

MATERIALS AND METHODS

Labeled compound

^{14}C -Trichloroethylene (1,2- ^{14}C , specific activity 9.30 mCi/mmol) of 98% purity by gas-liquid radiochromatography was obtained from New England Nuclear, Dreieichenhain, FRG.

Animals

Male NMRI-mice weighing about 25 g were used. The animals were kept at room temperature and given a commercial pellet diet

(AB Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum*.

Autoradiography

^{14}C -Trichloroethylene was diluted with unlabeled, analytical grade trichloroethylene to a final solution of $2.5\ \mu\text{Ci}/\mu\text{l}$. Mice were administered $10\ \mu\text{l}$ of the solution by inhalation for 10 minutes as described in Chapter I. The animals were killed by CO_2 anoxia at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h and 48 h after the inhalation and subject to autoradiography in three steps as described in Chapter I.

Elimination study

^{14}C -Trichloroethylene was diluted with unlabeled, analytical grade trichloroethylene to a final solution of $0.135\ \mu\text{Ci}/\mu\text{l}$. Mice were administered $10\ \mu\text{l}$ of the solution by inhalation for 10 minutes. The elimination of radioactivity by various routes was followed for 8 hours in the metabolic cage whereafter the remaining radioactivity in several organs was determined as described in Chapter I.

RESULTS AND DISCUSSION

Autoradiography

Low-temperature autoradiography (Fig. V:2) of trichloroethylene immediately after inhalation shows an even distribution of radioactivity but with a remarkable high uptake of radioactivity in the brain and body fat. High levels of radioactivity are also

