values of four mice $\pm$ S.E.M.

tissue	methylene chloride		chloroform		carbon tetrachloride	
	dpm/g	total radio-activity (dpm)	dpm/g	total radio-activity (dpm)	dpm/g	total radio-activity (dpm)
brain	2237 $\pm$ 86	833 $\pm$ 27	40006 $\pm$ 1375 ⁽²⁾ 3281 $\pm$ 130 ⁽³⁾	4295 $\pm$ 172 ⁽²⁾ 991 $\pm$ 63 ⁽³⁾	4844 $\pm$ 476	1909 $\pm$ 191
liver	17085 $\pm$ 1340	22207 $\pm$ 1598	34082 $\pm$ 1088	46345 $\pm$ 1083	28854 $\pm$ 974	38919 $\pm$ 852
lung	11687 $\pm$ 969	2120 $\pm$ 263	40605 $\pm$ 2885	7309 $\pm$ 238	20130 $\pm$ 1164	3536 $\pm$ 388
spleen	5622 $\pm$ 400	560 $\pm$ 115	13914 $\pm$ 1074	1209 $\pm$ 119	6547 $\pm$ 366	479 $\pm$ 65
kidney	7558 $\pm$ 728	2827 $\pm$ 328	37070 $\pm$ 930	16661 $\pm$ 933	12548 $\pm$ 288	5043 $\pm$ 159
pancreas	5562 $\pm$ 495	892 $\pm$ 73	9874 $\pm$ 943	1876 $\pm$ 191	6784 $\pm$ 364	1218 $\pm$ 63
thymus	5148 $\pm$ 486	353 $\pm$ 47	11993 $\pm$ 1058	765 $\pm$ 107	3608 $\pm$ 183	239 $\pm$ 7
testis	2434 $\pm$ 97	413 $\pm$ 61	4471 $\pm$ 303	762 $\pm$ 53	6179 $\pm$ 576	1056 $\pm$ 76
white fat	4502 $\pm$ 434	-	7846 $\pm$ 569	-	14522 $\pm$ 1853	-
brown fat	4642 $\pm$ 470	-	8102 $\pm$ 307	-	12966 $\pm$ 1037	-
blood	2158 $\pm$ 339 ⁽¹⁾	-	6059 $\pm$ 648 ⁽¹⁾	-	2827 $\pm$ 245 ⁽¹⁾	-

(1) dpm/ml (2) cerebellum (3) cerebrum

chloride. The lung radioactivity levels are also relatively high, especially after inhalation of ^{14}C -chloroform. Slightly higher levels of radioactivity than in the blood are also registered in the spleen, pancreas and thymus after inhalation of all three ^{14}C -labeled solvents.

The uptake of methylene chloride, chloroform and carbon tetrachloride vapors by mice was high which is to be expected if it is measured for a short period of time during the beginning of an inhalation when equilibrium between the solvent concentrations in the inspiratory air and in the alveolar air and arterial blood is far from being established. However, in spite of the short period of time and high solvent concentrations used, the uptake seems to follow the blood solubility of the three solvents (Appendix 2) since chloroform with the largest blood/gas solubility coefficient showed the largest uptake and carbon tetrachloride, which is poorly soluble in blood, showed the smallest uptake.

Large proportions of the doses of ^{14}C -methylene chloride and ^{14}C -carbon tetrachloride were exhaled as volatile radioactivity trapped in cold toluene, which agrees with the results of earlier animal studies showing that methylene chloride and carbon tetrachloride are exhaled unchanged to a large extent (102, 246, 312, 377, 409). No attempts were made in this study to fractionate the exhaled volatile material although volatile compounds such as chloroform and hexachloroethane are recognized biotransformation products of carbon tetrachloride (57, 133, 312) and an unidentified volatile radioactive compound was

