SRC TR 81-568

SECOND DRAFT

HEALTH RISK ASSESSMENT

DOCUMENT FOR TOLUENE

Prepared by:

Center for Chemical Hazard Assessment
Syracuse Research Corporation
Merrill Lane
Syracuse, New York 13210

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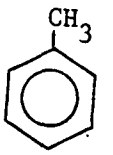
1. EXECUTIVE SUMMARY

1.1 ENVIRONMENTAL SOURCES, FATE, AND LEVELS

Toluene, a homolog of benzene that contains a single methyl group, is a clear, colorless liquid at room temperature. The molecular formula of toluene is C_7H_8 and the molecular weight is 92.13. The structural formula is given below.



Other physical properties of toluene include a melting point of -95°C, a boiling point of 110.6°C, a flash point of 4.44°C, a vapor pressure of 28.7 torr at 25°C, and a density of 0.8669 g/ml at 20°C. Toluene is slightly soluble in both fresh and salt water (535 mg/l and 379 mg/l, respectively) at a temperature of 25°C. The physical properties of toluene would indicate that toluene in the environment is likely to be present in the air, and that toluene originally present in water may be transferred to the atmosphere. Toluene can undergo photochemical reactions, particularly under atmospheric smog conditions. In aqueous media under the conditions of water chlorination, toluene may be chlorinated followed by subsequent hydrolysis to benzaldehyde. This reaction may account for the benzaldehyde detected in some finished drinking waters.

The general population may be exposed to toluene through inhalation of air, ingestion of food or water, or through dermal exposure. The four largest sources of emission of toluene to the atmosphere are, in descending order of importance, automobile use, industrial use of toluene as a solvent, coke ovens, and toluene-producing industries. Other than exposure via the air, toluene has been detected in drinking water and the flesh of edible fish. Dermal exposure to toluene is

only important in the workplace. The estimated quantities of toluene taken in by the general public from each source are between a trace and 94 mg/week by inhalation (depending on whether an individual resides in an urban or rural area or near an industry that uses toluene) and 0.0 to 0.75 mg/week from food and water. Occupational exposure (up to 18,000 mg/week) or cigarette smoking (14 mg/week from 140 cigarettes) will increase an individual's exposure to toluene. Although there are technical problems with estimating inhalation exposure to toluene, there is reasonable agreement between the values obtained by dispersion modeling and those obtained from calculations using monitoring data.

The total amount of toluene produced in the United States in 1978 was 3595 million kg. The majority (96.5%) is produced by catalytic reformation from selected petroleum fractions, and the remainder is produced from pyrolytic cracking, and as a recovered by-product of styrene production and coke oven emission. This value of 3595 million kg is for isolated toluene and accounts for only 11% of the total toluene produced, the remaining 89% of the toluene produced is not isolated as pure toluene but is a benzene-toluene-xylene mixture used in gasoline. Other uses of toluene are feed stock for the production of benzene and other chemicals, as a gasoline additive, and as a solvent.

Activities associated with automobiles (marketing and evaporation of gasoline and automobile exhaust) are the largest single atmospheric source of toluene (677 million kg/year), with industries using toluene as a solvent (the paint and coating, adhesive, ink, and pharmaceutical industries) being the second largest emitter of toluene to the atmosphere (375 million kg/year). These two sources account for 75% of the toluene emitted to the atmosphere. The amount of toluene released to other media in the environment is small and is equal to approximately 0.15% of the total amount released to the atmosphere.

The preferred method for the monitoring of toluene in ambient air consists of sorbent collection, thermal elution, and GC-FID determination. For a 25 l sample, the detection limit is <0.1 ppb. Purge and trap with GC-photoionization detection is the most widely used method for the analysis of toluene in aqueous samples. With a 5 ml sample, the method has a detection limit of 0.1 ppb.

Toluene is the most prevalent aromatic hydrocarbon in the atmosphere, with average measured levels ranging from 0.14 to 59 ppb. Toluene has also been detected in surface waters and in treated wastewater effluents at levels generally below 10 ppb. Concentration of toluene as high as 19 ppb has been detected in a drinking water supply. In a study of toluene, 95% of the sample contained less than 1 ppm of toluene. The atmosphere is the major environmental receiver for toluene. It has been estimated that approximately 124 million people in the U.S. are exposed to atmospheric toluene at a concentration level greater than 1 $\mu g/m^3$.

Toluene released to the aquatic or soil environment is at least partly removed by biodegradation. There is little information on the rate and extent of biodegradation in soil; however, in one study a half-life of between 20 and 60 min was observed in soil containing toluene-degrading bacteria and in a second study 20 to 60% of toluene was removed following percolation through 140 cm of sand. As a result of the limited number of studies available, the extent of toluene degradation in soil cannot be determined although studies with pure cultures indicate that a variety of bacteria and fungi can utilize toluene, and some pure cultures have been isolated that can use toluene as a sole source of carbon. Toluene is also readily biodegraded in aqueous media, both in surface water and during wastewater treatment; however, disappearance of toluene from aqueous media is mainly through evaporation and transport to the atmosphere. The conversion of toluene to compounds that can be utilized as sources of carbon and

energy suggests that toluene will be degraded rapidly by microbial species proliferating at the expense of the compound and will not accumulate significantly in the environment.

1.2 EFFECTS ON HUMANS

Exposures of humans to toluene have almost exclusively involved inhalation in experimental or occupational settings or during episodes of intentional abuse. The health effect of primary concern is dysfunction of the central nervous system (CNS). Acute experimental and occupational exposures to toluene in the range of 200-1500 ppm have elicited dose-related symptoms indicative of CNS depression, as well as impairments in reaction time and perceptual speed. Following initial CNS excitatory effects (e.g., exhilaration, lightheadedness), progressive development of narcosis has characterized acute exposures to excessive concentrations of toluene (i.e., levels approaching the air saturation concentration of approximately 30,000 ppm). Repeated occupational exposures to toluene over a period of years at levels of 200-400 ppm have resulted in some evidence of neurologic effects, and chronic exposure to mixtures of solvent vapors containing predominantly toluene at levels of 30-100 ppm have resulted in impaired performance on tests for intellectual and psychomotor ability and muscular function. Prolonged abuse of toluene or solvent mixtures containing toluene have, on occasion, led to residual or permanent CNS effects.

Early reports of occupational exposures ascribed myelotoxic effects to toluene, but the majority of recent evidence indicates that toluene is not toxic toward the blood or bone marrow. The myelotoxic effects previously attributed to toluene are currently considered to be the result of concurrent exposure to benzene, which was typically present as a contaminant. Acute exposures to toluene have not resulted in any definite effects on heart rate or blood pressure.

Liver enlargement was reported in an early study of painters exposed to 100-1100 ppm toluene for 2 weeks to more than 5 years, but this effect was not associated with clinical evidence of liver disease or corroborated in subsequent studies. Chronic occupational exposure to toluene or intensive exposure via glue or thinner sniffing has generally not been associated with abnormal liver function. Evidence of renal dysfunction has been observed in workers who were accidentally overexposed to toluene and in toluene abusers, but a single occupational study of women exposed to 60-100 ppm toluene for over 3 years did not report abnormal urinalysis findings. Several reports have recently appeared that associate deliberate inhalation of toluene with metabolic acidosis.

Dysmenorrhea has been reported in women exposed for over 3 years to 60-100 ppm toluene and concommitantly to 20-50 ppm gasoline in a "few" working places. Disturbances of menstruation have also been reported in female workers exposed concurrently to toluene, benzene, and xylene, and to toluene and other unspecified solvents.

Single short-term exposures to moderate levels of toluene have, on occasion, been reported to cause transitory eye and respiratory tract irritation, but irritative effects have generally not been observed in workers exposed repetitively to toluene. Dermal contact with toluene may cause skin damage due to its degreasing action.

1.3 ANIMAL STUDIES

The most pronounced effect of toluene in animal studies is on the central nervous system. Acute exposure to inhalation of high levels of toluene has been linked with depression of activity. Levels below 1000 ppm vapor have little or no effect on gross observations of behavior, although lower levels have been observed to have an effect using more sensitive methods of assay, i.e., detection of changes in cognition and brain neuromodulator levels.

Although early studies suggested toluene induced myelotoxicity, most studies using toluene that contained negligible amounts of benzene have not produced injury on blood-forming organs; however, three Russian and one Japanese study have reported leukocytosis, impaired leukopoiesis, or chromosomal damage in the bone marrow.

Inhalation of concentrations of up to 1085 ppm toluene for 6 weeks or 300 ppm for 24 months and ingestion of 590 mg toluene/kg body weight for 6 months produced no liver damage; however, several studies noted increase of liver weight or slight histological change suggestive of possible liver damage at higher levels of exposure or in animals treated by the intraperitoneal route.

Renal injury was noted in rats, dogs, and guinea pigs after subacute inhalation of toluene vapors at doses in excess of 600 ppm in three studies, while no renal damage was found in other subacute and subchronic studies in which rats, dogs, guinea pigs, and monkeys inhaled vapors up to a concentration of 1085 ppm or ingested 590 mg toluene/kg body weight.

Although no effect was observed in the lungs of rats, guinea pigs, dogs, or monkeys after exposure to 1085 ppm toluene vapor intermittently for 6 weeks, in rats after inhalation of up to 300 ppm toluene for 24 months, or in rats after ingestion of 590 mg toluene/kg body weight for 6 months, other studies noted irritation effects in the respiratory tract in dogs, guinea pigs, and rats. Sensitization of the heart in mice, rats, and dogs was reported after inhalation of toluene.

The acute oral toxicity (LD50) of toluene in rats is in the range of 6.0 to 7.5 g/kg, which indicates only slight toxicity in this species. An acute dermal toxicity (LD50) was reported to be 14.1 ml/kg in the rabbit. Slight to moderate irritation was noted in rabbit and guinea pig skin and the rabbit cornea. An

LC50 in the range of 5500 to 7000 ppm was reported in mice and of 4050 ppm in rats.

1.4 PHARMACOKINETICS

Toluene is readily absorbed from the respiratory tract. Studies with humans indicate that the total amount of toluene absorbed is proportional to the concentration of toluene in inspired air, the length of exposure, and pulmonary ventilation, which in turn depends upon the level of physical activity. Approximately 50% of the amount inspired is retained in the body. Absorption of toluene from the gastrointestinal tract is probably fairly complete, based on excretion data from experimental animals. Toluene is absorbed less readily through the skin than through the respiratory or gastrointestinal tracts.

Animals given toluene orally or by inhalation had high concentrations of toluene in their adipose tissue and bone marrow, and moderately high concentrations of toluene and its metabolites in liver and kidney. These results are reasonable based on tissue-blood partition coefficients and known routes of metabolism and excretion.

The initial step in the metabolism of toluene is side-chain hydroxylation by the hepatic mixed-function oxidase system, followed by oxidation to benzoic acid. Benzoic acid is then conjugated with glycine to form hippuric acid and excreted in the urine. In both humans and animals, 60 to 75% of the absorbed toluene can be accounted for as hippuric acid in the urine, regardless of the dose or whether the chemical was administered orally or by inhalation. Much of the remaining toluene is exhaled unchanged. The excretion of toluene and its metabolites is rapid; the major portion occurs within 12 hours of oral administration or the end of inhalation exposure.

1.5 CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY

Inhalation exposure to toluene at concentrations of up to 300 ppm for 24 months did not produce an increased incidence of neoplastic, proliferative, inflammatory, or degenerative lesions in various organs of rats relative to unexposed controls. Other studies indicate that toluene is not carcinogenic when applied topically to the shaved skin of laboratory animals and that it does not promote the development of skin tumors following initiation with DMBA.

Toluene has been shown to be non-mutagenic in a battery of microbial, mammalian cell, and whole organism test systems. The Russian literature reported chromosome aberrations in the bone marrow cells of rats exposed subcutaneously and via inhalation to toluene, but these findings have not been corroborated in other studies of rats following intraperitoneal injection of toluene, in human lymphocytes exposed to toluene in culture, or in lymphocytes from workers chronically exposed to toluene.

Toluene has been reported to induce cleft palates in mice following oral exposure, but it was not teratogenic in mice or rats following inhalation exposure. Embryotoxic effects (increased incidence of skeletal anomalies and signs of retarded skeletal development, low fetal weights) and increased maternal toxicity were, however, noted in some of the rats and mice exposed via inhalation.

1.6 EFFECTS ON ECOSYSTEMS

The effects of toluene on ecosystems have been studied using aquatic organisms, microbiologic organisms, and higher plants. In algae, toluene can both stimulate and inhibit growth, depending on the species of algae and the concentration of toluene. The no-effect level for most algal species is 10 mg/l. Significant toxic effects of toluene in fish, except during accidental spills, are unlikely because of the rapid volatilization of toluene from water. Toluene

has only a low bioconcentration potential and is metabolized and rapidly lost from fish, which indicates that toluene is unlikely to biomagnify through the aquatic food chain. Toluene can impart an unpleasant taste to fish that inhabit contaminated water. In both microorganisms and higher plants, toluene can disrupt cell membranes as a result of its solvent action and cause toxic or lethal effects. Except in cases of intentional application or accidental spills, toluene is unlikely to be present at levels that would cause adverse effects on the ecosystem. Even after accidental spills, toluene would volatilize rapidly and thus limit adverse effects.

1.7 RISK ASSESSMENT

Considerable information is available on the effects of toluene on humans and experimental animals after inhalation exposures. Based on these data, approximate dose-response relationships and estimates of acceptable daily intake (ADI) can be proposed. The data on oral exposure are much less satisfactory, although one acceptable subchronic oral study using rats is available. No information on dermal exposures suitable for use in human risk assessment was encountered.

Based on a few studies involving controlled exposures of humans to toluene vapors as well as some reports of occupational incidents and voluntary abuse ("glue sniffing"), the dose-response relationships for the acute effects in humans of single short-term exposures to toluene can be estimated as:

10,000-30,000 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.
300-800 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.
100-300 ppm	:	Detectable signs of incoordination may be expected during exposure periods up to 8 hours.
200 ppm	:	Mild throat and eye irritation.
50-100 ppm	:	Subjective complaints (fatigue or headache) but probably no observable impairment of reaction time or coordination.
>37 ppm	:	Probably perceptible to most humans.

Because of the deficiencies in the studies on which these estimates are based as well as variations in sensitivity to toluene that may be expected in the human population, these estimates should be regarded as approximations only. Nonetheless, the weight of the evidence suggests that the precision of the estimates is likely to be about $\pm$50%.

The subchronic and chronic inhalation data lend themselves less to the definition of dose-response relationships. Most of the reports on human exposures failed to define precisely levels or durations of exposure, involved relatively small numbers of exposed individuals, and did not adequately control exposure to other toxic agents. The animal data are of little use in supporting the human data because humans appear to be more sensitive to toluene than the experimental animals on which data are available.

An ADI for humans on inhalation exposure can be derived from the available human data using the current Threshold Limit Value (TLV) of 100 ppm. Using an uncertainty factor of 10, the ADI is estimated to be 2.69 mg/kg body weight. Given the limitations and inconsistencies in the human data, a reasonable upper limit would be 5.38 mg/kg and a lower limit would be 0.27 mg/kg.

For oral exposures, an ADI can be derived from a single subchronic study using rats. Because the information on the effects of oral exposures is scanty, an uncertainty factor of 1000 is applied to the results of this study and the ADI is estimated at 0.59 mg/kg body weight, which is probably more protective than predictive of a toxic threshold.

Information on the dermal toxicity of toluene cannot be used quantitatively for human risk assessment. Qualitatively, dermal exposure to toluene can cause skin damage, as is the case with many solvents, but systemic signs of intoxication are likely to occur only in cases of gross overexposure.

Based on the available exposure estimates, the only group at possible high risk from toluene are workers who are exposed at or near the TLV. For non-occupational exposures, there seems to be a safety margin of about 6 between the ADI for oral exposure and the current worst-case levels of exposure. Although this is reassuring, uncertainties over the carcinogenic, and teratogenic effects of toluene should be a matter of concern and future research.

2. INTRODUCTION

At the April 18, 1980 meeting of the Toxic Substances Priorities Committee, a decision was made to develop a multimedia integrated risk assessment document for toluene. One of the primary objectives of this undertaking was to minimize or eliminate inter-agency and inter-office duplication of risk assessment documentation projects. This document on toluene will serve as a pilot to test the feasibility and value of the multimedia integrated approach to environmental risk assessment. Toluene was chosen for this pilot study primarily because of its inclusion on a variety of program office priority lists, since it is a chemical produced in large quantity and exposure to the compound is widespread. Development of the toluene documentation project was directed by EPA's Environmental Criteria and Assessment Office, ORD, Research Triangle Park - Project Officer, Mr. Mark Greenberg.

In addition to the present document for toluene, two other recent reports contain valuable health and environmental effects data on toluene. The first is The Alkyl Benzenes, published in 1980 by the Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences, National Research Council. The second recent review, developed by the U.S. EPA in 1980, is the Ambient Water Quality Critiera for Toluene, EPA Report 440/5-80-075.

3. PHYSICAL AND CHEMICAL PROPERTIES

Toluene is a homolog of benzene in which one hydrogen atom has been replaced by a methyl group. Some of the relevant physical and chemical properties of toluene are described below.

3.1 SYNONYMS AND TRADE NAMES

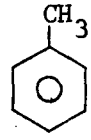
Toluol
Phenylmethane
Methylbenzene
Methylbenzol
Methacide

3.2 IDENTIFICATION NUMBERS

Chemical Abstracts Service (CAS) No.: 108-88-3

Registry of Toxic Effects of Chemical Substances (RTECS) No.: XS5250000

3.3 STRUCTURE, MOLECULAR FORMULA, AND MOLECULAR WEIGHT



Molecular Formula: C_7H_8

Molecular Weight: 92.13

3.4 PHYSICAL PROPERTIES

3.4.1 Description

Toluene is a clear, colorless liquid at ambient temperature that has a benzene-like odor. It is both volatile and flammable (The Merck Index, 1976).

3.4.2 Other Physical Properties

Melting Point (Weast, 1977):	-95°C
Boiling Point (Weast, 1977):	110.6°C
Density (g/ml, 20°C) (Weast, 1977):	0.8669
Specific Gravity (15.6/15.6°C) (Cier, 1969):	0.8623

Vapor Pressure (25°C) (Weast, 1977):	28.7 torr
Vapor Density (air = 1) (Weast, 1977):	3.20
Percent in Saturated Air (760 mm, 26°C) (Walker, 1976):	3.94
Density of Saturated Air-Vapor Mixture (760 mm (air = 1), 26°C) (Walker, 1976):	1.09
Solubility (Sutton and Calder, 1975):	
Fresh water (25°C)	534.8 mg/l
Sea water (25°C)	379.3 mg/l
Flammable Limits (percent by volume in air) (Walker, 1976):	1.17-7.10
Flash Point (closed cup) (Walker, 1976):	40°F
Autoignition Temperature (Walker, 1976):	552°C
Log Octanol-Water Partition Coefficient (Tute, 1971):	2.69
Odor Threshold in Air (Walker, 1976):	
Coke derived	4.68 ppm
Petroleum derived	2.14 ppm
Surface Tension (20°C) (Walker, 1976):	28.53 dynes/cm
Liquid Viscosity (20°C) (Walker, 1976):	0.6 cp
Refractive Index (68°F) (Cier, 1969):	1.49693
Conversion Factor (in air, 25°C):	1 ppm ≡ 3.77 mg/m^3 1 mg/m^3 ≡ 0.265 ppm

3.4.3 Significance of Physical Properties with Respect to Environmental Behavior

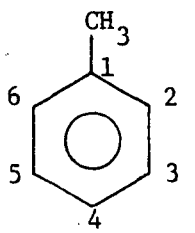
The volatility of toluene as indicated by its relatively high vapor pressure is indicative that a substantial fraction of environmental toluene is likely to be present in the vapor phase mixed with air. The relatively high volatility of toluene combined with its low solubility in water may lead to intermedia transfer of toluene from water to the air phase. The details of the environmental fate of toluene as determined by its physical and chemical properties are discussed in Section 6.

The log octanol-water partition coefficient for toluene may have significance in determining its affinity toward organics in soil and aquatic organisms. The details of the bioconcentration factor for toluene based on the octanol-water

partition coefficient value also are discussed in Section 9. The knowledge of physical properties such as flammable limits and flash point are important for the safe handling and transport of toluene; data on density and solubility may be necessary for health effect studies.

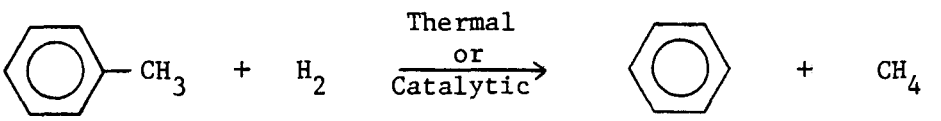
3.5 CHEMICAL PROPERTIES

Toluene undergoes substitution reactions, either on the aliphatic side group ($-CH_3$) or on the benzene ring. These substitutions occur exclusively at the ortho (1,2) and para (1,4) positions marked in the following figure:



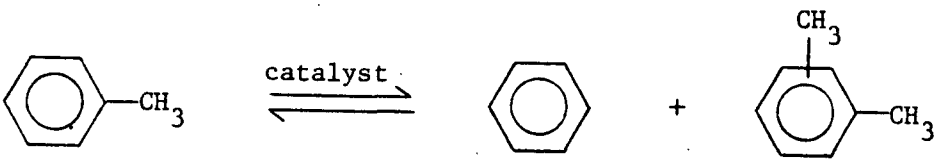
Nitration, sulfonation, halogenation, methylation, and chloromethylation are some examples of substitution reactions. These reactions occur at a rate between 467 and 2.1 times faster with toluene than with benzene (Cier, 1969).

The methyl group in toluene is susceptible to dealkylation to produce benzene (Bradsher, 1977).

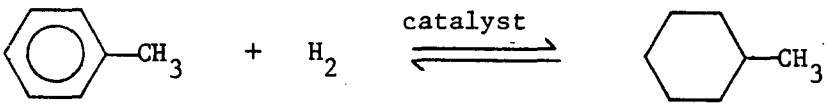


At one time, the most significant use of toluene was in the production of benzene by the above reaction (Cier, 1969).

Toluene undergoes a reversible disproportionation and transalkylation reaction in the presence of a catalyst (Cier, 1969).

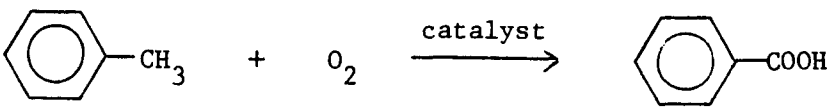


Hydrogenation of toluene takes place readily to produce methylcyclohexane (Cier, 1969).

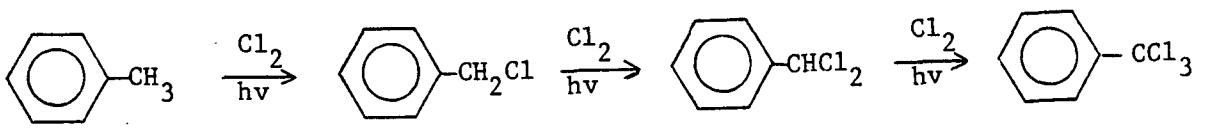


The reverse process of dehydrogenation of methylcyclohexane is the principal mode of toluene manufacture. Methylcyclohexane is found in petroleum fractions, along with other naphthenes (Cier, 1969).

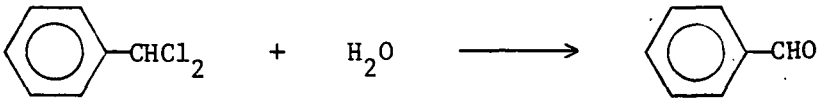
Oxidation of toluene under catalytic conditions yields benzoic acid as a principal product (Cier, 1969).



Chlorination of toluene under actinic light conditions yields methyl substitution products (Cier, 1969).

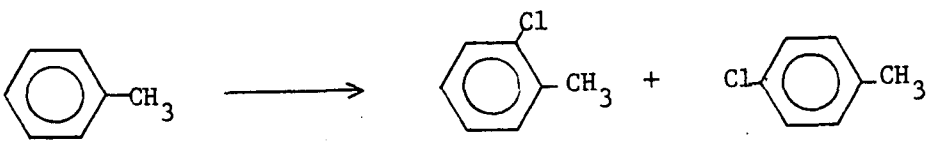


The hydrolysis of benzalchloride produces benzaldehyde (Gait, 1967).



The above reaction may have some significance with respect to chlorination of drinking water. The presence of benzaldehyde and benzoic acid detected in drinking water (U.S. EPA, 1980) may be due to the oxidation of toluene found in drinking water.

In the presence of catalysts and in the absence of light, chlorination produces o- and p-chlorotoluene (Cier, 1969).



In the vapor phase, toluene is relatively unreactive toward RO_2 radicals and O_3 found in the troposphere. It is, however, relatively more reactive toward OH radicals. The products of the reaction are normally benzaldehyde and cresols (Brown et al., 1975). This reaction may have significance with respect to the fate of toluene in the atmosphere and is discussed in detail in Section 6.1.

Toluene forms azeotropes with a number of solvents, including paraffinics, naphthenics, and alcoholic hydrocarbons. Azeotropes are important in the purification of toluene, in solvent technology, and in the recovery of toluene from reaction mixtures (Cier, 1969).

Toluene is marketed principally as nitration grade (1°, boiling range of 1°C), pure commercial grade (2°C), and all other grades. Generally accepted quality standards for the first two grades are given by the American Society for

Testing and Materials (Cier, 1969). The actual concentration of toluene is not stipulated in these specifications. However, the nitration grade (1°) and pure commercial grade (2°) toluene are of 99.5% to 100% and 98.5% to 99.4% purity, respectively (USITC, 1980). All other grades include toluene used as solvent grade and for blending aviation and motor gasoline. The non-fuel toluene (solvent grade) is of 90% to 98.4% purity (USITC, 1980).

Commercial toluene may contain benzene as an impurity. Therefore, all health effect studies involving toluene should specify the quality of toluene used for experimentation. If benzene is present in the toluene, it must be demonstrated that the observed health effects are not wholly or partly due to benzene. Because of this contamination, it may also be necessary to determine the amount of benzene released to the environment due to industrial usage of toluene.

In general, toluene is quite stable in air, and most of the chemical reactions discussed above require specialized conditions. While some of the reactions may have environmental significance, the majority of the chemical reactions discussed above are conducted under conditions of commercial and research applications.

4. PRODUCTION, USE, AND RELEASES TO THE ENVIRONMENT

4.1 MANUFACTURING PROCESS TECHNOLOGY

Toluene is produced primarily from three sources: (1) petroleum refining processes, (2) by-product of styrene production, (3) by-product of coke-oven operation.

4.1.1 Petroleum Refining Processes

Low levels of toluene are present in crude petroleum. Toluene is, however, produced principally from petroleum by two processes: (1) catalytic reforming and (2) pyrolytic cracking.

4.1.1.1 Catalytic Reforming

The largest quantity of toluene produced in the United States is generated in the catalytic reforming process. The total estimated toluene produced in this process in 1978 was 3110 million kg. This represented about 87% of the total toluene produced in the United States in 1978 (see Table 4-1).

Catalytic reforming involves the catalytic dehydrogenation of selected petroleum fractions which are rich in naphthenic hydrocarbons to yield a mixture of aromatics and paraffins. The proportions of aromatics and paraffins in the reformate depend on the feedstock used and the severity of the reforming operation (Cier, 1969). At present, reforming operations are geared primarily to produce a benzene-toluene-xylene (BTX) reformate from which the individual aromatics are recovered (Cier, 1969). Toluene is isolated from the reformate by distillation followed by washing with sulfuric acid and redistillation. Only a small fraction of catalytic reformate, however, is utilized for isolating toluene. The unseparated toluene in catalytic reformate is used for gasoline blending.

Table 4-1. U.S. Production of Isolated Toluene in 1978 (Slimak, 1980)

Production Process	Amount Produced (10^6 kg)	Percent of Total
Catalytic reforming	3110	86.5
Pyrolytic cracking	324	9
Styrene by-product	135	3.8
Coke oven by-product	26[a]	0.7
TOTAL	3595	100

[a]This value does not include toluene obtained from tar distillers.

4.1.1.2 Pyrolytic Cracking

The second largest quantity of toluene originates from pyrolytic cracking. Of the total isolated toluene produced in the United States in 1978, approximately 9% (324 million kg) was obtained from this source (see Table 4-1).

When heavier hydrocarbons, such as hydrocarbon condensates, naphtha, and gas oil, are pyrolytically cracked for the manufacture of olefins, pyrolysis gasoline is produced as a by-product. The amount of pyrolysis gasoline produced depends on the feedstock and the manufacturing conditions (Mara et al., 1979). The by-product pyrolysis gasoline contains a high percent of aromatics. Toluene can be isolated from pyrolysis gasoline by distillation, removal of any olefins and diolefins, and redistillation. Not all pyrolysis gasoline produced in the United States is utilized for the production of isolated toluene.

4.1.2 By-Product of Styrene Production

When styrene is produced by the dehydrogenation of ethylbenzene, some toluene is also synthesized as a by-product. The toluene isolated from the by-product is not suitable for chemical and solvent use. Therefore, toluene obtained from this source is used either for gasoline blending or as feed for the manufacture of benzene by the hydrodealkylation process (Mara et al., 1979). In 1978, approximately 135 million kg of isolated toluene, which was about 4% of the total, was obtained from the by-product of styrene production (see Table 4-1).

4.1.3 By-Product of Coke-Oven Operation

The production of coke by the high-temperature carbonization of coal yields coal-tar and crude light oil as by-products. Both of these by-products contain some toluene. The production of toluene from distillation of coal-tar is minimal (Mara et al., 1979). Some toluene, however, is isolated from crude light oil. As shown in Table 4-1, approximately 26 million kg of toluene were isolated from

coal-derived toluene in the year 1978. This amounted to about 0.7% of the total isolated toluene produced during the same year.

4.2 PRODUCERS

Of the total toluene produced in the United States for internal consumption, only about 11% is isolated as toluene (see Table 4-2). The remainder stays in gasoline as a benzene-toluene-xylene (BTX) mixture. The total amount of toluene available in the United States in 1978, both isolated and non-isolated, is shown in Table 4-2.

The identification of isolated toluene producers, their estimated toluene producing capacity, and the estimated amount of toluene produced in 1978 from catalytic reforming, pyrolytic cracking, and styrene by-product are shown in Tables 4-3 through 4-5. The identification of the producers of isolated toluene from coke-oven by-product is given in Table 4-6. However, the capacity for isolated toluene production and the actual amount of toluene produced are not given because the data are unavailable.

During 1979, the production of toluene from coke-oven operators had a reported increase of 17.6% over 1978 (USITC, 1980). The production of toluene from petroleum refiners has been reported to have decreased by 4.3% during the same period (USITC, 1980). This caused a net decrease of 4.2% of the overall isolated toluene production in 1979 over 1978 (USITC, 1980).

4.3 USERS

As mentioned in Section 4.2, most of the toluene produced as BTX mixture is never isolated but remains in various refinery streams for use in gasoline. Isolated toluene, on the other hand, is used for different purposes. The consumption of isolated toluene in different usage is shown in Table 4-7. The fluctuating but largest single use of isolated toluene is in the production of benzene through the hydrodealkylation (HDA) process. The fluctuation in the use

Table 4-2. Isolated and Non-Isolated Toluene Available in the United States in 1978 (Slimak, 1980)

Source	Quantity (10^6 kg)	
	Isolated	Non-Isolated as BTX
Catalytic reforming	3,110	27,000
Pyrolytic cracking	324	197
Styrene by-product	135	NA[a]
Coke oven by-product	26	96
Imports	192	
Exports	-364	NR[b]
SUBTOTAL	3,423	27,293
TOTAL	30,716	

[a] NA = not applicable.

[b] NR = not reported.

Table 4-3. Producers of Isolated Toluene from Catalytic Reforming in 1978 (Slimak, 1980)

Company and Location	Toluene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
Amerada Hess - St. Croix, VI	460	310
American Petrofina - Big Spring, TX	164	110
Beaumont, TX	125	84
Ashland Oil - Catlettsburg, KY	99	67
N. Tonawanda, NY	39	26
Arco - Houston, TX	125	84
Wilmington, CA	49	33
Charter Oil - Houston, TX	39	26
Coastal States - Corpus Christi, TX	56	38
Commonwealth - Penuelas, PR	395	266
Crown - Pasadena, TX	46	31
Exxon - Baytown, TX	411	277
Getty - Delaware City, DE	--[a]	NA[b]
El Dorado, KS	20	13
Gulf - Alliance, LA	194	130
Philadelphia, PA	92	62
Port Arthur, TX	49	33
Kerr McGee - Corpus Christi, TX	148	100
Marathon - Texas City, TX	72	49
Mobil - Beaumont, TX	280	189
Monsanto - Chocolate Bayou, TX	33	22
Pennzoil - Shreveport, LA	--[c]	NA
Phillips - Sweeney, TX	33	22
Guayama, PR	335	226
Quintana-Howell - Corpus Christi, TX	56	38
Shell - Deer Park, TX	197	133

Table 4-3. Producers of Isolated Toluene from Catalytic Reforming in 1978 (Slimak, 1980) (Cont'd)

Company and Location	Toluene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
Sunoco - Corpus Christi, TX	138	93
Marcus Hook, PA	151	102
Toledo, OH	247	166
Tulsan, OK	66	44
Tenneco - Chalmette, LA	115	78
Texaco - Port Arthur, TX	92	62
Westville, NJ	132	89
Union Oil - Lemont, IL	56	38
Union Pacific - Corpus Christi, TX	99	67
TOTAL	4613	3108

[a] 1980 capacity for this producer was 85 million kg.

[b] NA = not applicable.

[c] 1980 capacity for this producer was 72 million kg.

Table 4-4. Producers of Isolated Toluene from Pyrolysis Gasoline (Slimak, 1980)

Company and Location	Toluene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
Arco - Chanelview, TX	105	76
Commonwealth - Penuelas, PR	49	36
Dow - Freeport, TX	13	9.4
Gulf - Cedar Bayou, TX	66	48
Mobil - Beaumont, TX	16	15
Monsanto - Chocolate Bayou, TX	132	96
Union Carbide - Taft, LA	66	48
TOTAL	447	328.4

Table 4-5. Producers of Isolated Toluene from Styrene By-Product (Slimak, 1980)

Company and Location	Styrene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
American Hoechst - Baton Rouge, LA	400	16
Arco - Beaver Valley, PA	100	4
Cos-Mar - Carville, LA	590	24
Dow - Freeport, TX	660	26
Midland, MI	140	5.5
El Paso Natural Gas - Odessa, TX	68	2.7
Gulf - Donaldsville, LA	270	11
Monsanto - Texas City, TX	680	27
Standard Oil (Indiana) - Texas City, TX	380	15
Sunoco - Corpus Christi, TX	36	1.4
U.S. Steel - Houston, TX	54	2.2
TOTAL	3400	134.8

Table 4-6. Producers of Isolated Toluene from Coke-Oven Crude Light Oils (Slimak, 1980)

Plant	Location
Armco	Middletown, OH
Ashland Oil	Catlettsburg, KY N. Tonawanda, NY
Bethlehem Steel	Bethlehem, PA Sparrows Pt., MD
CF and I	Pueblo, CO
Interlake	Toledo, OH
Jones and Laughlin	Aliquippa, PA
Lone Star	Lone Star, PA
Republic Steel	Youngstown, OH Cleveland, OH
U.S. Steel	Clairton, PA Geneva, UT

Table 4-7. Consumption of Isolated and Non-Isolated Toluene in Different Usages (Revised from Slimak, 1980)

Usage	Amount Used/year (10^6 kg)	Percent of Total Use in Each Category
Non-isolated gasoline as BTX	27,293	100
Isolated benzene dealkylation	1,675	40.2
Gasoline back-blending	1,465	35.1
Solvent for paint and coatings	263	6.3
Solvent for adhesives, inks, and pharmaceuticals	132	3.2
Toluene diisocyanate	200	4.8
Xylene	98	2.4
Benzoic acid	65	1.6
Benzyl chloride	36	0.9
Vinyl toluene	25	0.6
Miscellaneous others	39	0.9
Net export	172	4.1
TOTAL	4,170	100.1

of isolated toluene exists because the HDA process is used as an effective means of balancing supply and demand for benzene (Mara et al., 1979). The U.S. producers of benzene through the HDA process, their capacity, and the amount produced are shown in Table 4-8.

The second largest use of isolated toluene is back-blending into gasoline for increasing the octane ratings. Approximately 1465 million kg of isolated toluene representing 35.1% of 1978 consumption were used for gasoline back-blending.

The third major use of toluene is in solvent applications, with the major use being in the paint and coatings industry. Significant amounts also are used in adhesives, inks, pharmaceuticals, and other formulated products. With the establishment of federal and state laws limiting the emission of aromatic solvents in the workplace and in the general environment, the demand for toluene as a solvent declined significantly since 1975 (Mara et al., 1979). Identification of specific users of toluene as a solvent is difficult because the users are too widespread.

Another major use of isolated toluene is as a raw material in the production of toluene diisocyanate (TDI), benzyl chloride, benzoic acid, xylene, and vinyl toluene. Manufacture of phenol, cresols, toluene sulfonic acids, nitrotoluenes, terephthalic acid, caprolactam, and styrene are some of the other minor uses of isolated toluene (Mara et al., 1979). A small amount of isolated toluene (6.6 million kg, $<1\%$ of total) is used for the manufacture of p-cresol (Slimak, 1980). The latter compound is used primarily for the manufacture of the pesticide 2,6-di-tert-butyl-p-cresol (BHT). Judging from the percent of toluene used in the manufacture of BHT, its emission from this source should be considered insignificant.

Table 4-8. Consumers of Toluene for the Manufacture of Benzene by HDA Process (Anderson et al., 1980)

Company and Location	Toluene Used (10^6 kg)	Benzene Production Capacity (10^6 kg)
American Petrofina - Port Arthur, TX	59	77
Big Spring, TX	103	130
Ashland Oil - Catlettsburg, KY	91	120
Coastal States - Corpus Christi, TX	156	200
Commonwealth - Penuelas, PR	298	380
Crown - Pasadena, TX	59	77
Dow - Freeport, TX	65	84
Gulf - Alliance, LA	122	160
Philadelphia, PA	52	67
Monsanto - Alvin, TX	103	130
Phillips - Guayam, PR	103	130
Quintana-Howell - Corpus Christi, TX	191	250
Shell - Odessa, TX	18	23
Sunoco - Corpus Christi, TX	52	67
Toledo, OH	163	210
Tulsa, OK	39	50
TOTAL	1674	2155

The identification of primary users of toluene as a chemical intermediate, their production capacity, and the amount produced is shown in Tables 4-9 and 4-10. It should be pointed out that the amount of isolated toluene used in the United States in 1978 (excluding net export) adds up to 4000 million kg according to Table 4-7. However, Table 4-2 shows that the total amount of toluene available for internal consumption during the same period (excluding net export) was only 3600 million kg. This discrepancy is due to the fact that Table 4-7 is based on data that are only estimates and the data in Table 4-2 are obtained from the manufacturers who reported their net toluene production to the U.S. International Trade Commission.

4.4 ENVIRONMENTAL RELEASE

The three primary sources of toluene release or emission to the environment are from: production, usage, and inadvertent sources.

4.4.1 Emission from Production Sources

Toluene can be released into the environment during its production as process losses, fugitive emissions, and storage losses. Process emissions are those that originate from the reaction and distillation vents deliberately used for venting gases. Storage emissions originate from losses during loading and handling of the product used for manufacturing processes and storage of the final product. Fugitive emissions are those that have their origin in plant equipment leaks. The air emission factors used to estimate the total emission of toluene from different production sources have been obtained from Mara et al. (1979) and the values are given in Table 4-11.

