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# Health Assessment Document for Toluene

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## (Part 2 of 2 parts: Sections 13-18 & References)

# 13. PHARMACOKINETIC CONSIDERATIONS IN HUMANS AND IN ANIMALS

## 13.1. ROUTES OF EXPOSURE AND ABSORPTION

For humans, the most common routes of exposure to toluene are through the respiratory tract and the skin. Toluene is absorbed readily through the respiratory tract. In experimental exposures of humans to toluene conducted by Astrand and coworkers (1972; also reported in Astrand, 1975), toluene was detected in arterial blood during the first 10 seconds of exposure. Toluene was supplied in the inspired air at 100 or 200 ppm through a breathing valve and mouthpiece. Unless otherwise specified, in the experiments reported here, human subjects breathed toluene vapor from some type of respiratory apparatus. In resting subjects, the concentration of toluene in arterial blood increased rapidly during the first 10 minutes of exposure and then began to level off, approaching an apparent steady state by 30 minutes. The concentration of toluene in alveolar air (i.e., an air sample taken at the end of a normal expiration) increased concomitantly.

Alveolar and arterial concentrations of toluene were proportional to the concentration in inspired air. At the end of 30 minutes of exposure to 100 or 200 ppm (0.375 or 0.750 mg/ℓ) toluene, the concentration of toluene in alveolar air (mg/ℓ) was 18% of that in inspired air (mg/ℓ), while the concentration in arterial blood (mg/kg) was 270% of that in inspired air (mg/ℓ) (Astrand et al., 1972; Astrand, 1975). The ratio between arterial blood and alveolar air concentrations was 15, which is similar to the in vitro blood/air partition coefficients (at 37°C) of 14.6, 15.6, and 15.6 reported for human blood by Sato et al. (1974b), Sherwood (1976), and Sato and Nakajima (1979a), respectively.

According to Veulemans and Masschelein (1978a), subjects' lung clearances (i.e., the virtual volume of inspired air from which all available toluene is

absorbed per unit time) decreased during exposure at rest, reaching an apparent steady state 9 to 13 minutes from the beginning of exposure. Lung clearance = $(C_i-C_e)/C_i \times \dot{V}_e$ where $C_i$ is the concentration of toluene in inspired air (mg/ℓ), $C_e$ is the concentration of toluene in expired air (mg/ℓ), and $\dot{V}_e$ is the respiratory minute volume (ℓ/min). Lung clearance varied less among individuals than did the concentration in expired air.

Nomiyama and Nomiyama (1974a) measured the pulmonary retention ($(C_i-C_e)/C_i \times 100$) of volunteers exposed to about 115 ppm toluene for 4 hours. The subjects may have been fairly sedentary because the authors did not mention exercise. Retention at the end of 1 hour was approximately 52% and decreased to 37% at the end of 2 hours, remaining constant at that level for the remaining 2 hours. These results suggest a slower approach to steady-state concentrations in expired or alveolar air than was indicated by the time courses obtained for lung clearance by Veulemans and Masschelein (1978a) or for alveolar air concentrations by Astrand et al. (1972). The results also suggest a lower percentage of uptake or retention than was reported by Veulemans and Masschelein (1978a) and others as will be presented subsequently. The reasons for these discrepancies are unclear.

Exercise affected the absorption of toluene through the respiratory tract. In the experiments of Astrand and coworkers (Astrand et al., 1972; Astrand, 1975), exercise greatly increased the concentrations of toluene in arterial blood and alveolar air of the subjects during exposure, and these concentrations did not level off as soon in exercising subjects as in resting subjects. The concentrations of toluene in arterial blood and alveolar air were approximately the same at 30 minutes of exposure to 200 ppm during rest as at 30 minutes of exposure to 100 ppm during light exercise (50 watts). At 30 minutes exposure to 100 or 200 ppm (0.375 or 0.750 mg/ℓ) toluene, the concentrations in milligrams

per liter expressed relative to the concentration in inspired air (mg/ℓ) were 33% for alveolar air and 620% for arterial blood at exercise of 50 watts, and 47% for alveolar air and 725% for arterial blood at exercise of 150 watts. The ratio of arterial to alveolar concentration remained about the same as at rest. Thus, alveolar concentrations appeared to reflect arterial concentrations during exposure to 100 to 200 ppm toluene at rest and various intensities of exercise.

The inhalation of 4% $CO_2$ by resting subjects during exposure to 100 ppm toluene increased their alveolar ventilation (ℓ/min) and the concentrations of toluene in their arterial blood and alveolar air (Astrand et al., 1972). The increased toluene concentration in blood and alveolar air were similar to those obtained with a corresponding increase in alveolar ventilation during exercise. Because exercise increased both alveolar ventilation and heart rate while $CO_2$ increased only alveolar ventilation, the effect of exercise on toluene absorption appears to be due to increased alveolar (or pulmonary) ventilation.

In the experiments of Veulemans and Masschelein (1978a), the "steady state" lung clearances of 6 different subjects during exposure to 50 ppm toluene at rest and at workloads of 25 and 50 watts on a bicycle ergometer correlated well ($r^2 = 0.96$) with their respiratory minute volumes. Lung clearance was determined from the regression line to be equal to 0.47 $\dot{V}_e$. The uptake rate in milligrams per minute, which equals lung clearance times the inhaled concentration, therefore was equal to 0.47 $\dot{V}_e C_i$ (where $C_i$ is expressed in mg/ℓ) and total uptake in milligrams equaled 47% of the total amount inhaled. Lung clearances and respiratory minute volumes doubled with an exercise intensity of 25 watts and tripled with an exercise intensity of 50 watts over the corresponding values at rest (Veulemans and Masschelein, 1978a).

Carlsson and Lindqvist (1977) found that the uptake of toluene by 7 male subjects exposed to 100 ppm for 30 minutes (0.375 mg/ℓ) during rest or various

levels of exercise (50, 100, and 150 watts on a bicycle ergometer) correlated inversely ($r^2$ = 0.72) with the alveolar concentration determined at the end of 30 minutes exposure, as described by the following equation:

$$\% \text{ Uptake} = -0.63 \frac{\text{alveolar concentration (mg/ℓ) x 100}}{\text{inspired concentration (mg/ℓ)}} + 72.9$$

This relationship is logical and applies to other solvents as well (Astrand, 1975; Ovrum et al., 1978). Percent uptake was determined on the basis of the total amount of toluene inhaled and exhaled during the entire exposure period (i.e., the expired air was collected continuously throughout exposure, and thus was a mean value). The uptake ranged from about 47 to 67% at rest and from about 36 to 57% at an exercise level of 150 watts. This group of men comprised 3 thin, 1 slightly overweight, and 3 obese subjects (Carlsson and Lindqvist, 1977).

Ovrum and coworkers (1978), monitoring four workers exposed to toluene in a printing plant, found good agreement between the value for percent uptake determined directly from the total amounts of toluene inspired and expired during a sampling period and the value determined indirectly from the instantaneous concentrations in alveolar and inspired air, using the equation given in the preceeding paragraph. Percent uptake determined by the direct method was 47% and by the indirect method was 51%. The total uptake of toluene that would occur during exposure to 80 ppm (0.3 mg/ℓ) for an 8 hour work day was calculated using the mean value for pulmonary ventilation of 16 ℓ/min measured for these 4 workers and a percent uptake of 50. The total uptake amounted to approximately 1150 mg (Ovrum et al., 1978).

The percent uptake values determined by Carlsson and Lindqvist (1977) and by Ovrum et al. (1978) are in reasonable agreement with those previously reported in abstracts from the foreign literature: 54% average uptake during 5 hours' exposure to 271 to 1177 µg/ℓ (Srbova and Teisinger, 1952) and 72% initial retention

decreasing to 57% retention towards the end of 8 hours of exposure to 100 to 800 µg/ℓ (Piotrowski, 1967).

Another factor, in addition to exercise, that has been reported to affect the absorption of toluene through the respiratory tract is the amount of adipose tissue in the body. Carlsson and Lindqvist (1977) found that mean alveolar air concentrations were slightly higher in 3 thin men than in 3 obese men at the end of 30 minutes of exposure to 100 ppm (0.375 mg/ℓ) toluene during rest or exercise. The ranges, however, overlapped. Conversely, the total uptake of toluene during 30 minutes of exposure (determined as previously described) was lower for the thin subjects than for the obese ones (Table 13-1). The thin subjects had a mean adipose tissue content of 6 kg and the obese ones had a mean adipose tissue content of 44 kg. It appears, from Figure 6 in the Carlsson and Lindqvist (1977) paper, that the obese men inspired a greater total quantity of toluene than did the thin men. Because the concentrations of toluene in the inspired air were the same for both thin and obese subjects, pulmonary ventilation must have been greater in the obese ones. Thus the differences in uptake between the thin and obese men may have been at least partially due to greater ventilation (respiratory minute volume) in the obese subjects rather than to their adipose tissue per se. Veulemans and Masschelein (1978a) reported finding no correlation between a subject's content of adipose tissue and uptake of toluene during exposures to 50 to 150 ppm toluene lasting about 4 hours. Astrand and coworkers (1972) stated that they found no systematic differences between male subjects (N = 11, adipose tissue 5.7 $\pm$ 1.5 kg, mean $\pm$ S.D.) and female subjects (N = 4, adipose tissue 13.3 kg, mean; 9.6 to 20.2 kg, range) in alveolar air and arterial blood concentrations of toluene.

Dahlmann and coworkers (1968a, 1968b) investigated the absorption of toluene contained in cigarette smoke through the mouths and respiratory tracts of

TABLE 13-1

Uptake of Toluene in Thin and Obese Men During Exposure to a Toluene Concentration of 375 mg/m$^3$ (100 ppm)[a,b]

| Number of Subjects | Adipose Tissue (kg) | Uptake (mg) | | | |
|---|---|---|---|---|---|
| | | | Exercise | | |
| | | Rest | 50 W | 100 W | 150 W |
| Thin (N = 3) | | | | | |
| Mean | 6.0 | 61 | 148 | 193 | 228 |
| Range | 1.4-10.7 | 55-69 | 133-158 | 168-211 | 181-271 |
| Slightly overweight (N = 1) | 22.8 | 71 | 179 | 246 | 299 |
| Obese (N = 3) | | | | | |
| Mean | 44.0 | 84 | 198 | 258 | 319 |
| Range | 35.1-49.0 | 72-73 | 183-206 | 237-275 | 258-358 |

[a]Source: Carlson and Lindqvist, 1977

[b]The subjects were exposed during one 30 minute period of rest and three consecutive 30 minute periods of exercise in order of increasing intensity. A 20 minute pause without exposure occurred between rest and exercise. Expired air was collected continuously during exposure.

volunteers. The uptake of toluene from smoke that stayed in the subject's mouth for 2 seconds or less and was not inhaled was 29%; uptake when the smoke was inhaled into the lungs was 93%. It is unclear whether each subject was exposed to a single puff of smoke, the smoke from 1 cigarette (8 puffs), or the smoke from 2 cigarettes.

During inhalation exposure of resting subjects, the concentration of toluene in peripheral venous blood (from the cubital vein of the arm) attained apparent steady state more slowly than did lung clearance or concentrations in alveolar air or arterial blood and was more variable among subjects than were the above mentioned values (Veulemans and Masschelein, 1978a; 1978b; Astrand et al., 1972; Sato and Nakajima, 1978). Peripheral venous concentrations appeared to level off during the second or third hour of exposure. Von Oettingen (1942a, 1942b) had observed that toluene concentrations in subjects' peripheral venous blood at the end of eight hours of exposure were roughly proportional to the concentrations of toluene (200 to 800 ppm) in the atmosphere of the exposure chamber. Veulemans and Masschelein (1978b) reported that the steady-state concentrations of toluene in peripheral venous blood were correlated with the rate of uptake at different inspired concentrations (50, 100, and 150 ppm) ($r^2$ = 0.73) and at different levels of rest and exercise ($r^2$ = 0.74). In both instances, the relationship between peripheral venous concentrations and uptake rate was:

Venous concentration (mg/ℓ) = 0.3 min/ℓ x uptake rate (mg/min).

The concentration of toluene in peripheral venous blood of exercising subjects increased more rapidly and appeared to reach steady-state values sooner than in resting subjects (Astrand et al., 1972; Veulemans and Masschelein, 1978b).

Absorption through the respiratory tract has been studied less extensively in experimental animals than in humans. The initial uptake of a relatively low

concentration of toluene was found to be approximately 90% in dogs inhaling toluene (Egle and Gochberg, 1976). Varying the ventilatory rate from 5 to 40 inhalations per minute, the tidal volume from 100 to 250 mℓ, or the concentration of toluene from 0.37 to 0.82 µg/ℓ (approximately 100 to 220 ppm) had no significant effect on the animals' initial respiratory uptake. Toluene was readily absorbed from the upper as well as from the lower respiratory tract. The dogs were anesthetized with sodium pentobarbital for these experiments and breathed toluene from a recording respirometer for 1 to 2 minutes. The percent uptake was calculated from the total amounts of toluene inhaled and exhaled during the 1 to 2 minute exposure.

Von Oettingen and coworkers (1942b) found that the concentration of toluene in the peripheral venous blood of dogs at the end of 8 hours of exposure was proportional to the concentration of toluene (200, 400, or 600 ppm) in the air of the exposure chamber. As previously described, similar observations had been made with humans.

Mice exposed singly to an extremely high initial concentration of methyl-$^{14}$C-toluene in a closed chamber for 10 minutes retained about 60% of the radioactivity when removed from the chamber at the end of the exposure (Bergman, 1979). This value is a rough approximation of absorption because some of the toluene may have been adsorbed to the animals' fur. A substantial portion of the retained dose appears to have been absorbed, however, as shown by its subsequent excretion in the urine (Section 13.4.). The initial concentration of toluene in the chamber (10 µℓ evaporated in a volume of about 30 mℓ, or about 77,000 ppm) would have been above the saturation concentration even if the temperature had been as high as 30°C (saturation concentration = 48,900 ppm at 30°C) (Verschueren, 1977). Bergman (1979) noted that exposure to toluene under these conditions markedly reduced the respiratory rate of the mice and attributed this

reduction to irritation. It seems more likely that the decreased respiratory rate was due to narcosis.

Absorption of toluene also occurs through the skin. Dutkiewicz and Tyras (1968a, 1968b), in experiments with humans, measured the absorption of liquid toluene into the skin of the forearm and found the rate of absorption to be 14 to 23 $mg/cm^2/hr$. This rate was calculated from the difference between the amount of toluene introduced under a watch glass affixed to the skin and the amount remaining on the skin at the end of 10 to 15 minutes. Absorption of toluene from aqueous solutions during immersion of both hands was 160 to 600 $\mu g/cm^2/hr$ and was directly proportional to the initial concentration of toluene (180 to 600 mg/ℓ). From these results, Dutkiewicz and Tyras (1968a, 1968b) calculated that the absorption of toluene through the skin of both hands during contact with a saturated aqueous solution of toluene for 1 hour could be in the same range as absorption through the respiratory tract during 8 hours of exposure to 26.5 ppm (0.1 mg/ℓ) toluene.

Sato and Nakajima (1978) found, however, that the maximum toluene concentration (170 μg/ℓ) in the blood of subjects who immersed one hand in liquid toluene for 30 minutes was only 26% of the concentration (650 μg/ℓ) in blood of subjects who inhaled 100 ppm toluene vapor for 30 minutes. Blood was collected from the cubital vein of the (unexposed) arm at intervals during and after exposure. Sato and Nakajima (1978) suggested that some of the toluene that penetrates the stratum corneum may be subsequently given off into the air, rather than entering the systemic circulation. Toluene appears to pass slowly from the skin into the bloodstream after penetrating the skin. Guillemin et al. (1974) reported that the elimination of toluene in alveolar air sometimes increased during the first 20 minutes after the termination of exposure of both hands to liquid toluene, and Sato and Nakajima (1978) noted that the maximum levels of

toluene in venous blood were maintained for about 15 minutes after the end of exposure.

Absorption of toluene vapor through the skin does not appear to result in a significant contribution to the body burden of toluene as compared to absorption through the respiratory tract. In experiments conducted by Riihimaki and Pfaffli (1978), volunteers wearing light, loose-fitting clothing and respiratory protection were exposed to 600 ppm toluene for 3.5 hours. The subjects remained at rest except for 3 exercise periods, each lasting for 10 minutes, which occurred at 0.5, 1.5, and 2.5 hours of exposure. The exercise was sufficient to stimulate perspiration and raise the skin temperature slightly, conditions which are thought to enhance percutaneous absorption. The concentration of toluene in peripheral venous blood, measured at the end of 1, 2, and 3 hours of exposure, was constant at approximately 100 µg/ℓ.

Riihimaki and Pfaffli (1978) compared total uptake through the skin (calculated from the amount of toluene exhaled assuming that 16% of absorbed toluene is exhaled) with theoretical uptake through the respiratory tract (assuming pulmonary ventilation of 10 ℓ/min and retention of 60%) at the same (600 ppm) level of exposure. They estimated that uptake through the skin was approximately 1% of the theoretical uptake through the respiratory system.

In similar experiments conducted by Piotrowoski (1967, reviewed in NIOSH, 1973), subjects exposed dermally to 1600 $mg/m^3$ (427 ppm) toluene for 8 hours had no increase in urinary excretion of a metabolite (benzoic acid) of toluene. Based on this result, Piotrowoski (1967) concluded that absorption of toluene through the skin would not exceed 5% of absorption through the respiratory tract under the same conditions.

The absorption of toluene from the gastrointestinal tract appears to occur more slowly than through the respiratory tract, but appears to be fairly complete

based on experiments with animals. The concentration of radioactivity in the blood of adult male rats reached a maximum 2 hours after gastric intubation of 100 µl 4-$^{3}$H-toluene in 400 µl peanut oil (Pyykko et al., 1977). The oil may have retarded absorption. Based on the percentages of the dose excreted unchanged in the expired air and as hippuric acid in the urine of rabbits, toluene appears to be completely absorbed from the gastrointestinal tract (El Masri et al., 1956; Smith et al., 1954).

## 13.2. DISTRIBUTION

Toluene is highly soluble in lipid and sparingly soluble in water, as indicated by the partition coefficients in Table 13-2. Judging from the fluid/air partition coefficients for water, plasma, and blood, much of the toluene in blood may be associated with the lipid and lipoprotein components, including the cellular elements. The tissue/blood partition coefficients for fatty tissues were very high (113 for adipose tissue and 35 for bone marrow); for other tissues, they ranged from about 1 to 3.

Little is known about the tissue distribution of toluene in humans. During inhalation exposure to 50 to 200 ppm toluene, the slow approach to steady-state of peripheral venous concentrations as compared to arterial concentrations (described under absorption) indicates that equilibration with the tissues may take at least 2 to 3 hours. Concentrations in peripheral venous blood do not, however, reflect the discharge of toluene to the tissues as fully as would concentrations in central venous blood. A teenage boy who died from sniffing glue had the following levels of toluene in his tissues: heart blood, 11 mg/kg; liver, 47 mg/kg; brain, 44 mg/kg; and kidney, 39 mg/kg (Winek et al. 1968; also reported in Winek and Collum, 1971).

TABLE 13-2

Partition Coefficients for Toluene at 37°C

| | Partition Coefficient | Reference |
|---|---|---|
| I. Fluid/Air or Material/Air | | |
| Water | 2.23 | Sato and Nakajima, 1979a |
| Oil, olive | 492 | |
| Blood, Human | 15.6 | |
| Fat, human, peritoneal | 1296 | |
| Oil, olive | 1380 | Sherwood, 1976 |
| Lard | 1270 | |
| Blood, human | 15.6 | |
| Blood, human | 14.64 | Sato et al., 1974a, 1974b |
| Blood, rabbit | 10.41 | |
| Plasma, rabbit | 16.99 | |
| II. Tissue[a]/Blood (Rabbit) | | |
| Liver | 2.58 | Sato et al., 1974a, 1974b |
| Kidney | 1.54 | |
| Brain | 3.06 | |
| Lung | 1.92 | |
| Heart | 2.10 | |
| Muscle, femoral | 1.18 | |
| Bone marow, red[b] | 35.43 | |
| Fat, retroperitoneal | 113.16 | |

[a]Homogenates.

[b]20% fat by volume.

Several laboratories have investigated the tissue distribution of toluene and its metabolites in animals exposed by inhalation to relatively high concentrations of toluene. The concentrations of toluene in liver, brain, and blood of mice exposed to 15 mg/ℓ (3950 ppm) toluene for 3 hours in a dynamic exposure chamber rose continuously throughout the exposure period, as shown previously in Figure 12-1. Concentrations of toluene reached 625 mg/kg in liver, 420 mg/kg in brain, and 200 mg/kg in blood at the end of exposure (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a). Exposure of mice to 40 mg/ℓ (10,600 ppm) toluene for 10 minutes resulted in lower tissue and blood concentrations. Intermittent exposure to 40 mg/ℓ in cycles of 5 minutes on, 10 minutes off or 10 minutes on, 20 minutes off for a total of 3 hours produced tissue and blood levels approximately 3 times higher than those produced by the single 10 minute exposure to 40 mg/ℓ and similar to those produced by the 3 hour exposure to 15 mg/ℓ. The intermittent exposures were an attempt to simulate solvent abuse (e.g., glue sniffing) by humans (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981b).

After adult male rats were exposed by inhalation to radioactively-labeled toluene, the highest concentrations of radioactivity were found in their white adipose tissue (Carlsson and Lindqvist, 1977; Pyykko et al., 1977). In the experiments of Pyykko and coworkers (1977) the concentration of radioactivity reached a maximum in all tissues but white adipose tissue within 15 to 30 minutes after the end of 10 minutes of exposure to 4600 ppm 4-$^{3}$H-toluene. The concentration in white adipose tissue reached a maximum one hour after the end of exposure. In the experiments of Carlsson and Lindqvist (1977), a similar increase in the concentration of radioactivity in white adipose tissue occurred during the first hour after cessation of exposure for 1 hour to 1.950 mg/ℓ (550 ppm) methyl-$^{14}$C- toluene. No such increase occurred in other tissues.

Carlsson and Lindqvist (1977) found that after white adipose tissue, the next highest concentrations of radioactivity occurred in adrenals and kidneys, followed by liver, cerebrum, and cerebellum. At the end of exposure, white adipose tissue contained a 6-fold higher concentration of radioactivity than did cerebrum or cerebellum. Pyykko et al. (1977) reported that after white adipose tissue, the next highest concentration of radioactivity was found in brown adipose tissue, followed in order of decreasing concentrations by adrenal, stomach, liver and kidney, brain and other tissues, blood, and bone marrow. The loss of radioactivity from adipose tissue and bone marrow appeared to occur more slowly than the loss from other tissues (Pyykko et al., 1977). Radioactivity in the tissues presumably represented toluene and its metabolites.

Bergman (1979), using three-step whole-body autoradiography, investigated the distribution of toluene, its metabolites, and covalently bound reactive intermediates in mice exposed to an extremely high concentration of methyl-$^{14}$C-toluene. This work was briefly described in a previous report (Bergman, 1978). The mice were exposed singly to a very high initial concentration of toluene for 10 minutes in a closed chamber, as described in Section 13.1., and sacrificed at intervals thereafter. Low temperature autoradiography, performed at -80°C, allowed the detection of both volatile radioactivity (representing toluene) and non-volatile radioactivity (representing metabolites). In a second step, sections were dried and heated to remove volatile material before autoradiography, thus permitting detection of non-volatile metabolites only. In the third step, sections that had been dried and heated were then extracted to remove water-soluble and lipid-soluble radioactivity, presumably leaving only the radioactivity that was covalently bound to proteins and nucleic acids.

Low temperature autoradiography performed immediately after exposure revealed high levels of radioactivity in adipose tissue, bone marrow, and spinal

nerves, with some radioactivity also present in the brain, spinal cord, liver, and kidney (Bergman, 1979). Bergman reported that the adrenal did not contain high concentrations of radioactivity, but he did not discuss whether radioactivity was found in the stomach.

The only radioactivity visible in dried, heated sections appeared in the liver, kidney, and blood (Bergman, 1979). This indicates that significant amounts of metabolites had already been formed by the end of exposure, and that the radioactivity in fat and nervous tissue was due to the parent compound. Similarly, as early as 8 minutes after intraperitoneal injection of 290 μg $^{14}$C-toluene/kg into mice, the majority of radioactivity in the kidney (78%) and liver (64%) and about half the radioactivity in blood (48%) was reported to represent non-volatile metabolites, while most of the radioactivity in brain and virtually all in the adipose tissue was volatile and thus represented toluene itself (Koga, 1978). The methods used in Koga's study are unclear because the text of the paper is in Japanese, with only the figures, tables, and summary in English. Bergman (1979) reported that no radioactivity was detected in autoradiograms prepared from dried, heated, and extracted sections, indicating an absence of covalent binding.

As had been observed in the studies of Pyykko et al. (1977) and Carlsson and Lindqvist (1977), radioactivity disappeared from the tissues relatively quickly after exposure was terminated. The distribution patterns observed in mice killed more than four hours after exposure were the same on low temperature autoradiograms as on dried, heated sections. Thus, the radioactivity remaining in the tissues at this time represented non-volatile metabolites. At eight hours after exposure only the kidney and the intestinal contents had detectable radioactivity (Bergman, 1979).

Oral administration of 4-$^{3}$H-toluene (100 µl toluene in 400 µl peanut oil by intubation) to adult male rats produced a pattern of tissue distribution similar to that produced by inhalation exposure (Pyykko et al., 1977). Distribution appeared to be delayed, however, by absorption from the digestive tract. Maximum tissue concentrations occurred 2 to 3 hours after administration for most tissues and 5 hours after administration for adipose tissue.

In summary, toluene was preferentially accumulated in adipose tissue and was retained in adipose tissue and bone marrow, which is reasonable on the basis of the high tissue/blood distribution coefficients of these tissues. Toluene and its metabolites were found in relatively high concentrations in tissues active in its metabolism and excretion (i.e., liver and kidney). Levels in brain relative to those in other tissues were perhaps lower than would be expected on the basis of the tissue/blood distribution coefficients reported by Sato et al. (1974a, 1974b). Tissue distribution was similar after inhalation and oral exposure.

## 13.3. METABOLISM

Toluene is thought to be metabolized in humans and in animals by the pathways outlined in Figure 13-1. Some of the absorbed toluene is excreted unchanged in the exhaled air, but the major portion is metabolized by side-chain oxidation to benzoic acid, which is conjugated with glycine to form hippuric acid and then excreted in the urine. Small amounts of benzoic acid may be conjugated with glucuronic acid. Minor amounts of toluene undergo ring hydroxylation, probably via arene oxide intermediates, to form o-cresol and p-cresol, which are excreted in the urine as sulfate or glucuronide conjugates.

Humans exposed to toluene by inhalation exhaled about 16% of the absorbed toluene after exposure was terminated, according to Nomiyama and Nomiyama (1974b) and Srbova and Teisinger (1952, 1953), or 4%, according to Veulemans and

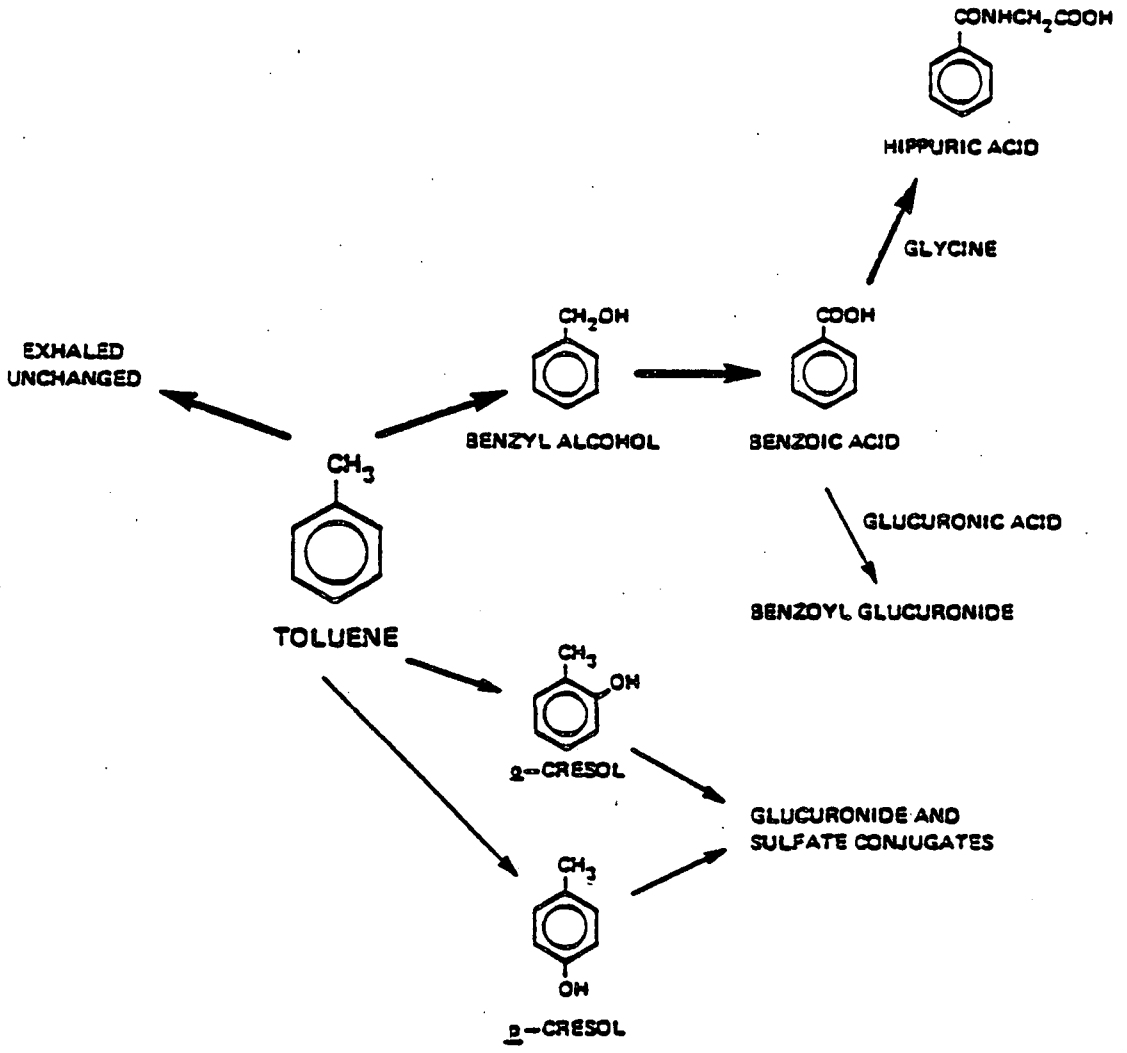


Figure 13-1. Metabolism of Toluene in Humans and Animals (Adapted from Laham, 1970)

Masschelein (1978a). Volunteers inhaling 50 to 150 ppm toluene for about 4 hours during rest or exercise excreted 60 to 70% of the absorbed dose as hippuric acid in the urine during and after exposure (Veulemans and Masschelein, 1979). A similar value was obtained when subjects were exposed to toluene (67 ppm) and xylene (83 ppm) simultaneously for 3 hours; 68% of the absorbed toluene was excreted as urinary hippuric acid during and after exposure (Ogata et al., 1970). Srbova and Teisinger (1953) reported that although most of the benzoic acid in the urine of subjects who inhaled 0.271 to 2.009 mg/ℓ toluene (72 to 532 ppm) was excreted as hippuric acid, 10 to 20% was excreted as a glucuronide conjugate.

The excretion of hippuric acid in the urine was elevated within 30 minutes of the initiation of inhalation exposure, indicating that the metabolism of toluene is rapid (Nomiyama and Nomiyama, 1978; Ogata et al., 1970; Veulemans and Masschelein, 1979). The maximum rate of hippuric acid formation from benzoic acid was reported by Amsel and Levy (1969) to be about 190 μmol/min, and it appeared to be limited by the availability of glycine (Amsel and Levy, 1969; Quick, 1931). Assuming retention of 60% of the inhaled concentration, Riihimaki (1979) estimated that uptake of toluene may saturate the conjugation capacity at a toluene concentration of 32 mmol/$m^3$ (780 ppm) during light work (pulmonary ventilation of 10 ℓ/min) or 11 mmol/$m^3$ (270 ppm) during heavy work (pulmonary ventilation of 30 ℓ/min).

o-Cresol, a compound which is often not detected in normal urine, was identified in the urine of workers exposed to 7 to 112 ppm toluene (Angerer, 1979; Pfaffli et al., 1979). The concentration of o-cresol in urine collected at the end of exposure was directly proportional to the time-weighted average exposure of the workers (Pfaffli et al., 1979). Angerer (1979) estimated that approximately 0.05% of the retained toluene had been metabolized to o-cresol. p-Cresol may also have been a metabolite of toluene as its concentration was

higher in the urine of workers exposed to toluene than in the urine of unexposed workers (Angerer, 1979). The difference, however, was not significant. Wiowode et al. (1979) reported finding m-cresol in addition to o-cresol and p-cresol in the urine of workers exposed to 280 ppm toluene. No m-cresol was detected in the urine of unexposed workers. No other studies of *in vivo* human or animal metabolism or *in vitro* microsomal metabolism reviewed for this document have detected m-cresol as a metabolite of toluene.

The concentration of phenol has been reported to be slightly elevated in the urine of exposed workers as compared to controls (Angerer, 1979; Szadkowski et al., 1973). The origin of the increased phenol excretion was thought to be the small amount of benzene present in industrially-used toluene (Angerer, 1979).

The metabolism of toluene has been more fully studied in animals than in humans. The initial step in the metabolism of toluene to benzoic acid appears to be side-chain hydroxylation of toluene to benzyl alcohol by the microsomal mixed-function oxidase system. Toluene has been shown to produce a type I binding spectrum with cytochrome P450 from rats and hamsters, indicating that it is probably a substrate for the mixed-function oxidase system (Canady et al., 1974; Al-Gailany et al., 1978). When incubated with rabbit hepatic microsomes, toluene was metabolized primarily to benzyl alcohol (Daly et al., 1968) and small amounts of benzyl alcohol have been detected in the urine of rats given toluene orally (Bakke and Sheline, 1970).

Additional evidence that toluene is metabolized by mixed-function oxidases has been obtained by Ikeda and Ohtsuji (1971) who demonstrated that the induction of hepatic mixed-function oxidases by pretreatment of adult female rats for four days with phenobarbital increased the metabolism of toluene. When given 1.18 mg toluene/kg body weight intraperitoneally, phenobarbital-pretreated

(induced) rats had greatly elevated urinary excretions of hippuric acid and decreased concentrations of toluene in the blood compared to non-induced rats given the same dose of toluene. Induced rats had high levels of benzoic acid in the blood; non-induced rats had none (blood was obtained at decapitation).

The increased metabolism of toluene by induced rats appeared to reflect an increase in side-chain hydroxylation of toluene, because the activity of hepatic side-chain hydroxylase, assayed *in vitro* with the model substrate p-nitro toluene, was significantly increased per gram of liver. The *in vitro* oxidation of the resultant alcohol (p-nitrobenzyl alcohol) to the acid (p-nitrobenzoic acid) was not affected. The conjugation of benzoic acid with glycine, measured *in vivo* as the total amount of hippuric acid excreted after benzoic acid administration, was also unaffected (Ikeda and Ohtsuji, 1971).

It has been assumed (Ikeda and Ohtsuji, 1971; Nomiyama and Nomiyama, 1978; NRC, 1980), by analogy with the metabolism of the model substrate p-nitrotoluene (Gillette, 1959), that benzyl alcohol is metabolized to benzaldehyde by alcohol dehydrogenase and that benzaldehyde in turn is oxidized to benzoic acid by aldehyde dehydrogenase. These enzymes are both found in the soluble fraction from liver. Benzaldehyde itself has not been detected in the urine or expired air of animals given toluene orally (Smith et al., 1954; Bakke and Sheline, 1970). Metabolism of toluene probably occurs primarily in the liver. This assumption is based on the previously discussed tissue distribution of metabolites, the demonstrated metabolism of toluene by liver microsomal preparations, and by analogy with the metabolism of other xenobiotics.

Rabbits intubated with 300 mg toluene/kg body weight eliminated approximately 18% of the dose in the expired air (Smith et al., 1954) and, in another study from the same laboratory, excreted about 74% of the dose as hippuric acid in the urine (El Masri et al., 1956). These results are similar to those

obtained with humans who inhaled toluene. None of the toluene appeared to be converted to benzoyl glucuronide (Smith et al., 1954), although about 14% of an oral dose of benzoic acid was excreted by rabbits as the glucuronide conjugate (Bray et al., 1951).

Toluene metabolism appears to be rapid in animals, as shown by the appearance of metabolites in the livers, kidneys, and blood of mice within minutes of exposure to toluene (Bergman, 1979; Koga, 1978) (discussed in Section 13.2.) and by the increased urinary excretion of hippuric acid in rabbits within 0.5 hour of the initiation of inhalation exposure (Nomiyama and Nomiyama, 1978). As was previously mentioned for humans, the rate of conjugation of benzoic acid with glycine may be limited in animals by the availability of glycine. Administration of glycine to dogs exposed by inhalation to 200, 400, or 600 ppm toluene enhanced the rate of hippuric acid excretion (Von Oettingen, 1942b). At the end of 8 hours of exposure to 600 ppm toluene, the concentrations of toluene in peripheral venous blood from glycine-treated dogs were lower than the concentrations in dogs that had not been treated with glycine. No such difference was observed at the two lower exposure levels. This result suggests that conjugation of benzoic acid with glycine may have limited metabolic elimination at the highest level of exposure. The level of exposure at which glycine treatment produced a difference in venous blood levels of toluene is similar to that (780 ppm) calculated by Riihimaki (1979) for saturation of the glycine conjugation capacity of humans.

A minor pathway for the metabolism of toluene is ring hydroxylation by mixed-function oxidases. Incubation of toluene with rat or rabbit liver microsomes resulted in the production of small amounts of o-cresol and p-cresol (Daly et al., 1968; Kaubisch et al., 1972). The migration of deuterium when toluene was labeled in the 4-position and a comparison of the rearrangement products of

arene oxides of toluene with the cresols obtained by microsomal metabolism of toluene indicated that arene oxides are intermediates in the metabolism of toluene to o- and p-cresols (Daly et al., 1968; Kaubisch et al., 1972).

Because phenols, including cresols, are eliminated in the urine as sulfate conjugates, thereby increasing the excretion of organic sulfates and decreasing the excretion of inorganic sulfate, investigators have used urinary sulfate excretion after toluene administration as an indicator of cresol formation. Oral doses of 350 mg toluene/kg body weight produced no increase in organic sulfate excretion in rabbits (Smith et al., 1954). In rats, high doses (2.2 and 4.3 g/kg) of toluene, administered orally, resulted in slight but significant decreases in the ratio of inorganic sulfate to total sulfate in the urine, while lower doses did not (Gerarde and Ahlstrom, 1966). This would appear to be a relatively insensitive and nonspecific assay for metabolism to cresols.

Bakke and Sheline (1970) analyzed urinary phenols (after hydrolysis) from male rats placed on purified diets containing neomycin, which reduced the urinary levels of naturally occurring phenols. Toluene, administered orally in a dose of 100 mg/kg body weight, was metabolized to o-cresol (0.04 to 0.11% of the dose) and p-cresol (0.4 to 1.0% of the dose).

Metabolism to cresols is of concern because of the putative arene oxide intermediates, which are highly reactive and may bind to cellular macromolecules. Very little toluene is metabolized via this pathway, however, and the studies already discussed in the distribution section indicate that binding of toluene metabolites to proteins and nucleic acids does not occur to any significant extent.

Van Doorn and coworkers (1980) have reported detecting small amounts of a mercapturic acid, tentatively identified as benzylmercapturic acid (N-acetyl-S-benzyl-L-cysteine), in the urine of male rats treated with toluene. Approxi-

mately 0.4 to 0.7% of a dose of 370 mg/kg toluene body weight, administered intraperitoneally, was recovered as the mercapturic acid. The concentration of glutathione in the liver was decreased slightly by administration of toluene. Benzylmercapturic acid would arise from conjugation with glutathione of an electrophilic product of side-chain oxidation of toluene.

The metabolism of toluene appears to result in its detoxification. The length of the sleeping time produced by high doses of toluene (1.18 to 1.45 g/kg intraperitoneally) was decreased in phenobarbital-induced female rats to 50% or less of the sleeping time of controls (Ikeda and Ohtsuji, 1971). Similar results were obtained with male mice (Koga and Ohmiya, 1968). Phenobarbital-induced animals, however, did not have significantly different mortality rates than controls when given high doses of toluene (Ikeda and Ohtsuji, 1971; Koga and Ohmiya, 1968). Male mice given various inhibitors of drug metabolism (SKF 525A, cyanamide, and pyrazole) 30 minutes before the injection of toluene had sleeping times that were significantly longer than those of control mice and had higher mortality rates than did control mice (Koga and Ohmiya, 1978).

## 13.4. EXCRETION

In both humans and animals, toluene is rapidly excreted as the unchanged compound in expired air and as a metabolite, hippuric acid, in the urine. Most of the absorbed toluene is excreted within 12 hours of the end of exposure.

The concentrations of toluene in exhaled air and in arterial and venous blood of human subjects declined very rapidly as soon as inhalation exposure was terminated (Astrand et al., 1972; Carlsson and Lindqvist, 1977; Ovrum et al., 1978; Sato et al., 1974b; Veulemans and Masschelein, 1978a, 1978b). Sato et al. (1974b) reported that semilogarithmic plots of toluene concentrations in alveolar air and in peripheral venous blood versus time after the end of exposure

suggested that desaturation occurred in three exponential phases: an initial rapid phase, followed by an intermediate phase and then a slow phase. The data were obtained from 3 male subjects who inhaled 100 ppm toluene for 2 hours (Sato et al., 1974b; clarified in Sato and Nakajima, 1979b). The desaturation curves were resolved graphically into three components, and constants were determined by the least squares method. The rate coefficients and corresponding half-lives ($t_{1/2}$) for the decay of toluene in peripheral venous blood were 0.355 $min^{-1}$ ($t_{1/2}$ = 1.95 minutes), 0.0197 $min^{-1}$ ($t_{1/2}$ = 35.2 minutes), and 0.00339 $min^{-1}$ ($t_{1/2}$ = 204 minutes). Rate coefficients and half lives for the decay of toluene in alveolar air were 0.437 $min^{-1}$ ($t_{1/2}$ = 1.59 minutes), 0.0262 $min^{-1}$ ($t_{1/2}$ = 26.5 minutes), and 0.00313 $min^{-1}$ ($t_{1/2}$ = 221 minutes).

Because the rate coefficient for the rapid phase was derived from only two points (at 0 and 5 minutes), the second of which belonged with the intermediate phase, Sato et al. (1974b) noted that the coefficient for the rapid phase involved some error. The data of Sato et al. (1974b) indicate that the decay of toluene concentrations in peripheral venous blood was more gradual than that in expired air. Similar conclusions have been reported by Astrand et al. (1972), and Veulemans and Masschelein (1978b). Astrand et al. (1972) have reported that peripheral venous concentrations declined more gradually than did arterial concentrations.

Veulemans and Masschelein (1978a) and Nomiyama and Nomiyama (1974b) found the excretion curves for toluene in expired air to be adequately described as the sum of 2 exponential terms rather than 3. Subjects for these studies were exposed to 50, 100, or 150 ppm toluene for about 4 hours. The sampling regimens differed from that of Sato et al. (1974b), in that Veulemans and Masschelein (1978a) did not begin monitoring expired air as soon after exposure ended, and Nomiyama and Nomiyama (1974b) sampled expired air infrequently during the period

used by Sato et al. (1974b) to determine the first two exponential phases. Rate coefficients for the rapid and slow phases were calculated by Veulemans and Masschelein (1978a) to be 0.340 $min^{-1}$ and 0.00608 $min^{-1}$, respectively, using a curve-fitting computer program. These rate coefficients corresponded to half-lives of 2.04 and 114 minutes. Nomiyama and Nomiyama (1974b) reported rate coefficients for the rapid phase of 5.10 $h^{-1}$ ($t_{1/2}$ = 8.16 minutes) for men and 3.22 $h^{-1}$ ($t_{1/2}$ = 12.9 minutes) for women; the rate coefficient for the slow phase was 0.335 $h^{-1}$ ($t_{1/2}$ = 124 minutes) for both sexes.