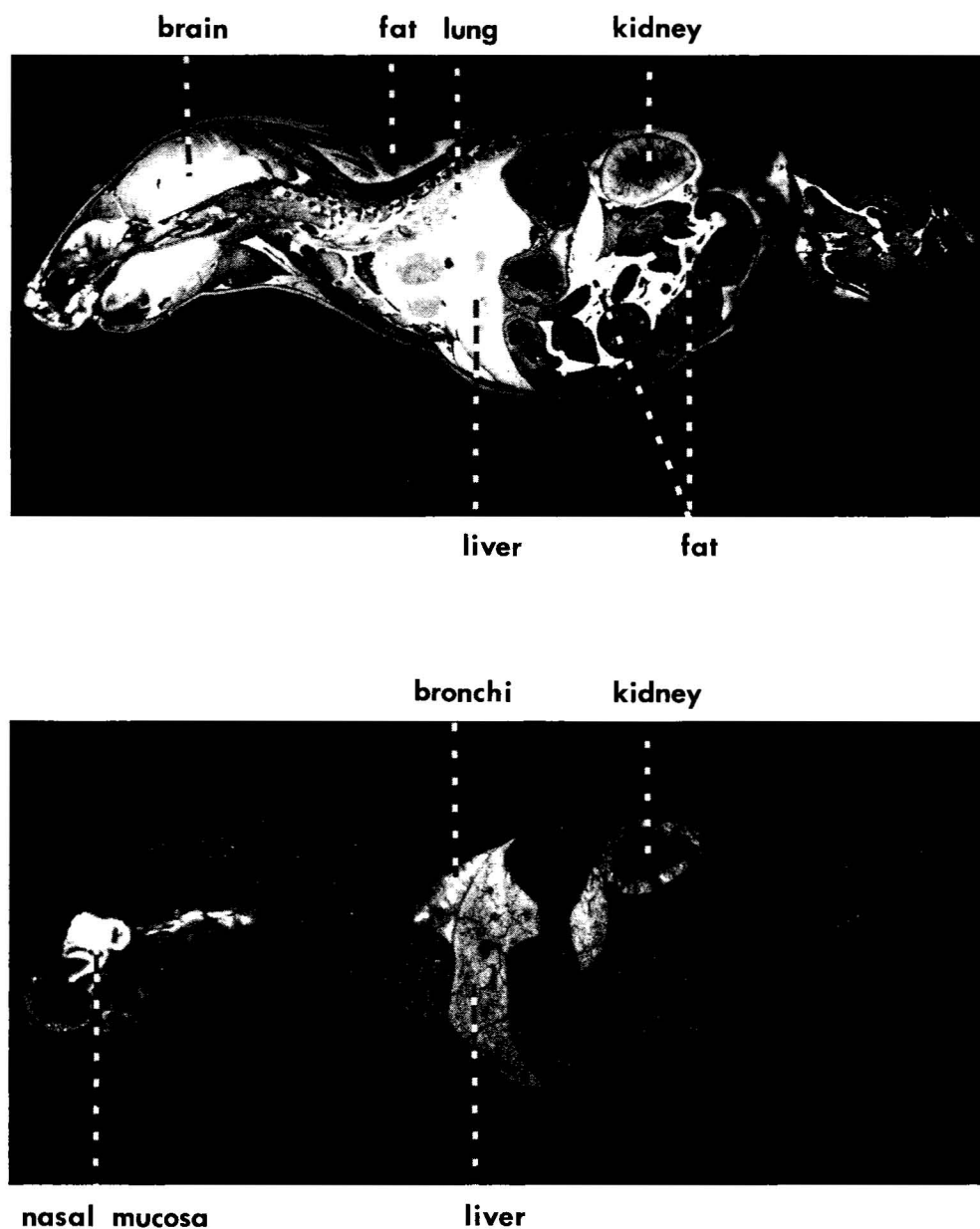


Fig. V:2. Whole-body autoradiograms of a mouse immediately after inhalation of $10\ \mu\text{l}$ ^{14}C -trichloroethylene for 10 min. Hemisection exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). Note the high uptake of volatile radioactivity in the brain and fatty tissues. Metabolites are visible in the bronchi, liver and kidney.

seen in the blood, liver and lung. All radioactivity in the brain, fatty tissues and blood and a large part of the radioactivity in the liver, lung and kidney is volatile, which is seen upon comparison with an autoradiogram obtained at -20°C (Fig. V:2). Non-volatile radioactivity is seen in the bronchi, liver and kidney already immediately after the inhalation. Thirty minutes after inhalation (Fig. V:3) the brain is essentially cleared of radioactivity whereas high amounts of volatile radioactivity may still be registered in body fat by low-temperature autoradiography. Non-volatile radioactivity has increased in the liver, kidney and blood and may also still be seen in the bronchi. The level of non-volatile metabolites of trichloroethylene increased in the kidney up to about 4 hours and in the liver up to about 8 hours after inhalation, whereafter almost constant levels of radioactivity could be registered. Volatile radioactivity was registered in body fat for 4 hours with traces of radioactivity still visible 8 hours after inhalation. Metabolites of trichloroethylene were excreted mainly via the kidney but some excretion of metabolites in the bile was also obvious as shown by the radioactivity present in the intestinal contents from 30 minutes up to 8 hours after inhalation. The non-volatile radioactivity registered in the bronchi, liver and kidney was not completely extractable from the tissues at any survival time. Starting at about 1 hour after inhalation there was also a slight incorporation of non-extractable radioactivity into tissues with a rapid cell turnover such as the bone marrow, thymus and gastro-intestinal mucosa and into tissues with a high rate of protein synthesis such as the spleen, salivary glands and exocrine pancreas. Fig. V:4 shows the distribution