Based on the emission factors indicated in Table 4-11, the amount of toluene emitted into the atmosphere from the four production sources has been estimated in Table 4-12. Atmospheric releases of toluene from each source shown in Table 4-12 are from production of both isolated and non-isolated toluene. It is

Table 4-9. Producers of Toluene Diisocyanate (TDI) in 1978 (Mara et al., 1979)

Company and Location	TDI Capacity (10^6 kg)	Toluene Used (10^6 kg)
Allied Chemical - Moundsville, WV	36	20
BASF Wyandotte - Geismar, LA	45	25
Dow Chemical - Freeport, TX	45	25
Du Pont - Deepwater, NJ	32	17
Mobay Chemical - Baytown, TX	59	32
New Martinsville, WV	45	25
Olin - Astabula, OH	14	7
Lake Charles, LA	45	25
Rubicon Chemical - Geismar, LA	18	10
Union Carbide - S. Charleston, WV	25	13
TOTAL	364	199

Table 4-10. Other Toluene Chemical Intermediate Users in 1978 (Mara et al., 1979)

Company and Location	Production Capacity (10^6 kg)	Toluene Used (10^6 kg)
	Xylene Producers	
Arco - Houston, TX	89	48
Sunoco - Marcus Hook, PA	92	50
TOTAL	181	98
	Benzoic Acid Producers	
Kalama - Kalama, WA	64	33
Monsanto - St. Louis, MO	5	2
Velsical - Beaumont, TX	23	12
Chattanooga, TN	27	14
Pfizer - Terre Haute, IN	3	1
Tenneco - Garfield, NJ	7	3
TOTAL	129	65
	Benzyl Chloride Producers	
Monsanto - Bridgeport, NJ	36	16
Sauget, IL	36	16
Stauffer - Edison, NJ	5	3
UOP - E. Rutherford, NJ	1	0.5
TOTAL	78	35.5
	Vinyl Toluene Producers	
Dow - Midland, MI	27	25

Table 4-11. Toluene Air Emission Factors from Production Sources (Mara et al., 1979)

Source	Emission Factor (kg lost/kg produced)			
	Process	Storage	Fugitive	Total
Catalytic reforming	0.00002	0.00006	0.00002	0.0001
Pyrolytic cracking	0.00015	0.00060	0.00015	0.0009
Styrene by-product	0.00001	0.00060	0.00015	0.00076
Coke oven by-product	0.00050	0.00060	0.00015	0.00125

Table 4-12. Estimated Atmospheric Toluene Emissions from Four Major Production Sources

Production Source		Total Amount Produced (million kg/yr)	Total Emission Factor	Total Emission (10^3 kg/yr)
Catalytic reforming	- Isolated	3,110	0.0001	3,011
	- Non-isolated	27,000		
Pyrolytic cracking	- Isolated	324	0.0009	469
	- Non-isolated	197		
Styrene by-product		135	0.00076	103
Coke oven by-product	- Isolated	26	0.00125	153
	- Non-isolated	96		
TOTAL				3,736

assumed that the air emission is dependent only on the manufacturing process and is the same for both isolated and non-isolated toluene from the same process.

The manufacturing processes may lead also to toluene release to other media. The release of toluene to water from petroleum refineries performing catalytic reforming and pyrolytic cracking processes is assumed to be negligible because the concentration of toluene has been determined to be below the quantification limit in more than 90% of discharged water from the refineries (Slimak, 1980).

Coking operations, however, can lead to toluene release in other media. The wastewaters from coking plants have the following distribution (Slimak, 1980):

Direct discharge: 33%

Publicly Owned Treatment Works (POTW): 25%

Quenching: 40%

Deep well injection: 2%

Two-thirds of the wastewater from the quenching operation is recirculated and actually not discharged. Therefore, only 73% of the total wastewater containing toluene is actually discharged to the environment.

The average volume of effluents produced from coke-oven operation (Slimak, 1980), the toluene concentration in these effluents (Slimak, 1980), and the emission factors in these effluents are given in Table 4-13.

For a total coke production of 44×10^9 kg in 1978 (Slimak, 1980), the total amount of toluene discharged in wastewater amounted to $44 \times 10^9 \times 4.43 \times 10^{-6} \times 0.73 = 142 \times 10^3$ kg. Some toluene in wastewater may finally enter other media because of the following reason. Wastewater from the quenching operation is sent to sumps that generate only solid and gaseous wastes (Slimak, 1980). Therefore, the distribution of total released toluene in wastewater can be estimated as given in Table 4-14.

Table 4-13. Toluene Emission Factors in Wastewater from Coke Oven Operation (Slimak, 1980)

Effluent	Liters of Effluent Produced/kg Coke	Toluene Conc. (mg/l)	Emission Factor
Waste ammonia liquor	0.16	3.1	0.496×10^{-6}
Final cooler blow down	0.13	17.0	2.21×10^{-6}
Benzol plant wastes	0.20	8.6	1.72×10^{-6}
TOTAL			4.43×10^{-6}

Table 4-14. Toluene Released in Different Media from Coke-Oven Wastewater

Medium	Percent of Total Released	Amount released/yr (10^3 kg)
Air	20	28
Water	33	47
Land	22	31
POTW	25	36

4.4.2 Emission from Toluene Usage

The emission of toluene from various usages has been estimated from emission factors and the amounts used. The values for the emission factors obtained from Mara et al. (1979) are shown in Table 4-15.

The atmospheric emission of toluene from its usage in gasoline as non-isolated BTX and the isolated form (for back-blending) has already been included in Table 4-12. The emission factor for miscellaneous uses has been assumed to be the average of other toluene usages excluding its use as solvent. All the toluene used in paint and coatings has been assumed to be ultimately released to the atmosphere (Mara et al., 1979). Therefore, an emission factor of 1.0 has been estimated for this usage. Fifteen percent of the toluene used as a solvent for adhesives, inks, and pharmaceuticals is recovered for fuel use (Mara et al., 1979). The remainder is emitted to the atmosphere. Hence, an emission factor of 0.85 has been assumed for this usage.

Based on the emission factors given in Table 4-15, the estimated toluene emissions from its various usages are shown in Table 4-16.

It can be concluded from Table 4-16 that, among the different usages of toluene, the maximum emission occurs from solvent application.

The released toluene from the different user sources shown in Table 4-16 has been assumed to enter only one medium, air. The use of toluene as a solvent, however, has been found to produce toluene in wastewater (Slimak, 1980). Table 4-17 shows the total estimated release of toluene to aqueous media from its use as a solvent in different industries.

4.4.3 Emission from Inadvertent Sources

Because gasoline consumes a vast amount of total toluene produced (see Table 4-7), this use constitutes the largest source of environmental emission of toluene. The emission of toluene from its use in gasoline can occur from three

Table 4-15. Toluene Emission Factors for Its Usages (Mara et al., 1979)

Usage	Emission Factor (kg lost/kg used)			
	Process	Storage	Fugitive	Total
Benzene production	0.00005	0.00010	0.00005	0.00020
Solvent for paint and coatings	NA[a]	NA	NA	1.0
Solvent for adhesives, ink, pharmaceuticals, and others	NA	NA	NA	0.85
Toluene diisocyanate	0.00077	0.00032	0.00019	0.00128
Xylene production	0.00005	0.00010	0.00005	0.00020
Benzoic acid	0.00100	0.00040	0.00010	0.00150
Benzyl chloride	0.00055	0.00030	0.00015	0.00100
Vinyl toluene	0.00055	0.00030	0.00015	0.00100
Miscellaneous	NA	NA	NA	0.00100

[a]NA = not applicable.

Table 4-16. Estimated Toluene Emission from Different Uses

Source	Amount Used/yr (10^6 kg)	Emission Factor	Total Emission/yr (10^3 kg)
Benzene production	1675	0.0002	335
Solvent for paint and coatings	263	1.0	263,000
Solvent for adhesives, inks, pharmaceuticals, and others	132	0.85	112,000
Toluene diisocyanate	200	0.00128	256
Xylene production	98	0.0002	20
Benzoic acid	65	0.00150	98
Benzyl chloride	36	0.0010	36
Vinyl toluene	25	0.0010	25
Miscellaneous others	39	0.0010	39
TOTAL	2533		375,809

Table 4-17. Toluene Released in Aqueous Media from Use as a Solvent in Various Industries (Slimak, 1980)

Source	Toluene Conc. in Wastewater (μg/ℓ)	Percent Occurrence	Wastewater Discharged (10^6 ℓ/d)	Amount of Toluene Released (10^3 kg/yr)[a]
Ink formulating	1600	87	0.092	0.038
Textile products	14	46	2000	3.8
Gum and wood chemicals	2000	78	0.11	0.17
Paint formulating	990	87	2.8	0.72
Leather tanning	78	25	200	1.2
Pharmaceuticals	515	62	250	24
TOTAL				29.9

[a]Based on 300 operating d/yr.

distinct sources: evaporation from its use in the automobile, evaporation from marketing activities (handling and transfer of bulk quantities), and emission from automobile exhaust.

Other inadvertent sources of toluene emissions into the environment include transportation, spills into surface water and land, other manufacturing processes that do not attempt to produce toluene, different combustion sources, and cigarette smoke. The inadvertent release of toluene from other manufacturing processes occurs primarily from feedstock contamination, by-product formation, and the use of oil. An example of the latter source is in the manufacture of acrylonitrile in which wastewater ponds are covered with oil to control the release of volatile organics.

The release of toluene into different media from various inadvertent sources is shown in Table 4-18. Because of the volatility of toluene, intermedia transfers of the compound will possibly change the emission values given in Table 4-18.

4.4.4 Sum of Emissions from All Sources

The emissions of toluene into different media from all sources are given in Table 4-19. The estimates also include toluene emission from coke production which remains unrecovered. The emission of toluene from coke oven operation is based on an emission factor of 0.00024 (Mara et al., 1979) and an estimated coke production of 44×10^9 kg (Slimak, 1980) for the year 1978.

It is evident from Table 4-19 that the toluene released into the environment predominantly enters one medium, the atmosphere. The three largest sources of toluene emission in descending order are auto exhaust, solvent use, and evaporative loss from automobile and service stations. A large amount of toluene from land and water spills is also likely to enter air as a result of evaporation. The large figure for the combined release of toluene into the atmosphere explains the

Table 4-18. Toluene Emission from Different Inadvertent Sources (Slimak, 1980)

Source	Environmental Release (10^3 kg/yr) Air	Water	Land
Gasoline marketing	19,000	---	---
Automobile gasoline evaporation[a]	18,000	---	---
Automobile exhaust[a]	640,000	---	---
Transportation spills:			
Oil	---	400	5.6
Gasoline	---	680	230
Toluene	---	2.2	11
Propylene oxide manufacture	36	---	---
Polychloroprene manufacture	460	---	---
Ethylene-propylene rubber manufacture	90	---	---
Ethylene-propylene terpolymer production	4,200	---	---
Wood preserving industry	---	6.3	---
Insulation board manufacture	---	neg.	---
Hardboard manufacture	---	neg.	---
Acrylonitrile manufacture	59	---	---
Combustion processes:			
Coal refuse piles	4,400	---	---
Stationary fuel combustion	13,000	---	---
Forest fires	7,000	---	---
Agricultural burning	1,000	---	---
Structural fires	< 1,000	---	---
Cigarette smoke	53	---	---
Others	8	---	---
TOTAL	708,306	1,089	247

[a]According to the estimates of McGinnity (1981), the yearly vehicular toluen emission amounts to 820,000 x 10^3 kg.

Table 4-19. Total Yearly Release of Toluene into Different Media

Source	Environmental Release (10^3 kg/yr)			
	Air	Water	Land	POTW
Production	3,764	47	31	36
Usage	375,809	30	NA[a]	NA
Inadvertent	708,306	1,089	247	NA
Coke production	10,560	NA	NA	NA
TOTAL	1,098,439	1,166	278	36

[a]NA = not available.

reason for its presence as the aromatic hydrocarbon of highest concentration in the ambient atmosphere (see Chapter 7).

4.5 USE OF TOLUENE IN CONSUMER PRODUCTS

The consumer products shown in Table 4-20 may contain some toluene. The percent of toluene in these products also is indicated in the same table. The emission of toluene into the environment from this source is already included under Section 4.4.2.

Information available through the Food and Drug Administration (FDA) (Bolger, 1981) shows the following: of the 19,500 cosmetic products registered with the FDA through August 14, 1979, 664 products contain varying percents of toluene. One of the products contains more than 50% toluene, 166 products contain 25-50% toluene, 492 products contain 10-25% toluene, 1 product contains 1-5% toluene, and 4 products contain 0.1% or less toluene. The use of toluene is related to nail base coats, nail enamel, nail polish removers, and other manicure products.

Table 4-20. Consumer Product Formulations Containing Toluene (Gleason et al., 1969)

Product	Percent Toluene Content
China cement, solvent type	20 to 30
Contact rubber cement	may contain toluene
Microfilm cement, cotton base	27 to 30
Model cement	up to 20 to 25
Plastic cement, polystyrene	24
Shoe cement	may contain toluene
Tire repair, bonding compounds	> 80
Paint brush cleaners	contain 25 to 90 BTX
Stain, spot, lipstick, rust removers	may contain toluene
Nail polish	35
De-icers, fuel antifreeze	30
Fabric dyes	$\leq$ 60
Indelible inks	may contain toluene
Marking inks	80 to 90
Stencil inks	40 to 60
Solvents and thinners	may contain toluene

5. ABATEMENT PRACTICES IN INDUSTRY

The four major potential sources of toluene release to the environment, in order of importance (see Section 5.4.4), are (1) inadvertent sources, such as vehicular emissions and losses during gasoline transfer, (2) solvent use in paint, coating adhesives, and inks, (3) coke production, and (4) manufacturing sites such as petroleum refineries and chemical plants. Therefore, institution of pollution control devices for these four major industries can be expected to produce a large impact on the overall toluene level in the environment.

5.1 ABATEMENT PRACTICES FOR INADVERTENT SOURCES

The two major sources of vehicular emissions of toluene in the atmosphere are exhaust emissions and evaporative emissions from the gas tank and the carburetor. Crankcase emissions have been essentially eliminated through the use of positive crankcase ventilation technologies (U.S. EPA, 1980b).

The installation of catalytic converters on automobiles has resulted in significant reduction of hydrocarbon emissions from automobiles. Generally, tailpipe catalysts control systems remove unsaturated and aromatic hydrocarbons, including toluene, more efficiently than paraffinic hydrocarbons (U.S. EPA, 1980b). Therefore, both the photochemical reactivity and the mass of hydrocarbons emitted are reduced by the catalytic converter systems.

Evaporative emissions from automobiles have been reduced through the use of adsorption regeneration carbon canister technologies (U.S. EPA, 1980b). Such systems are, however, more effective for regular grade gasoline containing 25% to 27% aromatics than for premium grade unleaded gasoline containing 43% aromatics (U.S. EPA, 1980b).

Most of the current diesel exhaust emission studies are concerned with emission controls through either engine design or the use of fuel additives. Other control options, such as catalytic reactors, appear to be viable.

Other major sources of automobile emissions are losses from spilled gasoline and losses during fuel transfer. The former can be reduced by educating the public about the necessity of restricting spillage both for economic and environmental reasons. The loss of gasoline during fuel transfer is already controlled in most areas of the country by incorporating vapor recovery systems.

5.2 ABATEMENT PRACTICES FOR SOLVENT USAGE

Solvent vapors originating from industrial usage of toluene in coatings and thinners can be controlled or recovered by the application of condensation, compression, adsorption, or combustion principles. Control efficiencies of 90% or greater are possible by activated carbon adsorption provided particulates are removed from the contaminated airstream by filtration before the airstream enters the carbon bed (U.S. EPA, 1980b).

When recovery of the vapor is not desired, an incineration method can be used for controlling emissions. The choice between direct flame and catalytic incineration methods must be based on economic factors and on local emission standards.

Control of toluene emissions from gravure printing can be done in a number of ways (U.S. EPA, 1980b). Process modifications involving microwave, infrared, electron beam, or ultraviolet drying and subsequent recovery of organic vapors will reduce emissions from organic vapors. Another alternative is to replace inks containing organic solvents with aqueous or solventless inks. Incineration of the exhaust gases by thermal or catalytic methods provides another method of emission control. Last, solvent vapors can be adsorbed in activated carbon as a method of controlling toluene vapor emissions into the atmosphere.

5.3 ABATEMENT FOR COKE OVEN EMISSIONS

Hydrocarbon emissions result from the burning of the stripped coke oven gas for the under-firing of the coke batteries. The combustion exhaust gases from each oven are combined together and vented through a common stack. Improvement of combustion efficiency of the coke batteries would be a proper method of control (U.S. EPA, 1980b).

5.4 ABATEMENT FOR EMISSIONS FROM MANUFACTURING SITES

Current technology for the control of gaseous hydrocarbon emissions from manufacturing sites takes the form of charcoal adsorption, direct flame or catalytic incineration, chemical sorbents, vapor condensation, process and material change, and improved maintenance (U.S. EPA, 1980b). The feasibility of sorbing organics by the wet scrubbing method using selected aqueous surfactant systems as opposed to plain water has been demonstrated (Matunas et al., 1978). Organic removal as high as 90% to 95% can be attained by utilizing this method. Condensation of organics by the removal of heat may be an expensive method since refrigeration must be used for the removal of heat from gases (Matunas et al., 1978).

5.5 ABATEMENT PRACTICES FOR RAW AND FINISHED WATERS

No information could be found on this subject. Treatment of water with activated carbon, however, is expected to remove toluene from drinking waters.

5.6 ECONOMIC BENEFITS OF CONTROLLING TOLUENE EMISSIONS

There is no significant geographical area in the United States in which ambient concentrations of alkylbenzenes are known to be harmful to plants or animal lives (NRC, 1980); however, as reactive hydrocarbons, they can contribute to the formation of photo-chemical smog that is known to be harmful to life and property. Brookshire et al. (1979) selected residential properties in six pairs of selected neighborhoods and found the property value could increase on the

average of $504 annually if the air quality were improved. The authors ascribed about one-half of the enhanced value to respondent-perceived aesthetic benefits (visibility) and the other half to perceived health benefits. Thayer and Schulz (1980) extrapolated the results of Brookshire et al. (1979) to the entire south coast air basin of California and concluded that the urban benefits from improved air quality amounted to between $1.6 billion and $3 billion in the basin. The benefits that an improved air quality would provide for commercial agriculture in southern California can be added to the urban benefits described above. Adams et al. (1980) examined the economic impact of ambient oxidants upon 14 selected crops in the region. They extrapolated their results of these 14 crops to all southern California commercial agricultural products and predicted a $250 million benefit to be derived from control of oxidants in the air.

All of the cost benefits discussed above are based on total pollutants in air. It is not possible to project the portion of these benefits that may be attributable to control of toluene pollution alone. For a detailed description of the cost benefits of controlling alkylbenzene pollution, interested readers are referred to a recent NRC (1980) document.

6. ENVIRONMENTAL FATE, TRANSPORT, AND PERSISTENCE

The environmental fate, transport, and transformation of toluene in three different media--air, water, and soil are individually discussed below.

6.1 AIR

6.1.1 Fate in Air

Toluene can be persistent in the atmosphere. It is, therefore, a prime candidate for short- and long-range transport away from urban emission sources. The dispersion of toluene from a point source to the ambient atmosphere can be theoretically modeled by using dispersion equations. One such modeling method has been used in the Integrated Exposure Analysis Section (Section 10) to determine the transport characteristics of toluene.

The atmospheric toluene concentration downwind from one of the largest U.S. automobile manufacturing plants was measured by Sexton and Westburg (1980). At a point 6 km from the plant site, the toluene concentration was found to be 20.5 ppb. The concentration of toluene was still 15.1 ppb at a point 18 km downwind.

The primary mode of toluene removal from the atmosphere is probably via photochemical reactions, which occur during the course of its transport. Toluene itself does not absorb light at wavelengths longer than 295 nm. The solar spectrum in the troposphere does not contain much light of wavelengths shorter than 295 nm. Therefore, toluene can absorb only insignificant amounts of sunlight in the lower atmosphere, but a charge-transfer complex between toluene and molecular oxygen absorbs light of wavelengths to at least 350 nm. According to Wei and Adelman (1969), it is the photolysis of this complex that may be responsible for some of the observed photochemical reactions of toluene.

Toluene is apparently removed from the atmosphere entirely through free radical chain processes (NRC, 1980). Of the free radicals in the atmosphere,

hydroxy (•OH), atomic oxygen (O), and peroxy ($•HO_2$ or $•RO_2$, where R is an alkyl or acyl group) radicals are potential initiators for the removal of toluene. An additional reactive species is ozone. The rate constants for the reaction of these species with toluene and their relative significance for toluene removal are given in Table 6-1.

It is obvious from Table 6-1 that reactions with hydroxy radicals are the most important processes for the removal of toluene from the atmosphere. Based upon an estimated daytime hydroxy concentration given in Table 6-1 and a rate constant for the reaction of •OH radicals with toluene of 5.5×10^{-12} cm^3 $mol^{-1}sec^{-1}$ (Atkinson et al., 1978), the chemical lifetime of toluene in daylight hours has been estimated to be 50 hours (NRC, 1980). This value is subject to considerable uncertainty and may vary on a day-to-day basis by as much as an order of magnitude depending on solar intensity, temperature, and local trace gas composition of the atmosphere.

The reaction products formed from toluene under simulated atmospheric conditions are not known with certainty. According to the study of O'Brien et al. (1979), the gaseous products of the reaction are o-cresol, m- and p-nitrotoluene, benzyl nitrate, and benzaldehyde. Of these products, o-cresol and benzaldehyde are the major components, each composing about 8% of the total product yield. The mechanisms by which these products are formed are shown in Figure 6-1.

It is obvious that the reaction proceeds via addition of •OH radicals to the ring or by abstraction of hydrogen from the methyl side chain. Several investigators have determined the relative importance of both reaction pathways. From the amounts of reaction products formed, it was determined that the addition mechanism is of much greater significance than the abstraction mechanism (Kenley et al., 1978; O'Brien et al., 1979; Hoshino et al., 1978).

Table 6-1. Rate Constants for Reactions of Toluene with Reactive Species in the Atmosphere[a] (NRC, 1980)

Species	Estimated Average Daytime Annual Concentration ppm	Rate Constant, $ppm^{-1}\ min^{-1}$	Rate of Toluene Removal, ppm/min	Fraction of Hydroxyl Rate
Hydroxyl radical	4×10^{-8}	9.5×10^{3}	3.7×10^{-4}	1
Atomic oxygen	3×10^{-9}	1.1×10^{2}	3.3×10^{-7}	10^{-3}
Peroxy radical	1×10^{-4}	2.5×10^{-7}	2.5×10^{-11}	4×10^{-8}
Ozone	3×10^{-2}	5×10^{-7}	1.5×10^{-8}	5×10^{-5}

[a]Modified from Hendry, 1979.

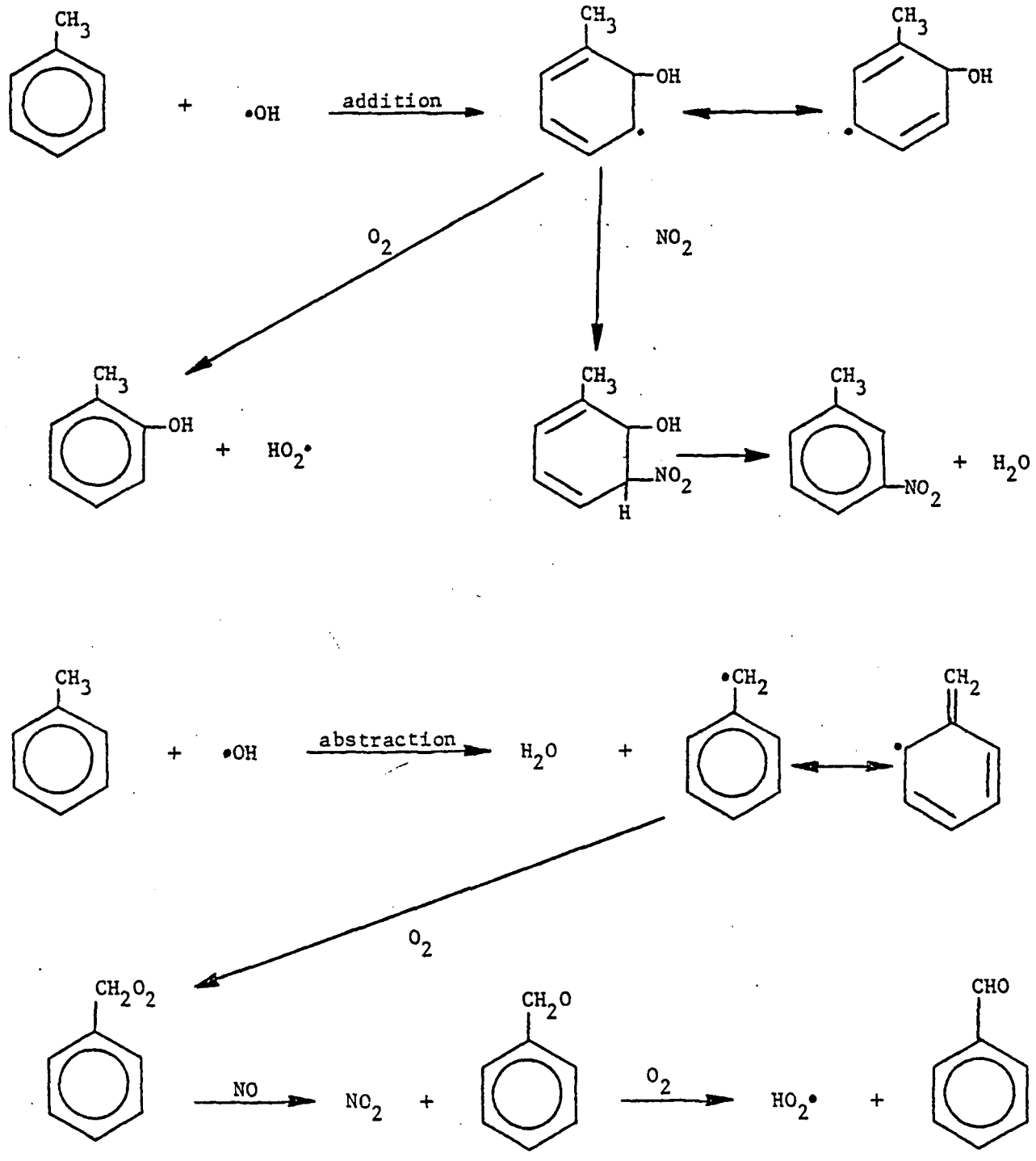


Figure 6-1. Proposed Reaction Pathways of Toluene Under Atmospheric Conditions (NRC, 1980)

Other reaction products are also formed from toluene reactions under simulated atmospheric conditions. Some of the ring fragmentation products formed are acetylene, acetaldehyde, and acetone. The total yield of these products is much less than 1%. Formaldehyde and formic acid are also formed, but their yields are not known. A measurement of the total gas phase carbon showed that 60% of the oxidation products from the photodecomposition of toluene left the gas phase and deposited on the walls of the reaction vessel or formed an aerosol (NRC, 1980). The distribution of the products between gas and condensed phases (aerosol) in the open atmosphere is still not clear.

In addition to the above photooxidation products, photolysis of toluene in polluted atmospheres (containing NO_x) yields ozone and fairly high amounts of peroxyacetylnitrate (PAN) (5% to 30% nitrogen yield) and peroxybenzoylnitrate (PBzN) (0% to 5% nitrogen yield) (NRC, 1980). The mechanism of PAN formation is either by the fragmentation of the aromatic ring or by the secondary reactions involving products of toluene photolysis. PBzN is formed by the photooxidation of benzaldehyde produced from the photooxidation of toluene (NRC, 1980). The formation of the peroxy compounds is significant because these products are strong eye irritants, oxidizing agents, and may induce plant damage (NRC, 1980). For an excellent review of the photochemical fate of toluene in the atmosphere, the reader is referred to a recent NRC document (NRC, 1980).

6.1.2 Transport

The volatility of toluene and its low solubility in water permit it to volatilize from water surfaces to the atmosphere (MacKay and Wolkoff, 1973). Studies of actual and simulated oil spills in seawater indicate that virtually all hydrocarbons smaller than C_{15} will be lost to the atmosphere within a few days (McAuliffe, 1977). The reverse process, that is, transfer of toluene from air to hydrosphere through rain, is also known to occur (Walker, 1976); however,

washout should not be considered to be a significant removal process for toluene from air (NRC, 1980).

6.2 AQUATIC MEDIA

6.2.1 Fate

Sauer et al. (1978) concluded from their studies of the coastal waters of the Gulf of Mexico that toluene and other alkylbenzenes are persistent in the marine environment. The probable modes of toluene loss or transformation from the aquatic environment are discussed below.

Oxidation: Reaction of toluene in water with hydroxy radicals generated from the irradiation of hydrogen peroxide produces benzaldehyde, benzyl alcohol, and cresols (Jefcoate et al., 1969). No data were found in the literature from which a relevant rate of oxidation of toluene in the aquatic environment could be determined.

It has been observed (Carlson et al., 1975) that toluene may form small amounts of chlorine-substituted products during chlorination under conditions used for water renovation. The extent of chlorination increases with the decrease of pH and increase of contact time. At a water temperature of 25°C and a chlorine concentration of 7×10^{-4} M, the percent chlorine uptake was determined to be 11.1% and 2.9% at water pH of 3 and 7, respectively (Carlson et al., 1975). With other conditions remaining the same, no chlorine uptake was observed at water pH of 10.1.

Hydrolysis: No data have been found that would support any role of hydrolysis in the fate of toluene in the aquatic medium.

Bioaccumulation: No measured steady-state bioconcentration factor (BCF) is available for toluene but, using the equation of Veith et al. (1979) and the measured octanol-water partition coefficient (as opposed to the theoretical value for log BCF of 2.69 [Chiou et al., 1977]), the U.S. EPA (1980b) has

estimated the BCF as 27.1. A factor of 3.0/7.6 = 0.395 has been used to adjust the estimated BCF from the 7.6% lipids on which the Veith et al. (1979) equation is based to the 3% lipids that is the weighted average for consumed fish and shellfish in the United States. Thus, the weighted average BCF for toluene from edible aquatic organisms consumed by Americans has been calculated to be 27.1 x 0.395 = 10.7.

In one experiment (Roubal et al., 1978), coho salmon (Oncorhynchus kisutch) and starry flounder (Platichthys stellatus) were exposed to a soluble fraction of a crude oil containing aromatic hydrocarbon in a flowing seawater. It was found that alkylated aromatics accumulated in tissues to a greater degree than unsubstituted derivatives. In both species, accumulations of substituted benzenes increased with increased alkylation. The tissues were not analyzed for toluene because of inadequate analytical procedures. It was, however, determined that the bioconcentration factors in starry flounder for C_4 and C_5 substituted benzenes were as high as 2600 and as low as near zero (concentration in fish tissue was below detection limit of 0.05 ppm) for xylenes. Substantial variations in BCF for individual hydrocarbons were found in both species. The muscle of coho salmon, which has a higher lipid content than starry flounder, showed a lower BCF. It was concluded (Roubal et al., 1978) that factors other than lipid content were more important in the observed species differences in the BCF values.

6.2.2 Transport

The primary fate-determining processes of toluene in aqueous media appear to be its intermedia transport processes (U.S. EPA, 1979). The details of the transport processes are discussed below.

Water to Air: Although there are no experimentally determined evaporation rates of toluene from water, there are theoretical models available for

predicting the rate of evaporation of slightly-soluble materials from aqueous solution (Mackay and Wolkoff, 1973; Liss and Slater, 1974; Mackay and Leinonen, 1975; Dilling, 1977). The most accurate of these is based on the mass transfer coefficients for the liquid and vapor phases reported by Liss and Slater (1974) and the Henry's law constant (the equilibrium concentration of a solute in air divided by its concentration in water for a solute as calculated by its solubility, vapor pressure, and molecular weight (Mackay and Leinonen, 1975). Based on these, Mackay and Leinonen (1975) reported the calculated evaporation half-life for toluene from 1-m deep water to be 5.18 hours.

The intramedia transfer of toluene in water can be calculated from this half-life value. If the $t_{1/2}$ and the current velocity are assumed to be 5.18 hours and 1 m/sec, respectively, the distance downstream that water in a river would flow before the volatilization of 50% toluene is:

$$5.18 \text{ hour} \times 1 \text{ m/sec} \times 3600 \text{ sec/hour} = 18{,}648 \text{ m}$$

Similarly, Henry's law coefficient (H) can be used to determine toluene concentration in air phase over seawater. If the height of the air and water columns are assumed to be the same, the Henry's law coefficient can be given as:

$$H = \frac{[\text{toluene}]_{gas}}{[\text{toluene}]_{liq}} = 0.349 \text{ for seawater (NRC, 1980)}$$

Thus, if equilibrium were attained, only 26% of toluene would be present in the gas phase above seawater.

In shallow or deep waters where stratification occurs, it is likely that the atmospheric mixing layer is 10 to 100 times deeper than the aquatic mixing layer (NRC, 1980). In such water, 78% or 97%, respectively, of the toluene would exist in the gas phase.

Water to Soil: The importance of this transport process can be evaluated by experimentally determining the toluene content in sediments of surface water

contaminated with toluene. Theoretical modeling can also be used for this purpose. Using the U.S. EPA's multi-compartment Exposure Analysis Modeling System (EXAMS), ADL (1980) has determined that bottom sediments account for over 90% of the total toluene discharged into surface waters under steady-state conditions. The values for the distribution of toluene between surface water and sediment as determined by the EXAMS modeling do not agree with experimental results of Jungclaus et al. (1978). Jungclaus et al. (1978) determined the toluene content in the water and sediments of a river receiving wastewater containing toluene. Although many other compounds were found to accumulate in the sediments, toluene was not one of these compounds. More research in this area is needed to explain this discrepancy between the EXAMS modeling and the experimental results.

6.3 SOIL

6.3.1 Fate

Toluene probably exists in soils in the sorbed state. The sorption of toluene by clay minerals (bentonite and kaolinite) was studied by El-Dib et al. (1978) and was found to follow Freundlich's adsorption isotherm. These authors also found that the adsorption capacity increased as the pH value decreased.

The fate of toluene in soil has not been thoroughly investigated. It can, however, be anticipated that a part of toluene in soil will undergo intermedia transfer to air and water, and a part will undergo intramedia transfer. The part that stays in soil may participate in chemical reactions (including photochemical reactions) and biological degradation and transformation. The relative importance of intermedia transfer and chemical and biological reactions of toluene in soils is not accurately known.

Investigations of Wilson et al. (1980) indicate that volatilization, biodegradation, and biotransformation processes dominate the fate of toluene in

soils. The intermedia transfer of toluene from soil to water is probably not an important pathway. No data could be found in the existing literature searched that would support any role of chemical reactions in determining the fate of toluene in soils. The intermedia transport of toluene and its biological fate in soils have been separately discussed below.

6.3.2 Transport

6.3.2.1 Soil to Air

Laboratory experiments of Wilson et al. (1980) show that 40% to 80% of toluene applied to the surface of sandy soils will volatilize to air. The volatilization rate is dependent on the nature of soil. The volatilization rate may be significantly lower for soils with high organic contents due to their sorption properties (ADL, 1980). This phenomenon may be especially important with municipal sludges that normally contain high organic substances.

6.3.2.2 Soil to Water

The transfer of toluene from soil to ground or surface waters can be of importance with regard to the possibility of contamination of these water bodies and their subsequent use as sources of drinking waters. Unfortunately, very little information is available on this subject. From the investigations of Wilson et al. (1980), it can be concluded that the transport of toluene from soil to water is probably not a major transfer pathway. These investigators showed that 0% to 20% of the applied toluene on a sandy soil system could be elicited through a column of 140-cm height. The leaching of toluene from landfill sites that contain soil originated partly from municipal sludges can be expected to be even lower. The higher organic content of these soils may retard the aqueous elution process due to higher sorption properties of the soils toward toluene.

6.4 ENVIRONMENTAL PERSISTENCE

6.4.1 Biodegradation and Biotransformation

6.4.1.1 Mixed Cultures

The study of the disappearance of toluene in soil began nearly 75 years ago. Stormer (1908) and Wagner (1914) showed that toluene was susceptible to bacterial decomposition in the soil. Gray and Thornton (1928) and Tausson (1929) isolated soil bacteria that utilized toluene as a sole carbon source. Claus and Walker (1964) found that the half-life of toluene in soil inhabited with toluene-degrading bacteria was 20 to 60 minutes. Wilson et al. (1980) indicated that from 20 to 60% of toluene eluted through 140 cm of sandy soil biodegraded. The authors stated that the process was probably very sensitive to the soil type and therefore may or may not be an important removal process of toluene from a particular soil system.

More literature, however, exists on the biodegradation of toluene in aquatic environments. In a report prepared by the Arthur D. Little Company (1981), the biodegradation of toluene in lakes, rivers, and ponds was discussed using the U.S. Environmental Protection Agency's (U.S. EPA) Multicompartment Exposure Analysis Modeling System (EXAMS). The report stated that the biodegradation of toluene accounted for 0.31, 4.81, 0.36, 0.09, and 18.47% of the total toluene loss in oligotrophic lakes, eutrophic lakes, clean rivers, turbid rivers, and ponds, respectively. Sontheimer (1980) also reported the rate of toluene disappearance from Rhine River water but did not specify the rate of disappearance. Using the standard dilution method and filtered wastewater effluent as the seed to determine the biochemical oxygen demand (BOD), the biodegradability (percentage bio-oxidized) of toluene ranged from 63 to 86% after up to 20 days (Price et al., 1974; Bridie et al., 1979).

Matsui et al. (1975) found, that in activated sludge acclimated to various organic compounds, the total organic carbon (TOC) removal efficiency for toluene was 60% while the chemical oxygen demand (COD) was 72% for 24 hours. The authors concluded, however, that although toluene was a readily biodegradable compound, in this experiment disappearance was mainly due to evaporation. Using the Warburg technique, Lutin et al. (1965) reported a 40% degradation of toluene in activated sludge after 144 hours. In comparison, 63% of the benzene was degraded in the same time. The degradation of toluene in benzene-acclimated activated sludge reached 17.2% of the theoretical BOD after 6 hours and 48% after 192 hours (Malaney and McKinney, 1966). Toluene was the most biodegradable of a number of alkylbenzenes tested by these authors, who also found that the introduction of a methyl group to benzene retarded the initial (6 hour) rate of oxidation of toluene but not the extent of degradation compared to benzene. Marion and Malaney (1964) exposed activated sludge to 500 mg/l of toluene from three municipal plants and reported that unacclimated sludge showed little ability to oxidize benzene and toluene after 6 hours and that after 72 hours, less than 11% oxidation had taken place (compared to 44.7% reported by Malaney and McKinney, 1966). However, one sludge sample acclimated to benzene oxidized greater than 30% of the toluene after 180 hours.

The degradation of toluene has also been studied in mixed cultures of bacteria. Chambers et al. (1963), using phenol-adapted bacteria, reported 38% degradation of toluene after 180 minutes. In another study, Declev and Damyanova (1977) grew sludge cultures in either phenol, xylene, or toluene as the sole carbon source and found that phenol-adapted bacteria proved less able to degrade xylene and toluene, while toluene-adapted cells showed greater versatility in being able to oxidize phenol and xylene.

6.4.1.2 Pure Cultures

Fungi and bacteria have been shown to utilize toluene (Smith and Rosazza, 1974). In the course of studying the effects of toluene on microbial activity, Kaplan and Hartenstein (1979) discovered that 6 of 7 fungi imperfecti, 7 of 13 basidiomycetes, and 6 of 14 bacteria grew with 0.1 or 0.05% toluene as the sole carbon source. The addition of yeast extract increased the amount of toluene-utilizing microorganisms. In contrast, no oil-utilizing or hydrocarbon-degrading fungi grew on toluene as the sole carbon source (Davies and Westlake, 1979). Using an oxygen electrode to measure oxidation, Buswell and Jurtshuk (1969) found that resting cells of an n-octane-utilizing Corynebacterium sp. oxidized only 7% of the available toluene compared to 100% oxidation of n-octane. Toluene did not serve as a growth substrate in this experiment. Kapraleck (1954) isolated a Pseudomonas-type bacteria from the soil of a petroleum deposit that utilized toluene. Pseudomonas and Achromobacter spp. from soil used toluene as the sole carbon source for growth (Claus and Walker, 1964; Gibson and Yeh, 1973). Smith and Rosazza (1974) reported that bacteria and yeast hydroxylated toluene. In contrast, Nei et al. (1973) found little oxidation of toluene by phenol-utilizing yeast.

