

TOXICITY OF TOLUENE

The knowledge of toluene toxicity up to 1965 has been summarized by Browning (51). The article by Hayden *et al.* (175) reviews more recent research on the toxicology of toluene. Like benzene, toluene is a CNS depressant, causing narcosis and anesthesia in both animals and humans at acute exposure to high concentrations. Animal experiments generally indicate that toluene is more acutely toxic than benzene. Svirbely *et al.* (434) found a minimal lethal concentration of 5300 ppm in mice during exposure for 7 hours compared to 10400 ppm for benzene. Gerarde (145) found toluene to have a higher acute oral toxicity than benzene in rats, whereas Wolf *et al.* (475) states the oral LD₅₀ of toluene and benzene in rats to be 7.0 and 5.6 g/kg, respectively. An atmosphere of 35000-45000 ppm toluene was fatal to rabbits in 40 minutes (66). Von Oettingen *et al.* (300) exposed humans to varying concentrations of toluene (50-800 ppm) for 8 hours and noted an increase in CNS effects with increasing concentrations. Drowsiness and mild headache occurred at 50 ppm. Exposure to higher concentrations added nausea, muscular weakness, fatigue and mental confusion to the CNS symptoms. Exposure to 800 ppm resulted in severe fatigue, extreme nausea, confusion, lack of self control, incoordination and staggering gait. After-effects lasted for several days. Longley *et al.* (256) estimate that human exposure to 10000-30000 ppm toluene causes anesthesia in 1 minute. Recently, even brief exposures to quite low concentrations of toluene ($380 \text{ mg/m}^3 = 100 \text{ ppm}$) have been shown to cause statistically significant impairment of psychomotor and perceptual performance tests indicative

of disturbed CNS function (137). Toluene has also been ranked among the potentially cardiotoxic agents, inducing "sudden sniffing death" in humans (175) and animals (440) at acute exposure to high concentrations.

Chronic exposure to toluene may cause permanent CNS damage. Grabski (159) describes a case of cerebellar degeneration after deliberate inhalation of toluene for 6 years. Knox and Nelson (228) examined the same patient after 8 additional years of toluene sniffing and found signs of permanent encephalopathy as evidenced by changes in the EEG and pneumo-encephalogram. The patient showed ataxia, tremors, emotional lability and mental changes. A recent report by Boor and Hurtig (46) describes a case of reversible cerebellar ataxia after occupational toluene exposure and a case of persistent cerebellar ataxia after toluene addiction for 5 years. The latter case showed slurred speech, nystagmus and inability to stand or walk. A brain scan revealed diffuse widening of the cortical and cerebellar sulci. Electromyograms and nerve conduction velocities were normal, indicating absence of peripheral nervous system effects. Another case of toluene habituation showed recurrent headaches and personality changes. An abnormal EEG was interpreted as diffuse encephalopathy (365). Toluene was a minor component of jet fuel reported to cause psychiatric symptoms, impairment of psychologic test performances and EEG changes in Swedish jet motor factory workers (226). Toluene was the major component in paints reported to affect visual intelligence and memory as well as emotional reactivity in Finnish car painters (169). Toluene was also present in glues causing peripheral neuropathies in

glue sniffers (17, 157), although this disease was considered to be caused by the hexacarbons also used in glues. Animal experimentation with toluene has also revealed neurotoxic effects. Dogs administered toluene intermittently for 4-6 weeks showed shrinkage and hyperchromicity of cortical neurons and loss of myelin. The cerebellar folia showed abnormalities suggesting a decrease and degeneration in Purkinje cells (28). Rats exposed to 4000 ppm toluene, 2 h/day for 60 days showed decreased learning abilities, interpreted as damage to cognitive processes in the CNS (191). EEG changes have also been found in rats exposed to toluene (438).

The ability of toluene to cause other kinds of organ injuries is a matter of varying opinion. Scattered early reports suggest a benzene-like effect on the bone marrow and the blood picture after occupational toluene exposure (51). However, the toluene used may be safely assumed to have been contaminated with as much as 20% benzene (46). Relatively recent studies, where the purity of the toluene was ascertained, have showed panmyelopathy (142) and granulocytopenia (225) after intensive exposure to toluene for less than a year. Increased levels of enzymes such as alkaline phosphatase in leukocytes in toluene exposed workers have been described (134, 151) but the significance of the enzymatic changes in the development of blood dyscrasias is uncertain. Chromosome studies of peripheral lymphocytes did not reveal any differences between toluene exposed workers and controls (132). Speck and Moeschlin (412) administering toluene to rabbits found no cytopenia or disturbed DNA-synthesis in the bone marrow in contrast to the results from their study of benzene

(279). Animal studies have all failed to show toluene hematoxicity (124, 144, 198, 475).