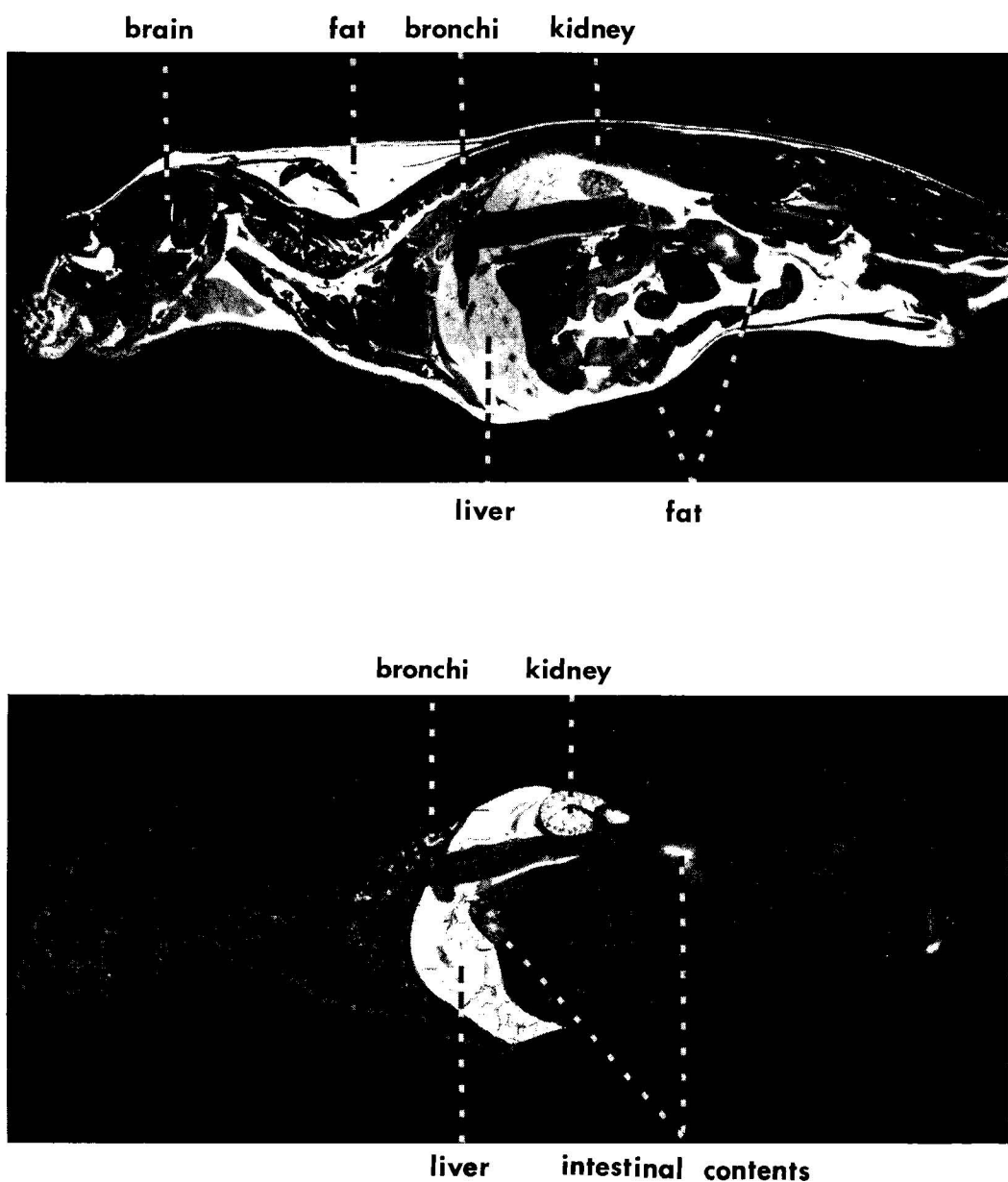


Fig. V:3. Whole-body autoradiograms of a mouse 30 min after inhalation of 10 μ l ^{14}C -trichloroethylene for 10 min. Hemisection exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). High amounts of volatile radioactivity are still present in the body fat. The level of metabolites has increased in the liver and kidney.