The metabolic pathway for the bacterial oxidation of toluene has been studied with soil microorganisms (Figure 6-2) and reviewed by Gibson (1971) and Subramanian et al. (1978). On the basis of simultaneous adaptation studies, Kitagawa (1956) concluded that Pseudomonas aeruginosa oxidized toluene via benzyl alcohol and benzaldehyde to benzoic acid and then to catechol. This pathway was supported by the investigations of Nozaka and Kusunose (1968). A Mycobacterium sp. also produced benzoic acid from toluene (Atkinson and Newth, 1968), as did a methanotrophic bacterium (Methylosinus trichosporium) (Higgins et al., 1980).

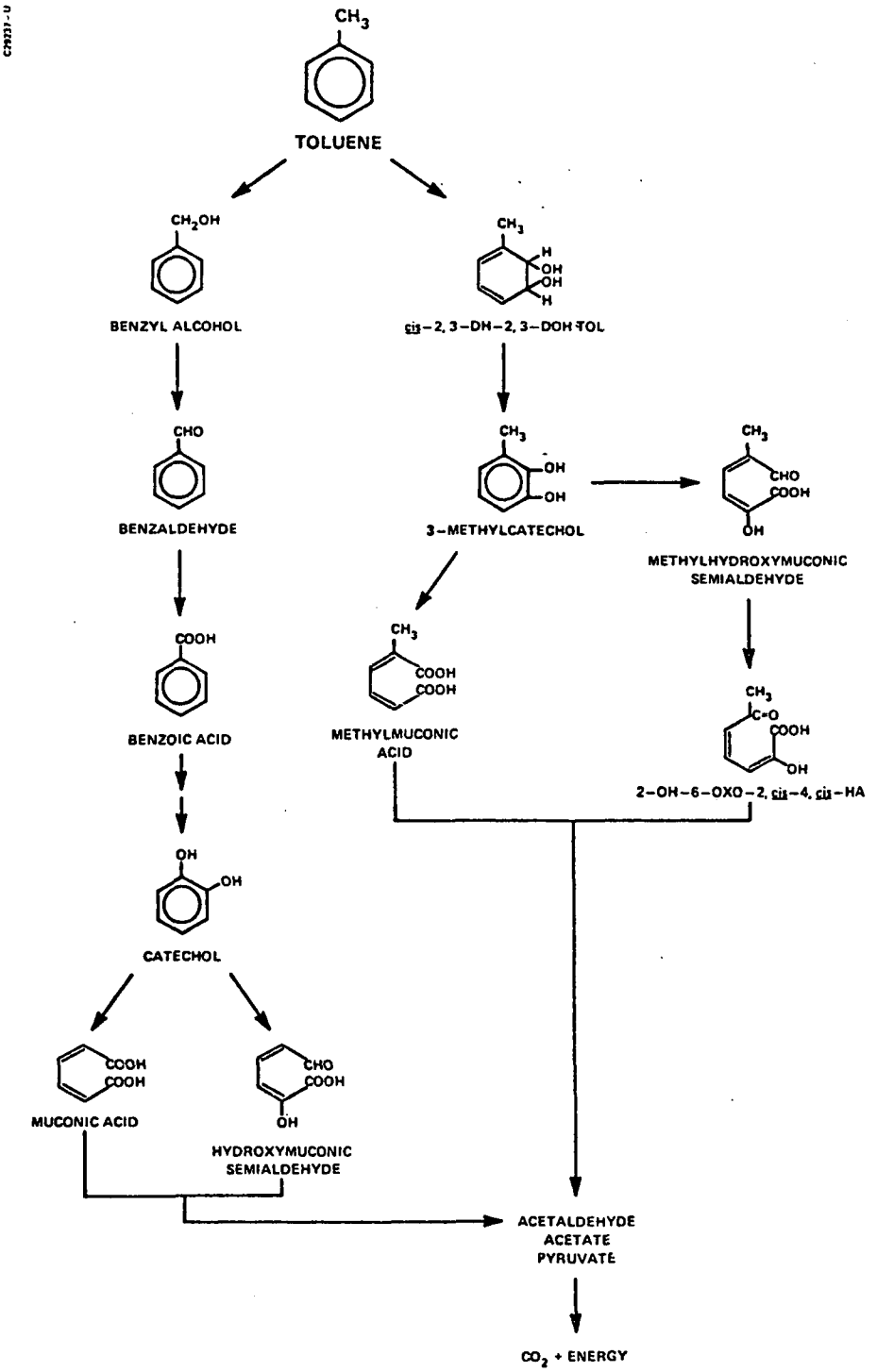


Figure 6-2. Microbial Metabolism of Toluene (prepared by Syracuse Research Corporation)

An alternative pathway was proposed by Claus and Walker (1964) using a Pseudomonas sp. and an Achromobacter sp. isolated from soil that used toluene as a sole carbon source for growth. These investigators found that washed cell suspensions oxidized toluene to 3-methylcatechol, indicating that the methyl moiety was not oxidized, as occurred in the pathway proposed by Kitagawa (1956). A similar oxidation product was found by Nozaka and Kustnose (1969) using Pseudomonas mildenbergii cell-free extracts. Gibson et al. (1968a) also reported the detection of 3-methylcatechol from toluene by Pseudomonas putida. An oxidation product preceding 3-methylcatechol was found in cultures of a mutant strain of P. putida (strain 39/D) (Gibson et al., 1968b, 1970). This new product was identified as (+)-cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (cis-2,3-dihydro-2,3-dihydroxytoluene) (cis-2,3-DH-2,3-DOH TOL) (Kobal et al., 1973). The catechol and 3-methylcatechol can be then cleaved by ortho cleavage to yield the corresponding muconic acids or by meta cleavage to yield the corresponding hydroxymuconic semialdehydes (Bayly et al., 1966). Methylmuconic acid was formed from toluene oxidation by a soil bacterium Nocardia corallina (Jamison et al., 1969). The semialdehydes are further converted to 2-hydroxy-6-oxo-2,cis-4,cis-heptadienoic acid (2-OH-6-OXO-2,cis-4,cis-HA) and then to acetate, pyruvate, and acetalydehyde and to CO_2 and energy (Bayley et al., 1966). The conversion of toluene to compounds that can be utilized as sources of carbon and energy suggests that toluene will be degraded rapidly by microbial species proliferating at the expense of the compound and will not accumulate significantly in the environment.

The enzymes responsible for toluene degradation are carried on plasmids (Williams and Worsey, 1976; Saunders, 1977). Williams and Worsey (1976) isolated 13 bacteria from soil, all of which carried the toluene-degrading plasmids, suggesting that the plasmid-borne gene responsible for toluene degradation is wide

spread in the soil microbial population. The plasmid can also be transposed into other hosts, further increasing the number of toluene-degrading bacteria (Broda et al., 1977; Jacoby et al., 1978). The toluene plasmid in Pseudomonas putida coded for the metabolism of toluene to the corresponding alcohol and aldehyde via the meta pathway, to the semialdehyde and further products (Worsey and Williams, 1975; Worsey et al., 1978). A plasmid coding for both toluene and xylene degradation in a Pseudomonas sp. has recently been isolated and characterized (Yano and Nishi, 1980). Broda et al. (1977) have speculated that the ortho pathway of toluene degradation is probably chromosomally coded.

7. ENVIRONMENTAL AND OCCUPATIONAL CONCENTRATIONS

Monitoring data for the concentration of toluene has been divided into two subsections, one pertaining to the environmental levels and the other to the occupational levels.

7.1 ENVIRONMENTAL LEVELS

Toluene has been detected in the following environmental media: (1) air, (2) aqueous media, (3) sediments, (4) solid wastes and leachates, and (5) edible aquatic organisms.

7.1.1 Air

Toluene is the most prevalent aromatic hydrocarbon present in ambient air. Atmospheric levels of toluene in different locations in the United States and other parts of the world are given in Table 7-1.

From the experimental measurements of the toluene-to-benzene ratio, Pilar and Graydon (1973) concluded that the major source of toluene in urban air with high traffic volume is automobile emission. Recently, Pellizzari (1979) has measured toluene levels near manufacturing and refining sites in the United States. The ratio of toluene to benzene in these sites indicates that besides automobile emission, manufacturing processes are probably a factor in ambient toluene concentration at many of the sites.

It can be inferred from Table 7-1 that the atmospheric concentration of toluene in urban areas not containing toluene manufacturing or gasoline refining sites are in the same range as the sites containing these industries. It can also be concluded from Table 7-1 that the concentration of toluene has declined significantly in the past 15 years in Los Angeles, presumably as a result of automotive emission controls. The concentration of toluene in many urban areas

Table 7-1. Atmospheric Concentrations of Toluene

Location	Year	Concentration, ppb	
		Average	Highest, or Range
Manufacturing or Refining Sites:			
Baton Rouge, LA	NR[a]	0.14[b]	0.03-0.23
Birmingham, AL	NR	2.0	0.21-4.7
El Dorado, AR	NR	11.0[b]	2.5-13.6
Elizabeth, NJ	NR	17.0[b]	1.9-39.1
El Paso, TX	NR	4.9[b]	0.05-18.8
Houston, TX	NR	1.6[b]	0.21-2.93
Magma, UT	NR	0.35[b]	0.23-0.43
S. Charleston, WV	NR	0.05[b]	0.04-0.07
Upland, CA	NR	7.3[b]	0.78-14.8
Other Urban Areas:			
Los Angeles, CA	1963-65	59[c]	NR
	1966	37[d]	129
	1967	30[e]	50
	1968	39[f]	NR
	1971	50[e]	NR
	1973	22[c]	NR
	1979	11.7[g]	1.1-53.4
Azusa, CA	1967	14[e]	23
Riverside, CA	1970-71	NR[h]	9-18
Denver, CO	1973	9[i]	74
Phoenix, AZ	1979	8.6[g]	0.54-38.7
Oakland, CA	1979	3.1[g]	0.15-16.9
Albany, NY	NR	1.3[k]	NR
Troy, NY	NR	1.0[k]	NR
Newbury Park CA	1978	NR[r]	0.7-13
Tuscaloosa, AL	1977	38	24-85[s]

Table 7-1. Atmospheric Concentrations of Toluene (Continued)

Location	Year	Concentration, ppb	
		Average	Highest, or Range
Rural and Remote Areas:			
Brethway-Gunderson Hill, WA	1971	0.1[l]	NR
Camel's Hump, VT	1971	1.0[l]	NR
Hell's Canyon, ID	1971	0.3[l]	NR
Moscow Mt., ID	1971	0.2[l]	NR
Point Reyes, CA	1971	0.2[l]	NR
Grand Canyon, AZ	NR	Trace[b]	Trace
Talladega Nationa Forest, AL	1977	0.4	0.2-1.3
Global:			
Zurich, Switzerland	NR	39[m]	NR
Toronto, Canada	1971	30[n]	188
Berlin, W. Germany	1975-76	27[o]	2.4-94.2
Stockholm, Sweden	NR	NR[p]	0-2.7
The Hauge, Netherland	1974	18[c]	54

[a]NR: Not reported.
[b]Pellizzari, 1979.
[c]Leonard et al., 1976.
[d]Lonneman et al., 1968.
[e]Altshuller et al., 1971.
[f]Kopcznski et al., 1972. A single measurement was made.
[g]Singh et al., 1979.
[h]Stephens, 1973.
[i]Russell, 1977.
[k]Atwicker et al., 1977.
[l]Robinson et al., 1973.
[m]Grob and Grob, 1971.
[n]Pilar and Graydon, 1973.
[o]Lahmann et al., 1977.
[p]Johansson, 1978.
[r]Hester and Meyer, 1979.
[s]G. Holzer et al., 1977.
[t]Burgardt and Jeltes, 1975.

in the United States in recent years ranged from less than 0.1 ppb to as much as 50 ppb, averaging approximately 1 to 10 ppb. In remote locations of the United States, the value averaged approximately 0.3 ppb in 1971, but the current level may be lower as indicated by the toluene concentration at Grand Canyon.

Sexton and Westberg (1980) monitored the air near an automotive painting plant at Jamesville, Wisconsin, to investigate the effect of emission from paint solvents on atmospheric toluene level. The toluene concentration downwind within 1.6 km of the plant was 160 ppb. The concentration of toluene was still 20.5 ppb, 22.9 ppb, 17.5 ppb, and 15.1 ppb at distances 6 km, 10.5 km, 13.5 km, and 16.5 km, respectively, downwind from the plant. These concentrations are about 10 to 15 times higher than the background toluene concentrations of 1.5 ppb determined at a distance of 1.6 km upwind of the plant. These concentrations are also comparable to or higher than most of the values given in Table 7-1.

In response to numerous complaints from residents about illness and odors in the vicinity of a chemical solvent reclamation plant in Maryland, Smoyer et al. (1971) monitored the valley air surrounding the plant. A toluene concentration as high as 11 ppm was registered in the valley air. Both this result and the more recent investigation of Sexton and Westberg (1980) indicate that processes involving solvent use of toluene may result in high emission of toluene in the vicinity of these sources.

7.1.2 Aqueous Media

Toluene has been monitored in a number of aquatic media including: (1) surface waters, (2) industrial wastewater, (3) water from publicly-owned treatment works (POTW), (4) underground waters, (5) municipal drinking waters, and (6) rainwater. The toluene levels in each of the media have been discussed separately.

7.1.2.1 Surface Waters

Information regarding toluene levels in surface water has been primarily obtained from the STORET system as given by Slimak (1980). Table 7-2 shows the toluene levels for major river basins in the United States. It is evident from Table 7-2 that 83% of all the monitored surface water contains toluene levels below a concentration of 10 ppb. The concentration of toluene in surface waters of the central region (Lake Erie, upper Mississipi, Lake Michigan, etc.) are higher than surface waters from other regions. This higher level of toluene cannot be attributed to the emission from production sites since the central region contains only 8 of the 38 major production sites. Surface waters from Texas, which contains 20 of the 38 production sites, showed lower levels of toluene. This indicates that production processes may not be the major source of toluene emission in surface waters.

Recent studies of the coastal waters of the Gulf of Mexico have shown that aromatic hydrocarbons comprise 80% to 90% of the total dissolved volatile hydrocarbons ($< C_{14}$) at most sampling sites (Sauer et al., 1978). The volatile hydrocarbons, however, were only a few percent of the total dissolved hydrocarbons. The concentration of toluene in surface waters at several sites in the Gulf of Mexico ranged from 4.5 ng/l to 376.0 ng/l, while the average was 61.4 ng/l.

7.1.2.2 Industrial Wastewaters

Table 7-3 shows the levels of toluene in industrial effluents as stored in the STORET system (Slimak, 1980). It can be concluded from Table 7-3 that 85% of the effluents showed toluene concentrations of less than 10 ppb. Fifteen of the reporting stations showed toluene concentration in excess of 100 ppb.

Wastewaters from a speciality chemicals manufacturing plant were analyzed by Jungclaus et al. (1978). The concentration of toluene in the wastewater was reported to be in the range of 13 to 20 ppm. Similarly, wastewater from one tire

Table 7-2. Distribution of U.S. Surface Waters Within a Certain Toluene Concentration Range (U.S. EPA, 1980)

	Number of Observations	Percent Sample in the Toluene Concentration Range, ppb			
		<10	10.1-100	100.1-1000	>1000
Northeast	1		100		
North Atlantic	14	100			
Southeast	110	93	4	4	
Tennessee River	16	81	6	6	6
Ohio River	54	98		2	
Lake Erie	2		100		
Upper Mississippi	18	67	22	11	
Lake Michigan	30	20	77	3	
Missouri River	34	44	53	3	
Lower Mississippi	8	88		13	
Colorado River	3	100			
Western Gulf	15	100			
Pacific Northwest	80	99	1		
California	5	100			
Great Basin	1	100			
Puerto Rico	1	100			
Unlabeled	1	100			
TOTAL	393	83	14	3	---

Abbreviation: IA = insignificant amount.

Table 7-3. Percent Distribution of U.S. Wastewaters Within a Certain Toluene Concentration Range (U.S. EPA, 1980).

Effluent Discharged	Number of Observations	Percent Sample in the Toluene Concentration Range, ppb			
		<10	10.1-100	100.1-1000	>1000
Northeast	103	84	9	4	3
North Atlantic	48	88	6	6	
Southeast	100	87	10	3	
Tennessee River	28	96	4		
Ohio River	70	84	11	3	1
Upper Mississippi	64	69	30	2	
Lake Michigan	6	100			
Missouri River	16	100			
Colorado River	1	100			
Western Gulf	1	100			
Pacific Northwest	45	91	7	2	
TOTAL	482	85	11	3	1

manufacturing company was analyzed by Jungclaus et al. (1976) and was found to contain approximately 10 ppm of toluene. Both of these values are among the highest values reported in Table 7-3.

Analysis of raw wastewater and secondary effluent from a textile manufacturing plant was reported to contain toluene as one of the predominant compounds (Rawlings and Samfield, 1979). The toluene concentrations in 22 wastewater samples and 22 secondary effluent samples were in the range of 0.5 to 300 ppb (Rawlings and Samfield, 1979). Effluents from a paper mill in Hiro Bay, Japan, were analyzed for organic matter. It was determined that toluene constituted 1% of the total chloroform extractables from the effluent (Yamaoka and Tanimoto, 1977).

Toluene has also been detected in a variety of industrial wastewaters. Table 7-4 shows the frequency of toluene detection in industrial wastewaters (U.S. EPA, 1980).

7.1.2.3 Publicly-Owned Treatment Works (POTW)

A pilot study of two POTW's, one handling more organic pollutant than the other, was conducted for the U.S. EPA (1979). Toluene was detected in 100% of the influent samples and 95% of the final effluent samples from the plant containing more organic pollutants. The maximum and median toluene concentrations in the influent sample from this plant were 440 ppb and 13 ppb, respectively. The influent sample at the other plant had maximum and median toluene concentrations of 37 ppb and 10 ppb, respectively. The frequency of toluene occurrence at this plant was 76% for the influent and 71% for the final effluent sample.

The state of Ohio (U.S. EPA, 1977) conducted a survey of toxic substances in 2 municipal wastewater treatment plants. The toluene concentration in the wastewater of the plant dealing primarily with domestic wastewater ranged between 1 ppb and 5 ppb. The treated effluent from the same plant, on the other hand,

Table 7-4. Detection Frequency of Toluene in Industrial Wastewaters (U.S. EPA, 1980)

Industry	Frequency of Detection (No. Found/No. Samples)
Soap and Detergents	1/20
Adhesives and Sealants	2/11
Leather Tanning	19/81
Textile Products	56/121
Gum and Wood Products	14/18
Pulp and Paper	4/98
Timber	58/285
Printing and Publishing	50/109
Paint and Ink	48/94
Pesticides	23/147
Pharmaceuticals	38/95
Organics and Plastics	306/723
Rubber	15/67
Coal Mining	53/249
Ore Mining	6/72
Steam Electric Power Plants	32/84
Petroleum Refining	18/76
Iron and Steel	43/431
Foundries	2/54
Electroplating	5/18
Nonferrous Metals	21/173
Coil Coating	2/12
Photographic	9/25
Inorganic Chemical	10/107
Electrical	1/35
Auto and Other Laundries	9/56
Phosphates	1/33
Plastic Processing	1/1
Procelain Enameling	2/19
Landfill	3/17
Mechanical Products	23/35
Pubicly-Owned Treatment Works	11/40

showed a concentration of 1 ppb. About 87% of the influent from the other plant which treated industrial-domestic wastewater showed the presence of toluene in the concentration range of 8 ppb to 150 ppb. The frequency of toluene detection in the treated effluent from the same plant amounted to 36%. The toluene concentrations in these treated effluents ranged from 1 ppb to 10 ppb.

7.1.2.4 Underground Water

The New York State Department of Health and the United States Geological Survey examined 39 wells in 1978 for organic contamination in groundwater (Slimak, 1980). Toluene was detected in 85% of the wells tested. However, the toluene concentration in these waters was below 10 ppb.

Toluene concentration in well water can be obtained from the data recorded in STORET (U.S. EPA, 1980). Of the 143 monitored data, only 3 indicated the presence of toluene in the concentration range of 42 ppb to 100 ppb. All of these 3 wells were in the vicinity of landfill sites.

7.1.2.5 Drinking Water

Toluene has been detected in raw water and in finished water supplies of several communities in the United States. Levels of up to 11 ppb were found in finished water from the New Orleans area (U.S. EPA, 1975a). In a nationwide survey of water supplies from 10 cities, 6 were discovered to be contaminated with toluene (U.S. EPA, 1975b). Concentrations of 0.1 and 0.7 ppb were measured in 2 of these water supplies. Toluene was detected in 1 of 111 finished drinking waters during a second nationwide survey (U.S. EPA, 1977). In a subsequent phase of this survey, toluene was found in 1 raw water and 3 finished waters out of 11 supplies surveyed (U.S. EPA, 1977). A level of 19 ppb measured by gas chromatography/mass spectrometry was found in 1 of these finished waters, and 0.5 ppb was found in another.

Nineteen volatile organic compounds, including toluene, were detected at concentrations below 5 ppb in District of Columbia drinking water (Saunders et al., 1975). These investigators also found that the concentrations of the various contaminants in tap water vary from week to week, but the chemical composition remains the same.

7.1.2.6 Rainwater

Toluene has been detected in rainwater from Berlin, West Germany (Lahmann et al., 1977). The toluene content in the rainwater varied with sample collection points. The rainwater from a residential area, airport, and a busy traffic intersection showed toluene concentration of 0.13 ppb, 0.70 ppb, and 0.25 ppb, respectively.

7.1.3 Sediment

Toluene concentrations in sediment samples as recorded in STORET (U.S. EPA, 1980) show that 91% of the samples contain less than 10 ppb of toluene. The concentration of toluene exceeded 500 ppb in only 7% of the samples. Samples with higher concentrations of toluene were obtained from the vicinity of an industrial area in San Francisco.

Jungclaus et al. (1978) monitored the sediment from a river receiving industrial effluent from a specialty chemicals manufacturing plant containing toluene. However, these investigators could not detect the presence of toluene in the river sediment.

7.1.4 Edible Aquatic Organisms

Of the 59 monitored tissue samples that were recorded in the STORET system (U.S. EPA, 1980), 95% of the data showed toluene concentrations of less than 1 ppm. The maximum toluene concentration detected in 1 fish tissue was 35 ppm. Toluene was also detected in fish caught from polluted waters in the proximity of

petroleum and petrochemical plants in Japan (Ogata and Miyake, 1973). A concentration of 5 ppm was measured in the muscle of 1 such fish.

7.1.5 Solid Wastes and Leachates

Toluene has been detected in the air samples at a few landfill sites (U.S. EPA, 1980a) and in well water near a few landfill sites (U.S. EPA, 1980). However, no data regarding the level of toluene in solid wastes and their leachates could be found in the literature.

7.2 OCCUPATIONAL CONCENTRATIONS

Several reports describing the presence of toluene in occupational atmospheres were found in the literature. A toluene level of 10,000 to 30,000 ppm was reported in a merchant ship after it was internally sprayed with a toluene-containing insecticide (Longley et al., 1967). Two hours after the initial monitoring, concentrations ranging from 5000 to 10,000 ppm were still present in the atmosphere of the ship.

A monitoring program was instituted in response to a report of an epidemic solvent poisoning in a rotogravure plant in Milan, Italy. Solvent containing toluene was largely used in this plant as an ink solvent and diluent. The results of the monitoring showed that the concentration of toluene ranged from 0 to 277 ppm in different parts of the work areas (Forni et al., 1971). The determined toluene concentration at different parts of the plant during the period 1957 to 1965 is shown in Table 7-5.

In 1966, the above rotogravure plant was moved to a different location and the ventilation system of the plant was improved. Subsequent analysis for toluene showed annual mean concentrations at 156 ppm and 265 ppm near the folding machines and between the machine elements, respectively (Forni et al., 1971).

Table 7-5. Toluene Concentrations in Different Work Areas of a Rotogravure Plant in Milan, Italy (Forni et al., 1971)

Work Area	Toluene concentration, ppm	
	Range	Annual Mean
Center of Room	140-239	203
Folding Machines	56-277	203
Between Machine Elements	306-824	431

A study of 8 Japanese factories operating polychromic rotory processes for photogravure printing reported toluene concentrations in the range of 4 to 240 ppm in different work areas of the plants (Ikeda and Ohtsuji, 1969).

Toluene exposures to workers in 11 leather-finishing and rubber-coating plants have also been reported (Pagnotto and Lieberman, 1967). Toluene is used as lacquer thinners and stain removers in the leather finishing industry. In rubber-coating plants, the major source of toluene emission is the fabric-spreading machine areas. The concentration of toluene in work areas of these industries is shown in Table 7-6.

Toluene has been detected in other occupational atmospheres. For example, a toluene concentration of 0.18 ppm has been reported in a submarine atmosphere (Chiantella et al., 1966). The origin of toluene in this atmosphere has been speculated to be paint solvents and diesel fuel used in the submarine. Toluene has been detected in the atmosphere of M15 and M19 antitank mines (Jenkins et al., 1973). The origin of toluene in this atmosphere was attributed to mine casings.

A more recent study (Fraser and Rappaport, 1976) designed to determine the health effects associated with the curing of synthetic rubber simulated the vulcanization process in the laboratory. Toluene emission in the vulcanization area from this experiment amounted to 1.1 ppm. The actual field survey of different work areas of 10 large tire manufacturing plants across the United States was conducted by Van Ert et al. (1980). The toluene concentrations in different work areas measured by these investigators is shown in Table 7-7.

It can be concluded from Table 7-7 that the extrusion process area and the tire building process area are the 2 areas of tire manufacturing plants that account for the major toluene emissions from these plants.

Table 7-6. Toluene Concentrations in Work Areas of Leather Finishing and Rubber Coating Plants (Pagnotto and Lieberman, 1967)

Industry	Work Areas	Toluene Concentration, ppm	
		Range	Average
Leather finishing	Finishing Area	19-85	53
	Washing and Topping Area	29-195	112
Rubber Coating	Spreading Machines	34-120	73

Table 7-7. Toluene Concentrations in Selected Work Areas of Tire Manufacturing Plants (Van Ert et al., 1980)[a]

Work Area	No. of Plants Surveyed	Area Toluene Concentration, ppm	
		Mean	Range
Cement Mixing	8	2.9	0.2-7.7
Extrusion	4	14.0	3.3-50.0
Tire Building	2	8.0	2.5-13.4
Curing Preparation	3	0.6	0.1-1.1
Inspection and Repair	3	1.9	0.6-2.7
Warehouse	2	0.28	0.01-0.76

[a]All of the plants, with the exception of plants where the warehouse samples were taken, were surveyed during 1973-77. The warehouse samples were collected in 1977.

7.3 CIGARETTE SMOKE

The concentration of toluene in inhaled cigarette smoke is approximately 0.1 mg/cigarette (NRC, 1980; Dalhamn et al., 1968). Jerimini et al. (1976) determined the concentration of toluene in the sidestream smoke of cigarettes. When 30 cigarettes were inhaled in a 30 m^3 room and the concentration of toluene in room air was determined, it was found to be 0.23 ppm. This value corresponds to 0.87 mg of toluene in the sidestream smoke of each cigarette. Holzer et al. (1976) determined the toluene concentration in a 60 m^3 room and found an ambient toluene concentration of 40 ppb. When 1 cigarette was smoked in the room, the concentration of toluene rose to 45 ppb. This corresponds to 1.1 mg of toluene contribution from each cigarette. It seems from this discussion that the mainstream smoke of 1 cigarette contributes 0.10 mg toluene to the smoker. The sidestream smoke, on the other hand, may contain a higher amount of toluene.

8. ANALYTICAL METHODOLOGY

Toluene has been analyzed in a multiple of media including the following: (1) air, (2) waters, (3) soils and sediments, (4) crude oil and organic solvents, (5) biological samples, (6) some foods, and (7) cigarette smoke. The analytical methods for the determination of toluene in each of these media are individually discussed below.

8.1 AIR

In addition to the analysis of test mixtures of toluene in air for the evaluation of methods, toluene has also been determined in ambient air, occupational air, forensic air, and air containing the pyrolysis products of organic wastes.

8.1.1 Ambient Air

The determination of toluene in ambient air consists of two distinct steps: sampling and analysis.

8.1.1.1 Sampling

Toluene can be collected from ambient air in several different ways including grab sampling in aluminized plastic bags (Neligan et al., 1965), Tedlar bags (Altshuller et al., 1971; Lonneman et al., 1968), and glass containers (Schneider et al., 1978; Pilar and Graydon, 1973). Although the grab sampling is conceptually the simplest approach, this collection method without subsequent concentrative technique does not provide sufficient quantity of toluene for analytical detection and quantification. Since ambient samples contain toluene in the parts per billion range, preconcentration steps are often necessary.

Sample collection by cyrogenic procedures (Seifert and Ullrich, 1978) is an alternative method for the collection of toluene in ambient air; however, the drawbacks of this procedure include the inconveniences in sampling and sample

regeneration. Also, unless the moisture in air is removed, it condenses in the collection tube and may reduce or restrict the air flow through the collection tubes. Various drying agents, such as anhydrone, anhydrous K_2CO_3, ascarite, LiH, and molecular sieve can be used. It has, however, been demonstrated by Isidorov et al. (1977) that it is impossible to find a drying agent that will preferentially absorb the moisture from air without absorbing some of the trace organics.

Reversible sorption on various high surface area materials provides an excellent method for preconcentrative collection of toluene from ambient air. Since the moisture content in the air is normally 3 to 4 orders of magnitude higher than the total organics (Isidorov et al., 1977), the chosen sorbents must show little affinity toward moisture. Otherwise, the retention capacity of the sorbents will be reached much sooner than desired.

A number of sorbents such as Tenax GC (Holzer et al., 1977), various carbonaceous materials (Burghardt and Jeltes, 1975; Holzer et al., 1977; Isidorov et al., 1977), Polisorbimid (Isidorov et al., 1977), molecular sieves and spherisil (Ball, 1976), and Porapak Q (Johansson, 1978) have been successfully used. Typically, sampling is performed by drawing air through a trap containing the selected sorbent with battery-operated diaphragm pumps. The air flow through the trap is controlled by needle valves and measured by a previously calibrated rotometer. The trap is kept at ambient temperature to avoid condensation of water. At the end of the sampling, the trap-ends are closed with caps and transferred to the laboratory in a refrigerated state, to avoid sample loss.

8.1.1.2 Analysis

The method of analysis is usually dependent on the method of sample collection. The earlier investigators who used plastic bags or glass bottles for collection of grab samples utilized a trapping system for concentrating a relatively large volume (1 to 10 l) of sample before analysis. In this method, the

collected sample is allowed to flow through a cryogenic trap containing suitable sorbents. At the end of trapping, the coolant is removed from the trap and the trap is quickly heated to vaporize and transfer the trapped compounds into the gas chromatographic (GC) columns. The columns used by earlier investigators (Lonneman et al., 1968; Altshuller et al., 1971) for aromatic separations consisted of long open-tubular columns coated with m-bis(m-phenoxy-phenoxy)benzene combined with Apiezon grease on a packed dual column with SF-96 as the liquid phase (Pilar and Graydon, 1973).

The more recent methods, which use sorbents for trapping organics, connect the trap to a GC systems via multiple-port gas sampling valves. The trap is quickly heated and the desorbed organics are passed through the chromatographic columns. Since the collected samples contain a multitude of organics, capillary columns are normally used for the resolution of the organics. The Grob and Grob (1971) technique, involving the passage of the thermally desorbed organics through a small uncoated section of the capillary column cooled crypogenically, is used. When the collection is completed, this section of the capillary is quickly heated and the sample is separated on the remaining portion of the analytical column. A number of coating materials for capillary columns including Emulphor ON-870 (Holzer et al., 1977), UCON 50 HB 2000 or 5100 (Johansson, 1978), dinonyl phthalate (Isodorov et al., 1977), Al_2O_3 (Schneider et al., 1978), DC-550 (Louw and Richards, 1975), OV-17 and OV-101 (Pellizzari et al., 1976) have been used.

In one method, the method of thermal desorption of organics from the sorbents was replaced by solvent desorption (Burghardt and Jeltes, 1975). In this procedure, the organics sorbed on activated carbon were desorbed by CS_2. A part of the CS_2 was injected into a packed column GC containing a long column coated with 1,2,3-tri-(2'-cyanoethoxy)-propane.

The quantification of toluene separated by the GC columns is almost exclusively done by flame ionization detectors (FID). Confirmation of the authenticity of the GC peaks is often provided by coupled mass spectrometer (MS), with or without the aid of a computerized data system (Holzer et al., 1977; Pellizzari et al., 1976).

A continuous automated procedure for the determination of toluene in the ambient air was developed by Hester and Meyer (1979). This method needs no sample preconcentration prior to analysis. In this method, a small diaphragm pump activated by a timer automatically injects air into 1-ml gas-sampling (GS) loop of a GC every 10 minutes. The separating column was packed with Chromosorb P coated with N,N-bis(2-cyanoethyl)formamide. Since no concentration method was employed, the detector used had about two orders of magnitude higher sensitivity than flame ionization detectors. A photoionization detector was found to show the required sensitivity.

8.1.1.3 Preferred Method

The preferred method for the monitoring of toluene in ambient air consists of sorbent collection, thermal elution, and GC-FID determination. Collection by trapping toluene in a solid sorbent provides a concentration method during sample collection. Thermal desorption is preferred over solvent elution because of the higher sensitivity of the former method. Tenax GC is perhaps the most suitable sorbent for sample collection. The collection and thermal desorption efficiency of toluene is excellent with Tenax GC. The generation of artifacts during thermal elution with Tenax GC can largely be eliminated by proper clean up of the sorbent and GC conditioning procedure (Holzer et al., 1977). The greatest advantage of the ambient sorption-thermal elution method is its extreme simplicity and speed.

The separation and quantification of sorbent desorbed components can be achieved by GC-FID method. Although photoionization detectors (PID) may have higher sensitivity than flame ionization detectors, this higher level of sensitivity is not required when the samples are preconcentrated by solid sorbents. High resolution capillary columns are a necessity because of the observed complexity and low concentration of the samples. Of the different coating materials available, N,N-bis-(2-cyanoethyl)formamide and 1,2,3-tris(2-cyanoethoxy)-propane are probably most suitable for the separation of aromatic components.

8.1.1.4 Detection Limits

The detection limit of toluene in ambient air is dependent on the volume of air passed through the sorbent trap. For a 25-l sample, the detection limit is less than 0.1 ppb (Holzer et al., 1977) with a capillary column and flame ionization detector. When direct injection (1 ml) and GC-PID method are used, the detection limit for toluene is 0.3 ppb (Hester and Meyer, 1979).

8.1.2 Occupational Air

8.1.2.1 Sampling

The concentration of toluene in occupational air is normally much higher than in ambient air. Therefore, collection of samples in certain instances may not require a concentration step. The collection of samples by the grab method has been used by a number of authors (Tokunaga et al., 1974; Chovin and Lebbe, 1967).

Some of the earlier methods used liquid scrubbers for absorbing toluene from occupational air. A number of scrubbers, including potassium iodate in dilute sulfuric acid (Ministry of Labour, 1966), cooled organic solvents such as ethyl cellusolve acetate, dimethylformamide, and dimethyl sulfoxide in dimethyl formamide (Ogata et al., 1975), and nitrating solution (Chovin and Lebbe, 1967) have been used. In addition to the inherent limitations in its ability to overcome

the interferences, this method is not convenient for the collection of breathing zone samples.

The more recent methods used solid sorbents for the collection of toluene. Silica gel (Ogata et al., 1975; Tokunaga et al., 1974), activated carbon (Esposito and Jacobs, 1977; Fracchia et al., 1977; Reid and Halpin, 1968; Fraser and Rappaport, 1976; NIOSH, 1977) and Tenax GC (Nimmo andd Fishburn, 1977) are some of the sorbents used for this purpose. Aromatic hydrocarbons such as toluene are easily displaced from silica gel by water vapor, resulting in possible losses of toluene in humid atmospheres (NRC, 1980). Therefore, both activated carbon and Tenax GC are the two most frequently used sorbents for the collection of toluene from occupational air. The suitability of either of the sorbents is dictated by the method of sample analysis. When thermal desorption is used, Tenax GC is the preferred sorbent. On the other hand, activated carbon is preferred when solvent desorption is the method used.

8.1.2.2. Analysis

For grab samples, direct injections into a GC system via syringes or gas sampling loops have been applied (Tokunaga et al., 1974; Chovin and Lebbe, 1967). The separating columns used in these cases were packed columns with stationary liquid phases of either dioctyl phthalate (Tokunaga et al., 1974) or bis-(beta-cyanoethyl)formamide (Chovin and Lebbe, 1967). Flame ionization detectors were used for the quantification of toluene in both cases; however, this method is capable of analyzing toluene in work atmosphere at concentrations of around 10 ppm (Chovin and Lebbe, 1967).

Toluene collected by scrubber methods is usually analyzed by colorimetric methods. Irrespective of the different variations, most colorimetric methods show interferences from other chemically similar compounds (e.g., benzene, xylenes, ethylbenzenes) that are normally cocontaminants of toluene.

The first step in the analysis of toluene collected in solid sorbents is desorption. Two methods are usually available for desorption: thermal and solvent. Carbon disulfide is the most frequently used solvent for the desorption of toluene from solid sorbents (Esposito and Jacobs, 1977; Fracchia et al., 1977; Reid and Halpin, 1968; NIOSH, 1977; Van Ert et al., 1950), although some investigators have used other solvents (Ogata et al., 1975). Solvent desorption is the method of choice when activated carbon is used as the sorbent. Activated carbon has not only high efficiency of reversible toluene sorption, but it has almost quantitative toluene desorption efficiency with CS_2 (Fracchia et al., 1977). In the presence of other common organic solvents found in the work atmosphere (e.g., n-butanol, cellosolve acetate, butyl cellosolve, etc.), the CS_2 extraction efficiency decreases slightly, but addition of 5% methanol to CS_2 increases the desorption efficiency to almost quantitative value (Fracchia et al., 1977).

When Tenax GC or Chromosorb 102 is used as the sorbent, elution by thermal process is the method of choice (Nimmo and Fishburn, 1977). Although this method may require multiport sampling valves and a cyrogenic sample trap for the transfer of samples from the sorbent trap to the GC system, it has certain advantages not available to solvent elution. Since this method utilizes the whole sample for quantification, it has higher sensitivity than the thermal desorption method.

The quantification of toluene eluted from solid sorbents is almost always done by GC-FID method. A number of packed GC columns have been used for this purpose. Dioctyl phthalate (Tokunaga et al., 1974), UCC W-982 (Nimmo and Fishburn, 1977), N,N-bis(2-cyanoethyl)formamide (Esposito and Jacobs, 1977), dinonyl phthalate (Ogata et al., 1975), and Porapak Q (NIOSH, 1977) are some of the liquid phases used for chromatographic separations.

Other methods of analysis, such as high pressure liquid chromatography (HPLC) on a reverse phase column with methanol-water as the mobile phase and ultraviolet (UV) detection, have been attempted (Esposito and Jacobs, 1977), but the sensitivity of detection was poor.

Methods involving the use of detection tubes have been applied for the determination of toluene in occupational air (Tokunaga et al., 1974). The accuracy of the detector tubes for toluene quantification is rather poor, particularly in the presence of other organic vapor (Tokunaga et al., 1974). Therefore, the detector tubes are suitable for the rough estimation of toluene concentration in the work atmosphere.

A simple directly-combined GC-IR (infrared) system was developed to detect low molecular weight hydrocarbons in air (Louw and Richards, 1975). In this method, the grab sample is directly injected into a GC and the effluent from the GC column is split in a certain ratio (1:49). The major portion of the effluent is directed toward a cold trap (-50°C) to freeze the organics. At the end of the trapping process, the trap is quickly heated and the released gases are allowed to pass through a microlight pipe gas cell of an IR detector. This method has been claimed to detect 14-19 μg of each sample component present in air (Louw and Richards, 1975); however, no field samples have been analyzed with this system.

8.1.2.3 Preferred Method

The preferred method for monitoring toluene in occupational air can be either the NIOSH (1977) method of activated carbon sorption and CS_2 desorption or Tenax GC sorption and thermal desorption. The quantification of desorbed toluene by GC-FID is still the method of choice. As in the case of ambient air samples, N,N-bis(2-cyanoethyl)formamide liquid phase will provide one of the best separations for the aromatics.

