

RESULTS AND DISCUSSION

Autoradiography

Benzene. Benzene was rapidly and evenly distributed in the body immediately after inhalation. Low-temperature autoradiography (Fig. II:3) shows a very high uptake of radioactivity in body fat and bone marrow. Radioactivity is also present in the white matter of the brain, spinal cord, blood and liver. No blackening of the film was apparent at autoradiography of dried, evaporated sections. It may therefore be concluded that the radioactivity registered by low-temperature autoradiography immediately after inhalation was due to benzene only. Benzene was rapidly cleared from the CNS; no radioactivity was registered in the brain or spinal cord 30 minutes after inhalation. However, low-temperature autoradiography revealed considerable amounts of benzene in body fat still 30 minutes after inhalation. At this survival time, radioactivity was also registered in the liver and kidney by autoradiography of dried, evaporated sections, indicating formation of benzene metabolites. As evidenced by low-temperature autoradiography benzene was largely cleared from body fat in about 2 hours after which time autoradiograms obtained at -80°C and from

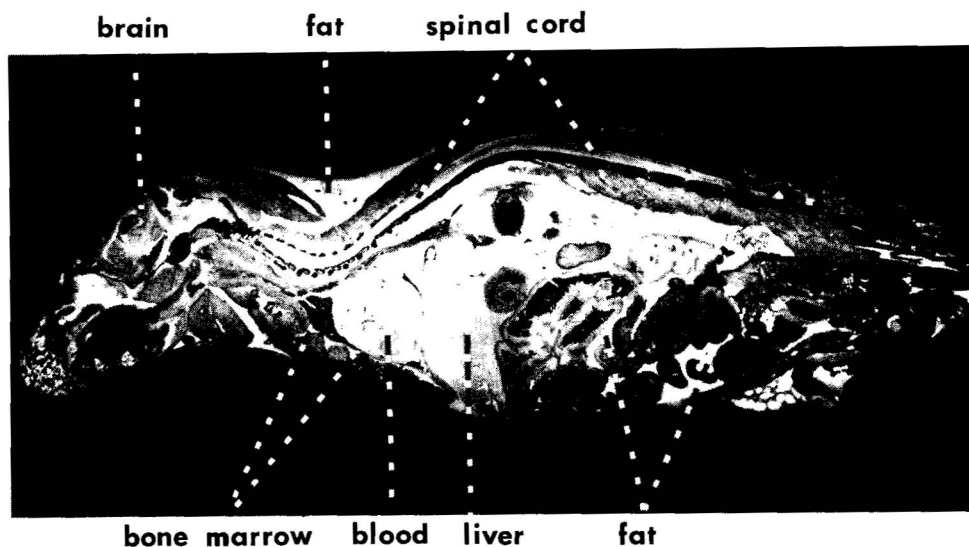


Fig. II:3. Whole-body autoradiogram of a mouse immediately after inhalation of 5 μ l ^{14}C -benzene for 10 minutes. Note the high uptake of radioactivity in the adipose tissues, blood spinal cord, bone marrow and white matter of the brain.

dried, evaporated sections at -20°C showed the same distribution picture. The distribution of benzene metabolites within the kidney was characterized by a very high radioactivity in the medullary region up to 4 hours after inhalation, which is indicative of a rapid urinary excretion of benzene metabolites. This was also shown by the high radioactivity in the urinary tract. At 8 hours after inhalation, radioactivity was seen only in the cortical region of the kidney where it remained firmly bound up to 48 hours after inhalation. The radioactive content of the liver, as shown by autoradiography at -20°C , gradually increased up to 4 hours after inhalation, whereafter a small amount could be registered firmly bound even 48 hours after inhalation. At 8 hours after inhalation and at all following survival times, the liver radioactivity

was unevenly distributed, suggesting a localized distribution within the organ. There was also evidence of a relatively large excretion of benzene metabolites in the bile. Radioactivity appeared in the intestinal contents at 1 hour after inhalation whereafter it increased and dominated the autoradiographic distribution picture up to 8 hours after inhalation. At 24 hours after inhalation the intestine was free of radioactivity. Benzene metabolites were not registered in significant amounts in any other organs except the lung, which showed quite a high uptake of radioactivity at short survival times, localized specifically to the bronchi at 2 hours, but rapidly cleared from this organ since no radioactivity could be registered in the lung at the following survival times. The same was true for the testicle, which showed some interstitially localized benzene metabolites up to 2 hours after inhalation. The amount of radioactivity in the bone marrow, as shown by autoradiography of dried, evaporated sections, was very low and at no survival time higher than that of peripheral blood. Thus, no selective localization of benzene metabolites was found in the bone marrow. All radioactivity registered by autoradiography of dried, evaporated sections was extractable at all survival times, except the radioactivity in the kidney cortex and liver (Fig. II:4).