Toluene has also occasionally been linked to liver and kidney injuries. Greenberg (160) examined painters exposed to 100-1100 ppm toluene in an airplane factory and found a three times higher frequency of hepatomegaly as compared to controls. There was no evidence of actual liver disease. Heavy chronic exposure, such as occurs in sniffing or accidentally in occupational settings, has resulted in cases of liver or kidney damage. Grabski's patient with cerebellar degeneration also showed hepatomegaly and impaired liver function (159). The liver of the patient of Gattner and May had a moderate parenchymal injury with some fatty infiltration and necrosis of single cells (142). A case of heavy occupational toluene poisoning showed myoglobinuria and renal failure (340) and two cases of toluene sniffing renal tubular acidosis, assumed to be due to a distal tubular defect (437). Since toluene is an extensively used solvent, it may very well be involved in glomerulonephritis, a newly recognized and frequently described condition after occupational exposure to solvent mixtures (175). This is believed to be an autoimmune disease, where formation of antibodies to the glomerular basement membrane may be demonstrated.

Animal studies of toluene have generally failed to demonstrate adverse liver or kidney effects. Guinea pigs injected intraperitoneally with 150-1200 mg/kg toluene showed no increase 24 hours later in serum ornithine carbamoyl transferase activity, an enzyme found exclusively in liver mitochondria, considered by the authors to be a very sensitive

test for liver injury (103). Upon histological examination some liver abnormalities were found at the highest dose level only. Reynolds (343) using a battery of biochemical tests found no hepatotoxicity in rats 2 hours after administration of 2600 μ mol toluene/100 g orally. Mice inhaling lethal concentrations of toluene showed moderate pulmonary and renal "irritation" at postmortem examination (434). Dogs chronically exposed to toluene developed congestion and focal hemorrhages in the liver, some alveolitis and pulmonary congestion, swelling of glomeruli and albuminuria (124). Bruckner and Peterson (52) exposed mice 3 hours daily to 4000 ppm toluene, 5 times weekly for as long as 8 weeks. No lung, liver or kidney injury was noted. Rats, guinea pigs, monkeys and dogs exposed 8 h/day, 5 days/week for 6 weeks or continuously for 90-127 days to 107 or 1085 ppm toluene showed no histological changes of heart, liver, lung, spleen or kidney (198).

ABSORPTION, DISTRIBUTION, BIOTRANSFORMATION AND ELIMINATION OF TOLUENE

Toluene is absorbed not only by inhalation but also from the gastrointestinal tract (146). Gerarde (146) and Browning (51) state that toluene is poorly absorbed through the skin. Dutkiewicz and Tyras (109, 110), however, found an absorption rate of 14-23 mg/cm²/h upon immersion of the hand and forearm in liquid toluene. They calculated that immersion of both hands in toluene for 1.5 minutes would result in the absorption of as much toluene as during an 8 hour inhalation exposure to 26.6 ppm. The pulmonary absorption of toluene vapors is very rapid. Åstrand *et al.* (23) and Carlsson and Lindqvist (64)

report a rapid increase in blood toluene concentration during the first 10-15 minutes of inhalation whereafter only a small increase could be noted. Pyykkö *et al.* (330) exposed rats to radioactive toluene orally or by inhalation. After inhalation exposure the maximum radioactivity in various tissues was reached in 15-30 minutes. After oral administration absorption was delayed with maximum tissue radioactivity levels appearing within 2-3 hours. Human experimental (23, 300) or occupational (435) toluene exposure show a good correlation between the concentration of toluene in the inhaled air and in the blood. Srbova and Teisinger (415) report an average retention of 53.2% in men inhaling an atmosphere of 0.3-1.2 mg/l toluene for 5 hours. According to Nomiyama and Nomiyama (294) 36.6% was retained by man during inhalation of 98-130 ppm toluene for 4 hours. The total respiratory tract retention of toluene in dogs inhaling 0.4-0.6 µg/ml toluene was 91-94% (116). The uptake of toluene increases considerably with increasing physical work load (23, 64) and body fat content (64). Some toluene is expired unchanged after exposure. Srbová and Teisinger (415) found 16.3% and Nomiyama and Nomiyama (295) 13.5% of the retained amount in the expired air after cessation of exposure. Rabbits dosed with 350 mg/kg toluene exhaled about 18% of the dose unchanged in 12-14 hours (397). Nomiyama and Nomiyama (295) found a two-exponential decay curve for toluene in the expired air of men with an initial very rapid drop in toluene breath concentration followed by a slower phase. Sato *et al.* (363) exposed men to 100 ppm toluene for 2 hours and measured blood and expired air toluene concentrations at different times during 5 hours after cessation of exposure. They were able to