found in the exhaled air of rats administered ^{14}C -methylene chloride (102). Approximately equal proportions of the administered dose of ^{14}C -chloroform were exhaled as volatile radioactivity and $^{14}\text{CO}_2$ in agreement with the results of Brown *et al.* (49), showing that mice biotransform chloroform to CO_2 to a large extent. In this study 28% of a dose of about 280 mg/kg ^{14}C -chloroform administered by inhalation was exhaled as $^{14}\text{CO}_2$ whereas Brown *et al.* (49) recovered 85% of an oral dose of 60 mg/kg ^{14}C -chloroform as $^{14}\text{CO}_2$. Thus, although the oral route of administration may make a larger proportion of the dose available for biotransformation, it seems as if there is a limit to the capacity of the mouse to biotransform chloroform to CO_2 , which may also be concluded from experiments performed with rats (243, 312). The extensive biotransformation of ^{14}C -chloroform also reflects itself in the relatively large amount of radioactivity eliminated in the urine as compared to the urinary radioactivity recovered after inhalation of ^{14}C -methylene chloride or ^{14}C -carbon tetrachloride (Table IV:1). Radioactive CO_2 was exhaled also after administration of ^{14}C -methylene chloride or ^{14}C -carbon tetrachloride in amounts roughly corresponding to those reported in earlier animal studies (102, 266, 312, 377). The results of the present study also add the mouse to the various animal species which have been shown to biotransform methylene chloride to CO (30, 63, 183, 235, 352), since about 0.6% of the dose of ^{14}C -methylene chloride was recovered as ^{14}CO in 8 hours. No radioactivity was recovered as ^{14}CO after administration of ^{14}C -chloroform, which supports earlier studies where CO could not be identified as a biotransformation product of chloroform *in vivo* (235, 350).

However, the formation of CO from chloroform *in vivo* cannot be excluded in view of its occurrence *in vitro* (7) and the fact that metabolically formed CO may be oxidized in the body to CO₂ (450) and therefore not exhaled as such.

Mice inhaling methylene chloride or chloroform were severely affected and showed a marked depression of respiration at the end of the inhalation period, which probably explains the low values of the elimination rates obtained for the initial 15 minute periods after which time the mice recovered from the narcotic effects of the solvents. The two-compartment pharmacokinetic models obtained for methylene chloride and chloroform from their elimination rate curves in this study would seem to support the results of studies found in the literature where two-compartment pharmacokinetics have been observed after experimental human exposure to methylene chloride (105, 118) or chloroform (136). However, in this study it seems more probable that the depressant effects of the solvents on the respiration have caused two initial parts of the elimination rate curves to merge into one since the first parts of the curves are extended over relatively long periods of time (Fig. IV:7). Disregarding the disparity of the experimental data, the time course and the equations obtained for the elimination rates of methylene chloride and chloroform in the exhaled air indicate a slower elimination of chloroform than of methylene chloride from the body, which is probably explained by the higher fat solubility and lower vapor pressure at body temperature of chloroform compared to methylene chloride (Appendices 1 and 2). The discrepancy between the elimination rate curves obtained for methylene chloride and chloroform on

one hand and carbon tetrachloride on the other makes comparisons of the three solvents in this respect difficult. The elimination of carbon tetrachloride could actually seem to be faster than that of methylene chloride or chloroform when judged from the mathematical constants obtained for its elimination rate in the exhaled air. However, as evident from the autoradiographic results and liquid scintillation counting data, carbon tetrachloride was still present in body fat in significant amounts 8 hours after inhalation (Fig. IV:5, Table IV:2), which, together with the lack of a plateau in its cumulative elimination curve (Fig. IV:6), could indicate that the pharmacokinetics of carbon tetrachloride may be more complex than those of a three-compartment model. The expiration of unchanged carbon tetrachloride is known both from animal experimentation (266, 409) and human poisoning (421) to take place during a remarkably long period of time after exposure. As suggested by Riley *et al.* (347), the "fat phase" of excretion of organic solvents may include several rates of long half-times since body fat is variable in composition and the fat depots may not be equally perfused. The pharmacokinetics of chloroform may also be of a more complex nature considering its long retention time in some parts of the nervous system as shown by low-temperature autoradiography.

The tissue radioactivity levels registered by liquid scintillation counting at 8 hours after inhalation are in accordance with the autoradiographic distribution pictures where the highest levels of radioactivity were noted in the liver, kidney and bronchi after administration of all three ¹⁴C-labeled solvents. In addition, after inhalation of

^{14}C -chloroform a high radioactivity level was observed by both techniques also in the cerebellum. The radioactivities registered in the spleen, pancreas and thymus after inhalation of the three ^{14}C -labeled solvents, slightly higher than those of the blood, may be assumed to represent the incorporation of one-carbon fragments into tissues with a rapid cell turnover or high rate of protein synthesis.