In the desaturation period, men and women expired 17.6 and 9.4%, respectively, of the total amount of toluene calculated to have been absorbed during exposure (Nomiyama and Nomiyama, 1974b). These values are close to what had been reported previously (i.e., 16%) by Srbova and Teisinger (1952, 1953) in abstracts from the foreign literature. Veulemans and Masschelein (1978a) estimated that about 4% of the toluene absorbed during exposure was subsequently excreted in the expired air. Unlike the continuous exposures employed in the other pertinent investigations, however, the exposure regimen employed by Veulemans and Masschelein (1978a) was discontinuous (i.e., four 50 minute periods of exposure separated by 10 minute intervals of nonexposure).

According to Veulemans and Masschelein (1978a) a much greater variability was observed for the excretion of toluene in expired air during the first four hours after the end of exposure than had been observed for the related lung clearances during exposure. This variability could be explained partially by differences in respiratory minute volume during the post-exposure period; the percent of absorbed toluene excreted in the expired air during the first 4 hours after exposure correlated positively with respiratory minute volume ($r^2$ = 0.71). Another factor that appeared to affect excretion was the amount of body fat, because there was a significant ($p < 0.025$) negative correlation between fat

content as measured by the index of Broca and the percent excretion in expired air after exposure at rest ($r^2$ = 0.2134). This indicates that less of the absorbed toluene would be excreted in the expired air of an obese person than in the expired air of a thin person during the first four hours of desaturation. Additionally, subjects who had been exposed to toluene while exercising expired less of the absorbed amount during the first four hours of desaturation than did subjects who had been exposed while resting (Veulemans and Masschelein, 1978a).

As previously described, 60 to 70% of the toluene absorbed by humans during inhalation can be accounted for as hippuric acid in the urine (Veulemans and Masschelein, 1979; Ogata et al., 1970). The excretion rate of hippuric acid in the urine of subjects inhaling 50, 100, or 150 ppm toluene increased during the first 2 hours, leveling off at about the third hour after initiation of exposure (Veulemans and Masschelein, 1979; Nomiyama and Nomiyama, 1978). Hippuric acid excretion (mg/hr) declined fairly rapidly after cessation of about four hours of exposure. Nomiyama and Nomiyama (1978), treating this decline as a monoexponential process, determined a half-life for hippuric acid in urine of 117 minutes for men and 74 minutes for women. Veulemans and Masschelein (1979) reported an initial, fairly rapid decrease with a half-life between 2.0 and 2.3 hours, followed by a more gradual return to baseline excretion levels by about 24 hours after the start of exposure.

The excretion rate of hippuric acid, measured at the end of about 4 hours of experimental exposure or 8 hours of occupational exposure, correlated reasonably well with the uptake rates (Veulemans and Masschelein, 1979) or total uptake (Wilczok and Bieniek, 1978) during exposure. At a given level of physical activity and exposure concentration, the intra- and interindividual variability in hippuric acid excretion was greater than that noted for uptake rates and was attributed to the variable baseline excretion of this compound because it was not

explained by other factors (body weight, body fat, cardiorespiratory parameters) (Veulemans and Masschelein, 1979). Exercise during exposure increased the rate of excretion of hippuric acid (Veulemans and Masschelein, 1979) in accordance with the increase in uptake rate.

Hippuric acid is a normal constituent of urine derived from benzoic acid and precursors of benzoic acid in the diet (Quick, 1931). Concentrations of hippuric acid in the urine of 101 workers not exposed to toluene ranged from 0.052 to 1.271 mg/mℓ (corrected to urine specific gravity of 1.024) and rates of excretion of hippuric acid ranged from 18.47 to 23.00 mg/hr for diuresis of greater than 30 mℓ/hr (Wilczok and Bieniek, 1978). Others have also reported great variability in the physiological concentrations of urinary hippuric acid (Ikeda and Ohtsuji, 1969; Imamura and Ikeda, 1973; Engstrom, 1976; Kira, 1977; Ogata and Sugihara, 1977; Angerer, 1979).

Volunteers exposed in a chamber to 200 ppm toluene for 3 hours followed by a 1 hour break and an additional 4 hours of exposure excreted hippuric acid as shown in Figure 13-2 (Ogata et al., 1970). This exposure regimen was chosen to simulate exposure in the workplace. After leveling off after approximately 3 hours of exposure, excretion increased again during the afternoon exposure. The rate of hippuric acid excretion remained elevated for about 2 hours after exposure was terminated and then declined almost to baseline levels by 18 hours after the end of exposure. The total quantity of hippuric acid excreted during the period lasting 26 hours from the initiation of exposure was directly proportional to the degree of exposure (ppm x time) up through the highest toluene concentration of 200 ppm and could be used to calculate exposure with a fairly high degree of accuracy. Less accurate for this purpose were excretion rates during exposure (i.e., total hippuric acid excreted during exposure ÷ time) and concentrations in urine, corrected for specific gravity. Concentrations of

hippuric acid in urine collected during the entire exposure period and corrected to a specific gravity of 1.024 were 0.30 ± 0.10, 2.55 ± 0.55, and 5.99 ± 1.20 mg/mℓ (mean ± standard deviation) for control, 100 ppm, and 200 ppm exposed subjects, respectively. Values for controls were lower and more uniform than those reported by others, as described previously.

Spot urine samples collected from workers after at least three hours of exposure to toluene (and from nonexposed workers at the same time) have not given as good a distinction between unexposed and exposed workers. Imamura and Ikeda (1973) have pointed out that the upper fiducial limit (P = 0.10) of normal hippuric acid concentrations, whether or not corrected for specific gravity, is so close to the lower fiducial limit of workers exposed to 100 ppm toluene (the Threshold Limit Value) that such a measurement would not be reliable in screening for overexposure. This conclusion was based on data reported by Ikeda and Ohtsuji (1969). The correlations between concentrations of toluene in workplace air and the concentration of hippuric acid in urine of individual workers have been relatively poor (Veulemans et al., 1979; Szadkowski, 1973; Ogata et al., 1971). The correlation between exposure concentration and excretion rate during exposure, although slightly better, was also poor: $r^2 = 0.096$ for the correlation with hippuric acid concentration (corrected for specific gravity) and $r^2 = 0.116$ for the correlation with rate of excretion of hippuric acid (Veulemans et al., 1979). Some of the variance in excretion rates was accounted for by differences in lung clearance, and, hence, uptake among workers (Veulemans et al., 1979).

Mice exposed to a very high initial concentration of methyl-$^{14}$C-toluene in a closed chamber for 10 minutes excreted about 10% of the absorbed dose as volatile material in the exhaled air and about 68% as unidentified compounds in the urine within 8 hours (Bergman, 1979). Details of exposure were discussed in

Section 13.1. In these experiments, volatile expired radioactivity (thought to represent the parent compound) was collected continuously in a trapping device. The total volatile radioactivity expired during each time interval was converted to the mean percent dose excreted per minute during that interval and plotted at the end of the interval. The resultant semilogarithmic plot of mean percent dose exhaled per minute versus time was a curve. Computerized non-linear regression analysis of the data according to the method of least squares yielded 3 exponential components with rate coefficients of 0.0659, 0.0236, and 0.0044 $min^{-1}$ corresponding to apparent half-lives of 10.5, 29.4, and 158.7 minutes, respectively.

The respiratory rates of the mice were, according to Bergman (1979), "remarkably reduced" during exposure, and hence probably were reduced during at least part of the post-exposure period. If respiratory minute volumes were also decreased, this would, on the basis of the observations of Veulemans and Masschelein (1978a), be expected to reduce the pulmonary excretion of toluene. The results of Bergman (1979) may therefore not be relevant to exposures at lower concentrations of toluene.

After inhalation exposure of rats or mice to toluene, the disappearance of toluene and its metabolites from blood and from most tissues, including brain, was rapid (Peterson and Bruckner, 1978; Carlsson and Lindqvist, Pyykko et al., 1977; Bergman, 1979) as described in Section 13.2. The exceptions were white adipose tissue, for which both accumulation and elimination were slow, and bone marrow, for which elimination was very slow (Carlsson and Lindqvist, 1977; Pyykko et al., 1977). By 24 hours after exposure to radioactively-labeled toluene, the concentration of radioactivity remaining in most tissues was less than 1% and that remaining in adipose tissue was about 5% of the initial whole-body concentration (Pyykko et al., 1977).

Rabbits exposed to toluene vapor at 350 ppm for 100 minutes or 4500 ppm for 10 minutes had increased rates of urinary hippuric acid excretion that reached maximum values 1.5 hours after exposure (Nomiyama and Nomiyama, 1978). Excretion rates returned to baseline levels at 7 hours after the initiation of exposure to 350 ppm for 100 minutes and at about 3 hours after the initiation of exposure to 4500 ppm for 10 minutes.

Dermal exposure of human subjects to toluene liquid or vapor resulted in the appearance of toluene in the expired air (Guilleman et al., 1974; Riihimaki and Pfaffli, 1978) as discussed in Section 13.1. The excretion of toluene in the expired air of subjects exposed to 600 ppm toluene for 3 hours appeared to consist of at least 2 exponential phases (Riihimaki and Pfaffli, 1978). The mean amount of toluene expired during the "quantitatively significant" portion of the excretion curve was calculated to be 45.9 μmole (4.23 mg) Riihimaki and Pfaffli, 1978). Piotrowski (1967, reviewed in NIOSH, 1973) found that subjects exposed dermally (with respiratory protection) to 1600 $mg/m^3$ (427 ppm) toluene for 8 hours had no detectable increase in urinary excretion of benzoic acid (presumably analyzed after hydrolysis of conjugates).

Oral administration of toluene to rabbits resulted in a pattern of excretion similar to that observed after inhalation exposure of humans. Rabbits (N = 2) intubated with 350 mg toluene/kg body weight expired 18% of the dose as the parent compound within 14.5 hours; less than 1% of the dose was eliminated in the expired air in the period from 14.5 through 35 hours after dosing (Smith et al., 1954). In similar experiments from the same laboratory, rabbits intubated with 274 mg toluene/kg body weight excreted an average of 74% of the dose in the urine as hippuric acid; excretion was complete with 24 hours of dosing (El Masry et al., 1956). The elimination of toluene and its metabolites from tissues and blood of rats given toluene orally (Pyykko et al., 1977) was similar to the

pattern already described after inhalation exposure (Pyykko et al., 1977) except that elimination after oral administration appeared to be delayed by a slower rate of absorption than had been observed for inhalation exposure.

The excretion of other metabolites of toluene (i.e., cresols, benzyl alcohol, glucuronide and sulfate conjugates, benzylmercapturic acid) in the urine of humans and animals has already been described in Section 13.3. With the possible exception of benzoylglucuronide (Srbova and Teisinger, 1953), none of these excreted metabolites represented more than about 1% of the total dose of toluene administered or absorbed (Angerer, 1979; Bakke and Sheline, 1970; Van Doorn et al., 1980; Smith et al., 1954). Trace amounts of toluene were eliminated in the urine of humans exposed to toluene (Srbova and Teisinger, 1952).

Biliary excretion of toluene or its metabolites appeared to be negligible. Rats given 50 mg $^{14}$C-toluene/kg body weight intraperitoneally excreted less than 2% of the administered radioactivity in the bile within 24 hours (Abou-El-Markarem et al., 1967).

Most of the experimental work on the disposition of toluene in humans and animals has focused on single exposures. The elimination of toluene is rapid enough that few investigators have studied its potential accumulation with repeated daily exposure. Ovrum and coworkers (1978) took samples of capillary blood daily before work from 8 printers exposed occupationally to 35 to 353 ppm toluene. No cumulative increase in blood concentrations of toluene was found during the course of a 5 day work week. Konietzko and coworkers (1980) observed, however, that toluene concentrations in peripheral venous blood tended to increase during the course of a 5 day work week, although the ranges overlapped (Table 13-3). Mean exposure concentrations, measured by a personal air sampling method, did not increase during the week. The blood samples were taken before work on Monday, Wednesday, and Friday from 8 workers exposed to 184 to 332 ppm

TABLE 13-3

Toluene Concentrations in Workplace Air and Peripheral Venous Blood of Exposed Workers[a,b]

| | | Monday | Tuesday | Wednesday | Thursday | Friday |
|---|---|---|---|---|---|---|
| | Toluene in air (ppm) | 225 (95-303) | 233 (153-383) | 209 (107-341) | 212 (92-314) | 203 (124-309) |
| First week | Toluene in blood before exposure (μg/mℓ) | 0.12 (0.09-0.24) | -- | 0.51 (0.28-0.82) | -- | 0.77 (0.29-1.67) |
| | Toluene in blood after exposure (μg/mℓ) | 3.63 (2.3-4.75) | -- | 6.69 (4.21-10.36) | --- | 6.70 (3.99-10.67) |
| | Toluene in air (ppm) | 285 (145-473) | 304 (190-521) | 309 (213-413) | 232 (125-451) | 191 (105-432) |
| Second week | Toluene in blood before exposure (μg/mℓ) | 0.27 (0.07-0.57) | -- | 1.00 (0.35-151) | -- | 1.21 (0.44-2.29) |
| | Toluene in blood after exposure (μg/mℓ) | 11.60 (6.99-17.10) | -- | 10.49 (3.24-20.31) | -- | 5.85 (1.94-9.78) |

[a]Source: Kometzko et al., 1980

[b]Means and (range) of eight workers

daily in a plastic processing factory. Concentrations in blood samples taken after work were highly variable and did not seem to follow a consistent pattern.

In an analysis of 3155 samples of urine taken in the course of biological monitoring from different workers on different days of the week and in different workplaces, Lenhert et al. (1978) observed that concentrations of hippuric acid in the urine did not vary with the day of the week except on Monday, when the concentrations were significantly higher than on other days. The authors conjectured that the elevation of hippuric acid concentrations on Mondays was a result of different eating habits on the weekend.

In experiments with dogs, exposure to 400 ppm for 7 hours a day for 5 consecutive days did not result in an increase in the total amount of hippuric acid excreted per day over the period of 5 days or change the time course of urinary excretion (Von Oettingen et al., 1942b). Nor did the concentration of toluene in peripheral venous blood sampled at the end of exposure increase with day of exposure.

## 13.5. SUMMARY

Toluene is readily absorbed through the respiratory tracts of humans and experimental animals, as would be expected from its blood/air partition coefficient of approximately 15 (Sato and Nakajima, 1979; Sato et al., 1974a, 1974b; Sherwood, 1976). The amount of toluene absorbed (uptake) is proportional to the concentration in inspired air, length of exposure, and pulmonary ventilation (respiratory minute volume) (Astrand et al., 1972; Astrand, 1975; Veulemans and Masschelein, 1978a).

The uptake of toluene by humans was about 50% of the amount inspired (Veulemans and Masschelein, 1978a; Carlsson and Lindqvist, 1977, Ovrum et al., 1978). Total uptake (absorption) can be approximated as follows: Uptake

= 0.5 $\dot{V}_e$ $C_i$ t, where $\dot{V}_e$ is the respiratory minute volume in ℓ/min, $C_i$ is the inspired concentration in mg/ℓ, and t is the length of exposure in minutes (Ovrum et al., 1978; Veulemans and Masschelein, 1978a). Because of its dependence on respiratory minute volume, the uptake of toluene is affected by the subjects' level of physical activity (Astrand et al., 1972; Astrand, 1975; Veulemans and Masschelein, 1978a; Carlsson and Lindqvist, 1977). A subject's content of adipose tissue had little or no effect on the uptake of toluene during exposures lasting four hours or less (Veulemans and Masschelein, 1978a; Astrand et al., 1972) except in the case of extremely obese individuals (Carlsson and Lindqvist, 1977), and even then the increased uptake may have been at least partly due to greater pulmonary ventilation in the obese subjects than in the thin ones. Under "steady state" conditions, peripheral venous concentrations of toluene correlated roughly with exposure concentrations. Inter- and intraindividual variability were high enough to make this an insensitive estimate of exposure concentration or uptake (Von Oettingen et al., 1942a, 1942b; Veulemans and Masschelein, 1978b).

Although toluene appears to be absorbed less readily through the skin than through the respiratory tract, percutaneous absorption of liquid toluene may be significant. The maximum toluene concentration in peripheral venous blood of subjects who immersed one hand in liquid toluene for 30 minutes was about 26% of the concentration in peripheral venous blood of subjects who inhaled 100 ppm toluene vapor for 30 minutes (Sato and Nakajima, 1978). Absorption of toluene vapor through the skin in humans, however, probably amounts to less than 5% of the total uptake through the respiratory tract under the same conditions of exposure (Riihimaki and Pfaffli, 1978; Piotrowski, 1967; reviewed in NIOSH, 1973). Absorption of toluene through the gastrointestinal tract appears to be fairly complete, based on the amounts of toluene and its metabolites excreted by

experimental animals after administration of toluene (Pyykko et al., 1977; El Masri et al., 1956; Smith et al., 1954).

Toluene appers to be distributed in the body in accordance with the tissue/blood distribution coefficients and its metabolic and excretory fate. Thus, toluene itself is found in high concentrations in adipose tissue and bone marrow, and toluene and its metabolites are found in moderately high concentrations in liver and kidney (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a; Carlsson and Lindqvist, 1977; Pyykko et al., 1977; Bergman, 1979). The time course of toluene concentrations in the brain appeared to correlate with behavioral effects (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a).

The major portion of inhaled or ingested toluene is metabolized by side-chain oxidation to benzoic acid, conjugated with glycine to form hippuric acid, and excreted in the urine. Regardless of the route of administration, dose, or species, 60 to 75% of the absorbed (inhalation) or administered (oral) toluene could be accounted for as hippuric acid in the urine (Veulemans and Masschelein, 1979; Ogata et al., 1970; El Masri et al., 1956). Much of the remaining toluene (9 to 18%) was exhaled unchanged (Nomiyama and Nomiyama, 1974b; Srbova and Teisinger, 1952, 1953; Smith et al., 1954). Two percent or less appeared in the urine as cresols and benzylmercapturic acid; these metabolites are of concern because they indicate formation of reactive intermediates that potentially could bind to tissue macromolecules. No evidence of covalent binding to tissue components has been detected, however, by autoradiography of mice that inhaled $^{14}C$-toluene (Bergman, 1979).

Most of the toluene absorbed by humans or animals after inhalation or oral exposure is excreted within 12 hours of the end of exposure (Ogata et al., 1970; Veulemans and Masschelein, 1979; Nomiyama and Nomiyama, 1978; Smith et al., 1954; Bergman, 1979). In experimental animals, elimination of toluene and its

metabolites from most tissues, including brain, was rapid; elimination from fat and bone marrow was slower (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a; Pyykko et al., 1977; Carlsson and Lindqvist, 1977).

In humans, the time course of desaturation after cessation of inhalation exposure appeared to consist of 3 exponential phases with half-lives of 1.95, 35.2, and 204 minutes for toluene concentrations in peripheral venous blood and 1.59, 26.5, and 221 minutes for toluene concentrations in alveolar air (Sato et al., 1974). Toluene concentrations in expired air or peripheral venous blood after the end of inhalation exposure were not reliable indicators of toluene uptake or of exposure concentrations because of the great variability among individuals (Veulemans and Masschelein, 1978a, 1978b; Astrand et al., 1972). Some of this variability, particularly in expired air concentrations, could be explained by differences in exercise load during exposure, in respiratory minute volumes after exposure, and in adipose tissue content (Veulemans and Masschelein 1978a, 1978b). Similarly, although the excretion of hippuric acid in the urine is roughly proportional to the degree of exposure to toluene, inter- and intra-individual variations in the physiological excretion of hippuric acid render quantitation of exposure or uptake from urinary hippuric acid concentration or excretion rates unreliable (Immamura and Ikeda, 1973; Veulemans et al., 1979; Veulemans and Masschelein, 1979; Ogata et al., 1971; Wilczok and Bienick, 1978; and others as reported in Section 13.4.).

# 14. CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY

## 14.1. CARCINOGENICITY

In the 24 month chronic inhalation study described in Section 12.2.2., CIIT (1980) concluded that exposure to toluene at concentrations of 30, 100, or 300 ppm did not produce an increased incidence of neoplastic, proliferative, inflammatory, or degenerative lesions in Fischer 344 male or female rats relative to unexposed controls. It should be noted, however, that the design of this study has been deemed inadequate in that the rats were not exposed to a maximum tolerated dose (MTD) of toluene (Powers, 1979).

The NCI/NTP Carcinogenesis Testing Program has initiated bioassays of commercial toluene in rats and mice exposed via inhalation and gavage (NTP, 1981). Prechronic testing is currently in progress.

Toluene has been utilized extensively as a solvent for lipophilic chemicals being tested for their carcinogenic potential when applied topically to the shaved skin of animals. Results of control experiments with pure toluene have been uniformly negative. Poel (1963), for example, applied toluene (volume not stated) to the shaved interscapular skin 3 times a week throughout the lifetime of 54 male SWR, C3HeB, and A/He mice and found no carcinogenic response. Coombs et al. (1973) treated the dorsal skin of 20 randomly bred albino mice with 1 drop of toluene (6 µℓ) twice a week for 50 weeks. There was no evidence of squamous papillomas or carcinomas in the mice one year following termination of exposure, although survival was only 35% (7 of 20). Doak et al. (1976) applied estimated toluene volumes of 0.05 to 0.1 mℓ/mouse to the backs of CF1, $C_3H$, and CBaH mice (approximately 25 mice of each sex of each strain) twice weekly for 56 weeks, and failed to elicit skin tumors or a significantly increased frequency of systemic tumors over untreated controls. It is not clear in these studies, however,

whether the toluene was applied under an occlusive dressing or allowed to evaporate. Lijinsky and Garcia (1972) did report a skin papilloma in 1 mouse and a skin carcinoma in a second mouse in a group of 30 animals that were subjected to topical applications of 16 to 20 μl of toluene twice a week for 72 weeks.

Frei and Kingsley (1968) examined the promoting effect of toluene in Swiss mice following initiation with 7,12-dimethylbenz[a]anthracene (DMBA). In this study, the ears of the mice were topically treated once with 0.1 ml of 1.5% DMBA in mineral oil and subsequently, beginning a week later, twice a week with the same volume of 100% toluene for 20 weeks. Results showed that 11 of 35 mice developed tumors (6 permanent, 5 regressing) compared with 8 of 53 negative controls treated with 100% mineral oil (Table 14-1). In 14 mice painted with 100% toluene but no DMBA initiator, 2 developed tumors (1 permanent, 1 regressing). In another study with an identical experimental design, Frei and Stephens (1968) similarly found that 100% toluene promoted a yield of tumors no different from that found in the controls (Table 14-1). In this study, a total of 7 tumors were found in 35 surviving mice treated with toluene following initiation with DMBA; the negative control group (DMBA followed by biweekly applications of mineral oil) had 8 skin tumors in 53 survivors after the 20 weeks.

## 14.2. MUTAGENICITY

14.2.1. Growth Inhibition Tests in Bacteria. The ability of toluene to induce DNA damage was evaluated in two studies by comparing its differential toxicity to wild-type and DNA repair-deficient bacteria (Fluck et al., 1976; Mortelmans and Riccio, 1980). Two species were tested with negative results: Escherichia coli W3110 (pol $A^+$) and p3478 (pol $A^-$) and Salmonella typhimurium SL4525 (rfa) ($rec^+$) and SL4700 (rfa) ($rec^-$). In the first study, Fluck et al. (1976) applied

TABLE 14-1

Epidermal Tumor Yield in 20 Week Two-Stage Experiments[a]

| DMBA | Promoting Agent | No. Surviving Mice | Tumor bearing survivors | Number of Tumors | | | Tumors per Survivor | Regressing Tumors (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Permanent | Regressing | Total | | | |
| + | None | 23[b] | NR | 0 | 0 | 0 | 0 | 0 | Frei and Kingsley, 1968 |
| + | 5% croton oil[e] | 33[b] | NR | 381 | 70 | 451 | 13.7 | 15.5 | |
| + | 100% toluene | 35[b] | NR | 6 | 5 | 11 | 0.31 | 45.4 | |
| + | 100% mineral oil | 53[b] | NR | 8 | 0 | 8 | 0.15 | 0 | |
| - | 5% croton oil[c] | 25[b] | NR | 1 | 2 | 3 | 0.11 | 66.6 | |
| - | 100% toluene | 14[b] | NR | 1 | 1 | 2 | 0.14 | 5.0 | |
| + | None | 23[d] | 4% | NR | NR | 1 | 0.04 | NR | Frei and Stephens, 1968 |
| + | 5% croton oil[c] | 33[e] | 88% | NR | NR | 352 | 10.7 | NR | |
| + | 100% | 35[d] | 11% | NR | NR | 7 | 0.2 | NR | |
| + | 5% croton oil | 53[e] | 11% | NR | NR | 8 | 0.15 | NR | |
| - | 5% croton oil[c] | 20[d] | 5% | NR | NR | 1 | 0.05 | NR | |
| - | 100% toluene | 14[d] | 0% | 0 | 0 | 0 | 0 | 0 | |

[a] Ears of Swiss mice treated once with 0.1 ml of 0.5% DMBA and subsequently, beginning 1 week later, twice a week with the promoting agent.

[b] Not specifically stated whether this is the number of surviving mice. Also, the number of mice at the start not stated.

[c] In mineral oil

[d] 30 mice at the start

[e] 60 mice at the start

NR = not reported

toluene (25 µℓ/plate) without metabolic activation directly to wells in the center of culture plates containing the E. coli and found no zones of growth inhibition with either strain. In the Mortelmans and Riccio (1980) study, growth inhibition was also found to be comparable with both the repair competent and deficient strains of the E. coli and S. typhimurium when sterile filter discs inoculated with 0.001 to 0.01 µℓ toluene were placed in the centers of culture plates; these assays were performed both with and without metabolic activation. Mortelmans and Riccio (1980) further found that toluene (0.001 to 0.01 µℓ/plate) was not differentially toxic to either strain of the E. coli or S. typhimurium in quantitative growth inhibition tests. In the quantitative assays, the toluene was preincubated in liquid suspension with the bacteria, with and without S-9 activation, prior to plating; following plate incubation, the numbers of surviving cells were counted (instead of recording measurements of diameters of zones of growth inhibition).

14.2.2. Tests for Gene Mutations

14.2.2.1. ASSAYS USING BACTERIA AND YEAST — Toluene has been reported to be non-mutagenic in the Ames Salmonella assay when tested with strains TA1535, TA1537, TA1538, TA98, and TA100 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980; Nestmann et al., 1980; Bos et al., 1981; Snow et al., 1981), and in the E. coli WP2 reversion to $trp^+$ prototrophy assay (Mortelmans and Riccio, 1980). The details of these studies are summarized in Table 14-2. All assays were performed in the presence and in the absence of Aroclor 1254-induced rat liver homogenate (S-9) and employed positive and negative controls. It should be noted that there may have been significant losses of toluene from the culture media during incubation in all but one of the aforementioned studies (Snow

TABLE 14-2

Microbial Mutagenicity Assays

| Type of Assay | Strain | Metabolic Activation[a] | Dose | Application | Response | Reference |
|---|---|---|---|---|---|---|
| **Reverse Mutation** | | | | | | |
| S. typhimurium | TA 98, 100, 1535, 1537, 1538 | yes and no | 0.001 to 5.0µl/ plate | Plate incorporation | - | Litton Bionetics, Inc., 1978a |
| S. typhimurium | TA98, 100, 1535, 1537, 1538 | yes and no | 0.004 to 0.031%[b] | Liquid suspension | - | |
| S. typhimurium | TA98, 100, 1535, 1537, 1538 | yes and no | 0.01 to 10µl/plate | Plate incorporation | - | Mortelmans and Riccio, 1980 |
| S. typhimurium | TA98, 100, 1535, 1537, 1538 | yes and no | 5 µl/plate | Plate incorporation | - | Westmann et al., 1980 |
| S. typhimurium | TA98, 100, 1535, 1537, 1538 | yes and no | 0.115 to 2.3 µl/ plate | Plate incorporation | - | Bos et al., 1981 |
| S. typhimurium | TA98, TA100 | yes and no[c] | 0.3 µl to 100 µl/ plate | Plate incorporation[d] | - | Snow et al., 1981 |
| | | yes and no[c] | 11 to 3764 ppm | Vapor exposure[e] | - | |
| E. coli | WP2 | yes and no | 0.01 to 10 µl/ plate | Plate incorporation | - | Mortelmans and Riccio, 1980 |
| S. cerevisiae | D7 | yes and no | 0.001 to 0.5%[f] | Liquid suspension | - | Mortelmans and Riccio, 1980 |
| **Mitotic Gene Conversion** | | | | | | |
| S. cerevisiae | D4 | yes and no | 0.001 to 5.0µl/ plate | Plate incorporation | - | Litton Bionetics, Inc., 1978a |
| | | yes and no | 0.13% to 1.1%[b] | Liquid suspension | - | |
| S. cerevisiae | D7 | yes and no | 0.001 to 5.0%[f] | Liquid suspension | - | Mortelmans and Riccio, 1980 |
| **Mitotic Crossing-Over** | | | | | | |
| S. cerevisiae | D7 | yes and no | 0.001 to 5.0%[f] | Liquid suspension | - | Mortelmans and Riccio, 1980 |

[a] Aroclor 1254-induced rat liver homogenate S-9 fraction
[b] 50% mortality at the highest dose
[c] The toluene was tested with toluene-induced S-9 as well as with Aroclor induced S-9.
[d] The plates were incubated in sealed plastic bags or chambers for part of a 72-hr incubation period; in the Aroclor-induced S-9 tests, the plates were removed from the bags after 48 hr, counted, incubated an addition 24 hr, and recounted; in the experiments with toluene-induced S-9 the plates were removed after 24 hr to prevent moisture and spreading problems, and then incubated an additional 48 hr before counting.
[e] The assays were run in a sealed incubation chamber with a second glass plate (open) which contained the toluene; after 24 hr the chambers were opened and the plates incubated for an additional 48 hr.
[f] 100% mortality at 0.1% and 0.5%

et al., 1981), particularly at the higher doses tested. Snow et al. (1981) conducted plate incorporation assays in sealed plastic bags and chambers as well as vapor exposures in desiccators to prevent excessive evaporation. The design of the Snow et al. (1981) study is also noteworthy, because the toluene was tested with toluene-induced rat liver S-9 fraction as well as with Aroclor-induced S-9.

Toluene, with and without metabolic activation, was also tested in S. cerevisiae for its ability to induce reversions to isoleucine independence in strain D7 (Mortelmans and Riccio, 1980), mitotic gene conversion to tryptophan independence in strains D4 (Litton Bionetic, Inc., 1978a) and D7 (Mortelmans and Riccio, 1980), and mitotic crossing over at the ade2 locus in strain D7 (Mortelmans and Riccio, 1980). Toluene did not elicit a positive mutagenic response in any of these tests (Table 14-2).

14.2.2.2. TK MUTATION IN L5178Y MOUSE LYMPHOMA CELLS — Litton Bionetics, Inc. (1978a) reported that toluene failed to induce specific locus forward mutation in the L5178y Thymidine Kinase (TK) mouse lymphoma cell assay. Toluene was tested at concentrations of 0.05 to 0.30 µl/ml, with and without mouse liver S-9 activation.

14.2.3. Test for Chromosomal Mutations

14.2.3.1 MICRONUCLEUS TEST IN MICE — It was recently reported by SRI International (Kirkhart, 1980) that the intraperitoneal administration of toluene to male Swiss mice failed to cause an increase in micronucleated polychromatophilic erythrocytes in the bone marrow. Doses of 250, 500, and 1000 mg/kg were administered to groups of 32 mice at 0 and 24 hours, with

sacrifices 30, 48, and 72 hours after the first dose (8 mice/sacrifice). Five hundred polychromatic erythocytes per animal were evaluated for the presence of micronuclei. The highest dose tested (1000 mg/kg) approximated the $LD_{50}$ for male mice (Koga and Ohmiya, 1978).

14.2.3.2. MOUSE DOMINANT LETHAL ASSAY -- Toluene was recently evaluated for its ability to induce dominant lethal mutations in sperm cells of CD-1 male mice (Litton Bionetics, Inc., 1981). Test mice (12 per dose) were exposed via inhalation to targeted exposure levels of 100 and 400 ppm 6 hours per day, 5 days per week for 8 weeks. Twelve negative control mice were exposed to filtered air in an identical exposure regimen, and 12 positive control mice were injected intraperitoneally with 0.3 mg/kg triethylenemelamine (TEM) on day 40 of the dosing schedule. Following treatment, the males were mated sequentially to 2 females per week for each of 2 weeks; 14 days after the midweek of mating, each female was sacrificed using $CO_2$ and the number of living and dead implantations were counted. The results of this study showed that toluene did not cause any significant reduction in the fertility of the treated males, and did not cause increases in either pre- or post-implantation loss of embryos when compared with the negative controls. A significant induction of dominant lethal mutations was observed in the positive control mice.

14.2.3.3. CHROMOSOME ABERRATION STUDIES -- Two reports from the Russian literature concluded that toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection (Dobrokhotov, 1972; Lyapkalo, 1973). In an analysis of 720 metaphases from the bone marrow of 5 rats that had been subcutaneously injected with 0.8 g/kg/day toluene for 12 days, Dobrokhotov (1972) found that 78 (13%) showed aberrations. Sixty-six percent of the

aberrations were chromatid breaks, 24% were chromatid "fractures", 7% were chromosome "fractures", and 3% involved multiple aberrations. The frequency of spontaneous aberrations in 600 marrow metaphases from 5 control rats injected with vegetable oil averaged 4.16% (65.8% were breaks and 32.4% were chromatid aberrations; no "fractures" or multiple injuries were recorded.). It was further found that similar administration of 0.2 g/kg/day of benzene induced a frequency of chromosomal damage (13.6%) comparable to that of 0.8 g/kg/day of toluene, and that when a mixture of 0.2 g/kg benzene and 0.8 g/kg toluene was injected daily for 12 days, the damage was approximately additive (33.33% aberrations). The significance of the positive clastogenic effects attributed to toluene is difficult to assess, however, because the purity of the sample employed was not stated, and because the distinction between chromatid breaks and fractures is unclear.

Lyapkalo (1973) administered 1 g/kg/day toluene to 6 rats and 1 g/kg/day benzene to 8 rats by subcutaneous injection for 12 days. Treatment with toluene reportedly resulted in chromosome aberrations in 11.6% of the bone marrow cells examined (84 aberrant metaphases/724 cells) compared with 3.87% (40/1033) in olive oil injected controls. The types of aberrations that were observed consisted of "gaps" (60.47%), chromatid breaks (38.37%) and isocromatid breaks (1.16%). Benzene caused a greater degree of chromosome damage than the toluene (57.2% of the cells examined had aberrant chromosomes (573/1002)), and the distribution of aberration types was different (44.72% "gaps", 50.94% chromatid breaks, 4.34% isochromatid breaks). The purity of the toluene used in this study was also not stated.

In a third Russian study, Dobrokhotov and Einkeev (1975) reported that rats exposed to 80 ppm (610 $mg/m^3$) toluene via inhalation, 4 hours daily for 4 months, showed damaged metaphase chromosomes in 21.6% of the bone marrow cells

analyzed. The percentage of metaphases with damaged chromosomes in bone marrow cells from air-exposed control rats was 4.02%. Inhalation of 162 ppm benzene caused damage to chromosomes in 21.56% of the marrow cells, and a mixture of the toluene and benzene (80 and 162 ppm, respectively) damaged chromosomes in an additive manner (41.21% of the cells were involved). Chromosome damage was also observed in all of the groups 1 and 2.5 months after the initial exposure, and one month after the end of exposure, the frequency of chromosome damage was still elevated. A total of 96 rats were used in this study, but the number of rats in each group was not stated; it should also be emphasized that the number of cells scored and the purity of the toluene used were not reported.

In contrast to the aforementioned Russian cytogenetics studies, Litton Bionetics, Inc. (1978) found that intraperitoneal injection of pure toluene into Charles River rats did not induce bone marrow chromosomal aberrations. Toluene was injected at dose levels of 22, 71, and 214 mg/kg in 2 different experiments. In 1 study, 5 rats were sacrificed at 6, 24, and 48 hours following injection of each dose; in a second study, 5 rats were dosed daily at each level for 5 days, and the rats were sacrificed 6 hours after injection of the last dose. Approximately 50 cells per animal were scored for damage. Dimethyl sulphoxide (DMSO; the solvent vehicle) administered intraperitoneally at 0.65 mℓ/rat was used as a negative control, and triethylenemelamine (TEM) in saline at 0.3 mg/kg was used as a positive control. The results of the bone marrow cytogenetic analyses following sacrifice are summarized in Table 14-3. It was also noted that none of the observed aberrations differed significantly in frequency or type from either concurrent or historical spontaneous values.

Gerner-Smidt and Friedrich (1978) reported that toluene at concentrations of 1.52, 152, and 1520 µg/mℓ did not influence the number of structural chromosomal aberrations in cultured human lymphocytes. Benzene and xylene at the same

TABLE 14-3

Rat Bone Marrow Cell Aberrations Following Intraperitoneal Injection of Toluene[a]

| Treatment[b] | Dose | Time of Sacrifice | No. of Animals | Total No. of Cells | Type and Frequency of Aberrations | | No. of Cells With One or More Aberrations | No. of Animals Without Aberrations | Mitotic Index[e] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Structural[d] | Numerical | | | |
| DMSO (Solvent) | 0.65 ml/rat | 6 h | 5 | 225 | 2f,1td | -- | 3 (1.3%) | 3 | 3.8 |
| | | 24 h | 5 | 250 | -- | -- | 0 (0.0%) | 5 | 6.0 |
| | | 48 h | 5 | 250 | 1tb,1f | -- | 2 (0.8%) | 4 | 6.1 |
| | | 6 h (SA)[c] | 5 | 227 | 1td | -- | 1 (0.4%) | 4 | 5.0 |
| Triethylene Melamine | 0.3 mg/kg | 24 h | 5 | 250 | 11tb,2sb,5af,45f, 26t,1r,10td,12>, 1pu,1qr,2ao,3tr | 2pp | 72 (28.8%) | 0 | 1.4 |
| Toluene | 22 mg/kg | 6 h | 5 | 250 | -- | -- | 0 (0.0%) | 5 | 3.4 |
| | | 24 h | 5 | 242 | -- | -- | 0 (0.0%) | 5 | 5.9 |
| | | 48 h | 5 | 250 | -- | -- | 0 (0.0%) | 5 | 7.0 |
| | | 6 h | 5 | 238 | 3f | -- | 2 (0.8%) | 3 | 6.3 |
| Toluene | 71 mg/kg | 6 h | 5 | 239 | 1td | 1pp | 2 (0.8%) | 4 | 2.5 |
| | | 24 h | 5 | 227 | 2td,1af,1f | -- | 4 (1.8%) | 3 | 4.3 |
| | | 48 h | 3 | 150 | -- | -- | 0 (0.0%) | 3 | 5.7 |
| | | 6 h | 5 | 212 | -- | -- | 0 (0.0%) | 5 | 3.3 |
| Toluene | 214 mg/kg | 6 h | 5 | 250 | 1f | 2pp | 3 (1.2%) | 3 | 4.5 |
| | | 24 h | 5 | 250 | -- | 1pp | 1 (0.4%) | 4 | 3.6 |
| | | 48 h | 5 | 250 | 1tb,1td | -- | 2 (0.8%) | 3 | 5.4 |
| | | 6 h (SA)[d] | 5 | 250 | 1td,3af | -- | 2 (0.8%) | 3 | 5.4 |

[a]Source: Litton Bionetics, Inc., 1978a

[b]The toluene used was 99.96 wt. % pure (ethylbenzene, 0.03%; p-xylene, <0.01%; m-xylene, <0.01%; sulfur, 0.4 ppm) (Foulo, 1981).

[c]SA = subacute study; rats were dosed daily for 5 days, with sacrifice 6 hours after the last dose

[d]af = acentric fragment (2 tid); f = fragments; pp = polyploid; pu = pulverized chromosome; qr = quadriradial; r = ring; sb = chromosome break; t = translocation; tb = chromatid break; td = chromatid deletion; tr = triradial; > = greater than 10 aberrations

[e]Based on a count of at least 500 cells per animal

concentrations also had negative clastogenic effects but toluene (152 and 1520 μg/mℓ) and xylene (1520 μg/mℓ) caused a significant cell growth inhibition which was not observed with benzene. The data from this study cannot be adequately evaluated, however, because the source and purity of the toluene were not stated, no positive control experiments were performed, no metabolic activation system was employed, and the type of chromosome damage scored was not specified.

Peripheral blood lymphocytes of toluene-exposed rotogravure workers have also been examined for chromosome aberrations with negative results. In one study, Forni and coworkers (1971) examined the lymphocyte chromosomes from 34 workers from a single plant and 34 controls from outside the plant matched for age and sex. Ten of the workers were exposed daily to minimum concentrations of 131 to 532 ppm benzene for 2 to 7 years and subsequently to toluene in the general range of 200 to 400 ppm for 14 years; 24 of the workers were exposed only to toluene for 7 to 15 years. (The ink solvent used in this plant was changed from benzene to toluene which contained some xylene, but reportedly no benzene, after an outbreak of benzene poisoning in 1954.) No significant differences were found between the toluene and control groups in frequencies of stable and unstable chromosome aberrations or in chromosome counts (Table 14-4). Approximately 100 metaphases from each subject or control were scored. The proportion of chromosome changes were significantly higher statistically in the benzene/toluene group compared with controls, and in the benzene/toluene group relative to the toluene group.

Maki-Paakkanen et al. (1980) recently found no evidence of clastogenicity in cultured peripheral blood lymphocytes from 32 printers and assistants from 2 different rotoprinting factories who had a history of exposure to pure toluene (benzene concentration, $\leq$0.05%; average benzene concentration, 0.006%) at 8 hour time-weighted average (TWA) concentrations of 7 to 112 ppm. The average age of

TABLE 14-4

Frequency of Unstable and Stable Chromosome Changes and Chromosome Counts in Subjects Exposed to Benzene or Toluene or Both[a]

| Expsoure Subjects | No. of Cases | Age Range | Total Cells Counted | % Cells | | % Cells With Chromosome Number | | |
|---|---|---|---|---|---|---|---|---|
| | | | | $C_u$[b] | $C_s$[c] | <46 | 46 | >46 (Polyploid) |
| Benzene (+ toluene) | 10 | 36-54 | 964 | 1.66(1.87)[d,e,f] | 0.62[e,f] | 13.1 | 86.0 | 0.9(0.52) |
| Toluene | 24 | 29-60 | 2,400 | 0.80(0.83)[d] | 0.08 | 14.3 | 85.4 | 0.3(0.29) |
| Control subjects | 34 | 25-60 | 3,262 | 0.61(0.67) | 0.09 | 10.2 | 89.5 | 0.3(0.3) |

[a]Source: Forni et al., 1971

[b]Cells with "unstable" chromosome aberrations (fragments, dicentrics, ring chromosomes). The presence of each fragment was considered as one break, the presence of a dicentric or ring chromosome as two breaks.

[c]Cells with "stable" chromosome changes (abnormal monocentric chromosomes due to deletions, translocations, etc., trisomies)

[d]Numbers in parentheses show percentage of calculated breaks.

[e]Difference from toluene group was significant ($P < 0.05$)

[f]Difference from control was significant ($P < 0.01$)

the workers was 34.2 years and the average length of employment was 14.6 years. Results of analyses showed that when frequencies of chromosome aberrations were compared with those of 15 unexposed research institute workers, there were no significant differences (Table 14-5). Similarly, no significant deviations were observed in the frequencies of aberrations in relation to duration of exposure.