fit their experimental data to a three-compartment pharmacokinetic model. Compared to the decay curve obtained for benzene in the same study, the initial rate constant for toluene elimination was somewhat higher but the third curve phase, assumed to represent the elimination from fatty tissues, showed a lower rate constant, probably reflecting the higher fat solubility of toluene. The study by Åstrand *et al.* (23) also demonstrates the initial very rapid fall in air and blood toluene concentrations after the end of exposure.

Very few distribution studies of toluene may be found in the literature. Fabre (120) exposed rabbits by inhalation to 1 mg/l toluene, 6 h/day for 106 days. Five days after the last exposure the highest toluene concentrations were found, in decreasing order, in the cerebellum, spleen, bone marrow, fur, blood and liver. Rabbits exposed to 5 mg/l toluene, 6 h/day for 16 days and killed 4 days after the last exposure had high toluene levels in the fur, brain, spleen, adrenals, blood and liver. The concentration in brain tissue was about three times higher than in the liver. Body fat was not examined. In a study by Fabre *et al.* (124) dogs were exposed to an atmosphere of 7.5 mg/l toluene for 4 months followed by 10 mg/l for 2 months, 8 h/day, 6 days/week. Two dogs were sacrificed 12 hours after the last exposure. The largest concentration of toluene was found in the adrenals (20 µg/g) followed by the cerebellum (19 µg/g), the bone marrow and cerebrum (18 µg/g), the liver (14 µg/g) and the blood (9 µg/g). The concentration in fat tissue was not measured. The distribution of toluene in rats has been the subject of two recent studies. Carlsson and Lindqvist (64) exposed rats to 550 ppm ¹⁴C-labeled toluene for

1 hour and measured the radioactivity levels in the liver, kidney, adrenal tissue, white adipose tissue, cerebrum and cerebellum at different times after exposure. Immediately after exposure the radioactivity in white adipose tissue was six times higher than in brain tissue and four times higher than in the liver. The radioactivity in white adipose tissue was a little higher 1 hour than immediately after exposure while the radioactivity in the other tissues was considerably lower. Six hours after exposure tissue radioactivity levels had dropped to almost zero in the brain and adrenal tissues while some radioactivity was still present in the adipose tissue, the kidney and the liver. The authors did not distinguish between toluene and toluene metabolites. All radioactivity levels were expressed as μg toluene/g tissue although it is very probable that the radioactivity in several of the examined organs was due largely to toluene metabolites. Pyykkö *et al.* (330) compared the distribution of ^3H -toluene in rats after gastric intubation or inhalation of 20 $\mu\text{l/l}$ toluene for 10 minutes. Radioactivity was measured in several organs at different times after exposure. In the inhalation experiments, toluene was rapidly distributed to various tissues. In all tissues except white adipose tissue the maximum concentration was reached 15-30 minutes after exposure. White adipose tissue reached its maximum radioactivity level at 1 hour after exposure. The white adipose tissue radioactivity level was higher than all other tissue levels at all times of measurement. The radioactivity in all tissues decreased rapidly; 24 hours after exposure 1% or less of the initial radioactivity was found in tissues other than white adipose tissue where 3% could be registered. The

distribution pattern after oral administration of 100 μ l ^3H -toluene was similar to that obtained after inhalation but delayed due to the slower absorption of toluene from the gastro-intestinal tract than from the lungs. The radioactivity levels were measured in toluene extracts of tissue homogenates. Since toluene also dissolves benzoic acid, the main toluene metabolite, the registered radioactivities did not represent the amounts of toluene only. Human cases of fatal toluene poisoning showed high brain, blood, kidney and liver levels (472).