Intraperitoneal injection of ^{14}C -benzene did not change the distribution pattern of benzene metabolites at 4 hours as compared to the distribution 4 hours after inhalation. Stimulation of benzene biotransformation with phenobarbital or depletion of glutathione with diethyl maleate likewise did not change the autoradiographic distribution picture of

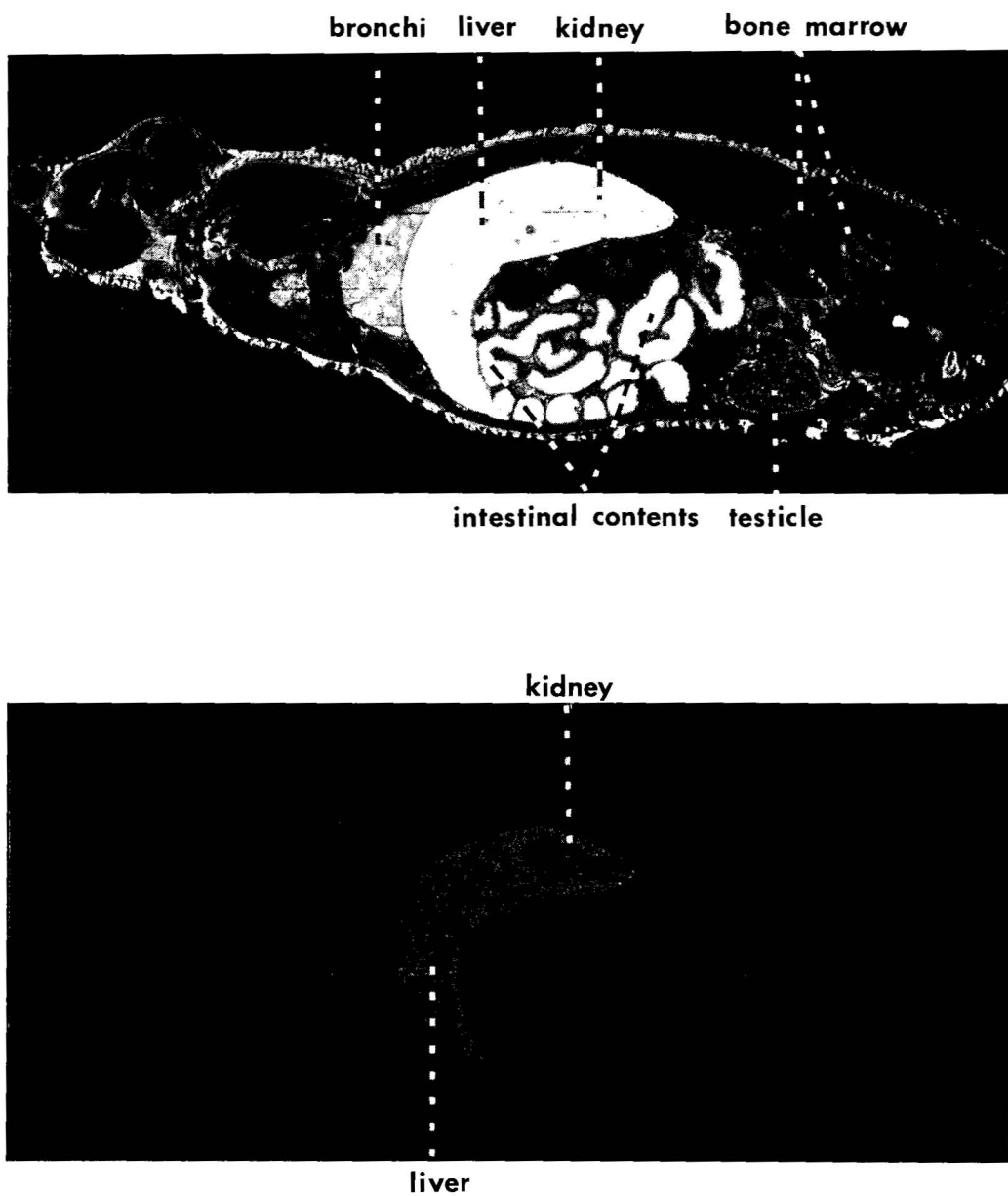


Fig. II:4. Whole-body autoradiograms of a mouse 2 h after inhalation of 5 μ l ^{14} C-benzene for 10 minutes. Dried, evaporated section (upper) and dried, evaporated and extracted section (lower). High concentrations of benzene metabolites are seen in the bronchi, liver, kidney, intestinal contents and testicle. Metabolites are firmly bound in the kidney cortex and liver.

benzene metabolites.

Autoradiography at -20°C after repeated intraperitoneal injections of benzene for up to 18 days did not reveal any accumulation of benzene metabolites in the tissues, including the bone marrow, except a slight increase in firmly bound radioactive material in the liver and kidney cortex. Autoradiography of dried, evaporated and extracted sections showed firmly bound benzene metabolites only in the liver and kidney.

After intravenous injection of ^{14}C -phenol, high amounts of radioactivity appeared in the liver and kidney already 5 minutes after the injection. The radioactivity was rapidly excreted both via urine and bile, most of it during the first 8 hours. Some radioactivity was still present in the liver and kidney after 48 hours. No specific tissue localizations of phenol were noted.

Toluene. Autoradiography of toluene at -80°C immediately after inhalation shows a high uptake of radioactivity in the body fat, bone marrow and spinal nerves and also some radioactivity in the brain and spinal chord (Fig. II:5). Radioactivity is also present in the liver and kidney. These localizations, except the liver and kidney, are not visible at autoradiography of dried, evaporated sections (Fig. II:5). Thus, metabolites of toluene have already been formed immediately after inhalation and the radioactivity in the CNS, spinal nerves and fatty tissues may be assumed to be due to toluene itself. Toluene was relatively rapidly cleared from nervous tissues; 30 minutes after inhalation only traces of radioactivity were