8.1.2.4 Detection Limit

The detection limit for toluene by carbon sorption-CS_2 desorption method depends on the volume of air sampled. Concentrations as low as 0.1 ppm toluene in a rubber tire manufacturing factory have been detected by this method (Van Ert, 1980). For a 100-ml sample, the Tenax GC sorption-thermal desorption method showed a detection limit of 0.5 ppb (Nimmo and Fishburn, 1977).

8.1.3 Forensic Air

In suspected arson cases, the method of Twibell and Home (1977) can be applied to speculate or even confirm the cause of fire. According to this method, nickel wires (curie point 358°C) coated with finely-divided activated carbon with the aid of an inert adhesive (cement binder LQ/S6) are suspended in the atmosphere under test for 1-2 hours at room temperature. The apparatus is connected to a GC-FID system, and the wires are heated by induction heating. The resulting chromatographic profile obtained from the desorbed gases can be compared with different fire accelerant residues (e.g., gasoline). Although the method is not quantitative, it has been claimed to show a better sensitivity than the method of hot headspace analysis (Twibell and Home, 1977).

8.1.4 Gaseous Products from Pyrolysis of Organic Wastes

The gaseous products from a pilot plant burning such organic wastes as wood shavings, solid municipal wastes, and rice hull were analyzed by Brodowski et al. (1976). The method consisted of collecting grab samples in stainless steel sampling bulbs and injecting 0.5 ml of the gas into a GC. The separating columns were dual stainless steel columns packed with Porapak QS modified with terephthalic acid. Evidently, the method does not have high sensitivity of detection. The toluene concentration of the pilot plant gaseous products was determined to be 0.2 to 0.3 mol % by this method (Brodowski et al., 1976).

8.2 WATER

Toluene has been determined in a number of aqueous media including surface waters, industrial wastewaters, water from publicly-owned treatment works (POTW), underground water, drinking water, and rainwater.

8.2.1 Sampling

Water samples other than industrial wastewater samples are generally collected by the grab method. In the case of industrial discharges where the discharge parameters are dependent on the operating process, continuous samples using a commercial composite sampler have been used (Rawlings and Samfield, 1979). The preservation and handling of the aqueous samples after collection are especially important for volatile components. The samples are collected in glass bottles that are filled to overflow and sealed with teflon-backed silicon rubber septa and screw caps. It has been demonstrated that simple samples in non-reactive matrix (e.g., drinking water, ground water) collected in the above fashion can be held under ambient conditions from 10 to 22 days without significant loss of volatile compounds (Bellar and Lichtenberg, 1979); however, wastewater samples should be adjusted to a pH of 2 by adding dilute hydrochloric acid. Any free chlorine should be neutralized by the addition of 35 mg of sodium thiosulfate per 1 ppm of free chlorine (Federal Register, 1979) before the samples are collected in glass bottles. The samples must be iced or refrigerated during transportation and storage. All such wastewater samples should be analyzed within 7 days of collection (Federal Register, 1979).

8.2.2 Analysis

Although direct injection (Jungclaus et al., 1978) and solvent extraction (Yukiho and Terumi, 1977; Jungclaus et al., 1976) methods have been used to determine the concentration of organics including toluene in industrial wastewaters, these methods are not suitable for toluene determination in other media.

Even in wastewater, both of these methods have questionable accuracy. The direct aqueous injection method does not have good sensitivity and the solvent extraction method is likely to provide low recovery since some of the volatile components will be lost during the concentrative evaporating step.

The three most commonly used methods for toluene analysis in aqueous media are (1) purge and trap, (2) headspace, and (3) sorption on solid sorbents. Each of these methods is individually discussed below.

8.2.2.1 Purge and Trap

Purge and trap is the most widely used method for the analysis of toluene in aqueous media. It has been used for the determination of toluene in drinking waters (Bertsch et al., 1975; Lingg et al., 1977; Ryan and Fritz, 1978), in wastewaters (Bellar and Lichtenberg, 1979; Rawlings and Samfield, 1979; Jungclaus et al., 1978), and in rainwater (Seifert and Ullrich, 1978). The U.S. Environmental Protection Agency recommends the use of this method for toluene analysis in wastewater (Federal Register, 1979).

In this method, an inert gas (helium) is bubbled through a water sample via a glass frit contained in a specially designed purging chamber. The aromatics released into the vapor phase are swept through and trapped in a sorbent tube. After the purging and trapping is completed, the trap is transferred to the injection port of a GC. The trap is heated and backflushed into a GC system, where the separation of the volatiles takes place. Both packed (Bellar et al., 1979; Lingg et al., 1977; Federal Register, 1979) and capillary columns (Dowty et al., 1979; Bertsch et al., 1975) employing a variety of liquid phases have been used. The resolution of components can be expected to be better with capillary columns.

The detection of the GC column effluents can be done either by flame ionization detector (Dowty et al., 1979) or photoionization detector (Federal

Register, 1979). The use of photoionization detector will provide better selectivity and sensitivity of detection. The confirmation of GC peaks is usually provided by mass spectrometry aided by a computerized data system (Lingg et al., 1977; Dowty et al., 1979; Bellar et al., 1979).

A number of variations of the purge-trap method (Grob and Zucher, 1976; Lingg et al., 1977; Dowty et al., 1979; Bellar et al., 1979) involving the variation of water volume, the temperature of the purging system, the stripping rate, the duration of stripping, the nature of sorbent, and the method of desorption (thermal versus solvent) are available. Using a 5-ml sample size and flame ionization detection, Dowty et al. (1979) determined the lower detection limit for toluene to be 0.1 ppb by this method. The detection limit can be further lowered if a larger volume of sample (Lingg et al., 1977) or photoionization detection method is used. The purge-trap method is the preferred method for the monitoring of toluene both in drinking and wastewater samples.

8.2.2.2 Headspace Analysis

This method has not been frequently applied for the analysis of field samples; however, the method was standardized with water samples spiked with model compounds (Vitenberg et al., 1977; Drozd et al., 1978).

In the method of Drozd et al. (1978), a known volume (50 ml) of water is introduced into a specially designed enclosed glass apparatus (100 ml) and the system is thermostatically maintained at 40°C. After the system attains equilibrium (30 minutes), a known volume of headspace vapor is introduced into a capillary GC system via a trapping system consisting of a short cooled (-70°C) precolumn coated with OV-101 (Grob and Grob technique). The separating colum was coated with squalene.

The method of headspace analysis in the past had faced problems owing to the difficulty in establishing a calibration procedure. The partition coefficient

of a component between gas and liquid phases is dependent on the total ionic strength in solution. Therefore, the same concentrations of a component present in two aqueous solutions of different ionic strengths but otherwise identical conditions, will not produce the same equilibrium vapor pressure. This problem of a calibration curve has been largely obviated through the development of a standard addition method (Drozd et al., 1978). Water samples containing toluene in the parts per billion range can be quantified by this method (Drozd et al., 1978) with a reasonable accuracy; however, the method may not be applicable for drinking water samples where the concentration may be lower than 1 ppb.

8.2.2.3 Sorption on Solid Sorbents

This method is rarely used for the monitoring of toluene in aqueous samples. The applicability of the method was explored by Pfaender (1976), and Ryan and Fritz (1978) utilized the method for monitoring toluene in drinking water.

The method consists of passing a known volume of water through a sorbent such as XAD-2 (Pfaender, 1976) or XAD-4 (Ryan and Fritz, 1978). The sorbed organics including toluene are desorbed either by solvent extraction (Pfaender, 1976) or by thermal desorption (Ryan and Fritz, 1978) and injected onto a GC-FID system for component separation and quantification. In the thermal desorption method of Ryan and Fritz (1978), the use of a trap consisting of a Tenax GC precolumn to eliminate the excess water showed a good sensitivity for the method. The recovery of toluene was nearly 90% when the concentration in drinking water ranged from 1-10 ppb. For the quantification of toluene in water by this method, the recovery of toluene from the sorbent should be known.

8.3 SOILS AND SEDIMENTS

8.3.1 Sampling

Bottom sediment samples can be collected either by Hopper-dredge or by clam-type dredge samplers (U.S. EPA, 1979). Hopper-dredge collected samples

generally contain more water than clam-type dredge-collected samples. Bottom sediment samples can also be collected using a core sampler (U.S. EPA, 1979).

For volatile organic analysis, the samples should be collected in screw-capped glass containers lined with aluminum foil (Jungclaus et al., 1978) or in glass hypovials with crimped aluminum seals and teflon-backed septa (U.S. EPA, 1979). For best results, the container should be filled to maximum capacity to reduce the amount of headspace and should be transported and stored at wet ice temperature (U.S. EPA, 1979).

The method of soil sampling is given in detail by de Vera et al. (1980). The soil samples should be taken in a grid pattern over the entire site. A scoop can be used for collection of soil samples up to 8 cm deep. To sample beyond this depth, a soil auger or Veihmeyer soil sampler, as described by de Vera et al. (1980), should be used. After the sample is transferred into glass containers to a maximum capacity, the container must be tightly capped with contamination-free lids to prevent loss of volatile components and to exclude possible oxidation. The samples should be refrigerated (4°C) during transport and storage.

8.3.2 Analysis

Very few reliable methods are available for the analysis of volatile organics in soil and sediment samples. Solvent extraction methods using highly volatile solvents are not likely to be successful. The evaporative concentration step of this method would result in the loss of volatile organics. Headspace analysis, which has few provisions to concentrate the organics, will produce unreasonably high detection limits.

A modification of the purge and trap method has been suggested by the U.S. EPA (1979) for the analysis of soil and sediment samples. The modified purge and trap apparatus used for this purpose is described by the U.S. EPA (1979). The sample, contained in a specially-designed glass vial, is heated at 80°C and

purged with helium gas. The desorbed organics are trapped in a Tenax GC column. At the end of trapping, the Tenax GC column is inserted in the injection port of a GC, and the thermally desorbed organics are analyzed by GC-FID as in the case of water and wastewater samples. The recovery of toluene was determined to vary between 32% and 44% when 0.1 µg to 3.0 µg of toluene was spiked onto a specially prepared soil matrix. Although the recoveries were low, they were found to be linear and reproducible (U.S. EPA, 1979). Data on spiked environmental samples showed much higher recoveries (80-100%).

With the purge-trap system described, the minimum detection limit of 0.1 ppb can be attained. Thus, the method showed at least two orders of magnitude higher sensitivity than headspace analysis (U.S. EPA, 1979).

8.4 CRUDE OIL AND ORGANIC SOLVENTS

Benzene and toluene concentration in petroleum crude and other fossil fuel samples can be determined by a method developed by Grizzle and Coleman (1979). In this method, the sample is directly injected into a GC system containing two columns in series. The effluent from the first column containing aromatics is separated into individual fractions by the second column. Quantification of the separated components is done by a flame ionization detector.

A combination of liquid chromatography (silica gel column) and GC-FID method was employed by Fett et al. (1968) routinely to determine toluene in hydrocarbon solvents.

8.5 BIOLOGICAL SAMPLES

Toluene or its metabolites have been determined both in blood and in urine samples. These methods of analysis are discussed below.

8.5.1 Blood

Toluene in blood has been determined by GC analysis of headspace samples (Premel-Cabie et al., 1974; Anthony et al., 1978). According to this method,

blood is equilibriated with air in a closed container at a fixed temperature. The headspace gas is injected into a GC-FID system for detection of toluene. The method can be used for quantification of toluene in blood by the standard addition method as described in subsection 8.2.2.2.

8.5.2 Urine

In the body, toluene is mainly oxidized to benzoic acid which, after conjugation with glycine, is eliminated as hippuric acid in the urine. Hippuric acid may be formed from other metabolic processes besides toluene metabolism.

Hippuric acid in urine can be determined by a number of methods including colorimetry (Umberger and Fioresse, 1963) and UV spectrometry (Pagnatto and Lieberman, 1967); however, one of the better methods of hippuric acid analysis in urine was developed by Caperos and Fernandez (1977). According to this method, the hippuric acid in acidified urine is extracted with ethyl acetate. The extracted hippuric acid is esterfied with 1-p-tolyltriazene. The dried ester is dissolved in chloroform and quantified by GC-FID. The recovery of hippuric acid by this method is determined from the recovery of an added internal standard. The sensitivity of the method with 0.5 ml urine was determined to be 5 mg/l.

8.6 FOODS

A headspace GC technique for quantification and a GC-MS technique for confirmation were used to determine trace amounts of toluene in plastic containers (Hollifield et al., 1980). The sample, taken in a specially enclosed vial, was heated at 90°C for 2 hours and 2 ml of headspace gas was injected into a GC system. The principle of standard addition was used for the quantification of toluene. Toluene present in parts per billion range can be determined by this method.

8.7 CIGARETTE SMOKE

The concentration of toluene both in sidestream smoke (Jerimini et al., 1976) and mainstream smoke (Dalhamn et al., 1968) has been determined. For the determination of toluene in mainstream smoke, standard cigarettes were smoked by machine under standardized conditions (a 2-second 35-ml puff once every minute). The mainstream smoke is collected in a cold trap (Dalhamn et al., 1968). The contents of the cold trap can be introduced into the GC by multiport valves and analyzed by GC-FID for toluene determination.

Toluene determination in sidestream smoke can be accomplished by adopting the sampling and analysis technique of Holzer et al. (1976). The sidestream smoke can be collected by drawing the smoke through a solid sorbent tube packed with Tenax GC. The Tenax GC sorbent tube can be thermally eluted onto a glass capillary column for the determination of toluene content. Adoption of a cold trap for splitless injection of the sample into the capillary column (Grob and Grob technique) will enhance the sensitivity and accuracy of the method. Additional confirmation of the GC peaks can be done by interfacing the GC with a MS (Holzer et al., 1976).

9. EXPOSED POPULATIONS

The number of people exposed to various sources of toluene can be divided into three categories, namely, general population, occupational group, and cigarette smokers. The breakdown of general population subjected to inhalation exposure of toluene from various sources of emissions can be obtained by performing a population analysis around each source. A computer program was used by Anderson et al. (1980) to extract site-specific population patterns from the U.S. Census figures standardized to 1978 population levels. The number of general population exposed to various levels of toluene from different sources of emission as calculated by Anderson et al. (1980) is shown in Table 9-1. For an explanation of the breakdown of the source variety shown in Table 9-1, see subsection 8.1.1.

The exposed population count shown in Table 9-1 is derived from the geographical coordinate of each location. Error in the geographical coordinates of a source and population center will cause errors in population count. In addition, the population count figures obtained from U.S. Census Bureau is subject to undercounting. The result of this undercounting will be lower population exposure estimates than the actual case.

No estimate of the number of general population exposed to toluene from ingestion of foods and drinking waters can be given. Toluene has been detected in only a small fraction of total drinking water supplies and foods that have been monitored. The number of people consuming the contaminated waters and foods is not known at the present time.

According to the estimate of the Department of Health, Education, and Welfare (1977), more than 4.8 million people per year are occupationally exposed

Table 9-1. Population Distribution and Inhalation Exposure Levels of Toluene from Different Sources (Anderson et al., 1980)

Concentration Level	Number of People Exposed From: Specific Point Sources	Prototype Point Sources	Area Sources
>100	0	159	58,347
100 - >50	0	2,841	446,793
50 - >25	34	10,200	12,348,504
25 - >10	475	22,700	42,478,913
10 - >5	1,434	33,900	66,368,769
5 - >2.5	6,103	75,200	0
2.5 - >1	19,781	240,000	0
1 - >0.5	39,064	246,000	0
0.5 - >0.25	95,883	350,000	0
0.25 - >0.1	269,883	1,229,000	0
0.1 - 0	34,316,299	0	34,977,809
Subtotals	34,748,633	2,210,000	158,679,135
Total	195,637,768		

to toluene. Toluene ranks fourth among all other agents listed in terms of the number of people exposed to any single agent.

The number of people in the U.S. exposed to toluene through cigarette smoke has been estimated to be 56 million during the year 1978[a]. This figure which considers the exposure to the smokers only, is bound to be an underestimate since it does not include passive smokers.

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[a]This figure is based on the following assumptions of the total population of 225 million, 21.4% are under age 13 (Dept. Commer., 1979) and do not smoke. Teenagers in the age group 13 years to 17 years constitute 7.6% of the total population (Dept. Commer., 1979). Of the 7.6% of the teenagers, only 11.7% are assumed to be smokers (PHS, 1980). Of the remaining population, 51% are assumed to be females and 49% to be males (Dept. Commer., 1979). The percent of female and male smokers over age 17 are assumed to be 30.4% and 37.4%, respectively (PHS, 1980).

10. INTEGRATED EXPOSURE ANALYSIS

Exposure is the contact between a subject of concern and an agent such as a chemical, biological, or physical entity. The magnitude of the exposure is determined by measuring or estimating the amount of an agent available at the exchange boundaries, that is, lung, gut, and skin, during some specified time. Exposure assessment is the qualitative estimation or quantitative determination of the magnitude, frequency, duration, and route of exposure. Exposure assessments are often combined with environmental and health effects data in performing risk assessments. The exposure of an agent may lead to the intake of some of the agent. Uptake or an absorbed dose is the amount of the intake which is absorbed by the subject.

The assessment of human health risks from exposure to any environmental pollutant requires knowledge of (1) the dosage of the pollutant received by the exposed human population and (2) the effect of the pollutant on human health. Because the purpose of this section is not to develop a health effects model, no attempt will be made to address such parameters as population characteristics (e.g., age, sex, occupation, racial background), population habits (e.g., food habits, recreational habits, product-use habits), and population groupings (e.g., the aged, pregnant women, children, other high health risk groups). Instead, this section will attempt to derive the human exposure of toluene received from all sources of emissions.

In order to make an exposure asessment, one must consider the following: route of entry; magnitude of exposure; frequency of exposure; and duration of exposure. The general population may be exposed to toluene through the following three routes: (1) inhalation of air; (2) ingestion of water and foods; and (3) exposure through skin. The next step toward an integrated exposure analysis

combines the estimation of environmental concentrations with the description of the exposed population to yield exposure profiles and exposure pathway analysis.

Certain segments of population may be exposed to toluene through occupational exposure and cigarette smoking. Because exposure of this segment of the population falls under a special category, these scenarios will be discussed separately. It should be mentioned that this section does not include toluene exposure from the use of consumer products. As has been mentioned in Subsection 10.5, some consumer products contain high percentages of toluene. Undoubtedly, the use of these consumer products would lead to various degrees of toluene exposure in the general population; however, no data are available from which estimates of toluene exposure from consumer products could be derived.

10.1 EXPOSURE VIA INHALATION

Estimation of toluene exposure via inhalation can be done in two ways. The exposure can be estimated from the total nationwide toluene emission data by the use of mathematical models simulated to reflect the actual environmental setting. The exposure can also be estimated from actual monitoring data. Estimating exposure on the basis of monitoring data is often a preferred method because these data directly provide the environmental distribution of toluene; however, this method has its own limitations. Although the monitoring data available for toluene are more abundant than those available for many other organic chemicals, they do not include many exposure scenarios. The monitoring data may not provide information on the extent of concentration variation due to chemical reactivity (e.g., photoreaction, oxidation in the atmosphere, etc.). These data also do not yield relationships between materials balance of the emitted toluene and the environmental concentration distribution in an area. Therefore, the approach toward exposure estimation in this section has utilized

both the available ambient monitoring data and the theoretical dispersion modeling of toluene emission data.

10.1.1 Theoretical Modeling

The estimation of inhalation exposure to toluene among different segments of the general population involves the following computational tasks: (1) estimation of annual average toluene concentration in the air at different distances from the emission sources and (2) estimation of the population distribution around each source of emission (available through the U.S. Census Bureau). This part has already been discussed in Section 7.

The performance of the first task requires the following data: (1) emission inventories of toluene, which are already available (see Subsections 10.4.1 and 10.4.4); (2) atmospheric reactivity of toluene; (3) meteorological data, which are available through the U.S. or local weather bureau; and (4) a dispersion equation to estimate concentration distribution of toluene.

Toluene concentration downwind from a source can be estimated using the following dispersion equation of Turner (1969):

$$C(X,0,0) = \frac{Q}{\pi\sigma_y\sigma_z U_w} \quad \exp \quad \frac{-h^2}{2\sigma_y^2}$$

where

$C(X,0,0)$ = concentration of toluene at various x coordinates and at zero y and z coordinates (mg/m^3)

Q = emmission rate (mg/s)

σ_y = horizontal dispersion coefficient of the plume concentration distribution

σ_z = vertical dispersion coefficient of the plume concentration distribution

U_w = wind speed (m/s) (w = the heat of the source)

h = the effective stack height; i.e., the sum of the stack height and plume rise (m)

Assuming U_w = 5 m/s; Q = 200 x 10^6 kg/year = 6.34 x 10^3 mg/s; plume height = 10 m and 20 m; and the values of σ_y and σ_z from the following equation (Anderson et al., 1980):

$$\sigma_z \text{ (m)} = 0.06x(1 + 0.0015x)^{-1/2}$$

$$\sigma_y \text{ (m)} = 0.08x(1 + 0.0001x)^{-1/2}$$

one can calculate the concentration of toluene at different distances from the source, as given in Table 10-1.

The calculations of the values in Table 10-1 for toluene distribution from a stationary source do not consider the chemical reactivity of toluene in the atmosphere and the effect of plume temperature on the concentration distribution of toluene. A more detailed calculation that incroporates these two variables, as well as building wake effect (enhanced dispersion due to buildings), has been made for the estimation of spatial concentration of toluene from the major stationary and mobile sources of toluene emission (Anderson et al., 1980).

The dispersion equation developed by Anderson et al. (1980) was used to compute annual average concentration pattern of toluene from each point source. A computer program was used to evaluate these concentration patterns from the given meteorological and emission data. Because there are numerous sources of emission, the sources were divided into three types, which are defined below.

Specific Point Sources: These sources were treated using parameters appropriate to each source. These sources included emissions from production sources and from chemical intermediate users.

General Point Sources: For such sources, a prototype analysis was done and the results were multiplied by the estimated number of sources. These sources included emissions from gasoline marketing, from the coke-oven industry, and from isolated and non-isolated toluene producers (not included in the previous categories).

Table 10-1. Concentration of Toluene (mg/m^3) at Different Distances (m) From A Source Emitting 200 Million kg/Year Toluene (Slimak, 1980)

Plume Height (m)	100	500	1,000	1,500	5,000	10,000
10	1.36	0.45	0.15	0.12	0.02	0.01
20	0.003	0.31	0.13	0.10	0.02	0.01

Area Sources: Such sources were treated as emission per unit area over identified areas. These sources included mobile emission, emission from solvent use, and emissions from miscellaneous sources.

The three equations used to calculate the spatial concentration distribution of toluene from all sources are given in considerable detail in Anderson et al. (1980); interested readers are referred to that document. The final results of the calculations of Anderson et al. (1980) led to the estimate of spatial concentration range of toluene around different sources of emissions. These values are given in Table in Section 7.

Anderson et al. (1980) listed the following factors that could cause uncertainties in their calculated exposure levels given in Table 10-2:

Emission Estimates Errors: Some of these are (1) error in the estimates of production and use of toluene; (2) the assumption that all plants operate at the same capacity; (3) omission of certain emission sources; (4) error in derivation of emission factors and, in certain cases, the use of a uniform emission factor, which implies that all these plants have similar emission controls. It is difficult to project whether the emission estimates used by Anderson et al. (1980) will lead to higher or lower exposure estimates. This can be done, however, by comparing these estimates with the experimentally determined concentration patterns obtained from sources that are reasonably isolated from other sources.

Concentration Pattern Errors: The concentration patterns used in the exposure computations were obtained through atmospheric dispersion modeling. Any deviations in these estimates from the true pattern directly affect the exposure results. Many assumptions were used in calculating the concentration distribution. The exposure errors will be more severe in the case of prototype point sources where a prototype model was used for cal

Table 10-2. Population Distribution and Inhalation Exposure Levels of of Toluene From Different Sources (Anderson et al., 1980)

Concentration Level ($\mu g/m^3$)	Number of People Exposed From		
	Specific Point Sources	Prototype Point Sources	Area Sources
>100	0	159	58,347
100 - >50	0	2,841	446,793
50 - >25	34	10,200	12,348,504
25 - >10	475	22,700	42,478,913
10 - >5	1,434	33,900	68,368,769
5 - >2.5	6,103	75,200	0
2.5 - >1	19,781	240,000	0
1 - >0.5	39,064	246,000	0
0.5 - >0.25	95,560	350,000	0
0.25 - >0.1	269,883	1,229,000	0
0.1 - 0	34,316,299	0	34,977,809
Subtotals	34,748,633	2,210,000	158,679,135
Total		195,637,768	

culating exposure from all other similar sources. The same can be said about the exposure estimates from area sources where a box model method that incorporated a number of uncertainties was used.

Interpolation Errors: The interpolation of population and concentration patterns used to develop patterns of exposure can introduce errors.

With the available information, it is not possible to quantify any of the errors described above. The theoretical model may provide qualitative insights in certain instances to predict whether the exposure estimate is either too high or too low compared to the actual values.

10.1.2 Inhalation Exposure Based on Monitoring Data

Exposure of the general population to toluene by inhalation can occur under a wide range of exposure scenarios. Because it may be considered impractical to measure toluene concentration from all possible exposure scenarios, an attempt has been made to develop a few of the most prevalent exposure scenarios.

The four largest sources of toluene emission, in descending order, are automobile use (exhaust emission, engine evaporative loss, gasoline marketing evaporative loss); industry sites using toluene as a solvent; coke oven sites, and toluene production sites (see Subsection 10.4.4). In place of dispersion modeling, one can use the monitoring data from each of the four sites to evaluate the four different exposure scenarios. The difficulty with this approach is that the available monitoring data were often developed for sites with various degrees of intermixing between these exposure scenarios. Therefore, inhalation exposure has been classified under three scenarios--the urban areas; areas containing the user sites; and rural or remote areas. In this manner, the exposure estimates developed may be representative of a broad range of the possible exposure scenarios. It should be remembered that the urban areas may contain sites with

high automobile use, production and other manufacturing sites, and coke-oven sites.

Human exposure to toluene through inhalation of urban air is shown in Table 10-3. The concentration of toluene in urban areas in the United States in recent years ranged from 0.1 $\mu g/m^3$ to 204 $\mu g/m^3$ (see Table 7-1). The intake estimate is based on a breathing rate of 1.2 m^3/hour for an adult during waking hours and 0.4 m^3/hour during sleeping hours (Slimak, 1980). It is also assumed that the sleeping period for an adult is 8 hours/day. This results in an inspired volume of 1.2 x 16 x 7 + 0.4 x 8 x 7 = 156.8 m^3/week.

Near user sites, the range of toluene concentration has been assumed to be 5.5-600 $\mu g/m^3$. This range corresponds to the measured value of Sexton and Westberg (1980) near an automotive painting plant (see Subsection 7.1.1). The concentration of toluene at a distance 18 km from the plant measured 55.5 $\mu g/m^3$--a value 10 times higher than the background concentration (Sexton and Westberg, 1980). Therefore, even workers who commute more than 18 km from the plant are susceptible to inhale toluene concentration in the range of 5.5-600 $\mu g/m^3$ for the entire 168 hours in a week. The toluene concentrations near manufacturing sites range from 0.1 to 147 $\mu g/m^3$. The estimated toluene exposure range from the manufacturing and user sites shown in Table 10-3 is based on a concentration range of 0.1 to 600 $\mu g/m^3$.

In rural and remote areas, the concentration of toluene has been reported to be in the range of a trace to 3.8 $\mu g/m^3$ (see Table 7-1). These concentrations were determined in 1971. The current level may be lower than this range as indicated by the toluene concentration mentioned recently at Grand Canyon. The estimated toluene exposure in rural and remote areas is shown in Table 10-3.

It should be remembered that Table 10-3 shows the amount of toluene inspired per week by humans around certain exposure scenarios and not the amount absorbed.

Table 10-3. Toluene Exposure Under Different Exposure Scenarios

Scenario	Observed Range of Concentration	Frequency of Exposure	Total Volume Exposed or Amount Consumed	Inhalation or Ingestion Rate (mg/wk)
General Population				
Inhalation				
Urban areas	0.1-204 μg/m^3	168 h/wk	156.8 m^3	0.02-32
Rural and remote areas	trace-3.8 μg/m^3	168 h/wk	156.8 m^3	trace 0.6
Areas near manufacturing and user sites	0.1-600 μg/m^3	168 h/wk	156.8 m^3	0.02-94
Ingestion				
Drinking water	0-19 μg/l	2 l/d	14 l	0-0.3
Food	0-1 mg/kg	6.5 g/d	45.5 g	0-0.45
Occupational Group				
Inhalation	377,000 μg/m^3	40 h/d	48 m^3	18,100
Dermal	0-170 μg/l[a]	0-30 min/wk	5.9 l	0-1.0
Cigarette Smokers				
Inhalation	0.1 mg/cigarette	20 cigarettes/d	140 cigarettes	14

Abbreviations: h = hour; wk = week; d = day; min = minute.

[a]This value represents exposure to blood due to dermal contact.

Only a certain fraction of the toluene inhaled is absorbed by human organs. Also, part of the absorbed toluene is rapidly excreted from the body.

10.2 INGESTION EXPOSURE BASED ON MONITORING DATA

No theoretical modeling method is available for estimating toluene exposure from ingestion. Therefore, the exposure estimate from this source has been attempted by using the limited monitoring data that are available.

10.2.1 Exposure from Drinking Water

The concentrations of toluene in drinking water range from 0-19 µg/l (see Subsection 7.1.2.5). The concentration of toluene measured in well waters in New York State was below 10 µg/l (see Subsection 7.1.2.4). Therefore, a concentration range of 0 to 19 µg/l has been used for exposure assessment shown in Table 10-3. A consumption rate of 2 l/day has also been assumed for exposure asessment.

10.2.2 Exposure from Edible Aquatic Organisms

The concentration range of toluene in edible aquatic organisms has been assumed to be 0-1 mg/kg, based on the level of toluene found in fish tissues (Subsection 7.1.4). On the basis of these data and the assumption that the per capita consumption of aquatic organisms in the United States is approximately 6.5 g/day (Stephan, 1980), the exposure range of toluene from food is shown in Table 10-3.

10.3 OCCUPATIONAL EXPOSURE

Occupational exposure to toluene can take place from two scenarios--inhalation of air containing toluene, and skin contact with toluene or other solvent mixtures containing toluene. The concentration of toluene in the air of working atmosphere has been assumed to be 377,000 µg/m^3. This value corresponds to the OSHA (Occupational Safety and Health Administration) recommended workroom air standard of 100 ppm toluene vapor as a time-weighted average (TWA) exposure

for an 8-hour work day (OSHA, 1973). Based on the above assumptions, the inhalation exposure of toluene by occupational groups as shown in Table 10-3 far exceeds that for any other group.

Sato and Nakajima (1978) studied the absorption of toluene through human skin. These investigators immersed one hand of 5 male subjects in pure toluene for 30 minutes and monitored the blood levels of toluene. A peak concentration of 170 µg/l of blood was observed after a 30-minute immersion. This maximum concentration was maintained for 10-15 minutes after exposure had ended and decreased thereafter.

Although the standard set forth by OSHA (1973) requires all workers handling toluene to wear gloves, it is conceivable that short-term exposure of bare skin to toluene takes place under certain circumstances. For assessment of exposure through skin as shown in Table 10-3, a maximum concentration of 170 µg/l in blood and a blood volume of 5.9 l for an adult male have been assumed. It has also been assumed that the skin exposure duration does not exceed 30 minutes/week. It should also be recognized that the value for blood concentration through dermal contact given in Table 10-3 does not represent the total exposure value as it ignores exposure to other organs.

10.4 CIGARETTE SMOKERS

The concentration of toluene in inhaled cigarette smoke has been determined to be 0.1 mg/cigarette (see Subsection 7.3). In assessing toluene exposure from cigarette smoking, it was assumed that an individual smokes 20 cigarettes (per pack) per day. On the basis of these assumptions, it can be predicted from Table 10-3 that cigarette smoking may be the second largest source of human exposure to toluene.

10.5 LIMITATIONS OF EXPOSURE ASSESSMENT BASED ON MONITORING DATA

As discussed earlier, exposure assessment on the basis of monitoring data has the following limitations:

(1) The limited monitoring data do not provide information for estimating exposure under different exposure scenarios. Even when some data are available, they may be inadequate and even susceptible to error. It is very difficult to assess the errors in the monitoring data.

(2) The monitoring data often do not relate to the source of emissions in terms of material balancing of the amount emitted and the concentration measured.

(3) The population distribution around the monitoring area is rarely provided in these data.

(4) The estimate for toluene exposure to the general population from food and drinking water as given in Table 10-3 is very crude. Toluene has been detected in only a small fraction of total drinking water supplies monitored (see Subsection 7.1.2.5). The exposure estimate does not specify either the number of people or the locations where people are exposed to toluene from drinking water. The same can be said with respect to toluene exposure from food.

10.6 COMPARISON BETWEEN EXPOSURE DATA BASED ON THEORETICAL AND EXPERIMENTAL VALUES

If the concentration values ranging from 0 $\mu g/m^3$ to greater than 100 $\mu g/m^3$ are combined with the value of 156.8 m^3 for inspired volume of air per week, an inhalation exposure estimate as shown in Table 10-4 can be developed.

A comparison of inhalation exposure data shown in Table 10-4 which are based on dispersion equations, with inhalation exposure data in Table 10-3, which are derived from monitored concentrations, shows reasonable agreement between the two sets of data. The monitoring data estimate toluene inhalation by the general population in urban areas to be 0.02-32 mg/week. The exposure data developed from dispersion equations estimate this value to be in the range of zero to greater than 15.7 mg/week.

Table 10-4. Exposed Population and Exposed Amount of Toluene From Dispersion Modelling (Slimak, 1980)

Concentration Level ($\mu g/m^3$)	Exposed Concentration mg/week
>100	>15.7
100-10	15.7-1.6
10-1	1.6-0.15
1-0.1	0.15-0.02
0.1-0	0.02-0

11. EFFECTS ON HUMANS

Exposures of humans to toluene have almost exclusively involved inhalation, and the effect of greatest concern is dysfunction of the central nervous system. The exposures may be classified into three groups: occupational exposures, experimental studies and deliberate inhalation of toluene or toluene-containing substances ("glue sniffing"). It should be noted that occupational exposures and glue sniffing often involve complex mixtures of solvents, and that in the older studies, benzene was a common contaminant to toluene. In evaluating the effects of toluene exposures, the purity of the compounds used must be considered.

Glue sniffers inhale the vapors from a wide variety of volatile hydrocarbons (usually poorly defined mixtures) contained in products such as glues and thinners for their euphoric or intoxicating effects. The most popular of these products contain toluene, and toluene is the hydrocarbon most frequently implicated as the cause of the adverse effects associated with deliberate inhalation. The practice has been extensively reviewed (Massengale, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b; Gellman, 1968; Wyse, 1973; Linder, 1975; Faillace and Guynn, 1976; Oliver and Watson, 1977; Walter et al., 1977; Watson, 1979). Excessive levels of toluene are generally inhaled over a short time interval, and repeated inhalation of the vapors is associated with the development of tolerance and psychological dependence. The most common methods of inhalation involve (1) placing the solvent in a plastic bag and inhaling the fumes, (2) soaking a rag or handkerchief with the solvent and sniffing the rag, or (3) sniffing the solvent from a container. The concentrations of toluene inhaled under these conditions can approach 30,000 ppm (i.e., saturation concentration at 20°C), and may be regarded as a type of maximum tolerated dose.

11.1 EFFECTS ON THE NERVOUS SYSTEM

11.1.1 Central Nervous System

11.1.1.1 Acute Effects

Experimental exposures of up to 800 ppm toluene have produced acute dose-related symptoms of central nervous system (CNS) depression (Von Oettingen et al., 1942a, 1942b; Carpenter et al., 1944). Von Oettingen et al. (1942a, 1942b) provided what is generally acknowledged to be the most complete description of the effects of pure toluene (benzene $\leq$ 0.01%) on the CNS. In single 8-hour exposures, 3 human subjects were subjected to concentrations of toluene in an exposure chamber that ranged from 50-800 ppm (Table 11-1). A maximum of two exposures a week were performed over an 8-week period, and a number of these exposures were to pure air; exposures to the different levels of toluene were replicated only 1 to 4 times within the 8 weeks. The effects that were observed are also summarized in Table 11-1. Subjective complaints such as fatigue, muscular weakness, confusion, impaired coordination, and enlarged pupils and accommodation disturbances were reported at levels of 200 ppm. These effects increased in severity with increases in toluene concentration, until at 800 ppm the subjects experienced severe fatigue, pronounced nausea, mental confusion, considerable incoordination and staggering gait, strongly impaired accommodation to light, and after-effects (muscular fatigue; nervousness and insomnia) that lasted for several days.

Carpenter and coworkers (1944) exposed 2 male subjects to known concentrations of toluene (purity not stated) for periods of 7 to 8 hours and noted slight exhilaration at 200 ppm, and lassitude, nausea, and hilarity at 400 ppm. Lassitude, hilarity, verbosity, and boisterousness occurred at 600 ppm (anorexia and listlessness were reported as after-effects), and transitory headaches, extreme lassitude, scotomata (areas of depressed vision), verbosity, slight

Table 11-1. Effects of Controlled 8-hour Exposures to Pure Toluene on Three Human Subjects[a] (Von Oettingen et al., 1942a, 1942b)

Concentration	No. of Exposures	Effects
0 ppm (control)	7	No complaints or objective symptoms, except occasional moderate tiredness toward the end of each exposure, which was attributed to lack of physical exercise, unfavorable illumination, and monotonous noise from fans.
50 ppm	2	Drowsiness with a very mild headache in 1 subject. No aftereffects.
100 ppm	4	Moderate fatigue and sleepiness (3), and a slight headache on one occasion (1).
200 ppm	3	Fatigue (3), muscular weakness (2), confusion (2), impaired coordination (2), paresthesia of the skin (2), repeated headache (1), and nausea (1) at the end of the exposure. In several instances the pupils were dilated, accommodation to light was impaired, and the fundus of the eye was engorged. Aftereffects included fatigue, general confusion, moderate insomnia, and restless sleep in all 3 subjects.
300 ppm	2	Severe fatigue (3), headache (2), muscular weakness and incoordination (1), and slight pallor of the eyeground (2). Aftereffects included fatigue (3) and insomnia (1).
400 ppm	2	Fatigue and mental confusion (3), headache, paresthesia of the skin, muscular weakness, dilated pupils, and pale eyeground (2). Aftereffects were fatigue (3), skin paresthesia (1), headache (1), and insomnia (2).
600 ppm	1	Extreme fatigue, mental confusion, exhilaration, nausea, headache and dizziness (3), and severe headache (2) after 3 hours of exposure. After 8 hours' exposure, the effects included considerable incoordination and staggering gait (3), and several instances of dilated pupils, impaired accommodation and pale optic discs; aftereffects included fatigue and weakness, nausea, nervousness and some confusion (3), severe headache (2), and insomnia (2). Fatigue and nervousness persisted on the following day.
800 ppm	1	Rapid onset of severe fatigue and, after 3 hours, pronounced nausea, confusion, lack of self-control, and considerable incoordination and staggering gait in all 3 subjects. Also, accommodation to light was strongly impaired (1) and optic discs were pale (2). All 3 subjects showed considerable aftereffects, lasting at least several days, which included severe nervousness, muscular fatigue, and insomnia.

aExposures were twice weekly for 8 weeks. The number of subjects affected is noted in parentheses.

nausea, and "inebriation" were found at 800 ppm. Marked unsteadiness was also observed in the subjects during exposure to 800 ppm toluene. Steadiness was determined by a test that involved holding at arms' length a wire in a hole for 3 minutes; the percentage of time the wire was actually in contact with the side of the hole was determined, and compared with the normal value from each test session.

Short-term experimental exposures to toluene have also elicited increases in reaction time and reductions in perceptual speed (Ogata et al., 1970; Gamberale and Hultengren, 1972). Ogata and coworkers (1970) reported that 23 Japanese subjects given single exposures to 200 ppm toluene showed a prolongation of eye-to-hand reaction time, but no effect on flicker fusion frequency. Exposures were for 3 hours, or 3 hours and a 1-hour break period followed by 4 additional hours of exposure. No changes in either reaction time or flicker value were obvious at 100 ppm. It should be noted, however, that no other information regarding the design of these experiments was presented.