Toluene has been known since 1867 to be oxidized in the body to benzoic acid, which is conjugated with glycine to hippuric acid excreted in the urine (374). Several later animal studies have confirmed that 70-80% of an oral or injected dose of toluene is excreted as hippuric acid in the urine (397). Conjugation of benzoic acid with glucuronic acid may also occur (124). Von Oettingen *et al.* (300) found a correlation between the toluene concentration in the inhaled air and the urinary excretion of hippuric acid in humans. The excretion was complete 14 hours after cessation of exposure. Ogata *et al.* (303) exposed humans to 200 ppm toluene for 3-7 hours and recovered 68% of the absorbed toluene as hippuric acid in the urine measured up to 18 hours after exposure. Since hippuric acid is a normal constituent of human urine, due to the ingestion of benzoic acid in foods, excretion of hippuric acid may not be a reliable index of toluene exposure. However, linear relationships between toluene in air and hippuric acid excretion have been found (302, 303). Rats given 50 mg/kg ^{14}C -toluene excreted about 2% of the dose in the bile in

24 hours (2). The bile metabolites were not analyzed qualitatively, but ^{14}C -hippuric acid administered directly was not excreted in the bile. High doses (2.5 or 5.0 ml/kg) of toluene administered to rats by gastric intubation gave rise to an increase in urinary organic sulfate (147), which is indicative of phenol formation (146). Bakke and Scheline (29) identified o-cresol (0.04-0.11% of the dose) and p-cresol (0.4-1.0% of the dose) in rat urine collected for 48 hours after administration of 100 mg/kg toluene orally. Small amounts of urinary benzyl alcohol were also identified, suggesting that benzyl alcohol is an intermediate in toluene biotransformation (Fig. II:2). Szadkowski *et al.* (435) noted an increase of urinary phenols parallel to the increase in urinary hippuric acid after occupational toluene exposure. According to von Oettingen *et al.* (300) there was no biotransformation of toluene to phenols, as evidenced by unchanged urinary inorganic sulfate levels, after experimental human toluene exposure. Although benzoic acid may inhibit certain enzyme systems, it is not considered a very toxic substance (124). The relationship between a possible chronic organ toxicity, such as blood dyscrasias or hepatorenal toxicity, and formation of phenolic metabolites from toluene is not known. However, since only very small amounts of phenols are formed and these are rapidly conjugated and excreted, they are probably of little importance in both acute and chronic toluene toxicity (29).

XYLENE

Commercial xylene (dimethylbenzene), $C_6H_4(CH_3)_2$, is a mixture of three isomers, ortho-, meta- and para-xylene. The composition of commercial xylene varies although m-xylene is always the dominating component. A typical xylene mixture consists of 45-70% m-xylene, 15-25% p-xylene and 10-15% o-xylene. Ethylbenzene and various non-aromatic hydrocarbons may also be present (51, 146). Like benzene and toluene, xylene is a clear, colorless and flammable liquid. The addition of two methyl groups to the benzene nucleus makes xylene less volatile but more fat soluble than benzene and toluene (Appendices 1 and 2). Xylene is used as a solvent, cleaning agent and degreaser. It is a constituent of aviation fuels and a starting material and intermediate in the chemical industry, especially in the production of phthalic acids, which are important in the manufacture of synthetic fibers, plastics and enamels (51,146). The U.S. production of xylene was about 2.7 million tons in 1977 (432).

TOXICITY OF XYLENE

Xylene is a narcotic with anesthesia and narcosis occurring after administration of high doses. Xylene induced narcosis in experimental animals is of slower onset but longer duration compared to benzene or toluene narcosis (146). Xylene is generally considered to be more acutely toxic and narcotic to animals than benzene (122, 146), but has been stated to be both more or less toxic than toluene (51, 146). Batchelor (cited by Fabre *et al.* (122)) found that xylene was more

acutely toxic to rats than benzene but less toxic than toluene when injected subcutaneously or intraperitoneally. Upon inhalation, benzene was lethal to rats at 2440 ml/m³ whereas xylene and toluene were lethal at 1600 ml/m³. Gerarde (145) and Wolf *et al.* (475) found xylene to possess a higher acute oral toxicity than benzene and toluene. A recent investigation of mixed xylenes gives a 4 hour LC₅₀ of 6700 ppm for rats (65). The narcotic and lethal doses of the individual xylene isomers have also been compared. According to Browning (51), the m- and p-xylenes are more narcotic than o-xylene with narcosis occurring at 2100-3500 ppm and 3500-10000 ppm, respectively. Gerarde (145) found o- and p-xylenes to be more acutely toxic orally than m-xylene. The lethal or narcotic concentration for humans is not known and there are few reports of cases of unconsciousness or death due to acute exposure, probably because nobody stays voluntarily in concentrated xylene vapors due to their highly irritating properties (51). The human odor threshold is about 1 ppm and some individuals experience eye, nose and throat irritation at about 200 ppm (65).