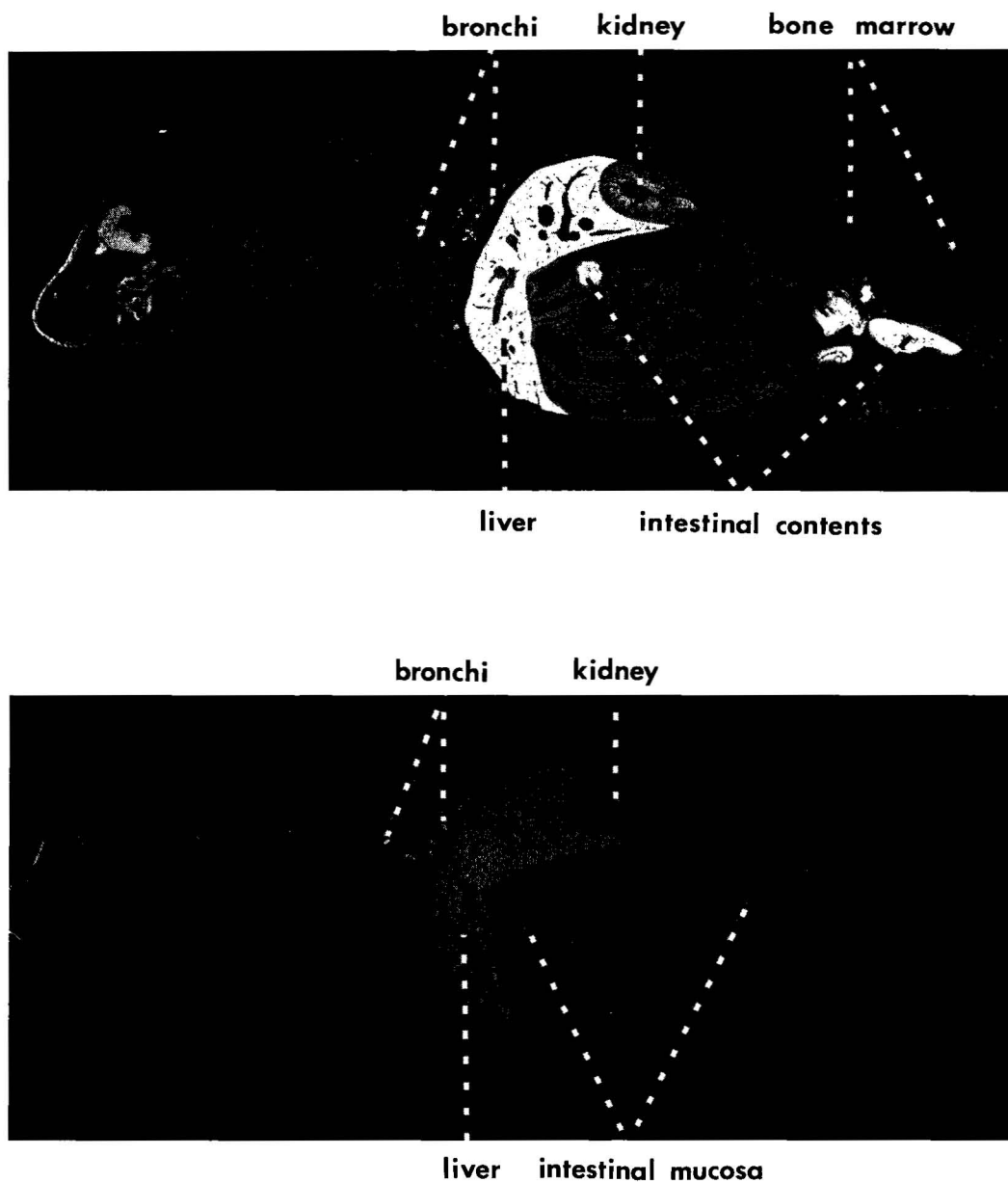


Fig. V:4. Whole-body autoradiograms of a mouse 8 h after inhalation of $10\ \mu\text{l}\ ^{14}\text{C}$ -trichloroethylene for 10 min. Dried, evaporated section (upper) and dried, evaporated and extracted section (lower). Metabolites are still present in the liver, kidney and bronchi. A relatively large proportion of the liver radioactivity is firmly bound. There is also a slight incorporation of radioactivity into tissues with a rapid cell turnover or a high rate of protein synthesis.

of total trichloroethylene metabolites and non-extractable metabolites 8 hours after inhalation. The liver radioactivity is still high and a large part of it is firmly bound. Within the time of the experiment, 48 hours, trichloroethylene metabolites were not cleared from the body but were still registered in the bronchi, liver, kidney and tissues with a rapid cell turnover or protein synthesis at 48 hours after inhalation.