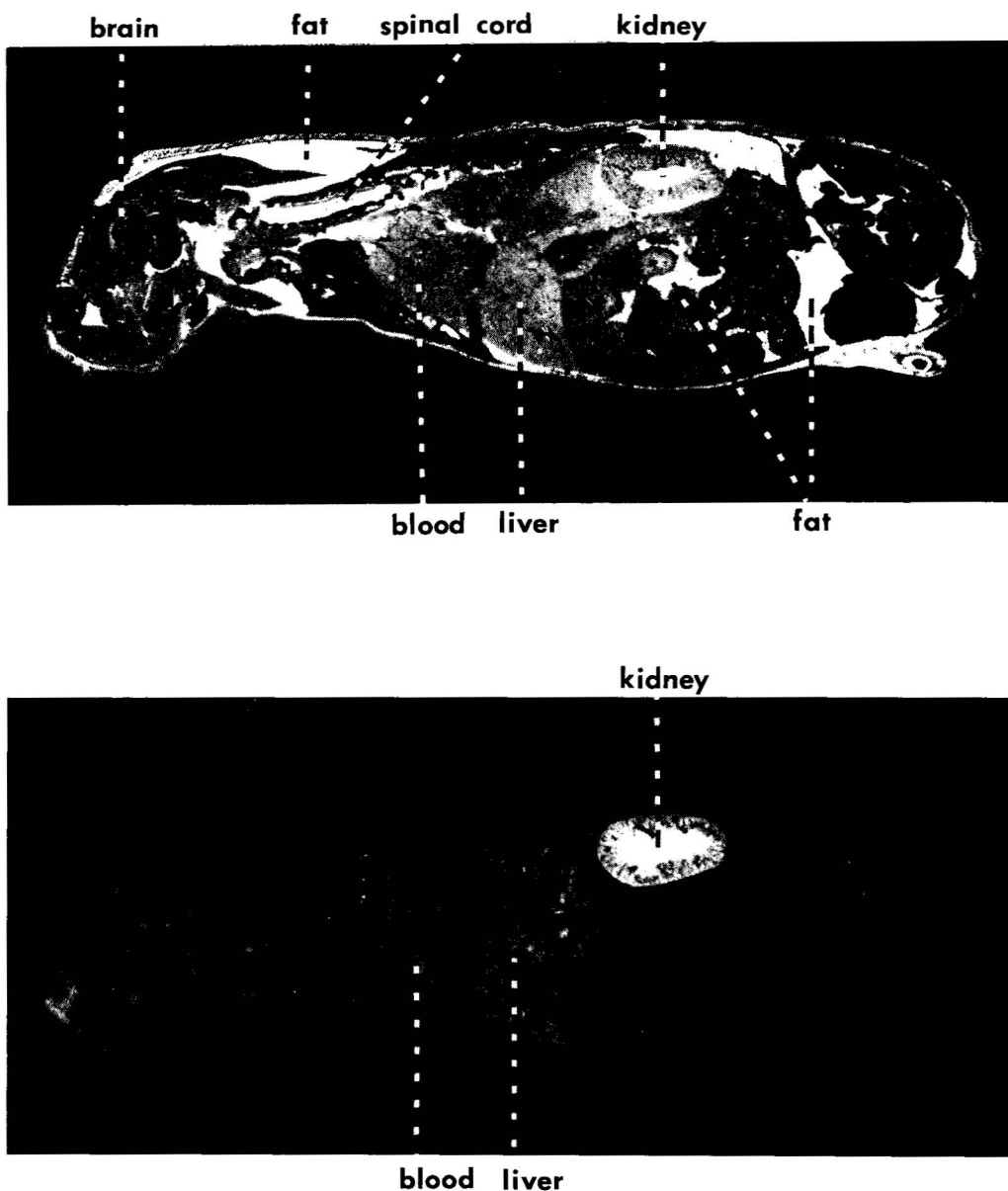


Fig. II:5. Whole-body autoradiograms of a mouse immediately after inhalation of 10 μ l ^{14} C-toluene for 10 minutes. Hemi-section exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). Note the high uptake of non-metabolized toluene in the body fat and CNS (upper as compared to lower autoradiogram). Metabolites are seen in the liver, kidney and blood (lower autoradiogram).

found in the white matter of the brain. All radioactivity in nervous tissues had disappeared 1 hour after inhalation. Toluene was still present in body fat 2 hours after inhalation but had been almost cleared from fat after 4 hours after which time autoradiograms obtained at -80°C and -20°C showed the same distribution pattern. Autoradiography of dried, evaporated sections at -20°C showed that toluene was rapidly biotransformed and excreted. Excretion took place mainly via the kidney which showed radioactivity up to 8 hours after inhalation. The liver radioactivity dropped rapidly; only traces were left 4 hours after inhalation. Still 8 hours after inhalation the intestinal contents showed some radioactivity. At 24 hours after inhalation all radioactivity had disappeared from the body. No specific tissue localizations of toluene were noted. Fig. II:6 shows that none of the metabolites formed from toluene are firmly bound to the tissues.

The distribution pattern of intravenously injected ^{14}C -benzoic acid closely resembles that of toluene metabolites (Fig. II:6). Benzoic acid was rapidly excreted from the body both via urine and bile. 24 hours after injection traces of radioactivity were visible only in the intestinal contents.

Xylene. As shown by low-temperature autoradiography, m-xylene is rapidly taken up in large amounts in the body fat and bone marrow and also in the brain, spinal cord and spinal nerves. m-Xylene was present in the CNS and spinal nerves for up to 1 hour after inhalation whereas the amount of m-xylene in fatty tissues was still large 4 hours after inhalation and still notable 8 hours after inhalation after which time auto-