Very little is known about chronic xylene toxicity. Human chronic exposure to high concentrations of xylene vapors is likely to produce irritation of the skin, mucuous membranes and eyes (51, 146). There are few reports of hematopoietic effects of chronic xylene exposure, reviewed by Browning (51), although these were probably caused by benzene often present in technical grade xylene. There may be signs of gastrointestinal disturbances, but liver and kidney injuries are rare (51).

Fabre *et al.* (122) exposed rabbits to 5 mg/l xylene for 40-55 days and rabbits and rats to 3 mg/l xylene for 110-130 days, 8 h/day, 6 days/week. The animals exposed to the higher concentration showed eye irritation, anorexia and weight loss and, at the end of the exposure period, respiratory difficulties and signs of nervous system effects. The blood picture was not significantly abnormal at the end of the exposures. At autopsy, vascular congestion was found in several organs such as the liver, kidney, heart, adrenals, lungs and spleen. The most prominent lesions were found in the kidneys, characterized by inflammation and commencing necrosis. Jenkins *et al.* (198) exposed rats, guinea pigs, monkeys and dogs to 78 or 780 ppm o-xylene repeatedly 8 h/day, 5 days/week over a 6 week period or continuously for 90-127 days. There was no loss of body weight in any of the animal species and no abnormal hematological findings. Sections of heart, lung, liver and kidney were all normal upon histopathological examination. Carpenter *et al.* (65) performed an extensive inhalation study of mixed xylenes. Rats and dogs inhaled 180, 460 or 816 ppm xylene 6 h/day, 5 days/week for 13 weeks. Differential blood counts, weight data, blood chemistry and microscopic examination of several organs revealed no adverse effects of inhalation of xylene. Divincenzo and Krasavage (103) found xylene to be of low hepatotoxicity as high doses of xylene, 1000 or 2000 mg/kg, were required to increase serum ornithine carbamoyl transferase in guinea pigs. Upon histological examination some lipid accumulation was noted but no necrosis. Xylene did not cause cytopenia or inhibition of DNA synthesis in rabbits dosed with 300 or 700 mg/kg (412).

ABSORPTION, DISTRIBUTION, BIOTRANSFORMATION AND ELIMINATION OF XYLENE

Xylene is rapidly absorbed into the blood by inhalation and may also be absorbed from the gastro-intestinal tract (146). Dutkiewicz and Tyras (110) found that liquid xylene was absorbed from the skin at a rate of $4.5\text{--}9.6\text{ mg/cm}^2/\text{h}$. Only a few per cent of the retained amount of xylene is exhaled unchanged after exposure. Sedivec and Flek (378) exposed humans to 0.2 or 0.4 mg/l of the different xylenes for 8 hours. The concentration of xylene in the expired air was followed for 48 hours after exposure to 0.4 mg/l during which time 5.3%, 5.8% and 3.5% of the retained amounts of o-, m- and p-xylene respectively were exhaled, most of it during the first 2 hours. The respiratory elimination curve was considered to consist of the sum of three exponential curves but it was not subject to mathematical treatment. Sedivec and Flek (378) also determined the retention of xylene to be around 64%, irrespective of the concentration in the inhaled air, the xylene isomer under study or the duration of exposure (15 minutes - 7 hours). In contrast, Senczuk and Orlowski (388) found a decrease in retention with increasing concentration of m-xylene in the inhaled air and increasing exposure time. The mean retention at a concentration of 100 mg/m^3 m-xylene inhaled by humans for 8 hours was 85%, whereas the mean retention was 75% at a concentration of 300 mg/m^3 and 71% at 600 mg/m^3 .