The autoradiographic results support the early findings of high fat concentrations of trichloroethylene (34, 121). At no survival time was radioactivity registered in nervous or adipose tissues by autoradiography of dried, evaporated sections. The hypnotic metabolites of trichloroethylene, trichloroethanol and/or chloral hydrate, which may be expected to be localized to nervous tissues, are both quite volatile and may be expected to have evaporated from the sections upon heating them to +50°C for 24 hours. The radioactivity registered by autoradiography of dried, evaporated sections may therefore be assumed to mainly represent trichloroacetic acid and other non-volatile metabolites. The autoradiographic findings are therefore also in contrast to the results of Fabre and Truhaut (121), who found large amounts of trichloroacetic acid in body fat. The brain level of volatile radioactivity immediately after inhalation, probably representing trichloroethylene itself to a large extent, was very high, which would be in accordance with the powerful narcotic action of trichloroethylene. The rapid disappearance of trichloroethylene from the brain supports the results of Savolainen *et al.* (368), who did not note any

accumulation of trichloroethylene in the brain of rats upon repeated exposure. The relatively long retention time in body fat would support the results of the same authors (368) that trichloroethylene could accumulate in body fat, which has also been predicted by Fernandez *et al.* (128) using a computerized model of trichloroethylene distribution and transfer in the body at repeated exposure. The slow excretion of trichloroacetic acid, as noted in both human and animal studies, in addition to firm binding of radioactivity, may contribute to the autoradiographic registration of trichloroethylene metabolites still 48 hours after exposure. The excretion of trichloroethylene metabolites in the bile, as noted in this study, has also been observed after human experimental exposure (36). The registration of firmly bound radioactivity not only in the liver but also in the bronchi and kidney is in accordance with recent results of covalent binding of radioactivity from ^{14}C -trichloroethylene to liver proteins *in vitro* and *in vivo* (15, 44, 451, 459). The fact that radioactivity from radiolabeled trichloroethylene may become covalently bound and that pretreatments of animals that increase trichloroethylene biotransformation increase the degree of binding *in vivo* as well as the hepatotoxicity (15) support a role of a reactive trichloroethylene metabolite as a hepatotoxic agent. The appearance of radioactivity in tissues with a rapid cell turnover or a high rate of protein synthesis indicate that a splitting of the C-C bond takes place during the biotransformation of trichloroethylene with the formation of one-carbon fragments that are incorporated into these tissues via normal metabolic pathways (cf. Chapter I). However, since the firmly bound liver radioactivity is clearly

higher than that of e.g. the gastro-intestinal mucosa, which is not characteristic of a one-carbon pool incorporable compound such as formaldehyde (Fig. III:4), it represents the firm binding of another trichloroethylene metabolite.

Elimination study

Uptake. The dose of ^{14}C -trichloroethylene administered to mice by inhalation as calculated from the difference between the amount of radioactivity added to the inhalation apparatus and the amount of radioactivity recovered from the apparatus by washings with scintillation fluid was $0.65 \pm 0.09 \mu\text{Ci}$ or $280.8 \pm 40.0 \text{ mg/kg}$ (mean value of four mice $\pm$ S.E.M.). The uptake of trichloroethylene was from this value calculated to be $47.6 \pm 6.8\%$ (mean value of four mice $\pm$ S.E.M.).

Elimination in the exhaled air. The amount of radioactivity exhaled as unchanged trichloroethylene during 8 hours after inhalation was $349898 \pm 49750 \text{ dpm}$ or $24.6 \pm 3.5\%$ of the calculated dose. During the same period of time $46770 \pm 2295 \text{ dpm}$ or $3.0 \pm 0.2\%$ of the dose was recovered as $^{14}\text{CO}_2$ (mean values of four mice $\pm$ S.E.M.). The time course of the cumulative elimination of unchanged ^{14}C -trichloroethylene and $^{14}\text{CO}_2$ in the exhaled air is shown in Fig. V:5. At the end of the experiment, the expiration of unchanged ^{14}C -trichloroethylene had almost ceased whereas the expiration of $^{14}\text{CO}_2$ was still evident. No radioactivity was recovered as ^{14}CO . The semilogarithmic plot of the elimination rate of trichloroethylene against time is shown in Fig. V:6. Computerized non-linear regression analysis gave a very good fit of the experimental data to a