In a more extensive study, Gamberale and Hultengren (1972) exposed 12 male subjects to 100, 300, 500, or 700 ppm toluene (via breathing valve and mouthpiece) during successive 20-minute exposure periods, and measured their performance on four tests of perceptual speed and reaction time at each level of exposure (Table 11-2). The tests were always made in the same sequence (i.e., Identical Numbers, Spokes, Simple Reaction Time, Choice Reaction Time) during the final 15 minutes of each exposure period. Toluene concentrations were increased from 100 to 300 ppm and from 500 to 700 ppm without interruption, but the increase from 300 to 500 ppm was made following a 5-minute interval without exposure. Menthol crystals contained in the mouthpiece tubing camouflaged the taste and the smell of the toluene. The 12 subjects were divided into two groups of equal size: subjects in one group were studied individually, first under

Table 11-2. Effect of Toluene Exposure on the Performance of Perceptual Speed and Reaction Time Tests[a] (Gamberale and Jultengren, 1972)

Performance Test	Concentration (ppm)	Mean Test Scores: Experimental Conditions	Mean Test Scores: Control (Air) Conditions	t-Value
Identical Numbers[b] (minutes)	100	5.62	5.53	+0.50
	300	5.25	5.29	-0.39
	500	5.13	5.04	+1.34
	700	5.19	4.80	+2.65*
Spokes[c] (seconds)	100	50.5	50.8	-0.08
	300	46.7	43.7	+1.18
	500	43.6	40.2	+1.28
	700	45.4	36.9	+2.51*
Reaction Time - Simple[d] (meters/second)	100	228	230	-0.31
	300	236	222	+2.35*
	500	246	219	+3.88**
	700	253	214	+4.81**
Reaction Time - Choice[e] (meters/second)	100	425	422	+0.34
	300	429	416	+1.99
	500	432	400	+2.91*
	700	442	408	+3.59**

Degrees of freedom = 11; *P < 0.05; **P < 0.01; ***P < 0.001

[a] 12 male subjects were exposed to toluene concentrations of 100, 300, 500, and 700 ppm during four successive 20-minute periods. The tests were performed at each concentration sequentially in the order listed. The number of times each test sequence was repeated was not stated.

[b] Perceptual speed: Identical Numbers. Subjects were instructed to underline the 3-digit number , from a total of 60 columns, that was identical to the number at the head of each column. Performance was measured as the time taken to complete the test.

[c] Perceptual speed: Spokes. Subjects were instructed to connect circles located at random on four pages and numbered from 1 to 20 in the correct numerical order using a pen. Performance was measured as the mean time taken for the four assignments.

[d] Simple Reaction Time. Subjects were instructed to respond to a signal from a lamp by pressing a pushbutton. Stimuli were administered at intervals of approximately 10 seconds, an acoustic warning signal was given 3 seconds prior to onset of stimuli, and 30 stimuli were given in each trial. Performance was measured as the mean reaction time for the last 20 stimuli administered.

[e] Choice Reaction Time: Stimulus/reply test as above, but there were three pushbuttons equipped with matching stimulus lamps. Stimulus administration followed a random sequence with the number of light signals evenly distributed among the lamps, but the trial and performance measurements were otherwise the same as for simple reaction time.

experimental conditions with exposure and then under control (atmospheric air containing menthol) conditions 7 days later, and subjects in the other group were studied under similar conditions but in the reverse order. The camouflage of the inspiratory air with menthol made it impossible for 11 of the 12 subjects to distinguish between exposure to toluene and exposure to pure air.

Results of the Gamberale and Hultengren (1972) study showed that both reaction time and perceptual speed were impaired during exposure to toluene as compared to exposure to pure air (Table 11-2). With respect to reaction time, a significant effect was noted upon exposure to 300 ppm toluene in one test (Simple Reaction Time), and a performance decrement which reportedly approached statistical significance at the 0.05 level was noted for the other test (Choice Reaction Time). Subject reaction time was further impaired at higher levels of exposure (500 and 700 ppm toluene), but no impairment in either reaction time test was noted for exposure to 100 ppm. (The 100 ppm reaction time no-effect level is consistent with the aforementioned results of Ogata *et al.*, 1970.) No statistically significant impairment in subject perceptual speed was observed until the concentration of toluene in the inspiratory air was 700 ppm. Because perceptual speed was unaffected at concentrations below 700 ppm, the authors suggested that the simpler CNS functions may be affected at lower levels of toluene exposure than the more complex functions.

Wineke *et al.* (1976) noted, in the Proceedings of the 2nd International Industrial and Environmental Neurology Congress (Prague, Czechoslovakia), that experimental exposure to 98 ppm toluene for 3 hours did not affect psychophysiological performance in 20 subjects. The parameters evaluated in this study included performance in a bisensory (auditory and visual) vigilance task, psychomotor performance, critical flicker frequency, and auditory evoked potentials. It should be noted that the available meeting abstract did not provide

any additional information on the experimental design, the nature of the psychophysiological tests, or the results of this study.

Gusev (1965) examined the effects of acute low-level toluene exposure on the electroencephalographic (EEG) activity of 4 human subjects who were trained to develop synchronous and well-marked alpha rhythms when stimulated by light. Toluene exposures of 1 mg/m^3 (approximately 0.27 ppm) for 6 minutes were reported to cause statistically distinct changes in EEG activity from the left temporal-occipital region in all subjects; these changes persisted through a 6-minute recovery period. It should be noted that the 1 mg/m^3 concentration is slightly lower than the odor threshold determined for toluene in the same experiment (1.5 mg/m^3; see subsection 11.7.2). Toluene concentrations of 0.6 mg/m^3 caused no variations in the electric potentials of the EEGs. Exposure sessions consisted of 10 separate observation periods in which inhalation of toluene (5 periods) alternated with inhalation of pure air (5 periods). A single period consisted of 18 one-minute cycles. Every cycle included the sequential presentation of a sound stimulus (10 seconds), a wait for the light stimulus (7 seconds), the presentation of the light stimulus (18 seconds), and an interval of active physical exercise (25 seconds) for recovery of normal EEG rhythm. Of the 18 minutes allotted for EEG recording in each period, 3 minutes were used for training, the next 3 minutes for background observations, the following 6 minutes for the toluene exposure, and the final 6 minutes for recovery. It should be noted that no other studies have reported any effect on the CNS at such low levels of exposure, and that the purity of the toluene used was not stated.

Narcosis is the primary result of acute toluene exposure at high concentrations. A number of accounts of workers who were rendered unconscious by toluene vapor have been published in the medical literature (Lurie, 1949; Andersen and Kaada, 1953; Browning, 1965; Longley et al., 1967; Reisin et al., 1975). Most of

these cases have involved the entry of workmen into confined areas with poor ventilation and subsequent exposure to high levels of toluene during maintenance operations. Longley et al. (1967) described two episodes of acute toluene intoxication involving 26 men who were exposed in the holds of cargo ships. Toluene concentrations were estimated to have ranged from 10,000 ppm at waist level to 30,000 ppm at floor level, but it was emphasized that this estimate was purely conjectural. Effects at these concentrations ranged from exhilaration, lightheadedness, and cluminess and dizziness to collapse and unconsciousness. No deaths occurred and recovery was quite rapid, with no after-effects following removal from the contaminated atmosphere. The durations of the exposures were not indicated, but loss of consciousness occurred within minutes.

Episodes of toluene abuse are characterized by the progressive development of CNS symptoms. Toluene sniffers experience an initial excitatory stage that is typically characterized by drunkenness, dizziness, euphoria, delusions, nausea and vomiting, and, less commonly, visual and auditory hallucinations (Press and Done, 1967a, 1967b; Wyse, 1973; Lewis and Patterson, 1974; Hayden et al., 1977; Oliver and Watson, 1977; Barnes, 1979). As duration of exposure increases, symptoms indicative of CNS depression become evident: confusion and disorientation, headache, blurred vision and reduced speech, drowsiness, muscular incoordination, ataxia, depressed reflexes, and nystagmus. In extreme cases, loss of consciousness, possibly with convulsions (Helliwell and Murphy, 1979), occurs. The duration and severity of these effects vary greatly, depending upon the intensity of exposure; the duration may range from 15 minutes to a few hours (Press and Done, 1967b). Also, not all of the symptoms described are exhibited in any single sniffer, nor in any single episode of sniffing.

Winek et al. (1968) published partial results of an autopsy on an adolescent who had died as a result of sniffing toluene-containing model airplane glue. At

autopsy the cut surfaces of the lungs of this individual were found to be extremely frothy and congested, with diminished amounts of crepitation throughout the lung tissue. Other gross observations that were noted included some petechial hemorrhages in the larynx and upper trachea, firmness and congestion in the spleen, and a dark red brown color and congestion in the liver. No hemorrhages, obstructions, or ulcerations were seen anywhere in the gastrointestinal tract, and all other organs were unremarkable. The results of toxicological analyses of various body tissues for toluene are presented in Section 12.2. Congestion in various organs, swelling of the brain, subseromucous petechiae, and pulmonary edema were associated with 19 other cases of acute death from thinner intoxication (Chiba, 1969). The English abstract of this Japanese study indicated that toluene was the major component of the inhaled thinner. Nomiyama and Nomiyama (1978) described an instance in which 4 adolescents were found dead after sniffing 99% pure toluene in a car, but post-mortem results other than levels of toluene (blood and alveolar air) and hippuric acid (urine) were not presented. Sudden death due to solvent sniffing has been reported in at least 122 cases (Bass et al., 1970; Alha et al., 1973). These deaths have been attributed to severe cardiac arrhythmia, and are discussed in subsection 11.5 (Effects on the Heart).

11.1.1.2 Subchronic and Chronic Effects

Wilson (1943) described the effects of exposure to commercial toluene vapor on 100 workers (out of a total of 1000 workers) who showed symptoms severe enough to cause them to present themselves to a hospital for examination. The workers were exposed daily to toluene concentrations ranging from 50-1500 ppm for periods of 1 to 3 weeks, but the composition of the commercial formulation and the type of industry were not described. Also, it is unclear whether the remaining 900 workers evidenced any symptoms of toluene exposure. The

concentration of toluene was determined shortly after any exposed person appeared at the hospital with symptoms, and the patients were classified into groups by degree of exposure. The following effects were reported:

50 to 200 ppm (approximately 60% of the patients) - headache, lassitude, and loss of appetite. These symptoms were so mild that they were considered to be due primarily to psychogenic and other factors rather than to toluene fumes.

200 to 500 ppm (approximately 30% of the patients) - headache, nausea, bad taste in the mouth, anorexia, lassitude, slight but definite impairment of coordination and reaction time, and momentary loss of memory.

500 to 1500 ppm (approximately 10% of the patients) - nausea, headache, dizziness, anorexia, palpitation, and extreme weakness. Loss of coordination was pronounced and reaction time was definitely impaired.

Characteristic CNS symptoms have been described in foreign reports of workers exposed for longer durations to moderate levels of toluene. Parmeggiani and Sassi (1954) found signs of "nervous hyperexcitability" in 6 out of 11 paint and pharmaceutical industry workers who were exposed to 200-800 ppm toluene vapor for "many" years. Capellini and Alessio (1971) noted symptoms of stupor, nervousness, and insomnia in 1 worker who was employed for "diverse" years in preparing a toluene-containing mixture for use in the manufacture of V-belts. The mean atmospheric concentration of toluene in the mixing department was 250 ppm, with extremes of 210 ppm and 300 ppm. No CNS effects were observed, however, in 17 other workers who were exposed to 125 ppm toluene (range, 80-160 ppm) while engaged in the manufacture of the belts.

In a more extensive study, Suhr (1975) found no evidence of adverse neurological effects in a group of 100 rotogravure printers with at least 10 years of exposure to 200-400 ppm pure toluene (<0.3% benzene). Subjective complaints indicative of CNS depression (headache, giddiness, nervousness, irritability, sleeplessness, bodily fatigue and incoordination), abnormal reflex reactions,

and abnormal Sphallograph test results were not found to occur significantly more often in the printers than in an unexposed control group of equal size. The Sphallograph is an instrument that is used to detect slight disturbances of muscular coordination by sensing variations in the balance of two metal plates; a test person stands on the plates, and balance disturbances are detected by strain gauges.

The Suhr (1975) conclusion that chronic occupational exposure to 200-400 ppm toluene did not cause adverse neurological effects in the rotogravure workers is equivocal for several reasons. First, the nature of the control group used in this study is not defined, other than that they "were from the same firm and not exposed to toluene." Additionally, the worker and control groups were only roughly matched by groups for age distribution, years of exposure, and nature of workshift (i.e., 2- or 3-shift work). Second, the venous blood levels measured in the printing room workers at the end of their shifts indicate exposure to toluene levels of at least 300 ppm and possibly as high as 600 ppm. These levels are consistent with the reported air concentration measurements, which were made with a "measuring cell" device. It is not clear, however, when workers were examined for reflex reactions and Sphallograph measurements. If it was after or before the workshifts (as the data for the 33 Sphallograph groups would indicate), then blood levels of toluene may have declined significantly. Astrand et al. (1972) have shown major drops in levels within minutes after the removal of human subjects from exposure. Third, the Sphallograph appears to be a very infrequently used device in the United States; several behavioral toxicologists who were contacted by Syracuse Research Corporation (SRC) indicated that they have never heard of the instrument, and the device does not appear to have been described in standard texts. Suhr (1975) also cites the work of Pohl and Schmidle (1973), who tested the effects of "extreme" concentrations of 11 fre

quently used organic solvents in humans with the Sphallograph and found only minimal effects. This would argue that the Sphallograph is not a sensitive test for determining CNS effects of solvents. Last, until more is known concerning the exposures of the control group, the significance of the reportedly negative results of the subjective symptom survey is questionable.

Chronic occupational exposure to toluene has also been associated with behavioral changes. Munchinger (1963) diagnosed an "organic psychosyndrome" in 21% of a group of printers exposed on the average to 300 ppm toluene for 18 years (mean age, 42 years), and in 40% of a group of printers' helpers exposed to 430 ppm for 12 years (mean age, 44 years). The tests involved a total of 110 workers, but testing on control subjects was not performed. This syndrome was characterized by subjective memory, thinking, and activity disturbances. Results of the Rorschach testing were consistent with the psychosyndrome diagnosis in 83% of the cases. The Rorschach test and Knoepfel's 13-Error Test results in combination agreed with the diagnosis in 95% of the cases.

More recently, several groups of investigators have shown that long-term exposure to combinations of toluene and other common organic solvents caused impairments in visual intelligence and psychomotor performance of workers. In 1973, Lindstrom compared the psychological test performances of a group of 168 male workers who had been exposed to hydrocarbon solvents for 0.1-30 years (mean, 6 years) to those of an unexposed control group (N = 50). Twenty-six of the workers had been exposed primarily to toluene and 25 to a combination of toluene and xylene; the other workers (numbers in parentheses) were exposed primarily to trichloroethylene (44), tetrachloroethylene (8), "thinners" (44), and miscellaneous solvents (21). Exposure concentrations were not reported. Results showed that the solvent-exposed workers were inferior in performance to the

controls in sensorimotor speed performance, psychomotor performance, and visual accuracy as determined by standardized test procedures (e.g., Bourdon-Wiersma vigilance test, Santa Ana dexterity test, Mira psychomotor test). The performance of the workers on the Rorschach personality test was comparable to that of the control group.

Hanninen et al. (1976) compared the behavioral responses of a group of 100 car painters with those of 101 age-matched nonexposed subjects. The painters (mean age 35 ± 11 years) were exposed to different organic solvents for 1 to 40 years (mean, 14.8 ± 8.5 years), but, as detailed in Table 11-3, toluene was present in the greatest amount (30.6 ppm). A battery of tests included one test for verbal intelligence, three visual tests, five memory or learning tasks, four tests of psychomotor performances, and the Rorschach test for measuring personality changes (Tables 11-4 and 11-5). Results of this study showed significant differences between the exposed and reference group in almost all intellectual performances and memory tasks. Impairments in visual and verbal intelligence and in memory as well as a reduction of emotional reactivity as indicated by the Rorschach test were the predominant effects of solvent exposure (Tables 11-4 and 11-5). Differences in psychomotor performances between the exposed and control subjects were less consistent; impairments were seen only in some of the Santa Ana dexterity and finger tapping test scores, and reaction times were unaffected by exposure. It should be noted that in other studies, reaction time increased as a result of acute (Ogata et al., 1970; Gamberale and Hultengren, 1972) and subchronic (Wilson, 1973) exposures to toluene concentrations in excess of 200 ppm. The possible influence of differences in initial intelligence levels on the performance scores was controlled in the Hanninen et al. (1976) study by a separate comparison of the test results of 33 pairs of exposed and unexposed subjects who were matched for age and for intelligence.

Table 11-3. Mean Concentrations of Organic Solvents in the Breathing Zone of 40 Car Painters (Hanninen et al., 1976)

Solvent	Mean Concentration (ppm)
Toluene	30.6
Xylene	5.8
Butyl Acetate	6.8
White Spirit	4.9
Methyl Isobutyl Ketone	1.7
Isopropanol	2.9
Ethyl Acetate	2.6
Acetone	3.1
Ethanol	2.9

[a]Sampling Period = 1 hour; Number of Car Repair Garages = 6; Number of Samples = 54.

Table 11-4. Performance Tests: Means, Standard Deviations, and Significance Between the Group Means (Age-Matched) Groups (Hanninen et al., 1976)

Test	Means and Standard Deviations: Exposed (N = 100)	Means and Standard Deviations: Nonexposed (N = 150)	Significance of Differences (t-test)
WAIS[a] Similarities test[b]	19.4 ± 3.1	2.9 ± 2.1	***
WAIS Picture Completion[c]	14.9 ± 2.9	16.2 ± 2.3	***
WAIS Block Design[d]	34.6 ± 7.0	39.6 ± 5.6	***
Figure Identification[e]	32.0 ± 9.0	36.7 ± 9.8	***
WAIS and WMS[f] Digit Span[g]	10.6 ± 1.6	11.5 ± 1.8	***
WMS Logical Memory[h]	11.7 ± 3.7	13.9 ± 3.1	***
WMS Associate Learning[i]	15.3 ± 3.6	17.1 ± 2.6	***
Benton Test for Visual Reproduction	21.1 ± 3.1	22.6 ± 2.3	***
Benton Test for Visual Retention	8.2 ± 1.5	8.7 ± 1.3	*
SADT - right hand[j]	44.7 ± 5.7	47.5 ± 5.8	**
SADT - left hand[j]	42.3 ± 5.4	43.6 ± 5.1	
SADT - coordination with both hands[j]	29.0 ± 5.4	31.5 ± 5.7	**
Finger Tapping - right hand[k]	202.5 ± 29.2	209.6 ± 23.8	
Finger Tapping - left hand[k]	186.7 ± 28.5	196.4 ± 22.4	*
Reaction Time (Simple) - right hand	12.4 ± 2.9	11.9 ± 1.4	
Reaction Time (Simple) - left hand	12.1 ± 3.0	11.7 ± 1.4	
Reaction Time (Choice)	9.1 ± 1.8	9.1 ± 1.2	
Mira Test[l]	18.8 ± 3.8	20.3 ± 4.6	**[m]
Mira Test[l]	2.2 ± 1.0	2.0 ± 0.8	*

*P < 0.05; **P < 0.01; ***P < 0.001

[a]Wechsler Adult Intelligence Scale.

[b]Measures verbal intelligence and abstraction.

[c]Measures visual intelligence and observation.

[d]Measures visual intelligence and abstraction.

[e]Measures speed of perception and memory for visual details.

[f]Wechsler Memory Scale.

[g]Measures memory for digits.

[h]Measures verbal memory.

[i]Measures verbal memory and learning.

[j]Santa Ana Dexterity Test; measures psychomotor speed.

[k]Measures motor speed.

[l]Test for psychomotor behavior and psychomotor ability; two variables tested.

[m]Paired t-test.

Table 11-5. Rorschach Personality Test Variables: Means, Standard Deviations, and Significances Between the Group Means (Age-Matched Groups) (Hanninen et al., 1976)

Variable	Means and Standard Deviations		Significance of Differences (t-test)
	Exposed (N = 100)	Nonexposed (N = 101)	
Number of responses	13.6 ± 6.4	13.8 ± 4.5	
Number of rejections	0.7 ± 1.1	0.4 ± 1.0	**[a]
Average latency time of responses	16.4 ± 8.5	16.5 ± 8.1	
Adaptability	11.6 ± 3.1	12.1 ± 3.1	
Emotionality	8.8 ± 3.3	10.4 ± 3.2	***
Spontaneity	11.8 ± 2.4	11.9 ± 2.6	
Rational self-control	8.6 ± 2.8	7.3 ± 2.8	***[b]
Originality of perception	1.6 ± 1.7	1.5 ± 1.2	
Hostility	1.6 ± 1.6	2.4 ± 1.7	***
Anxiety	3.9 ± 2.0	3.8 ± 2.2	
Bodily Preoccupation	0.4 ± 0.8	0.8 ± 1.1	*[a]

*P < 0.05; **P < 0.01; ***P < 0.001

[a]Paired Chi Square-test for dichotomized scores.

[b]Paired t-test.

In a related study, Seppalainen et al. (1978) examined the same cohort of car painters studied by Hanninen and coworkers (1976) for neurophysiological effects. Results of EEG analysis on 102 solvent-exposed car painters and 102 nonexposed control subjects showed no increase in abnormalities (abnormal EEGs were encountered in 32 painters and 37 controls). It was noted, however, that the incidence of abnormal EEGs in both groups was higher than expected (approximately 10%) on the basis of EEG literature. It was further reported that 26 of the car painters had a complex of four subjective symptoms indicative of CNS disturbance (interrupted sleep, absentmindedness, easy to fall asleep when watching television, frequent headaches); this symptom complex was found only in 12 controls. EEG testing on the workers with these symptoms showed abnormalities in 46% (12/26) of the cases, but 26% (20/76) of those without the symptom complex also displayed EEG abnormalities. This difference was not statistically significant (Chi squared = 2.68)

Rouskova (1975) did observe changes in EEG response to photic stimulation in a group of 20 workers with a 13.5-year (average) history of exposure to higher concentrations of toluene (>250 ppm) and 1,1,1-trichloroethane (concentration not stated). Photic stimulation was applied in a series of rhythmic flashes, each lasting 10 seconds with intervals of 10 seconds between each flash series; frequences ranged from 1 to 30 per second. Evaluated as a normal response was the occurrence of EEG activity of the same frequency as stimulation or of a harmonic or a subharmonic multiple of that frequency lasting at least 1 second. Results showed that abnormal EEG responses were found in 18 of the 20 workers (90%), but in only 1 of 20 unexposed control subjects.

Residual effects indicative of cerebellar and cerebral dysfunction have been observed in a number of persons who had abused toluene or solvent mixtures containing toluene over a period of years (Grabski, 1961; Satran and Dodson,

1963; Knox and Nelson, 1966; Kelly, 1975; Boor and Hurtig, 1977; Weisenberger, 1977; Keane, 1978; Sasa et al., 1978; Tarsh, 1979; Malm and Lying-Tunell, 1980). Boor and Hurtig (1977) also described a case of cerebral involvement in an optician who regularly used toluene occupationally to clean eyeglasses and contact lenses in a small, unventilated room. Clinical signs in these individuals included ataxia, intention tremors, nystagmus, equilibrium disorders, positive Babinski reflex, impairment of speech and hearing, reduced vision, disturbance of concentration and memory, emotional lability, and psychosis. These reports, which are summarized in Table 11-6, indicate that the severity of the encephalopathic effects generally varied with the intensity and duration of exposure and that the effects were largely reversible, particularly when the exposures were not too extreme. Prolonged toluene abuse had, however, on occasion led to permanent encephalopathy and brain atrophy as evidenced by EEG and neuroradiological (pneumoencephalogram, angiogram) changes (Knox and Nelson, 1966; Boor and Hurtig, 1977; Sasa et al., 1978).

11.1.2 Peripheral Nervous System

Matsushita et al. (1975) found evidence of peripheral neuropathy in a group of 38 female shoemakers (mean age 20.7 $\pm$ 5.2 years) who had been exposed to a glue containing mainly toluene and "slight" gasoline for an average duration of 3 years and 4 months. The results of neurological and muscular function tests reportedly showed abnormal tendon reflexes, reduced grasping power of the dominant hand, and decreased finger tapping tempo in the exposed workers relative to a group of 16 unexposed control women (Table 11-7), but descriptions of the tests were not provided. A significant decrease in finger agility was also noted in the exposed shoemakers; agility of the fingers was estimated by measuring the time needed to move 25 "bulbs" using glass chopsticks. The average toluene concentration in the air varied with time of year from 60 to 100 ppm (range 15-

Table 11-6. Encephalopathic Effects of Chronic Toluene Abuse

Subject (Age)	Exposure History	Effects and Diagnosis	Reference
Male (33 years)	Regularly sniffed toluene for 14 years. Subject purchased a gallon of pure toluene every 4-6 weeks, and inhaled the toluene on an almost daily basis at frequent intervals throughout the day.	Patient initially examined after 6 years by Grabski; signs included ataxia, intention tremors, pyramidal signs and psychosis which were concluded to be consistent with cerebellar degeneration. After 8 more years of abuse, Knox and Nelson reexamined the patient and concluded that the syndrome was primarily a diffuse cerebral disorder based on findings of ataxia, tremors, limb incoordination, emotional lability, marked snout reflex, and positive Babinski toe reflex; cerebral atrophy was confirmed by EEG and pneumoencephalography.	Grabski, 1961; Knox and Nelson, 1966
Male (30 years)	10-year history of toluene abuse.	Recurrent headaches, "inappropriate" speech, brief episodes of memory loss, increased irritability, and exaggerated swings in mood. Unremarkable clinical and neurological exam, but nonspecific EEG changes were found that were regarded as consistent with diffuse encephalopathy.	Satran and Dodson, 1963
Female (19 years)	Almost daily sessions of prolonged paint sniffing for 1-1/2 years. Ingredients not specified but it was indicated that toluene was a common ingredient in all the brands sniffed. Previous 4-year history of multiple drug and solvent abuse.	Ataxia, intention tremors of hands and feet, incoordination, hallucinations. Normal EEG, brain scan, arteriography, and pneumoencephalography. The diagnostic impression was cerebellar dysfunction secondary to some toxic factor in the paint. Objective neurological improvement 5 months after sniffing was discontinued.	Kelly, 1975
Male (25 years)	10-year history of lacquer thinner (99% toluene) abuse; during the last 5 years he had spent virtually all his waking hours inhaling the vapors (1 gallon used every 2 weeks)	Ataxia, mildly slurred speech, nystagmus, and bilateral Babinski signs. Normal EEG, nuclide brain scan, electromyogram, and nerve conduction studies, but a computerized brain scan showed diffuse widening of the cortical and cerebellar sulci. Subjective improvement in condition following abstinence from exposure, but a neurological exam after 9 months was essentially unchanged.	Boor and Hurtig, 1977

Table 11-6. Encephalopathic Effects of Chronic Toluene Abuse (Cont.)

Subject (Age)	Exposure History	Effects and Diagnosis	Reference
Male (59 years)	Optician who frequently but intermittently used 99% toluene in a small unventilated room to clean eyeglasses and contact lenses. Unable to smell toluene because of chronic anosmia. Duration of exposure not stated.	Fatigue and clumsiness of the left side which got progressively worse. Occasional staggering and mildly slurred speech, disturbed concentration and memory. Normal neurological exam, EEG, and brain scans. Daily improvement without specific treatment following cessation of exposure.	Boor and Hurtig, 1977
Male (age not stated)	Habitual inhalation of paint thinner (toluene) on the job. Duration not stated.	Bizzare behavior prior to hospital admission. Admitted in an agitated, violent, nearly catatonic state.	Weisenberger, 1977
Male (27 years)	Sniffed unspecified glues and paint thinners for 10 years. From age 25, toluene was involved 4-5 times per week (200-300 ml/week used), and from age 26, he inhaled 4-7 times per day (100 ml/day used.	Arm and neck tremors, ataxia, incoordination, and equilibrium disorders. No abnormal psychiatric symptoms. Pneumoencephalographic and angiographical evidence of midbrain and cerebrum atrophy. Degeneration of the cerebellum suspected.	Sasa et al., 1978
Male (20 years)	3-year history of daily aerosol spray paint inhalation. Product contained copper, toluene, and xylene as solvents and isobutane propane and methylene chloride as propellants.	Reduced vision, poor color perception, constricted visual fields, normal optic fundi, impaired papillary response, ataxia, and nystagmus. Symptoms slowly subsided following cessation of paint sniffing.	Keane, 1978
Male (25 years)	Sniffed toluene for 4 months, starting while on the job using toluene as a solvent in the rubber processing industry.	Delusions and unpredictable behavior. Largactil prescribed because he was thought to have a schizophrenic illness. Symptoms disappeared and did not recur following termination of sniffing.	Tarsh, 1979
Female (18 years)	Inhaled pure toluene since age 12, regularly since age 16 (2 liters used per month). Sniffed more heavily than usual during the last 2 months.	Personality changes (apathy, irritability, emotional lability, carelessness), vomiting, difficulty in walking, and slurred speech 1-2 weeks before admission. Gait ataxia, incoordination, dysarthria, downbeat nystagmus, bilateral positive Babinski sign, visual and color sense loss, impaired concentration and abstracting ability upon admission. Symptoms consistent with mainly cerebellar-brain stem involvement and possibly optic neuritis. Symptoms decreased when she did not inhale toluene, and disappeared after 8 months.	Malm and Lying-Tunell, 1980

Table 11-7. Results of Neurological and Muscular Function Tests of Toluene-Exposed Female Shoemakers (Matsushita et al., 1975)

Test[a]	Exposed Group	Control Group
Abnormal tendon reflex:		
Biceps and triceps	6(16)[b]	3(19)
Patellar	14(37)*	1(6)
Ankle	7(18)**	0(0)
Pathological reflex	1(3)	0(0)
Grasping power (dominant hand)	11(29)**	1(6)
Tapping tempo (M ± S.D.)[c]	162.9 ± 16.6	168.6 ± 17.3
Cold pressure test	6(16)	2(13)
Postural hypotension	2(5)	1(6)
Cuff test (upper arm)	5(13)	1(6)
Dermatographism	5(13)	1(6)
Blocking test (M ± S.D.) (seconds)	68.2 ± 13.3	61.8 ± 13.7
Numbers investigated	38(100)	16(100)

Statistical significance (Chi Square- and t-tests): *P < 0.05; **P < 0.01; M = mean; SD = standard deviation.

[a]Numbers of subjects with abnormal scores reported.

[b]The percentage of subjects affected is indicated in the parentheses.

[c]Unit of measurement not stated.

200 ppm); in a "few" working places, gasoline ranged from 20-50 ppm. An increased urinary hippuric acid level among the exposed women (3.26 ± 0.82 mg/ml versus 0.35 ± 0.24 mg/ml for controls) supported the role of toluene as the causative agent in producing the toxic effects.

Electroneuromyographic measurements were made in the Seppalainen et al. (1978) study (described in Section 11.1.1) on 59 of the toluene-exposed car-painters and 53 referents with a similar age distribution for an indication of a possible peripheral neurotoxic effect of exposure. Maximum motor conduction velocity (MCV), conduction velocity of the slower motor fibers (CVSF), maximal sensory conduction velocity (SCV), and motor distal latencies were recorded from nerves in the upper and lower extremities (median, ulnar, deep peroneal, posterior tibial, and sural nerves). Results of these measurements showed that the mean conduction velocities and motor distal latencies of the car painters were almost identical to those recorded for the unexposed control group. In several instances, however, individual nerve conduction velocities were found to be slower than the normal historical value (not stated) for Seppalainen's laboratory. When the conduction velocities of the study group were compared with the historical values, abnormally slow MCVs or SCVs and/or prolonged motor distal latencies were found in 12 of the 59 painters, but in none of the 53 controls.

Although the two previous reports (Matsushita et al., 1975; Seppalainen et al., 1978) indicate a possible effect of toluene on the peripheral nervous system, toluene's role in the causation of human peripheral neuropathies has not been clarified. Reports of polyneuropathies in abusers exposed to excessive and prolonged concentrations of glues and solvents have appeared in the Japanese and American literature, but have in all cases involved mixtures of toluene and other solvents (Matsumura et al., 1972; Takenaka et al., 1972; Goto et al., 1974; Shirabe et al., 1974; Suzuki et al., 1974; Korobkin et al., 1975; Oh and Kim,

1976; Towfighi et al., 1976; Altenkirch et al., 1977). The cases described in these reports were characterized by the sudden onset and rapid progression of a symmetric, predominantly motor polyneuropathy (although sensory nerve involvement of the glove and stocking type has been reported), even after exposure has ceased. Symptoms included extremity weakness, numbness, paresthesia, marked amyotrophy, and occasional flaccid paresis. The collective results of electromyographic studies have shown signs of denervation with delayed nerve conduction velocity, and biopsies of nerves have shown axonal degeneration, demyelination, and enlargement of some axons with focal accumulation of neurofilaments. Muscle biopsies revealed extensive neurogenic atrophy.

The earlier reports regarded either n-hexane alone (Korobkin et al., 1975; Towfighi et al., 1976) or a combination of n-hexane and toluene (Matsumura et al., 1972; Goto et al., 1974; Shirabe et al., 1974; Suzuki et al., 1974) as the cause of glue sniffers' neuropathy. The following observations have been offered as evidence to indicate that n-hexane plays an important role in its etiology: (1) in many of the reported cases, neuropathy did not develop until the patients began to sniff glue products that contained n-hexane, and (2) it is known that continuous occupational exposure to n-hexane under poor ventilation conditions produces a neuropathy among workers that is clinically and pathologically similar to that observed among the glue sniffers. From a recent outbreak of polyneuropathy among 18 glue thinner sniffers in West Germany, however, Altenkirch et al. (1977) presented data that implicate methyl ethyl ketone (MEK) as the causative agent and argues against n-hexane and toluene as the causes. These data are summarized as follows (Altenkirch et al., 1977):

1. In a number of sniffing adolescents (1000-2000), no adverse neurological effects were observed during the abuse of a thinner with a high n-hexane (31%) and toluene (30%) content over a period of 7 years.

2. The clinical picture of neuropathy occurred when the n-hexane fraction had been decreased by approximately one-half (16%) and MEK (11%) had been added; the amount of toluene was not significantly changed (29%).

3. Individuals who had discontinued sniffing prior to the introduction of the new formulation or who had used only the old composition were not affected. Neuropathies occurred, however, after 3-4 months in sniffers who had used only the new mixture.

4. Sniffing even a relatively small amount of the MEK-containing composition led to neurotoxic damages, while comparatively large amounts of the old composition were tolerated for a long time without consequences.

5. After the MEK-containing thinner was taken off the market, new cases of the disease were not observed.

Altenkirch and coworkers (1977) further noted that the exact composition of the glues that contained n-hexane and toluene cited in many of the aforementioned reports is incompletely characterized, and concluded that it remains open to question whether n-hexane was the sole causative agent in those cases. It should be emphasized that no report was located in the literature in which peripheral neuropathy is attributed to the inhalation of toluene alone. Further, it is noteworthy that no sensory or neuromuscular involvement was detected in a patient who experienced permanent cerebral dysfunction following prolonged inhalation of 99% pure toluene (Boor and Hurtig, 1977).

11.2 EFFECTS ON THE BLOOD AND HEMATOPOIETIC TISSUE

11.2.1. Bone Marrow

The action of toluene on human bone marrow has been the subject of persistent controversy. Early reports of occupational exposures (generally prior to the 1950s) ascribed myelotoxic effects to toluene (Ferguson et al., 1933; Greenburg et al., 1942; Wilson, 1943), but the majority of recent evidence indicates that the chemical is not toxic to the blood or bone marrow. The myelotoxic effects previously attributed to toluene are generally regarded by recent investigators to be the result of concurrent exposure to benzene, which

was present as a contaminant. Banfer (1961) noted that it first became possible to supply industry with adequate quantities of "pure" toluene ($\leq$0.3% benzene) in 1955; earlier, workers were typically exposed to toluene that was derived from coal tar and contaminated with as much as 20% benzene.

Greenburg et al. (1942) found mild depression of erythrocyte levels, absolute lymphocytosis, macrocytosis, and elevation of the hemoglobin level and the mean corpuscular hemoglobin concentration in 61 airplane painters who had been exposed to 100-1100 ppm toluene for periods extending from 2 weeks to 5 years (Table 11-8). Exposure was also associated with liver enlargement in 13 of the 61 painters (Section 11.3), but not with abnormal leukocyte counts, differential leukocyte counts, reticulated erythrocyte counts, basophilic aggregation estimates, platelet counts, erythrocyte sedimentation rates, coagulation time, hematocrit values, erythrocyte fragility, or serum bilirubin levels. Approximately 75% of the painters were exposed to concentrations of 500 ppm or less, and the group had no known prior exposure to benzene. Because these blood changes are consistent with those of benzene poisoning, however, the contamination of the toluene vehicle in the paint with benzene cannot be precluded (NIOSH, 1973). Volatile components such as ethyl alcohol, ethyl acetate, butyl alcohol, and petroleum naphtha were present in quantity in the lacquers, dopes, and brushes used by the workers (Table 11-9).

In 1943, Wilson found that of approximately 1000 industrial workers (industry not stated) exposed to 50-1500 ppm of commercial toluene vapor for 1 to 3 weeks, 100 showed symptoms attributable to toluene intoxication. Ten of the 100 workers had been exposed to concentrations in excess of 500 ppm and showed signs of serious CNS depression (Section 11.1.1.1). In most of these 10 cases, all blood elements remained normal except for the red cell count, which was "usually" reduced. In 2 of the 10 cases, other blood elements were reduced as

Table 11-8. Results of Blood Examinations Performed on Toluene-Exposed Airplane Painters (Greenburg et al., 1942)

	Toluene-Exposed Workers	Unexposed Workers
Erythrocyte Count $<5.2 \times 10^6/mm$	13.1% (N = 61)	5.2% (N = 346)
Absolute Lymphocyte Count $>5000/mm^3$	20.4% (N = 59)	7.7% (N = 395)
Mean Corpuscular Volume $\geq 100\ \mu^3$	21.3% (N = 61)	7.2% (N = 111)
Hemoglobin $\geq$16g/100cc	29.5% (N = 61)	2.4% (N = 81)
Mean Corpuscular Hemoglobin 35 micromicrograms	13.1% (N = 61)	0% (N = 73)
Mean Corpsucular Hemoglobin Concentration % of cases >34%	34.4% (N = 61)	2.5% (N = 81)

Table 11-9. Analysis of Paint Used by Painters[a]
(Greenburg et al., 1942)

	Percentage in Mixture
Spray painters	
Primer (75% of paint used):	
Zinc chromate	10.8
Magnesium silicate	0.7
Synthetic resin	12.8
Driers (lead and cobalt compounds)	0.3
Xylene	5.8
Toluene	69.6
	100.0
Lacquer 1 (15% of paint used):	
Volatile portion:	
Ethyl alcohol	7.0
Ethyl acetate	18.0
Butyl alcohol	7.0
Butyl acetate	15.0
Petroleum naphtha	3.0
Toluene	50.0
	100.0
Nonvolatile:	
Nitrocellulose, synthetic resin, titanium oxide, ferrocyanide blue, iron oxide, carbon black, zinc oxide, etc. No lead compounds	
Lacquer 2 (10% of paint used):	
Volatile portion:	
Toluene	25.0
Xylene	33.0
Petroleum naphtha	42.0
	100.0
Novolatile:	
Resin, titanium oxide, zinc oxide, ultramarine blue, ferrocyanide blue, iron oxide, diatomaceous earth, amorphous silica, carbon black	

Table 11-9. Analysis of Paint Used by Painters[a]
(Greenburg et al., 1942)

	Percentage in Mixture
Brush painters	
Dope:	
Volatile portion:	
Ethyl acetate	16.5
Ethyl alcohol	3.2
Butyl acetate	16.5
Butyl alcohol	5.6
Petroleum naphtha	13.7
Toluene	44.5
	100.0
Nonvolatile:	
Nitrocellulose, glycol sebacate, aluminum, cadmium sulfide, barium sulfate	
Brush wash:	
Acetone	22.5
Ethyl alcohol	22.5
Toluene	55.0
1	100.0

[a]Dip painters used a primer only of the same composition as given for spray painters.

well (leukocytes, platelets, polymorphonuclear cells, reticulocytes), and sternal bone marrow biopsies showed partial degeneration of the blood-forming elements, which resulted in a diagnosis of aplastic anemia. No clinical blood changes were seen in the workers who had been exposed to the lower concentrations of toluene (i.e., ¢500 ppm).