In a report on chromosome aberrations of women in laboratory work, Funes-Cravioto et al. (1977) also presented data on 14 workers who were exposed to toluene in a rotogravure factory. Exposures ranged from 1.5 to 26 years and air measurements of toluene showed TWA values of 100 to 200 ppm, with occasional rises up to 500 to 700 ppm; the exposures were sufficient in most cases to elicit frequent headaches and fatigue, and occasional vertigo, nausea, and feelings of drunkenness. The workers had been exposed to toluene since approximately 1950; before 1958, it was stated that the toluene was probably contaminated by a "low" percentage of benzene. Results of lymphocyte analysis showed an excess of chromosome aberrations (abnormal chromosomes and breaks) in the 14 toluene-exposed workers relative to a control group of 42 adults. It should be noted, however, that only a small number of subjects were examined in this study and the exposure background (e.g., extent of exposure to benzene and other chemicals) of the group was not well characterized. The results of this study are presented in Table 14-6. The results of chromosome analyses of 8 other workers with definite exposure to benzene (concentration not measured) for 2 to 10 years prior to 1950, and subsequently to toluene as stated above, are included for comparison.

14.2.3.4. SISTER CHROMATID EXCHANGE -- Gerner-Smidt and Friedrich (1978) reported that in vitro exposure to toluene at concentrations of 15.2, 152, and 1520 μg/mℓ had no effect on the number of sister-chromatid exchanges (SCEs) in cultured human lymphocytes, but no positive control experiments were performed

TABLE 14-5

Effect of Occupational Toluene Exposure and Smoking on Chromosomal Aberrations and Sister Chromatid Exchanges[a]

| Occupational Toluene Exposure (yr) | No. of Subjects | Mean Age (yr) | Cells Analyzed[b] | Cells with Chromosomal Aberrations (%) — Gaps Excluded: Chromatid Type | Gaps Excluded: Chromosome Type | Gaps Excluded: Total | Gaps Included: Total | Sister Chromatid Exchanges (SCEs): Cells Analyzed[c] | Sister Chromatid Exchanges (SCEs): Mean per Subject per Cell[d] |
|---|---|---|---|---|---|---|---|---|---|
| Total Worker (14.6 yr average exposure) | 32 | 34.2[e] | -- | 1.0 | 0.5 | 1.5 | 2.5 | -- | 8.5 |
| Total Control | 15 | 34.2[e] | -- | 0.7 | 0.9 | 1.6 | 2.7 | -- | 8.9 |
| 0 (controls) | | | | | | | | | |
| Nonsmokers | 4 | 31.0 | 800 | 0.5 | 0.8 | 1.3 | 2.3 | 234 | 8.0 |
| Smokers | 11 | 35.5 | 1100 | 0.9 | 1.0 | 1.8 | 3.1 | 318 | 9.7** |
| Total | 15 | 34.3 | 1900 | 0.7 | 0.9 | 1.6 | 2.7 | 552 | 9.2 |
| 1-10 (mean, 8.0) | | | | | | | | | |
| Nonsmokers | 3 | 27.7 | 300 | 0.7 | 0.3 | 1.0 | 2.3 | 79 | 7.9 |
| Smokers | 10 | 28.2 | 1000 | 0.7 | 0.3 | 1.0 | 1.9 | 295 | 9.1*** |
| Total | 13 | 28.1 | 1300 | 0.7 | 0.3 | 1.0 | 2.0 | 374 | 8.8 |
| >10 (mean, 19.3) | | | | | | | | | |
| Nonsmokers | 11 | 38.5 | 1100 | 0.8 | 0.5 | 1.4 | 2.5 | 330 | 7.5 |
| Smokers[f] | 8 | 35.9 | 800 | 1.8 | 0.8 | 2.5 | 3.1 | 205 | 9.6*** |
| Total | 19 | 37.5 | 1900 | 1.2 | 0.6 | 1.8 | 2.8 | 535 | 8.3 |

[a]Source: Maki-Paakkanen et al., 1980

[b]100 cells analyzed per individual

[c]30 cells analyzed per individual

[d]Calculated from individual means

[e]Mean value

[f]SCEs were analyzed from 7 subjects: **P < 0.01 and *** P < 0.001 compared to nonsmokers in the group, one-tailed Student's t-test

yr = year

TABLE 14-6

Chromosome Aberrations in Rotoprinting Factory Workers[a]

| | Group[b] | | |
|---|---|---|---|
| | Control | Toluene | Benzene/Toluene |
| No. of Subjects | 49 | 14 | 8 |
| Age (year) | | | |
| Range | 0.16 to 63 | 23 to 54 | 54 to 65 |
| Mean | 24.4 | 37.2 | 61.3 |
| No. of Cells Analyzed | | | |
| Total | 5000 | 1,400 | 800 |
| Abnormal | | | |
| Total | 217 | 108 | 76 |
| Frequency range (%) | 0 to 20 | 2 to 15 | 4 to 17 |
| Mean frequency (%) | 4.3 | 7.7 | 9.5 |
| No. of Chromosomes Analyzed | | | |
| Total | 230,000 | 64,400 | 36,800 |
| Breaks | | | |
| Total | 233 | 124 | 95 |
| Range (per 100 cells) | 0 to 22 | 2 to 17 | 6 to 17 |
| Mean (per 100 cells) | 5.1 | 8.9 | 11.9 |

[a]Source: Funes-Cravioto et al., 1977

[b]Exposure details provided in accompanying text.

and no metabolic activation system was employed. Twenty-six cells/dose were scored for SCEs and cytotoxicity was observed at the highest dose. Evans and Mitchell (1980) concluded that toluene did not alter SCE frequencies in cultured Chinese hamster ovary (CHO) cells. In the latter study, CHO cells without rat liver S-9 activation were exposed to 0.0025 to 0.04% toluene for 21.4 hours, and CHO cells with activation were exposed to 0.0125 to 0.21% for 2 hours.

In an analysis of cultured peripheral blood lymphocytes from 32 rotogravure workers with daily chronic exposure to 8 hour TWA concentrations of 7 to 112 ppm pure toluene, Maki-Paakkanen et al. (1980) found no increase in SCEs relative to a group of 15 unexposed control subjects. The average age of the workers was 34.2 years and their average length of employment was 14.6 years. The SCE analysis was part of a study examining chromosomal aberrations in these workers; the exposure history of the subjects is described in more detail with the summary of the aberration findings (Section 14.2.4.1.), and the results of the SCE analyses are included in Table 14-5.

Funes-Cravioto et al. (1977) studied SCE formation in groups of 4 rotogravure printers, 12 laboratory technicians, and 4 children of female laboratory technicians. The printers had been exposed to benzene during the 1940's for 2 to 10 years and subsequently to toluene; exposure to benzene and toluene ranged from 2 to 26 years. TWA concentrations of toluene generally ranged from 100 to 200 ppm (occasionally to 500 to 700 ppm), but benzene concentrations were not measured. The technicians also had a history of exposure to toluene, but the exposures were poorly characterized (duration and concentrations not stated) and each had considerable concurrent exposure to other solvents as well, particularly benzene and chloroform. Results of peripheral lymphocyte analysis (20 cells/individual scored) showed a statistically significant increase in SCEs in the laboratory technicians and the children of female technicians, but not in

the exposed printers; however, due to the nature of the exposure, the increases noted cannot be exclusively attributed to toluene.

## 14.3. TERATOGENICITY

14.3.1. Animal Studies. Toluene was reported in a recent abstract to be teratogenic to CD-1 mice following oral exposure (Nawrot and Staples, 1979). Toluene was administered by gavage from days 6-15 of gestation at levels of 0.3, 0.5, and 1.0 mℓ/kg/day and from days 12 to 15 at 1.0 mℓ/kg/day. The vehicle used was cottonseed oil (0.5% of maternal body weight per dose). A significant increase in embryonic lethality occurred at all dose levels when administered on days 6 to 15, and a significant reduction in fetal weight was measured in the 0.5 and 1.0 mℓ/kg groups. Exposure to 1.0 mℓ/kg toluene on days 6 to 15 also significantly increased the incidence of cleft palate; this effect reportedly did not appear to be due merely to a general retardation in growth rate. When toluene was administered at 1.0 mℓ/kg on days 12 to 15, however, decreased maternal weight gain was the only effect observed. Maternal toxicity was not noted after exposure to toluene on days 6 to 15 at any dose level. It should be emphasized that the numbers of mice exposed and the numbers of fetuses examined were not stated in the available abstract of this study; a complete copy of this report is not available for review but has been submitted for publication.

Hudak and Ungvary (1978) recently concluded that toluene was not teratogenic to CFLP mice or CFY rats when administered via inhalation according to the following schedule:

| | Dose | Days of Pregnancy | Duration |
|---|---|---|---|
| CFPL mice | 133 ppm (500 mg/m$^3$) | 6-13 | 24 hours/day |
| | 399 ppm (1500 mg/m$^3$) | 6-13 | 24 hours/day |
| CFY rats | 266 ppm (1000 mg/m$^3$) | 1-21 | 8 hours/day |
| | 399 ppm (1500 mg/m$^3$) | 1-8 | 24 hours/day |
| | 399 ppm (1500 mg/m$^3$) | 9-14 | 24 hours/day |

It was found that the entire group of mice exposed to 399 ppm toluene died within 24 hours. Toluene administered to rats at 399 ppm also had an effect on maternal survival, but none of the exposures adversely affected the incidence of external or visceral malformations in either species relative to air-exposed controls (Table 14-7). An increased incidence of skeletal anomalies (fused sternebrae, extra ribs) was observed, however, in the rats exposed continuously to 399 ppm toluene on days 9 to 14, and signs of retarded skeletal development (including poorly ossified sternebrae, bipartite vertebra centra, and shortened 13th ribs) were found in the rats exposed on days 1 to 8 (399 ppm) and during the entire period of pregnancy (days 1 to 21) at 266 ppm for 8 hours/day. An embryotoxic effect of toluene was further indicated by low fetal weights in the mice, and in the rats exposed on days 1 to 8 of pregnancy. Fetal loss (percent of total implants), mean litter size, mean placental weight, and maternal weight gain were unaffected by exposure in either species.

In a more recent teratogenicity study, groups of 20 CFY rats were exposed to 266 ppm (1000 mg/m$^3$) toluene, 125 ppm (400 mg/m$^3$) benzene, or a combination of these concentrations of toluene and benzene vapor for 24 hours/day on days 7 to 14 of gestation (Tatrai et al., 1980). A group of 22 rats inhaling pure air served as controls, and the fetuses were examined on day 21 of pregnancy. The results of the toluene exposures in this study are consistent with those of Hudak and Ungvary's continuous 399 ppm toluene exposures with rats on days 9 to 14 of gestation. Tatrai et al. (1980) concluded that continuous exposure to 266 ppm toluene was not teratogenic (no external, internal, or skeletal malformations

TABLE 14-7

Teratogenicity Evaluation of Toluene in CFY Rats and CF1P Mice[a]

| | Rats | | | | | Mice | | |
|---|---|---|---|---|---|---|---|---|
| | Air Inhalation | Toluene | | Air Inhalation | Toluene | Air Inhalation | Toluene | |
| | Days 1 to 21 8 h/d | 266 ppm Days 1 to 21 8 h/d | 399 ppm Days 1 to 8 24 h/d | Days 9 to 14 24 h/d | 399 ppm Days 9 to 14 24 h/d | Days 6 to 13 24 h/d | 133 ppm Days 6 to 13 24 h/d | 399 ppm Days 6 to 13 24 h/d |
| No. pregnant animals examined | 10 | 10 | 9 | 26 | 19 | 14 | 11 | 0 |
| No. pregnant animals died | 0 | 0 | 5 | 0 | 2 | 0 | 0 | 15 |
| Maternal weight gain[b] (g) | 46.6 | 44.1 | 44.0 | 46.9 | 41.8 | -- | -- | |
| No. live fetuses | 111 | 133 | 95 | 388 | 213 | 124 | 112 | 0 |
| No. resorbed fetuses | 8 | 3 | 6 | 15 | 18 | 6 | 10 | 0 |
| No. dead fetuses | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Fetal loss (%) | 6.7 | 2.2 | 5.9 | 4.1 | 7.8 | 6.1 | 8.2 | 0 |
| Mean litter size | 11.1 | 13.3 | 10.6 | 13.4 | 11.2 | 9.0 | 10.2 | -- |
| Mean fetal weight (g) | 3.8 | 3.6 | 3.3* | 3.8 | 3.8 | 1.1 | 1.0* | -- |
| Mean placental weight (g) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | -- | -- | -- |
| Weight retarded fetuses[c] (%) | 7.2 | 16 | 46** | 6.9 | 17.3 | 6.5 | 27.6** | -- |
| External malformations[d] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -- |
| No. fetuses dissected[e] | 54 | 64 | 49 | 179 | 110 | 64 | 58 | 0 |
| Internal malformations[f] | | | | | | | | |
| Anophthalmia | 0 | 0 | 0 | 1 | 0 | 0 | 0 | -- |
| Hydrocephalus | -- | -- | 4 | -- | -- | -- | -- | -- |
| Hydronephorosis | 1 | 6 | 4 | 16 | 4 | 1 | 3 | -- |
| No. of Alizarin-stained fetuses | 57 | 69 | 42 | 169 | 102 | 60 | 54 | -- |
| Skeletal retardation signs[g] | 0 | 17** | 7** | 11 | 24** | 3 | 1 | -- |

TABLE 14-7 (cont.)

| | Rats | | | | | Mice | | |
|---|---|---|---|---|---|---|---|---|
| | Air Inhalation | Toluene | | Air Inhalation | Toluene | Air Inhalation | Toluene | |
| | Days 1 to 21 8 h/d | 266 ppm Days 1 to 21 8 h/d | 399 ppm Days 1 to 8 24 h/d | Days 9 to 14 24 h/d | 399 ppm Days 9 to 14 24 h/d | Days 6 to 13 24 h/d | 133 ppm Days 6 to 13 24 h/d | 399 ppm Days 6 to 13 24 h/d |
| **Skeletal anomalies** | | | | | | | | |
| Fused sternebrae | 0 | 0 | 0 | 2 | 7[aa] | 0 | 0 | -- |
| Extra ribs | 0 | 0 | 0 | 0 | 22[aaa] | 0 | 0 | -- |
| **Skeletal malformations**[b] | | | | | | | | |
| Missing vertebrae | 0 | 0 | 0 | 0 | 2 | 0 | 0 | -- |
| Brachimelia | 0 | 0 | 0 | 0 | 0 | 1 | 0 | -- |

[a] Source: Hudak and Ungvary, 1978

[b] Percent of starting body weight

[c] Percent of living fetuses weighing <3.3 g (rats) or 0.9 g (mice)

[d] Agnathia, brachimelia, missing tail

[e] The rats were sacrificed on day 21 of pregnancy, the mice on day 18

[f] Thymus hypoplasia also looked for

[g] Including poorly ossified sternebrae, bipartite vertebra centra, and shortened 13th ribs

[h] Fissura sterni and agnathia also looked for

[aa] $P < 0.01$ ($t$-test); [aaa] $P < 0.05$ (Mann Whitney U Test); [aaa] $P < 0.01$ (Mann Whitney U Test)

h = hour; d = day

were reported), although the exposures were associated with evidence of skeletal retardation (not detailed) and an increased incidence of extra ribs (Table 14-8). It was additionally found that the incidence of extra ribs was higher in the group exposed to toluene in combination with benzene than in the groups exposed to toluene alone. Maternal loss, maternal weight gain, number of litters, mean implantation/dam, placental weight, fetal loss, and fetal weight loss were not significantly affected by the toluene exposures. Exposure to 125 ppm benzene did cause decreases in maternal weight gain, placental weight and fetal weight, but these effects appeared to be inhibited by concurrent exposure to 266 ppm toluene. Further, it was reported that post-implantation fetal loss (the number of dead and resorbed fetuses relative to the number of total implantation sites in percent) was significantly increased in the group exposed to benzene in combination with toluene; fetal loss was not, as indicated earlier, affected by exposure to the toluene (or benzene) alone.

In a third inhalation study, Litton Bionetics, Inc. (1978b) reported no evidence of teratogenicity in the 20 day old fetuses of Charles River rats that were exposed to 100 or 400 ppm toluene vapor for 6 hours/day on days 6 to 15 of gestation. Histological examinations revealed no unusual incidence of visceral or skeletal abnormalities (Table 14-9); unusual skeletal variations were observed in a small but comparable number of fetuses from both the exposed and control groups, but these changes were in most cases attributed to retarded bone ossification and were not considered to be malformations as such. It was also noted that there were no maternal deaths during this study, and that the sex ratio of the offspring did not differ significantly between the treated and control groups.

In a brief abstract, Roche and Hine (1968) noted that toluene was not teratogenic to either the rat fetus or the chick embryo. Parameters evaluated

TABLE 14-8

Teratogenic Effects of Exposure to Toluene, Benzene, and a Combination of Toluene and Benzene in CFY Rats[a]

| Inhalation on days 7 to 14 of pregnancy 24 h/d | Air | Toluene 266 ppm ($1000 mg/m^3$) | Benzene 125 ppm ($400 mg/m^3$) | Toluene/Benzene 266 ppm + 125 ppm (1000 mg/$m^3$ / 400 mg) | Significance of Interaction |
|---|---|---|---|---|---|
| Number of females | | | | | |
| treated | 21 | 20 | 20 | 20 | |
| died | — | — | — | — | — |
| non pregnant | 1 | 2 | 3 | 1 | |
| total resorption | — | — | — | — | |
| Number of liters | 21 | 18 | 17 | 19 | |
| Mean implantation/dam | 14.0 | 14.4 | 14.6 | 13.8 | |
| Maternal weight gain in % of starting body weight | 68.82 ±2.40 | 65.82 ±2.13 | 46.74*** ±2.69 | 53.94*** ±1.84 | $p < 0.05$ |
| Relative liver weight (%) | 4.25 ±0.08 | 4.37* ±0.07 | 4.67* ±0.12 | 4.10 ±0.09 | $p < 0.01$ |
| Mean placental weight (g) | 0.58 ±0.006 | 0.60 ±0.006 | 0.48*** ±0.006 | 0.54*** ±0.004 | $p < 0.05$ |
| Number of fetuses | 294 | 259 | 248 | 262 | |
| live | 280 | 239 | 236 | 234 | |
| dead | — | — | 2 | — | |
| resorbed | 14 | 20 | 10 | 28 | |
| Mean fetal weight (g) | 3.94 ±0.02 | 3.91 ±0.02 | 3.16*** ±0.03 | 3.79** ±0.02 | $p < 0.001$ |
| Weight retarded fetuses in % of living fetuses | 2.8 | 3.3 | 57.6** | 9.8* | |
| External malformations | — | — | — | — | |
| Fetal loss/total implantation sites (%) | 4.7 | 7.7 | 4.8 | 10.7* | |
| No. Alizarine-stained fetuses | 142 | 121 | 122 | 118 | |
| Skeletal retarded fetuses in % of Alizarine-stained fetuses | 13 | 31* | 77*** | 39* | |

TABLE 14-8 (cont.)

| Inhalation on days 7 to 14 of pregnancy 24 h/d | Air | Toluene 266 ppm ($1000 mg/m^3$) | Benzene 125 ppm ($400 mg/m^3$) | Toluene/Benzene 266 ppm + 125 ppm (1000 mg/$m^3$/400 mg) | Significance of Interaction |
|---|---|---|---|---|---|
| Skeletal anomalies | | | | | |
| sternum misaligned | 4 | 4 | 5 | 1 | |
| asymmetric vertebra | 1 | — | 3 | 1 | |
| extra ribs | 1 | 7+ | 1 | 19** | |
| Skeletal malformations | — | — | — | — | |
| No. fetuses dissected | 138 | 118 | 114 | 116 | |
| Internal malformations | | | | | |
| polycystic lungs | 1 | — | — | — | |
| pyelectasia | 2 | 5 | — | 1 | |
| dystopia renis | — | 1 | — | — | |
| vesica giganta | — | 3 | 1 | 1 | |
| microphthalmia | — | — | — | 1 | |
| anophthalmia | — | — | 2 | — | |
| hydrocephalus internus | — | — | 3 | — | |

[a]Source: Tatrai et al., 1980

+ = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ± = SEM

TABLE 14-9

Teratogenicity and Reproductive Performance Evaluation in Rats Exposed to Toluene[a]

| | Dose (ppm) | | |
|---|---|---|---|
| | 0 | 100 | 400 |
| Pregnancy ratio (Pregnant/Bred) | 26/27 | 27/27 | 27/27 |
| No. pregnant rats that died | 0 | 0 | 0 |
| Live litters | 26 | 27 | 26 |
| Implantation sites (Left Horn/Right Horn) | 152/194 | 181/177 | 179/190 |
| Resorptions | 26 | 28 | 41[b] |
| Litters with resorptions | 13 | 20 | 17 |
| Dead fetuses | 0 | 1 | 0 |
| Litters with dead fetuses | 0 | 1 | 0 |
| Live fetuses/implantation site | 320/346 | 329/358 | 328/369 |
| Mean live litter size (fetuses) | 12 | 12 | 12 |
| Average fetal weight (g) | 3.6 | 3.5 | 3.8 |
| Number of fetuses examine for soft tissue (visceral) changes[c] | 108(51/57) | 105(47/58) | 104(51/53) |
| Number of fetuses examined for skeletal changes[d] | 212 | 221 | 224 |
| Number of fetuses with normal skeletal examinations | 139 | 150 | 158 |
| Fetuses with commonly encountered skeletal changes[e,f] | 67(20) | 62(20) | 58(20) |
| Fetuses with unusual skeletal variations[f,g] | 6(4) | 9(4) | 8(6) |

[a] Source: Litton Bionetics, Inc., 1978b
[b] The increase in total resorptions at this dose was attributed to the total resorption of the litter of one particular female.
[c] Numbers of male/females examined in parentheses.
[d] Four specimens from one litter were not examined (missing).
[e] A qualitative examination of the observations recorded for the fetuses indicates that bilateral ribs, unilateral ribs, and reduced ossification of various bones were the most frequently encountered changes.
[f] Number of litters in parenthesis.
[g] These were generally cases of more severe and extensive retarded ossification.

included body weight, bone length, and gross abnormalities, but no dose or exposure information or other quantitative data were provided.

Elovaara et al. (1979b) injected toluene into the air space of developing chicken eggs at doses of 5, 25, 50, and 100 µmol/egg on the 2nd and 6th days of incubation. Survival incidence after 14 days of incubation appeared to be influenced only after injection of toluene on day 6 at 100 µmol/egg; the "approximate $LD_{50}$" for toluene was judged to be in excess of 100 µmol/egg. Macroscopic examination on day 14 indicated that only 3 of 46 of the chick embryos treated with 5 to 100 µmol/egg of toluene were malformed; 1 displayed profound edema and 3 had skeletal abnormalities (musculoskeletal defects of the lower extremities, but not wings).

McLaughlin et al. (1964) injected toluene at dose levels of 4.3, 8.7, and 17.4 mg into the yolk sac of fresh fertile chicken eggs before incubation. Following incubation, the percentages of hatch at the 3 doses were, respectively, 85%, 25%, and 0%. Teratogenic effects were not observed in either the eggs that failed to hatch or in the chicks that did hatch.

14.3.2. Human Reports. Holmberg (1979) gathered information on exposure to noxious agents during the pregnancies of 120 mothers of children with congenital CNS defects and their matched-pair controls. The matched-control mother is the mother whose delivery immediately preceded that of the case mother in the same Finnish maternity welfare district. Results showed that 14 of the 120 case mothers had been exposed more often than control mothers (3/120) to organic solvents during the first trimester of pregnancy. Among the 14 exposed mothers, 2 had been exposed to toluene. One of the toluene-exposed mothers (age 18) had reportedly been exposed in the metal products manufacturing industry (no other details of exposure given), and gave birth to a child that died after 2 hours and

showed internal congenital hydrocephaly and agenesis of the corpus callosum upon autopsy; other findings included pulmonary hypoplasia and a diaphragmatic hernia. The other mother was exposed to toluene concomitantly with other solvents (xylene, white spirit, methyl ethyl ketone) during rubber products manufacturing; her child was hydranencephalic and died 24 days after birth. It was noted that in this case parental age (maternal, 42 years; paternal, 44 years) and a previous child with brain injury (born 20 years previously, died at age 4) were more likely than the recent exposure to have predisposed the more recent child to the defect.

Toutant and Lippman (1979) described the birth of a child with "nearly classic" fetal alcohol syndrome to a 20 year old primigravida whose major addiction was to solvents (reportedly, primarily toluene). This woman had a 14 year history of daily heavy solvent abuse (no details provided) and a 3 year history of alcohol intake of about a six-pack of beer weekly. On admission, she exhibited signs compatabile with severe solvent and/or alcohol abuse (ataxia, resting and intention tremors, mild diffuse sensory deficits, short-term memory loss, and poor intellectual functioning). The child was born at term, was small (10th percentile in weight, 5th percentile in head size), and exhibited abnormal features that included microcephaly, a flat nasal bridge, hypoplastic mandible, short palpebral fissures, mildly low-set ears, pronounced sacral dimple, sloping forehead, and uncoordinated arm movements. It was noted that although solvent abuse rather than alcohol predominated in this mother's addiction pattern, the case seemed no different from reports of fetal alcohol syndrome.

## 14.4. SUMMARY

CIIT (1980) concluded that exposure to 30, 100, or 300 ppm toluene for 24 months did not produce an increased incidence of neoplastic, proliferative,

inflammatory, or degenerative lesions in rats relative to unexposed controls; the highest dose tested was not, however, a minimum toxic dose. Other studies indicate that toluene is not carcinogenic when applied topically to the shaved skin of mice (Poel, 1973; Linsky and Garcia, 1972; Coombs et al., 1973; Doak et al., 1976), and that it does not promote the development of epidermal tumors following initiation with DMBA (Frei and Kingsley, 1968; Frei and Stephens, 1968).

Toluene has yielded negative results in a battery of microbial, mammalian cell, and whole organism test systems. The microbial assays conducted include differential toxicity testing with wild-type and DNA repair-deficient strains of E. coli and S. typhimurium (Fluck et al., 1976; Mortelmans and Riccio, 1980), reverse mutation testing with various strains of S. typhimurium, E. coli WP2, and S. cerevisiae D7 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980; Nestman et al., 1980), and mitotic gene conversion and crossing-over evaluation in S. cerevisiae D4 and D7 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980). Toluene also failed to induce specific locus forward mutation in the L5178Y Thymidine Kinase mouse lymphoma cell assay (Litton Bionetics, Inc., 1978a), was negative in the micronucleus test in mice (Kirkhart, 1980), and was negative in the mouse dominant lethal assay (Litton Bionetics, Inc., 1981). Sister-chromatid exchange (SCE) frequencies were not altered in Chinese hamster ovary cells (Evans and Mitchell, 1980) or in human lymphocytes (Gerner-Smidt and Friedrich, 1978) cultured with toluene, or in the peripheral lymphocytes cultured from workers with a history of chronic exposure to toluene (Funes-Cravioto et al., 1977; Maki-Paakkanen et al., 1980).

In the Russian literature, chromosome aberrations were reported in the bone marrow cells of rats exposed subcutaneously (Dobrokhotov, 1972; Lyapkalo, 1973) and via inhalation (Dobrokhotov and Einkeev, 1977) to toluene. These findings

were not corroborated, however, in a Litton Bionetics, Inc. (1978b) study in rats following intraperitoneal injection, in cultured human lymphocytes exposed to toluene in vitro (Gerner-Smidt and Friedrich, 1978), or in lymphocytes from workers chronically exposed to toluene (Forni et al., 1971; Maki-Paakkanen et al., 1980). Funes-Cravioto et al. (1977) did report an excess of aberrations in the lymphocytes from 14 printers exposed to 100 to 200 ppm toluene for 1 to 16 years, but it is probable that part of the exposure was to benzene-contaminated toluene.

Toluene was reported in a recent abstract from NIEHS to induce cleft palates at a level of 1.0 mℓ/kg following oral exposure to mice on days 6 to 15 of gestation (Nawrot and Staples, 1979); significant increases in embryolethality and decreases in fetal weight were noted as well at doses as low as 0.3 m/kg/day and 0.5 m/kg/day, respectively. The teratogenic effect reportedly did not appear to be due merely to the general retardation in growth rate. Three other studies concluded that toluene is not teratogenic in mice (Hudak and Ungvary, 1978) or rats (Hudak and Ungvary, 1978; Litton Bionetics, Inc., 1978b; Tatrai et al., 1980) following inhalation exposure. Embryotoxic effects (increased incidence of skeletal anomalies and signs of retarded skeletal development, low fetal weights) and increased maternal mortality were noted, however, in some of the rats and mice exposed via inhalation. Injection of toluene into the yolk sac (McLaughlin et al., 1964) or air space (Elovaara et al., 1979b) of chicken eggs before incubation or during development, respectively, did not result in teratogenic effects.

# 15. SYNERGISMS AND ANTAGONISMS AT THE PHYSIOLOGICAL LEVEL

## 15.1. BENZENE AND TOLUENE

Animal studies have shown that benzene and toluene may be metabolized by similar enzyme systems in parenchymal cells of the liver. In the studies of Pawar and Mungikar (1975), the activities of hepatic aminopyrine N-demethylase, NADPH-linked peroxidation, and ascorbate-induced lipid peroxidation were reduced, while acetanilide hydroxylase was increased by either benzene pretreatment or toluene pretreatment in male rats. Induction of aminopyrine N-demethylase and components of the electron transport system was seen when the animals were given phenobarbital (Pawar and Mungikar, 1975; Mungikar and Pawar, 1967a, 1967b). When phenobarbital was coadministered with benzene or toluene, the changes in the activity of these enzymes produced by single administration of the xenobiotics were attenuated (Pawar and Mungikar, 1975). That induction of hepatic enzymes by phenobarbital affects metabolism of toluene is indicated by the reduction of toluene toxicity (decreased narcosis) in female rats or male mice given phenobarbital prior to intraperitoneal injection of toluene (Ikeda and Ohtsuji, 1971; Koga and Ohmiya, 1978) and the accelerated excretion of toluene metabolites from female rats as described in Sections 12.3. and 12.4. (Ikeda and Ohtsuji, 1971).

The following studies indicate that toluene has the potential for altering the bioactivity of benzene when given in sufficiently large quantities. When benzene was given in combination with toluene, the conversion of benzene to its metabolites (phenols) was suppressed in rats (Ikeda et al., 1972) and in mice (Andrews et al., 1977). Ikeda et al. (1972) administered a mixture of benzene and toluene (equivalent to 110 mg benzene/kg and 430 mg toluene/kg) intraperitoneally to female rats and observed a reduced excretion of total phenols. When

a mixture of toluene and benzene (110 mg toluene/kg and 440 mg benzene/kg) was administered, hippuric acid excretion was reduced up to 4 hours after injection. Induction of hepatic microsomal enzymes by phenobarbital prior to administration of the mixture alleviated the suppression.

Andrews et al. (1977) co-administered 440 or 880 mg/kg benzene and 1720 mg/kg toluene intraperitoneally to mice and found a significant reduction in urinary excretion of benzene metabolites and a compensatory increase of pulmonary excretion of unmetabolized benzene. When toluene and benzene were coadministered by subcutaneous injection, toluene did not significantly change the total amount of benzene found in fat, liver, spleen, blood, or bone marrow, but it did reduce significantly the accumulation of metabolites in these tissues. Coadministration of toluene and benzene also counteracted benzene-induced reduction of red cell $^{59}Fe$ uptake in developing erythrocytes, suggesting that the myelotoxicity of benzene might be attenuated by toluene-inhibition of benzene metabolism in the bone marrow. In an in vitro study of a liver microsome preparation, Andrews and coworkers (1977) determined that toluene is a competitive inhibitor of benzene metabolism.

In the studies of Ikeda et al. (1972) and Andrews et al. (1977), however, benzene and toluene were given intraperitoneally in large amounts. Sato and Nakajima (1979b) used doses in the range of 24.2 to 390.6 mg/kg of benzene and 28.6 to 460.8 mg/kg of toluene to assess concentrations which might be found in the workplace. They found that when benzene was given to rats in the range of 24.2 to 97.7 mg/kg, there was no significant difference in the rate of disappearance of benzene from the blood whether the benzene was administered singly or in combination with an equimolar amount of toluene. At a dose of 390.6 mg/kg benzene, an equimolar dose of toluene delayed the disappearance of benzene from blood, and the excretion of phenol was reduced. A dose-dependent inhibition of

the metabolism of benzene by toluene was found. In a study of human exposure, inhalation of a mixture of 25 ppm benzene and 100 ppm toluene for 2 hours did not exert any influence on the disappearance rate of benzene and toluene in either blood or end-tidal (alveolar) air as compared to inhalation of either solvent singly. Desaturation curves (concentration versus time) for blood or end-tidal air obtained for each solvent after inhalation of the specified mixture were virtually superimposable on desaturation curves obtained after inhalation of the same solvent (25 ppm benzene or 100 ppm toluene) by itself. These results indicate that in the range of threshold limit value "the pharmacokinetic processes . . . of absorption, distribution, excretion, and metabolism of either benzene or toluene are not influenced by simultaneous exposure to the other" (Sato and Nakajima, 1979b). The data for the single-solvent exposures had been published previously (Sato et al., 1974b); details of the experiment with toluene were discussed in Section 12.4.

## 15.2. XYLENES AND TOLUENE

When 0.1 mℓ/kg or 0.2 mℓ/kg toluene was co-administered with similar doses of m-xylene intraperitoneally into male rats, the amounts of hippuric and m-methylhippuric acid excreted in urine over a period of 24 hours were not different from the amount of metabolites formed by single injection of toluene or m-xylene. The velocity of excretion of metabolites in the simultaneously injected group was slightly delayed in comparison with that in singly injected groups. Thus, simultaneous administration of the compounds does not significantly interfere with the metabolism of either compound (Ogata and Fujii, 1979).

To study the excretion kinetic interactions between toluene and xylene, Riihimaki (1979) determined the conjugation and urinary excretion of metabolites of toluene and m-xylene, benzoic acid and methylbenzoic acid, respectively, in

vivo in one man. Forty-one millimoles benzoic acid or 7.4 mmol methylbenzoic acid was ingested singly or in combination by one adult human male. In the 25 to 30 hours that urine was collected after ingestion, the total recovery of the ingested compounds with the exception of one sample (dose excreted in that case: 84%) indicated that all excretion took place via the kidneys. The combined intake of methylbenzoic acid and benzoic acid did not significantly affect conjugation or excretion of either metabolite. This study indicates that during simultaneous exposures to toluene and m-xylene, even at a relatively high level of occupational exposure, conjugation and excretion of metabolites are not likely to be rate-limiting steps except under conditions of limited availability of glycine.

## 15.3. TOLUENE AND OTHER SOLVENTS

Simultaneous intraperitoneal injection of 1.18 g/kg toluene with 0.91 g/kg n-hexane into female rats did not affect the concentrations of n-hexane in the blood nor was excretion of hippuric acid affected by coadministraton of n-hexane (Suzuki et al., 1974).

Coadministration of ethanol by ingestion and of toluene by inhalation (4000 mg toluene/$m^3$, 6 hours daily, 5 days a week for 4 weeks) into rats did not change the electrocardiogram, hematocrit values, or histological and histochemical structure of the heart. Toluene increased vascular resistance of the myocardium and reduced cerebral blood flow, while alcohol ingestion reduced arterial blood pressure, the cardiac index, and blood flow to the myocardium, kidney, skin, and carcass. Myocardial and cutaneous vascular resistance, as well as cerebral blood flow, increased after alcohol ingestion. It was concluded that combined exposure to the two substances produced additive effects on myocardial vascular resistance (Morvai and Ungvary, 1979). During subchronic exposure of

rats to toluene and ethanol, there is a potentiation of microsomal and mitochondrial changes in the liver (Hudak et al., 1978).

In their study of joint toxic action, Smyth et al. (1969) suggested that perchloroethylene is capable of enhancing the toxicity of toluene administered orally in rats. Withey and Hall (1975) observed that administration by intubation into rats of trichloroethylene and toluene in combinations of mixtures at five different dose levels revealed a departure from an additive model. They concluded that the effect of co-administration of the solvents could not be described in terms of synergism or potentiation until further studies were made.

Ikeda (1974) observed that coadministration of trichloroethylene and toluene (730 mg/kg and 430 mg/kg, respectively) by the intraperitoneal route into rats reduced the amounts of metabolites of both solvents compared with amounts excreted after administration of either solvent alone.

# 16. ECOSYSTEM CONSIDERATIONS

## 16.1. EFFECTS ON VEGETATION

16.1.1. Introduction. Toluene volatilizes rapidly from solutions (Mackay and Wolkoff, 1973). Most studies investigating the phototoxicity of toluene have been with algae. Of these studies, only one (Dunstan et al., 1973) was done under conditions that maintained a nearly constant concentration of toluene in the culture medium throughout the experiment. Other studies were done with culture vessels capped with metal caps or with cotton plugs, allowing the toluene to volatilize and escape from the exposure solutions. Even though steady-state concentrations are lacking, these studies do approximate situations in the environment where a point source of toluene exists to a body of water. The discussion of these studies will, therefore, be under the headings of "closed" and "open" experimental systems.

16.1.2. Effects of Toluene on Plants.

### 16.1.2.1. ALGAE

16.1.2.1.1. Closed System Studies -- Dunstan et al. (1975) exposed 4 marine algal species to toluene concentrations ranging from 1 to $10^5$ µg/ℓ. Axenic algal cultures were inoculated at 18°C and grown with a 12-hour light/dark cycle under cool-white fluorescent light (4000 µW/cm$^2$, 380 to 700 nm) in filtered enriched seawater. To minimize loss of toluene by vaporization, the 125 mℓ Erlenmeyer flasks were made airtight with rubber stoppers. Experiments were never run beyond a cell density at which $CO_2$ limitations might limit growth.

The four species used were the diatom, Skeletonema costatum; the dinoflagellate, Amphidinium carterae; the coccolithophorid, Cricosphaera carterae; and the green flagellate, Dunaliella tertiolecta.

To illustrate the difficulty of establishing absolute concentration when working with toluene, Dunstan et al. (1975) observed the toluene concentrations at three intervals in stoppered flasks (Table 16-1). Eighty-four percent of the theoretial initial concentration was lost at the beginning of the experiment during the handling and dispensing of the toluene into culture flasks, even when the toluene was rapidly dispensed under sterile conditions.

Figure 16-1 shows how toluene can both stimulate and inhibit algal growth depending on the species and the concentration of toluene. The dinoflagellate, Amphidinium carterae was inhibited at all concentrations of toluene (1 to $10^5$ µg/ℓ) from 20 to 50%. The other three species however, were stimulated by 1 to $10^4$ µg/ℓ, but higher concentrations of toluene either had no effect (Dunaliella tertiolecta) or became inhibitory (Skeletonema costatum and Cricosphaera carterae). This work indicated that one of the most significant environmental effects was in the short-term selection of certain phytoplanktonic species by the growth stimulation brought about by low levels of toluene. Dunstan et al. (1975) concluded that the differential growth of phytoplanktonic species within the phytoplankton population ultimately determines the community structure, its succession, and its trophic relationship.

Potera (1975) evaluated the effect of toluene on saltwater phytoplankton dominated by Chlorella sp. using Warburg manometry. Toluene inhibited photosynthesis 29% at 34 mg/ℓ and 35% at 342 mg/ℓ (at 20°C). Respiration (at 20°C) was inhibited 62% at 34 mg/ℓ and 16% at 342 mg/ℓ.

TABLE 16-1

Concentrations of Toluene in Stoppered Flasks[a]

| Time of Measurement | Percent of Theoretical Concentration |
|---|---|
| Theoretical initial concentration | 100 |
| Measured initial concentration | 16 |
| Concentration after 3 days of growth | |
| Stoppered flask | 14 |
| Cotton-plugged flask | 1 |

[a]Source: Dunstan et al., 1975

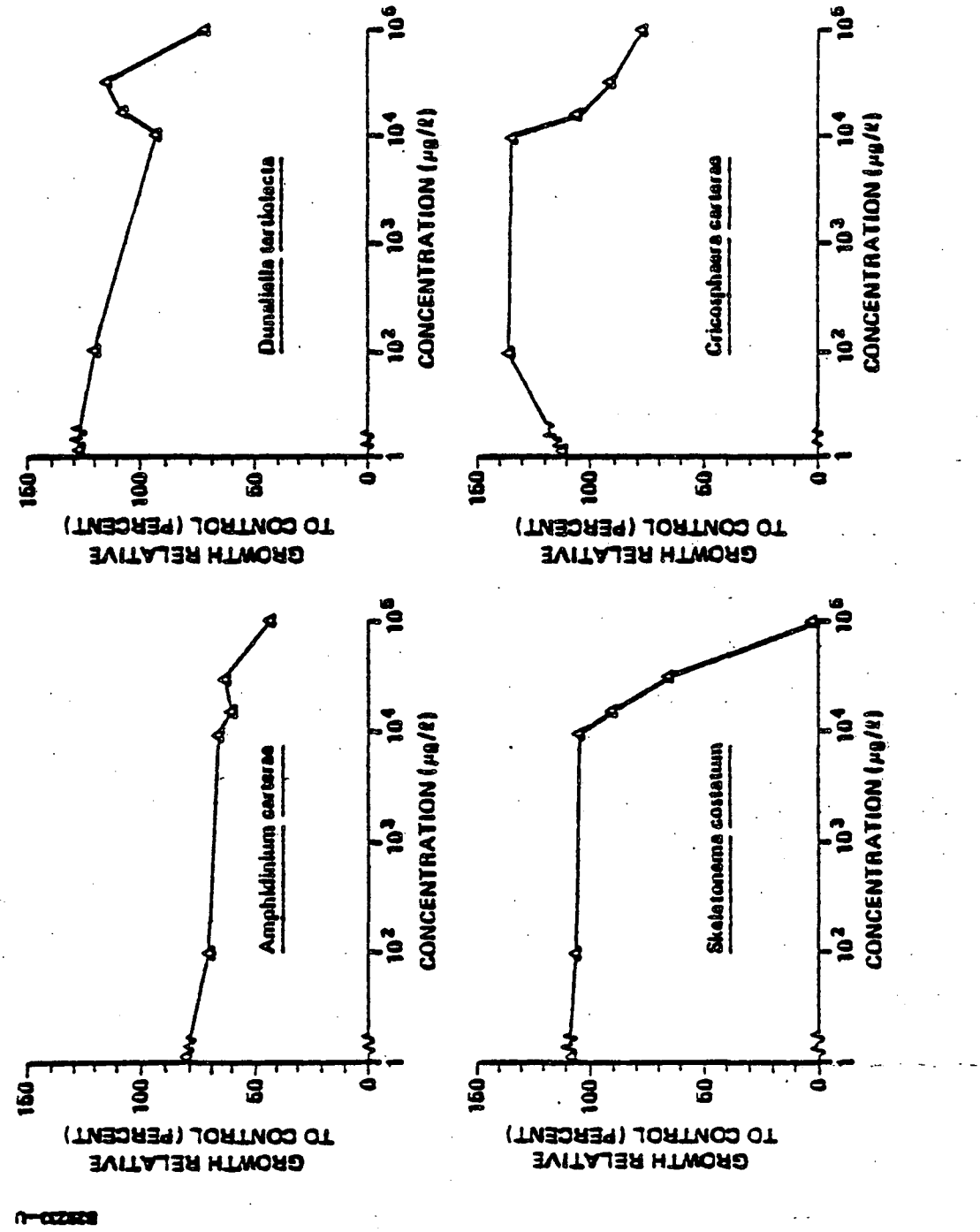


FIGURE 16-1

Phytoplankton Growth in Various Concentrations of Toluene (Organisms were grown in stoppered flasks. Growth, measured by cell numbers and in vivo chlorophyll, was determined on the 2nd and 3rd days of logarithmic growth. Concentrations of low molecular weight hydrocarbons are in theoretical values.)

Source: Dunstan et al., 1975

16.1.2.1.2. Open Studies -- Illustrative of the "open" type of experiment is that of Kauss and Hutchinson (1975). The freshwater alga, Chlorella vulgaris, was exposed to toluene for 10 days in 125 mℓ cotton-plugged Erlenmeyer flasks. Each flask was agitated to resuspend the cells daily. The concentrations listed in Figure 16-2 are nominal initial concentrations. In this open experiment, toluene was less toxic to the alga because the toluene concentration diminished by volatilization during the experiments. Comparison with controls revealed that a lag phase that lasted for one day existed between inoculation and commencement of growth for 50 and 100 mg/ℓ. Recovery was less rapid with 250 mg/ℓ. At concentrations approaching toluene saturation (i.e., 505 mg/ℓ), toluene was lethal to the cells.