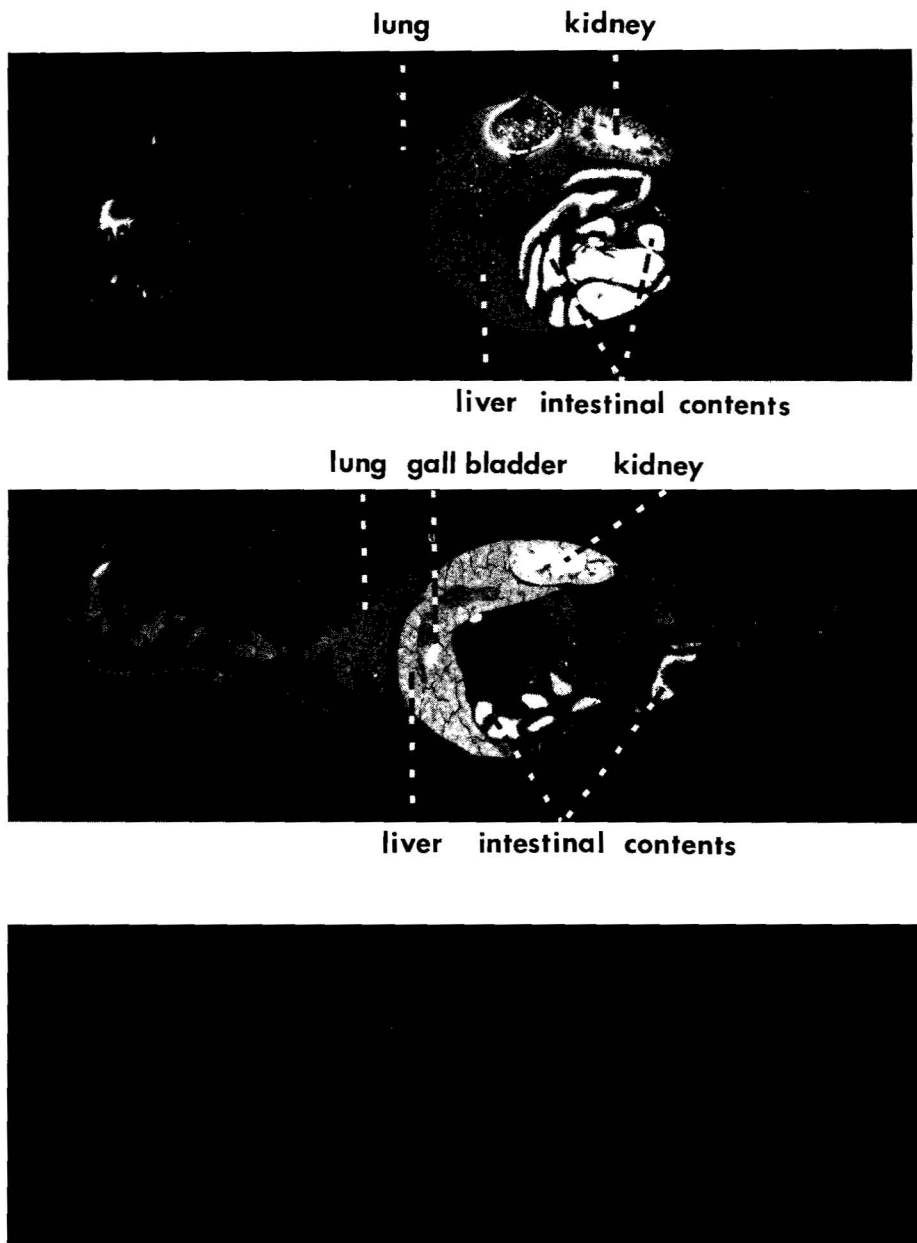


Fig. II:6. Autoradiograms of mice 1 h after injection of ^{14}C -benzoic acid (upper) and inhalation of $10\ \mu\text{l}$ ^{14}C -toluene for 10 minutes (middle: dried, evaporated section, lower: dried, evaporated and extracted section). The distribution of toluene metabolites is very similar to that of benzoic acid. All toluene metabolites are completely extractable.

radiograms obtained at -80°C and from dried, evaporated sections at -20°C showed the same distribution pattern. The liver, lung, kidney and blood also showed radioactivity immediately after inhalation both on autoradiograms obtained at -80°C and -20°C (Fig. II:7). The biotransformation of m-xylene and the excretion of metabolites via the kidney was rapid; at 8 hours after the inhalation little radioactivity was seen in the kidney. There was also a considerable excretion of m-xylene metabolites in the bile, visible as an increasing amount of radioactivity in the intestinal contents from 2 hours up to 8 hours after inhalation. The radioactivity in the lung was localized to the bronchi 2 hours after inhalation where it remained up to 24 hours after inhalation. At 24 hours traces of radioactivity also remained in the intestine but at 48 hours after inhalation no radioactivity could be registered in the body. None of the metabolites formed from m-xylene were firmly bound to the tissues (Fig. II:8).