Von Oettingen et al. (1942a, 1942b) were the first workers to document the effects of essentially pure toluene on human subjects. The toluene used was shown, on spectrophotometric analysis, to contain not more than 0.01% benzene. In this study, no significant changes in the total or differential white cell count were found in 3 volunteers following controlled 8-hour exposures to various concentrations of toluene within the range of 50-800 ppm. Not more than two exposure sessions were performed per week to provide sufficient time for recovery in between exposures, and the experiments were conducted over a period of 8 weeks (Section 11.1.1.1). Erythrocyte counts were not made.

Parmeggiani and Sassi (1954) concluded from a clinical study of 11 paint and pharmaceutical workers exposed to 200-800 ppm toluene and 13 others with exposure to a combination of toluene (150-1900 ppm) and butyl acetate (150-2400 ppm) that toluene had no particular injurious action on the bone marrow (or other organs). The English summary of this study indicated that the workers were exposed for "many" years, but the purity of the toluene was not reported. Among the workers in the two groups, 34% reportedly showed slight anemia (¢4,000,000 erythrocytes/mm^3), 26% had a mild neutropenia (¢3500/mm^3) with lymphocytosis (&2000/mm^3), and 45% of the cases showed a decrease in blood platelets (¢150,000/mm^3) not accompanied by evident signs of capillary fragility.

In a more recent investigation, Banfer (1961) examined 889 rotogravure printers and helpers who were exposed to the vapors of toluene-containing printing inks for at least 3 years. Four hundred seventy eight non-exposed

persons from two groups served as controls; one group was composed of 155 management workers from the same plant, and the second group was composed of 323 persons from outside the plant. The available commercial toluene used in these inks reportedly contained only traces of benzene (<0.3%); when 5 samples of the toluene were examined by Banfer, no traces of benzene were found, but the method of analysis and detection limits were not stated. Analysis of the room air for toluene was performed by infrared spectroscopy but limited to 5 samples taken from different sites on a single day. Ambient toluene concentrations were not specified but three of the samples were determined to be below the "MAK-Wert," the fourth sample was at the "MAK-Wert," and the fifth sample, taken near one of the presses, exceeded the "MAK-Wert" by 400 ppm. A translation of this study by NIOSH (1973) indicates that the "MAK-Wert" was 200 ppm. Hematologic examinations of the workers and controls did not reveal any significant changes in the total number of leukocytes, lymphocytes, granulocytes, or erythrocytes, or hemoglobin levels (Table 11-10). Sternal biopsies from 6 printers with white cell counts of less than 5000/cmm were normal.

Capellini and Alessio (1971) performed hematological examination on 17 workers who had been exposed for "diverse" years to 125 ppm toluene (range, 80-160 ppm) in a plant manufacturing V-belts for industrial machinery. Results showed that the hemoglobin values, red cell counts, white cell counts, and platelet counts of the workers were within the same limits as those of 19 nonexposed control subjects from the same plant. The benzene content of the toluene was not reported. Blood findings were also within normal limits in another worker employed in a different department who was exposed to mean toluene concentrations of 250 ppm (range, 210-300 ppm) and who demonstrated symptoms of CNS toxicity and conjunctival irritation.

Table 11-10. Hematologic Examination of 889 Rotogravure Workers (Banfer, 1961)

	Printers (N = 889)	Controls, Group 1[a] (N = 155)	Controls, Group 2[b] (N = 323)
Leukocytes, total			
counts > 8500/cmm	78 (8.77%)	11 (7.09%)	26 (8.04%)
counts < 5000/cmm	74 (8.32%)	18 (11.61%)	38 (11.76%)
counts < 4500/cmm	28 (3.15%)	4 (2.58)	12 (3.71%)
counts < 4000/cmm	3 (0.33%)	1 (0.64%)	1 (0.30%)
Lymphocytes			
< 35% total leukocytes	25 (2.81%)	3 (4.16%)	4 (1.32%)
total counts < 5000/cmm	889 (100%)	155 (100%)	323 (100%)
Granulocytes	889 (100%)	155 (100%)	323 (100%)
total counts > 2000/cmm			
Erythrocytes			
counts < 4 million/cmm	16 (1.79%)	3 (1.93%)	7 (2.10%)
Hemoglobin			
value < 13g/100ml	4 (0.45%)	4 (2.58%)	4 (1.23%)

[a]Unexposed management workers from the same plant

[b]Unexposed individuals not employed at the plant

In 1975, a report by the West German Association of Gravure Printers (Suhr, 1975) identified a study population of 100 printers with at least 10 years of exposure to pure toluene (<0.3% benzene) and an unexposed control group of equal size from the same plant. Analysis of air samples collected from the workplace indicated that the potential exposure to toluene ranged from 200-400 ppm. Blood analyses (hemoglobin, erythrocyte, leukocyte, thrombocytes, differential analysis) demonstrated no unusual frequency of abnormalities in either the exposed or control groups.

Matsushita et al. (1975) found no alterations in the specific gravity of whole blood, hemoglobin content, hematocrit, or white blood cell counts in a group of 38 female shoemakers who had been exposed to toluene (60-100 ppm average) and, in a "few" places, gasoline (range, 20-50 ppm) for an average duration of 3 years and 4 months. The hematological test results from the shoemakers were compared with those from an unexposed control group of 16 female workers. A significantly increased number of Mommsen's toxic granules were observed, however, in the neutrophils of the exposed workers. Thirteen of the 38 workers showed an abnormal appearance of the granules (mean number per neutrophil, 7.6 $\pm$ 5.6) compared with 1 of 16 controls (mean number per neutrophil, 3.8 $\pm$ 3.4).

Further evidence of the relative non-toxicity of toluene to the hematopoietic system was presented by Francone and Braier (1954). Toluene, because of its supposed myelotoxic action, was administered orally as a treatment for leukemia. It was found that daily doses of up to 10 g of toluene in olive oil for 3 weeks (to a total of 130 g) were tolerated by leukemia patients without complaints or evidence of side effects, but the treatment had no clinical effect on the leukemic process.

Hematological abnormalities have been infrequently reported in sniffers of toluene-based glues. In a total of 90 cases surveyed by four groups of investigators (Christiansson and Karlsson, 1957; Massengale et al., 1963; Barman et al., 1964; Press and Done, 1967b), there were no instances of anemia or lymphopenia, a single report of neutropenia, and 6 cases of eosinophilia of greater than 5%. Christiansson and Karlsson (1957) also performed bone marrow examinations on 17 individuals; 10 of these showed changes suggestive of disturbances in maturation of leukocytes, although these changes were not reflected in the peripheral blood of the same individuals. The individuals examined in this study were habituated to the inhalation of toluene-based paint thinners, rather than model glues as were the subjects in other surveys. In a fifth clinical survey of 89 glue sniffers, however, Sokol and Robinson (1963) found abnormalities of the blood in 68 of the cases. An effect on the white blood cells was indicated by findings of eosinophilia (25 subjects), leukocytosis (12 cases), and lymphopenia (4 subjects). Sokol and Robinson (1963) also reported low hemoglobin values in 20 subjects, basophilic stippling of erythrocytes in 42 of the patients, and noted the frequent occurrence of poikilocytosis (25 cases), anisocytosis (20 cases), hypochromia (14 cases), and polychromasia (10 cases). There is no obvious explanation for the discrepancy between the hematologic findings of Sokol and Robinson (1963) and those of the other investigators. Because none of the aforementioned cases deal with exposure to pure toluene, however, the abnormalities observed should be considered to be the possible result of contamination of the toluene by benzene or some other organic solvent.

Powers (1965) diagnosed 5 cases of acute aplastic anemia that were associated with glue sniffing in black adolescents with pre-existing sickle-cell disease. The 5 children had apparently used three different glues, two containing toluene and one containing acetone. All of these patients recovered

following transfusion and cessation of sniffing. A case of fatal aplastic anemia, uncomplicated by the presence of sickle-cell disease, was described in a sixth individual with a 3-year history of glue sniffing.

11.2.2 Blood Coagulation

Pacseri and Emszt (1970; cited in NIOSH, 1973) reported that an increase in the prothrombin time was found in 191 printers exposed to 170-340 ppm toluene (duration of exposure not stated). Two of the subjects showed a reduced number of red blood cells, but no other hematologic abnormalities were found in these workers. The benzene content of the toluene was not reported.

11.2.3 Phagocytic Activity of Leukocytes

It has been reported that the phagocytic activity of leukocytes from printing-plant workers exposed to toluene vapors was significantly reduced relative to a control population (Bansagi, 1968). There was no relationship, however, between the decrease in activity and the concentration of toluene in the air. The English summary of this study did not detail any of the exposure information or mention the benzene content of the toluene.

Friborska (1973; cited in NRC, 1980) noted increased concentrations of alkaline phosphatase and lactic acid dehydrogenase in leukocytes and increased acid phosphatase in both leukocytes and lymphocytes from workers who were routinely exposed to toluene. The authors associated these alterations with increased functional capacity of the cells.

11.2.4 Immunocompetence

Serum immunoglobulin level (Lange et al., 1973a) and leukocyte agglutinins (Lange et al., 1973b) were studied in a group of 35 workers with a history of exposure to benzene, toluene, and xylene. The duration of exposure ranged from 1-21 years and the concentration of these compounds in the air ranged from 0.011-0.17 mg/l, 0.08-0.23 mg/l, and 0.12-3.0 mg/l, respectively. Serum IgG and IgA

levels were found to be significantly lower in the solvent-exposed workers than in nonexposed controls, although IgM levels tended to increase (Lange et al., 1973a). Lange and coworkers (1973b) also found that 10 of the 35 workers had leukocyte agglutinins for autologous leukocytes, and demonstrated an increase of leukoagglutination titer in human sera after incubation with benzene, toluene or xylene; this suggested that some workers exposed simultaneously to these aromatic compounds may exhibit allergic blood dyserasias. In another group of workers (N = 79) with a similar history of exposure to benzene, toluene, and xylene (i.e., levels and durations of exposure comparable to those of the workers examined by Lange et al.), Smolik et al. (1973) found a decreased level of serum complement. It should be noted that in all of the aforementioned studies, the specific solvent(s) responsible for the changes was not identified.

11.3 EFFECTS ON THE LIVER

Greenberg et al. (1942) found enlarged livers in 13 out of 61 airplane painters (21%) who were exposed to 100 to 1100 ppm toluene for from 2 weeks to more than 5 years. Toluene was the major solvent used in the paints, although significant quantities of other volatile components were present (Table 10-9); these workers reportedly had no history of inhalation exposure to any other toxic volatile solvents, including benzene. This incidence of liver enlargement was 3 times that observed in a control group of 430 workers who had never been exposed to toluene, but it cannot be correlated with exposure level because only the numbers of workers exposed at different exposure levels (and not hepatomegaly incidences) were reported. The liver enlargement was diagnosed by palpitation, and in no cases were the livers tender. There was also no correlation between the enlarged livers and either clinical or laboratory evidence of disease, and it was suggested that the enlargement might have been compensatory in nature.

Greenburg and coworkers' (1942) finding of hepatomegaly has not been substantiated in subsequent studies of workers with histories of occupational toluene exposure. Parmeggiani and Sassi (1954) found a comparable incidence (27%) of enlarged livers in a group of 11 paint and pharmaceutical production workers exposed to 200-800 ppm toluene for "many" years and in a control group of unexposed workers from the same plant. Normal liver function, as determined by electrophoresis, serum colloid stability testing, and galactose tolerance testing, was also observed in the exposed workers. Capellini and Alessio (1971) observed no changes in "the function of the liver" in 17 workers exposed for "diverse" years to mean atmospheric concentration of 125 ppm toluene (range, 80-160 ppm) in a plant manufacturing V-belts for industrial machinery. Liver function was evaluated by determinations of total serum protein and protein electrophoresis.

More recently, Suhr (1975) also found comparable, but high, incidences of enlarged livers and elevated liver enzymes in a group of 100 gravure printers with at least 10 years' exposure to 200-400 ppm pure toluene (benzene $\leq$0.3%), and in a control group of 100 workers from the same company who had not been exposed to toluene. It should be noted that the nature and history of the control group was not defined in any greater detail. Enlargement of the liver was established in 22% of the printers and 20% of the control group, and liver enzyme assays showed that about half of all test persons (50% of the printers, 51% of the controls) had increases in serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), glutamic dehydrogenase (GLDH), or gamma glutamyl transferase levels. It was concluded that because of the equal distribution of affected persons in both groups, the deviations in these parameters could not be attributed to toluene exposure. The cause of the hepatomegaly and liver enzyme deviations was not further investigated. Blood alcohol determinations before

and after the workshifts indicated comparably elevated levels in both the printers and control group (less than half of the 100 subjects in each group were tested; approximately half of the tested subjects had levels between 0.01 and 0.1%); but the significance of this finding is unclear because of the small number of subjects tested, because only single blood alcohol determinations were performed on each subject, and because the data was presented ambiguously.

Other studies have reported significant effects on indices of liver function in groups of toluene-exposed workers. In an examination of 94 rotogravure printers with a history of exposure to 18-500 ppm toluene and of a reference group of 30 municipal clerks, Szadlowski et al. (1976) found significant reduction in bilirubin and alkaline phosphatase in the exposed group, but no difference from controls in SGOT, SGPT, leucinamino-peptidase, or cholinesterase levels. The 94 rotogravure workers were divided into four groups depending upon the intensity of exposure to toluene. The mean exposure levels, durations of exposure and ages of the groups were, respectively (Szadkowski et al., 1973): Group 1 (N = 68) - 300 ppm, 7.3 $\pm$ 5.3 years, 32 years; Group 2 (N = 4) - 426 ppm, newly appointed on day of investigation, 24.3 years; Group 3 (N = 11) - 82 ppm, 5.6 $\pm$ 5.2 years, 42.9 years; Group 4 (N = 11) - 18 ppm, 8.5 $\pm$ 4.4 years, 35.8 years. Blood alcohol levels ranged from 0.02% to 0.07% in the exposed workers.

Trevisan and Chiesura (1978) performed the following hepatic function tests on 47 subjects who were exposed occupationally to toluene via inhalation: bilirubin, SGOT, gamma glutamyl transpeptidase (GGT), alkaline phosphatase (AP), ornithine-carbamyl transferase (OCT), Quick's test, and protein measurement. All tests gave normal results with the exception of GGT, which was reportedly above normal in 34% of the cases. In a group of 12 subjects controlled before and after toluene entered in the working operation, mean GGT activity increased

2-fold after exposure, with no effects on any of the other tests. Although GGT has proved to be a very sensitive screening enzyme for slight changes in liver function (Dragosics et al., 1976), it should be noted that these data were presented in abstract form and no information on exposure or type of occupation was presented.

English summaries of two Polish studies of women with histories of occupational exposure to toluene indicated abnormalities in the glucoprotein, serum mucoid and haptoglobin patterns of 53 women (Kowal-Gierczak et al., 1969), and changes in the serum levels of iron and copper and urinary excretion of porphyrin in 51 women (Cieslinska et al., 1969). Clinical signs of liver function impairment were not observed in these subjects, but the changes were interpreted by the investigators to indicate a hepatotoxic effect of toluene. The concentrations of toluene, durations of exposure, and the possibility of exposure to other chemicals were not discussed in summaries that were reviewed.

Intensive exposure to toluene via glue or thinner sniffing appears to have a minimal effect on the liver. Results of hepatic function tests (SGOT, SGPT, AP, bilirubin, sulfobromophthalein excretion, serum proteins, cephalin flocculation) on a total of 179 sniffers who were examined in early clinical surveys were essentially unremarkable (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b). Christiansson and Karlsson (1957) did detect liver enlargement in 5 out of 32 Swedish lacquer thinner sniffers, but other signs of liver function were normal. More recently, Litt and coworkers (1972) found elevated SGPT and AP levels in 2% and 5%, respectively, of a group of 982 glue sniffers.

Grabski (1961) described an individual who had abused pure toluene for 6 years and showed signs of cerebellar degeneration, hepatomegaly, and impaired liver function. Complete series of liver function tests were normal, however, in

an optometrist and a glue sniffer exposed independently to 99% pure toluene, both of whom also exhibited encephalopathic effects (Boor and Hurtig, 1977). Reversible hepatorenal damage was diagnosed in an individual with a 3-year history of inhaling a cleaning fluid that contained 80% toluene (other components not known) coupled with alcohol ingestion (O'Brien, 1971); the hepatic effect was indicated by elevated serum bilirubin and AP.

11.4 EFFECTS ON THE KIDNEYS

Exposure to mean concentrations of 60-100 ppm toluene and 20-50 ppm gasoline in a "few" working places for an average duration of 3 years and 4 months did not result in any abnormal urinalysis findings, except for excretion of hippuric acid, in 38 female shoemakers (Matsushita et al., 1975). Proteinuria and hematuria were noted, however, in a worker who was exposed to concentrations of toluene sufficient to cause unconsciousness while cleaning the inside of a tank that was coated with an emulsion of 45% toluene and 27% DDT (Lurie, 1949).

Reisin and coworkers (1975) published a report concerning the development of severe myoglobinuria and non-oliguric acute renal failure in a paint factory laborer who was exposed to pure toluene by skin contact and aspiration when a hose burst. The patient had inhaled sufficient amounts of toluene to cause a loss of consciousness for 18 hours and subsequent development of chemical pneumonitis and sustained superficial burns on approximately 10% of his body surface area. Acute renal failure apparently developed from the lack of fluid intake accompanied by heavy myoglobinuria rather than from a direct effect of toluene. The early administration of intravenous fluids and diuretics, and the use of hemodialysis led to complete recovery.

Pyuria, hematuria, and proteinuria have been the most frequently observed signs of renal dysfunction associated with the deliberate inhalation of toluene-based glues (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and

Robinson, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b). The clinical findings observed in 159 cases surveyed between 1957 and 1967 are tabulated in Table 11-11. These indications of renal dysfunction have not been universally observed in glue sniffers, are generally transient, and follow closely the intensive exposures (Press and Done, 1967b).

O'Brien et al. (1971) more recently described a case of reversible hepatorenal damage in a 19-year old male who had a 3-year history of glue sniffing while employed in the sign-painting trade. Prior to hospital admission, the subject had spent 6 hours inhaling a cleaning fluid that contained 80% toluene (the other components were not identified). Upon admission, the patient was vomiting and anuric, and after 8 hours, periorbital edema and subconjunctival hemorrhages developed. Blood concentration of toluene was determined to be 160 ppm. Other evidence of renal damage included hematuria, proteinuria, elevated serum creatinine, and renal insufficiency required peritoneal dialysis. The effects of these exposures on hepatic function are discussed in Section 11.3 (Effects on the Liver).

Although serious involvement of the kidney with human intoxication by toluene has not been stressed in the early literature, several reports have recently appeared that associate deliberate inhalation of toluene with metabolic acidosis (Taher et al., 1974; Fischman and Oster, 1979a; Kroeger et al., 1980; Bennett and Forman, 1980; Moss et al., 1980). The cases of acidosis described by these investigators (Table 11-12) are characterized by serious electrolyte abnormalities (hypokalemia, hyperchloremia), and are related primarily to toluene's ability to impair hydrogen ion secretion in the distal renal tubule (distal renal tubular acidosis). In addition to findings compatible with distal renal tubule acidosis, Moss et al. (1980) found pathologically increased excretions of amino acids, glucose, phosphate, uric acid, and calcium that indicated

Table 11-11. Renal Function Investigations of Glue Sniffers[a]
(Adapted from Press and Done, 1967b)

Number of Patients	Pyuria	Hematuria	Proteinuria	Clearances	Azotemia	Reference
32	All 32 urine samples "normal"; details not given	ND[b]	ND	ND	ND	Christiansson and Karlsson, 1957
27	0	2	0	ND	0	Massengale et al., 1963
89[c]	32	14	12	ND	ND	Sokol and Robinson, 1963
15	0	0	1	PSP[d] 0/13	0/7	Barman et al., 1964
16	6	3	5/13	Urea 1/7	0/9	Press and Done, 1967b

[a]Exposure were to toluene-containing plastic cements except in the Christiansson and Karlsson (1957) study, in which the subjects examined had sniffed paint thinner.

[b]ND = not determined.

[c]Urinary abnormalities were found in 67 of the 89 glue sniffers.

[d]Phenosulfonphthalein clearance in 2 hours.

Table 11-12. Toluene Induced Metabolic Acidosis

Subject/Age	Exposure History	Symptoms	Clinical Findings	Reference
Male (23 yr)	Sniffed glue and pure toluene intermittently for 6 yr.	Several episodes of muscle weakness following prolonged (e.g., 4-7 d) inhalation sessions. One instance of flaccid paralysis.	Hypokalemia with hyperchloremic metabolic acidosis. Elevated urinary pH. Toluene detected in blood.	Taher et al., 1974
Female (20 yr)	Two 3- to 5-d episodes of sniffing aerosol paint containing 60% toluene within 4 wk.	Nausea.	Hyperchloremic acidosis. Elevated urinary pH. Toluene detected in blood.	Taher et al., 1974
Female (17 yr)	Sniffed transmission fluid containing 100% toluene for 5 d.[a]	Persistent vomiting.	High anion gap[b] metabolic acidosis.	Fischman and Oster, 1979a
Female (21 yr)	Intermittently sniffed transmission fluid containing 100% toluene for at least 5 yr.[a]	Hospitalized on 6 occasions within a 16 mo. period. Severe weight loss (18 kg) at first admission. Recurrent symptoms of vomiting, muscle weakness, and lethargy. After the 6th episode, patient died of cardiopulmonary arrest.	Hypokalemia. Hyperchloremic metabolic acidosis and high urinary pH on 1st and 6th admissions. High anion gap metabolic acidosis on the other admissions.	Fischman and Oster, 1979a
Female (25 yr)	Frequent sniffing of transmission fluid containing 100% toluene during a 5-yr period.[a]	Persistent vomiting, lethargy, and muscle weakness.	Normal anion gap hyperchloremic metabolic acidosis with severe hypokalemia.	Fischman and Oster, 1979a
Male (23 yr)	Sniffed toluene on a "regular" basis for 5 yr. Form not specified.	Hospitalized 4 times within 15 mo. History of vomiting, flank pain, and paralysis of the lower extremities.	Recurrent uretal and renal calculi (4 stones total). Hyperchloremic metabolic acidosis and hypokalemia. Acidic urine.	Kroeger et al., 1980
Female (27 yr)	Daily inhalation of glue for 9 mo.	Lethargy, weakness, and ataxia. Microscopic hematuria and sterile pyuria.	Hyperchloremic metabolic acidosis, hypokalemia, hypocalcemia, hypophosphatemia, and hypouricemia. Increased excretion of 14 amino acids and glucose.	Moss et al., 1980
Four individuals (details not stated)	Glue or paint sniffers (details not stated).	Not stated.	Hyperchloremic metabolic acidosis with hypobicarbonatemia.	Moss et al., 1980
Male (22 yr)	Abused a lacquer thinner (99% toluene) for 8 yr.	Abdominal pain, vomiting, generalized weakness, and diminished reflexes.	Hypokalemic and hypochloremic metabolic acidosis.	Bennett and Forman, 1980

Abbreviations: yr = year; d = day; wk = week; mo. = month.

[a] Toluene is not ordinarily a component of transmission fluid (Fischman and Oster, 1979b).

[b] Anion gap is defined as serum Na - ($Cl + HCO_3$) in milliequivalents per liter.

proximal tubule dysfunction consistent with Fanconi's syndrome. Kroeger et al. (1980) reported the case of a patient with toluene-induced renal tubular acidosis who developed recurrent urinary calculi. It should be noted that each of the subjects who developed acidosis had a history of multiple toluene abuse and, although the acute consquences of renal tubular acidosis associated with toluene sniffing were on occasion life threatening, these effects were completely reversible with abstinence from toluene exposure. These symptoms also responded promptly to electrolyte repletion therapy with potassium chloride and sodium bicarbonate.

Fischman and Oster (1979a) found a high anion gap metabolic acidosis with hypokalemia in two patients who had sniffed 100% toluene; this condition is reportedly indicative of an increased production of acid by the body. Although it was noted that renal failure, ketonemia, and elevated lactate levels could have accounted in part for the abnormal increases in anion gap, it was suggested that the acid metabolites of toluene (e.g., benzoic and hippuric acids) may have caused the high anion gap metabolic acidosis.

Clinical manifestations associated with the reported metabolic alterations included nausea, lethargy, ataxia, muscular weakness, and paralysis (Table 11-12). NRC (1980) noted that some of these manifestations may mimic those usually attributed to the effects of toluene on the CNS, and that altered pH and electrolyte balance may be more commonly responsible for the manifestations of toluene abuse than is usually recognized. In particular, hypokalemia often produces significant muscular weakness including flaccid paralysis.

11.5 EFFECTS ON THE HEART

Ogata et al. (1970) found an apparent decrease in the pulse rate of 23 volunteers exposed to 200 ppm toluene for periods of 3 hours or of 7 hours with one break of 1 hour, but no effect at 100 ppm. Systolic and diastolic blood

pressure were not affected by exposure. Exposure to 100 and 200 ppm toluene for 30 minutes did not, however, have any effect on the heart rates or electrocardiograms of 15 other subjects during either rest or light exercise (Astrand et al., 1972). Other studies have shown that experimental exposure to toluene at levels of 100-700 ppm for 20 minutes (Gamberale and Hultengren, 1972) or 50-800 ppm for 8 hours (Von Oettingen et al., 1942a, 1942b) did not cause any definite effects on heart rate or blood pressure. Suhr (1975) noted that the pulse rates and blood pressures of a group of 100 printers with a 10-year history of exposure to 200-400 ppm toluene and those of an unexposed control group of identical size were similar at the beginning and end of work shifts.

Sudden deaths that were not due to suffocation secondary to solvent sniffing but rather were attributed to a direct effect of the solvent itself have been reported in at least 122 cases (Bass, 1970; Alha et al., 1973). Toluene, benzene, and gasoline have been individually implicated in a small number of these deaths (10, 6, and 4 cases, respectively), but the volatile hydrocarbons most frequently involved were trichloroethane and fluorinated aerosol propellants. Severe cardiac arrhythmia resulting from light plane anesthesia seems to be the most likely explanation for the cause of the sudden sniffing deaths. Bass et al. (1970) noted that stress, vigorous activity, and hypoxia in combination with sniffing appear to increase the risk of death.

11.6 EFFECTS ON MENSTRUATION

Dysmenorrhea was reported by 19 out of 38 Japanese female shoemakers (mean age, 20.7 years) who were exposed to mean toluene concentrations of 60-100 ppm for an average duration of 3 years and 4 months (Matsushita et al., 1975). In an unexposed control group of 16 women from the same plant, this effect was noted in 3 individuals (19%). It should be noted that these women were concomitantly exposed to 20-50 ppm of gasoline in a "few" working places.

Michon (1965) reported disturbances of menstruation in a group of 500 women (age 20-40 years) who had been exposed to a mixture of benzene, toluene, and xylene in the air of a leather and rubber shoe factory. The concentration and component distribution of this mixture were not specified, but it was stated in the English summary of this study to be within permissible occupational limits established at the time in Poland (100 mg/m^3 (31 ppm) for benzene, 250 mg/m^3 (67 ppm) for toluene, and 250 mg/m^3 (58 ppm) for xylene). When the menstrual cycles of the exposed women were compared with those of 100 women from the same plant with no exposure to these hydrocarbons, prolonged and more intense menstrual bleeding was found in the exposed group. The regularity of the cycle was not affected.

It has also been noted in the English summary of a Russian study that occupational exposure to average concentrations of 25-350 mg/m^3 toluene and other solvents, through the use of organosiliceous varnishes in the manufacture of electric insulation materials, caused a high percentage of menstrual disorders (Syrovadko, 1977). The newborn of these women were reportedly more often underweight and experienced more frequent fetal asphyxia and "belated" onset of nursing.

11.7 EFFECTS ON THE RESPIRATORY TRACT AND THE EYES

11.7.1 Effects of Exposure

Carpenter et al. (1944) observed that 2 male subjects who were exposed to toluene for 7-8 hours experienced transitory mild throat and eye irritation at 200 ppm, and lacrimation at 400 ppm. Parmeggiani and Sassi (1954) found irritation of the upper respiratory tract and conjunctiva in 1 of 11 paint and pharmaceutical product workers who were exposed to 200-800 ppm toluene for "many" years. In the studies of Von Oettingen et al. (1942) and Wilson (1943), however, no complaints of respiratory tract discomfort were recorded in volunteers or

workers exposed to levels of toluene as high as 800-1500 ppm for 8-hour periods (Section 11.1, Effects on the Nervous System). In two episodes of accidental poisoning on ships that involved estimated short-term exposures to 10,000-30,000 ppm toluene, Longley et al. (1967) recorded no complaints of respiratory tract or eye irritation among 26 men.

Three workers accidentally splashed with toluene have transient epithelial injury to the eyes that consisted of moderate conjunctival irritation and corneal damage with no loss of vision (McLaughlin, 1946; Grant, 1962, both cited in NIOSH, 1973). Complete recovery generally occurred within 48 hours. The results of opthalmologic examinations of 26 spray painters who were exposed to toluene at levels of 100-1000 ppm for 2 weeks to more than 5 years were reported to be negative (Greenburg et al., 1942); results were not published, the examinations in each case consisted of a "history of ocular complaints, visual acuity, fundus, pupil and slit lamp investigation of the media of the eye.

Raitta and coworkers (1976) found lens changes in a group of 92 car painters who were exposed to a mixture of organic solvents for 1 to 40 years (mean 15 ± 9 years). Of the organic solvents detected in the breathing zones of the workers, toluene was present in the greatest amounts (30.6 ppm). This study was part of a large investigation performed to evaluate the effects of chronic solvent exposure on the nervous system of the car painters (Hanninen et al., 1976; Seppalainen et al., 1978) (Section 11.1.1.2); the mean concentrations of the other solvents present in the air are included in the summary of the Hanninen study (Table 11-3). Among the 92 car painters (mean age 34.9 ± 10.4 years, range 21-64 years), 2 had been operated on for a cataract and 46 had ocular changes that consisted mainly of lens opacities and/or nuclear sclerosis. To eliminate the influence of age on the development of the lens changes, the painters were compared with age-matched unexposed railroad engineers; 69 age-matched pairs

were generated for comparison. Results showed that in 27 instances, more lens changes were present in the car painters than in the age-matched engineers, and in 4 instances, there were more changes in the engineers (Table 11-13). In the remaining 38 pairs, both the painters and the unexposed engineers had similar lens changes. The lens changes were further found to occur with increased frequencies after 10 years of exposure (Table 11-13).

11.7.2 Sensory Thresholds

Gusev (1965) investigated the olfactory threshold for toluene in 30 subjects with a total of 744 observations. The minimum perceptible concentration was found to be within 0.40-0.85 ppm (1.5-3.2 mg/m^3) and the maximum imperceptible concentration within 0.35-0.74 ppm (1.3-2.8 mg/m^3). In sniff tests with 16 subjects (8 male, 8 females), May (1966) determined the minimum perceptible concentration to be a much higher 37 ppm (140 mg/m^3); toluene was found to be clearly perceptible at 70 ppm. In the latter study, the number of observations used to establish the average values were not stated.

Odor thresholds and sensory responses to inhaled vapors of Toluene Concentrate were recently determined by Carpenter et al. (1976b). Toluene Concentrate is a hydrocarbon mixture containing 45.89% toluene, 38.69% paraffins, 15.36% naphthenes, and 0.06% benzene. The most probable concentration for odor threshold, determined in two trials with 6 subjects, was 2.5 ppm. Based on sensory thresholds for irritation (eye, nose, throat), dizziness, taste, and olfactory fatigue, 6 of 6 volunteers indicated their willingness to work for 8 hours in a concentration of 480 ppm (corresponding to 220 ppm of toluene). Only 3 subjects thought they could work in an atmosphere containing 930 ppm (corresponding to about 427 ppm toluene).

Table 11-13. Frequency of Lens Changes and Distribution by Exposure Time in 69 Age-Matched Pairs of Car Painters and Railway Engineers (Raitta et al., 1976)

Result	Frequency of Lens Changes (no. pairs)	Distribution of Lens Changes by Years of Exposure		
		≤ 10	11-20	>21
Car painters had fewer changes than the engineers	4	3	1	0
No noticeable difference between the pairs	38	22	13	3
Car painters had more changes than the engineers	27	6	17	4

11.8 EFFECTS ON THE SKIN

Toluene is poorly absorbed through the skin (Section 13.1), and has an affinity for fat. When toluene is applied to the skin, its degreasing action will remove natural lipids, possibly causing dryness, fissures, and contact dermatitis (Gerarde, 1960; Browning, 1965).

Malten et al. (1968) found that exposure of human forearm skin for 1 hour on 6 successive days to toluene (volume and conditions not stated) resulted in injury to the epidermal stratum corneum (horny layer). The skin damage was assayed by measurements of water vapor loss, and daily measurements following the exposures indicated that regeneration took about 4 weeks.

Koilonychia and hapalonychia of the fingernails (conditions in which the nails are, respectively, concave and soft, uncornified) were observed in 6 of 16 cabinet makers who were dermally exposed to a thinner mixture that contained 30% toluene, 30% xylene, and 40% methyl alcohol (Ancona-Alayon, 1975). These deformities involved primarily the thumb, index, and middle fingernails, and were attributed to the practice of cleaning metal parts on furniture with solvent-soaked rags and unprotected hands. Most of the affected workers had an average exposure of 2 years.

11.9 SUMMARY

Exposures of humans to toluene have almost exclusively involved inhalation in experimental or occupational settings or during episodes of intentional abuse, and the health effect of greatest concern is dysfunction of the central nervous system.

Single eight-hour experimental (Von Oettingen et al., 1942a, 1942b; Carpenter et al., 1944) and subchronic occupational (Wilson, 1942) exposures to toluene in the range of 200-300 ppm have elicited subjective symptoms indicative of CNS depression (e.g., fatigue, nausea, muscular weakness, mental confusion,

and impaired coordination). These types of effects were generally dose-dependent, and increased in severity with increasing toluene concentration. Acute experimental exposures to toluene have also caused objective increases in reaction time at 200-300 ppm (Ogata et al., 1970; Gamberale and Hultengren, 1972), and decreases in perceptual speed at 700 ppm (Gamberale and Hultengren, 1972). Gusev (1965) observed disturbances of EEG activity in several subjects exposed to 0.27 ppm toluene for 6-minute intervals, but this effect does not have any apparent toxicological significance.

Short-term accidental workplace (Lurie, 1949; Andersen and Kaada, 1953; Browning, 1965; Longley et al., 1967; Reisin et al., 1975) and deliberate (Press and Done, 1967a, 1967b; Wyse, 1973; Lewis and Patterson, 1974; Hayden et al., 1977; Oliver and Watson, 1977; Barnes, 1979; Helliwell and Murphy, 1979) inhalation exposures to excessive levels of toluene (i.e., levels approaching air saturation concentrations of 30,000 ppm) have initially resulted in CNS stimulatory effects such as exhilaration, lightheadedness, dizziness, and delusions. As exposure durations increase, narcotic effects characteristic of CNS depression progressively develop, and, in extreme cases, collapse, loss of consiousness, and death (Winek et al., 1968; Chiba, 1969; Nomiyama and Nomiyama, 1978) have occurred.

Chronic occupational exposure to toluene has been associated with "nervous hyperexcitability" (Parmeggiani and Sassi, 1954) and subjective memory, thinking, and activity disturbances (Munchinger, 1963) in workers exposed, respectively, to concentrations of 200-800 ppm and 300-430 ppm. No evidence of adverse neurological effects have been reported, however, in other studies of printers exposed to 200-400 ppm toluene (Suhr, 1975) or manufacturing workers exposed to 80-160 ppm toluene (Capellini and Alessio, 1971), although the negative findings in the fomrer study are equivocal and symptoms of stupor,

nervousness, and insomnia were noted in one worker exposed to 210-300 ppm toluene in the latter study. Exposure to mixtures of varpors of organic solvent containing predominately low-levels of toluene (approximately 30 ppm) for an average of 15 years has produced a greater incidence of CNS symptoms and impaired performance on tests for intellectual and psychomotor ability and memory in car painters (Hanninen et al., 1976; Seppalainen et al., 1978). Matsushita et al. (1975) reported impaired performance in neurological and muscular function tests in female shoemakers who had been exposed to 15-200 toluene for an average duration of over 3 years, but these workers were exposed to "slight" levels of gasoline. Changes in EEG response to photic stimulation were reported by Rouskova (1975) in workers exposed to >250 ppm toluene and unspecified levels of 1,1,1-trichloroethane for an average of 13.5 years.

Residual effects indicative of cerebellar and cerebral dysfunction have been observed in a number of persons who had abused toluene or solvent mixtures containing toluene over a period of years (Grabski, 1961; Satran and Dodson, 1963; Knox and Nelson, 1966; Kelly, 1975; Boor and Hurtig, 1977; Weisenberger, 1977; Keane, 1978; Sasa et al., 1978; Tarsh, 1979; Malm and Lying-Tunell, 1980). These effects were largely reversible upon cessation of exposure, but prolonged toluene abuse has, on occasion, led to permanent encephalopathy and brain atrophy (Knox and Nelson, 1966; Boor and Hurtig, 1977; Sasa et al., 1978). Reports of polyneuropathies in abusers of glues and solvents have appeared in the literature, but have in all cases involved mixtures of toluene and other solvents such as n-hexane and methyl ethyl ketone (Matsumura et al., 1972; Takenaka et al., 1972; Goto et al., 1974; Shirabe et al., 1974; Suzuki et al., 1974; Korobkin et al., 1975; Oh and Kim, 1976; Towfighi et al., 1976; Altenkirch et al., 1977).

Early reports of occupational exposures (generally prior to the 1950s) ascribed myelotoxic effects to toluene (Greenburg et al. 1942; Wilson, 1943), but

the majority of recent evidence indicates that toluene is not toxic towards the blood or bone marrow (Von Oettingen et al., 1942a, 1942b; Parmeggiani and Sassi, 1954; Banfer, 1961; Capellini and Alessio, 1971; Suhr, 1975; Matsushita et al., 1975). When administered orally to leukemia patients, it has been further reported that toluene had no effect on the leukemic process (Francone and Braier, 1954). Hematological abnormalities have been infrequently reported in sniffers of toluene-based glues and thinners (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967b). Other investigators have noted increases in prothrombin time (Pacseri and Emszt, 1970), decreases in progocytic activity of leukocytes (Bansagi, 1968), and increased enzyme concentrations in leukocytes and lymphocytes (Friborska, 1973) of workers who were exposed to toluene. Decreases in serum immunoglobin and complement levels (Lange et al., 1973a; Smolik et al., 1973) and leukocyte agglutinins (Lange et al., 1973b) have been reported in workers exposed simultaneously to benzene, toluene, and xylene.

Liver enlargement was reported in an early study of painters with exposures to 100-1100 ppm toluene for 2 weeks to more than 5 years (Greenburg et al., 1942), but this effect was not associated with chemical evidence of liver disease or corroborated in subsequent studies of workers (Parmeggiani and Sassi, 1954; Suhr, 1975). Chronic occupational exposure to toluene has generally not been associated with abnormal liver function (Greenberg et al., 1942; Parmeggiani and Sassi, 1954; Capellini and Alessio, 1971; Suhr, 1975), although reductions in serum bilirubin and alkaline phosphatase (Szadlowski et al., 1976) and gamma glutamyl transpeptidase (Trevisan and Chiesura, 1978) have been noted. Intensive exposure to toluene via glue or thinner sniffing appears to have a minimal effect on the liver (Christiansson and Karlsson, 1957; Grabski, 1961; Massengale

et al., 1963: Sokol and Robinson, 1963; Barman et al., 1964; Boor and Hurtig, 1977; Press and Done, 1967a, 1967b).