Table 16-2 summarizes the toxic effects of toluene on algae. In assessing the toxicity of toluene to algae, both the inherent toxicity of toluene and the exposure time need to be considered. The no-effect concentration for most algal species studied appears to be at the 10 mg/ℓ level. The evaporation rate from solution (fresh or saltwater) however, rapidly diminishes the exposure concentration of toluene (Dunstan et al., 1975). The toxicity of toluene is more closely approximated by levels of 100 mg/ℓ in "open" systems, as shown by Kauss and Hutchinson (1975).

16.1.2.2. EFFECTS ON HIGHER PLANTS -- Currier (1951) exposed barley, tomatoes, and carrots to toluene vapor. Air at a flow rate of 11.5 ℓ/min passed through a small vaporizing chamber containing the toluene and into the top of a bell jar containing the plants. The concentration of toluene in the vapor chamber was varied by changing the temperature of the toluene. The concentration of vapor in the air was determined by measuring the amount of toluene evaporated per unit of time. Three tomatoes, 20 carrots, and 12 barley seedlings were

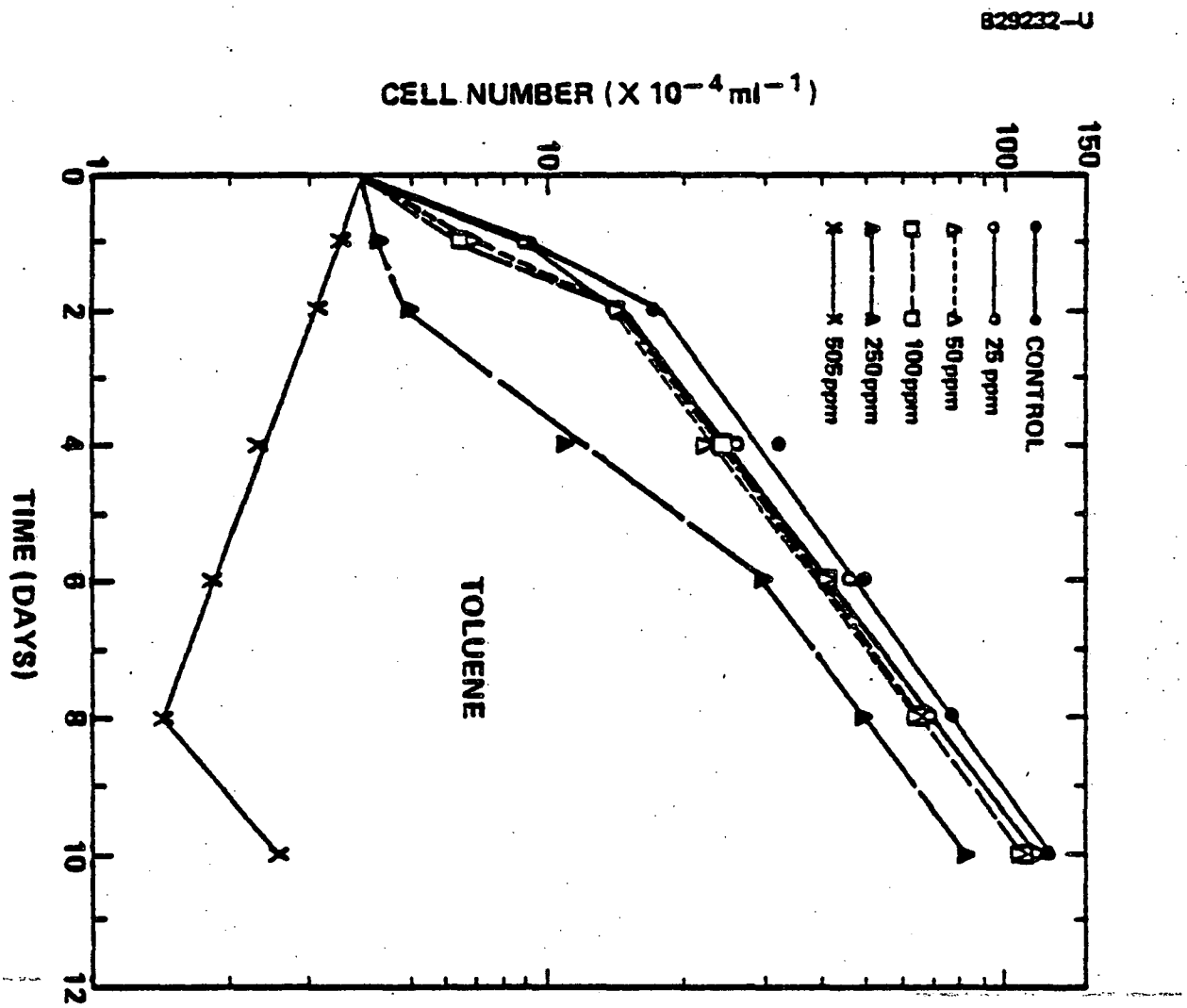


FIGURE 16-2

Growth of Chlorella vulgaris in Medium Containing Toluene (Data plotted are the average of three replicates. Lines of best fit were determined using regression coefficients. Numbers represent initial hydrocarbon concentration on a parts per million basis. The arrow on the ordinate indicates starting cell concentration.)

Source: Kauss and Hutchinson, 1975

TABLE 16-2

Toxic Effects of Toluene to Algae

| Species | Concentration | Effect | Reference |
|---|---|---|---|
| | | FRESHWATER | |
| Chlorella vulgaris | 245 mg/ℓ | 24 h $EC_{50}$ (cell number) | Kauss and Hutchinson, 1975 |
| Chlorella vulgaris | 250 mg/ℓ | 96 h no-effect conc. (cell number) | Kauss and Hutchinson, 1975 |
| Microcystis aeruginosa | 105 mg/ℓ | 8 d no-effect conc. (chlorophyll a) | Bringmann and Kuhn, 1978 |
| Scenedesmus quadricauda | >400 mg/ℓ | 8 d no-effect conc. (chlorophyll a) | Bringmann and Kuhn, 1978 |
| | | SALTWATER | |
| Amphidinium carterae | <0.001 mg/ℓ | 2 to 3 d no-effect conc. (cell number and chlorophyll) | Dunstan et al., 1975 |
| Dunaliella tertiolecta | 10 mg/ℓ | 2 to 3 d no-effect conc. (cell number and chlorophyll) | Dunstan et al., 1975 |
| Skeletonema costatum | 10 mg/ℓ | 2 to 3 d no-effect conc. (cell number and chlorophyll) | Dunstan et al., 1975 |
| Cricosphaera carterae | 10 mg/ℓ | 2 to 3 d no-effect conc. (cell number and chlorophyll) | Dunstan et al., 1975 |
| Ectocarpus sp. | 1730 mg/ℓ | inhibits asexual spore germination | Skinner, 1972 |
| Enteromorpha sp. | 1730 mg/ℓ | inhibits asexual spore germination | Skinner, 1972 |

h = hour; conc. = concentration; d = day.

tested 32, 32, and 14 days respectively after planting. Plants were exposed in the gas chamber for 1/4, 1/2, 1, and 2 hours. The type and extent of injury were recorded after one month to allow for a recovery period. Temperature of the plants was held at 25°C.

Results showed that toxic effects of toluene vapor were influenced by exposure period and dosage (Table 16-3). Toluene was observed to be toxic at concentrations of 6.4 to 12.0 mg/ℓ after 15 minutes of exposure (Currier, 1951). Fifteen minutes of exposure at 12 mg/ℓ toluene produced a 50, 0, and 60% injury to tomato, carrot, and barley, respectively. The effects of the exposures on flower and fruit development were not determined. For lethality to occur at 12.0 mg/ℓ, barley required 1 hour, tomato 2 hours, and carrot over 2 hours. The toxicity appeared to vary markedly within a narrow limit. By lowering the concentration of toluene from 12.0 to 6.4 mg/ℓ, the percentage of injury to barley after a two hour exposure was reduced from 100% (lethal) to 15%. At 24.1 mg/ℓ, toluene was only twice as toxic to barley seedlings as at 12.0 mg/ℓ after a 30 minute exposure.

Toluene entered the plant rapidly through the cuticle and stomata. Symptoms of injury included a darkening of the tips of the youngest leaves, presumably as a result of leakage of sap into the cellular spaces (Currier, 1951). This darkening spread to the older leaves. There was a loss of turgor, with draping stems and leaves. In bright sunlight, the chlorophyll was destroyed.

Toluene is classified as a contact poison that quickly kills the plant tissue with which it comes in contact (Currier, 1951). This material is not accumulated in plants nor is it translocated. The mechanism of toxicity involves disorganization of the outer membrane of the cell due to solvent action on the lipoid constituents, resulting in disruption of photosynthesis, respiration, and turgor pressure.

TABLE 16-3

Toxic Effects of Toluene Vapor on Carrots, Tomatoes, and Barley[a]

| Material | Concentration | Percent Injury[b] Exposure Time (h) | | | |
|---|---|---|---|---|---|
| | | 1/4 | 1/2 | 1 | 2 |
| Tomato | 12.0 mg/l | 50 | 60 | 75 | 100 |
| Carrot | 12.0 mg/l | 0 | 50 | 75 | 75 |
| Barley | 12.0 mg/l | 60 | 50 | 98 | 100 |
| Barley | 6.4 mg/l | 0 | 25 | 15 | 15 |
| Barley | 24.1 mg/l | ND | 100 | 100 | ND |

[a]Source: Currier, 1951

[b]0% = no effect; 100% = lethal 1 month after exposure.

h = hour; ND = not determined.

## 16.2. BIOCONCENTRATION, BIOACCUMULATION, AND BIOMAGNIFICATION POTENTIAL

Limited information is available concerning toluene's potential for accumulating in aquatic organisms and aquatic food chains. Possible pathways of toluene uptake are directly from water (bioconcentration) and from both water and food (bioaccumulation). Biomagnification occurs if the concentration of a compound in an organism increases with its trophic level as a result of passage through food chains.

Nunes and Benville (1979) studied the uptake and depuration of toluene and other monocyclic aromatic components of the water-soluble fraction (WSF) of Alaskan Cook Inlet crude oil in Manila clams (Tapes semidecussata). Clams were exposed for eight days to a constant WSF concentration under continuous-flow exposure conditions. The toluene concentration in water was measured daily. The toluene concentration in a pooled sample of 10 clams was measured at 2, 4, 6, and 8 days. At the end of the exposure period, remaining clams were transferred to clean-flowing seawater and pooled tissue samples were analyzed for toluene after 1, 7, and 12 days of depuration. The data are provided in the following tabulation:

| | | Toluene Concentration (ppm) | |
|---|---|---|---|
| | Days | Water | Tissue |
| Exposure | | | |
| | 1 | 1.2 | -- |
| | 2 | 1.3 | 2.3 |
| | 3 | 1.7 | -- |
| | 4 | 1.4 | 2.2 |
| | 5 | 1.2 | -- |
| | 6 | 0.9 | 0.87 |
| | 7 | 1.0 | -- |
| | 8 | 1.1 | 2.0 |
| Depuration | | | |
| | 1 | -- | 3.30 |
| | 7 | -- | 0.80 |
| | 14 | -- | 1.10 |

The mean water concentration during the uptake period was 1.2 ppm toluene. Tissue concentrations reached a maximum by two days of exposure and remained relatively constant except for a temporary decline on day six. The average tissue concentration during the exposure period was 1.5 ppm. The calculated bioconcentration factor (BCF) is 1.25 (which is equivalent to 1.5 ppm in tissue and 1.2 ppm in water). The depuration study showed that toluene was lost rapidly during the first week of depuration, but that a significant concentration of toluene remained in the clams by two weeks after beginning depuration.

Hansen et al. (1978) investigated the uptake and depuration of $^{14}$C-toluene by blue mussels (Mytilus edulis). Groups of mussels were exposed under static conditions to four concentrations of $^{14}$C-toluene for up to eight hours, followed by exposure to clean recirculating seawater for up to 192 hours. The $^{14}$C-toluene concentration in water and tissue (pooled sample from four mussels) was measured by liquid scintillation counting at 1, 2, 4, and 8 hours after beginning the uptake phase and periodically in tissue during the depuration phase.

The $^{14}$C-toluene concentration in tissue exceeded the water concentration by one hour at all exposure concentrations except the highest (40 µl/kg = ppm), which was toxic as shown by closure of the mussels at this concentration (Hansen et al., 1978). Equilibrium was reached by four hours in all groups. The BCF values at eight hours, expressed as the tissue concentration divided by the mean water concentration, were as follows:

| Water concentration (µl/kg) | BCF |
|---|---|
| 0.05 | 3.8 |
| 0.4 | 5.7 |
| 4.0 | 3.6 |
| 4.0 | 3.6 |

The BCF values, which averaged 4.2, seemed to be independent of the exposure concentration, indicating that accumulation was proportional to the level in

water (Hansen et al., 1978). More than half of the accumulated $^{14}$C-toluene was eliminated by one hour after the depuration phase began at all exposure concentrations. The depuration time by which no $^{14}$C-toluene was detectable in tissue was one hour in the mussels exposed to 0.05 µℓ $^{14}$C-toluene/kg, four hours for those exposed to 0.4 µℓ/kg, 120 hours for those exposed to 4 µℓ/kg, and 192 hours for the animals exposed to 40 µℓ/kg.

Lee et al. (1972) reported that the same species of mussel (Mytilus edulis) took up 3 to 10 µg of $^{14}$C-toluene per mussel (average dry weight tissue = 0.3 g) during static exposure for an unspecified period of time to 0.1 to 0.5 mg/ℓ. Using tissue toluene concentrations of 10 to 33 µg/g, the BCF is calculated to have been between 66 and 100. Because these values are based on dry tissue weights rather than wet weight, they are considerably higher than those reported by Nunes and Benville (1979) and Hansen et al. (1978).

Berry (1980) investigated the uptake of $^{14}$C-toluene by bluegill sunfish (Lepomis macrochirus) and crayfish (Orconectes rusticus). The exposure solutions were prepared by adding 1 mℓ of $^{14}$C-toluene to 100 ℓ of water for the fish experiment and by adding 1 mℓ $^{14}$C-toluene to 10 ℓ of water for the crayfish experiment. A group of 40 animals was added after thorough mixing of the solutions. Duplicate water samples and 2 to 4 animals were taken at 0, 0.5, 1, 2, 4, 8, 12, 16, 20, 24, and 48 hours after beginning exposure. The $^{14}$C-toluene concentration, expressed as nanograms per milligram (= ppm), was determined in water and in 7 (crayfish) or 9 (fish) tissues or organs by liquid scintillation counting. The BCF for each tissue was also calculated. Analysis of water samples showed that the toluene concentration in water decreased at a much greater rate in the crayfish experiment than in the bluegill experiment (89% versus 51% loss by 48 hours). The maximum BCF of bluegill tissues ranged from about 3 for brain to 45 for spleen. Fish muscle tissue was not analyzed. The

maximum BCF for most fish tissues was reached by eight hours. The maximum BCF of crayfish tissues ranged from about 8 for muscle to 140 for hepatopancreas. The BCF values increased throughout the 48 hour exposure period for all tissues except testes and muscle. These results indicate that toluene is accumulated above the water concentration by many tissues in these two species. The BCF of eight in the edible portion (muscle) of crayfish is considered to be a minimum value because of the rapidly decreasing toluene exposure concentration during this experiment.

Berry et al. (1978) also measured the uptake of $^{3}$H-toluene by fed and unfed mosquito (Aedes aegypti) larvae and the uptake of $^{3}$H-toluene by fed larvae in the presence or absence of benzene. The larvae were exposed to an initial concentration of 0.5 mℓ $^{3}$H-toluene/ℓ water. Duplicate water samples and 2 to 5 larvae were taken at 1, 2, 4, 8, 12, 16, 20, and 24 hours and counted individually by liquid scintillation counting. Maximum $^{3}$H-toluene counts per minute (cpm) were equal in fed and unfed larvae, but were reached more quickly (one hour versus four hours) by the fed animals. The $^{3}$H-toluene counts per minute values in larvae, expressed as the percentage of initial water counts, were greater during the first four hours in the benzene and toluene mixture than in the solution containing toluene alone. BCF values cannot be calculated because the authors expresssed $^{3}$H-toluene uptake as counts per minute per larvae rather than counts per minute per gram. The weight of the larvae was not provided. Interpretation was also complicated by rapid loss of $^{3}$H-toluene (half-time about four hours) during the uptake period. It is likely, however, that uptake by ingestion of toluene adsorbed to food particles can be a significant route of accumulation in aquatic organisms.

Ogate and Miyake (1973) identified toluene as the cause of offensive odor in the flesh of grey mullet (Mugil japanicus) taken from a harbor receiving efflu-

ents from refineries and petrochemical industries. Toluene was identified in seawater and fish tissue by gas chromatography, infrared (IR) and ultraviolet (UV) absorption, and mass spectrometry. The toluene concentration in most fish was not quantified; however, the flesh of one mullet with an offensive odor contained 5 ppm toluene. Additional experiments showed that toluene was accumulated by caged eels kept for ten days in several locations in the harbor to an average of 2.4 times the water concentration. These eels had the same offensive odor as mullet collected from the harbor. In another experiment, four eels were exposed in seawater to which a mixed solution of benzene, toluene, and xylenes was added daily for five days. The concentration of each chemical was then measured in seawater, muscle, and liver. The results with toluene were as follows:

| | Fish No. | Toluene Concentration (ppm) | BCF |
|---|---|---|---|
| Muscle | 1 | 11.2 | 0.70 |
| | 2 | 2.6 | 0.16 |
| | 3 | 5.1 | 0.32 |
| | 4 | 30.8 | 1.91 |
| | Mean | 12.4 | 0.77 |
| Liver | 1 | 9.0 | 0.56 |
| | 2 | 2.5 | 0.16 |
| | 3 | 5.2 | 0.32 |
| | 4 | 2.5 | 0.16 |
| | Mean | 4.8 | 0.30 |
| Water | -- | 16.1 | -- |

The results indicate that BCF in muscle was equal to or greater than the BCF in liver and that tissue concentrations rarely exceeded the water concentration.

In later experiments, Ogata and Miyake (1978) found that eels (Anguilla japonica) accumulated toluene to whole-body concentrations greater than the water concentration in freshwater. For this study, the authors studied the uptake and elimination of toluene by eels exposed in freshwater to crude oil.

The animals were exposed for ten days to a recirculating oil suspension (50 ppm, w/v) which was renewed every day. During this period, the toluene concentration was measured in pooled groups of 5 eels taken on 1, 5, and 10 days after beginning exposure. The concentration of toluene in water was measured each day at 1, 3, 6, 9, 14.5, and 24 hours after preparing the crude oil suspensions. The remaining eels were then transferred to clean seawater and sampled after 3, 5, and 10 days of depuration. The average toluene concentration in water during the uptake period was 0.130 ppm. The concentration in eels was 0.641 ppm after 1 day, 1.547 ppm after 5 days, and 1.718 ppm after 10 days. The respective BCF values were 4.9, 11.9, and 13.2. A semilogarithmic plot of the logarithm of tissue concentration versus time indicated that equilibrium had not quite been reached by ten days. The depuration phase of the experiment showed that tissue concentration decreased rapidly from 1.718 ppm at the beginning of depuration to 0.315 ppm after 3 days, 0.121 ppm after 5 days, and 0.035 ppm after 10 days. A semilog plot showed that toluene was eliminated in 2 phases. The elimination half-time during the first phase, lasting from 0 to 5 days, was 1.4 days. About 93% of the accumulated toluene was eliminated by the end of this period. The remaining toluene was eliminated at a somewhat slower rate, with about 2% of the accumulated toluene remaining after ten days of depuration.

The only information found concerning food-chain transfer of toluene is provided by Berry and Fisher (1979), who exposed mosquito larvae (Aedes aegypti) to $^{14}$C-toluene for 3 hours and then fed them to bluegill sunfish (Lepomis macrochirus). In duplicate experiments, each of 25 fish in separate containers were fed with 10 contaminated larvae. The mean level of radioactivity in 10 larvae was 736 cpm in the first experiment and 3196 cpm in the second experiment. Internal organs (spleen, gall bladder, liver, stomach, intestine, and kidney) from 5 fish, sampled at each interval of 1, 4, 8, 24, and 48 hours after feeding,

were analyzed for radioactivity by liquid scintillation counting. Radioactivity was expressed as counts per minute per organ rather than on a weight basis. The only organ that had counts per minute values significantly greater than background levels was the stomach at 1, 4, and 8 hours after feeding. The authors concluded that an insignificant amount of toluene, if any, leaves the digestive tract to be accumulated in other organs of sunfish. The validity of this conclusion is unknown because the dose was so low that absorption, if it had occurred, could not have been differentiated from background counts and because the counts were not expressed on a tissue weight basis, even in the stomach.

In summary, the available information indicates that the primary path of toluene uptake in aquatic organisms is direct absorption from water. The reported or calculated BCF values for edible portion or whole organism ranged between <1 to about 14, indicating that toluene has a low bioconcentration potential. These BCF values are lower than the value predicted on the basis of the relationship established between octanol-water partition coefficient (P) of lipophilic compounds and steady-state BCF (Veith et al., 1979). This relationship, expressed by the equation "log BCF = (0.85 log P) - 0.70," would predict a BCF of 39, using a log P value of 2.69 for toluene (see Subsection 3.4.2.).

Low bioconcentraton potential, rapid depuration, and the ability of fish to metabolize toluene all indicate that toluene is unlikely to biomagnify through aquatic food chains. Aquatic organisms do accumulate toluene, however, and concentrations in edible species from polluted areas have reached levels that cause organoleptic effects in humans (Ogata and Miyake, 1973).

## 16.3. EFFECTS ON MICROORGANISMS

Toluene has been used for quite some time as an antimicrobial agent. Sabalitschka and Preuss (1954) sterilized a urine sample containing Escherichia

coli and Pseudomonas fluorescens within 24 hours with 4000 mg/ℓ toluene. Threshold concentrations for toluene have been established by Bringmann and Kuhn (1959, 1976, 1977, 1980) for various microorganisms. These investigators reported values of 29 mg/ℓ for P. putida, 200 mg/ℓ for E. coli, and greater than 450 mg/ℓ for the ciliated protozoan Uronema parduczi. Partial sterilization of soil was achieved by adding toluene to the soil (Pochon and Lajudie, 1948).

The effects of toluene on bacterial activity and growth have also been studied. As measured by methane evolution rates, 20 mg/ℓ toluene increased the growth rate of bacteria in sewage sludge deposits, while 200 mg/ℓ produced a toxic effect (Barash, 1957). Similarly low levels of toluene allowed good growth of P. putida and Nocardia sp., while saturation levels (515 mg/ℓ at 20°C) were toxic (Gibson, 1975). Depending on the concentration (173 to 17,300 mg/ℓ), a rotifer (Dicranophorus forcipatus) was unaffected, or temporarily inhibited, or permanently inhibited by toluene (Erben, 1978). Death and disintegration of rumen ciliates occurred between 460 and 645 mg/ℓ of toluene (Eadie et al., 1956). At sublethal concentrations (1000 and 6000 mg/ℓ), toluene caused a negative chemotactic response or totally inhibited the chemotatic response of all marine bacteria tested (Mitchell et al., 1972; Young and Mitchell, 1973). Although the effects were reversible, the authors of the 1972 paper expressed concern that the inhibition could seriously undermine the capacity of the marine microflora to control the self-purification processes in the sea. Beck and Poschenrieder (1963) found that high concentrations of toluene (50 to 100,000 mg/g of soil) suppressed soil microflora activity. In addition, they found that gram-positive bacilli sporeformers, streptomycetes, and cocci were especially resistant, while gram-negative bacteria were sensitive.

Toluene has been shown to affect the integrity of the microbial cell wall and cytoplasmic membrane (Dean, 1978). Thompson and Macleod (1974) reported that

marine pseudomonad cells washed and suspended in 0.5 M NaCl were lysed by treatment with 20,000 mg/ℓ toluene and released 95% of the cells' alkaline phosphatase. Because the cells remained intact with 0.05 M $MgSO_4$ and 20,000 mg/ℓ toluene, the authors concluded that Mg ions prevented cellular disruption by strengthening the integrity of the cell wall. Woldringh (1973) established that a 2500 mg/ℓ solution of toluene partially dissolved the inner cytoplasmic membrane of E. coli and displaced nuclear material to the periphery of the cell. DeSmet et al. (1978) reported that at 100,000 mg/ℓ toluene, the cytoplasmic membrane was completely disorganized. The presence of Mg ions at lower toluene concentrations (up to 10,000 mg/ℓ), however, prevented extensive damage to the cytoplasmic membrane and loss of intracellular material; thus, permeability depended on the integrity of the outer membrane (DeSmet et al., 1978). Deutscher (1974) found that the effects of toluene treatment were dependent on various cultural conditions including pH, temperature, Mg ion concentration, and age of the culture. Temperature-dependent effects of toluene treatment were also reported by Jackson and DeMoss (1965). Toluene changed the asymmetric unit membrane profile to a symmetric profile in vegetative cells of Bacillus subtilis and caused gaps in the membrane to appear (Silva et al., 1978). Fan and Gardner-Eckstrom (1975) found that toluene-treated Bacillus megaterium cells liberated a membrane protein essential for peptidoglyca synthesis and that this protein could be added back to the membrane to reconstitute peptidoglycan synthesis. Toluene at 86,000 mg/ℓ induced the autolysis of Saccharomyces cerevisiae, the release of UV absorbing substances from the cells, and the deacylation of phosphoplipids (Ishida, 1978). At saturation concentrations of toluene, however, no cytolysis of yeast occurred (Lindenberg et al., 1957). Scholz et al. (1959) noted that toluene-treated yeast cells accumulated hexosephosphates. Bucksteeg (1942) found that the concentration of toluene and time

of exposure determined its effect on Cytophaga sp. and Azotobacter chroococcum. The lower the concentration, the longer the contact time needed to produce lethal effects. Azotobacter was more resistant than the Cytophaga sp. Bucksteeg theorized that toluene affected the physical and chemical constitution of the cell. An alteration in plaque morphology in two coliphages ($T_6$rt and $T_3$) occurred with 1% toluene (Brown, 1957).

The ability of toluene to disrupt cell membranes led to the use of this compound as an unmasking agent in microbial research to assay a variety of enzymes (Herzenberg, 1959; Dobrogosz and DeMoss, 1963; Levinthal et al., 1962). The in vitro assays using toluene have been used to make enzymes within a cell accessible to exogenous substrates (Jackson and DeMoss, 1965; DeSmet et al., 1978). Generally, toluene treatment makes the cells permeable to low molecular weight compounds (such as deoxynucleoside triphosphate dNTP) and several macromolecules while remaining impermeable to proteins larger than approximately 50,000 daltons (Deutscher, 1974; DeSmet et al., 1978). Several investigators have used these findings to study DNA replication in bacteria (E. coli, B. subtilis), bacteriophage (E. coli, $T_4$), and diatoms (Cylindrotheca fusiformis) after treating the organisms with 0.1 to 1% toluene in solution (Miller et al., 1973; McNicol and Miller, 1975; Moses and Richarson, 1970; Matsushita et al., 1971; Winston and Matsushita, 1975; Sullivan and Valeani, 1976). Other uses of toluene treated cells are in studying the synthesis of heteroribonucleotides, RNA, and peptidoglycan and the repair synthesis of DNA (DeSmet et al., 1978; Moses and Richardson, 1970; Segev et al., 1973; Winston and Matsushita, 1975). Burger (1971) showed that toluene-treated E. coli cells continued DNA replication, but only in that chromosomal region that was about to be replicated in vitro. Toluene-treated cells can also be used to study the effects of various

antibiotics in cell growth and DNA replication (Hein, 1954; Burger and Glaser, 1973).

Although the exact mechanisms of toluene-induced disaggregration of cell membranes are not known, Jackson and DeMoss (1965) state that the mechanisms fall into two classes: (1) a disaggregrating (autolytic) enzyme(s) perhaps synthesized in the presence of toluene or (2) a direct denaturation of cell membrane constituents such as phospholipids; a condition inhibited by stabilizing factors such as divalent cations (e.g., Mg).

# 17. EFFECTS ON AQUATIC SPECIES

## 17.1. GUIDELINES FOR EVALUATION

Evaluation of the available information concerning the effects of toluene on aquatic organisms must take into account several factors. A primary consideration for evaluation of toxicity test results is toluene's high volatility. The half-life for volatilization of toluene from a water column one m deep has been reported to be between approximately 30 minutes (Mackay and Wolkoff, 1973) and 5 hours (Mackay and Leinonen, 1975). Benville and Korn (1977) analyzed the toluene concentration in test containers during a 96 hour static toxicity test and showed that the percentage of toluene lost was 48% by 24 hours, 53% by 48 hours, and greater than 99% by 72 hours. Korn et al. (1979) reported that toluene was lost at a greater rate from bioassay containers at 12°C (99% loss by 72 hours) than at 8°C (>99% loss by 96 hours) or at 4°C (75% loss by 96 hours). Potera (1975) found that the observed half-life of toluene in bioassay containers was 16.5 $\pm$ 1.13 hours. The rate of volatilization of toluene from water varies with the amount of mixing, temperature, surface area to volume ratio, and other factors. Adsorption to sediments and suspended particles may decrease evaporative loss and result in greater persistence of toluene. Although adsorption may lower the concentration of dissolved toluene in the water column, binding to sediment and suspended matter may increase the effective exposure concentration to benthic and filter-feeding organisms.

Most of the reported aquatic toxicity studies with toluene have used a static exposure technique. In most cases, the $LC_{50}$ has been calculated on the basis of initial nominal (unmeasured) or initial measured concentrations. The test organisms in these static experiments however, are exposed to rapidly

decreasing toluene concentrations. Most of the reported acute static toxicity studies show little or no change in the $LC_{50}$ value between 24 and 96 hours. This lack of change indicates that most, if not all, of the mortalities in these tests occurred during the first 24 hours when toluene concentrations were highest. In contrast, those flow-through studies that reported acute $LC_{50}$ values at more than one exposure period showed that $LC_{50}$ values decreased significantly with time.

Numerous other factors may affect the results of toxicity tests with toluene. It has been shown that the acute toxicity of toluene is affected in some cases by temperature and salinity (Section 17.3.). These effects on toxicity may be due to effects on the test organisms (metabolism, uptake, stress, etc.), effects on the physicochemical behavior of toluene (solubility, volatilization, etc.), or interactive effects of both. For example, toluene is less soluble in saltwater than in freshwater and is both more soluble and more volatile at higher temperatures. Laboratory results may also be influenced by the loading ratio (gram organism per liter water); dissolved oxygen concentration; age, health, and species of test organisms; and other exposure conditions, all of which may interact to affect the results in an unpredictable manner.

Prediction of environmental effects from laboratory results must consider the influence of the variables associated with laboratory tests and with the natural variability intrinsic to the aquatic environment. Results of static acute toxicity tests with volatile compounds such as toluene may approximate the acute toxic effects that may occur in nature to the same species during accidental spills, because toluene concentrations rapidly decrease in both situations. Flow-through acute toxicity tests may provide some insight into the expected effects of a short-term but constant release of toluene into the aquatic environment, as might occur in areas receiving refinery or petrochemical effluents. Neither static nor flow-through acute toxicity tests can predict the

received refinery and petrochemical effluents, the effects of such low level chronic pollution in natural aquatic habitats are unknown.

## 17.3. LABORATORY STUDIES OF TOXICITY

17.3.1. Lethal Effects. The lethal effects of toluene have been reported for numerous species of freshwater and marine fish and invertebrates. The acute $LC_{50}$ for 22 species of freshwater and marine animals ranged between 3 and 1180 ppm (Table 17-1). All but four of the $LC_{50}$ values were determined in static tests. Of the four flow-through $LC_{50}$ tests, only two utilized measured toluene concentrations. No information was found concerning the effects of toluene on amphibians.

17.3.1.1. FRESHWATER FISH — The earliest investigation of toluene toxicity to freshwater fish was conducted by Shelford et al. (1917), who reported that one hour of exposure to 61 to 65 mg/l toluene was lethal to orange spotted sunfish (Lepomis humilis). This test was conducted under static conditions at 20°C in freshwater of unspecified temperature and composition.

Degani (1943) conducted static toxicity tests with 15 day old lake trout (Salvelinus namaycush) fry and 1.5 g mosquitofish (Gambusia affinis) in dechlorinated tapwater at 17 to 18°C using 3 to 5 fish per container (2 liter volume). The time to death at a nominal exposure concentration of 90 ppm toluene was 390 minutes for trout and 47 minutes for mosquitofish. The time to death of trout fry exposed to 50 ppm toluene was 258 minutes.

Wallen et al. (1957) also conducted static acute toluene toxicity tests with female mosquitofish (Gambusia affinis) of unspecified size in turbid pond water (150 ppm turbidity as measured by Jackson turbidimeter, pH 7.5 to 8.5,

chronic effects of low level toluene pollution. In addition, acute toxicity tests usually determine the concentration of toxicant which kills or affects 50% of the test population. $LC_{50}$ or $EC_{50}$ values, therefore, represent concentrations which are toxic to half the population and provide no information concerning the concentration which will have no adverse effects during acute or chronic exposure.

## 17.2. EFFECTS OF ACCIDENTAL SPILLS

No information was found concerning the effects of accidental spills of toluene per se on aquatic organisms; however, toluene is one of the major aromatic components of crude oil and such refined petroleum products as diesel fuel, gasoline, and jet fuel, all of which have been released in large amounts to the aquatic environment during spills.

The long term ecological impact of accidental spills of toluene is unknown. In spill situations, most of the toluene would probably evaporate rapidly. For instance, McAuliffe (1976) reported that toluene, benzene, and xylene could be found in the water under crude oil slicks only during the first 30 minutes after spillage. In contrast, spills in areas of shallow water and restricted water flow, such as in certain portions of estuaries, lakes, and streams, have a greater potential for causing acute mortalities because the toluene may reach higher dissolved concentrations and may persist longer through adsorption to sediments. Toluene is an acute toxic to many aquatic species at concentrations well below its water solubility, and lethal exposure may well occur during spills in shallow water.

Although chronic, low-level pollution by toluene has been reported in a Japanese river (Funasoka et al., 1975) and a harbor (Ogate and Miyake, 1973) that

TABLE 17-1

Acute Toxicity of Toluene to Fish and Aquatic Invertebrates

| Species | Temp. (°C) | Type Test | LC50 24 h | LC50 48 h | LC50 72 h | LC50 96 h | No Effect Concentration | Reported Concentration Units | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| FISH | | | | | | | | | | |
| Freshwater | | | | | | | | | | |
| Ide | 20±1 | SU | --- | 70 | --- | --- | 52 | mg/ℓ | Lab 1, 100% kill at 88 mg/ℓ. | Juhnke and Ludemann, 1978 |
| (Leuciscus idus melanotus) | 20±1 | SU | --- | 422 | --- | --- | 365 | | Lab 2, 100% kill at 470 mg/ℓ. Tests were supposedly conducted under identical conditions. | |
| Mosquitofish (Gambusia affinis) | 17 to 22 | SU | 1340 | 1260 | --- | 1180 | 560 | ppm | Tests were conducted in aerated turbid pond water. | Wallen et al., 1957 |
| Goldfish (Carassius auratus) | 20±1 | SH | 58 | --- | --- | --- | --- | mg/ℓ | Test was conducted in tap water (pH 7.8) | Bridie et al., 1979 |
| Goldfish (Carassius auratus) | 25 | SU | 57.7 (48.9 to 68.8) | 57.7 (48.9 to 68.8) | --- | 57.7 (48.9 to 68.8) | --- | mg/ℓ | Test was conducted in soft water. | Pickering and Henderson, 1966 |
| Goldfish (Carassius auratus) | 17 to 19 | FH | 41.6 (32.0 to 71.7) | 27.6 (21.6 to 36.0) | 25.3 (20.1 to 31.9) | 22.80 (17.1 to 30.0) | --- to | ppm | Tests were conducted under flow-through conditions in soft dechlorinated tap water. The test was continued to 720 h (30 d) at which time the $LC_{50}$ (and 95% confidence interval) was 14.6 (10.7 to 20.0) ppm. | Brenniman et al., 1976 |
| Fathead minnow (Pimephales promelas) | 25 | SU | 46.3 (37.0 to 59.4) | 46.3 (37.0 to 59.4) | --- | 34.3 (22.8 to 45.9) | --- | mg/ℓ | Tests were conducted in soft water. | Pickering and Henderson, 1966 |
| Fathead minnow (Pimephales promelas) | 25 | SU | 56.0 (44.7 to 67.1) | 56.0 (46.7 to 67.1) | --- | 42.3 (33.5 to 53.5) | --- | mg/ℓ | Tests were conducted in hard water. | |

TABLE 17-1 (cont.)

| Species | Temp. (°C) | Type Test | $LC_{50}$ 24 h | 48 h | 72 h | 96 h | No Effect Concentration | Reported Concentration Units | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Bluegill sunfish (Lepomis macrochirus) | 25 | SU | 24.0 (18.9 to 30.5) | 24.0 (18.9 to 30.5) | --- | 24.0 (18.9 to 30.5) | --- | mg/l | Tests were conducted in hard water. | Pickering and Henderson, 1966 |
| Bluegill sunfish (Lepomis macrochirus) | MR | SU | 16.6 (15.0 to 19.1) | 13.3 (11.6 to 14.8) | 12.7 (11.5 to 14.5) | 12.7 (11.5 to 14.5) | 10.0 | ppm | Only these data cited in U.S. EPA, 1980. | U.S. EPA, 1978 |
| Guppies (Poecilia reticulata) | 25 | SU | 62.8 (55.0 to 73.7) | 61.0 (52.8 to 71.9) | --- | 59.3 (50.9 to 70.3) | --- | mg/l | Tests were conducted in hard water. | Pickering and Henderson, 1966 |
| Zebrafish (Brachydanio rerio) | 20±1 | FW | --- | 25 to 27 | --- | --- | --- | mg/l | Tests were conducted in closed aquaria with dechlorinated hard tap water at a flow rate of 6 l/h. | Slooff, 1978<br>Slooff, 1979 |
| Medaka (Oryzias latipes) | 25±2 | SU | 80 (mean= 80) | 20 to 135 (mean= 63) | | 23 to 110 (mean= 58) | ≤16 | mg/l | Range and mean of $LC_{50}$ values for different stage embryos | Stoss and Haines, 1979 |
| Medaka (Oryzias latipes) | 25±2 | SU | 44 | 36 | | 32 | --- | mg/l | $LC_{50}$ values for fry. The 168 h. $LC_{50}$ was 23 mg/l. | Stoss and Haines, 1979 |
| Coho salmon fry (Oncorhynchus kisutch) | --- | FH | --- | --- | --- | 9.36 | --- | µl/l | Unparasitized | Holes, 1980 |
| | --- | FH | --- | --- | --- | 3.08 | --- | µl/l | Parasitized | Holes, 1980 |
| MARINE | | | | | | | | | | |
| Coho salmon (Oncorhynchus kisutch) | 8 | SU | --- | 22.4 | 22.4 | 22.4 | 10 | ppm | Tests were conducted in artificial salt-water (pH 8.1, 30‰ salinity). | Morrow et al., 1975 |
| Pink salmon fry (Oncorhynchus kisutch) | 12 | SW | 5.4 (4.4 to 6.5) | --- | --- | --- | --- | ppm | Tests were conducted according to methods of Korn et al., 1979. | Thomas and Rice, 1979 |

TABLE 17-1 (cont.)

| Species | Temp. (°C) | Type Test | $LC_{50}$ 24 h | $LC_{50}$ 48 h | $LC_{50}$ 72 h | $LC_{50}$ 96 h | No Effect Concentration | Reported Concentration Units | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Pink salmon (Oncorhynchus kisutch) | 4 | SM | --- | --- | --- | 6.41 (5.73 to 7.18) | --- | µl/l | Tests were conducted with salmon fry acclimated to 28°/oo seawater at different temperatures. | Korn et al., 1979 |
| | 8 | SM | --- | --- | --- | 7.63 (6.86 to 8.48) | --- | | | |
| | 12 | SM | --- | --- | --- | 8.09 (7.45 to 8.78) | --- | | | |
| Striped bass (Morone saxatilis) | 16 | SM | 7.3 | --- | --- | 7.3 | --- | µl/l | Tests were conducted in 25°/oo salinity seawater with juvenile fish. | Benville and Korn, 1977 |
| Sheepshead minnow (Cyprinodon variegatus) | NR | SU | >277 <485 | >277 <485 | --- | >277 <485 | 277 | ppm | Data only cited in U.S. EPA, 1980. | U.S. EPA, 1978 |
| INVERTEBRATES | | | | | | | | | | |
| Freshwater | | | | | | | | | | |
| Water flea (Daphnia magna) | 22±1 | SU | 310 (240 to 420) | 310 (240 to 420) | --- | --- | 28 | mg/l | Test was conducted with reconstituted well water (hardness 72±6 mg/l as $CaCO_3$, pH 7.0±0.2) in containers sealed with plastic wrap. | LeBlanc, 1980 |
| Water flea (Daphnia magna) | 23 | SU | --- | 60 | --- | --- | --- | mg/l | Test was conducted in natural water (pH 7.5, hardness 214 mg/l). | Bringmann and Kuhn, 1959 |
| Mosquito larvae (Aedes aegypti) | 25±1 | SM | 21.52 (21.36 to 21.68) | --- | --- | --- | 9.95 | ppm | Test was conducted with distilled water. | Berry and Brammer, 1977 |
| Marine | | | | | | | | | | |
| Brine shrimp nauplii (Artemia salina) | 24.5 | SU | 33 | --- | --- | --- | --- | mg/l | Test was conducted with artificial seawater. | Price et al., 1974 |

TABLE 17-1 (cont.)

| Species | Temp. (°C) | Type Test | $LC_{50}$ 24 h | 48 h | 72 h | 96 h | No Effect Concentration | Reported Concentration Units | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Bay shrimp (Crago franciscorum) | 16 | SM | 12 (10 to 13) | --- | --- | 4.3 (3.1 to 5.8) | --- | µl/l | Tests were conducted with 25°/oo salinity seawater. | Benville and Korn, 1977 |
| Shrimp (Eualus spp.) | 4 | SM | --- | --- | --- | 21.4 (19.5 to 23.5) | --- | µl/l | | Korn et al., 1979 |
| | 8 | SM | --- | --- | --- | 20.2 (17.9 to 22.8) | | µl/l | | Korn et al., 1979 |
| | 12 | SM | --- | --- | --- | 14.7 (13.1 to 16.6) | | µl/l | | Korn et al., 1979 |
| Grass shrimp (Pagaemonetes pugio) | 20 | SM | 20.2 (16.3 to 22.5) | --- | --- | --- | --- | mg/l | Adults at 15°/oo salinity. | Potera, 1975 |
| | 20 | SM | 17.2 (14.9 to 19.4) | --- | --- | --- | --- | mg/l | Adults at 25°/oo salinity. | Potera, 1975 |
| | 10 | SM | 37.6 (35.0 to 40.3) | --- | --- | --- | --- | mg/l | Adults at 15°/oo salinity. | Potera, 1975 |
| | 10 | SM | 38.1 (36.1 to 39.6) | --- | --- | --- | --- | mg/l | Adults at 25°/oo salinity. | Potera, 1975 |
| Grass shrimp (Pagaemonetes pugio) | 20 | SM | 30.6 (21.3 to 44.5) | --- | --- | --- | --- | mg/l | Larvae at 15°/oo salinity. | Potera, 1975 |
| | 20 | SM | 25.8 (18.8 to 34.6) | --- | --- | --- | --- | mg/l | Larvae at 25°/oo salinity. | Potera, 1975 |

TABLE 17-1 (cont.)

| Species | Temp. (°C) | Type Test | $LC_{50}$ 24 h | $LC_{50}$ 48 h | $LC_{50}$ 72 h | $LC_{50}$ 96 h | No Effect Concentration | Reported Concentration Units | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Grass shrimp (Palaemonetes pugio) | NR | SU | --- | --- | --- | 9.5 | --- | mg/l | --- | Neff et al., 1976 |
| Mysid shrimp (Mysidopsis bahia) | NR | SU | 64.8 (50.9 to 82.5) | 56.3 (43.0 to 70.8) | 56.3 (43.0 to 70.8) | 56.3 (43.0 to 70.8) | 27.7 | ppm | Data only cited in U.S. EPA, 1980. | U.S. EPA, 1978 |
| Dungeness crab (Cancer magister) | NR | FU | --- | 170 | --- | 28 | --- | mg/l | Larvae. | Caldwell et al., 1976 |
| Copepod (Hitocra spinipes) | 20 | SM | 24.2 (19.8 to 30.2) | --- | --- | --- | --- | mg/l | 15°/oo salinity. | Potera, 1975 |
| | 20 | SM | 74.2 (52.0 to 100.5) | --- | --- | --- | --- | mg/l | 25°/oo salinity. | Potera, 1975 |
| Pacific oyster (Crassostrea gigas) | 20 to 21.5 | SU | --- | 1050 | --- | --- | --- | mg/l | Larvae. | Legore, 1974 |

Temp. = temperature; h = hour; d = day; NR = not reported.

methyl orange alkalinity < 100 ppm, temperature 17 to 22°C). For these toxicity tests, ten fish per concentration were added immediately after addition of different amounts of toluene to the bioassay containers (15 liter volume). The test solutions were constantly aerated and mortalities were recorded daily for 96 hours. The 24, 48, and 96 hour $LC_{50}$ values were 1340, 1260, and 1180 ppm, respectively. These values were estimated on the basis of the initial nominal toluene concentrations. Because the test containers were vigorously aerated, it is probable that the actual toluene concentrations decreased rapidly during the exposure period. It was also observed that the turbidity of the toluene solutions decreased from 150 to 100 ppm over the 96 hour exposure period. At concentrations of 560 ppm and below, all fish appeared to be unaffected. The remainder of the test results are presented below:

| Concentration (ppm) | Percent Mortality (N = 10) | | |
|---|---|---|---|
| | 24 h | 48 h | 96 h |
| ≤ 560 | 0 | 0 | 0 |
| 1,000 | 20 | 30 | 40 |
| 1,800 | 80 | 80 | 100 |
| 3,200 | 80 | 90 | 100 |
| 5,600 | 100 | 100 | 100 |
| 10,000 | 100 | 100 | 100 |

Pickering and Henderson (1966) investigated the acute toxicity of toluene to fathead minnows (Pimephales promelas), bluegill sunfish (Lepomis macrochirus), goldfish (Carassius auratus), and guppies (Lebistes reticulatus = Poecilia reticulata). The length and weight of the fish used for testing were 3.8 to 6.4 cm and 1 to 2 g for the first 3 species and 1.9 to 2.5 cm and 0.1 to 0.2 g for guppies. Each test utilized 10 fish per concentration or control in either 10 l (minnows, sunfish, goldfish) or 2 l (guppies) of soft water (pH 7.5, alkalinity 18 mg/l, EDTA hardness 20 mg/l) made by mixing 5 parts of hard natural spring water with 95 parts of distilled demineralized water. In addition, fathead minnows were tested (10 fish/concentration) in the hard spring water (pH

8.2, alkalinity 300 mg/ℓ, EDTA hardness 360 mg/ℓ) to investigate the effect of these water characteristics on toluene toxicity. All tests were conducted at 25°C. The test solutions were not aerated, and dissolved oxygen concentrations were measured but not reported. The 24, 48, and 96 hour $LC_{50}$ values and their 95% confidence limits, as calculated by the moving average-angle method of Harris (1959) using initial nominal toluene concentrations, are presented in Table 17-1. The 96 hour $LC_{50}$ values increased in the order of bluegill sunfish (24.0 mg/ℓ), fathead minnow (34.3 mg/ℓ in soft water, 42.3 mg/ℓ in hard water), goldfish (57.7 mg/ℓ), and guppies (59.3 mg/ℓ). The 96 hour $LC_{50}$ for fathead minnows in soft water was not significantly different from the 96 hour $LC_{50}$ for the same species in hard water. Comparison of the 95% confidence limits of the 96 hour $LC_{50}$ values in soft water for the 4 species indicated that the $LC_{50}$ values were not significantly different between fathead minnows and bluegill sunfish or between goldfish and guppies. Both fathead minnows and bluegill sunfish had 96 hour $LC_{50}$ values significantly lower than goldfish and guppies. The 96 hour $LC_{50}$ was not significantly different from the 24 hour $LC_{50}$ for any of the species tested in soft water.