Fabre *et al.* (122) studied the distribution of a xylene mixture in rabbits exposed by inhalation of 3 mg/l, 8 h/day, 6 days/week for 130 days. The highest concentrations of xylene

were found in the adrenals (148 $\mu\text{g/g}$), the bone marrow (130 $\mu\text{g/g}$), the spleen (115 $\mu\text{g/g}$), the brain (100 $\mu\text{g/g}$) and the blood (91 $\mu\text{g/g}$). The kidneys, liver, heart and lungs contained 50-85 μg xylene/g.

The investigation of benzene and toluene biotransformation by Schultzen and Naunyn from 1867 (374) also reports the biotransformation of xylene in dogs and humans. An oral dose of xylene was found to be oxidized at one of the methyl groups to toluic acid which was conjugated with glycine to toluric acid excreted in the urine. Bray *et al.* (47) studied the metabolism of the three xylenes after oral administration to rabbits. An average of 60% of a dose of 0.9-1.8 g o-xylene was found to be oxidized to o-toluic acid, which was excreted in the urine mainly unconjugated but also as a glucuronic acid conjugate. 81 and 88% respectively of a 1.7 g dose of m- or p-xylene was oxidized to m- and p-toluic acids excreted chiefly as glycine conjugates, only small amounts being excreted free or conjugated with glucuronic acid. The excretion was complete within 24-48 hours. There was also evidence of hydroxylation of the aromatic nucleus giving rise to phenolic metabolites excreted in the urine as sulfate or glucuronic acid conjugates. In a later investigation, the same authors established the conversion of xylene to xylenols (48). 2-4% of the administered amounts of xylene isomers were recovered in the urine as non-acidic phenols excreted mainly in conjugated form. Fabre *et al.* (123) investigated the biotransformation of the three xylenes in the rat, guinea pig and rabbit. The animals received 0.4 ml xylene/kg/day orally for a week and urine was collected daily for a period of 10 days. Hydrolyzed urine samples from all

animals contained the three toluic acids. A glucuronic acid conjugate of 3,4-xyleneol was identified after administration of o-xylene and p-xylene gave rise to 2,5-xyleneol. Also m-xylene was biotransformed to phenolic metabolites. The authors also suggested that hydroxytoluic acids may be formed during the biotransformation of xylenes. Bakke and Scheline (29) confirmed the presence of phenolic compounds in the urine of rats collected for 48 hours after oral administration of 100 mg/kg of the three xylenes. 0.1% of the o-xylene dose was recovered as 3,4-xyleneol. Very small amounts of 2,3-xyleneol were also identified. 0.9% of the m-xylene dose was biotransformed to 2,4-xyleneol and 1.0% of the p-xylene dose to 2,5-xyleneol. In addition, a small amount of 2-methylbenzylalcohol was found in the urine of rats receiving o-xylene, suggesting that methylbenzylalcohols are intermediates in xylene biotransformation (Fig. II:2). Sedivec and Flek (378) identified the same xyleneols in human urine after exposure to the three xylenes. The xyleneols accounted for a very small part of the amount of xylenes retained, more than 95% being recovered as toluric acids. No free toluic acids were excreted and no glucuronic acid conjugates of toluic acids identified. The excretion of toluric acids in the urine was rapid, 95% of the total amount excreted in 24 hours, including the 8 hour exposure period, was excreted during the first 10 hours. Senzcuk and Orlowski (388) made a similar observation, the maximum amount of m-toluric acid was found in the urine collected during the last 2 hours of an 8 hour exposure to m-xylene. They also found a linear relationship between the amount of m-xylene retained at exposure to 100, 300 or 600