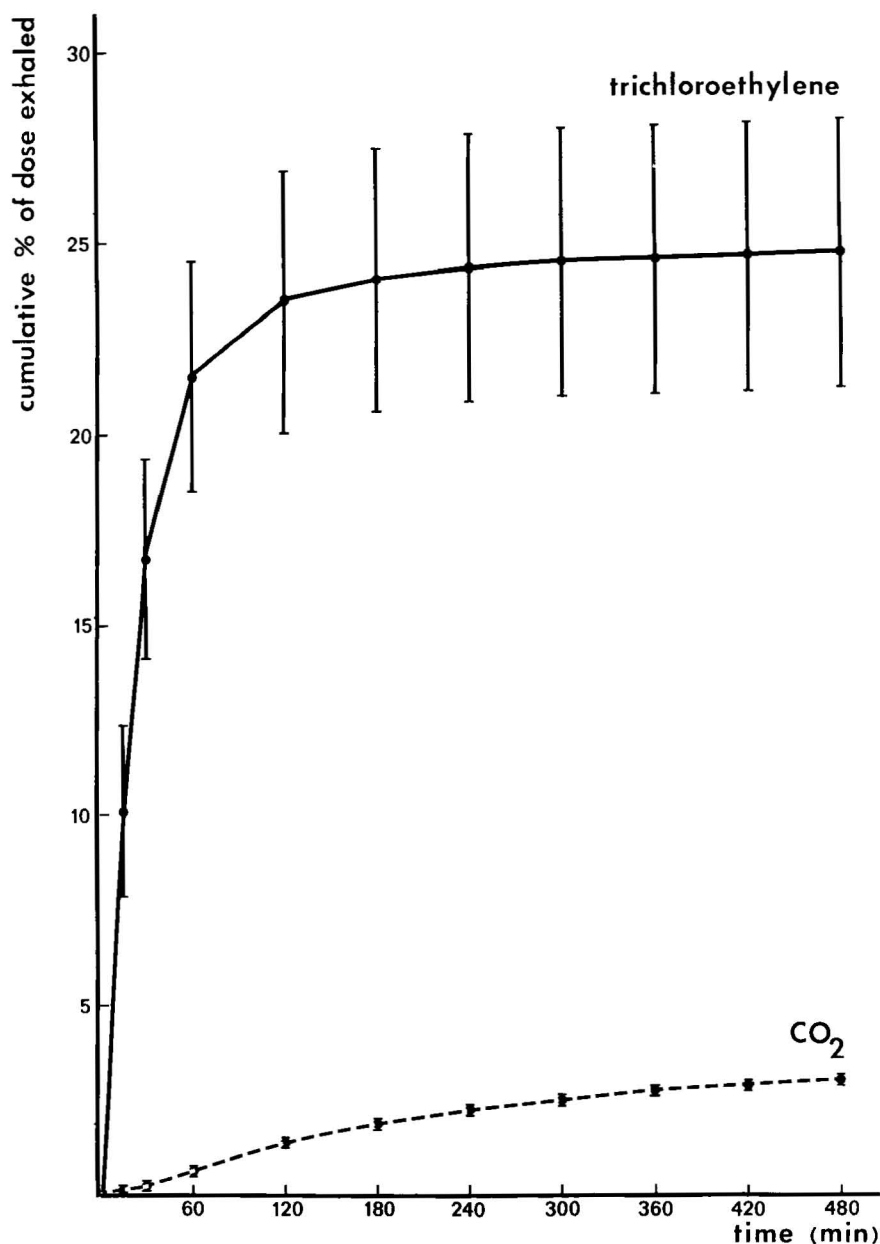


Fig. V:5. The cumulative elimination of ^{14}C -trichloroethylene and $^{14}\text{CO}_2$ in the exhaled air. Mean values of four mice $\pm$ S.E.M.

three-exponential curve with an initial very rapid decrease of elimination rate (0-90 min) followed by a slower decrease (90-270 min) and an even slower decrease (270-450 min). The computer calculated equation for the curve was:

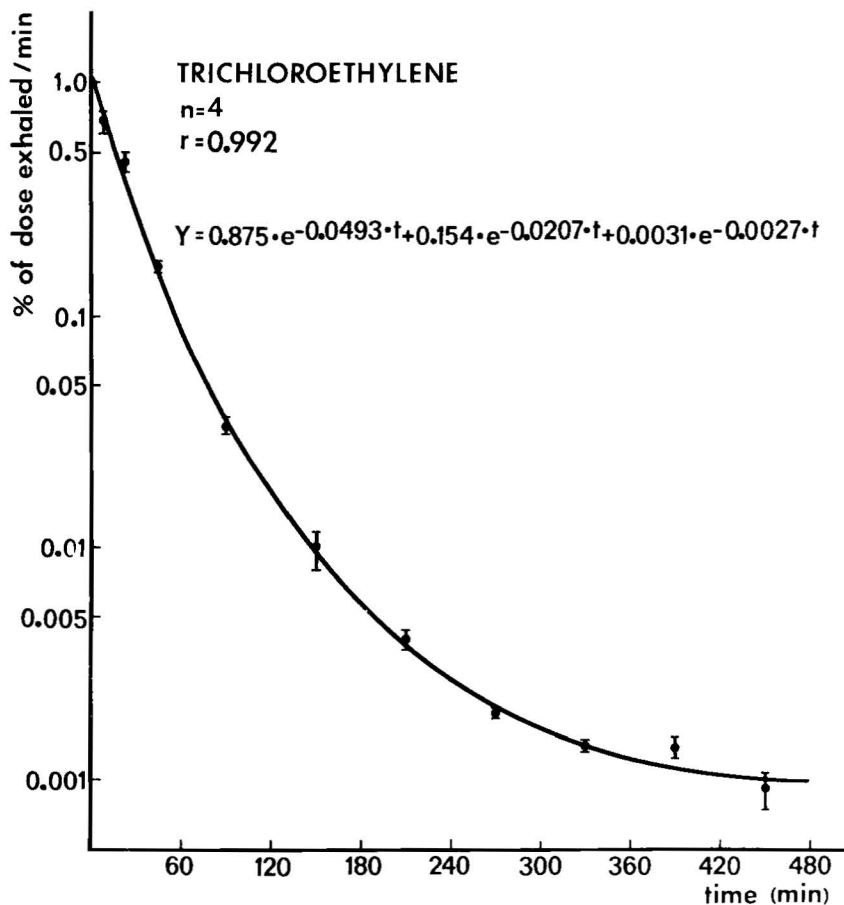


Fig. V:6. Elimination rate curve for trichloroethylene in the expired air. Mean values of four mice $\pm$ S.E.M.

$$Y = 0.875 \cdot e^{-0.0493 \cdot t} + 0.154 \cdot e^{-0.0207 \cdot t} + 0.0031 \cdot e^{-0.0027 \cdot t}$$

with apparent half-lives of:

$$t_1 = 14.1 \text{ min}, t_2 = 33.4 \text{ min}, t_3 = 260.3 \text{ min}$$

Elimination in urine. In 8 hours after inhalation 583568 ± 20552 dpm or $41.1 \pm 1.5\%$ of the dose was recovered in the urine (mean value of four mice $\pm$ S.E.M.).

Tissue radioactivity levels. The remaining radioactivity in

Table V:1. The retention of radioactivity in selected tissues of mice 8 h after inhalation of ^{14}C -trichloroethylene (dose: $4.80 \pm 0.68 \mu\text{l} = 1420.0 \pm 202.1 \cdot 10^3 \text{ dpm}$). Mean values of four mice $\pm$ S.E.M.

tissue	dpm/g	total radioactivity (dpm)
brain	5081 ± 193	2032 ± 96
liver	69162 ± 5577	89614 ± 6656
lung	21508 ± 784	4586 ± 357
spleen	8661 ± 441	1216 ± 97
kidney	21162 ± 2165	7781 ± 1255
pancreas	12422 ± 1485	1756 ± 527
thymus	11968 ± 1128	674 ± 111
testis	10625 ± 1150	1970 ± 319
white fat	17728 ± 1207	-
brown fat	12608 ± 1568	-
blood	$24878 \pm 2432^{(1)}$	-

(1) dpm/ml

various tissues 8 hours after inhalation is shown in Table V:1. The radioactivity levels are generally low although a notably high amount of radioactivity is still present in the liver.