Exposure to mean concentrations of 60-100 ppm toluene for over 3 years did not result in abnormal urinalysis findings in female shoemakers (Matsushita et al., 1975), but clincial case reports have described proteinuria and hematuria (Lurie, 1949; O'Brien et al., 1971) and myoglobenuria and renal failure (Reisin et al., 1975) in workers who were accidentally overexposed to toluene. Pyria, hematuria, and proteinuria have been the most frequently observed signs of renal dysfunction associated with the deliberate inhalation of toluene-based glues, but these effects have not been universally observed in glue sniffers (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b). Several reports have recently appeared that associate deliberate inhalation of toluene with metabolic acidosis (Taher et al., 1974; Fischman and Oster, 1979a; Koeger et al., 1980; Bennett and Forman, 1980; Moss et al., 1980).

Acute experimental exposure to toluene within the range of 50-800 ppm have not caused any definite effects on heart rate or blood pressure (Von Oettingen et al., 1942a, 1942b; Ogata et al., 1970; Astrand et al., 1972; Gamberale and Hultengren, 1972). Toluene has been implicated in a small number of sudden deaths due to solvent sniffing which appear to result from cardiac arrhythmias (Bass, 1970; Alha et al., 1973), but trichloroethane and fluorinated aerosol propellants have most frequently been associated with these deaths.

Dysmenorrhea has been reported in a significant number of female shoemakers exposed to 60-100 ppm toluene and concomitantly to 2050 ppm gasoline in a "few" working places for an average duration of 3 years and 4 months (Matsushita et al., 1975). Disturbances of menstruation have also been reported in women exposed concurrently to toluene, benzene, and xylene in the workplace (Michon,

1965), and in women exposed occupationally to toluene and other unspecified solvents (Syrovadko, 1977).

Minimum perceptible concentrations of toluene have been determined to be 0.40-0.85 ppm (Gusev, 195) and 37 ppm (May, 1966), but the reasons for this discrepancy are not apparent. Toluene has been reported to cause transitory eye and respiratory tract irritation as a result of 8-hour exposures in the range of 200-800 ppm (Carpenter et al., 1944; Parmeggiani and Sassi, 1954; Capellini and Alessio, 1971), but no complaints of respiratory tract discomfort were recorded in volunteers or workers exposed to levels as high as 800-1500 ppm for 8-hour periods in other studies (Von Oettingen et al., 1942; Wilson, 1943). No complaints of respiratory tract or eye irritation were recorded in men accidentally exposed to 10,000-30,000 ppm toluene for brief durations (Longley et al., 1967).

Opthalmologic examinations of spray painters who were exposed to 100-1000 ppm toluene for 2 weeks to more than 5 years were normal (Greenburg et al., 1942), but Ratta et al. (1976) found lens changes in a group of car painters exposed concurrently to approximately 30 ppm toluene and much lower concentrations of other solvents for an average of 15 years. The little information that is available on the dermal toxicity of toluene indicates that moderate contact may cause skin damage due to its degreasing action (Gerarde, 1960; Browning, 1965; Molten et al., 1968).

12.1 SPECIES SENSITIVITY

Information on the toxic effects of chronic exposure to low levels of toluene may be more relevant to greater numbers of people than information on acute toxicity from the viewpoint of industrial health. However, for those rare exposures to high levels, e.g., "glue sniffing", data obtained from acute toxicity studies are valuable. In the sections to follow consideration will be given to acute, as well as chronic, studies.

Inhalation has been a principal route of exposure in humans; therefore, animal studies have centered on intoxication by this route. In all species studied the progressive symptoms typically found after increasingly higher doses were irritation of the mucous membranes, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death. Cats appeared to be more resistant than dogs and rabbits. Rats and mice were less resistant than dogs or rabbits (see Tables 12-1 and 12-2).

12.1.1 Acute Exposure to Toluene

12.1.1.1 Acute Inhalation

Carpenter et al. (1976b) reported 100% mortality in rats exposed to 4 hours' inhalation of 12,000 ppm of "toluene concentrate" comprising a mixture of paraffins, naphthenes, and aromatics (45.9% toluene and 0.06% benzene). Tremors were seen in 5 minutes and prostration in 15 minutes. At 6300 ppm, inhalation produced head tremors in 1 hour and prostration in 2 hours, while only slight loss of coordination was seen after 4 hours at 3300 ppm. A calculated LC50 of 8800 ppm for a 4-hour period of inhalation was reported in this study. Inhalation of a thinner containing less toluene (≈33%) and only 0.01% benzene, elicited less toxic symptoms at a similar range of doses in rats in a companion study by the same laboratory (Carpenter et al., 1976a).

Table 12-1. Acute Toxicity of Toluene

Route	Species	Dose	Effect	Reference
inhalation	rats	4,000 ppm for 4 h	1/6 dead	Smyth et al., 1969a
inhalation	rats	24,400 ppm for 1.5 h	60% mortality	Cameron et al., 1938
inhalation	rats	12,200 ppm for 6.5 h	50% mortality	Cameron et al., 1938
inhalation	rats	13,269 ppm	Lethal dose	Faustov, 1958
inhalation	rats	12,000 ppm for 4 h ("toluene concentrate")	Lethal dose	Carpenter et al., 1976b
inhalation	rats	6,300 ppm for 4 h ("toluene concentrate")	Head tremors in 1 h Prostration in 2 h, normal 3 h after exposure	Carpenter et al., 1976b
inhalation	rats	3,300 ppm for 4 h ("toluene concentrate")	Slight loss of coordination	Carpenter et al., 1976b
inhalation	rats	1,700 ppm for 4 h ("toluene concentrate")	No-effect-level	Carpenter et al., 1976b
inhalation	rats	8,800 ppm for 4 h ("toluene concentrate")	LC50	Carpenter et al., 1976b
inhalation	mice	24,400 ppm for 1.5 h	10% mortality	Cameron et al., 1938
inhalation	mice	12,200 ppm for 6.5 h	100% mortality	Cameron et al., 1938
inhalation	Swiss mice	5,320 ppm for 7 h (less than 0.01% benzene present)	LC50	Svirbely et al., 1943
inhalation	mice	6,942 ppm for 6 h (99.5% purity)	LC50	Bonnet et al., 1979
inhalation	mice	6,634 ppm	LC50	Faustov, 1958
inhalation	mice	9,288 ppm	Lethal dose	Faustov, 1958
inhalation	cats	7,800 ppm for 6 h ("toluene concentrate")	Progressive signs: slight loss of coordination, mydriasis, and slight hypersensitivity to light within 20 min Prostration - 80 min Anesthesia - 2 h One death during 14 d observation period	Carpenter et al., 1976b
inhalation	guinea pigs	4,000 ppm for 4 h	2/3 dead within a few days	Smyth and Smyth, 1928
inhalation	rabbits	5,500 ppm	Lethal within 40 min	Carpenter et al., 1944
inhalation	dogs	850 ppm for 1 h	Increased respiration rate, decreased respiration volume	von Oettingen et al., 1942b

Table 12-1. Acute Toxicity of Toluene (Cont'd)

Route	Species	Dose	Effect	Reference
inhalation	mice	8,600 ppm, 15,000 ppm ("toluene concentrate")	50% reduction respiratory rate	Carpenter et al., 1976b
inhalation	mice	5,000 ppm "toluene concentrate"	No-effect-level on respiratory rate	Carpenter et al., 1976b
inhalation	dogs n = 2	760 ppm "toluene concentrate" 6 h/d x 2 d rested for 4 d, exposed again for 3 d	Weight loss of 1.1 kg in 1 dog, otherwise normal	Carpenter et al., 1976b
inhalation	dogs n = 2	1,500 ppm "toluene concentrate" 6 h/d x 3 d	Slight lacrimation and head tremors	Carpenter et al., 1976b
oral	rats	7.53 g/kg (6.73-8.43)	LD50	Smyth et al., 1969a
oral	Wistar rats adult	7.0 g/kg	LD50	Wolf et al., 1956
oral	Sprague-Dawley rats (150-200 g)	5.58 g/kg (5.3-5.9 g/kg)	LD50	Withey and Hall, 1975
oral	rats 14-d-old, both sexes young adults older adults	 3.0 ml/kg (2.6 g/kg) 5.4 ml/kg (5.5 g/kg) 7.4 ml/kg (6.4 g/kg)	 LD50 LD50 LD50	Kimura et al., 1971
i.p.	rats and mice	2.0 cc/kg (1.7 g/kg)	Lethal dose	Cameron et al., 1938
i.p.	rats	0.75 cc/kg (0.7 g/kg)	Apathy	Batchelor, 1927
i.p.	rats	1.75 to 2.0 cc/kg (1.5 g/kg to 1.7 g/kg)	Death from respiratory failure	Batchelor, 1927
i.p.	rats (both sexes)	800 mg/kg at 26°C 530 mg/kg at 8°C 255 mg/kg at 36°C	Approximate lethal dose	Keplinger et al., 1959
i.p.	mice (male)	1.15 g/kg in olive oil (1.04-1.31 g/kg) (graded doses between 0.79 and 1.65 g/kg)	LD50 Observed for 24 h Cause of death: respiratory failure	Koga and Ohmiya, 1978
i.p.	mice (female)	1.64 g/kg	LD50	Ikeda and Ohtsuji, 1971
i.p.	mice	4 g/kg	Lethal dose	Tsuzi, 1956
i.p.	guinea pigs	2.0 ml pure solvent (1.7 g)	6/10 dead after 2 h All dead after 6 h	Wahlberg, 1976

Table 12-1. Acute Toxicity of Toluene (Cont'd)

Route	Species	Dose	Effect	Reference
s.c.	rats and mice	5-10 cc/kg (4.3-8.2 g/kg)	Lethal dose	Cameron et al., 1938
i.v.	rabbits	0.15 cc/kg (.13 g/kg) 0.20 cc/kg (.17 g/kg)	13% mortality 100% mortality	Braier, 1973
dermal (single application)	rabbits	14.1 ml/kg	LD50	Smyth et al., 1969a
dermal, abdomen	rabbits	uncovered application	Slight irritation	Smyth et al., 1969a
dermal	rabbits	10 to 20 applications of undiluted toluene to rabbit ear and bandaged to shaved abdomen	Perceptible erythema, thin layer of devitalized tissue which exfoliated No effect on gross appearance, behavior, or weight	Wolf et al., 1956
dermal	guinea pigs	1 ml for 16 h	Karyopyknosis, karyolysis, perinuclear edema, spongiosis, junctional separation, cellular infiltration in dermis, no liver and kidney damage	Kronevi et al., 1979
dermal	guinea pigs	2.0 ml, covered	Completely absorbed by 5th to 7th d No mortality up to 4 wk Weight less than controls for wk 1-3, no difference at wk 4	Wahlberg, 1976
corneal	rabbits	0.005 ml	Moderately severe injury	Smyth et al., 1969a
corneal	rabbits	0.005 ml	Moderately severe injury	Carpenter and Smyth, 1946
corneal	rabbits	2 drops	Perceptible irritation of conjunctival membranes No corneal injury	Wolf et al., 1956

Abbreviations: h = hour; min = minute; d = day; wk = week; i.p. = intraperitoneal; s.c. = subcutaneous; i.v. = intravenous; n = number; ns = not specified.

Table 12-2. Subchronic Effects of Toluene

Species	Route	Dose	Effect	Reference
Rat	Inhalation	1600 ppm 18-20 h/d	Initial effect of instability and incoordination, conjunctivitis, lacrimation, and sniffles; then narcosis	Batchelor, 1927
Rat	Inhalation	1600 ppm 18-20 h/d x 3 d	Mild twitching; drop in body temperature; death. Histology: severe cloudy swelling of kidneys, no effect on liver, heart, or testes	Batchelor, 1927
Rat	Inhalation	1250 ppm 18-20 h/d	Slight instability and incoordination; mucous membrane irritation	Batchelor, 1927
Rat	Inhalation	620 ppm, 1100 ppm 18-20 h/d	No-effect-level on symptoms; hyperplasia of bone marrow	Batchelor, 1927
Rat	Inhalation	1000 ppm solvent mixture (50-60% benzene, 30-35% toluene, 4% xylene) 7 h/d x 5 d x 28 wk	No effect on body weight; lymphopenia followed by leucocytosis and lymphocytosis; transient changes in blood picture before or after each daily exposure; splenic hemosiderosis greater than that found after inhalation of benzene only: slight to moderate reduction 2-1/2 wk after exposure. Narrowing of peri-follicular collars of cells in sleen, no fat in livers and kidney; iron-negative pigment in kidneys of few animals.	Svirbely et al., 1944
Rat	Inhalation	240, 480, 980 ppm "toluene concentrate" 6 h/d x 5 d/wk x 65 d	No effect on red blood cell count white blood cell count, hematocrit, hemoglobin, total and differential white count, blood urea nitrogen, SGOT, SGPT, alkaline phosphatase, or body weight.	Carpenter et al., 1976b

Table 12-2. Subchronic Effects of Toluene (Cont'd)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	3184 ppm 4 h/d x 30 d	Increased levels of SGOT, SGPT, β-lipoproteins decreased levels of glutathione, catalase, peroxidase, total cholesterol	Khinkova, 1974
Rat	Inhalation	200 ppm , 600 ppm 7 h/d x 5 d x 6 wk	No narcosis; body weight normal; no significant change in WBC count, RBC count,or hemoglobin during weekly sampling; increase in percentage of segmented cells; histological changes: slight pulmonary irritation; few casts in straight collecting tubules in rats at 600 ppm; no change in liver, spleen, heart, and bone marrow	von Oettingen, 1942b
Rat	Inhalation	2500 ppm , 5000 ppm 7 h/d x 5 d x 5 wk	Transient decrease in body weight; hyperactivity, marked incoordination, recovery after cessation of exposure; mortality in 5000 ppm group 18/25; increased bleeding time; blood picture: total leucocytes reduced after each exposure; pulmonary lesions occurred earlier than in group exposed to 200 or 600 ppm; casts in renal tubules in all rats within 2 wk of exposure; rest of histology same as 200 and 600 ppm	von Oettingen et al., 1942b

Table 12-2. Subchronic Effects of Toluene (Cont'd)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	300 ppm; 6 h/d x 5 d/wk x 15 wk	Increase of hepatic enzymes (cytochrome P-450, ethoxy-coumarin O-deethylase increased; UDP glucuronslytransferase increased only at end of exposure)	Elovaara et al., 1979
Rat, guinea pig, dog, monkey	Inhalation	107 ppm continuously for 90 d; 1085 ppm 8 h/d, 5 d/wk x 6 wk	No effect on leukocytes, hemoglobin, or hematocrit; no effect on liver, kidney, lungs, spleen or heart; no effect on brain or spinal cord of dogs and monkeys	Jenkins et al., 1970
rats n=4-6 animals	inhalation	7 consecutive cycles daily, 5 d/wk x 8 wk: each cycle, 10 min to 1200 ppm followed by 20 min solvent-free internal	Depression of body weight; increased SGOT, LDH levels; no effect on BUN levels Depression of kidney, brain, and lung weights. Histology: no effect on brain, lung, liver heart, or kidney, no sign of lipid vacuolation in liver	Bruckner and Peterson, 1981a
Dogs n=2 experimental, 1 control	Inhalation	2000 ppm 8 h/d x 6 d/wk x 4 mo, and then 2660 ppm 8 h/d, 6 d/wk x 2 mo	Death on days 179 and 180; slight nasal and ocular irritation; motor incoordination and paralysis of extremities during terminal phase; congestion in lungs, hemorrhagic liver, reduced lymphoid follicles and hemosiderosis in spleen; hyperemic renal glomeruli; albumin in urine	Fabre et al., 1955

Table 12-2. Subchronic Effects of Toluene (Cont'd)

Species	Route	Dose	Effect	Reference
Dogs	Inhalation	200, 400, 600 ppm 3 8-h exposures for 1 wk, then 5 x 7-h for 1 wk and finally 850 ppm for 1 hr	No effect on circulation, spinal pressure; increase of respiratory rate, small increase of minute volume, decrease of respiratory volume	von Oettingen et al., 1942b
Dogs	Inhalation	400 ppm; 7 h/d x 5 d	Moderate temporary lymphocytosis	von Oettingen et al., 1942b
mice n=4-6 animals	Inhalation	7 consecutive cycles daily, 5 d/wk x 8 wk: each cycle, 10 min. to 12,000 ppm followed by 20 min. solvent-free interval	Depression of body weight gain; no effect on LDH; decreased BUN levels; SGOT levels increased (not significantly) depression of kidney, brain and lung weights; Histology: no effect on brain, lung, liver, heart, or kidneys; no sign of lipid vaculoation in liver.	Bruckner and Peterson, 1981a
Mice	Inhalation	4000 ppm 99.9% pure toluene for 3 h	No effect on LDH activity significant increase of SGOT 24 h post exposure only	Bruckner an dPeterson, 1981b
Mice	Inhalation	4000 ppm 99.9% pure toluene for 3 h/d x 1, 3, or 5 d	SGOT levels increased after 1 and 3 days of treatment; no effect 24 h after 5 d	Bruckner and Peterson, 1981b
Mice	Inhalation	4000 ppm 99.9% pure for 3 h/d x 5 d/wk x 8 wk	Depression of body weight gain during first 7 wk; increased liver-to-body weight ratio after 4 wk exposure, no effect at 1, 2, or 8 wk; no increase in kidney, brain, and lung; SGOT activity increased after 4 wk of exposure, and 2 wk post-exposure, but not 2 wk of exposure, or 8 wk; no change in BUN. Histology: no effect on heart, lung, kidney, brain and liver	Bruckner and Peterson, 1981b

Table 12-2. Subchronic Effects of Toluene (Cont'd)

Species	Route	Dose	Effect	Reference
Mice	Inhalation	1, 10, 100, 1000 ppm 6 h/d x 20 d	No effect on body weight; 1 and 10 ppm caused increase of RBC count on 10th day; recovery on day 20; 100 ppm, 1000 ppm - decrease of RBC count; all doses - increase (40-70%) of WBC count on day 10; recovery for all doses except 1000 ppm; 10 ppm-1000 ppm - decrease of thrombocytes; histology: 100 ppm - slight decrease in density of bone marrow cells and in megakaryocytes and red cell elements; 1000 ppm - slight hypoplasia of red cell elements; slight to moderate disturbance in maturity of neutrophils and thrombocytes, moderate increase of reticulocytes; no change in brain, lung, liver, spleen, or kidney.	Horiguchi and Inoue, 1977
Guinea pig	Inhalation	1250 ppm 4 h/d x 6 d/wk (18 exposures)	Prostration, marked liver and renal degeneration, marked pulmonary inflammation	Smyth and Smyth, 1928
		1000 ppm 4 h/d x 6 d/wk (35 exposures)	No symptoms; slight toxic degeneration in liver and kidney	
Inhalation	CFY rats (both sexes)	265 ppm 6 h/d x 5 d/wk x 1, 3 or 6 mo	Bromsulphthalein retention decreased; Cytochrome P-450 increased independent of period of exposure; SGOT and SGPT activity unaffected	Ungvary et al., 1980

Table 12-2. Subchronic Effects of Toluene (Cont'd)

Species	Route	Dose	Effect	Reference
	CFY rats (males)	929 ppm 8 h/d x 5 d/wk x 1 wk, 6 wk, 6 mo.	Cytochrome P-450 increased independent of exposure period; no effect on SGOT or SGPT; aniline hydroxylase and aminopyrine N-demethylase activity; cytochrome b_5 concentrations increased. Histological effects: dilation of cisternae of rough endoplasmic reticulum; increase of autophagons bodies which was dose and time dependent; retarded growth of females but not males glycogen content decreased	
	CFY rats (males)	298, 796, 1592 ppm 8 h/d x 5 d/wk x 4 wk	Cytochrome P-450 increased with dose	
Rats	Subcutaneous	1 cc/kg x 21 d	Slight induration at injection site; 5-14% loss of body weight; transient slight drop in RBC and WBC counts; hyperplasia of bone marrow; moderate hyperplasia of malpighian corpuscle in spleen; marked pigmentation of spleen; focal necrosis in liver, slight cloudy swelling in kidney; no effect on heart, testes, or lungs	Batchelor, 1927

Table 12-2. Subchronic Effects of Toluene (Cont'd)

Species	Route	Dose	Effect	Reference
Guinea pig	Subcutaneous	0.25 cc/d x 30-70 d	Local necrosis at injection site; survival period: 30-70 days; polypnea and convulsions during last days of survival; hemorrhagic, hyperemic, and sometimes degenerative changes in lungs, kidneys, secondary adrenals, liver, and spleen	Sessa, 1948
Rabbit	Subcutaneous	1 cc/kg x 6 d	Transient slight granulopenia followed by granulocytosis; no change in bone marrow	Braier, 1973
		4 cc/kg	More marked effect on granulocytes; all rabbits dead by end of second day; no effect on bone marrow	
Rats	Oral	118 mg/kg/d, 354 mg/kg/d, 590 mg/kg/d x 138 d	None; parameters observed: body and organ weights, adrenals, pancreas, femoral bone marrow, lungs, heart, liver, kidney, spleen, testes, bone marrow, BUN, blood counts	Wolf et al., 1956

Abbreviations: h = hour; d = day; wk = week; SGOT = serum glutamic oxalacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WBC = white blood cell; RBC = red blood cell; UDP = uridine 5'-phosphate; BUN = blood urea nitrogen; mo = month.

In a study by Smyth et al. (1969), inhalation of 4000 ppm technical grade toluene for 4 hours produced 1 death in 6 rats. In an early study Batchelor (1927) noted that inhalation of 1600 ppm of toluene for 18-20 hours daily produced initial effects of instability and incoordination, conjunctivitis, and lacrimation, then narcosis and mild twitching. A drop in body temperature in rats, followed by death occurred after 3 days of exposure. At necropsy, a severe cloudy swelling of the kidneys was found. In this study there were no effects on liver, heart, or testes, although hyperplasia of the bone marrow was noted, suggesting possible contamination of the solvent with benzene.

In the study of Cameron et al. (1938), a concentration of 24,400 ppm of toluene produced a mortality of 60% and 10% in rats and mice, respectively, after 1.5 hours of exposure. In another group of rats and mice exposed to 1/2 the concentration but for a longer period, 6.5 hours, the mortality was 50% and 100%, respectively. These two species are probably equally sensitive. Other studies of mice include that of Svirbely et al. (1943), in which the LC50 in Swiss mice was a concentration of 5320 ppm for 7 hours, and that of Bonnet et al. (1979), in which an LC50 of 6942 ppm for 6 hours of exposure was noted.

In the study of Carpenter et al. (1976b), 4 cats survived exposure to inhalation of 7800 ppm "toluene concentrate" for 6 hours, but during exposure they showed progressive signs of toxicity, including slight loss of coordination, mydriasis, and slight hypersensitivity to light within 20 minutes, prostration within 80 minutes, and light anesthesia within 2 hours. All survived the exposure, and only 1 cat died during the 14-day observation period.

Inhalation of 4000 ppm toluene (purified by distillation) for 4 hours daily was lethal within a few days to 2 of 3 guinea pigs. The other animal was severely prostrated. Under the same regimen, animals exposed to less than 1/3 of this concentration (1250 ppm) for 6 days a week survived 3 weeks of exposure,

although they were severely affected. At 1000 ppm, guinea pigs were not affected even after 35 exposures, although there were slight toxic degenerative changes in the liver and kidney (Smyth and Smyth, 1928).

Carpenter et al. (1944) reported that inhalation of a concentration of about 55,000 ppm was lethal for 6 rabbits in about 40 minutes (range of 24 to 62 minutes).

Von Oettingen et al. (1942b) observed that inhalation of 850 ppm of toluene containing 0.01% benzene for 1 hour by 6 dogs produced an increase of respiratory rate and a decrease of respiratory volume. Exposure to 1500 ppm of "toluene concentrate" for 6 hours daily for 3 days produced only slight lacrimation and head tremors in dogs. Reduction of the concentration to 1000 ppm did not alleviate the head tremors (Carpenter et al., 1976b).

Bruchner and Peterson (1981) found an age-dependent sensitivity in rats and mice. Mice, 4 weeks of age, were found to be more susceptible to exposure of 2600 ppm toluene vapor for 3 hours than 8 and 12 week old animals.

12.1.1.2 Acute Oral Toxicity

An LD50 of 7.53 g/kg and 7.0 g/kg body weight for a single oral dose in rats has been reported by Smyth et al. (1969a) and Wolf et al. (1956), respectively. Withey and Hall (1975) found 5.58 g/kg to be the LD50 in male Sprague-Dawley rats. Immature 14-day-old Sprague-Dawley rats were more sensitive than young or mature adult male rats of the same strain to the acute effects of toluene (analytical grade) in the studies of Kimura et al. (1971). These investigators determined an oral LD50 of 3.0 ml/kg body weight, 6.4 ml/kg body weight, and 7.4 ml/kg body weight for each group, respectively. This age-dependent sensitivity was also noted by exposure to inhalation (see Section 12.1.1.1). Cameron et al. (1938), however, reported that very young rats were more resistant to toluene than adult animals of the Wistar strain. Thirty-three

percent of a group of 12 9-day-old rats survived 5.25 hours of exposure to air saturated with toluene, in contrast to 100% mortality in the same period in a group of adult rats.

Based on the results of their studies on the oral toxicity of toluene in animals of different age groups, Kimura et al. (1971) suggested a maximum permissible limit for a single oral dose of 0.002 ml/kg body weight. This was obtained by taking 1/1000 of the dose giving the first observable gross signs of drug action on the central nervous system.

12.1.1.3 Acute Effects from Intraperitoneal Injection

Mortality is produced by a single intraperitoneal injection of toluene in the range of 0.8 to 1.7 g/kg in rats and mice. In a series of doses of toluene graduated between 0.79 and 1.65 g/kg and diluted in olive oil, Koga and Ohmiya (1978) determined an LD50 of 1.15 g/kg body weight in male mice. Respiratory failure was the main cause of death in these animals. An LD50 of 1.64 g/kg was reported in female mice by Ikeda and Ohtsuji (1971). Whether the disparity is due to interlaboratory differences or whether a sexual difference in sensitivity exists has not been tested. In rats 0.75 cc/kg produced apathy, while 1.75-2.0 cc/kg produced death from respiratory failure (Batchelor, 1927); 2.0 cc/kg was a lethal dose in rats, mice (Cameron et al., 1938), and guinea pigs (Wahlberg, 1976).

Savolainen (1978) observed that after an intraperitoneal injection of radiolabeled toluene, concentration of the label in the central nervous system (CNS) was highest in the cerebrum. The content of label in the CNS was undetectable by 24 hours after injection, which may be a simulation of acute toluene intoxication where clinical signs of toxicity are lost within 24 hours.

A temperature-dependent sensitivity to the solvent was observed in adult rats of both sexes by Keplinger et al. (1959). At 26°C the lethal dose was

800 mg/kg while at 8°C and 36°C, lethal doses were 530 mg/kg and 225 mg/kg, respectively. The toxicity of toluene is greater in hot and cold environments. Whether increased susceptibility to the solvent is caused by the stress of altered environmental temperature or by altered physiological processes, e.g., absorption, diffusion, distribution, or metabolic rate, is unknown.

12.1.1.4 Acute Effects From Subcutaneous Injection

Ranges of 1.25 to 2.0 cc/kg and 5 to 10 cc/kg have been found to produce mortality in rats and mice, respectively, when injected subcutaneously (Batchelor, 1927; Cameron et al., 1938). Braier (1973) reported that 4 cc toluene/kg toluene injected into rabbits produced marked transient granulopenia within 24 hours and marked granulocytosis and ensuing death in all animals by the end of the second day. A slight area of induration was seen at the injection site.

12.1.1.5 Acute Effects from Intravenous Injection

Intravenous injection of 0.2 cc/kg produced 100% mortality in rabbits (Braier, 1973).

12.1.1.6 Acute and Subacute Effects of Percutaneous Application

Repeated application of undiluted solvent to the rabbit ear or shaved skin of the abdomen produced slight to moderate irritation (Wolf et al., 1956; Smyth et al., 1969a) and increased capillary permeability locally (Delaunay et al., 1950). Continuous cutaneous contact in the guinea pig resulted in slower weight gain, karyopyknosis, karyolysis, spongiosis, and cellular infiltration in the dermis within 16 hours (Kronevi et al., 1979; Wahlberg, 1976). Application to the abdominal skin of the rat produced hemoglobinuria (Schutz, 1960). Slight irritation of conjunctival membranes but no corneal injury (Wolf et al., 1956) or moderately severe injury (Carpenter and Smyth, 1946; Smyth et al., 1969a) followed direct application to the eye.

12.1.2 Subchronic and Chronic Exposure to Toluene

Subchronic and chronic exposures to toluene in animals reveal little toxic effect with the exception of the study of Fabre et al. (1955) in 2 dogs subjected to much higher concentrations. Svirbely et al., (1944) found that repeated inhalations of 1000 ppm of a solvent mixture containing 30-35% toluene, 50-60% benzene, and a small amount of xylene for 28 weeks (7 hours/day, 5 days/week) had no effect on body weight in rats or dogs. There was no significant increase of liver volume, and no fat was found in the liver or kidneys; however, narrowing of perifollicular collars was observed in the spleen (see Table 12-2). Splenic hemosiderosis was greater than that found after exposure to benzene (Svirbely et al., 1944).

Neither continuous exposure to 107 ppm toluene for 90 days nor repeated exposure to 1,085 ppm for 6 weeks (8 hours/day, 5 days/week) affected liver, kidney, lungs, spleen, or heart in 30 rats, 30 guinea pigs, 4 dogs, or 6 monkeys. In addition, there were no effects of treatment seen in the brain or the spinal cord of dogs or monkeys. No significant change was observed in any of the hematologic parameters (hemoglobin, hematocrit, or leucocyte count). All animals except 2 of 30 treated rats survived exposure, and all gained body weight with the exception of the monkeys (Jenkins et al., 1970).

Similarly, repeated inhalation of 240, 480, or 980 ppm of "toluene concentrate" for 13 weeks (6 hours/day, 5 days/week) produced no treatment-related organ damage in rats or dogs. SAP, SGPT, SGOT, and blood urea nitrogen (BUN) activities were normal. Prior treatment with toluene did not render the animals either more susceptible or more resistent to a subsequent challenge dose of 12,000 ppm (Carpenter et al., 1976b).

Fabre et al. (1955) exposed 2 dogs for 8 hours daily, 6 days a week, to inhalation of 7.5 mg/l (2000 ppm) pure toluene for 4 months and then to 10 mg/l

(2660 ppm) for 2 months. Slight nasal and ocular irritation occurred at the lower concentration. Motor incoordination preceding paralysis of the extremities occurred in the terminal phase. Death occurred on days 179 and 180. There was no effect on gain in body weight, on the bone marrow, adrenal glands, thyroid, or pituitary gland. Congestion in the lungs, hemorrhagic liver, a decrease of lymphoid follicles, and hemosiderosis in the spleen were observed. Glomeruli of the kidney were hyperemic, and albumen was found in the urine.

In a recent chronic 24-month study (CIIT, 1980) where Fischer 344 rats of both sexes were exposed to 30, 100, or 300 ppm 99.98% pure toluene for 6 hours/day, 5 days/week, a battery of clinical chemistry tests (BUN, SAP, SGPT), hematologic studies, and urinalyses (specific gravity, blood, ketones, protein, and pH) (see Table 12-3) revealed normal levels in the treated animals except for two hematologic parameters in the female. Females exposed to 100 or 300 ppm showed significantly reduced hematocrit levels, while the mean corpuscular hemoglobin concentration was significantly increased in females exposed to 300 ppm. Body weights in males of the treatment groups were significantly higher than body weights of controls from approximately week 48 until termination of the study, while body weights of females in the treatment group were higher than body weights of controls from week 70 until the final 4 weeks of the study when the effect disappeared (see Table 12-4). No dose-response relationship was noted. Mortality in the treatment groups did not differ from controls (14.6%). Although a variety of proliferative, degenerative, and inflammatory lesions were observed in various organs, the lesions occurred with equal frequency in all control and treatment groups, and the authors concluded that no tissue changes could be attributed to toluene inhalation. Neoplasms were observed frequently in the lungs and liver, as well as in the endocrine organs, lymphoreticular system,

Table 12-3. 24-Month Chronic Exposure of Fischer 344 Rats Exposed 6 Hours/Day, 5 Days/Week, to Toluene by Inhalation (CIIT, 1980)

Group	Number of Animals	WBC (10^3/cu mm)	RBC (10^6/cu mm)	HB (g/DL)	HCT (%)	MCV (Cu. Mic.)	MCH (µµg)	MCHC (%)
			18 Months of Exposure (Males)					
Control	89	6.03	8.757	16.56	43.10	50.4	18.87	38.04
30 ppm	89	9.96*	8.766	16.61	42.42	49.6	18.90	38.82
100 ppm	89	6.54	8.700	16.47	41.93	49.5	18.91	38.93
300 ppm	90	6.53	8.894	16.80	42.34	48.8**	18.85	39.30**
			24 Months of Exposure (Males)					
Control	89	7.51	9.866	18.91	51.78	51.2	19.24	37.87
30 ppm	89	8.66	8.736	16.58	46.51	52.5	19.05	36.33
100 ppm	89	8.13	9.925	18.47	51.61	50.7	18.67	38.84
300 ppm	90	7.50	9.407	18.33	47.35	50.9	19.44	39.33
			18 Months of Exposure (Females)					
Control	90	4.04	8.022	15.67	41.70	53.0	19.49	37.26
30 ppm	90	4.59	7.956	15.77	41.25	52.8	19.77	37.90*
100 ppm	90	3.91	7.915	15.75	40.83	52.7	19.85*	38.24*
300 ppm	90	4.21	8.010	15.78	41.20	52.4	19.63	37.98
			24 Months of Exposure (Females)					
Control	90	4.93	8.397	16.46	44.99	54.7	19.50	36.10
30 ppm	90	5.40	8.274	15.89	43.06	53.3	19.11	36.42
100 ppm	90	5.74	8.076	15.94	42.47*	53.9	19.68	37.08
300 ppm	90	4.87	8.090	15.86	42.02**	53.1	19.52	37.46*

*Statistically significant difference from control ($P < 0.05$)
**Statistically significant difference from control ($P < 0.01$)
Abbreviations: WBC = white blood cell count; RCB = red blood cell count; HB - hemoglobin concentration; DL = 100 milliliters; HCT = hematocrit; MCV = mean corpuscular volume; Mic. = micron; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

Table 12-4. 24-Month Chronic Exposure of Fischer 344 Rats Exposed 6 Hours/Day, 5 Days/Week, to Toluene by Inhalation (CIIT, 1980)

Group	Number Animals	Mean Body Weight in Grams, Weeks of Exposure						Total Weight Change
		0	26	52	78	100	104	
Males								
Control	89	141	340	384	426	430	430	286
30 ppm	89	141	349*	396**	445**	456**	454**	314**
100 ppm	89	141	351**	404**	447**	454**	452**	312**
300 ppm	90	142	341	403**	446**	451**	445	304**
Females								
Control	90	109	203	213	214	260	265	156
30 ppm	90	109	191**	211	246**	272**	273*	164
100 ppm	90	109	194	211	248**	272**	275	166
300 ppm	90	109	195**	211	248**	271**	272	163

*Statistically significant difference from control ($P < 0.05$)
**Statistically significant difference from control ($P < 0.01$)

mammary gland, integument, testis, and uterus. Chronic progressive nephropathy was present in the urinary system (CIIT, 1980).

Although this study was comprehensive and is the only chronic study of toluene in laboratory adrenals, there are several deficiencies in this study which might becloud interpretation. The high spontaneous incidence (16%) of mononuclear cell leukemia in aging Fischer 344 rats reported by Coleman and coworkers (1977) suggests that this strain may be inappropriate for the study of a chemical that might be myelotoxic. A high testicular interstitial cell tumor incidence (66.2% reported by Coleman et al., 1977 and 85% bilaterial tremors reported by Mason et al., 1971) atuomatically removes this organ from any assessment of carcinogenicity, although this might not be a target organ for toluene. Therefore it would be an irrelevant point. The low mortality of rats in this study (14.6%) differs from the mortality rate (up to 25%) associated with maintaining these animals under barrier conditions (NCI, 19 a,b,c). If these animals were not raised under barrier conditions (which is not stated), then still higher mortality rates could be expected in this age group of Fischer 344 rats. No quality assurance of the study was extant after 6 months into the chronic study (CIIT public review of toluene study, May 12, 1981).

A higher exposure level, 1000 ppm, was dropped from this study based on a pilot investigation which revealed that body weight loss might interfere with maintenance of these animals for 24 minutes. Lack of a group at this level, where behavioral and central nervous system effects have been reported, or a group at some intermediate level precluded information on a possible effect/no-effect level.

In the only subchronic oral study, female rats fed up to 590 mg toluene/kg by intubation for periods of up to 6 months did not show ill effects as determined by gross appearance, growth, periodic blood counts, analysis for blood urea

nitrogen, final body and organ weights, bone marrow counts, or histopathological examination of adrenals, pancreas, lungs, heart, liver, kidney, spleen, and testis (Wolf et al., 1956).

12.2 EFFECTS ON LIVER, KIDNEY, AND LUNGS

Organ effects in the kidney and, possibly in the liver and lungs after higher doses, have been reported.

12.2.1. Liver

No histological damage was observed after subchronic and chronic inhalation of 1000 ppm of a solvent mixture containing 30-35% toluene for 28 weeks, 980 ppm of "toluene concentrate" for 13 weeks, 1085 ppm of toluene for 6 weeks, and 300 ppm of 99.98% pure toluene for 24 months in a variety of species in studies described in Subsection 12.1.2 (Svirbely et al., 1944; Carpenter et al., 1976b; Jenkins et al., 1970; CIIT, 1980). Furthermore, no liver damage was detected in female rats after subchronic daily oral doses of 590 mg/kg for 6 months (Wolf et al., 1956). Two preliminary reports (abstracts of presentations) from the laboratory of Bruckner and Peterson noted no effect on hepatorenal function. In a regimen mimicking solvent "sniffing," male rats and mice were exposed to 12,000 ppm toluene for 7 10-minute periods (with 20-minute solvent-free periods intervening) 5 days/week for 8 weeks. No organ pathology was found. Lactic dehydrogenase, SGPT activities, BUN content, and liver triglyceride content were normal (Bruckner and Peterson, 1978). In another study, inhalation of 4000 ppm toluene (3 hours/day, 5 times weekly) for up to 8 weeks failed to reveal toluene induced hepatorenal injury by a battery of toxicological tests (SGOT activity, BUN levels, urinary glucose and protein concentration, and urinary cell count) and upon histopathological examination of the liver, kidney, and lung (Bruckner and Peterson, 1976).

Although these early reports revealed no effect on SGOT activity or BUN levels in mice and rats, a recent paper (Bruckner and Peterson, 1981b) noted an increase in SGOT activity in mice and rats during intermittent exposure to 1200 ppm toluene (see Section 12.3). Increase in LDH activity was seen in rats and decrease in BUN levels was seen in mice. No histological changes were observed, but an increase of organ weight to body weight was found.

In a study in which reagent grade toluene that was dissolved in corn oil was injected intraperitoneally in doses of 150, 300, 600, or 1200 mg/kg into adult male guinea pigs, there was no change in serum ornithine carbamyl transferase activity at any dose level in blood collected 24 hours later. Histological examination revealed no liver abnormalities or lipid accumulation with the exception of the highest dose where there was evidence of lipid accumulation (Divincenzo and Krasavage, 1974).

Two hours after male rats (weighing 150 to 300 g) were administered 2600 µmol/100 g body weight of toluene in mineral oil by gavage, there was no evidence of injury to the microsomal function of the liver. There was no effect on protein synthesis, cell sap RNA, glucose 6-phosphatase, oxidative demethylase, nicotinamide adenine dinucleotide phosphate (NADPH) neotetrazolium reductase, or lipid conjugated diene content of microsomes (Reynolds, 1972). Inhalation of 300 ppm toluene (6 hours/day, 5 days/week) for 15 weeks slightly increased cytochrome P-450 content in liver, appreciably enhanced ethoxycoumarin o-deethylase, and at the end of exposure increased UDP glucuronyltransferase activity. The content of toluene in perirenal fat tended to decrease during continued exposure, while the presence of toluene in the brain was detected throughout exposure. The diminution of toluene content in perirenal fat at the same time that drug metabolizing enzymes increased suggests an adaptation to continued presence of thee solvent (Elovaara et al., 1979).