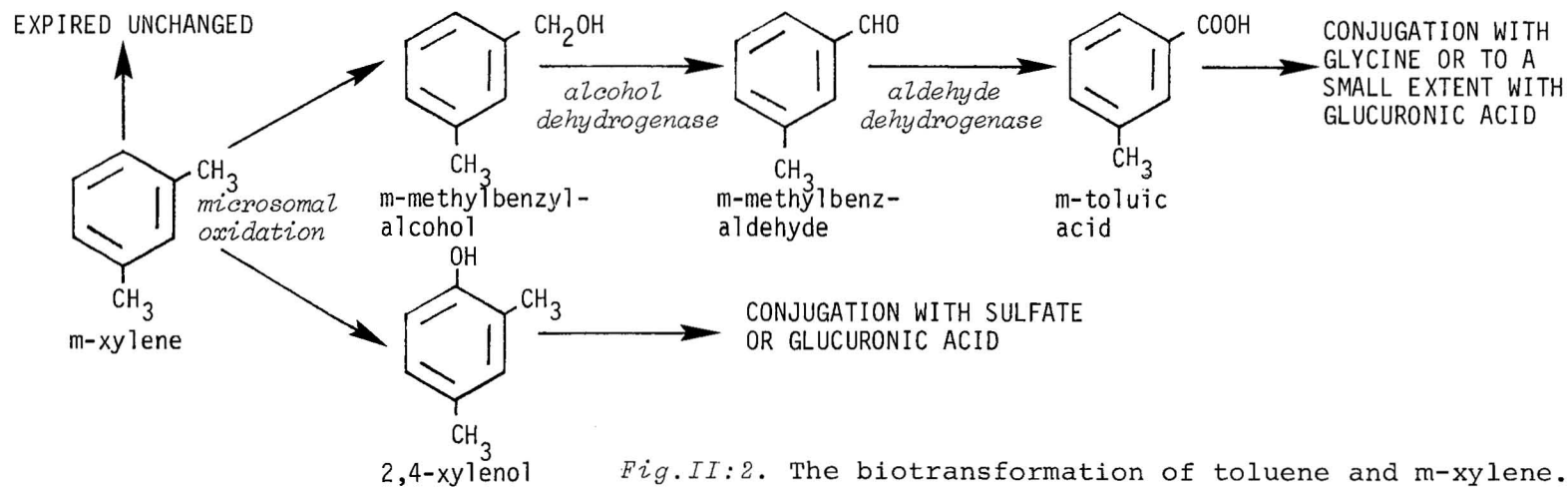
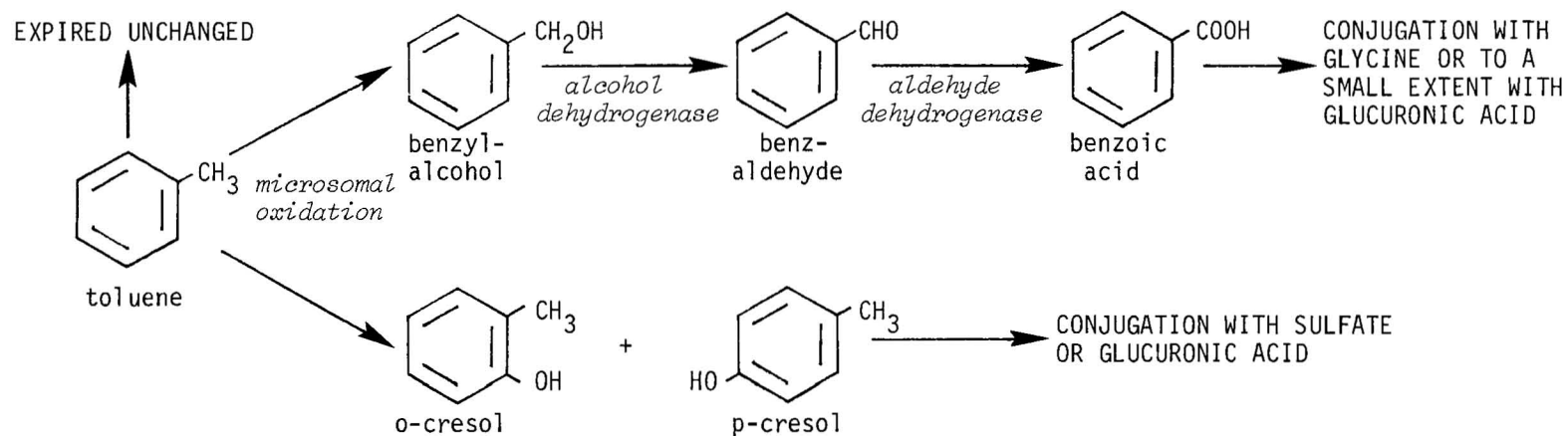


Fig.II:2. The biotransformation of toluene and m-xylene.

mg/m³ and the amount of toluric acid excreted during the last 2 hours of exposure. Investigations *in vitro* have shown that p-xylene is biotransformed by the microsomal fraction fortified with soluble fraction of rabbit lung and liver to p-toluic acid (61). Rat liver and lung microsomes hydroxylate p-xylene to p-methylbenzylalcohol, which is oxidized further to p-toluic acid by enzymes in the hepatic soluble fraction but not in the pulmonary soluble fraction (172).