Generally, comparisons with distribution studies of benzene, toluene and xylene found in the literature are difficult to make due to the extremely varying exposure conditions used in earlier studies and the lack of distinction between the distribution of the solvents themselves and their metabolites. Radioactivity in the bone marrow, adipose and nervous tissues were registered by low-temperature autoradiography only, showing that the three solvents themselves are distributed to these tissues. High concentrations of benzene in the cerebellum, bone marrow and brain have been found in rabbits inhaling

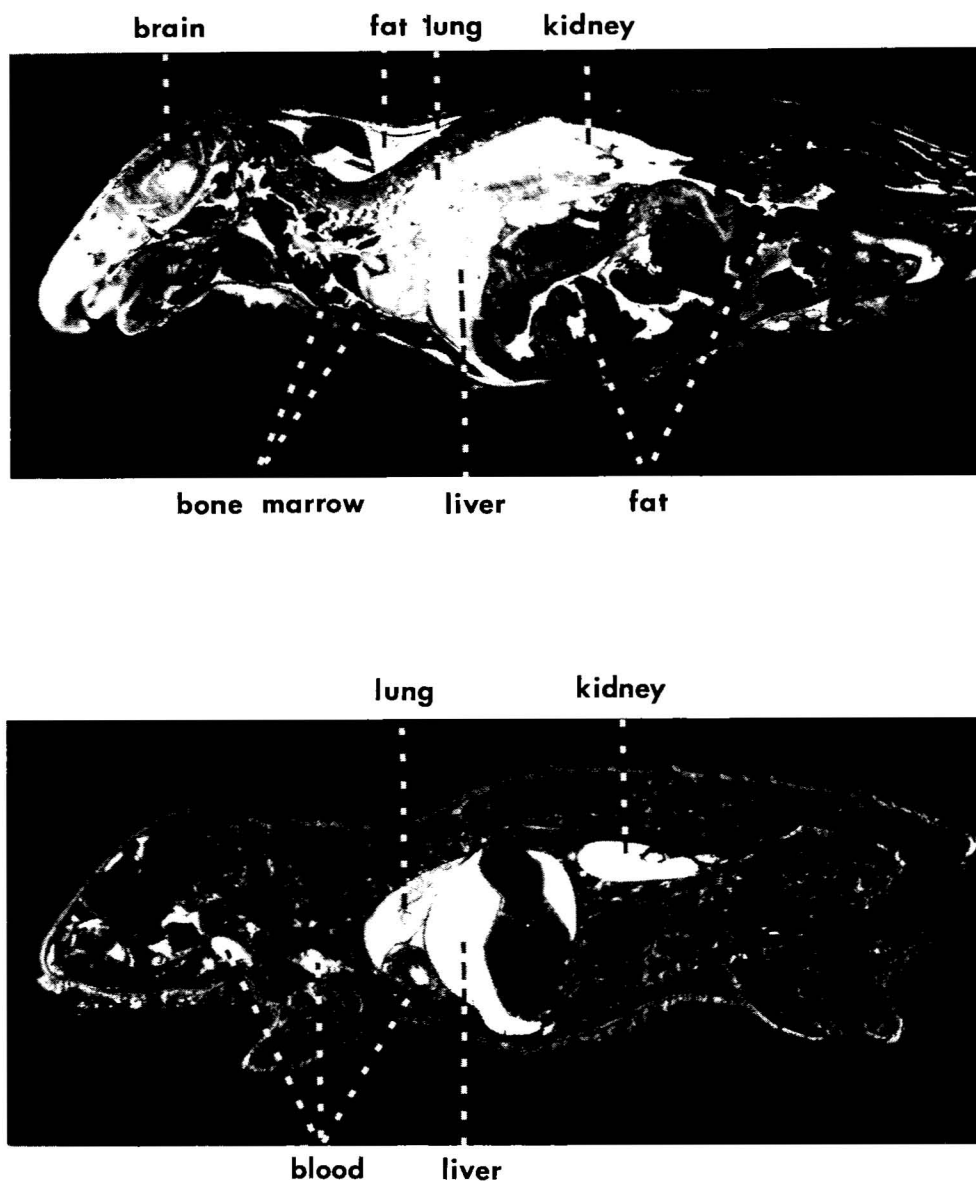


Fig. II:7. Whole-body autoradiograms of mice immediately after inhalation of 10 μ l ^{14}C -m-xylene for 10 minutes. Hemisection exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). Note the high uptake of m-xylene in the body fat, bone marrow, spinal nerves and white matter of the brain (upper as compared to lower autoradiogram). As shown by autoradiography at -20°C , a large part of the radioactivity in the liver, kidney, blood and lung is due to non-volatile m-xylene metabolites.