Static acute $LC_{50}$ values for bluegill sunfish have also been reported by the U.S. EPA (1978, cited in U.S. EPA, 1980). The 24, 48, 72, and 96 hour $LC_{50}$ values were 16.6, 13.3, 12.7, and 12.7 ppm, respectively. No effects were observed at or below 10 ppm. Additional information concerning these tests was not available.

Berry (1980) mentioned that the upper non-lethal toluene concentration for bluegill sunfish (Lepomis macrochirus) was 8.7 mg/ℓ. The duration of exposure and lowest lethal concentration were not specified.

Bridie et al. (1979) and Brenniman et al. (1976) also investigated the acute toxicity of toluene to goldfish. Bridie et al. (1979) used goldfish of

slightly greater weight (mean 3.3 g, range 2.3 to 4.3 g) than Pickering and Henderson (1966) to determine the static 24 hour $LC_{50}$. In this test, 6 fish per concentration were exposed without aeration to a toluene series in 25 ℓ of tapwater that had a pH of 7.8 and contained (in milligrams per liter): $Cl^-$ = 65; $NO_2^-$ = 0; $NO_3^-$ = 4; $SO_4^{2-}$ = 35; $PO_4^{3-}$ = 0.15; $HCO_3^-$ = 25; $SiO_2$ = 25; $NH_4^+$ = 0; Fe = 0.05; Mn = 0; $Ca^{2+}$ = 100; $Mg^{2+}$ = 8; and alkali as $Na^+$ = 30. The toluene concentration was measured at the beginning and end of the test. The 24 hour $LC_{50}$, obtained by interpolation from a graph of the logarithm of concentration versus percent mortality, was 58 mg/ℓ, which is the same as the 24 hour $LC_{50}$ for goldfish reported by Pickering and Henderson (1966).

Much larger goldfish (length, 13 to 20 cm; weight, 20 to 80 g) were used by Brenniman et al. (1976) to determine the acute toxicity of toluene under flow-through exposure conditions. The $LC_{50}$ values were determined by exposing 6 fish per 38 ℓ aquarium to three toluene concentrations (and a control) in dechlorinated soft tapwater (methyl orange alkalinity = 34 ppm as $CaCO_3$; phenolphthaline alkalinity = 37 ppm as $CaCO_3$; total hardness = 80 ppm as $CaCO_3$; calcium = 21.6 ppm; magnesium = 5.3 ppm; $SiO_3$ = 8 ppm; chromium = <0.002 ppm; pH 7.0 $\pm$ 0.3; temperature 17 to 19°C) at a flow rate calibrated to renew the test chamber volumes every 1.5 hours. This flow rate was sufficient to maintain dissolved oxygen concentrations at $\geq$7 ppm and to maintain constant toluene concentrations, as measured by continuous monitoring at 210 nm by spectrophotometer. The 24, 48, 72, and 96 hour $LC_{50}$ values, calculated by probit analysis, were 41.6, 27.6, 25.3, and 22.8 ppm, respectively. Although most of the fish died during the first 24 hours, the 96 hour $LC_{50}$ was significantly lower than the 24 hour $LC_{50}$. These $LC_{50}$ values are somewhat lower than those reported by Pickering and Henderson (1966) and Bridie et al. (1979) for goldfish tested under static conditions. In addition, the $LC_{50}$ values reported by Pickering and Henderson

(1966) did not decrease significantly from 24 to 96 hours. These differences are probably due to a rapid decline in the toluene concentration through evaporation in the static tests in contrast to constant toluene concentrations in the flow-through test. Brenniman et al. (1976) continued their flow-through exposure test for 30 days, at which time the $LC_{50}$ had decreased to 14.6 ppm. These results emphasize the fact that static acute toxicity tests may seriously underestimate the acute toxicity of toluene and that chronic effects may occur at concentrations which are considerably lower than those which cause acute effects.

Juhnke and Ludemann (1978) investigated the static acute toxicity of toluene to the ide (Leuciscus idus melanotus) using comparable procedures in two different laboratories. The toxicity tests were conducted according to the methods of Mann (1975, 1976), i.e. 48 hours of exposure with 10 fish (1.5 ± 0.3 g, 5 to 7 cm) per concentration in tapwater (pH 7-8, hardness 268 ± 54 mg/l) at 20 ± 1°C. The 48 hour $LC_0$ (0% mortality), $LC_{50}$, and $LC_{100}$ (100% mortality) values determined at each laboratory were as follows:

| | 48 Hour Lethal Concentration Values (mg/ℓ) | | |
|---|---|---|---|
| | $LC_0$ | $LC_{50}$ | $LC_{100}$ |
| Laboratory 1 | 52 | 70 | 88 |
| Laboratory 2 | 365 | 422 | 470 |

Although it was stated that these tests were conducted under comparable conditions, the results were clearly different. The concentration that caused no deaths of fish in laboratory 2 (365 mg/ℓ) was about 4 times higher than the concentration that killed all fish in laboratory 1 (88 mg/ℓ). The authors did not discuss the reasons for the difference in results.

Slooff (1978, 1979) reported that the 48 hour $LC_{50}$ of toluene to zebrafish (Brachydanio rerio) was 25 to 27 mg/ℓ. This test was conducted under flow-

through (6 ℓ/hr) exposure conditions using 10 fish per concentration in 10 ℓ sealed aquaria and dechlorinated tapwater (20 ± 1°C; pH 8.0 ± 0.2; hardness 180 ± 1.8 mg/ℓ as $CaCO_3$).

The acute effects of toluene on parasitized and unparasitized coho salmon (Oncorhynchus kisutch) fry were studied by Moles (1980). The parasitized fry were artificially infected before toluene exposure with glochidial larvae of the freshwater mussel, Anodonta oregonensis. Toluene exposure was conducted under flow-through conditions, using five measured concentrations and 20 fish per concentration. The temperature and characteristics of the water used were not specified. The 96 hour $LC_{50}$, as calculated by probit analysis, was 9.36 μℓ/ℓ (ppm) for unparasitized fish and 3.08 μℓ/ℓ for fish parasitized with a mean number of 69 glochidia per fish. The $LC_{50}$ values were significantly different, indicating that parasitized fish were less resistant to the effects of toluene.

Stoss and Haines (1978) investigated the effects of static exposure to toluene on the survival of fertilized eggs and newly hatched fry of the medaka, Oryzias latipes. Groups of ten eggs or fry were exposed in loosely capped vials containing 20 mℓ of the exposure medium (synthetic rearing medium: pH 7.6; akalinity 99 mg/ℓ as $CaCO_3$) at 23 ± 2°C. Toluene concentrations were prepared by diluting a water-soluble extract of 10 mℓ toluene/ℓ medium. In order to determine the sensitivity of different stages of embryo development, tests were begun with eggs of various ages after fertilization. Tests with fry were all begun within 24 hours after hatching. Nominal initial toluene concentrations were used for calculation of $LC_{50}$ values. The $LC_{50}$ values for embryos varied with length of exposure and the age at time of introduction. The mean 24, 48, and 96 hour $LC_{50}$ values for all ages of embryos were 80, 63, and 54 mg/ℓ. The range of $LC_{50}$ values was 20 to 135 mg/ℓ at 48 hours and 23 to 110 mg/ℓ at 96 hours

(Stoss, personal communication). Early (≤3.5 hours old) and late (≥192 hours old) embryos had significantly lower $LC_{50}$ values at each exposure period than embryos of intermediate age at time of introduction. The 24, 48, 96, and 168 hour $LC_{50}$ values for fry were 44, 36, 32, and 23 mg/ℓ, respectively (Stoss, personal communication). These values were lower than the mean embryo $LC_{50}$ values for the same exposure period; however, fry $LC_{50}$ values were greater than the $LC_{50}$ values for the susceptible early and late stage embryos and lower than most of the $LC_{50}$ values for intermediate stage embryos. Stoss and Haines (1978) also investigated the sublethal effects of toluene on hatching time and induction of developmental abnormalities. These sublethal effects are discussed in Section 17.3.2.1.

17.3.1.2. MARINE FISH -- Morrow et al. (1975) studied the effects of toluene on young coho salmon (Oncorhynchus kisutch) that had been acclimated to artificial seawater (30 °/oo (parts per thousand) salinity; 8°C; pH 8.1) for up to 2 weeks. A static exposure technique was used in which toluene was added directly to exposure aquaria containing fish and 73 ℓ of seawater (≤1 g fish/ℓ water) to give nominal concentrations of 0, 1, 10, 50, and 100 ppm toluene. The average weight of the fish used during triplicate tests ranged from 5 g/fish in the fall of the year to nearly 40 g/fish in the spring. The mortality data provided in the paper are given below:

| Concentration (ppm) | No. of Tests | No. of Fish per Concentration | Percent Mortality | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 h | 24 h | 48 h | 72 h | 96 h |
| 0 | 3 | 30 | 0 | 7 | 7 | 13 | 13 |
| 1 | 3 | 30 | 0 | 7 | 7 | 13 | 13 |
| 10 | 3 | 30 | 0 | 0 | 0 | 3 | 10 |
| 50 | 1 | 10 | 0 | 90 | 100 | 100 | 100 |
| 100 | 3 | 30 | 0 | 93 | 100 | 100 | 100 |

Using 2 x 2 contingency table analysis, the authors determined that mortal-

ity was significantly different from control mortality at 50 and 100 ppm, but not at 10 and 1 ppm. The reasons for control mortality were not discussed but may have been due to salinity stress; the authors mentioned that smaller fish adapted less easily to seawater than larger fish. In order to incorporate these data into Table 17-1, the $LC_{50}$ values were calculated as the geometric mean of 50 ppm (mortality = 100%) and 10 ppm (mortality corrected for control mortality = 0%). This value for the 48, 72, and 96 hour $LC_{50}$ was 22.4 ppm. The authors state that fish exposed to 50 and 100 ppm toluene exhibited rapid, violent, and erratic swimming within 15 to 20 minutes, followed by "coughing," loss of equilibrium, and death of most fish within the first few hours.

The acute effects of toluene on another species of salmon in seawater were investigated by Korn et al. (1979). Pink salmon (Onchorhynchus gorbuscha) fry, weighing about 0.35 g each, were acclimated to natural seawater (6 to 8°C; 26 to 28 °/oo salinity). Groups of fry were then acclimated to 4, 8, or 12°C for determination of the 96 hour $LC_{50}$ at 3 temperatures. Each toxicity test was conducted with 10 to 15 fry per concentration (<1 g fish/l water). Fish were added to the test containers after addition of an appropriate amount of toluene in water stock solution. The containers were not aerated until after the first 48 hours of exposure to minimize evaporative loss. Even so, analysis showed that toluene decreased to nondetectable levels by 72 hours at 12°C and by 96 hours at 8°C and to 25% of the initial concentration by 96 hours at 4°C. The 96 hour $LC_{50}$ values, estimated by probit analysis using initial measured concentrations expressed as microliters per liter toluene (= ppm), were 6.4 at 4°C, 7.6 at 8°C, and 8.1 at 12°C. The 95% confidence intervals of the 4°C and 12°C $LC_{50}$ values did not overlap, indicating that temperature affected the toxicity of toluene. There was no significant difference between 24 and 96 hour $LC_{50}$ values because almost all deaths occurred within the first 24 hours of exposure. The effect of

temperature may have been caused by greater sensitivity of the fish at the lower temperature and/or by the longer persistence of toluene at the lower temperature.

Thomas and Rice (1979) used the previously described techniques of Korn et al. (1979) to determine the static 24 hour $LC_{50}$ of toluene with somewhat larger (1 to 2 g, 4.5 to 5.5 cm) pink salmon fry at 12°C in seawater. The 24 hour $LC_{50}$ (and 95% confidence interval) was 5.4 (4.4 to 6.5) ppm, which is significantly different from the 96 hour $LC_{50}$ value of 8.1 ppm (7.5 to 8.8) obtained with younger fry at 12°C by Korn et al. (1979). The reasons for this difference cannot be determined from the information provided.

A similar static exposure technique was used by Benville and Korn (1977) in their study of the acute toxicity of toluene to juvenile striped bass (Morone saxatilis) in seawater (25 °/oo salinity, 16°C). The test was initiated by adding different amounts of saturated toluene in water stock solution to the test aquaria, each containing 10 fish. Toluene concentrations were measured at the beginning of the test and every 24 hours thereafter to the end of the test. The 24 and 96 hour $LC_{50}$ values were both 7.3 µℓ/ℓ (ppm). Almost all mortalities occurred within 6 hours. The average percent loss of toluene was 40% by 24 hours, 53% by 48 hours, and >99% by 72 hours.

The only other information available concerning the lethal effects of toluene on marine fish is provided in a U.S. EPA unpublished study (1978, cited in U.S. EPA, 1980). The 24, 48, and 96 hour static acute $LC_{50}$ values for sheepshead minnows (Cyprinodon variegatus) were all reported to be greater than 277 ppm and less than 485 ppm. The no-effect concentration was 277 ppm. No other information concerning these results was available.

17.3.1.3. FRESHWATER INVERTEBRATES -- Berry and Brammer (1977) investigated the acute static toxicity of toluene to fourth-instar larvae of the

mosquito, Aedes aegypti. The larvae were reared from eggs and tested in distilled water at 25 ± 1°C. For each of four replicate tests, duplicate groups of 20 larvae each were exposed to 14 toluene concentrations. The mortality data were pooled (160 larvae/concentration) to calculate the 24 hour $LC_{50}$ by probit analysis. Initial exposure concentrations were determined by gas-liquid chromatography. The 24 hour $LC_{50}$ (± standard error) was 21.52 ± 0.16 ppm. The highest concentration (± standard error) that caused no mortality over the 24 hour exposure period was 9.95 ± 1.30 ppm.

Berry (1980) mentioned that the upper non-lethal toluene concentration for crayfish (Orconetes rusticus) was 104.4 mg/ℓ. The duration of exposure and lowest lethal concentration were not specified.

The acute toxicity of toluene has also been determined with the cladoceran, Daphnia magna, by Bringmann and Kuhn (1959) and by LeBlanc (1980). Bringmann and Kuhn (1959) reported a 48 hour $LC_{50}$ of 60 mg/ℓ. This static test was conducted with first instar (<24 hours old) Daphnia magna in natural freshwater (pH 7.5; hardness 214 mg/ℓ) at 23°C.

LeBlanc (1980) conducted static tests with first instar (<24 hours old) animals in deionized well water reconstituted to a total hardness of 72 ± 6 mg/ℓ as $CaCO_3$ and a pH of 7.0 ± 0.2 at 22 ± 1°C. Three groups of 5 daphnids each were exposed to each of at least five toluene concentrations and uncontaminated water in covered 250 mℓ beakers containing 150 mℓ of test solution. The 24 and 48 hour $LC_{50}$ values (and 95% confidence intervals), based on initial nominal concentrations, were both 310 (240 to 420) mg/ℓ. The "no discernible effect concentration" was 28 mg/ℓ. This $LC_{50}$ value is considerably higher than that reported by Bringmann and Kuhn (1959). The reasons for this difference cannot be determined from the data provided.

17.3.1.4. MARINE INVERTEBRATES -- Price et al. (1974) determined the static 24 hour $LC_{50}$ of toluene to brine shrimp nauplii (Artemia salina) in artificial seawater (27.87 g/ℓ NaCl; 1.36 g/ℓ $CaSO_4$; 3.17 g/ℓ $MgSO_4 \cdot 7H_2O$; 8.42 g/ℓ $MgCl_2$; 0.79 g/ℓ KCl; 0.16 g/ℓ $MgBr_2 \cdot 6H_2O$) at 24.5°C. Groups of 30 to 50 newly hatched brine shrimp were exposed to 5 toluene concentrations in 100 mℓ seawater. The estimated 24 hour $LC_{50}$, based on initial nominal concentrations, was 33 mg/ℓ.

Bay shrimp (Crago franciscorum) were shown by Benville and Korn (1977) to be somewhat more sensitive to toluene. The 24 hour static $LC_{50}$, determined in natural seawater (25 °/oo salinity) at 16°C, was 12 µℓ/ℓ (ppm). The 96 hour $LC_{50}$ for this species (4.3 µℓ/ℓ) was significantly lower than the 24 hour $LC_{50}$ (non-overlapping 95% confidence limits). These values were calculated from initial measured toluene concentrations.

Korn et al. (1979) investigated the effects of temperature on the acute toxicity of toluene to another genus of shrimp (Eualus spp.). Shrimp (0.8 g; 6 cm long) were acclimated to the test temperatures in natural 26 to 28 °/oo salinity seawater for 4 days and then exposed in groups of 10 to 15 animals to a series of toluene concentrations, prepared by dilution of a saturated water solution. The tissue loading in the test containers was less than 1 g/ℓ. Measurement by UV spectrophotometry showed that toluene concentrations decreased to nondetectable levels by 72 hours at 12°C and by 96 hours at 8°C, and to 25% of the initial concentration by 96 hours at 4°C. The 96 hour $LC_{50}$ values, calculated from initial measured toluene concentrations, were 21.4 µℓ/ℓ at 4°C, 20.2 µℓ/ℓ at 8°C, and 14.7 µℓ/ℓ at 12°C. The 96 hour $LC_{50}$ values at 4°C and 8°C were not significantly different (overlapping 95% fiducial limits) from each other, but both were significantly higher than the 96 hour $LC_{50}$ at 12°C. This trend of greater toxicity at higher temperatures was opposite to the relationship

found by these authors for pink salmon fry (Section 17.3.1.2.) and by Potera (1975) for grass shrimp (see below). The reasons for this difference could not be established but may have been due to some combination of effects of temperature on persistence of toluene in water, altered toluene uptake and metabolic rates, and possible interaction of toluene toxicity and temperature stress. The authors concluded that temperature affected the toxicity of toluene to these species of shrimp and salmon but that it would be impossible to predict the effects of temperature change on the toxicity of toluene to other species.

Potera (1975) investigated the effects of temperature (10 and 20°C), salinity (15 and 25 °/oo), and life stage (larvae and adults) on the static 24 hour $LC_{50}$ of toluene to the grass shrimp, Palaemonetes pugio. The 24 hour $LC_{50}$ values, based on measured initial concentrations, ranged from 17.2 to 38.1 mg/ℓ.

As shown by overlapping 95% confidence intervals (Table 12-1), there was no significant difference in $LC_{50}$ values between adults and larvae at the same salinity and temperature, or between adults tested at the same temperature but at different salinities. The $LC_{50}$ was significantly lower at 20°C, however, than at 10°C for adults tested at either 15 °/oo or 25 °/oo salinity. The time to produce narcosis in at least 50% of adult shrimp at 20°C was less than 30 minutes at initial exposure concentrations of 19.8 mg/ℓ and greater. Recovery of more than 90% of exposed shrimp could occur if shrimp were transferred to clean water after exposure to up to 30 mg/ℓ for 30 minutes.

Potera (1975) also determined the 24 hour $LC_{50}$ for the copepod, Nitocra spinipes, at a temperature of 20°C and at salinities of either 15 °/oo or 25 °/oo. The 24 hour $LC_{50}$ values from replicate tests were 24.4 at 15 °/oo salinity and 74.2 mg/ℓ at 25 °/oo salinity. These values were significantly different (non-overlapping 95% confidence intervals). Potera (1975) suggested

that the lower salinity may have stressed the copepods, resulting in a lower $LC_{50}$ value.

Neff et al. (1976) also determined the static 96 hour $LC_{50}$ of toluene to grass shrimp, Palaemonetes pugio. This value, based on initial nominal concentrations, was 9.5 mg/ℓ, which is lower than the 24 hour $LC_{50}$ values reported by Potera (1975).

Caldwell et al. (1976) determined the 48 and 96 hour $LC_{50}$ of toluene to larval stages of the dungeness crab (Cancer magister) under flow-through exposure conditions. The 48 and 96 hour $LC_{50}$ values were 170 and 28 mg/ℓ, respectively.

Static acute $LC_{50}$ values for mysid shrimp (Mysidopsis bahia) have been reported by the U.S. EPA (1978, cited in U.S. EPA, 1980). The 24 and 48 to 96 hour $LC_{50}$ values were 64.8 and 56.3 ppm, respectively. The "no effect" concentration was 27.7 ppm. Additional information concerning this test was not available.

The 48 hour static $LC_{50}$ of toluene to larvae of the Pacific oyster (Crassostrea gigas) was reported to be 1050 mg/ℓ (LeGore, 1974). This test was conducted with filtered seawater (25.3 to 30.8 °/oo salinity) at 20 to 21.5°C using 30,000 larvae per exposure concentration.

17.3.2. Sublethal Effects.

17.3.2.1. FISH -- Very little information is available concerning the sublethal effects of toluene exposure on fish. Morrow et al. (1975) studied the effects of several aromatic hydrocarbons, including toluene, on the levels of $Na^+$ and $K^+$ in the blood of young coho salmon (Oncorhynchus kisutch) in seawater. Static exposure to 30 ppm toluene caused a small increase in these blood cations,

reaching a maximum at about two hours after beginning exposure. The $Na^+$ concentration returned to the control level by three hours. Blood $K^+$ decreased after two hours but was still elevated at four hours, the last sampling period. The toluene exposure concentration of 30 ppm was sufficient to cause some mortalities and behavioral effects. The authors suggested that toluene increased membrane permeability, particularly in the gills. In the hypertonic seawater medium, this change would result in ion influx and water loss in the fish, perhaps accounting for the initial rise in blood ion concentration.

Brenniman et al. (1979) conducted a series of experiments to determine the effects of toluene exposure on blood gas physiology, hippuric acid content, and histopathology of goldfish (Carassius auratus). The fish used in these experiments were exposed to two or more toluene concentrations under flow-through conditions using dechlorinated tapwater.

For the pathology study, groups of six fish were exposed for up to 30 days to 0, 5, 10, and 21 ppm toluene (Brenniman et al., 1979). No gross or microscopic lesions were observed in fish during the first week of exposure. After the first week, ascites developed in 3 fish at 21 ppm and in 2 fish at 10 ppm. In exposed fish that survived 15 to 30 days, about 50% had a white epidermal exudate of unknown origin, and some fish at all toluene concentrations had gross lesions in gill, liver, or gall bladder. Excessive mucus production in gills occurred in all fish at 21 and 10 ppm and in 50% of the fish at 5 ppm. Microscopic lesions were found in gills (fusion), liver (decreased cytoplasmic nuclear ratio), and kidney (tubular vacuolization) of many exposed fish but not in control fish. Exposed fish did not eat food and had livers which were paler and smaller than control fish.

For the blood gas study, groups of 3 or 4 fish were exposed for 4 hours to 0, 60, or 80 ppm toluene (Brenniman et al., 1979). The blood samples were analyzed

for pH, percent oxygen saturation, partial pressures of carbon dioxide ($p_{CO_2}$) and oxygen ($p_{O_2}$), and bicarbonate. The results are presented below:

| Toluene Conc. (ppm) | Mean Values | | | | |
|---|---|---|---|---|---|
| | $P_{O_2}$ | $P_{CO_2}$ | pH | $O_2$-Saturation (%) | Bicarbonate |
| 0 | 42.33 | 11.50 | 7.56 | 48.67 | 9.83 |
| 60 | 16.25[a] | 23.25[a] | 6.90[a] | 27.00[a] | 5.10 |
| 80 | 15.63[a] | 19.27 | 6.96[a] | 20.33[a] | 4.17[a] |

[a] $P < 0.05$ when compared to control.

Toluene exposure caused significant changes in all parameters (Brenniman et al., 1979). The authors suggested that the decreased $p_{O_2}$, increased $p_{CO_2}$, and resultant acid-base imbalance may have been due to lowered $O_2$ and $CO_2$ exchange at the gills. Two proposed mechanisms for impaired gas exchange were lowered respiratory rate and gill damage. The former mechanism is less likely because sublethal toluene exposure has been shown to increase the respiratory rate in fish (Slooff, 1978, 1979; Thomas and Rice, 1979). The latter mechanism is supported by the authors' observation that toluene caused excess mucus production and fusion of gill lamellae in gills.

The whole-fish content of hippuric acid was measured in fish exposed in groups of 6 fish to 0, 5, 10, or 21 ppm toluene for 96 hours (Brenniman et al., 1979). This experiment was conducted to determine whether the fish were able to metabolize toluene ultimately to hippuric acid, as occurs in mammals (Chapter 12.). The results, presented below, indicated that hippuric acid was elevated at all the toluene concentrations tested and that this metabolic pathway occurs in goldfish.

| Toluene Concentration (ppm) | Mean Hippuric Acid Concentration (ppm) |
|---|---|
| 0 | 1539.50 |
| 5 | 3608.67[a] |
| 10 | 3536.67[a] |
| 21 | 2829.17[a] |

[a] $P < 0.05$ when compared to control.

The pattern of decreasing hippuric acid concentration with increasing toluene concentration was attributed to increasing stress and lower metabolic efficiency as toluene concentration increased. Hippuric acid was elevated above the control levels, however, even at the highest toluene concentration.

The only other information available relevant to toluene metabolism in fish is provided by Ohmori et al. (1975), who investigated the comparative *in vitro* metabolism of a toluene analog, p-nitrotoluene, by liver homogenates of rats and eels. The species of eel was not specified. Both species were able to metabolize p-nitrotoluene (PNT) to p-nitrobenzoic acid (PNB acid), via oxygenation of PNT to p-nitrobenzyl alcohol (PNB alcohol), to p-nitrobenzaldehyde (PNB aldehyde), and finally to PNB acid. The rate of the overall reaction (PNT to PNB acid) in eel liver, however, was only 34% (at 25°C) to 46% (at 37°C) of the rate in rat liver. The rate of formation of PNB alcohol from PNT in eel liver was 29% (at 25°C) to 16% (at 37°C) of the rate in rat liver. This step was the rate-limiting step for the overall reaction because the formation of PNB acid from PNB alcohol was faster in eels than in rats.

Thomas and Rice (1979) measured the effects of flow-through toluene exposure on the respiratory rate and oxygen consumption of pink salmon (*Oncorhynchus gorbuscha*) fry at two temperatures (4°C, 12°C) in seawater. The fish were placed in sealed chambers fitted with a water inlet and outlet, mesh electrodes (for measuring opercular breathing rate), and oxygen electrodes (for measuring oxygen concentration of inflowing and outflowing water). After

determining the 24 hour $LC_{50}$ (5.38 ppm), the authors exposed fry to several toluene concentrations, expressed as percentages of the $LC_{50}$. Significant increases in opercular breathing rate at 12°C occurred at exposure concentrations of 94 and 69% of the $LC_{50}$, but not at 45 or 30% of the $LC_{50}$. The breathing rate remained elevated throughout the 15 hour exposure period only at 94% of the $LC_{50}$, at which concentration 6 of 23 fish died. The breathing rate at a toluene exposure concentration of 69% of the $LC_{50}$ reached a maximum at three hours and returned to control level by 15 hours. Additional experiments showed that exposures to 71% of the $LC_{50}$ increased oxygen consumption. The percent increase in both oxygen consumption and breathing rate was greater at 4°C than at 12°C. The authors suggested that these effects were due to the energy requirements for metabolism of toluene and that this requirement was greater at the lower temperature. The threshold for an effect on breathing rate at 12°C was estimated to be about 46% of the $LC_{50}$, or about 2.5 ppm.

Slooff (1978, 1979) conducted similar experiments to determine the sensitivity of a biological monitoring system using fish respiratory rates as an indicator of water pollution by toluene and other chemicals. Adult rainbow trout (mean weight 56 g) were acclimated to dechlorinated tapwater at 20 $\pm$ 1°C and tested individually in sealed flow-through chambers equipped with stainless steel mesh electrodes for measuring breathing rate. After the normal breathing rate for a fish over a three day period had been determined, toluene contaminated water was added continuously and the breathing rates were monitored over a period of 48 hours. Measurements were taken at the same time of day during the pre-exposure and exposure periods. A toxic effect was considered to have occurred if the respiration frequency of at least 75% of the test fish exceeded the predetermined individual normal frequencies measured at the same hourly interval. The lowest toluene concentration that caused an increase in respiratory rate was

2.5 mg/ℓ. This concentration is identical to the estimated threshold concentration for an effect on breathing rate in pink salmon (Thomas and Rice, 1979).

Leung and Bulkley (1979) investigated the effects of 100 μℓ/ℓ toluene on the rate of opercular movement by eight day old embryos of the Japanese medaka, Oryzias medaka. The basal (unexposed) rate was determined for each of three embryos and then toluene was added to the culture medium to obtain a nominal concentration of 100 μℓ/ℓ. The rate was then determined for each embryo at about five minute intervals for 40 minutes. The average rate before exposure was zero movements/minute. The average of 8 counts (each 1 minute long) over 40 minutes after beginning exposure was 2.28 movements/minute. The standard deviation was so great, however, that this increase was not statistically significant.

The sublethal effects of toluene on medaka were also investigated by Stoss and Haines (1978). The exposure techniques and lethal effects reported by these authors have been discussed in Section 17.3.1.1. Static exposure of eggs to initial nominal concentrations of 41 and 82 mg toluene/ℓ resulted in a significant delay in time to hatching and a decrease in the proportion of embryos that hatched successfully. Exposure to 41 mg/ℓ and greater caused numerous developmental abnormalities, including disruption of cell cleavage patterns, deformation of eyes, appearance of isolated blood islands in the circulatory system, and abnormal heart structure, tail flexures, and visceral organ formation and placement. No abnormalities were observed in embryos exposed to 16 mg toluene/ℓ.

The only other information available concerning sublethal toluene effects on fish is provided in a U.S. EPA unpublished study (1978, cited in U.S. EPA, 1980). An embryo-larval subchronic test with the sheepshead minnow (Cyprinodon variegatus) in seawater showed that toxic effects were observed at a toluene concentration of 7.7 ppm, but not at 3.2 ppm. The type(s) of toxic effects were not specified in the U.S. EPA (1980) report, which was simply a data compilation.

The 96 hour $LC_{50}$ for this species was between 277 and 485 ppm (Section 17.3.1.2.). The ratio between acute and sub-chronic toxicity was between 36 and 152, indicating that chronic effects occur at concentrations much lower than acute effects.

In summary, the lowest toluene concentration shown to cause sublethal effects in fish was 2.5 ppm, the concentration which caused an increased breathing rate in trout (Slooff, 1978, 1979) and salmon (Thomas and Rice, 1979). This value is somewhat below the lowest acute $LC_{50}$ value reported for any fish species (3.08 ppm for coho salmon, see Table 17-1). An embryo-larval test with sheepshead minnow (U.S. EPA, 1980) showed that subchronic toxic effects occurred at 7.7 ppm but not at 3.2 ppm and that the ratio between the acute $LC_{50}$ and subchronic toxicity for this species was between 36 and 152. Although acute-chronic ratios may vary greatly among species, this information suggests that chronic toxic effects may occur in coho salmon and other sensitive species at concentrations well below 3 ppm.

17.3.2.2. INVERTEBRATES -- Berry et al. (1978) conducted a series of experiments to determine the effects of 24 hours of exposure to sublethal concentrations of water-soluble fractions (WSFs) of gasoline, benzene, xylenes, and toluene on oxygen consumption by fed and unfed larval stages of the mosquito, Aedes aegypti. Control experiments with untreated animals showed that there was no significant difference in $O_2$ consumption between fed and unfed larvae. Treatment with the WSF of 1 mℓ/ℓ gasoline, however caused an increased $O_2$ consumption in fed, but not unfed, larvae relative to untreated controls. Treatment of fed larvae with individual WSFs of benzene (1 mℓ/ℓ), xylenes (0.3 mℓ/ℓ), or toluene (0.1 to 0.5 mℓ/ℓ) had no effect on $O_2$ consumption relative to fed controls. A WSF mixture of benzene, xylenes, and toluene and a mixture of

benzene and toluene (0.2 mℓ/ℓ for each compound) caused significant increases in $O_2$ consumption. Exposure to a WSF mixture of benzene and xylenes or toluene and xylenes (0.2 mℓ/ℓ for each compound) had no effect. The authors also conducted experiments on the uptake of $^3H$-labeled toluene in fed and unfed animals, as well as uptake of $^3H$- toluene by fed larvae in the presence or absence of benzene (Section 15.3.). Maximum $^3H$-toluene counts were equal in fed and unfed larvae, but were reached more quickly (one hour versus four hours) by the fed animals. The $^3H$-toluene counts in larvae, expressed as the percentage of the initial water counts, were greater in the benzene and toluene mixture than in the solution containing toluene alone. The authors concluded that the effects of gasoline on $O_2$ consumption were due to the enhanced uptake and synergistic effects of toluene and benzene, two of the major aromatic components of gasoline. They also suggested that the presence of food accelerated the uptake of toluene through absorption of toluene to the consumed food particles.

Blundo (1978) investigated the effects of toluene on the swimming activity and survival of barnacle (Balanus eburneus) larvae. Groups of larvae were exposed for one hour in specially constructed tubes to 10, 20, 30, 40, 50, 60, 70, 80, and 90% of the water soluble fraction (WSF) made by saturating seawater with toluene. The tubes were designed so that actively swimming photopositive larvae would be attracted to light at the top of the tube. After one hour of exposure, the inactive larvae were collected from the bottom of the tubes and stained with a vital dye (neutral red) to determine percent mortality. The remaining portion, containing the active larvae, was then collected and counted. The interpolated concentration that immobilized 50% of the larvae was 12.5% of the WSF. All larvae were immobilized at 30% WSF and higher. About 33-1/3% of the larvae were immobilized at 10% WSF, the lowest concentration tested. The percent mortality of the immobilized larvae ranged from about 3% at 10% WSF to a maximum

of 12% at 90% WSF. The author also measured the effects of WSFs that had been aged in covered containers for one day in a refrigerator or exposed to air for up to 3 days. The percent WSF that immobilized 33-1/3% of the larvae was 10% in the fresh solution, 37.5% in the refrigerated solution, and 90% in the evaporated solution. Additional experiments showed that aeration of the WSF for six hours lowered the toxicity to the same extent as three days of exposure to air.

Bakke and Skjoldal (1979) investigated the effects of toluene on activity, survival, and physiology of the isopod, Cirolana borealis. For determination of median effective times ($ET_{50}$, partial or complete narcotization as endpoint), groups of 15 isopods were exposed in duplicate to nominal initial concentrations of 0, 0.0125, 1.25, 5.7, 12.5, 25, and 125 ppm toluene for 4 days. The exposure medium (33.5 to 34.5 °/oo salinity seawater at 8 to 10°C) was changed every 2 days. The interpolated or extrapolated ET50 values were as follows:

| Toluene Concentration (ppm) | $ET_{50}$ (hours) |
|---|---|
| 0 | — |
| 0.0125 | — |
| 1.25 | — |
| 5.7 | 400 |
| 12.5 | 69 |
| 25 | 28 |
| 125 | 3 |

No effects on activity were observed in animals exposed to 1.25 ppm or less (Bakke and Skjoldal, 1979). The authors also investigated the recovery of isopods after exposure for varying periods to 12.5 or 125 ppm toluene. Exposure to 125 ppm for one hour caused complete inactivity, but all animals recovered within 12 hours after transfer to clean water. Exposure for 2 or more hours to 125 ppm caused partial or complete mortality. All isopods could recover after exposure to 12.5 ppm for 30 hours but not longer. Additional experiments showed that there was no significant effect of 4 days of exposure to up to 5.7 ppm

toluene on oxygen consumption, ATP concentration, or energy charge. Exposure to 12.5 ppm resulted in a progressive decrease in ATP level and energy charge over eight days of exposure, at which time all organisms had died. Exposure to the rapidly lethal concentration of 125 ppm toluene showed no effect on ATP level or energy charge. These results with 12.5 and 125 ppm were essentially the same as those reported by the authors in a previous paper (Skjoldal and Bakke, 1978). Bakke and Skjoldal (1979) concluded that the effect of toluene on activity was much more sensitive as an indicator of sublethal toluene toxicity than its effects on respiration, ATP level, and energy charge.

In summary, the lowest toluene concentration shown to cause sublethal effects in invertebrates was 5.7 ppm, the concentration which caused narcotization of isopods (Bakke and Skjoldal, 1979). This concentration is somewhat higher than the 96 hour $LC_{50}$ of 4.3 ppm for bay shrimp (see Table 17-1) reported by Benville and Korn (1977). The latter concentration is the lowest reported to have toxic effects on freshwater or marine invertebrates. Although the chronic toxicity of toluene to aquatic invertebrates has not been studied, it is probable that chronic effects could occur in sensitive invertebrate species at concentration below 4.3 ppm. This conclusion is supported by the fact that chronic effects in fish occurred at concentrations well below the acutely toxic concentrations (Section 17.3.2.2.).

# 18. HEALTH EFFECTS SUMMARY

## 18.1. EXISTING GUIDELINES AND STANDARDS

18.1.1. Air. The Occupational Safety and Health Administration (OSHA) currently limits occupational exposure to toluene to 200 ppm as an 8 hour time-weighted-average (TWA), with an acceptable ceiling concentration of 300 ppm (40 CFR 1910.1000); the acceptable maximum peak above the ceiling concentration is 500 ppm for a maximum duration of 10 minutes. The National Institute for Occupational Safety and Health (NIOSH, 1973) currently recommends an exposure limit of 100 ppm as an 8 hour TWA with a ceiling of 200 ppm. An 8 hour TWA concentration of 100 ppm is also recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1980) as a Threshold Limit Value (TLV) for toluene; the short-term (15 minute) exposure limit recommended by the ACGIH is 150 ppm. ACGIH (1980) has further noted that there may be significant contribution to the overall exposure by the cutaneous route.

Threshold limit values that have been established for occupational exposure to toluene in other countries are listed as follows (Verschueren, 1977):

| | | |
|---|---|---|
| USSR | 13 ppm (50 $mg/m^3$) | 1972 |
| Czechoslavakia | 52 ppm (200 $mg/m^3$) | 1969 |
| West Germany (BDR) | 200 ppm (750 $mg/m^3$) | 1974 |
| East Germany (DDR) | 52 ppm (200 $mg/m^3$) | 1973 |
| Sweden | 98 ppm (375 $mg/m^3$) | 1975 |

There are no standards for general atmospheric pollution by toluene in the United States, although a National Ambient Air Quality Standard specifies that nonmethane hydrocarbons shall not exceed 0.24 ppm (160 $\mu g/m^3$) as a maximum 3 hour average concentration (6 to 9 a.m.), more than once per year (40 CFR 50). Ambient air quality standards have, however, been promulgated for toluene in

other countries. These foreign standards are summarized as follows (Verschueren, 1977):

| Country | Concentration | Averaging Time |
|---|---|---|
| USSR | 0.15 ppm (0.6 mg/m$^3$) | 20 min |
| | 0.15 ppm (0.6 mg/m$^3$) | 24 hr |
| West Germany (BRD) | 15 ppm (60 mg/m$^3$) | 30 min |
| | 5 ppm (20 mg/m$^3$) | 24 hr |
| East Germany (DDR) | 0.5 ppm (2.0 mg/m$^3$) | 30 min |
| | 0.15 ppm (0.6 mg/m$^3$) | 24 hr |
| Bulgaria | 0.15 ppm (0.6 mg/m$^3$) | 20 min |
| | 0.15 ppm (0.6 mg/m$^3$) | 24 hr |
| Hungary | 13.3 ppm (50.0 mg/m$^3$) | 30 min |
| | 5.3 ppm (20.0 mg/m$^3$) | 24 hr |
| Hungary (protected areas) | 0.16 ppm (0.6 mg/m$^3$) | 30 min |
| | 0.16 ppm (0.6 mg/m$^3$) | 24 hr |
| Yugoslavia | 0.16 ppm (0.6 mg/m$^3$) | 20 min |
| | 0.16 ppm (0.6 mg/m$^3$) | 24 hr |

18.1.2. Water. The Committee on Safe Drinking Water of the National Academy of Sciences concluded in 1977 that toluene and its major metabolite, benzoic acid, were relatively nontoxic, and that there was insufficient toxicological data available to serve as a basis for setting a long-term ingestion standard (NAS, 1977). It was recommended that studies be conducted to produce relevant information. Toluene has recently been considered for a second time by a reorganized Toxicology Subcommittee of the Safety Drinking Water Committee of the National Academy of Sciences (U.S. EPA, 1980), but the results of the deliberations of this group have not yet been made public.