The approximately 48% uptake of trichloroethylene by mice at inhalation obtained in this study roughly agrees with values reported in the literature (5, 36, 410, 411). The expiration of unchanged trichloroethylene by mice (about 25% of the dose) is considerably less than that reported for rats (about 80% of

the dose) receiving large doses orally (88). A certain increase of the amount exhaled by rats was also noted in the same study with increasing dose and it has been observed that humans exposed to increasing concentrations of trichloroethylene exhale increasing amounts after cessation of exposure (218, 424, 429). Humans exposed to 54 or 97 ppm trichloroethylene exhaled 8.4% of the retained amount (129), whereas exposure to 0.54-1.0 mg/l (100-244 ppm) resulted in the expiration of 19% (410) and exposure to 252-380 ppm in the expiration of 23.1% of the retained amount (295). Thus, there seems to be a limit to the extent of trichloroethylene biotransformation both in animals and in man as evident by an increased expiration of unchanged trichloroethylene at high doses. Furthermore, the pharmacokinetics of trichloroethylene seem to be dose-dependent since the rate of trichloroethylene expiration is dependent upon duration of exposure, which determines the dose administered at exposure to equal atmospheric concentrations (429). However, similar three-compartment pharmacokinetics as observed in this study have been observed or postulated in previous studies (128, 129, 163, 364). The exhalation of trichloroethylene by mice was an initially very rapid process with 95% of the total amount exhaled in 8 hours recovered during the first 2 hours. An initially very rapid decrease of trichloroethylene concentrations in the exhaled air during the first 1-2 hours after exposure followed by a slower decrease during the next 2-5 hours followed by a very slow decrease with trichloroethylene present in the exhaled air for as long as 88 hours (424) has been observed in several studies and the three-exponential elimination curve supposed to represent 1/the elimination of

trichloroethylene mainly from blood and well-perfused tissues 2/the elimination mainly from less perfused tissues and 3/the elimination of the residual amount still present in body fat. The model seems to agree quite well with the autoradiographic distribution pictures where trichloroethylene itself was rapidly cleared from well-perfused tissues such as the brain, liver and kidney. The radioactivity registered at short survival times in the liver and kidney was to a large extent non-volatile. Trichloroethylene was registered in body fat for about 8 hours but the elimination curve and its accompanying pharmacokinetic compartment theory indicate that amounts too small to be registered by autoradiography, within the film exposure time used, may have been retained in adipose tissues for a longer period of time.

No reports of the formation of CO_2 from trichloroethylene seem to exist in the literature although this has been suggested (32, 36). Interestingly, according to the suggestions, the splitting of the C-C bond would result in the simultaneous formation of chloroform, which is a recognized hepato-renal toxic agent and also produces liver tumors in mice (119). The formation of chloroform could possibly contribute to the sometimes observed toxic effects of trichloroethylene on the liver and kidneys and perhaps also to the explanation of the recent observation that prolonged trichloroethylene exposure produced hepatocellular carcinoma in mice whereas this was not observed in rats (468). Metabolically formed chloroform would be exhaled to a certain extent, but chloroform was not identified in the exhaled air of rats after administration of trichloroethylene (88). The splitting of the C-C bond to CO_2 and possi-

bly chloroform may be a unique biotransformation pathway of trichloroethylene in mice. Moreover, the formation of $^{14}\text{CO}_2$ from ^{14}C -trichloroethylene also explains the slight incorporation of radioactivity into tissues with a rapid cell turnover or a high rate of protein synthesis via the one-carbon pool (cf. Chapter III) as observed by autoradiography. No formation of ^{14}CO from ^{14}C -trichloroethylene was observed in this study although this was recently shown to occur in microsomal preparations from rats pretreated with compounds that increase trichloroethylene biotransformation (447). The production of CO may be significant only in circumstances of enhanced trichloroethylene biotransformation. Metabolically formed CO may also be oxidized *in vivo* to CO_2 (450) and therefore not exhaled as such. The urinary excretion of trichloroethylene metabolites may be assumed not to be complete in 8 hours after inhalation. Both trichloroethanol and trichloroacetic acid may be identified in the urine for several days after a single exposure although the major part of the urinary trichloroethanol is usually recovered within 24 hours and that of trichloroacetic acid within 48 hours (129, 219, 411). The relatively high blood level of radioactivity 8 hours after inhalation (Table V:1) probably represents trichloroacetic acid bound to plasma proteins. In accordance with the autoradiographic distribution picture (Fig. V:4), the highest level of radioactivity was found in the liver 8 hours after inhalation (Table V:1).