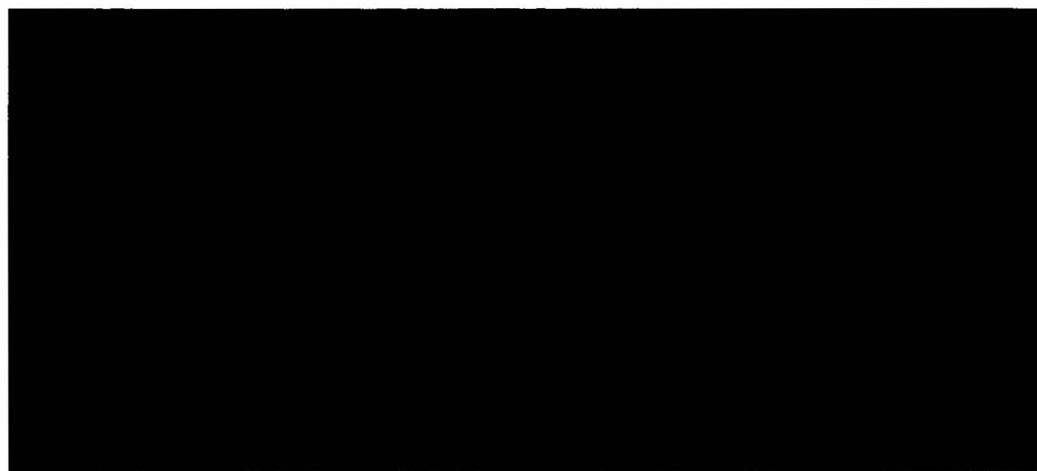
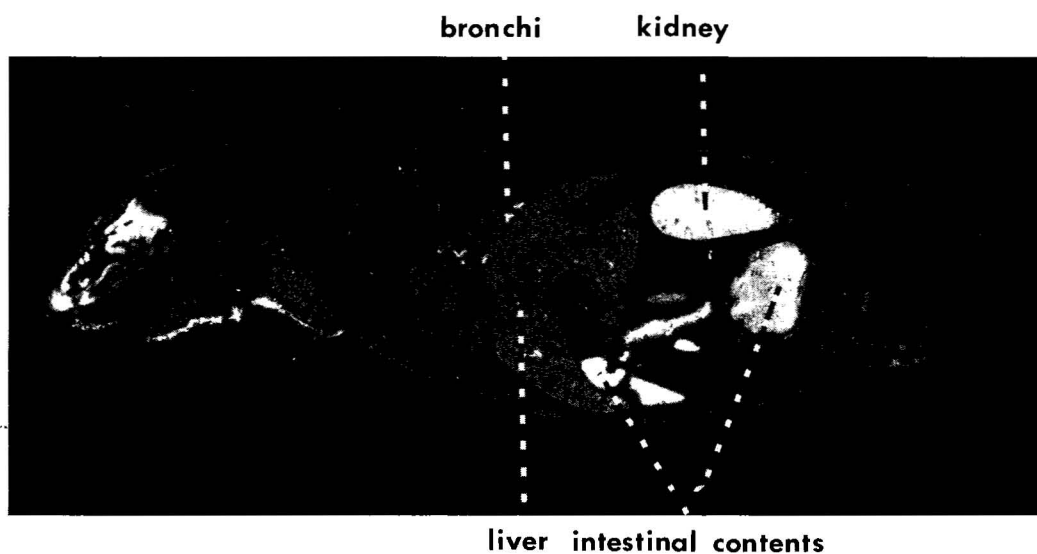


Fig. II:8. Whole-body autoradiograms of a mouse 2 h after inhalation of 10 μ l ^{14} C-m-xylene for 10 minutes. Dried, evaporated section (upper) and dried, evaporated and extracted section (lower). High concentrations of m-xylene metabolites are seen in the kidney, bronchi and intestinal contents. All metabolites are extractable.