The U.S. EPA (1980) has recently derived an ambient water criterion level for toluene of 14.3 mg/ℓ. This criterion is intended to protect humans against the toxic effects of toluene ingested through water and contaminated aquatic organisms, and is based on an Acceptable Daily Intake (ADI) calculated from the

maximum-no-effect dose reported in the Wolf et al. (1956) subchronic oral study in rats and an uncertainty factor of 1000. The criterion level for toluene can alternatively be expressed as 424 mg/l if exposure is assumed to be from the consumption of fish and shellfish products alone.

18.1.3. Food. Toluene has been approved by the Food and Drug Administration for use as a component of articles intended for use in contact with food (i.e., an indirect food additive). Articles that contain residues of toluene may be used in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. The use of toluene in the food industry is summarized as follows:

| | |
|---|---|
| Component of adhesives | 21 CFR 175.105 |
| Adjuvant substance in resinous and polymeric coatings for polyolefin films used as food contact surfaces | 21 CFR 175.320 |
| Component of the uncoated or coated surfaces of paper and paperboard articles intended for use with dry foods | 21 CFR 176.180 |
| Used in the formulation of semirigid and rigid acrylic and modified acrylic plastic articles | 21 CFR 177.1010 |
| Additive for cellophane (residue limit 0.1%) | 21 CFR 177.1200 |
| Additive for 1,4-cyclohexylene dimethylene terephthalate and 1,4-cyclohexylene dimethylene isophthalate copolymer | 21 CFR 172.1240 |
| Solvent for 4,4'-isopropylidenediphenol-epichlorohydrin resins with a minimum molecular weight of 10,000 (residue limit $\leq$1000 ppm in the finished resin) | 21 CFR 177.1440 |
| Solvent for polysulfide polymer-polyepoxy resins | 21 CFR 177.1650 |

| | |
|---|---|
| Solvent for poly(2,6-dimethyl-1,4-phenylene)oxide resins (residue limit 0.2% by weight) | 21 CFR 177.2460 |
| Blowing agent adjuvant used in the manufacture of foamed polystyrene (residue limit ≤0.35% by weight of finished framed polystyrene) | 21 CFR 178.3010 |

Toluene has also been exempted from the requirement of a tolerance when it is used as a solvent or cosolvent in pesticide formulations which are applied to growing crops (40 CFR 180.1001).

## 18.2. INHALATION EXPOSURES

As detailed in Chapter 11 of this report, many studies have reported the effects on humans of inhalation exposures to toluene. Because most of these studies involved relatively small numbers of human subjects, they failed to precisely define the levels or durations of the exposures, and/or did not consider the potential role of exposures to other toxicants. None of these studies would be suitable for human risk assessment if taken individually. In combination, however, they constitute a considerable body of human experience and provide a relatively consistent pattern of dose-response relationships.

18.2.1. Effects of Single Exposures. The effects on humans of single exposures to toluene for periods of up to eight hours are relatively well documented. Data on both toluene glue sniffers (Press and Done, 1967a, 1967b; Wyse, 1973; Lewis and Patterson, 1974; Helliwell and Murphy, 1979; Hayden et al., 1977; Oliver and Watson, 1977; Barnes, 1979) and workers accidentally exposed to high levels of toluene (Lurie, 1949; Browning, 1965; Longley et al., 1967; Reisen et al., 1975) indicate that exposure to air saturated or nearly saturated with toluene can cause a spectrum of effects, from lightheadedness to unconsciousness, in a very short period of time. Deaths attributed to the deliberate inhalation of toluene have been reported in at least 24 cases (Winek et al., 1968; Chiba, 1969;

Nomiyama and Nomiyama, 1978). Although most of these reports do not provide quantitative exposure estimates, glue sniffers are probably exposed to nearly saturated air-vapor mixtures of about 30,000 ppm toluene. The occupational report of Longley et al. (1967) indicated that a loss of consciousness occurred within minutes after exposure to atmospheres estimated to contain 10,000 ppm toluene at waist level and 30,000 ppm toluene at floor level. The acute inhalation toxicity data on experimental mammals, summarized in Table 12-1, suggest that exposure periods of several hours to toluene levels greater than 4000 ppm may be lethal. Based on the results of longer term human studies discussed below, short exposures to concentrations of up to 1500 ppm are not likely to be lethal (Wilson, 1943; Ogata et al., 1970, see following discussion). The single report by Gusev (1965) of effects on EEG activity in 4 individuals exposed to 0.27 ppm for 6 minute intervals may be a subtle indication of the perception of toluene at this low level but does not have any apparent toxicologic significance.

For single exposure periods that approximate a normal working day (7 to 8 hours), von Oettingen et al. (1942a, 1942b) and Carpenter et al. (1944) provide relatively consistent information on sublethal dose-response relationships. As summarized previously in Table 10-1, von Oettingen et al. (1942a, 1942b) noted a range of subjective complaints from 8 hour exposures to toluene concentrations ranging from 50 ppm (drowsiness) to 800 ppm (severe fatigue, nausea, incoordination, etc., with after effects lasting at least several days). Although the terminology used by Carpenter et al. (1944) is somewhat different from that used by von Oettingen, the effects noted seem comparable over the common exposure range (200 to 800 ppm). Although the consistency between these two studies is reassuring, it should be noted that even combined both studies involve exposures of only five individuals who were placed on multiple

exposure/recovery schedules. The impact that such multiple exposures could potentially have on the results cannot be determined. Given the small number of individuals involved in the exposures to toluene, an attempt to generalize for the human population a detailed dose-response gradient comparable to that presented in Table 11-1 does not seem justifiable. When these studies are considered along with the results of Ogata and coworkers (1970) and Gamberale and Hultengren (1972) however, it seems reasonable to conclude that exposure periods of 8 hours or less to toluene concentrations below 100 ppm may result in mild subjective complaints (fatigue or headache) but are not likely to induce observable effects. Concentrations above 100 ppm may cause impaired reaction time (200 ppm x 3 hours, Ogata et al., 1970; 300 ppm x 20 minutes, Gamberale and Hultengren, 1972). At concentrations of 300 to 800 ppm and above, gross signs of incoordination may be expected (von Oettingen et al., 1942a, 1942b; Carpenter et al., 1944).

Accidental acute overexposure to toluene may be limited to some extent by the organoleptic or irritant properties of the compound. Gusev (1965) reports ranges of maximum imperceptible concentrations and minimum perceptible concentrations of 0.35 to 0.79 ppm and 0.40 to 0.85 ppm, respectively. May (1966) reports a minimum perceptible concentration of 37 ppm. The reasons for this discrepancy between the Russian and American values are not apparent. Although the Russian study entailed a total of 30 subjects and 744 observations and the American report involved 16 individuals (number of observations not specified), it is unlikely that the difference in the reported detectable levels is due simply to sample size. In any event, toluene appears to be detectable in the air at levels below those causing impaired coordination (i.e., >100 ppm). In addition, Carpenter and coworkers (1944) reported that toluene caused mild throat and eye irritation at 200 ppm and also caused lacrimation at 400 ppm.

In summary, the estimated dose-response relationships for the acute effects of single short-term exposures to toluene are presented below:

| | | |
|---|---|---|
| 10,000 to 30,000 ppm | : | Onset of narcosis within a few minutes. Longer exposures may be lethal. |
| >4,000 ppm | : | Would probably cause rapid impairment of reaction time and coordination. Exposures of 1 hour or longer might lead to narcosis and possibly death. |
| 1,500 ppm | : | Probably not lethal for exposure periods of up to 8 hours. |
| 300 to 800 ppm | : | Gross signs of incoordination may be expected during exposure periods up to 8 hours. |
| 400 ppm | : | Lacrimation and irritation to the eyes and throat. |
| 100 to 300 ppm | : | Detectable signs of incoordination may be expected during exposure periods up to 8 hours. |
| 200 ppm | : | Mild throat and eye irritation. |
| 50 to 100 ppm | : | Subjective complaints (fatigue or headache) but probably no observable impairment of reaction time or coordination. |
| >37 ppm | : | Probably perceptible to most humans. |

From the above discussion, it should be evident that these approximations are crude composites and contain several areas of uncertainty and overlap.

18.2.2. Effects of Intermittent Exposures Over Prolonged Periods. Limited information is available on the effects of subchronic or chronic continuous exposures to toluene on humans or experimental animals. Most of the studies either involve occupational exposures or are designed to mimic occupational exposures. Consequently, while the data described below may be directly applicable to estimating effects from occupational exposures, an additional element of uncertainty must be considered in any attempt to estimate the effects of continuous exposures that may occur from ambient air.

Wilson (1943) provides the only acceptable data on the effects of repeated occupational exposures to toluene over a period of weeks (Section 11.1.1.2.). In this study, the workers were classified into three groups by the levels of toluene to which they were exposed: 50 to 200 ppm, 200 to 500 ppm, and 500 to 1500 ppm. The effects noted at the various levels were essentially the same as those seen in single exposures. In the low exposure group, the reports of headache and lassitude are consistent with symptoms noted by von Oettingen and coworkers (1942a, 1942b) over the same range of exposure. Although Wilson (1943) did not attribute these effects to toluene exposure, his failure to include an unexposed control group makes this judgment questionable in view of the von Oettingen data. In the middle and high exposure groups, the reports of headache, nausea, and concentration-related impairment of coordination and reaction time are also consistent with the symptoms reported by von Oettingen and coworkers (1942a, 1942b) and Carpenter and coworkers (1944) for short-term single exposures. The major discomforting feature of the Wilson (1943) report is that it involved only 100 out of a total of 1000 workers. It is unclear whether the remaining 900 workers evidenced any symptoms of toluene exposure.

The only other study that reports effects of repeated exposures to toluene for relatively short periods of time is that presented by Greenburg and coworkers (1942). In this study, repeated occupational exposures to toluene at levels of 100 to 1100 ppm for periods of 2 weeks to 5 years were associated with enlarged livers in 13 of 61 airplane painters. This incidence of liver enlargement was reported to be 3 times that of a control group of 430 workers not exposed to toluene. Because Greenburg and coworkers (1942) were not able to associate liver enlargement with clinical or laboratory evidence of disease, because the painters were also exposed to significant quantities of other volatile paint components (Table 11-9), and because the liver effect has not been corroborated

by other investigators (e.g., Parmeggiani and Sassi, 1954; Suhr, 1975), the hepatomegaly reported by Greenburg should be given relatively little weight in risk assessment.

Other reports of repeated occupational exposures to toluene involve periods of several years. For mean exposure levels above 200 ppm, all of the available studies except that of Suhr (1975) report some evidence of neurologic effects (Capellini and Alessio, 1971; Parmeggiani and Sassi, 1954; Munchinger, 1963; Rouskova, 1975).

The Suhr (1975) study involved a group of 100 printers exposed to 200 to 400 ppm toluene for over 10 years. Compared to a group of 100 non-exposed individuals, no significant differences were seen in symptoms of central nervous system (CNS) depression or Sphallograph tests, which are designed to measure muscular coordination. An interpretation of the significance of the Suhr (1975) study is confounded, however, by several factors. As discussed in Sections 11.1.1.2. and 11.3., the limitations of this study include an undefined control group, uncertainties involving the time of reflex reaction and sphallograph testing (i.e., blood toluene levels may have declined significantly if the workers were examined before or after the work shifts), and the use of an apparently unvalidated device (sphallograph) for the detection of slight disturbances of muscular coordination.

The other studies that do report effects at equal or higher levels of exposure can be challenged for various reasons. The report of "nervous hyperexcitability" in 6 of 11 exposed to 200 to 800 ppm toluene for "many years" (Parmeggiani and Sassi, 1954) does not seem to be characteristic of toluene intoxication. This report is from the Italian literature, however, and a full text translation has not yet been made available for this review. The Capellini and Alessio (1971) study, which associated stupor, nervousness, and insomnia

with occupational exposure to 250 (210 to 300) ppm toluene for several years, involved only a single worker. The "organic psychosyndrome" diagnosed by Munchinger (1963) in workers exposed to 300 and 430 ppm toluene for 18 and 12 years, respectively, is supported by the results of Rorschach tests and Knoepfel's 13-Error tests. Because Munchinger did not use a control group, however, the utility of this study is limited. The changes in EEG response to photic stimulation that were reported by Rouskova (1975) in workers exposed to >250 ppm toluene for an average of 13.5 years also involved exposure to unspecified levels of 1,1,1-trichloroethane. Thus, the interpretation of the discrepancies between the study by Suhr (1975) and these other reports is problematic. Considering the relatively well documented CNS effects of single exposures to toluene at levels above 200 ppm (Section 18.1.1.) and the effects noted by Wilson (1943) at comparable levels for much shorter periods of time, it would seem imprudent to accept the Suhr (1975) data as a "no-observed-effect level" for human risk assessment.

An alternative approach could be to use the study by Capellini and Alessio (1971) in which no CNS or liver effects were noted in a group of 17 workers occupationally exposed to 125 (80 to 160) ppm toluene for "diverse years." In addition to the problems of small sample size, failure to precisely define the duration of exposure, and lack of a control group, the use of this study is compromised by reports of effects in two other groups of workers at lower levels of toluene exposure. Matsushita and coworkers (1975) reported impaired performance in neurological and muscular function tests in a group of 38 female shoemakers who had been exposed to 15 to 200 ppm toluene for an average of 3 years and 4 months. In addition, 19 of 38 exposed women, compared to 3 of 16 in the control group, complained of dysmenorrhea. The second group of workers was composed of 100 car painters who had been occupationally exposed to an average of

30.6 ppm toluene for an average of 14.8 years. As reported by Hanninen and coworkers (1976) and Seppalainen and coworkers (1978), the exposed workers had a greater incidence of CNS symptoms and impaired performance on tests for intelligence and memory, as well as for visual and verbal ability. Both of the studies on this group of workers used control groups of approximately 100 unexposed individuals. The major problem with the reports of adverse effects on the female shoemakers and male car painters is that both groups were exposed to other potentially toxic agents. The female shoemakers were exposed to "slight" levels of gasoline (Matsushita et al., 1975) and, as detailed in Table 11-3, the male car painters were exposed to several other organic solvents.

The subchronic and chronic data on experimental mammals are of only limited use in helping to resolve the uncertainties in the human data. Jenkins and coworkers (1970), and CIIT (1980) report no-observable-effect levels (NOELs) in experimental mammals 1085 ppm (8 hours per day, 5 days per week for 6 weeks) and 300 ppm (6 hours per day, 5 days per week for 24 months), respectively. For reasons discussed in detail in Section 12.1.2., the CIIT study is not considered appropriate for human risk assessment; interpretation of this study is complicated by the absence of quality assurance throughout the study, the use of an inappropriate strain of rats for study of myelotoxicity, and the fact that the highest level tested was not a maximum tolerated dose. As discussed above in this section, a NOEL of 1085 ppm is contradicted by human experience, suggesting that humans are more sensitive than experimental mammals to toluene exposure. Similarly, the continuous-exposure NOEL of 107 ppm for 90 days in rats, guinea pigs, dogs, and monkeys (Jenkins et al., 1970) does not, in itself, negate the concerns with effects reported in humans at lower levels.

## 18.3. ORAL EXPOSURES

Very little information is available on the acute, subchronic, or chronic effects of toluene in experimental mammals. As summarized in Table 12-1, acute oral $LD_{50}$s in adult rats range from 5500 mg/kg to 7530 mg/kg. Using the cubed root of the body weight ratios for interspecies conversion (U.S. EPA, 1980c; Freireich et al., 1966; Rall, 1969), an approximate lethal dose for humans can be estimated at 983 mg/kg (5500 mg/kg • (70 kg • 0.4 kg)$^{1/3}$). The conversion factor, as used here, assumes that humans are more sensitive than rats, which, as discussed above, is consistent with the available data on inhalation exposure. This estimate of the approximate lethal dose is also consistent with the report by Francone and Braier (1954) that leukemia patients were able to tolerate cumulative doses of up to 130,000 mg of toluene given over a 3 week period (approximately 88 mg/kg/day).

The only subchronic oral data are reported in the study by Wolf and coworkers (1956), indicating a NOEL in rats at 590 mg/kg/day, given five days per week for six months.

## 18.4. DERMAL EXPOSURES

Studies on the dermal toxicity of toluene are not adequate for quantitative risk assessment. Qualitatively, the little information that is available suggests that moderate dermal contact with liquid toluene (i.e., exposure of human forearm skin to toluene for 1 hour on 6 successive days) may cause skin damage but does not result in overt signs of toxicity (Malten et al., 1968). Similarly, the acute and subchronic data on toluene exposure in experimental mammals do not suggest that toluene is a potent toxicant on dermal contact. A method for quantitatively using such data to estimate equivalent human dose-response relationships, however, has not been fully formulated or validated.

As discussed in Section 13.1., exposure to toluene vapor results in relatively little dermal absorption compared to absorption across the lungs.

## 18.5. RESPONSES OF SPECIAL CONCERN

18.5.1. Carcinogenicity. CIIT (1980) concluded that exposure to 30, 100, or 300 ppm toluene for 24 months did not produce an increased incidence of neoplastic, proliferative, inflammatory, or degenerative lesions in Fischer 344 rats; however, the high spontaneous incidence (16%) of mononuclear cell leukemia in aging Fischer 344 male rats has been reported by Coleman and coworkers (1977), suggesting that this strain may be inappropriate for the study of a chemical that might be myelotoxic. Also, the design of the study has been deemed inadequate in that the highest level tested was not a minimum lethal dose (Powers, 1979).

Other studies suggest that toluene is not carcinogenic when applied topically to the shaved skin of animals. Toluene is used extensively as a solvent for lipophilic chemicals being tested for carcinogenic potential; negative control studies employing 100% toluene have not elicited carcinogenic effects. Also, no evidence of a promotion effect was noted when toluene was painted on the skin of mice twice weekly for 20 weeks following initiation with 7,12-dimethylbenz-[a]-anthracene (Frei and Stephens, 1968; Frei and Kingsley, 1968).

The above data are not adequate for assessing the potential carcinogenicity of toluene with great assurance and they cannot be used for supporting carcinogenicity as a valid biologic endpoint in quantitative risk assessment.

18.5.2. Mutagenicity. Toluene has yielded negative results in a battery of microbial, mammalian cell, and whole organism test systems as indicated in the following:

Differential Toxicity/DNA Repair Assays
- Escherichia coli
- Salmonella typhimurium

Reverse Mutation Testing
- Salmonella typhimurium (Ames test)
- Escherichia coli WP2 assay
- Saccharomyces cerevisiae D7

Mitotic Gene Conversion/Crossing Over
- Saccharomyces cerevisiae D4, D7

Thymidine Kinase Assay
- L5178Y mouse lymphoma cells

Micronucleus Test
- mouse

Dominant Lethal Assay
- mouse

Sister-Chromatid Exchange
- cultured CHO cells
- human lymphocytes in vitro
- human lymphocytes in vivo (workers)

In the Russian literature, chromosome aberrations were reported in the bone marrow cells of rats exposed subcutaneously (Dobrokhotov, 1972; Lyapkalo, 1973) and via inhalation (Dobrokhotov and Einkeev, 1977) to toluene. These findings were not corroborated in a Litton Bionetics, Inc. (1978b) study in rats following intraperitoneal injection, in cultured human lymphocytes exposed to toluene in vitro (Gerner-Smidt and Friedrich, 1978), or in lymphocytes from workers chronically exposed to toluene (200 to 400 ppm, Forni et al., 1971; 7 to 112 ppm toluene, Maki-Paakanen et al., 1980). Differences in doses employed may account, at least in part, for these conflicting results. Funes-Cravioto et al. (1977) did report an excess of aberrations in the lymphocytes from 14 printers exposed to TWA concentrations of 100 to 200 ppm for 1 to 16 years, but it is probable that part of the exposure was to benzene-contaminated toluene. Also, the number of workers was small in this study.

18.5.3. Teratogenicity. Toluene was reported in a recent abstract from NIEHS to induce cleft palates at a level of 1.0 mℓ/kg (approximately 866 mg/kg) following oral exposure to mice on days 6 to 15 of gestation (Nawrot and Staples, 1979). This effect reportedly did not appear to be due merely to a general retardation in growth rate. Levels of 0.3 and 0.5 mℓ/kg (approximately 260 and 433 mg/kg) toluene had no teratogenic effect, but the number of mice exposed and number of fetuses examined were not stated. Nawrot and Staples (1979) also noted a significant increase in embryonic lethality at all dose levels and a significant reduction in fetal weight at the two higher dose levels. No frank signs of maternal toxicity were seen at any dose level; however, at the highest dose, decreased maternal weight gain was reported in mice exposed on days 12 to 15 of gestation. A complete copy of this report has not been made available for review but has been submitted for publication.

Three other studies have concluded that toluene is not teratogenic in mice (Hudak and Ungvary, 1978) or rats (Hudak and Ungvary, 1978; Litton Bionetics, 1978b; Tatrai et al., 1980) following inhalation exposure. Hudak and Ungvary (1978) and Tatrai et al. (1980) have noted, however, an increased incidence of skeletal anomalies and signs of retarded skeletal development in the rats that were not considered malformations as such. Embryotoxicity was also indicated by low fetal weights in mice and some rats (Hudak and Ungvary, 1978). At the high exposure levels in the study by Hudak and Ungvary (1978), increased maternal mortality was noted in rats (399 ppm, 24 hours/day, days 1 to 8) and mice (399 ppm, 24 hours/day, days 6 to 13). No increased maternal mortality was noted by either Hudak and Ungvary (1978) or Tatrai et al. (1980) at lower exposure levels in rats (266 ppm, 8 hours/day, days 1 to 21; 266 ppm, 24 hours/day, days 7 to 14) or mice (133 ppm, 24 hours/day, days 6 to 13). In the study by Litton Bionetics, Inc. (1978b), no signs of maternal toxicity were noted in rats exposed to 100 or 400 ppm, 6 hours/day, on days 6 to 15 of gestation.

The extrapolation of these results to define potential human risk is an uncertain process. The dose that produced cleft palates in mice on oral exposure, 866 mg/kg, is only slightly higher than the NOEL in rats, 590 mg/kg/day.

Although inhalation exposure to toluene have not been shown to be teratogenic, embryotoxicity is an endpoint of concern. The effects noted in rats and mice at the high exposure level (400 ppm) in the study by Hudak and Ungvary (1978) may be of limited use in human risk assessment because of the occurrence of maternal mortality. The lowest effect level not associated with maternal mortality was 133 ppm, 24 hours/day, on days 6 to 13, which caused low fetal weights in mice. No fetal effects were noted in the study by Litton Bionetics, Inc. (1978b), however, when rats were exposed to 100 ppm or 400 ppm, 6 hours/day, on days 6 to 15 of gestation, or in the Tatrai et al. (1980) study when rats were continuously exposed to 266 ppm toluene on days 7 to 14. As is the case with oral exposure studies, a quantitative approach for using this type of data in human risk assessment has not been validated.

## REFERENCES


Abou-el-Makarem, M.M. et al. (1967). Biliary excretion of foreign compounds. Benzene and its derivatives in the rat. Biochem. J. 105:1269-1274.

ACGIH (American Conference of Governmental Industrial Hygienists) (1979). Documentation of the Threshold Limit Values for Substances in Workroom Air, 3rd ed., 1971, with supplements through 1979. Cincinnati, OH: ACGIH, p. 348.

ACGIH (American Conference of Governmental Industrial Hygienists) (1980). Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1980. Cincinnati, OH: ACGIH, pp. 30.

Adams, R.M.; Crocker, T.D.; and Thanavibulchai, N. (1980). An Economic Assessment of Air Pollution Damages to Selected Annual Crops in Southern California. U.S. Environmental Protection Agency, Washington, DC, 27 pp.

A.D. Little (1981). Exposure Assessment of priority pollutants: Toluene. Draft report prepared by Arthur D. Little, Inc., Cambridge, MA, for the U.S. Environmental Protection Agency, Research Triangle Park, NC.

Al-Gailany, K.A.S.; Houston, J.B.; and Bridges, J. W. (1978). The role of substrate lipophilicity in determining Type 1 microsomal P450 binding characteristics. Biochem. Pharmacol. 27(5):783-88.

Alha, A.; Korte, T.; and Teahu, M. (1973). Solvent sniffing death. Z. Rechtsmed. 72:299-305.

Altenkirch, J.; Mager, J.; Stoltenburg, G.; Helmbrecht, J. (1977). Toxic polyneuropathies after sniffing a glue thinner. J. Neurol. 214(2):137-52.

Altshuller, A.P.; Lonneman, W.A.; Sutterfield, F.D.; and Kopczynski, S.L. (1971). Hydrocarbon composition of the atmosphere of the Los Angeles Basin--1967. Environ. Sci. Tech. 5:1009.

Altwicker, E.R.; Whitby, R.A.; and Stasiuk, W.N. (1977). Ambient hydrocarbon levels at two elevated and some street level sites. Proc. Int. Clean Air Congr., 4th. Taken from: Chem. Abst. 88:141039q, 1978.

Ancona-Alayon, A. (1975). Occupational koilonychia from organic solvents. Contact Dermatitis 1:367-269.

Amsel, L.P. and Levy, G. (1969). Drug biotransformation interactions in man. II. A pharmacokinetic study of the simultaneous conjugation of benzoic and salicylic acids with glycine. J. Pharm. Sci. 58(3):321-326.

Anderson, G.E.; Liu, C.S.; Holman, H.Y.; and Killus, J.P. (1980). Human Exposure to Atmospheric Concentrations of Selected Chemicals, Publication No. unavailable. Prepared by Systems Applications, Inc., San Rafael, CA, under Contract No. EPA 68-02-3066. U.S. Environmental Protection Agency, Research Triangle Park, NC.

Andersson, K.; Fuxe, K.; Toftgard, R.; Nilsen, O.G.; Eneroth, P.; and Gustafsson, J.A. (1980). Toluene-induced activation of certain hypothalamic and median-eminence catecholamine nerve-terminal systems of the male-rat and its effects on anterior pituitary hormone secretion. Toxicol. Letters 5(6):393-398.

Andrews, L.S.; Lee, E.W.; and Kocsis, J.J. (1975). Effects of toluene on the disposition of benzene in the mouse. Pharmacol. 17(2):500.

Andrews, L.S.; Lee, E.W.; Witmer, C.M.; Kocsis, J.J.; and Snyder, R. (1977). Effects of toluene on the metabolism, disposition and hemopoietic toxicity of (3H)benzene. Biochem. Pharmacol. 77(4):293-300.

Angerer, J. (1979). Occupational chronic exposure to organic solvents. VII. Metabolism of toluene in man. Int. Arch. Occup. Environ. Health 43(1):63-67.

Anthony, R.M.; Bost, R.O.; Thompson, W.L.; and Sunshine, I. (1978). Paraldehyde, toluene, and methylene chloride analysis by headspace gas chromatography. J. Anal. Toxicol. 2:262-264.

Aranka, H.; Zsuzsa, B.; and Gyorgy, U. (1975). [Experimental study of the hepatotoxic effect of toluol. I. Histological and histochemical studies.] Morphol. Igazsagugyi Orv. Sz. 15(3):209-17. (In Hung.)

Astrand, I. (1975). Uptake of solvents in the blood and tissues of man. A review. Scand. J. Work Environ. Health 1(4):199-218.

Astrand, I.; Ehrner-Samuel, H.; Kilbom, A.; and Ovrum, P. (1972). Toluene exposure. I. Concentration in alveolar air and blood at rest and during exercise. Work Environ. Health 72(3):119-30.

Atkinson, J.H., and Newth, F.H. (1968). Microbiological transformation of hydrocarbons. Microbiol., Proc. Conf. 68:35-45.

Atkinson, R., et al. (1978). Rate constraints for reaction of OH radicals and ozone with cresols at 300 ± 1=K. J. Phys. Chem. 82:2759. (Cited in Syracuse Research Corporation, 1980.)

Baker, A.B., and Tichy, F.Y. (1953). The effects of the organic solvents and industrial poisonings on the central nervous system. Proc. Assoc. for Research in Nervous and Mental Disease 32:475-505.

Bakke, O.M., and Scheline, R.R. (1970). Hydroxylation of aromatic hydrocarbons in the rat. Toxicol. Appl. Pharmacol. 16:691-700.

Bakke, T., and Skjoldal, H.R. (1979). Effects of toluene on the survival, respiration, and adenylate system of a marine isopod. Mar. Pollut. Bull. 10(4):111-15.

Ball, H. (1976). Some new aspects in air pollutants analysis of hydrocarbons by automatic gas-chromatography. Z. Anal. Chem. 282:301-305.

Banfer, W. (1961). [Studies on the effect of pure toluene on the blood picture of photogravure printers and helper workers.] Zentralbl. Arbeitsmed. 11:35-40. (In Ger.) (Cited in NIOSH, 1973.)

Bansagi, J. (1968). [Effect of toluene on the phagocytic activity of white blood cells in printers.] Munkavedelem 14:26-8. (In Hungarian; summarized in Chem. Abst. 69:89544a, 1968.)

Barash, V.A. (1957). The influence of some mineral and organic substances on methane fermentation in sewage sludges. Vsesoyuz. Nauch.-Issledovatel. Inst. Vodosnabshen., Kanalizats., Gidrotekh. Sooruzhenii i Inzhener. Gidrogeol., Materialy Soveshchaniya, pp. 105-14.

Barman, M.L.; Siegel, N.B.; Beedle, D.B.; and Larson, R.K. (1964). Acute and chronic effects of glue sniffing. Calif. Med. 100:19-22.

Barnes, G.E. (1979). Solvent abuse: A review. Int. J. Addict. 14:1-26.

Bass, M. (1970). Sudden sniffing death. J. Amer. Med. Assoc. 212:2075.

Batchelor, J.J. (1927). The relation toxicity of benzol and its higher homologues. Am. J. Hyg. 7:276-98.

Battig, K., and Grandjean, E. (1964). Industrial solvents and avoidance conditioning in rats. Arch. Environ. Health 9:745-49.

Bayly, R.C. et al. (1966). The metabolism of cresols by species of Pseudomonas. Biochem. J. 101:293-301.

Beck, T. and Poschenrieder, H. (1963). Experiments concerning the action of toluene on the microflora in soils. Platn Soil 18:346-357.

Bellar, T.A.; Budde, W.L.; and Eichelberger, J.W. (1979). The identification and measurement of volatile organic compounds in aqueous environmental samples. In: Monitoring Toxic Substances. ACS Symposium Series, pp. 49-62.

Bellar, T.A., and Lichtenberg, J.J. (1979). Semiautomated headspace analysis of drinking waters and industrial waters for purgeable volatile organic compounds. In: Measurement of Organic Pollutants in Water and Wastewater, ASTM STP 686. Van Hall, C.E., editor. Philadelphia, PA: American Society for Testing and Materials, pp. 108-129.

Bennett, R.H., and Forman, H.R. (1980). Hypokalemic periodic paralysis in chronic toluene exposure. Archives of Neurology 37(10):673.

Benville, P.E., Jr., and Korn, S. (1977). The acute toxicity of six monocyclic aromatic crude oil components to striped bass (Morone saxatilis) and bay shrimp (Crago franciscorum). Calif. Fish Game 63(4):204-209.

Bergman, K. (1978). Application of whole-body autoradiography to distribution studies of organic solvents. Int. Symp. Control Air Pollut. Work. Environ. Pt. 2, pp. 128-39.

Bergman, K. (1979). Whole-body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents. Scand. J. Work Environ. Health. 5:263 pp.

Berry, W.O. (1980). A comparative study of the uptake of toluene by bluegill, sunfish Lepomis macrochirus and crayfish Orconectes rusticus. Environ. Pollut. 80:109-19.

Berry, W.O., and Brammer, J.D. (1977). Toxicity of water-soluble gasoline fractions to fourth-instar larvae of the mosquito Aedes aegypti L. Environ. Pollut. 13(3):229-34.

Berry, W.O.; Brammer, J.D.; and Bee, D.E. (1978). Uptake of water-soluble gasoline fractions and their effect on oxygen consumption in aquatic stages of the mosquito (Aedes aegypti L.). Environ. Pollut. 15(1):1-22.

Berry, W.O., and Fisher, J.W. (1979). Transfer of toluene $^{14}C$ from mosquito larvae to bluegill sunfish. Bull. Environ. Contam. Toxicol. 23(6):733-36.

Bertsch, W.; Anderson, E.; and Holzer, G. (1975). Trace analysis of organic volatiles in water by gas chromatography-mass spectrometry with glass capillary columns. J. Chromatogr. 112:701-718.

Blundo, R. (1978). The toxic effects of the water soluble fractions of No. 2 fuel oil and of three aromatic hydrocarbons on the behavior and survival of barnacle larvae. Contrib. Mar. Sci. 21:25-37.

Bolger, M. (1981). Private communication from between M. Greenburg, ECAO, EPA and M. Bolger, Toxicologist, Food and Drug Administration, Washington, DC, April 13, 1981.

Bonnet, P; Raoult, G.; and Gradiski, D. (1979). Lethal concentration 50 of main aromatic hydrocarbons. Arch. Maladies Prof., de medicine du travail et de Securite Sociale 40(8-9):805-810.

Boor, J.W., and Hurtig, H.I. (1977). Persistent cerebellar ataxia after exposure to toluene. Ann Neurol. 2(5):440-42.

Bos, R.P.; Brouns, R.M.E.; Van Doorn, R.; Theuws, J.L.G.; and Henderson, P.T. (1981). Non-mutagenicity of toluene, o-, m-, and p-xylene, o-methylbenzyl alcohol and o-methylbenzyl sulfate in the Ames assay. Mutat. Res. 88(3):273-279.

Bradsher, C.K. (1977). Toluene. In: McGraw-Hill Encyclopedia of Science and Technology, 4th ed. New York: McGraw-Hill Book Co., Vol. 13, pp.

Brajer, L. (1973). Comparative study of isocyclic hydrocarbons in animals and in man. Haematologica 58(7-8):491-500.

Bray, H.G.; Thorpe, W.V.; and White, K. (1951). Kinetic studies of the metabolism of foreign organic compounds. Biochem. J. 48:88-96.

Brenniman, G.R.; Anver, M.R.; Hartung, R.; and Rosenberg, S.H. (1979). Effects of outboard motor exhaust emissions on goldfish (Carassius auratus). J. Environ. Pathol. Toxicol. 2(6):1267-281.

Brenniman, G.; Hartung, R.; and Weber, W.J., Jr. (1976). A continuous flow bioassay method to evaluate the effects of outboard motor exhausts and selected aromatic toxicants on fish. Water Res. 10(2):165-69.

Bridie, A.L. et al. (1979). BOD and COD of some petrochemicals. Water Research 13:627-30.

Briggs, G.A. (1970). Some Recent Analyses of Plum Rise Observations. Proc. of International Air Pollution Conference, December 1970, Washington, D.C. (Cited by Anderson et al., 1980.)

Bringmann, G., and Kuhn, R. (1959). The toxic effects of waste water on aquatic bacteria, algae, and small crustaceans. Gesundheis-Ingerieur 80:115. (Cited in McKee and Wolf, 1963.)

Bringmann, G., and Kuhn, R. (1976). Comparative results of the damaging effects of water pollutants against bacteria (Pseudomonas putida) and blue algae (Microcystic aeruginosa). Gas-Wasserfach, Wasser-Abwasser 117(9):41-13.

Bringmann, G.; Gottfried, ; and Kuhn, R. (1977). Limiting values for the damaging action of water pollutants to bacteria (Pseudomonas putida) and green algae (Scenedesmus quadricauda) in the cell multiplication inhibition test. Z. Wasser Abwasser Forsch. 10(3-4):87-98.

Bringmann, G., and Kuhn, R. (1978). Grenzwerte der Schadwirking wasser-gefahrdender stoff gegen blaualgen (Microcystis aeruginosa) und grunalgen (Scenedesmus quadricauda) in zellvermehrungschemmtest. Vom Wasser 50:45-60.

Bringmann, G., and Kuhn, R. (1980). Bestimmung der biologischen schadwirkung wassergefahodender stoffe gegen protozoen. II. Bakterinpressende ciliaten. Z. Wasser Abwasser Forsch. 13(1):26-31.

Broda, P.; Bayley, S.; Duggleby, C.J.; Worsey, M.J.; and William, P.A. (1977). Plasmid-coded degradation of toluene and xylenes in soil pseudomonads. In: Plasmids. Medical and theoretical aspects. Mitsuhashi, S., Rosival, L., and Kremery, V., eds. Berlin: Springer-Verlag KG., pp. 403-406.

Brodowski, P.T.; Wilson, N.B.; and Scott, W.J. (1976). Chromatographic analysis of gaseous products from pyrolysis of organic wastes with a single column. Anal. Chem. 48(12):1812-813.

Brookshire, D.S.; d'Arge, R.C.; Schulze, W.D.; and Thayer, M. (1979). Methods for valuing aesthetics and health effects in the south coast air basin: An overview. Paper presented at the 72nd Annual Meeting of the Air Pollution Control Association, June 24-28, 1979, Cincinnati, OH, 27 pp.

Brown, A. (1957). Alterations of plaque morphology in some caliphages. J. Bacteriol. 73:585-587.

Brown S.L.; Chan, F.Y.; Jones, J.L.; Liu, D.H.; McCaleb, K.E.; Mill, T.; Kapios, K.N.; and Schendel, D.E. (1975). Research Program on Hazard Priority Ranking of Manufactured Chemicals, Phase II--Final Report, chemicals 1-20. Prepared by Stanford Research Institute, Menlo Park, CA. National Science Foundation, Washington, D.C. Available from: National Technical Information Service, Springfield, VA (NTIS PB 263 161).

Browning, E. (1965). Toxicity and Metabolism of Industrial Solvents. New York: Elsevier Publishing Co., pp. 66-76.

Bruckner, J.V., and Peterson, R.G. (1976). Evaluation of toluene toxicity utilizing the mouse as an animal model of human solvent abuse. Pharmacol. 18(2):244.

Bruckner, J.V., and Peterson, R.G. (1978). Effect of repeated exposure of mice and rats to concentrated toluene and acetone vapors. Toxicol. Appl. Pharmacol. 45(1):359.

Bruckner, J.V., and Peterson, R.G. (1981a). Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmocodynamics. Toxicol. Appl. Pharmacol. (In press.)

Bruckner, J.V., and Peterson, R.G. (1981b). Evaluation of toluene and acetone inhalant abuse. II. Model development and toxicology. Toxicol. Appl. Pharmacol. (In press.)

Bucksteeg, W. (1942). The effect and mode of action of toluene on the bacterial cell. Zentr. Bakt. Parasitenk. 105:209-13.

Burger, R.M. (1971). Toluene-treated Escherichia coli replicate only that DNA which was about to be replicated in vivo. Proc. Nat. Acad. Sci. 68(7):2124-126.

Burger, R.M., and Glaser, D.A. (1973). Effect of nalidixic acid on DNA replication by toluene-treated Escherichia coli. Proc. Nat. Acad. Sci. 70(7):1955-958.

Burghardt, E., and Jeltes, R. (1975). Gas chromatographic determinaton of aromatic hydrocarbons in air using a semi-automatic preconcentration method. Atmos. Environ. 9:935-940.

Buswell, J.A., and Jurtshuk, P. (1969). Microbial oxidation of hydrocarbons measured by oxygraphy. Appl. Mikrobiol. 64:215-22.

Caldwell, R.S.; Caldarone, E.M.; and Mallon, M.H. (1976). Effects of a Seawater-soluble Fraction of Cook Inlet Crude Oil and its Major Aromatic Components on Larval Stages of the Dungeness Crab, Cancer magister dana. In: Fate and Effects of Petroleum Hydrocarbon in Marine Organisms and Ecosystems. Pergamon Press, NY, pp. 210-220.

Cameron, G.R.; Paterson, J.L.H.; de Saram, G.S.W.; and Thomas, J.C. (1938). The toxicity of some methyl derivatives of benzene with special reference to pseudocumene and heavy coal-tar naphtha. J. Path. Bact. 46:95-107.

Canady, W.J.; Robinson, D.A.; and Colby, H.D. (1974). Partition model for hepatic cytochrome P-450-hydrocarbon complex formation. Biochem. Pharmacol. 21(21):3075-78.

Capellini, A., and Alessio, L. (1971). [The urinary excretion of hippuric acid in workers exposed to toluene.] Med. Lavoro 62:196-201. (In Ital.)

Caperos, J.R., and Fernandez, J.G. (1977). Simultaneous determinaton of toluene and xylene metabolites in urine by gas chromatography. Brit. J. Ind. Med. 34:229-233.

Carlson, R.M.; Carlson, R.E.; Kopperman, H.L.; and Caple, R. (1975). Facile incorporation of chlorine into aromatic systems during aqueous chlorination processes. Environ. Sci. Technol. 9(7):674-675.

Carlsson, D., and Lindquist, T. (1977). Exposure of animals and man to toluene. Scand. J. Work, Environ. Health 3(3):135-43. Taken from: Chem. Abst. 88:115931p, 1978.

Carpenter, C.P.; Geary, D.L., Jr.; and Myers, R.C. (1976). Petroleum hydrocarbon toxicity studies. X. Animal and human response to vapors of '50 Thinner.' Toxicol. Appl. Pharmacol. 36(3):427-42.

Carpenter, C.P. et al. (1976). Petroleum hydrocarbon toxicity studies. XIII. Animal and human response to vapors of toluene concentrate. Toxicol. Appl. Pharmacol. 36:473-90.

Carpenter, C.P., Shaffer, C.B., Weil, C.S., and Smyth H.F., Jr. (1944). Studies on the inhalation of 1,3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. J. Ind. Hyg. Toxicol. 26:69-78.

Carpenter, C.P., and Smythe, H.F. (1946). Chemical burns of the rabbit cornea. Amer. J. Opthalmol. 29:1363-1372.

Chambers, C.W. et al. (1963). Degradation of aromatic compounds by phenol-adapted bacteria. J. Water Pollut. Cont. Fed. 35(12):1517-528.

Chemical Industry Institute of Toxicology (CIIT) (1980). A twenty-four month inhalation toxicology study in Fischer-344 rats exposed to atmospheric toluene. Executive Summary and Data Tables. October 15, 1980.

Chiantella, A.J.; Smith, W.D.; Umstead, M.E.; and Johnson, J.E. (1966). Aromatic hydrocarbons in nuclear submarine atmosphere. Amer. Ind. Hyg. Assoc. J. March-April, pp. 186-92.

Chiba, R. (1969). Sudden death from thinner. Nichidai Igaku Zasshi 28:982-998. Taken from: Chem. Abst. 72:64867g, 1969.

Christiansson, G., and Karlsson, B. (1957). "Sniffing" - berusningssatt bland barn. Svensk Lakartidn 54:33. (In Swedish; cited in Press and Done, 1967b.)

Cier, H.E. (1969). Toluene. In: Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed. Standen, A., editor. New York: John Wiley and Sons, Inc., Vol. 20, p. 528.

Cieslinska, A.; Kowal-Gierczak, B.; Kuczynska-Sekieta, K.; Malolepszy, J.; and Wrzyszcz, M. (1969). [Serum iron and copper levels in subjects with chronic toluene exposure.] Pol. Tyg. Lek. 24:1848-850. (In Pol.)

Chenoweth, M.B. (1946). Ventricular fibrillation induced by hydrocarbons and epinephrine. J. Ind. Hyg. Toxicol. 28:151.

Chiou, C.T.; Freed, V.H.; Schmedding, D.W.; and Kohnert, R.L. (1977). Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 11(5):475-578.

Chovin, P., and Lebbe, J. (1967). Chromatography of aromatic hydrocarbons. I. The determination of gas chromatography of aromatic hydrocarbons in the air of working environments. Occup. Health Rev. 19(1-2):3-10.

Claus, D., and Walker, N. (1964). The decomposition of toluene by soil bacteria. J. Gen. Microbiol. 36:107-22.

Colotla, V.A.; Bautish, S.; Lorenzana-Jimenez, M.; and Rodriguez, R. (1979). Effects of solvents on schedule-controlled behavior. Neurobehaviorial Toxicol. 1(1):113-118.