MATERIALS AND METHODS

Labeled compounds

¹⁴C-Benzene (uniformly labeled, specific activity 75-107 mCi/mmol) and ¹⁴C-toluene (methyl-¹⁴C, specific activity 17.5-26.4 mCi/mmol) of 98-99% purity by gas-liquid radiochromatography were obtained from the Radiochemical Centre, Amersham, England. ¹⁴C-m-Xylene (ring-¹⁴C, specific activity 1.59 mCi/mmol) 99% pure by gas-liquid radiochromatography was obtained from New England Nuclear, Dreieichenhain, FRG.

¹⁴C-Phenol (uniformly labeled, specific activity 34 mCi/mmol) 97-98% radiochemically pure by gas-liquid radiochromatography and thin-layer chromatography and ¹⁴C-benzoic acid (ring-¹⁴C, specific activity 45 mCi/mmol) 97-99% radiochemically pure by thin-layer chromatography were obtained from the Radiochemical Centre, Amersham, England.

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 -Benzene was diluted with unlabeled analytical grade benzene to a final solution of $2.0\ \mu\text{Ci}/\mu\text{l}$. ^{14}C -Toluene and ^{14}C -m-xylene were diluted with unlabeled analytical grade toluene and xylene respectively to final solutions of $1.0\ \mu\text{Ci}/\mu\text{l}$. Mice were administered $10\ \mu\text{Ci}$ of the solvents ($5\ \mu\text{l}$ benzene, $10\ \mu\text{l}$ toluene and xylene) 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 whole-body autoradiography in three steps as described in Chapter I.

Mice were given ^{14}C -benzene by intraperitoneal injection at two dose levels with or without pretreatment with phenobarbital or diethyl maleate. ^{14}C -Benzene was diluted with unlabeled analytical grade benzene and olive oil. Two mice were given 1 ml/kg benzene (10% benzene in olive oil, $10\ \mu\text{Ci}$) and 3 ml/kg benzene (30% benzene in olive oil, $10\ \mu\text{Ci}$) respectively. Two mice were given an intraperitoneal injection of 0.3 ml/kg diethyl maleate (6% diethyl maleate in olive oil) 30 min prior to injection of benzene (1 ml/kg or 3 ml/kg, $10\ \mu\text{Ci}$). Two mice were given intraperitoneal injections of 100 mg/kg sodium phenobarbital (20 mg/ml sodium phenobarbital in distilled water 96 h, 72 h, 48 h and 24 h prior to injection).

tion of benzene (1ml/kg or 3 ml/kg, 10 μ Ci). All mice were killed 4 h after the benzene injection. Whole-body autoradiography was performed with 20 or 60 μ m dried and evaporated sections as described in Chapter I.

An additional series of 6 mice was given daily intraperitoneal injections of 1 ml/kg ^{14} C-benzene (25% benzene in sesame oil, 5 μ Ci). One animal was killed every third day 4 hours after the last injection. Autoradiography was performed with dried, evaporated 20 or 60 μ m sections as well as with dried, evaporated and extracted 20 μ m sections as described in Chapter I.

^{14} C-Phenol and ^{14} C-benzoic acid were dissolved in distilled water and unlabeled analytical grade phenol and benzoic acid were added to give solutions of 6.25 mg/ml phenol (50 μ Ci/ml) and 2.5 mg/ml benzoic acid (50 μ Ci/ml). Mice were injected intravenously in a tail vein with 0.2 ml of the solutions, corresponding to a dose of about 50 mg/kg phenol (10 μ Ci) and 20 mg/kg benzoic acid (10 μ Ci). The animals were killed 5 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h and 48 h after the injection. Autoradiography was performed with dried 20 or 60 μ m sections as described in Chapter I.

Elimination studies

^{14} C-Benzene was diluted with unlabeled analytical grade benzene to a final solution of 0.260 μ Ci/ μ l. ^{14} C-Toluene and ^{14} C-m-xylene were diluted with unlabeled analytical grade toluene and xylene to final solutions of 0.145 μ Ci/ μ l and 0.116 μ Ci/ μ l, respectively. Mice were administered 5 μ l ^{14} C-benzene, 10 μ l

^{14}C -toluene or 10 μl ^{14}C -xylene by inhalation for 10 minutes.

The elimination of radioactivity by various routes was followed for 8 hours in the metabolic cage after which time the remaining radioactivity levels in several organs were determined as described in Chapter I.