benzene (120). Dogs inhaling benzene showed high body fat and bone marrow concentrations (373), which was also noted in mice after subcutaneous injection (20). Early distribution studies of toluene in rabbits indicated high bone marrow and nervous tissue concentrations (120, 124). Studies with ^{14}C -toluene administered by inhalation (64, 330) or gastric intubation (330) showed high brain and fat radioactivity levels. The only distribution study of xylene that seems to exist in the literature (122) reported high brain and bone marrow concentrations. The high affinity of benzene, toluene and xylene for fat points out fatty tissues as storage sites for the solvents in the body, which may be of importance even more during chronic solvent exposure. However, as shown by low-temperature autoradiography, the major part of the radioactivity registered in fatty tissues disappears relatively rapidly after cessation of exposure. The time during which the solvents may be autoradiographically registered in fat follows their fat solubility which increases and their vapor pressure at body temperature which decreases in the order benzene, toluene, xylene (Appendices 1 and 2). Benzene may thus be registered in body fat for about 2 hours, toluene for about 4 hours and xylene for as long as 8 hours. The order of increasing fat solubility and decreasing vapor pressure is also reflected in the time during which radioactivity may be seen in the CNS after inhalation of the three solvents. Benzene is thus cleared from the CNS in less than 30 minutes whereas toluene and xylene may be seen in the CNS up to 30 minutes and 1 hour, respectively. The much higher concentration of the three solvents in body fat than in nervous tissue after inhalation and the more rapid

fall in benzene, toluene and xylene concentrations in nervous tissue than in body fat has been observed for toluene in two recent distribution studies (64, 330). The rapid elimination of the three solvents from body fat indicates that there would be a relatively slight accumulation in the body upon chronic exposure although this would be expected to occur more easily with xylene than with benzene or toluene. The study by Schrenk *et al.* (373) showed no increase in tissue benzene levels when dogs were exposed to the same concentration for increasing periods of time. The ability of benzene, toluene and xylene to pass into the CNS probably explains their common narcotic action. The suggestion by Savolainen (366) that metabolites of organic solvents may be involved in their neurotoxicity is not supported by autoradiography since no metabolites were registered in nervous tissue. Likewise, the findings of Parke and Williams (310) that benzene metabolites may be found in fat, of Andrews *et al.* (20) that benzene metabolites may be found in both brain and fat, some of them irreversibly bound (406) and the results from early and more recent distribution studies reporting high spleen and adrenal levels of benzene (20, 120), toluene (64, 120, 124, 330) and xylene (122) are not supported by autoradiography.

Several early studies also note the presence of benzene, toluene and xylene in the liver and kidney (120, 122, 124, 373). Later studies, where radiolabeled benzene (20) and toluene (64, 330) were used, also report the presence of radioactivity in the liver and kidney. Whole-body autoradiography of dried, evaporated sections as compared to low-temperature autoradiography reveals that a large part of

the radioactivity in the liver and kidney is present in non-volatile form, very probably representing metabolites of benzene, toluene and xylene. All three compounds were rapidly biotransformed with metabolites of toluene and xylene visible already immediately after inhalation in the liver and kidney. Urinary excretion dominated the autoradiographic distribution picture of all three compounds at early survival times but biliary excretion was also obvious, especially at late survival times. The varying fat solubility and vapor pressure, reflected in the time during which the three compounds could be registered in adipose tissues, clearly also influenced the time course of elimination of the metabolites. Excretion of benzene metabolites in the urine appeared to be largely complete within about 4 hours and in the bile within 8 hours, whereas toluene metabolites could be registered for 8 hours in both the kidney and intestinal contents. Xylene, being more slowly released from fat, gave rise to metabolites visible in the kidney for about 8 hours and in the intestinal contents for about 24 hours. The rapid urinary excretion of benzene metabolites has been reported for the rabbit (310, 311), mouse (20) and several other animal species (355). The biliary excretion of benzene metabolites in the mouse as shown by autoradiography seems to be larger than that reported for the rat (2) and recently for the mouse (20). The similarly rapid urinary excretion of toluene (397) and xylene (47) metabolites in animals has also been reported. Toluene metabolites and radioactivity from ^{14}C -benzoic acid have also been found to be excreted in the bile (2) although to a lesser extent than is indicated by autoradiography.