Contreras, C.M.; Gonzalez-Estrada, T.; and Zarabozo, D. (1979). Petit mal and grand mal seizures produced by toluene or benzene intoxication in the cat. Electroencephalogr. Clin. Neurophysiol. 46(3):290-301.

Coombs, M.M.; Shatt, T.S.; and Croft, C.J. (1973). Correlation between carcinogenicity and chemical structure in cyclopenta[a]phenanthrenes. Cancer Research 33:832-37.

Currier, H.B. (1951). Herbicidal properties of benzene and certain methyl derivatives. Hilgardia 20(19):383-406.

Dalhamn, T.; Edfors, M.-L.; and Rylander, R. (1968a). Mouth absorption of various compounds in cigarette smoke. Arch. Environ. Health 16(6):831-35.

Dalhamn, T.; Edfors, M.L.; and Rylander, R. (1968b). Retention of cigarette smoke components in human lungs. Arch. Environ. Health 17:746-748.

Daly, J.; Jerina, D.; and Witkop, B. (1968). Migration of deuterium during hydroxylation of aromatic substrates by liver microsomes. I. Influence of ring substituents. Arch. Biochem. Biophys. 128(2):517-27.

Davies, J.S.; Westlake, D.W.S. (1979). Crude oil utilization by fungi. Can. J. Microbiol. 25(2):146-56.

Dean, B.J. (1978). Genetic toxicology of benzene, toluene, xylenes, and phenols. Mutat. Res. 47:75-97.

Dechev, G.D., and Damyanova, A.A. (1977). Functional investigation of bacterial composition of active sludge. Bulgarska Akademiiana Naukite. Doklaly Bolgarskoi Akndemiia Nauk. 30(10):1475-478.

Degani, J.G. (1943). Studies of the toxicity of ammunition plant wastes to fishes. Am. Fish Soc. Trans. 73:45-51.

Delaunay, A.; Lebrun, J. Fouequier, E.; and Wang, H.-S. (1950). Action and mechanism of action of toluene and related compounds on the permeability of blood capillaries. Compt. Red. Soc. Biol. 144:58-9.

De Smet, M.J.; Kingma, J.; and Witholt, B. (1978). The effect of toluene on the structure and permeability of the outer and cytoplasmic membranes of Escherichia coli. Biochim. Biophys. Acta 506(1):64-80.

Deutscher, M.P. (1974). Preparation of cells permeable to macromolecules by treatment with toluene. The tRNA nucleotidyltransferase. J. Bacteriol. 118(2):633-639.

de Vera, E.R.; Simmons, B.P.; Stephens, R.D.; and Storm, D.L. (1980). Samplers and Sampling Procedures for Hazardous Waste Streams, Publication No. EPA-600/2-80-018. Prepared by California Dept. of Health Services, Berkeley CA under Grant No. R804692010. Municipal Environmental Research Lab, U.S. EPA, Cincinnati, OH, Jan. 1980.

Dilling, W.L. (1977). Interphase transfer processes. II. Evaporation rates of chloro methanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous theoretical predictions. Environ. Sci. Technol. 11(4):405-409.

Divincenzo, G.D., and Krasavage, W.J. (1974). Serum ornithine carbamyl transferase as a liver response test for exposure to organic solvents. Am. Ind. Hyg. Assoc. J. 35:21-9.

Doak, S.M.A. et al. (1976). The carcinogenic response in mice to the topical application of propane sultone to the skin. Toxicology 6:139.

Dobrogosz, W.J., and DeMoss, R.D. (1963). Induction and repression of L-Arabinose isomerase in Pediococcus pentosaceus. J. Bacteriol. 85:1350-365.

Dobrokhotov, V.B. (1972). The mutagenic influence of benzene and toluene under experimental conditions. Gig. Sanit. 37:36-39. (In Russian; evaluation based on an English translation provided by the U.S. EPA.)

Dobrokhotov, V.B., and Enikeev, M.I. (1975). Mutagenic effect of benzene, toluene, and a mixture of these hydrocarbons in a chronic experiment. Gig. Sanit. 1:32-4. (In Russian with English summary; evaluation based on an English translation provided by the U.S. EPA.)

Dowty, B.J.; Antoine, S.R.; and Laseter, J.L. (1979). Quantitative and qualitative analysis of purgeable organics by high-resolution gas chromatography and flame ionization detection. In: Measurement of Organic Pollutants in Water and Wastewater, ASTM STP 686. Van Hall, C.W., editor. Philadelphia, PA: American Society for Testing and Materials, pp. 24-35.

Dragosics, B.; Ferenci, P.; Pesendorfer, F.; and Wewalka, F.G. (1976). Gamma-glutamyltranspeptidase (GGTP): Its relationship to other enzymes for diagnosis of liver disease. Progress in Liver Disease 5:436-449.

Drozd, J.; Novak, J.; and Rijks, J.A. (1978). Quantitative and qualitative headspace gas analysis of parts per billion amounts of hydrocarbons in water. A study of model systems by capillary-column gas chromatography with splitless sample injection. J. Chromatogr. 158:471-482.

Dunstan, W.M., et al. (1975). Simulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. Mar. Biol. 31:305-310.

Dutkiewicz, T., and Tyras, H. (1968a). The quantitative estimation of toluene skin absorption in man. Arch. Gewerbepath Gewerbehyg. 24:253-57.

Dutkiewicz, T., and Tyras, H. (1968b). Skin absorption of toluene, styrene, and xylene by man. Brit. J. Ind. Med. 25(3):243.

Eadie, J.M.; Mann, S.O.; and Oxford, A.E. (1956). Survey of physically active organic infusoricidal compounds and their soluble derivatives with special reference to their action on the rumen microbial system. J. Gen Microbiol. 14:122-33.

Egle, J.L., and Gochberg, B.J. (1976). Respiratory retention of inhaled toluene and benzene in the dog. J. Toxicol. Environ. Health 1(3):531-38.

El-Dib, M.A., et al. (1978). Role of adsorbents in the removal of soluble aromatic hydrocarbons from drinking water. Water Res. 12:1131. (Cited in Syracuse Research Corporation, 1980.)

El Masri, A.M.; Smith, J.N.; and Williams, R.T. (1956). Studies in detoxification. The metabolism of alkylbenzenes, n-propylbenzene, and n-butylbenzene with further observations on ethylbenzene. Biochem. J. 64:50-6.

Elovaara, E.; Hemminki, K.; and Vainio, H. (1979). Effects of methylene chloride, trichloroethane, trichloroethylene, tetrachloroethylene and toluene on the development of chick embryos. Toxicology 12(2):111-19.

Elovaara, E. et al. (1979b). Effects of methylene chloride, trichloroethane, trichloroethylene, tetrachloroethylene and toluene on the development of chick embryos. Toxicology 12(2):111.

Engstrom, K.; Husman, K.; and Rantanen, J. (1976). Measurement of toluene and xylene metabolites by gas chromatography. Int. Arch. Occup. Environ. Health 36(3):153-60.

Erben, R. (1978). Effects of some petrochemical products on the survival of Dicranophorus forcipatus O. F. Muller (Rotatoria) under laboratory conditions. Verein Limnol. 20:1988-991.

Esposito, G.S., and Jacobs, B.W. (1977). Chromatographic determination of aromatic hydrocarbons in ambient air. Amer. Ind. Hyg. Assoc. 38:401-407.

Evans, E.L., and Mitchell, A.D. (1980). An Evaluation of the Effect of Toluene on Sister Chromatid Exchange Frequencies in Cultured Chinese Hamster Ovary Cells. Prepared by SRI Internationa, Menlo Park, CA, under Contract No. 68-02-2947 for the U.S. Environmental Protection Agency, Research Triangle Park, NC.

Fabre, R. et al. (1955). Recherches toxicalogiques sur les solvents de remplacement du benzenede. Archives Maladies Professionalles de Medicine du Travail et de Securite Sociale 16:197-215. (Cited in Bergman, 1979.)

Faillace, L.A., and Guynn, R.W. (1976). Abuse of organic solvents. Phychosomatics 17(4):188-89.

Fan, D.P., and Gardner-Eckstrom, H.L. (1975). Passage of a membrane protein through the walls of toluene-treated Bacillus megaterium cells. J. Bact. 123:717-23.

Faustov, A.S. (1958). Toxicity of aromatic hydrocarbons. I. Comparative toxicity of some aromatic hydrocarbons. II. Some problems of the toxic-hygienic properties of aromatic hydrocarbons. Trudy Voronezh. Med. Inst. 35:247-255, 257-262.

Faustov, A.S. (1967). The determination of standard levels of toluene in industrial air. Gig. Sanit. 32(9):105-107.

Federal Register (1979). Purgeable Aromatics--Method 602. Federal Register 44(233):69474-9478.

Ferguson, T.; Harvey, W.F.; and Hamilton, T.D. (1933). An inquiry into the relative toxicity of benzene and toluene. I. Hyg. 33:547-575.

Fett, E.R.; Christoffersen, D.J.; and Snyder, L.R. (1968). Routine detemination of benzene, toluene, ethylbenzene and total aromatics in hydrocarbon solvents by a combination of liquid and gas chromatography. J. Gas Chromatogr. 6:572-576.

Fischman, C.M., and Oster, J.R. (1979). Toxic effects of toluene. A new cause of high anion gap metabolic acidosis. J. Am. Med. Assoc. 241(16):1713-715.

Fluck, E.R. et al. (1976). Evaluation of a DNA polymerase-deficient mutant of E. coli for the rapid detection of carcinogens. Chem. Biol. Inter. 15:219.

Fodor, G.G.; Schlipkoeter, H.W.; and Zimmermann, M. (1973). The Objective Study of Sleeping Behavior in Animals as a Test of Behavioral Toxicity. In: Adverse Effects of Environmental Chemicals and Phychotropic Drugs. Quantitative Interpretation of Functional Tests. Germany: Elsevier Science Publishing Co. Vol. 1, pp. 115-123.

Forni, A.; Pacifico, E.; and Limonta, A. (1971). Chromosome studies in workers exposed to benzene or toluene or both. Arch. Environ. Health 22(3):373-78.

Fowle, J. (1981). Draft report on mutagenicity of toluene. Prpared for the Reproductive Effects Assessment Group, Office of Health and Environmental Assessment, U.S. EPA.

Fracchia, M.; Pierce, L.; Graul, R.; and Stanley, R. (1977). Desorption of organic solvents from charcoal collection tubes. Amer. Ind. Hyg. Assoc. J. 38:144-146.

Francone, M.P., and Braier, L. (1954). [The basis for the substitution of benzene by the higher homologues in industry.] Med. Lavoro 45:29-32. (In Ital.)

Fraser, D.A., and Rappaport, S. (1976). Health aspects of the curing of synthetic rubbers. Environ. Health Perspect. 17:45-53.

Frei, J.V., and Kingsley, W.F. (1968). Observations on chemically induced regressing tumors of mouse epidermis. J. Natl. Cancer. Inst. 41:1307-313.

Frei, J.V., and Stephens, P. (1968). The correlation of promotion of tumor growth and of induction of hyperplasia in epidermal two-stage carcinogenesis. Brit. J. Cancer 22:83-92.

Freireich, E.J.; Gehan, E.A.; Rall, D.P.; Schmidt, L.H.; and Skipper, H.E. (1966). Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother. Rep. 50:219.

Friborska, A. (1973). Some cytochemical findings in the peripheral white blood cells in workers exposed to toluene. Folia Haematol. (Leipzig.) 99:233. (Cited in NRC, 1980.)

Funasaka, R.; Ose, Y.; and Sato, T. (1975). Offensive odor of fish from the Niagara River. III. Aromatic hydrocarbons as one of the offensive-odor substances. Eisei Kagaku 21(2):93-100. Taken from: Chem. Abst. 83:173356n, 1975.

Funes-Craviota, F. et al. (1977). Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. Lancet. 2:322.

Gait, A.J. (1967). Heavy Organic Chemicals. Oxford, England: Pergamon Press, Ltd., 249 pp.

Gamberale, F., and Hultengren, M. (1972). Toluene exposure. II. Phychophysiological functions. Work Environ. Health 9(3):131-39.

Geller, I.; Hartmann, R.J.; Randle, S.R.; and Gause, E.M. (1979). Effects of acetone and toluene vapors on multiple schedule performance of rats. Pharm. Biochem. Behavior 11:395-399.

Geller, I.; Randle, S.; and Hartmann, R. (1978). Effects of acetone and toluene on fixed-ratio, fixed-interval responding in the rat. Pharmacol. Biochem. Behav. 20(3):404.

Gellman, V. (1968). Glue sniffing among Winnipeg school children. Can. Med. Assoc. J. 98:411-13.

Gerarde, H.W. (1956). Toxicological studies on hydrocarbons. II. A comparative study of the effect of benzene and certain mono-alkylbenzenes on hemopoiesis and bone marrow metabolism in rats. Arch. Ind. Health 13:468-74.

Gerarde, H.W. (1959). Toxicological studies on hydrocarbons. III. Arch. Ind. Health 19:403-18.

Gerarde, H.W. (1960). Toxicology and Biochemistry of Aromatic Hydrocarbons. New York: Elsevier Publishing Co., pp. 141-50.

Gerarde, H.W., and Ahlstrom, D.B. (1966). Toxicologic studies on hydrocarbons. XI. Influence of dose on the metabolism of mono-n-alkyl derivatives of benzene. Toxic. Appl. Pharm. 9:185-190.

Gerner-Smidt, P., and Friedrich, U. (1978). The mutagenic effect of benzene, toluene and xylene studied by the SCE technique. Mutat. Res. 58(2-3):313.

Gibson, D.T. (1971). Microbial oxidation of aromatic hydrocarbons. Crit. Rev. Microbiol. 1(2):199-223.

Gibson, D.T. (1975). Microbial degradation of hydrocarbons. In: The Nature of Seawater: Report of the Dahlem Workshop on the Nature of Seawater, Goldberg, E.D. (ed.), Berlin, pp. 667-696. (Cited in NRC, 1980.)

Gibson, D.T.; Hensley, M.; Yoshioka, H.; and Mabry, T.J. (1970). Oxidative degradation of aromatic hydrocarbons by microorganisms. III. Formation of (+)-cis-2,3-dihydroxy-1-m-ethyl-4,6-cyclohexadiene from toluene by Pseudomonas putida. Biochem. 9(7):1626-630.

Gibson, D.T.; Koch, J.R.; Schuld, C.L.; and Kalio, R.E. (1968a). Oxidative degradation of aromatic hydrocarbons by microorganisms. I. Enzymatic formation of catechal from benzene. Biochem. 7(7):2653.

Gibson, D.T.; Koch, J.R.; Schuld, C.L.; and Kalio, R.E. (1968b). Oxidative degradation of aromatic hydrocarbons by microorganisms. II. Metabolism of halogenated aromatic hydrocarbons. Biochem. 7(11):3795-802.

Gibson, D.T., and Yeh, W.K. (1973). Microbial degradation of aromatic hydrocarbons. Microbiol. Degradation Oil Poll., Workshop, pp. 33-38.

Gilette, J.R. (1959). Side chain oxidation of p-nitrotoluene: I. Enzymatic oxidation of p-nitrotoluene. J. Biol. Chem. 234:139-143.

Gleason, M.N.; Gosselin, R.E.; Hodge, H.C.; and Smith, R.P. (1969). Clinical Toxicology of Commercial Products: Acute Poisoning, 3rd ed. Baltimore, MD: Williams and Wilkins Co., pp. VI.1-132. (Cited in Slimak, 1980.)

Goto, I.; Matsumura, M.; and Inoue, N. (1974). Toxic polyneuropathy due to glue sniffing. J. Neurol. Neurosurg. Psychiat. 37(7):848-53.

Grabski, D.A. (1961). Toluene sniffing producing cerebellar degeneration. Am. J. Psychiatry 118:461-62.

Grant, W.M. (1962). Toxicology of the eye. Springield, IL, Charles C. Thomas, pp. 544-45. (Cited in NIOSH, 1973.)

Gray, P.H.H., and Thornton, H.C. (1928). Soil bacteria that decompose certain aromatic compounds. Zbl. Bakt. (Abst. 2) 73:74. (Cited in Claus and Walker, 1964.)

Greenburg, L.; Mayers, M.R.; Heimann, H.; and Moskowitz, S. (1942). The Effects of exposure to toluene in industry. J. Amer. Med. Assoc. 118:573-578.

Grob, K., and Grob, G. (1971). Gas-liquid chromatographic/mass spectrometric investigation of $C_6$-$C_{20}$ organic compounds in an urban atmosphere. J. Chromatogr. 62:1-13. (Cited in Syracuse Research Corporation, 1980.)

Grob, K., and Zurcher, F. (1976). Stripping of trace organic substances from water: Equipment and procedure. J. Chromatogr. 117:285-294.

Guillemin, M.; Murset, J.C.; Lob, M.; and Riquez, J. (1974). Simple method to determine the efficiency of a cream used for skin protection against solvents. Brit. J. Ind. Med. 31(4):310-16.

Gusev, I.S. (1965). Reflective effects of microconcentrations of benzene, toluene, xylene and their comparative assessment. Hyg. Sanit. 30:331-35.

Gusev, I.S. (1967). Comparative toxicity of benzene, toluene and xylene. Biol. Deistvie Gig. Znachemie Atmos. Zagryaznenii 10:96-108. Taken from: Chem. Abst. 69:17711e, 1967.

Hanninen, H.; Eskelinen, L; Husman, K.; and Nurminen, M. (1976). Behavioral effects of long-term exposure to a mixture of organic solvents. Scand. J. Work Environ. Health 2(4):240-55.

Hansen, N.; Jensen, V.B.; Appelquist, H.; and Morch, E. (1978). The uptake and release of petroleum hydrocarbons by the marine mussel Mytilus edulis. Prog. Water Technol 10(5-6):351-59.

Harris, E.K. (1959). Confidence limits for the LC50 using the moving average-angle method. Biometrics 15:424-432. (Cited in Pickering and Henderson, 1966).

Hayden, J.W.; Peterson, R.G.; and Bruckner, J.V. (1977). Toxicology of toluene (methylbenzene): Review of current literature. Clin. Toxicol. 11(5):549-59.

Hein, H. (1954). Bakteriologische Untersuchungen mit neomycin und nebacetin. Arznemittel-Forschung 4:282-287.

Helliwell, M., and Murphy, M. (1979). Drug-induced neurological disease (letter). Brit. Med. J. 1(6173):1283-284.

Hendry, D.G. (1979). Reactions of aromatic hydrocarbons in the atmosphere. Herron, J.T.; Huie, R.E.; and Hodgeson, J.A., eds. Chemical Kinetics Data Needs for Modeling the Lower Troposphere: Proceedings of a Workshop held at Reston, VA, May 15-17, 1978. NBS Special Publication 557. U.S. Dept. of Commerce, NBS, Washington, DC. Pp. 85-91. (Cited in Syracuse Research Corporation, 1980.)

Herzenberg, L.A. (1959). Studies on the induction of beta-galactosidase in a cryptic strain of Escherichia coli. Biochimica et Biophysica Acta 31:525-39.

Hester, N.E., and Meyer, R.A. (1979). A sensitive technique for measurement of benzene and alkylbenzenes in air. Environ. Sci. Technol. 13(1):107-109.

Higgins, I.J. et al. (1980). New findings in methane-utilizing bacteria highlight their importance in the biosphere and their commercial potential. Nature 286:561.

Hirokawa, T. (1955). Studies on the poisoning by benzol and its homologues III. Experimental studies on the sexual differences of blood picture. Jap. J. Med. Sci. Biol. 8:279-81.

Hollifield, H.C.; Breder, C.V.; Dennison, J.L.; Roach, J.A.; and Adams, W.S. (1980). Container-derived contamination of maple syrup with methyl methacrylate, toluene, and styrene as determined by headspace gas-liquid chromatography. J. Assoc. Off. Anal. Chem. 63:173-177.

Holmberg, P.C. (1979). Central-nervous-system defects in children born to mothers exposed to organic solvents during pregnancy. Lancet 2:177-79.

Holzer, G.; Oro, J.; and Bertsch, W. (1976). Gas chromatographic-mass spectrometric evaluation of exhaled tobacco smoke. J. Chromatogr. 126:771-85.

Holzer, G.; Shanfield, H.; Zlatkis, A.; Bertsch, W.; Juarez, P.; Mayfield, H.; and Liebich, H.M. (1977). Collection and analysis of trace organic emissions from natural sources. J. Chromatogr. 142:755-764.

Horiguchi, S., and Inoue, K. (1977). Effects of toluene on the wheel-turning activity and peripheral blood findings in mice - an approach to the maximum allowable concentration of toluene. J. Toxicol. Sci. 2(4):363-72.

Hoshino, M.; Akimoto, H.; and Okuda, M. (1978). Photochemical oxidation of benzene, toluene, and ethylbenzene initiated by hydroxyl radicals in the gas phase. Bull. Chem. Soc. Japan 51:718-724. Taken from: Chem. Abst. 88:169346v, 1978.

Hudak, A.; Bors, Z.; Ungvary, G.; and Folly, G. (1976). Reversibility and interaction with hepatic regeneration of toluene induced liver injury. Acta Morphol. Acad. Sci. Hung. 24(1-2):153-66.

Hudak, A.; Szeberenyi, S.; Molnar, J. Cseh, I.; Suveges, M.; Folly, G.; Manyai, S.; and Ungvary, G. (1978). Effect on liver of chronic exposure to toluene and ethanol in rat. Acta Physiol. Acad. Sci. Hung. 51(1-2):128.

Hudak, A., and Ungvary, G. (1978). Embryotoxic effects of benzene and its methyl derivatives: Toluene and xylene. Toxicology 11:55.

Ideda, M. (1974). Reciprocal metabolic inhibition of toluene and trichloroethylene in vivo and in vitro. Int. Arch. Arbeitsmed. 33(2):125-30.

Ikeda, T., and Miyake, H. (1978). Decreased learning in rats following repeated exposure to toluene: Preliminary report. Toxicol. Lett. 1(4):235-39.

Ikeda, M., and Ohtsuji, H. (1967). Significance of urinary hippuric acid determination as an index of toluene exposure. Brit. J. Ind. Med. 26:244-46.

Ikeda, M., and Ohtsuji, H. (1969). Significance of urinary hippuric acid determination as an index of toluene exposure. Brit. J. Ind. Med. 26(3):244-46.

Ikeda, M., and Ohtsuji, H. (1971). Phenobarbital-induced protection against toxicity of toluene and benzene in the rat. Toxicol. Appl. Pharmacol. 20(1):30-43.

Ikeda, M.; Ohtsuji, H.; and Imamura, T. (1972). In vivo suppression of benzene and styrene oxidation by coadministered toluene in rats and effects of phenobarbital. Xenobiotica 2(2):101-106.

Imamura, T., and Ikeda, M. (1973). Lower fiducial limit of urinary metabolite level as an index of excessive exposure to industrial chemicals. Brit. J. Ind. Med. 30:289-92.

Ishida-Ichimasa, M. (1978). Degradation of lipids in yeast (Saccharomyces cerevisiae) at the early phase of organic solvent-induced autolysis. Agric. Biol. Chem. 42(2):247-51. Taken from: Chem. Abst. 88:164824q, 1978.

Ishikawa, T.T., and Schmidt, H., Jr. (1973). Forced turning induced by toluene. Pharmacol. Biochem. Behav. 1(5):593-95.

Isidorov, V.A.; Zenkevich, I.G.; and Ioffe, B.V. (1977). Investigation of new sorbents for the gas-chromatographic-mass-spectrometric determination of traces of volatile organic compounds in the atmosphere. Translated from Doklady Akademii Nauk SSSR 235(3):618-621. Available from: Plenum Publishing Corporation, New York.

Jackson, R.W., and DeMoss, J.A. (1965). Effects of toluene on Escherichia coli. J. Bacteriol. 90(5):1420-425.

Jacoby, G.A.; Rogers, J.E.; Jacob, A.E.; and Hedges, R.W. (1978). Transposition of Pseudomonas toluene-degrading genes and expression in Escherichia coli. Nature 274(5667):179-80.

Jamison, V.W.; Raymond, R.L.; and Hudson, J.O. (1969). Microbial hydrocarbon cooxidation. III. Isolation and characterization of an alpha.,.alpha.'-dimethyl-cis,cis-muconic acid-producing strain of Nocardia cornallina. Appl. Microbiol. 17(6):853-56.

Jefcoate, C.R.E., et al. (1969). Oxidation of some benzenoid compounds by Fenton's reagent and the ultraviolet irradiation by hydrogen peroxide. J. Chem. Soc. B.: 1013. (Cited in Syracuse Research Corporation, 1980.)

Jenkins, L.J., Jr.; Jones, R.A.; and Siegel, J. (1970). Long-term inhalation screening studies of benzene, toluene, o-xylene, and cumene on experimental animals. Toxicol. Appl. Pharmacol. 16:818-23.

Jenkins, T.F.; O'Reilly, W.F.; Murrmann, R.P.; Leggett, D.C.; and Collins, C.I. (1973). Analysis of Vapors Emitted from Military Mines. Report No. CRREL-SR-193, Cold Regions Research and Engineering Lab, Hanover, NH, September 1973.

Jermini, C.; Weber, A.; and Grandjean, E. (1976). Quantitative determination of various gas-phase components of the side-stream smoke of cigarettes in the room air as a contribution to the problem of passive smoking. Int. Arch. Occup. Environ. Health 36:169-81.

Johansson, I. (1978). Determination of organic compounds in indoor air with potential reference to air quality. Atmos. Environ. 12:1371-377.

Juhnke, I., and Ludemann, D. (1978). Results of research with 200 chemical compounds on acute fish toxicity with the golden orfe test. Z.f. Wasser-und Abwasser-Forschung 11(5):161-164.

Jungclaus, G.A.; Games, L.M.; and Hites, R.A. (1976). Identification of trace organic compounds in tire manufacturing plant wastewaters. Anal. Chem. 48(13):1894-896.

Jungclaus, G.A.; Lopez-Avila, V.; and Hites, R.A. (1978). Organic compounds in an industrial wastewater: A case study of their environmental impact. Environ. Sci. Technol. 12(1):88-96.

Kaplan, D.L., and Hartenstein, R. (1979). Problems with toluene and the determination of extracellular enzyme activity in soils. Soil Biol. Biochem. 11(4):335-38.

Kaubisch, N.; Daly, J.W.; and Jerina, D.M. (1972). Arene oxides as intermediates in the oxidative metabolism of aromatic compounds. Isomerization of methyl-substituted arene oxides. Biochem. 11:3080-88.

Kauss, P.B., and Hutchinson, T.C. (1975). Effects of water-soluble petroleum components on the growth of Chlorella vulgaris. Environ. Pollut. 9(3):157-174.

Keane, J.R. (1978). Toluene optic neuropathy. Ann. Neurol. 4(4):390.

Kelly, T.W. (1975). Prolonged cerebellar dysfunction associated with paint sniffing. Pediatrics 56:605-606.

Kenley, R.A.; Davenport, J.E.; and Hendry, D.G. (1978). Hydoxyl radical reactions in the gas phase. Products and pathways for the reacton of OH with toluene. J. Phys. Chem. 82:1095-1096.

Keplinger, M.L.; Lanier, G.E.; and Deichmann, W.B. (1959). Effects of environmental temperature on the acute toxicity of a number of compounds in rats. Toxicol. Appl. Pharmacol. 1:156-61.

Khinkova, L. (1974). Experimental data on the toxicity of some organic solvents used in the furniture industry. Tr. Inst. Khig, Okhr. Tr. Prof. Zabol. 22(1):133-140. Taken from: Chem. Abst. 88:1170j, 1978.

Kim, N.K. (1981). Air pollution evaluations using risk assessment methodology. Air Pollut. Contr. Assoc. 31(2):120-122.

Kimura, E.T.; Ebert, D.M.; and Dodge, P.W. (1971). Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19(4):699-704. Taken from: Chem. Abst. 75:139139u, 1971.

Kira, S. (1977). Measurement by gas chromatography of urinary hippuric acid and methylhippuric acid as indices of toluene and xylene exposure. Brit. J. Ind. Med. 34(4):305-309. Taken from: Chem. Abst. 88:84137c, 1978.

Kirkhart, B. (1980). Micronucleus Test on Toluene. Prepared by SRI International, Menlo Park, CA, under Contract No. 68-02-2947 for the U.S. Environmental Protection Agency, Research Triangle Park, NC.

Kitagawa, M. (1956). Studies on the oxidation mechanism of methyl group. J. Biochem. (43(4):553-63.

Knox, J.W., and Nelson, J.R. (1966). Permanent encephalopathy from toluene inhalation. N. Eng. J. Med. 275:1494-496.

Kobal, V.M.; Gibson, D.T.; Davis, R.E.; and Garza, A. (1973). X-ray determination of the absolute stereochemistry of the initial oxidation product formed from toluene by Pseudomonas putida 39/D. J. Amer. Chem. Soc. 95(13):4420-4421.

Koga, K. (1978). Distribution, metabolism and excretion of toluene in mice. Folia Pharmacol. Jpn. 74(6):687-98.

Koga, K., and Ohmiya, Y. (1978). Potentiation of toluene toxicity by hepatic enzyme inhibition in mice. J. Toxicol. Sci. 3(1):25-9.

Kojima, T., and Kobayashi, H. (1975). Toxicological study on toluene poisoning by inhalation. Toluene poisoning in the hypoxic atmosphere. Nippon Hoigaku Zasshi 29(2):82-7. (Cited in NRS, 1980.)

Konietzko, H.; Keilbach, J.; and Drysch, K. (1980). Cumulative effects of daily toluene exposure. Int. Arch. Occup. Environ. Health 46(1):53-58.

Kopczynski, S.L. et al. (1972). Photochemistry of atmospheric samples in Los Angeles. Environ. Sci. Technol. 6:342. (Cited in Syracuse Research Corporation, 1980.)

Korn, S.; Moles, D.A.; and Rice, S.D. (1979). Effects of temperature on the median tolerance limit of pink salmon and shrimp exposed to toluene, naphthalene, and Cook Inlet crude oil. Bull. Environ. Contam. Toxicol. 21(4-5):521-25.

Korobkin, R. et al. (1975). Glue sniffing neuropathy. Arch. Neurol. 32:158-62.

Kowal-Gierczak, B.; Kuczynska-Sekieta, K.; Cieslinska, A.; Wrzyszcz, M.; and Malolepszy, J. (1969). [Some biochemical tests in subjects occupationally exposed to toluene.] Pol. Tyg. Lek 24:1682-685. (In Pol.)

Kroeger, R.M.; Moore, R.J.; and Lehman, T.H. (1980). Recurrent urinary calculi associated with toluene sniffing. J. Urol. 123(1):89-91.

Krivanek, N., and Mullin, L.S. (1978). Comparison of conditioned avoidance and unconditioned reflex tests in rats exposed by inhalation to carbon monoxide, 1,1,1,-trichloroethane, toluene, or ethanol. Toxicol. Appl. Pharmacol. 45(1):357-58.

Kronevi, T.; Wahlberg, J.; and Holmberg, B. (1979). Histopathology of skin, liver, and kidney after epicutaneous administration of five industrial solvents to guinea pigs. Environ. Res. 19(1):56-69.

Laham, S. (1970). Metabolism of industrial solvents. Ind. Med. 39:237-40.

Lahmann, E.; Seifert, B.; and Ullrich, D. (1977). The Pollution of Ambient Air and Rain Water By Organic Components of Motor Vehicle Exhaust-Gases, Proc. Int. Clear Air Congr., 4th, pp. 595-97.

Lange, A. et al. (1973a). Serum immunoglobulin levels in workers exposed to benzene, toluene, and xylene. Inter. Arch. fuer Arbeitsmedizia 31(1):37-44.

Lange, A. et al. (1973b). Leukocyte agglutinins in workers exposed to benzene, toluene and xylene. Int. Arch. Arbeitsmed. 31:45-50.

LeBlanc, G.A. (1980). Acute toxicity of priority pollutants to water flea (Daphnia magna). Bull. Environ. Contam. Toxicol. 24:684-691.

Lee, R.L. et al. (1972). Petroleum hydrocarbons: Uptake and discharge by the marine mussel Mytilus edulis. Science 177:344-46.

LeGore, R.S. (1974). The Effect of Alaskan Crude Oil and Selected Hydrocarbon Compounds on Embryonic Development of the Pacific Oyster Crassostrea gigos. Ph.D. Dissert. Univ. Wash. 190 pp. (Cited in U.S. EPA, 1980.)

Leonard, M.J. et al. (1976). Effects of the motor vehicle control program on hydrocarbons in the central Los Angeles atmosphere. J. Air Pollut. Cont. Assoc. 26:359. (Cited in Syracuse Research Corporation, 1980.)

Leung, T.S., and Bulkley, R.V. (1979). Effects of petroleum hydrocarbons on length of incubation and hatching success in the Japanese Medaka. Bull. Environ. Contam. Toxicol. 23:236-243.

Levinthal, C. et al. (1962). Reactivation and hybridization of reduced alkaline phosphatase. Proc. Nat. Acad. Sci. 48:1230-237.

Lewis, P.W., Patterson, D.W. (1974). Acute and chronic effects of the voluntary inhalation of certain commercial volatile solvents by juveniles. J. Drug Issues 4(2):162-75.

Lijinsky, W., and Garcia, H. (1972). Skin carcinogenesis tests of hydrogenated derivatives of anthracene and other polynuclear hydrocarbons. Z. Krebstorsch. Klin. Onkol. 77:226. (Cited in U.S. EPA, 1980.)

Lindenberg, B.A.; Massin, M.; and Gauchat, G. (1957). Cytolysis of yeast caused by narcotics considered as an indifferent physical phenomenon. Compt. Rend. Soc. Biol. 151:1369-372. Taken from: Chem Abst. 52:13856d, 1958.

Linder, R.L.; Lerner, S.E.; and Wesson, D.R. (1975). Solvent sniffing: A continuing problem among youth. Proc. West Pharmacol. Soc. 18:371-74.

Lindstroem, K. (1973). Psychological performances of workers exposed to various solvents. Work Environ. Health 10(3):151-55.

Lingg, R.D.; Melton, R.G.; Kopfler, F.C.; Coleman, W.E.; and Mitchell, D.E. (1977). Quantitative analysis of volatile organic compounds by GC-MS. J. Amer. Water Works Assoc. 69(11, pt. 1):605-12.

Liss, P.S., and Slater, P.G. (1974). Flux of gases across the air-sea interface. Nature 247:181-184.

Litt, I.F.; Cohen, M.I.; Schonberg, S.K.; and Spigland, I. 1972. Liver disease in the drug-using adolescent. J. Pediatr. 81:238-42.

Litton Bionetics, Inc. (1978a). Mutagenicity Evaluation of Toluene. Final Report. Submitted to the American Petroleum Institute, Washington, D.C. in May 1978. LBI Project No. 20847. Litton Bionetics, Inc., Kensington, MD. 150 pp.

Litton Bionetics, Inc. (1978b). Teratology Study in Rats. Toluene. Final Report. Submitted to the American Petroleum Institute, Washington, D.C. in January 1978. LBI Project No. 20698-4. Litton Bionetics, Inc., Kensington, MD. 17 pp.

Litton Bionetics, Inc. (1981). Mutagenicity Evaluation of Toluene--Mouse Dominant Lethal Assay. Final Report. Submitted to the American Petroleum Institute, Washington, D.C. in January 1981. LBI Project No. 21141-05. Litton Bionetics, Inc., Kensington, MD. 15 pp.

Longley, E.O.; Jones, A.T.; Welch, R.; and Lomaev, O. (1967). Two acute toluene episodes in merchant ships. Arch. Environ. Health 14:481-87.

Lonneman, W.A.; Bellar, T.A.; and Altshuller, A.P. (1968). Aromatic hydrocarbons in the atmosphere of the Los Angeles Basin. Environ. Sci. Technol. 2(11):1017-1020.

Louw, C.W., and Richards, J.F. (1975). A simple directly combined gas chromatographic-infrared spectrometric system for identification of low molecular weight hydrocarbons. Appl. Spectrosc. 29:15-24.

Lurie, J.B. (1949). Acute toluene poisoning. S. Africa Med. J. 23:233-36.

Lutin, P.A.; Cibulka, J.J.; and Malaney, G.W. (1965). Oxidation of selected carcinogenic compounds by activated sludge. Purdue Univ., Eng. Bull, Ext. Ser. 118:131-145.

Lyapkalo, A.A. (1973). Genetic activity of benzene and toluene. Gig. Tr. Prof. Azbol. 17:24-28. (In Russian with English summary; evaluation based on an English translation provided by the U.S. EPA.)

Mackay, D., and Leinonen, P.J. (1975). Rate of evaporation of low-solubility contaminants from water bodies to atmosphere. Environ. Sci. Technol. 9:1178-1180.

Mackay, D., and Wolkoff, A.Q. (1973). Rate of evaporation of low-solubility contaminants from water bodies to atmosphere. Environ. Sci. Technol. 7:611. (Cited in Syracuse Research Corporation, 1980.)

Maki-Paakkanen, J. et al. (1980). Toluene exposed workers and chromosome aberrations. Jour. Toxicol. Environ. Health 6:775.

Malaney, G.W., and McKinney, R.E. (1966). Oxidative abilities of benzene-acclimated activated sludge. Water Sewage Works 113(8):302-309.

Malin, G., and Lying-Tunell, J. (1980). Cerebellar dysfunction related to toluene sniffing. Acta Neurol. Scand. 62(3):188.

Malm, G., and Lying-Tunell, U. (1980). Cerebellar dysfunction related to toluene sniffing. Acta Neurol. Scand. 62:188-190.

Malten, K.E.; Spruit, D.; and deKeizer, M.J.M. (1968). Horny layer injury by solvents. Berufsdermatosen 16:135-147.

Mann, H. (1975). The golden orfe test: German proposal for testing the action of chemical compounds on fish. Vom Wasser 44:1-13.

Mann, H. (1976). Comparative acute toxicity testing of water pollutants and wastewater with the golden orfe fish test: Experimental results from three ring tests. Z.f. Wasser-und Abwasser-Forschung 9:105-109.

Mara, S.J.; So, E.C.; and Suta, B.E. (1979). Uses, Sources, and Atmospheric Emissions of Alkylbenzene Derivatives, Final Report. Prepared by SRI International, Menlo Park, CA, under Contract No. 68-02-2835. U.S. Environmental Protection Agency, Research Triangle Park, NC.

Marion, C.V., and Malaney, G.W. (1964). Ability of activated sludge microorganisms to oxidize aromatic organic compounds. Proc. Indus. Waste Conf. 18:297-308.

Massengale, O.N.; Glaser, H.H.; LeLievre, R.E.; Dodds, J.B.; and Klock, M.E. (1963). Physical and psychologic factors in glue sniffing. N. Engl. J. Med. 269:1340-344.

Matsui, S. et al. (1975). Activated sludge degradability of organic substances in the waste water of the Kashima petroleum and petrochemical industrial complex in Japan. Prog. Water. Technol. 7:645-49.

Matsumura, M.; Snove, N.; and Ohnishi, A. (1972). Toxic polyneuropathy due to glue sniffing. Clin. Neurol. 12:290-296.

Matsumoto T.; Takeuchi, Y.; Tanaka, T.; and Maeda, K. (1971). Experimental studies on the chronic toluene poisoning. 3. Effects of toluene exposure on blood and organs in the rats. Sangyo Igaku. Jap. J. Indust. Health 13:501-506.

Matsushita, T. White, K.P.; and Sneoka (1971). Chromosome replication in toluenized Bacillus subtilis cells. Nature New Biol. 232:111-114. (Cited in Winston and Matsushita, 1975.)

Matsushita, T. et al. (1975). Hematological and neuro-muscular response of workers exposed to low concentration of toluene vapor. Ind. Health 13:115.

Matunar, F.C.; Trattner, R.B.; and Cheremisinoff, P.N. (1978). The absorption of organic compounds by wet scrubbing methods. Adv. Instrum. 331:307-314.

McAuliffe, G.D. (1976). Dispersal and Alteration of Oil Discharged on a Water Surface. In: Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms, Wolfe, D.A. (ed.). Pergamon Press, London, pp. 363-372.

McAuliffe, C.D. (1977). Evaporation and solution of $C_2$ to $C_{10}$ hydrocarbons from crude oils on the sea surface. Wolfe, D.A., ed. Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, New York, pp. 368-372. (Cited in Syracuse Research Corporation, 1980.)

McLaughlin, J. et al. (1964). Toxicity of fourteen volatile chemicals as measured by the chick embryo method. Am. Ind. Hyg. Assoc. Jour. 25:282.

McLaughlin, R.S. (1946). Chemical burns of the human cornea. Am. J. Ophthalmol. 29:1355-362.

McNichol, A.L., and Miller, R.C. (1975). Biological activity of Ty DNA synthesized in toluene treated Escherichia coli cells. J. Virol. 15:479-483.

The Merck Index: An Encyclopedia of Chemicals and Drugs, 9th ed. (1976). Windholz, M., editor. Rahway, NJ: Merck and Co., Inc., pp. .

Michon, S. (1965). [Disturbance of menstruation in women working in an atmosphere polluted with aromatic hydrocarbons.] Pol. Tyg. Lek. 20:1547-649. (In Polish with English summary.)

Miller, R.C.; Taylor, D.M.; McKay, K.; and Smith, H.W. (1973). Replication of Ty DNA in Escherichia coli treated with toluene. J. Virol. 12:1195-1203.

Ministry of Labour (1966). Methods for the Detection of Toxic Substances in Air, Booklet No. 4: Benzene, Toluene and Xylene, Styrene. London: Her Majesty's Stationery Office, pp. 1-12.

Mitchell, R.; Fogel, S.; and Chet, I. (1972). Bacterial chemoreception. Important ecological phenomenon inhibited by hydrocarbons. Water Res. 6(10):1137-140. Taken from: Chem. Abst. 78:53571d, 1973.

Moles, A. (1980). Sensitivity of parasitized Coho salmon fry to crude oil, toluene and naphthalene. Am. Fish. Soc., Trans. 109(3):293.

Morrow, J.E.; Gritz, R.L.; and Kirton, M.P. (1975). Effects of some components of crude oil on young Coho salmon. Copeia 2:326-31.

Mortelmans, K.E., and Riccio, E.S. (1980). In vitro Microbiological Gentoxicity Assays of Toluene. Prepared by SRI International, Menlo Park, CA, under Contract No. 68-02-2947 for the U.S. Environmental Protection Agency, Research Triangle Park, NC.

Morvai, V.; Albert, K.; Tolnai, A.; and Ungvary, G. (1978). Cardiovascular effects of long-term alcohol and toluene poisoning. Acta Phys. Acad. Sci. Hung. 51:(1-2):159.

Morvai, V., and Ungvary, G. (1979). Effects of simultaneous alcohol and toluene poisoning on the cardiovascular system of rats. Toxicol. Appl. Pharmacol. 50(3):381-89.

Morvai, V.; Hudak, A,; Varga, U.B. (1976). ECG changes in benzene, toluene and xylene poisoned rats. Acta Med. Acad. Sci. Hung. 33(3):275-86.

Moses, R.E., and Richardson, C.C. (1970). Replication and repair of DNA in cells of Escherichia coli treated with toluene. Proc. Nat. Acad. Sci. 67:674-81.

Moss, A.H.; Gabow, P.A.; Kaehny, W.D.; Goodman, S.I.; and Haut, L.L. (1980). Fanconi's syndrome and distal renal tubular acidosis after glue sniffing. Ann. Intern. Med. 92:69-70.

Munchinger, R. (1963). Der nachweis central nervoser storungen bei losungsmitt elexponierten Arbeitern. Excerpta Medica Series, Madrid; 16-21 2(62):687-89.

Mungikar, A.M., and Pawar, S.S. (1976). The effect of toluene, phenobarbital and 3-methylcholanthrene on hepatic microsomal lipid peroxidation. Curr. Sci. 45(1):22-4.

NAS (National Academy of Sciences) (1977). Drinking Water and Health. Safe Drinking Water Committee, Advisory Center on Toxicology, Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington, DC, p. 939. Available from: Printing and Publishing Office, National Academy of Sciences, 2101 Constitution Ave., Washington, DC 20418.

National Cancer Institute (1978). Bioassay of aniline hydrochloride for possible carcinogenicity. NCI-CG-TR-130. National Institute of Health, Bethesda, MD 20014, DHEW Publ. No. (NIH) 78-1385.

National Cancer Institute (1979a). Bioassay of 2,5-Dithiobiurea for Possible Carcinogenicity. National Institute of Health, DHEW Publ. No. (NIH) 79-1387.

National Cancer Institute (1979b). Bioassay of 3-Nitro-p-acetophenetide for Possible Carcinogenicity. National Institute of Health, DHEW Publication No. (NIH) 79-1388.

Nawrot, P.S., and Staples, R.E. (1979). Embryo-fetal toxicity and tertogenicity of benzene and toluene in the mouse. Teratology 19:41A. (abstract)

Neff, M.J.; Anderson, J.W.; Cox, B.A.; Laughlin, R.B.; Rossi, S.S.; and Tatem, H.E. (1976). Effects of petroleum on survival, respiration, and growth of marine animals. Proc. of the Symp. Amer. Univ., Washington, DC.

Nei, N.; Enatsu, T.; and Terui, G. (1973). Microbiological decomposition of phenols. IV. Oxidation of aromatic compounds by phenol-utilizing yeasts. Hakko Kogaku Zasshi 51:1-11. Taken from: Chem. Abst. 78:946392, 1973.

Neligan, R.E.; Leonard, M.J.; and Bryan, R.J. (1965). The gas chromatographic determination of aromatic hydrocarbons in the atmosphere. Reprint of paper presented to the Division of Water, Air, and Waste Chemistry, American Chemical Society, Atlantic City, NJ, September 12-17, 1965, 2 pp.

Nestmann, E.R.; Lee, G.G.-H.; Matula, T.I.; Douglas, G.R.; and Mueller, J.C. (1980). Mutagenicity of constitutents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay. Mutat. Res. 79:203-12.

Nimmo, P.M., and Fishburn, P.J. (1977). The characterization of odours by gas chromatography. In: Analytical Techniques in the Determination of Air Pollutants: A Symposium. Clean Air Society of Australia and New Zealand, pp. 44-48.

NIOSH (National Institute for Occupational Safety and Health) (1973). Criteria for a Recommended Standard. Occupational Exposure to Toluene. Final Report. Contract No. HSM-99-72-118. Available through NTIS, NTIS No. PB-222-219/8, 108 pp.

NIOSH (National Institute for Occupational Safety and Health) (1977). Toluene. In: NIOSH Manual of Analytical Methods, 2nd edition, Part II. Standards Completion Program Validated Methods, Vol. 3. NIOSH Publication No. 77-157-C, pp. 343-1 to 343-8. U.S. Dept. of Health, Education, and Welfare, Public Health Service, Center for Disease Control, NIOSH, Cincinnati, OH.

Nomiyama, K., and Nomiyama, H. (1974a). Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int. Arch. Arbeitsmed. 32(1-2):75-83.

Nomiyama, K., and Nomiyama, H. (1974b). Respiratory elimination of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int. Arch. Arbeitsmed. 32(1-2):85-91.

Nomiyama, K., and Nomiyama, H. (1978). Three fatal cases of thinner-sniffing, and experimental exposure to toluene in human and animals. Int. Arch. Occup. Environ. Health 41(1):55-64.

Nozaka, J., and Kusunose, M. (1968). Metabolism of hydrocarbons in microorganisms. I. Oxidation of p-xylene and toluene by cell-free enzyme preparations of Pseudomonas aeruginosa. Agr. Biol. Chem. 32(8):1033-1039.

Nozaka, J., and Kusunose, M. (1969). Metabolism of hydrocarbons in microorganisms. II. Degradation of toluene by cell-free extracts of Pseudomonas mildenbergii. Agr. Biol. Chem. 33(6):962-64.

NRC (National Research Council) (1980). The Alkyl Benzenes. Committee on Alkyl Benzene Derivatives, Board on Toxicology and Environmental Health Hazards; Assembly of Life Sciences, National Research Council. Washington, DC: National Academy Press.

NTP (National Toxicology Program) (1981). Chemicals on standard protocol. Bethesda, MD: NTP, Carcinogenesis Testing Program. Available from: Technical Information Resources Branch, Carcinogenesis Testing Program, NTP, Landow Bldg., Rm. A306, Bethesda, MD 20205.

Nunes, P., and Benville, P. (1979). Uptake and depuration of petroleum in the manila clam, Tapes semidecussath Reeve. Bull. Environ. Contam. Toxicol. 21(6):719-26.

O'Brien, E.T.; Yeoman, W.B.; and Hobby, J.A.E. (1971). Hepatorenal damage from toluene in a "glue sniffer." Brit. Med. J. 2:29-30.

O'Brien, J.R. et al. (1979). Interaction of oxides of nitrogen and aromatic hydrocarbons under simulated atmospheric conditions. Chapter 11, in Grosjean, D., ed. Nitrogenous Air Pollutants; Chemical and Biological Implications, Ann Arbor Science Publishers, Inc., Ann Arbor, MI. Pp. 189-220. (Cited in Syracuse Research Corporation, 1980.)

Ogata, M.; Asahara, H.; and Saeki, T. (1975). Sampling and analysis of some aromatic, aliphatic and chlorinated hydrocarbon vapours in air: A gas-liquid chromatographic and colorimetric method. Int. Arch. Arbeitsmed. 34:25-37.

Ogata, M., and Fujii, T. (1979). Urinary excretion of hippuric acid and m-methylhippuric acid after administration of toluene and m-xylene mixture to rats. Int. Arch. Occup. Environ. Health. 43(1):45-51.

Ogata, M., and Miyaki, Y. (1973). Identification of substances in petroleum causing objectionable odor in fish. Water Res. 7:1493-1504.

Ogata, M., and Miyake, Y. (1978). Disappearance of aromatic hydrocarbons and organic sulfur compounds from fish flesh reared in crude oil suspension. Water Res. 12(12):1041-44.

Ogata, M., and Sugihara, R. (1977). An improved direct colorimetric method for the quantitative analysis of urinary hippuric acid as an index of toluene exposure. Acta. Med. Okayama 31:235-242.

Ogata, M.; Takatsuka, Y.; Tomokuni, K.; and Muroi, K. (1971). Excretion of hippuric acid and m- or p-methylhippuric acid in the urine of persons exposed to vapors of toluene and m- or p-xylene in an exposure chamber and in workshops, with specific reference to repeated exposures. Brit. J. Ind. Med. 28(4):382-85.

Ogata, M.; Tomokuni, K.; Takatsuka, Y. (1970). Urinary excretion of hippuric acid and m- or p-methylhippuric acid in the urine of persons exposed to vapors of toluene and m- or p-xylene as a test of exposure. Brit. J. Ind. Med. 27(1):43-50.

Oh, S.J., and Kim, J.M. (1976). Giant axonal swelling in "huffer's" neuropathy. Arch. Neurol. 33(8):583-86.

Ohmori, S. et al. (1975). The metabolism and accumulation of petroleum components in fish, the side chain oxidation of p-nitrotoluene and p-nitrobenzyl alcohol in liver homogenates of the rat and eel. Physiol. Chem. Physics 7:477-

Oliver, J.S., and Watson, J.M. (1977). Abuse of solvents 'for hicks': A review of 50 cases. Lancet 1(8002):84-6.

Ovrum, P; Hultengren, M.; and Lindquist, T. (1978). Exposure to toluene in a photogravure printing plant. Concentration in ambient air and uptake in the body. Scand. J. Work, Environ. Health 4(3):237-45.

Pacseri, I., and Emszt, G. (1970). Medical aspects of the exposure to toluol. Munkavedelem 16:41-6. (In Hungarian; cited in NIOSH, 1973.)

Pagnotto, L.D., and Lieberman, L.M. (1967). Urinary hippuric acid excretion as an index of toluene exposure. Amer. Ind. Hyg. Assoc. J. 28:129-34.

Parmeggiani, L., and Sassi, C. (1954). [Occupational risk of toluene: Environmental studies and clinical investigations of chronic intoxication.] Med. Lavoro 45:574-83. (In Ital.)

Pawar, S.S.; Mungikar, A.M.; and Makhija, S.J. (1976). Phenobarbital induced effect on pulmonary and hepatic microsomal ethylmorphine N-demethylase and lipid peroxidation during oral intoxication of organic solvents in rats. Bull. Environ. Contam. Toxicol. 15(3):357-65.

Pellizzari, E.D. (1979). Information on the Characterization of Ambient Organic Vapors in Areas of High Chemical Pollution. Control No. 68-02-2721, Health Effects Research Lab, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC2, p. 134. (Cited in Syracuse Research Corporation, 1980.)

Pellizzari, E.D.; Bunch, J.E.; Berkley, R.E.; and McRae, J. (1976). Determination of trace hazardous organic vapor pollutants in ambient atmospheres by gas chromatography/mass spectrometry/computer. Anal. Chem. 48:803-807.

Peterson, R.G., and Bruckner, J.V. (1978). Measurement of toluene levels in animal tissues. In: Voluntary Inhalations of Industrial Solvents. C.W. Sharp and Carrol, L.T., editors. Rockville, MD: Nat. Inst. Drug Abuse 24:33-42.

Perry, R.A.,; Atkinson, R.; and Pitts, J.N., Jr. (1977). Kinetics and mechanism of the gas phase reaction of OH radicals with aromatic hydrocarbons over the temperature range 296-473°K. J. Phys. Chem. 81:296-304.

Pfaender, F.K. (1976) Analytic Methods Developed. ESE Notes 12(4):4-5.

Pfaffli, P.; Savolainen, H.; Kalliomaki, P.L.; and Kalliokoski, P. (1979). Urinary o-cresol in toluene exposure. Scand. J. Work Environ. Health 5(3):286-89.

Pickering, Q.H., and Croswell, H. (1966). Acute toxicity of some important petrochemicals to fish. J. Water Pollut. Contr. Fed. 38(9):1419-429.

Pickering, Q.H., and Henderson, C. (1966). Acute toxicity of some important petrochemicals to fish. J. Water Pollut. Contr. Fed. 38(9):1419-1429.

Pilar, S., and Graydon, W.F. (1973). Benzene and toluene distribution in Toronto atmosphere. Environ. Sci. Technol. 7(7):628-631.

Piotrowski, J. (1967). [Quantitative estimate of the absorption of toluene in people] Med. Pracy 18.213-223. (In Pol.) (Cited in NIOSH, 1973.)

Pochon, J., and Lajudie, J. (1948). Action of certain antiseptics on the normal microflora of the soil. Compt. Rend. 226:2091-92.

Poel, W.E. (1963). Skin as a test site for the bioassay of carcinogens and carcinogen precursors. Natl. Cancer Inst. Monogr. 10:611.

Pohl, K., and Schmilde, T. (1973). [Serum concentration and performance changes following repeated inhalation of eleven technical organic solvents.] Blutalkohol 10:95-120. (In Ger.)

Potera, G.T. (1975). The Effects of Benzene, Toluene, and Ethyl Benzene on Several Important Members of the Estuarine Ecosystem. Diss. Abstr. B 36(5):2010.

Powars, D. (1965). Aplastic anemia secondary to glue sniffing. N. Engl. J. Med. 273:700-702.

Powers, M.B. (1979). Memorandum for the Record from the NTP Chemical Selection Group, Toxicology Branch, CGT, DCCP, National Institute, Washington, D.C., May 25, 1979.

Premel-Cabic, A.; Cailleux, A.; and Allain, P. (1974). [Identification and quantification by gas chromatography of fifteen organic solvents in the blood.] Clin. Chim. Acta 56:5-11. (In Fr.)

Press, E., and Done, A.K. (1967a). Solvent sniffing. Physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. I. Pediatrics 39:451.

Press, E., and Done, A.K. (1967b). Solvent sniffing. Physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. II. Pediatrics 39:611.

Price, K.S.; Waggy, G.T.; and Conway, R.A. (1974). Brine shrimp bioassay and seawater BOD of petrochemicals. J. Water Pollut. Cont. Fed. 46(1):63-77.

Pryor, G.T.; Bingham, L.R.; and Howd, R.A. (1978). Behavioral toxicology in rats of a mixture of solvents containing substances subject to inhalation abuse by humans. Toxicol. Appl. Pharmacol. 45(1)252.

Public Health Service (1980). Smoking, Tobacco and Health, A Fact Book. U.S. Dept. of Health and Human Services, Public Health Service, Office on Smoking and Health.

Pyykko, K.; Tahti, H.; and Vapaatalo, H. (1977). Toluene concentrations in various tissues of rats after inhalation and oral administration. Arch. Toxicol. 38:169-76. Taken from: Chem. Abst. 88:45927r, 1978.

Quick, A.J. (1931). The conjugation of benzoic acid in the urine. J. Biol. Chem. 92:65-85.

Rall, D.P. (1969). Difficulties in extrapolating the results of toxicity studies in laboratory animals to man. Environ. Res. 2:360-367.

Rawlings, G.C., and Samfield, M. (1979). Textile plant wastewater toxicity. Environ. Sci. Technol. 13(2):160-64.

Raitta, C.; Husmann, K.; and Tossavainen, A. (1976). Lens changes in car painters exposed to a mixture of organic solvents. Albrecht v. Graefes Arch. Ophthal. 200(2):149-56.

Reid, F.H. and Halpin, W.R. (1968). Determination of halogenated and aromatic hydrocarbons in air by charcoal tube and gas chromatography. Amer. Ind. Hyg. Assoc. J. 29(4):390-96.

Reisin, E.; Teicher, A.; Jaffe, R.; and Eliahou, H.E. (1975). Myoglobinuria and renal failure in toluene poisoning. Brit. J. Indust. Med. 32(2):163-64.

Reynolds, E.S. (1972). Comparison of early injury to liver endoplasmic reticulum by halomethanes, hexachloroethane, benzene, toluene, bromobenzene, ethionine, thioacetamide, and dimethylnitrosamine. Biochem. Pharmacol. 21(19):2555-261.

Riihimaki, V., and Pfaffli, P. (1978). Percutaneous absorption of solvent vapors in man. Scand. J. Work Environ. Health 4(1):73-85.

Riihimaki, V. (1979). Conjugation and urinary excretion of toluene and m-xylene metabolites in a man. Scand. J. Work Environ. Health 5(2):135-42.

Robinson, E. et al. (1973). Nonurban, nonmethane low molecular weight hydrocarbon concentrations related to air mass identification. J. Geophys. Res. 78:5345. (Cited in Syracuse Research Corporation, 1980.)

Roche, S.M., and Hine, C.H. (1968). The tertogenicity of some industrial chemicals. Toxicol. Appl. Pharmacol. 12:327.

Roubal, W.T.; Stranaham, S.I.; and Malin, D.C. (1978). The accumulation of low molecular weight aromatic hydrocarbons of crude oil by coho salmon (Oncorhynchus kisutch) and starry flounder (Platichthys stellatus). Arch. Environ. Contam. Toxicol. 7:237-244.

Rouskova, V. (1975). Photic stimulation in early diagnosis of the effects of some harmful industrial substances on the central nervous system. Int. Arch. Arbeitsmed. 34(4):283-99.

Russel, P.A. (1977). Denver Air Pollution Study--1973. Proc. of a Symposium, Vol. II. Final Report, January 1974-June 1976. Report No. EPA/600/9-77/001. NTIS No. PB-264216. Atmospheric Chemistry and Physics Div., Denver Research Lab., Colorado Environmental Sciences Research Lab., Research Triangle Park, NC. (Cited in Syracuse Research Corporation, 1980.)

Ryan, J.P., and Fritz, J.S. (1978). Determination of trace organic impurities in water using thermal desorption by XAD resin. J. Chromatogr. Sci. 16:488-492.

Sabalitschka, T., and Preuss, J. (1954). Action of toluene on bacteria. Deut. Apoth.-Ztg. ver. Suddeut. Apoth-Ztg. 94:1226-1228.

Santodonato, J.; Basu, D.; and Howard, P.H. (1978). Health Effects Associated with Diesel Exhaust Emissions, Literature Review and Evaluation. EPA Publ. No. EPA-600/1-78-063. Prepared for U.S. EPA, HERL, NC.

Sasa, M.; Igarashi, S.; Miyazaki, T.; Miyazaki, K.; and Nakano, S. (1978). Equilibrium disorders with diffuse brain atrophy in long-term toluene sniffing. Arch. Otorhinolarngol. 221(3):163-69.

Sato, A.; Fukiwara, Y.; and Nakajima, T. (1974). Solubility of benzene, toluene and m-xylene in various body fluids and tissues of rabbits. Jap. J. Ind. Health 16(1):30.

Sato, A., and Nakajima, T. (1978). Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. Brit. J. Ind. Med. 35:43-9.

Sato, A., and Nakajima, T. (1979a). Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Brit. J. Ind. Med. 36(3):231-34.

Sato, A., and Nakajima, T. (1979b). Dose-dependent metabolic interaction between benzene and toluene in vivo and in vitro. Toxicol. Appl. Pharmacol. 48(2):249-56.

Sato, A., and Nakajima, T. (1978). Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. Brit. J. Ind. Med. 35(1):43-9.

Sato, A.; Nakajima, T.; Fujiwara, Y.; and Hirosawa, K. (1974). Pharmacokinetics of benzene and toluene. Int. Arch. Arbeitsmed. 33(3):169-82.

Satran, R., and Dodson, V. (1963). Toluene habitation - report of a case. N. Eng. J. Med. 263(13):219-20.

Saunders, J.R. (1977). Degradative plasmids. Nature 269:470.

Saunders, R.A.; Blachly, C.H.; Koracina, R.A.; Lamontagne, R.A.; Swinnerton, J.W.; and Saalfeld, F.E. (1975). Identification of volatile organic contaminants in Washington, D.C. municipal water. Water. Res. 99:1143-145.

Sauer, T.C., Jr. et al. (1978). Volatile liquid hydrocarbons in the surface waters of the Gulf of Mexico. Mar. Chem. 7:1-16. (Cited in Syracuse Research Corporation, 1980.)

Savolainen, H. (1978). Distribution and nervous system binding of intraperitoneally injected toluene. Acta Pharmacol. Toxicol. 43(1):78-80.

Savolainen, H. (1977). Some aspects of the mechanisms by which industrial solvents produce neurotoxic effects. Chem.-Biol. Interact. 18(1):1-10.

Savolainen, H., and Seppalainen, A.M. (1979). Biochemical and physiological effects of organic solvents on rat axon membranes isolated by a new technique. Neurotoxicol. 1/2:467-77.

Schneider, W.; Frohne, J.C.; and Bruderreck, H. (1978). Determination of hydrocarbons in the parts per $10^9$ range using glass capillary columns coated with aluminum oxide. J. Chromatogr. 155:311-327.

Scholz, R.; Schmitz, H.; Bucher, T.; and Lampen, J.O. (1959). Effect of nystatin on yeast. Biochem. Z. 331:71-86.

Segev, N.; Miller, C.; Sharon, R.; and Ben-Ishai, R. (1973). Exicision repair of ultraviolet radiation damage in toluene treated Escherichia coli. Biochem. Biophys. Res. Commun. 53(4):1242-245. Taken from: Chem. Abst. __:62056n,

Seifert, B., and Ullrich, D. (1978). Determination of organic pollutants by gas chromatography after cryogenic sampling. Stud. Environ. Sci. 1:69-72.

Seppalainen, A.M.; Husman, K.; and Martenson, C. (1978). Neurophysiological effects of long-term exposure to a mixture of organic solvents. Scand. J. Work Environ. Health 4(4):304-14. Taken from: Chem. Abst. 90:156383w, 1979.

Sessa, T. (1948). Histopathology in experimental chronic touene poisoning. Folia Med. (Naples) 31:91-105. Taken from: Chem. Abst. 42:1662b, 1948.

Sexton, K., and Westberg, H. (1980). Ambient hydrocarbon and ozone measurements downwind of a large automotive painting plant. Environ. Sci. Technol. 14:329. (Cited in Syracuse Research Corporation, 1980.)

Shelford, V.E. (1917). An experimental study of the effects of gas waste upon fishes with special reference to stream pollution. Bull. Ill. State Lab. Nat. Hist. 11:

Sherwood, R.J. (1976). Ostwald solubility coefficients of some industrially important substances. Brit. J. Ind. Med. 33(2):106-107.

Shigeta, S.; Aikawa, H.; Misawa, T.; and Kondo, A. (1978). Effect of single exposure to toluene on Sidman avoidance response in rats. J. Toxicol. Sci. 3(4):305-12.

Shirabe, T.; Tsuda, T.; Terao, A.; and Araki, S. (1974). Toxic polyneuropathy due to glue-sniffing: Report of two cases with a light and electron-microscopic study of the peripheral nerves and muscles. J. Neurol. Sci. 21(1):101-13.

Silva, M.T.; Sausa, J.C.F.; and Balassa, G. (1978). Ultrastructural effects of chemical agents and moist heat on Bacillus subtilis. I. Effects on vegetative cels. Am. Microbiol. 129B:363-375. (Cited in NRC, 1980.)

Singh, H.G. et al. (1979). Atmospheric Measurements of Selected Toxic Organic Chemicals. Interim Report prepared for U.S. EPA, Research Triangle Park, NC, by SRI, Menlo Park, CA. (Cited in Syracuse Research Corporation, 1980.)

Skinner, C.E. (1972). Role of algae in the deterioration of decorative and marine paints. FATIPEC Cong. 11:421-427.

Skjoldal, H.R., and Bakke, T. (1978). Relationship between ATP and energy charge during lethal metabolic stress of the marine isopod Cirolana borealis. J. Biol. Chem. 253(10):3355-356.

Sloof, W. (1978). Biological Monitoring Based on Fish Respiration for Continuous Water Quality Control. In: Aquatic Pollutants. Transformation and Biological Effects, Hutzinger, O., and Safe, S. (eds.), Pergamon Press. Vol. 1, pp. 501-506.

Sloof, W. (1979). Detection limits of a biological monitoring system based on fish respiration. Bull. Environ. Contam. Toxicol. 23(4-5):517-23.

Smith, J.N. et al. (1954). Studies in detoxication, 55. The metabolism of alkylbenzenes. a/Glucuronic acid excretion following the administration of alkylbenzenes; b/Elimination of toluene in the expired air of rabbits. Biochem. J. 56:317-20.

Smith, R.V., and Rosazza, J.P. (1974). Microbial models of mammalian metabolism. Aromatic hydroxylation. Arch. Biochem. Biophys. 161:551-558.

Smolik, R. et al. (1973). Serum complement level in workers exposed to benzene, toluene and xylene. Int. Arch. Arbeitsmed. 31:243-47.

Smoyer, J.C.; Shaffer, D.E.; and DeWitt, I.L. (1971). A program to sample and analyze air pollution in the vicinity of a chemical reclamation plant. Inst. Environ. Sci. Tech. Meet., Proc. 17:339-45.

Smyth, H.F., Jr.; Carpenter, C.P.; Weil, C.S.; Pozzani, U.C.; Striegel, J.A.; and Nycum, J.S. (1969). Range-finding toxicity data. VII. Amer. Ind. Hyg. Assoc. J. 30(5):470-76.

Smyth, H.F., Jr.; Weil, C.S.; West, J.S.; and Carpenter, C.P. (1969). Exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol. Appl. Pharmacol. 14(2):340-47.

Smyth, H.F., and Smyth, H.F., Jr. (1928). Inhalation experiments with certain lacquer solvents. J. Ind. Hyg. 10:261-71.

Snow, L.; MacNair, P.; and Casto, B.C. (1981). Mutagenesis testing of toluene in Salmonella strains TA100 and TA98. Report prepared for the U.S. EPA by Northrup Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709.

Sokol, J., and Robinson, J.L. (1963). Glue sniffing. Western Medicine 4:192.

Sollenberg, J., and Baldsten, A. (1977). Isotachophoretic analysis of mandelic acid, phenylglyoxylic acid hippuric acid and methylhippuric acid in urine after occupational exposure to styrene, toluene and/or xylene. J. Chromatogr. 132(3):469-76.

Sontheiner, H. (1980). Experience with riverbank filtration along the Rhine River. J. Amer. Water Works Assoc. 72:386-390.

Southworth, G.R. (1979). The role of volatilization in removing polycyclic aromatic hydrocarbons from aquatic environments. Bull. Environ. Contam. Toxicol. 21:507-514.

Speck, B., and Moeschlin, S. (1968). Effect of toluene, xylene, chloramphenicol, and thiouracil on bone marrow. Experimental autoradiographic study with thymidine-3H. Schweiz. Med. Wochenschr. 98(42):1684-686.

Srbova, J., and Teisinger, J. (1952). Absorption and elimination of toluene in man. Arch. Ind. Hyg. Occup. Med. 6:462.

Srbova, J., and Teisinger, J. (1953). Metabolism of toluene. Pracov. Lek. 5:259-263. Taken from: Chem. Abst. 49:3418e, 1955.

Stephens, E.R. (1973). Hydrocarbons in Polluted Air: Summary Report Coordinating Research Council Report CRC-APRAC-CAPA-5-68-1, NTIS No. PB-230993. Statewide Air Pollution Research Center, Univ. of California, Riverside, CA. (Cited in Syracuse Research Corporation, 1980.)

Stokinger, H.E., and Woodward, R.L. (1958). Toxicologic methods for establishing drinking water standards. J. Amer. Water Works Assoc., April, 1958.

Stormer, K. (1908). Uber die wirkung des schwefekkohlenstoffs und ahnlicler stoff auf den boden. Zbl. Bakt. (Abst. 2) 20:282. (Cited in Claus and Walker, 1964.)

Stoss, F.W., and Haines, T.A. (1979). The effects of toluene on embryos and fry of the Japanese medaka Oryzias latipes with a proposal for rapid determination of maximum acceptable toxican concentration. Environ. Pollut. 20(2):139-48.

Su, G., and Wurzel, K.A. (1981). A regulatory framework for setting air emission limits for noncriteria pollutants. J. Air Pollut. Contr. Assoc. 31(2):160-162.

Subramanian, V.; Sugumaran, M.; and Vaidyanathan, C.S. (1978). Double hydroxylation reactions in microorganisms. J. Indian Inst. Sci. 60(8):173-78.

Suhr, E. (1975). Comparative Investigation of the State of Health of Gravure Printers Exposed to Toluene. Gesellschaft zur Forderung des Tiefdrucks E.V., Weisbaden, Federal Republic of Germany. 92 pp.

Sullivan, C.W., and Volcani, B.E. (1976). Role of silican in diatom metabolism. VII. Silicic acid-stimulated DNA synthesis in toluene permeabilized cells of Cylindrothica lusiformis. Exptl. Cell Res. 98:23-30.

Sutton, C., and Calder, J.A. (1975). Solubility of alkylbenzenes in distilled water and seawater at 25=C. J. Chem. Eng. Data 2=(3):320- .

Suzuki, T.; Shimbo, S.; and Nishitani, H. (1974). Muscular atrophy due to glue sniffing. Int. Arch. Arbeitsmed. 33(2):115-23.

Svirbely, J.L.; Dunn, R.C.; and Von Oettingen, W.F. (1943). The acute toxicity of vapors of certain solvents containing appreciable amounts of benzene and toluene. J. Ind. Hyg. Toxicol. 25:366-73. Taken from: Chem. Abst. 39:4979, 1945.

Svirbely, J.L.; Dunn, R.C.; and Von Oettingen, W.F. (1944). J. Ind. Hyg. To icol. 26:37-46. Taken from: Chem. Abst. 38:4696, 1944.

Syracuse Research Corporation (1980). Hazard Assessment Report on Toluene. 1st Draft. Prepared for U.S. Environmental Protection Agency, Research Triangle Park, NC.

Syrovadko, O.N. (1977). Working conditions and health status of women handling organosiliceous varnishes containing toluene. Gig. Tr. Prof. Zabol. 12:15-9.

Szadkowski, D.; Pett, R.; Angerer, J.; Manz, A.; and Lehnert, G. (1973). Chronic solvent exposure at work. II. Harmful material levels in blood and excretion rates of metabolites in urine with the importance of environmental criteria for toluene exposed printers. Int. Arch. Arbeitsmed. 31(4):265-76.

Szadkowski, D. et al. (1976). Evaluation of occupational e posure to toluene. Medizinische Monatsschrift. 30(1): .

Taher, S.M.; Anderson, R.J.; McCartney, R.; Popvitzer, M.M.; and Schrier, R.W. (1974). Renal rubular acidosis associated with toluene sniffing. N. Engl. J. Med. 290:765-68.

Tahti, H.; Ruuska, J.; and Vapaatalo, H. (1977). Toluene toxicity studies on rats after 1 week inhalation exposure. Acta Pharmacol. Toxicol. 41(4):78.

Takenaka, S.; Tawara, S.; Ideta, T.; Okajima, T.; and Tokuomi, H. (1972). A case with polyneuropathy due to glue-sniffing. Clin. Neurol. 12:747.

Takeuchi, Y. (1969). Experimental studies on the toluene poisoning--chiefly on the findings of peripheral blood and adrenal gland. Ind. Health 7:31-45.

Takeuchi, Y., and Hisanaga, N. (1977). The neurotoxicity of toluene: EEG changes in rats exposed to various concentrations. Brit. J. Ind. Med. 34(4):314-24.

Takeuchi, Y., and Suzuki, H. (1975). Change of convulsion threshold of the rat exposed to toluene. Indust. Health 13:109-14.

Tarsh, M.J. (1979). Schizophreniform psychosis caused by sniffing toluene. J. Soc. Occup. Med. 29(4):131-33.

Tatrai, E.; Hudak, A.; and Ungvary, G. (1979). Simultaneous effect on the rat-liver of benzene, toluene, zylene and CCL4. Acta Physiol. Acad. Sci. Hung. 53(2):261.

Tausson, W.O. (1929). Uber die oxydation der benzolkohlenwaserstoffe durth bakterien. Planta 7:735. (Cited in Claus and Walker, 1964.)

Taylor, D.C., and Harris, W.S. (1970). Glue sniffing causes heart block in mice. Science 170:866-68.

Thayer, M., and Schulze, W.D. (1980). An Examination of Benefits and Costs of Achieving Ambient Standards in the South Coast Air Basin. U.S. Environmental Protection Agency, Washington, DC.

Thomas, R.E., and Rice, S. D. (1979). The effect of exposure temperatures on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, naphthalene, and water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil. Mar. Pollut. 79:39-52.

Thompson, L.M., and MacLeod, R.A. (1974). Biochemical localization of alkaline phosphatase in the cell wall of a marine pseudomonad. J. Bacteriol. 117(2):819-825.

Tokunaga, R.; Takahata, S.; Onoda, M.; Ishi-i, T.; Sato, K.; Hayashi, M.; and Ikeda, M. (1974). Evaluation of the exposure to organic solvent mixture. Comparative studies on detection tube and gas-liquid chromatographic methods, personal and stationary sampling, and urinary metabolite determination. Int. Arch. Arbeitsmed. 33:257-267.

Tommaso, S. (1948). Histopathology in experimental chronic toluene poisoning. Folia Med. 31:91-105. Taken from: Chem. Abst. 42:1662b, 1948.

Toutant, C., and Lippmann, S. (1979). Fetal solvents syndrome (letter). Lancet 1(8130):1356.

Towfighi, J.; Gonatas, N.K.; Pleasure, D.; Cooper, H.S.; and McCree, L. (1976). Glue sniffer's neuropathy. Neurology 26:238-43.

Trevisan, A., and Chiesura, P. (1978). Clinical research on the hepatotoxicity of toluene. Ital. J. Gastroenterology 10(3):210.

Tsuzi, K. (1956). Convulsion caused by phenol compounds. Kumamoto Med. 9:152-164. Taken from: Chem. Abst. 51:9909g, 1957.

Turner, D.B. (1969). Workbook of Atmospheric Dispersion Estimates. U.S. Dept. of Health, Education, and Welfare, Revised, 1969. (Cited in P. Walker, 1976.)

Tute, M.S. (1971). Principles and practice of Haasch analysis: A guide to structure-activity correlation for the medicinal chemist. Adv. Drug Res. 5:1- .

Twibell, J.D., and Home, J.M. (1977). Novel method for direct analysis of hydrocarbons in crime investigation and air pollution studies. Nature 268:711-713.

Umberger, J.C., and Fioresse, F.F. (1963). Colorimetric method for hippuric acid. Clin. Chem. 1:91-96.

Ungvary, G.; Hudak, A.; Bors, Z.; and Folly, G. (1976). The effect of toluene on the liver assayed by quantitative morphological methods. Exp. Mol. Pathol. 25(1):49-59.

Ungvary, G.; Hamori, J.; and Hudak, A. (1975). [Experimental study of the hepatotoxic effect of toluol. II. Electron microscopic and electron histochemical studies.] Morphol. Igazsagugyi Ory. Sz. 15(4):256-63.

Ungvary, G.; Manyai, S.; Tatrai, E.; Szeberenyi, S.; Cseh, R.J.; Molnar, J.; and Folly, G. (1980). Effect of toluene inhalation on the liver of rats--dependence on sex dose and exposure time. J. Hyg., Epidemiol., Microbiol., Immunol. 24:242-252.

U.S. Department of Commerce (1979). Statistical Abstract of the United States, 100th ed., National Data and Guide to Sources, Bureau of the Census.

U.S. Department of Health, Education, and Welfare (1977). National Occupational Hazard Survey, Vol. III. Survey Analysis and Supplemental Tables. U.S. Dept. of Health, Education, and Welfare, National Institute of Occupational Safety and Health, Div. of Surveillance, Hazard Evaluations, and Field Studies, Cincinnati, Ohio, p. 448.

U.S. EPA (U.S. Environmental Protection Agency) (1975a). New Orleans Area Water Supply Study. Analysis of Carbon and Resin Extracts. Prepared by the Analytical Branch, Southeast Environ. Res. Lab., Athens, GA, for the lower Mississippi River Branch, Surveillance and Analysis Division, Region VI. (Cited in Syracuse Research Corporation, 1980.)

U.S. EPA (U.S. Environmental Protection Agency) (1975b). Preliminary Assessment of suspended Carcinogens in Drinking Water. Report to Congress, Washington, D.C. (Cited in Syracuse Research Corporation, 1980.)

U.S. EPA (U.S. Environmental Protection Agency) (1977). National Organic Monitoring Survey, General Review of Results and Methodology: Phases I-III. (Cited in Syracuse Research Corporation, 1980.)

U.S. EPA (U.S. Environmental Protection Agency) (1979a). Chemistry Laboratory Manual for Bottom Sediments and Elutriate Testing. U.S. Environmental Protection Agency, Chicago, IL. Available from: National Technical Information Service, Springfield, VA (NTIS PB 294-596).

U.S. EPA (U.S. Environmental Protection Agency) (1979b). Fate of Priority Pollutants in Publicly Owned Treatment Works--Pilot Study, Publication No. EPA 440/1-79-300. Performed by Feiler, Burns and Roe Industrial Services Corp., Paramus, NJ.

U.S. EPA (U.S. Environmental Protection Agency) (1980). Ambient Water Quality Criteria for Toluene. Publication No. EPA440/5-80-075. U.S. Environmental Agency, Washington, D.C.

U.S. EPA (U.S. Environmental Protection Agency) (1980). STORET Water Quality Information System, October, 1980.

U.S. EPA (U.S. Environmental Protection Agency) (1980a). Priority Pollutant Frequency Listing Tabulations and Descriptive Statistics. Memo from D. Neptune, Analytical Programs to R.B. Schaffer, Director of Effluent Guidelines Div., January, 1980. (Cited in Slimak, 1980.)

U.S. EPA (U.S. Environmental Protection Agency) (1980b). Volatile Organic Compound (VOC) Species Data Manual, 2nd ed., Publication No. EPA-450/4-80-015. Office of Air, Noise, and Radiation, Office of Air Quality Planning and Standards, Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency (1980c). Guidelines and Methodology for the Preparation of Health Effect Assessment Chapters of the Ambient Water Quality Criteria Documents. U.S. EPA, Environmental Criteria and Assessment Office; Office of Health and Environmental Assessment; Office of Research and Development, Cincinnati, OH, November 28, 1980.

USITC (United States International Trade Commission) (1979). Synthetic Organic Chemicals: United States Production and Sales, 1978, USITC Publication 1001, USITC, Washington, D.C. 20436.

Van Doorn, R.; Bos, R.P.; and Brouns, R.M.E. (1980). Effect of toluene and xylenes on liver glutathione and their urinary excretion as mercapturic acids in the rat. Arch. Toxicol. 43(4):293-304.

Van Ert, M.D.; Arp, E.W.; Harris, R.L.; Symons, M.J.; and Williams, T.M. (1980). Worker exposures to chemical agents in the manufacture of rubber tires: Solvent vapor studies. Amer. Ind. Hyg. Assoc. J. 41:212-19.

Veith, G.D. et al. (1979). Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish Res. Board Can. 36:91. (Cited in Syracuse Research Corporation, 1980.)

Verschueren, K. (1977. Handbook of Environmental Data on Organic Chemicals. New York, NY: Van Nostrand Reinhold Company, pp. 592-596.

Veulemans, H., and Masschelein, R. (1978a). Experimental human exposure to toluene. I. Factors influencing the individual respiratory uptake and elimination. Int. Arch. Occup. Environ. Health 42(2):91-103. Taken from: Chem. Abst. 90:133618n, 1979.

Veulemans, H., and Masschelein, R. (1978b). Experimental human exposure to toluene. II. Toluene in venous blood during and after exposure. Int. Arch. Occup. Environ. Health 42(2):105-17. Taken from: Chem. Abst. 90:181040q, 1979.

Veulemans, H., and Masschelein, R. (1979). Experimental human exposure to toluene. III. Urinary hippuric acid excretion as a measure of individual solvent uptake. Int. Arch. Occup. Environ. Health 43(1):53-62.

Veulemans, H.; Van Vlem, E.; Janssens, H.; and Masschelein, R. (1979). Exposure to toluene and urinary hippuric acid excretion in a group of heliorotagravure printing workers. Int. Arch. Occup. Environ. Health 44(2):99-107.

Vitenberg, A.G.; Stolyarov, B.V.; and Smirnova, S.A. (1977). Gas-chromatographic determination of traces of aromatic hydrocarbons and alcohols in water by the equilibrium vapor analysis method. Vestn. Leningr. Univ., Fiz. Khim. 3:132-139.

Von Oettingen, W.F.; Neal, P.A.; and Donahue, D.D. (1942a). The toxicity and potential dangers of toluene--Preliminary report. J. Amer. Med. Assoc. 118:579-84.

Von Oettingen, W.F.; Neal, P.A.; Donahue, D.D.; Svirbely, J.L.; Baernstein, H.D.; Monaco, A.R.; Valaer, P.J.; and Mitchell, J.L. (1942b). The Toxicity and Potential Dangers of Toluene, with Special Reference to its Maximal Permissible Concentration. U.S. Public Health Serv. Pub. Health Bull. No. 279, 50 pp.

Wagner, R. (1914). Uber benzol bakterien. Z. Gar Physiol. 4:289. (Cited in Claus and Walker, 1964.)

Wahlberg, J.E. (1976). Percutaneous toxicity of solvents. A comparative investigation in the guinea pig with benzene, toluene and 1,1,2-trichloroethane. Ann. Occup. Hyg. 19(2):115-19. Taken from: Chem. Abst. 86:66415w, 1977.

Walker, P. (1976). Air Pollution Assessment of Toluene, Publication No. MTR-7215. Prepared by the Mitre Corp., McLean, VA, under Contract No. 68-02-1495. U.S. Environmental Protection Agency, Research Triangle Park, NC. Available from: National Technical Information Service, Springfield, VA (NTIS PB 256-735). (Cited in Syracuse Research Corporation, 1980.)

Wallen, I.E.; Green, W.C.; and Lasater, R. (1957). Toxicity to Gambusia affinis of certain pure chemicals in turbid waters. Sewage Indust. Wastes 29(6):695-711.

Walter P.V.; Maslyn, R.T.; Shaffer, G.P.; and Daniels, C.A. (1977). Glue sniffing: The continuing menace. Drug Forum 5(3):193-97.

Watson, J.M. (1979). Glue sniffing. Two case reports. Practitioner 222(1332):845-47.

Weast, R.C., editor (1977). CRC Handbook of Chemistry and Physics, 58th ed. Cleveland, OH: Chemical Rubber Co., pp.

Wei, K.S., and Adelman, A.H. (1969). The photooxidation of toluene. The role of an excited charge-transfer complex. Tetra. Lett. 38:3297. (Cited in Syracuse Research Corporation, 1980.)

Weisenberger, B.L. (1977). Toluene habituation. J. Occup. Med. 19(8):569-70.

Wilczok, T., and Bieniek, G. (1978). Urinary hippuric acid concentration after occcupational exposure to toluene. Brit. J. Ind. Med. 35(4):330-34.

Williams, P.A., and Worsey, M.J. (1976). Ubiquity of plasmids in coding for toluene and xylene metabolism in soil bacteria: Evidence for the existence of new TOL plasmids. J. Bacteriol. 125(3):818-28.

Wilson, J.T.; Enfield, C.G.; Dunlap, W.J.; Crosby, R.L.; Foster, D.A.; and Baskin, L.B. (1980). Transport and Fate of Selected Organic Pollutants in a Sandy Soil. Updated manuscript from Robert S. Kerr, Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, OK. (Cited in A.D. Little, Inc., 1981.)

Wilson, R.H. (1943). Toluene poisoning. J. Amer. Med. Assoc. 123:1106.

Winek, C.L., and Collom, W.D. (1971). Benzene and toluene fatalities. J. Occup. Med. 13:259-261.

Winek, C.L.; Wecht, C.H.; and Collom, W.D. (1968). Toluene fatality from glue sniffing. Penn. Med. 71:81.

Winneke, G.; Kastka, J.; and Fodor, G.G. (1976). Psychophysiological Effects of Low Level Exposure to Trichloroethylene and Toluene. In: Proceedings of the 2nd International Industrial and Environmental Neurology Congress, Prague, Czechoslovakia, 1974, (Klimkova-Deutschova, E. and Lukas, E., eds.) Univerzita Karlova Praha, p. 78.

Winston, S., and Matsushita, T. (1975). Permanent loss of chromosome initiation in toluene-treated Bacillus subtilis cells. J. Bacteriol. 123:921-927.

Withey, R.J., and Hall, J.W. (1975). Joint toxic action of perchloroethylene with benzene or toluene in rats. Toxicology 4(1):5-15.

Woiwode, W.; Wodarz, R.; Drysch, K.; and Weichardt, H. (1979). Metabolism of toluene in man: Gas chromatographic determination of o-, m-, and p-cresol in urine. Arch. Toxicol. 43:93-98.

Woldringh, C.L. (1973). Effects of toluene and phenethyl alcohol on the ultrastructure of Escherichia coli. J. Bacteriol. 114(3):1359-1361.

Wolf, M.A. et al. (1956). Toxicological studies of certain alkylated benzenes and benzene. Arch. Ind. Health 14:387.

Worsey, M.J., and Williams, P.A. (1975). Metabolism of toluene and xylenes by Pseudomonas putida (arvilla) mt-2. Evidence for a new function of the TOL plasmid. J. Bacteriol. 124(1):7-13.

Worsey, M.J.; Franklin, F.C.H.; and Williams, P.A. (1978). Regulation of the degradative pathway enzymes coded for by the TOL plasmid (pWWO) from Pseudomonas putida mt-2. J. Bacteriol. 134(3):757-64.

Wyse, D.G. (1973). Deliberate inhalation of volatile hydrocarbons: A review. Can. Med. Assoc. J. 108:71-4.

Yanaoka, Y., and Terumi, T. (1977). Organic matter in kraft pulp mill effluent in Hiro Bay. Nippon Kagaku Ikaishi No. 10:1554-559.

Yano, K., and Nishi, T. (1980). pKJI, a naturally occurring conjugative plasmid coding for toluene degradation and resistance to Streptomycin and sulfonamides. J. Bacteriol. 143(2):552.

Young, L.Y., and Mitchell, R. (1973). Negative chemotaxis of marine bacteria to toxic chemicals. Appl. Micro. 25(6):972-975.

Yushkevich, L.B., and Malipheva, M.V. (1975). Study of the bone marrow as an index of experimentally-induced poisoning with chemical substances (such as benzene and its homologs). Sanit. Toksikol. Metody Issled. Gig. 36: . (Cited in U.S. EPA, 1980.)