Continuous cutaneous contact with a dose of 2.0 ml toluene, which was completely absorbed within 5 to 7 days, produced no change in liver morphology (Wahlberg, 1976).

Although the studies just cited indicate the absence of toluene-induced toxicity, there are others which suggest a slight toxic effect. In a study by von Oettingen et al. (1942b) inhalation of concentrations of 600 to 5000 ppm toluene containing 0.01% benzene for 5 weeks (7 hours/day, 5 days/week) in rats caused an enlargement of the liver (increase of weight and volume) in a dose-dependent manner 16 hours after the last exposure. Histologically, there was a progressive decrease of density of the cytoplasm in the liver cells as the concentration of toluene increased. These observations were not seen in rats sacrificed 2 weeks after the last exposure. No evidence of hyperemia was seen in the liver. Matsumoto et al. (1971) reported an increase in liver weight and liver weight to body weight ratio in rats exposed 9 hours/day, 6 days/week for 43 weeks to 2000 ppm toluene vapor. This was not noted at lower doses (100 ppm or 200 ppm).

In the study of Fabre et al. (1955) 2 dogs exposed for 4 months (8 hours/day, 6 days/week) to inhalation of 7.5 mg/l (2000 ppm) pure toluene and, subsequently, to 10 mg/l (2660 ppm) for 2 months had hemorrhagic livers.

Tahti et al. (1977) observed that inhalation of 1000 ppm toluene 8 hours/day, for 1 week increased SGOT and SGPT activity and induced metabolic acidosis in rats.

Histological changes in the liver were found when male CFY rats were injected intraperitoneally with 0.05 or 0.1 ml/100 g body weight of analytical grade toluene for up to 4 weeks. There was a dose-dependent increase in the number of mitochondria per unit of cytoplasmic area in the liver. Total area, nuclear density, and nucleus/cytoplasmic ratio increased at the higher dosage.

Dose-dependent decreases in nuclear volume were seen after intraperitoneal or subcutaneous injection, with subcutaneous injection being less effective than intraperitoneal injection. The authors suggested that the considerable accumulation of mitochondria was related to increased metabolism of the liver and that oxidative detoxification of the solvent might involve mitochondrial enzymes as well as hepatic microsomal enzymes (Ungvary et al., 1976). In an earlier paper Ungvary et al. (1975) found that intraperitoneal or subcutaneous administration of toluene produced degenerative changes, i.e., separation of ribosomes and vacuolar dilation of the rough endoplasmic reticulum. In these studies the higher concentrations of toluene also decreased glycogen content. Following discontinuation of exposure, the hepatic changes indicating increased load on detoxification processes (increased succinate dehydrogenase (SDH) activity, increase of mitochondria and smooth endoplasmic reticulum, decreased glycogen content) as well as degeneration (dilation of endoplasmic reticulum, accumulation of autophagous vacuoles) rapidly regressed, indicating that the toxic and liver loading effects of toluene are reversible. The regenerative property of the liver after hepatectomy was not significantly affected by exposure to toluene (Hudak et al., 1976).

In a more recent study by Ungvary et al. (1980) where male CFY rats were exposed to inhalation of 265 ppm (6 hour daily), 929 ppm or 1592 ppm (8 hour daily), analytical grade toluene and female rats were exposed to lowest dose only (five times a wseek up to 6 months) growth was inhibited in male's at the higher concentration and in females only at the low dose. No abnormal histological changes were found in the liver. Liver weight was increased by treatment. Signs of adaptive compensation that were observed include proliferation of smooth endoplasmic reticulum, increased cytochrome P450 and cytochrome b_5 activity, increased aniline hydroxylase activity and aminopyrine N-demethylase activity.

These changes which were dose-dependent and reversible showed no or slight dependence on exposure time. There was no effect on SGOT or SGPT activity. The authors concluded from their latest studies that subchronic toluene exposure to toluene vapors has no specific hepatotoxic effect. The results of toluene inhalation corroborated earlier histological findings (by the intreperitoneal or subcutaneous route of this laboratory except that necrotic areas were not found after inhalation. Whether this reflects the different route of exposure or the higher concentration of toluene administered intraperitoneally has not been ascertained.

12.2.2 Kidney

No histological effects of renal toxicity were seen in subchronic inhalation studies (see Table 12-2) in mice exposed to 1000 ppm for 20 days (Honuguchi and Inoue, 1977), in rats, guinea pigs, dogs, or monkeys exposed to 1085 ppm for 6 weeks (Jenkins et al., 1970), in rats and mice exposed to 4000 ppm vapors for 8 weeks (Bruckner and Peterson, 11981b), or in chronic inhalation studies in rats exposed to 300 ppm for 24 months (CIIT, 1980). Neither was any effect of toluene observed in renal histology after subchronic oral dosing of 590 mg/kg for 138 days in rats (Wolf et al., 1956).

Pathological renal changes, however, have been observed in some studies. Von Oettingen et al. (1942b) found increasing numbers of casts in the collecting tubules of rat kidneys during inhalation of concentrations ranging from 600 ppm to 5000 ppm for 5 weeks (7 hours daily, 5 days/week). A few casts in the kidney were seen after the third week of exposure at 600 ppm and earlier in the higher doses. Appreciable fat in the convoluted tubules and hyaline casts in the collecting tubules of the kidney were observed in dogs after inhalation of 200 to 600 ppm for approximately 20 daily 8-hour exposures, then inhalation of 400 ppm for 7 hours/day, 5 days/week for 1 week, and finally to 850 ppm for 1 hour. In

the studies of Matsumoto et al., (1971) exposure of rats to inhalation of 2000 ppm for 8 hours/day, 6 days/week for 43 weeks produced histopathological findings of hyaline deplete in renal tubules. There was an increase of kidney weight and an increase of the ratio of kidney weight to body weight.

After inhalation of 7.5 mg/l toluene 8 hours/day, 6 days/week, for 4 months and followed by exposure to 10 mg/l during the remaining two months, hyperemic renal glomeruli and albuminuria were observed at autopsy in dogs by Fabre et al. (1955). Inhalation by guinea pigs of 1000 ppm (of distillation pure toluene) 4 hours/day, 6 days/week, for a total of 35 exposures produced slight toxic degeneration in the kidney. Eighteen exposures at a higher dose of 1250 ppm produced more marked degeneration (Smyth and Smyth, 1928). Degeneration of convoluted tubular epithelium in guinea pigs exposed by the subcutaneous route was reported in an abstract of a paper by by Sessa (1948).

12.2.3 Lungs

No histological damage of the lungs were seen after inhalation of 1000 ppm vapors for 20 days in mice (Hougnchi and Inoue, 1977), inhalation of 1085 ppm for 6 weeks in rats, guinea pigs, dogs, or monkeys (Jenkins et al., 1970), inhalation of 4000 ppm for; 8 weeks in rats and mice (Bruckner and Peterson, 1981b), 300 ppm for 24 months in rats (CIIT, 1980), a ingestion of 590 mg/kg for 138 days in rats (Wolf et al., 1956).

Irritative effects on the respiratory tract, however, have also been reported (Browning, 1965; Gerarde, 1959; Fabre et al., 1955; von Oettingen et al., 1942b).

Marked pulmonary inflammation was seen in guinea pigs after exposure to inhalation of 1250 ppm distillation pure toluene 4 hours daily, 6 days/week, for 18 exposures (Smyth and Smyth, 1928).

Hemorrhagic, hyperemic, and sometimes degenerative changes in the lungs have been observed in guinea pigs after a subcutaneous injection of 0.25 cc of toluene daily for 30 to 70 days as reported in an abstract (Sessa, 1948). Congestion in the lungs of dogs which had undergone repeated exposure to concentrations of 200 to 600 ppm toluene and to a final exposure by inhalation of 850 ppm for 1 hour, and pulmonary lesions in rats after 1 week of inhalation of 2500 ppm (7 hours/day, 5 days/week) were reported by von Oettingen et al., (1942b).

Congestion in the lungs was noted by Fabre et al. (1955) in dogs and in rabbits at the higher doses.

12.3 BEHAVIORAL TOXICITY AND CENTRAL NERVOUS SYSTEM EFFECTS

Excessive depression of the central nervous system has been linked with acute exposure to high levels of toluene. A concentration of 20,000 ppm toluene was lethal to rats after 30 to 50 minutes of exposure with death attributed to depression of the CNS (Kojima and Kobayashi, 1975, cited in NRS, 1980). Inhalation of 12,000 ppm of "toluene concentrate" containing 0.06% benzene was lethal to rats following tremors which appeared within 5 minutes of exposure and prostration which occurred within 15 minutes of exposure (Carpenter et al., 1976b).

A dose-related effect on instability, incoordination, and narcosis was found in rats exposed 18-20 hours daily to toluene concentrations of 1600 ppm and 1250 ppm. No symptoms were seen at 1100 ppm (Batchelor, 1927). Carpenter et al. (1976b) reported that rats were unaffected by exposure to inhalation of 1700 ppm of a "toluene concentrate" for 4 hours and suffered only slight incoordination at 3300 ppm. Dogs were unaffected by exposure to vapors of 760 ppm for 6 hours, but exhibited head tremors at 1500 ppm. After inhalation of 7800 ppm "toluene concentrate" for 6 hours, cats exhibited loss of coordination followed by

prostration and, finally, light anesthesia within 2 hours. All survived exposure.

Within 10 seconds after 1 intravenous injection of 0.07 cc toluene per kg body weight in 1 dog, generalized rigidity with hyperextension of the back was noted in a study made by Baker and Tichy (1953). Recovery occurred within 12 minutes. A second injection 5 days later produced a similar sudden rigidity. When a series of 10 doses of 0.07 cc toluene/kg was given intravenously every 3 to 5 days to another dog, the effect was rigidity in the animal and twitching of the extremities. Recovery occurred in 5 to 10 minutes. At necropsy after the last dose was given, cortical and cerebellar atrophy was found. Marked shrinkage and hyperchromaticity of many cortical neurons, patchy myelin pallor, and fragmentation, especially in perivascular areas, were found. Multiple fresh petechiae, especially in the white matter, was seen. There was a decrease and degeneration of Purkinje cells in the cerebellum (Baker and Tichy, 1953).

In the section on effects on humans (Section 10.1), inhalation of readily available thinners by young adults has been described as a prevalent practice which typically affects the CNS. Inhalation of solvent mixtures containing toluene in the laboratory rat have demonstrated similar effects. Inhalation of a mixture of solvents containing 25% methylene chloride, 5% methanol, 43% heptane, and 23% toluene for 10 minutes (60 to 226 mg/liter) caused a decrease in rearing and grooming, the appearance of ataxia, abnormal scratching, hind limb flaccid paralysis, and, finally, unconsciousness in male Fischer rats. Cumulative effects were noted with 4 intermittent 10 minute exposure periods with 15 minutes between exposures. When the interval between each exposure was increased to 40 minutes, recovery was almost complete (Pryor et al., 1978).

Subchronic exposure to a thinner containing toluene impaired acquisition of a complex behavior. Rats inhaled 50,000 ppm of a readily available commercial

paint thinner composed of 42% toluene, 25% methanol, 10% methyl iso-butyl ketone, and minor amounts of other solvents for 4, 8, or 16 weeks (twice-daily for 10-minute periods, 5 days a week) and then were observed for acquisition of temporal discrimination in a differential reinforcement of low rate schedule (DRL 20). In this test, the animal is rewarded for single responses, e.g., bar press made every 20 seconds. The results suggested that persistent inhalation of thinner vapors impaired temporal discrimination when the animals were tested within a relatively short time after the period of inhalation. However, responses in rats that had a period of rest after exposure did not differ from controls (Colotla and Bautista, 1979).

Studies in laboratory animals have shown that toluene contributes to the symptoms of thinner toxicity. In the studies of Peterson and Bruckner (1978), impairment of cognitive functional and muscular coordination were used to monitor CNS depression and narcosis. Behavioral performance (visual placing, grip strength, wire maneuver, tail pinch, and righting reflex) in mice exposed to 3980 ppm (15 mg/liter) toluene for 3 hours decreased over time of exposure, which was inversely correlated with toluene concentration in brain tissue. Concentration of toluene in the brain increased exponentially with the length of exposure and similarly decreased after termination of exposure, as did levels of toluene in liver and blood (see Figure 12-1). A single 10-minute exposure to a higher concentration (10,615 ppm) followed the pattern elicited by the lower concentration for a longer period. Recovery of behavioral performances occurred as solvent concentration in the brain decreased after exposure. Buckner and Peter (1981a) noted that ataxia, immobility in the absence of stimulation, hypnosis with difficult arousal and unconsciousness were apparent in mice at concentrations in blood of 40-75 µg/g, 75-125 µg/g, 125-150 µg/g and >150 lµg/g, respectively, as measured by the air bleb method.

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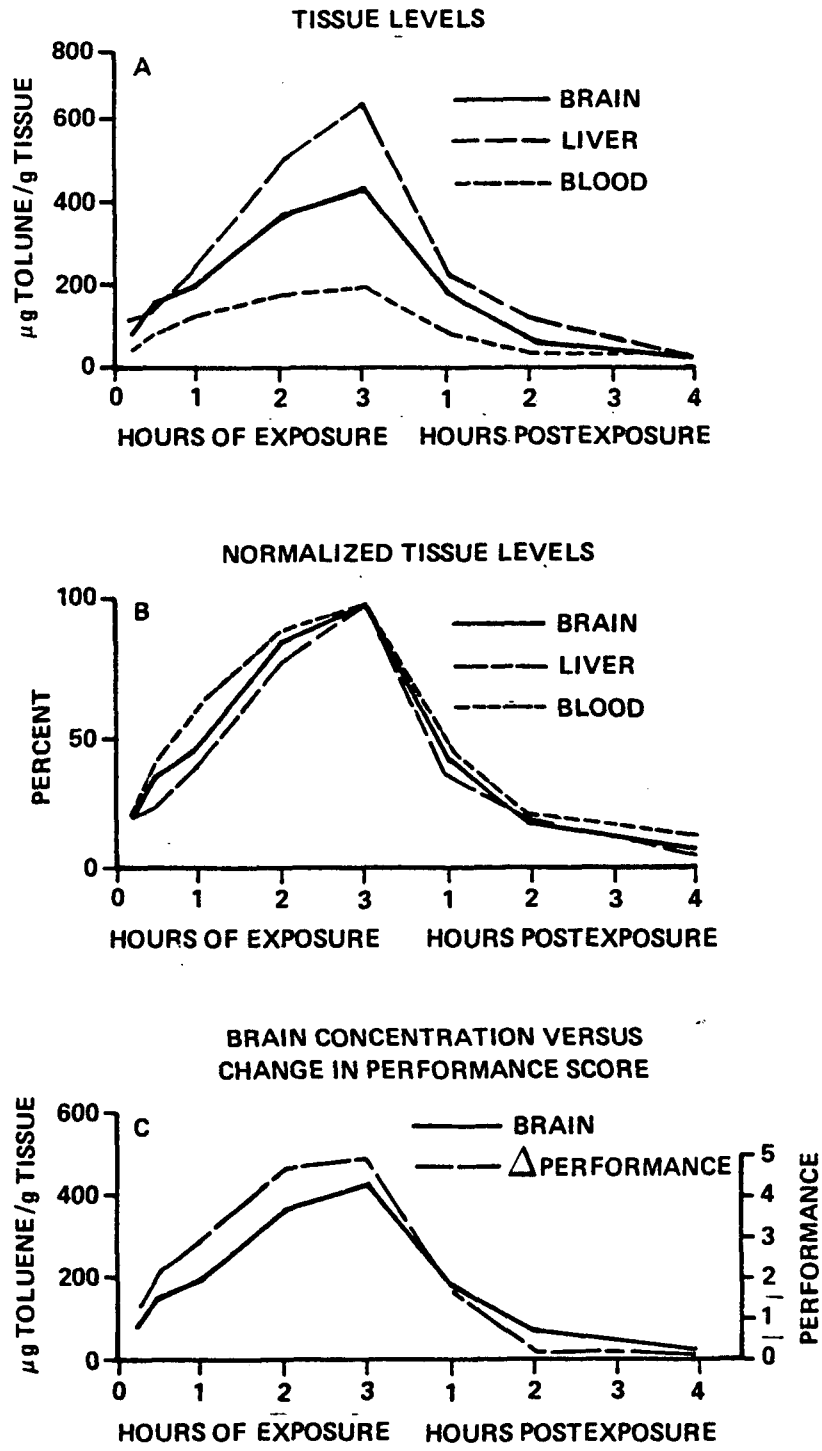

Figure 12-1. Toluene Levels in Tissue and Behavioral Performance (Mice were continuously exposed for 3 hours to an intoxicating concentration of toluene (15 mg per liter of air). Groups of animals were analyzed for air bleb concentration, reflex performance, and tissue levels after 15, 30, 60, 120, and 180 minutes of exposure and 1, 2, and 4 hours postexposure. Figure 12-1A shows toluene levels in liver, brain, and blood. Figure 12-1B shows toluene normalized to the highest mean level in each tissue. Figure 12-1C compares brain levels of toluene with change in performance of the animals. Lines represent means. N = 7 mice on all but 4 hours postexposure, in which case, N = 6.) (Peterson and Bruckner, 1978)

Bruckner and Peterson (1981b) observed that the onset of narcosis and the depth of CNS depression was dose-dependent. In mice exposed to inhalation of 12,000 ppm toluene the rapidity and depth of depression was greater than that of mice exposed to 5200 ppm. In the latter group these parameters exceeded those found in mice exposed to one-half the concentration (2600 ppm). Recovery was rapid. After exposure to 12,000 ppm for 20 minutes mean performance levels scored prior to exposure were resotred within approximately one-half hour in 4-week old rats.

A study was made by Peterson and Bruckner (1978) in mice to mimic the conditions typical of human solvent-sniffing abuses. During intermittent exposure to 10,615 ppm (5 minutes of exposure followed by 10 minutes without toluene or 10 minutes of exposure followed by 20 minutes without toluene) for approximately 3 hours or 11,942 ppm (10 minutes of exposure followed by 20 or 30 minutes without toluene) for approximately 3 hours, reflex performance became progressively lower throughout the experimental period for the regimens allowing 20 minutes or less toluene-free intervals. A 30-minute toluene-free interval between exposures permitted almost unimpaired performance indicating complete recovery between exposures (Peterson and Bruckner, 1978).

In a later acute study Bruckner and Peterson (1981a) exposed mice and rats to 7 consecutive cycles each cycle consisting of 10-minute exposure to inhalation of 12,000 ppm toluene followed by a 20-minute solvent-free recovery period. Unconditioned performance and reflexes of the animals were tested immediately prior to an following exposure. The mice showed almost complete recovery during the course of treatment while performance scores of rats exhibited a progressive decline. The authors speculated that the rapidity of recovery in mice might be attributed to the higher circulatory, metabolic, and respiratory rates of mice; that the increasing CNS depression seen in rats over

the 3-hour period of intermittent inhalation might recur from a progressive accumulation of the chemical. Substantial residual quantities in the brain 1 hour post exposure had been noted by the same authors in an earlier paper (Bruckner and Peterson, 1981a).

In a subchronic study, groups of 6 mice or 4 rats with comparable numbers of controls were subjected to 7 consecutive cycles (as described in the perceding paragraph) on a daily basis, 5 times a week for 8 weeks. Depression of body weight gain was seen in both rats and mice during 8 weeks of the intermittent toluene exposure. An increase in SGOT levels was noted in rats and mice but the increase in mice was not statistically significant. An increase in LDH was seen only in rats exposed to toluene at all sampling intervls. BUN levels in rats were unaffected by treatment whereas BUN levels in mice were consistently lower during the period of exposure. Recovery ocurred within 2 weeks post exposure. There were noe ffects on hair, lung, liver, heart, or kidney histology, although a depression in gain of age weights (kidney, brian, lung) was noted in treated mice and rats (Bruckner and Peterson, 1981a).

After a single exposure to 800 ppm toluene for 4 hours, unconditioned reflexes and simple behavior (corneal, grip, and righting reflexes, locomotor activity, and coordination) began to fail (Krivanek and Mullin, 1978). In these studies, male rats were exposed to concentrations of 0, 800, 1600, 3200, and 6400 ppm and tested at 0.5, 1, 2, and 4 hours during exposure and 18 hours after exposure (see Table 12-5).

Concentrations of toluene as low as 1 ppm administered 6 hours/day depressed wheel turning performance (a spontaneous activity) after 10 days of exposure in adult male mice (Horiguchi and Inoue, 1977). No effect on body weight was seen at any of the dosages used (1, 10, 100, and 1000 ppm) during the 20 daily exposures. However, there were alterations in blood elements in animals exposed to 10, 100, and 1000 ppm, which are noted in Section 12.5.

Table 12-5. Effect of Toluene on Behavior

Route	Species	Dose	Effect	Reference
inhalation	Wistar rats	574, 1148, 2296, and 4595 ppm	Deficit in multiple response schedule	Colotla and Bautista, 1979
inhalation	Sprague-Dawley rats	150 ppm for 0.5, 1, 2 or 4 h	Initial stimulation followed by depression in multiple response schedule	Geller et al., 1979
inhalation	rats (male)	550 to 800 ppm for 4 h/d x 2 wk	No effect on avoidance response	Battig and Grandjean, 1964
inhalation	rats	4000 ppm 2 h/d x 60 d	Multiple response schedule No effect on CRF or FR30 Deficit in DRL 12 sec schedule	Ikeda and Miyake, 1978
inhalation	rats (male)	3000 ppm for 4 h (no effect at 1000 ppm)	Deficit in conditioned avoidance response	Shigeta et al., 1978
inhalation	rats (male)	3200 ppm for 4 h	Deficit conditioned avoidance response	Krivanek and Mullin, 1978
		1600 ppm for 4 h	No-effect-level	
inhalation	rats (male)	800 ppm for 4 h	Deficit in unconditioned reflexes and simple behavior	Krivanek and Mullin, 1978
inhalation	rats	4-5 ml in 40-50 l of air for 1/2 h/d x 7.6 d	Induced forced turning	Ishikawa and Schmidt, 1973
i.p.	mice (male)	0.96 g/kg	Loss of righting reflex in 5/7 in 20.6 ± 1.6 min Interval from loss of righting reflex to recovery 35.0 ± 8.2 14.3% lethality in 24 h	Koga and Ohmiya, 1978
inhalation	mice	3980 ppm for 3 h 10,615 ppm for 10 min	Deficit in visual placing, grip strength, wire maneuver tail pinch, righting reflex	Peterson and Bruckner, 1978
inhalation	mice	4,000 ppm for 3 h/d x 5 d/wk for 8 wk	Deficit on an accelerating, rotating bar	Bruckner and Peterson, 1976
inhalation	mice (male)	1, 10, 100, 1,000 ppm for 6 h/d x 10 d	Deficit in wheel-turning	Horiguchi and Inoue, 1977
inhalation	mice	2650 ppm	Causes mice to fall on side	Faustov, 1958

Abbreviations: h = hour; d = day; wk = week; i.p. = intraperitoneal; min = minute; sec = second.

An exposure as small as 1 ppm of toluene suppressed wheel-turning activity whereas exposure to 100 ppm benzene approximated the depression caused by exposure to 10 ppm toluene; therefore, the narcotic action of toluene appears to be greater than benzene (Horiguchi and Inoue, 1977).

The positive findings at 1 ppm reported by Horiguchi and Inoue (1977) and the change of motor chronaxies in rats exposed continuously to 4 ppm toluene for 85 days (Gusev, 1967; cited by NRC) have been questioned in the NRC (1980) review as being at variance with negative effects observed in other experiments at much higher levels. For example, Ikeda and Miyake (1978) did not find any effect on spontaneous activity in their studies of repeated exposure to 4000 ppm toluene in rats. However, the behavioral tests of the latter authors were carried out 4 days after final exposure. Rapid recovery of behavior after exposure (Shigeta et al., 1978; Peterson and Bruckner, 1978; and Ishikawa and Schmidt, 1973) may explain the disparate results just cited.

A single exposure to 3000 ppm toluene for 4 hours disrupted established timing of bar pressing in a conditioned avoidance response test in adult male Wistar rats (Shigeta et al., 1978). Concentrations of 0 and 1000 ppm toluene did not affect this operant behavior. At 3000 ppm increased response and shortening of the inter-response-interval were noted, but no change in shock counts was seen. Behavioral recovery occurred 1 hour after exposure. Krivanek and Mullin (1978) reported a decrease in conditioned avoidance reflexes after inhalation by male rats of 3200 ppm toluene for 4 hours, but they reported no effect at dose levels of 1600 or 800 ppm.

In another study of operant behavior, Colotla and Bautista (1979) used rats that had been trained to reinforced bar pressing in a multiple schedule comprising fixed ratio (FR) 10 and differential reinforcement of low rates (DRL) 20-second components with 60-second time out between reinforcement periods. Five

trained adult Wistar rats were exposed to concentrations of 574, 1148, 2296, and 4595 ppm toluene. Test sessions were 36 minutes long. Control sessions intervened between solvent exposure sessions to assess recovery. A decrease in response of FR performance and an increase of frequency rate of the DRL component were observed with all doses in a dose-dependent manner. No residual effects were observed. An effect on behavioral rate was shown.

A lower concentration, 150 ppm toluene, for periods of 0.5, 1, 2, or 4 hours affected a multiple fixed ratio--fixed interval schedule of reinforcement in 3 male Holtzman, Sprague-Dawley rats. An initial enhancement of FR and FI rates occurred during shorter exposure periods followed by a decrease in rates during longer exposure periods (Geller et al., 1979); however, only a small number of animals was used, and the response was not uniform. Battig and Grandjean (1964) found no effect on acquisition or consolidation of an avoidance response after inhalation of toluene varying from 550 to 800 ppm, 4 hours/day for 2 weeks, by 6 adult male rats. Continued exposure at similar levels for another week effected a somewhat slower extinction of the avoidance response.

Repeated exposure of rats to inhalation of 4000 ppm toluene, 2 hours daily for 60 days, did not affect spontaneous locomotor activity, emotionality, or learning on 2 operant schedules: memory in a continuous reinforcement schedule (CRF) where every bar press was rewarded by food and extinction of a fixed ratio (FR 30) schedule where only a bar press every 30 seconds was rewarded. This exposure did impair learning on a third operant schedule, acquisition of a differential reinforcement of a low rate of responding (DRL 12 seconds) schedule that required the rat to allow at least 12 seconds between responses to receive a reward. Impaired performance was present 80 days after final exposure. Exposure to toluene appears to more seriously affect higher levels of cognition. Histological examination of the brain did not reveal any changes (Ikeda and Miyake, 1978).

Inhalation of 4000 ppm toluene by mice for 3 hours/day, 5 times weekly for up to 8 weeks, caused a steady deterioration of performance on an accelerating, rotating bar during the initial hour of each session of exposure. Solvent levels in blood and liver increased during each exposure session and decreased quickly after exposure (Bruckner and Peterson, 1976).

Circling (forced turning) was produced within a mean of 7.6 days in 90-day-old male Sprague-Dawley rats (n=10) by repeated toluene inhalation (4-5 ml in 40-50 liters of air) for 1/2 hour per day. After 15, 21, or 34 days of recovery, the rats were reexposed daily to toluene. When only 15 days of recovery had elapsed, the number of exposures required to elicit forced turning was significantly less than the number required to acquire the behavior originally. This effect was not seen when a longer period of recovery had elapsed. Thus, toluene has a residual effect. Furthermore, the effect is reversible. This turning was not associated with any histological lesions in the brain (Ishikawa and Schmidt, 1973).

The effect of toluene on electrical, as well as behavioral, parameters in the brain was studied by Contreras et al. (1979). Twenty cats were exposed for up to 40 days (10-minute periods, 7 days/week) to 25.5 to 204.7 mg/l/min (approximately 7,000 to 52,000 ppm) toluene administered through a tracheal cannula in increments of 25.5 mg/l/min with 10-minute recovery intervals between exposures. During the first seconds of acute intoxication at 12,000 ppm the behavior consisted of restlessness, polypnea, coughing, sneezing, and vegetative responses consisting of salivation, mydriasis, and lacrimation. Ataxia appeared 2 minutes later, ending with postural collapse. Changes of electrical activity at this point were found in the anterior lobe of the cerebellum, the amygdala, and the visual cortex. There was no behavioral response to light, sound, or pain stimuli (see Table 12-6).

Table 12-6. Central Nervous System Effects of Toluene

Route	Species	Dose	Effect	Reference
inhalation	cats	ca. 7,000 to 52,000 ppm 10 min/d x 40 d	Restlessness Autanomic nervous system stimulation, ataxia, collapse EEG changes Seizures	Contreras et al., 1979
inhalation	rats	1000, 2000, or 4000 ppm for 4 h	EEG changes Increased excitability Changed sleep cycle Increased pulse rate	Takeuchi and Hisanaga, 1977
inhalation	rats (male)	2000 ppm toluene for 8 h/d x 1 wk	Decreased threshold for Bemegride-induced convulsions	Takeuchi and Suzuki, 1975
inhalation	rats and mice	265 ppm	Threshold affecting CNS	Faustov, 1958
inhalation	rats (male) Sprague-Dawley n = 6	500 ppm 6 h/d x 3 d Killed 16-18 h after exposure	Increase of catecholamines in lateral palisade zone of median eminence	Andersson et al., 1980
		1000 ppm 6 h/d x 5 d decapitated 4 h after exposures	Increase of catecholamines in subependymal layer of median eminence Increase of FSH	

Abbreviations: min = minute; d = day; h = hour; wk = week; EEG = electroencephalogram; FSH = follicle-stimulating hormone; CNS = central nervous system.

Threshold dose for restlessness was approximately 7,000 ppm. No behavioral response to external stimuli occurred at approximately 39,000 ppm. Recovery from ataxia occurred 12 minutes after removal from exposure. With repeated exposure, at a concentration of 102.3 mg/l/minutes, hypersynchronous rhythms spread from the amygdala to the reticular formation, visual cortex, and cerebellum, and electrical activity appeared in the gyrus cinguli, which coincided with a display of hallucinatory behavior. These EEG and behavioral signs are similar to complex partial seizures in man (Contreras et al., 1979).

Takeuchi and Hisanaga (1977) found that 1000, 2000, or 4000 ppm toluene administered for 4 hours to groups of 4 or 5 male Wistar rats elicited changes in the sleep cycle, altered cortical and hippocampal EEG rhythms, and increased pulse rates. All phases of sleep were disturbed at a concentration of 2000 and 4000 ppm, while 1000 ppm deterred entry of sleep into the slow-wave phase but facilitated entry into the paradoxical phase.

A similar observation was made by Fodor et al. (1973), where an increased percentage of REM during sleep was found in female albino rats during exposure to 1000 ppm. A concentration of 1000 ppm decreased cortical and hippocampal components of the EEG (Takeuchi and Hisanaga, 1977). Exposure to 2000 ppm toluene increased cortical fast components and hippocampal components, whereas exposure to 4000 ppm increased the hippocampal fast component as well. At 4000 ppm excitability measured by rearing reactions (standing on hind legs) increased during the first hour of exposure, but this phase was followed by a depression and the rats were unable to stand or walk. Excitability increased again after exposure. At 2000 ppm only increased excitability was observed. At 1000 ppm excitability was not increased significantly. Myoclonic seizures were seen in both 2000 and 4000 ppm treated groups with greater frequency at the higher concentration.

Convulsion threshold after intraperitoneal injection of Bemegride was decreased significantly by preexposure to 2000 ppm toluene for 8 hours/day in 6 Sprague-Dawley male rats. The change was noted after 1 week of exposure. The convulsion threshold continued to decrease for 6 weeks of exposure. After 8 weeks of exposure the difference from the controls was not significant, although the convulsion threshold remained lower. The data suggest that toluene renders the CNS more susceptible to induction of a convulsion state. Body weights of these rats were lower than those of controls during the exposure period, although differences were not significant (Takeuchi and Suzuki, 1975).

Andersson et al. (1980) reported an increase of dopamine and noradrenaline in the median eminence after inhalation of 500 ppm and 1000 ppm toluene, respectively, by male rats. The higher levels also produced increases of noradrenaline turnover within the median eminence and the anterior periventricular and paraventricular hypothalamic nuclei. A significant increase of plasma levels of follicle-stimulating hormone (FSH) and a non-significant elevation of prolactin and corticosterone were also noted.

Although most studies, acute as well as chronic, indicate minor effects of toluene at concentrations under 1000 ppm and most reviews (NRC, 1980; EPA, 1980; NIOSH, 1973) have emphasized the negligible effects on the CNS at this level, several recent studies indicate that lower level exposures may not be innocuous. Horiguchi and Inoue (1977) found a decrement in performance during a simple task, Gusev (1967) found lengthened motor chronaxies at 4 ppm, Colotla and Bautista (1979) noted a decrement in operant behavior at concentrations of 574 ppm, and Anderson et al. (1980) reported histochemical changes in the brain at 500 ppm. In all of these studies, sensitive parameters of CNS activity were measured. Higher concentrations tended to affect more complex tasks. Furthermore, the studies of Andersson et al. (1980) indicate that 500 ppm affects an area of the

brain which regulates many vegetative, as well as reproductive, functions. These findings indicate that effects of toluene on the CNS at levels below 1000 ppm cannot be totally ignored.

12.4 EFFECTS ON OTHER ORGANS

12.4.1 Blood-Forming Organs

Myelotoxicity is an effect that has been attributed to toluene. Prior to the early 1940's it was believed that toluene had the same effect as benzene; however, in most of the earlier studies toluene was contaminated with benzene. Since then there have been studies indicating a lack of myelotoxicity and several which indicate a positive effect (Table 12-7).

One of the first studies using toluene free of benzene which demonstrated that it had no injurious effect on blood-forming organs was that of Von Oettingen et al. (1942b) in rats and dogs. Exposure of rats to 200 to 5000 ppm toluene contaminated with less than 0.01% benzene for 5-6 weeks (7 hours/day, 5 days/week) did not affect blood-forming organs, as indicated by the absence of anemia and changes in the bone marrow and spleen. Exposure to the higher concentrations of 2500 and 5000 ppm did produce a daily temporary shift in the blood picture, characterized by a decrease of lymphocytes and total white blood count with a moderate increase of segmented cells Table 12-8). Exposure of dogs to inhalation of 400 ppm toluene on five consecutive days for 7 hours daily produced no appreciable changes in the blood picture with the exception of a temporary lymphocytosis at the end of exposure (Von Oettinger et al., 1942b). Exposure of dogs to inhalation of higher concentrations of toluene containing less than 0.1% benzene (7.5 mg/l for 8 hours daily, 6 days weekly during 4 months and then 10 mg/l for the 2 remaining months) had no effect on the bone marrow (Fabre et al., 1955).

Table 12-7. Myelotoxicity Studies in Animals

Species	Route	Dose	Effect	Reference
Rats n=20/group	Inhalation	200, 600, 2500, 5000 ppm 7 h/d x 5 d x 5-6 wk	At highest doses: a temporary decrease of lymphocytes and total white blood cell count; no anemia; no effect on bone marrow or spleen	von Oettingen et al., 1942b
Rats n=15 Guinea pigs n=15 Dogs n=15 Monkeys n=3	Inhalation	107 ppm continuous exposure for 90 d or 1085 ppm 8 h/d, 5 d/wk, for 6 wk	No significant change in leukocyte count, hemoglobin, or hematocrit	Jenkins et al., 1970
Rats n=90 male + female	Inhalation	30, 100, 300 ppm 6 h/d x 5 d/wk x 24 mo	No effect on any hematological parameter except 2 parameters in females: at 100 or 300 ppm hematocrit was reduced, at 300 ppm mean corpuscular hemoglobin concentration was higher; no histopathology on any organ including spleen and bone marrow	CIIT, 1980
Rats Dogs	Inhalation	240, 480, 980 ppm 6 h/d x 5 d/wk x 65 d	No effect on red blood cell count, white blood cell count, hematocrit, hemoglobin, total and differential white count, SAP, SGPT, SGOT, or BUN; no effect on bone marrow.	Carpenter et al., 1976b

Table 12-7. Myelotoxicity Studies in Animals (Cont'd)

Species	Route	Dose	Effect	Reference
Dogs	Inhalation	400 ppm 7 h/d x 5 d	No change in blood picture; temporary lymphocytosis	von Oettingen et al., 1942b
Dogs	Inhalation	7.5 mg/l, 8 h/d x 6 d/wk x 4 mo, and then 10 mg/l, 8 h/d x 6 d/wk x 2 mo	No effect on bone marrow	Fabre et al., 1955
Rats	Subcutaneous	1 cc/kg body weight x 14 d	Normal leukocyte count, spleen, and bone marrow	Gerarde, 1960
Rats	Oral	118, 354, 590 mg/kg/d x 138 d	Normal bone marrow, spleen, bone marrow counts, blood count	Wolf et al., 1956
Mice	Inhalation	1, 10, 100, 1000 ppm 6 h/d x 20 d	Leukocytosis at all dose levels; 100, 1000 ppm: depressed red cell count; 10-1000 ppm: decreased thrombocyte count; 1000 ppm: trend toward hypoplasia in bone marrow	Horiguchi and Inoue, 1977
Donryu strain rats n=6/group	Inhalation	200, 1000, 2000 ppm 99.9% pure toluene for 32 wk	Significant retarded weight gain at 2 higher doses during initial 4 wk; no significant difference in hemoglobin hematocrit and total plasma protein; no significant increase of RBC; significant increase of leucocytes at highest dose at first week of exposure followed by recovery; eosinophile counts decreased rapidly in the first 2-4 weeks and the recovered; increase of Momonsin's toxic granules.	Takeuchi, 1969

Table 12-7. Myelotoxicity Studies in Animals (Cont'd)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	420 mg/m^3 4 h/d x 4 mo	Leukocytosis and chromosome damage in bone marrow	Dobrokhotov and Enikeev, 1977 (cited in EPA, 1980)
Rat	Subcutaneous	1 g/kg/d x 12 d	11.5% chromosome damaged cells vs. 3.9% in controls	Lyaphalo, 1973
Rat	Dermal	10 g/kg body weight/d	Impaired leukopoiesis	Yushkevich and Malysheva, 1975

Abbreviations: n = number; h = hour; d = day; wk = week; mo = month; SAP = serum alkaline phosphatase; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxalacetic transaminase; BUN = blood urea nitrogen.

Table 11-8. Weekly Blood Picture of Normal Rats and Rats Exposed to 600 and 2500 ppm of Toluene 7 Hours/Day, 5 Days/Week, for 5 Weeks (von Oettingen et al., 1942b)

Weeks	Number of Animals	Time	Million red blood cells	g/100 cc hemoglobin	Percent reticulocytes	Thousand white blood cells	Percent mononuclear cells	Percent segmented cells	Thousand total mononuclear	Thousand total segmented cells
NORMAL										
Preexposure period:										
First	5	A.M.	7.0	12.0	3.6	11.9	68	32	8.1	3.8
	15	P.M.								
Second	20	P.M.	6.2	11.3	4.0	16.4	69	31	11.3	5.1
Exposure period:										
First	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	6.2	12.0	6.5	17.9	70	30	12.5	5.4
Second	20	A.M.	6.6	11.8	3.6	14.0	65	35	9.1	4.9
	20	P.M.	6.5	10.7	3.9	17.5	64	36	11.2	6.3
Third	20	A.M.	6.7	11.5	4.8	15.9	70	30	11.1	4.8
	20	P.M.	6.2	10.9	4.2	16.2	66	34	10.7	5.5
Fourth	20	A.M.	6.7	12.8	4.4	18.3	73	27	13.4	4.9
	20	P.M.	6.4	11.2	4.7	15.5	65	35	10.1	5.4
Fifth	20	A.M.	6.5	11.5	6.6	17.6	66	34	11.6	6.0
	20	P.M.	6.1	10.1	6.2	18.2	59	41	10.7	7.5
2 Weeks After	9	A.M.	7.4	13.8	4.7	16.5	68	32	11.2	5.3
Exposure	9	P.M.	6.7	12.4	4.6	19.2	66	34	12.7	6.5
600 ppm										
Preexposure period:										
First	15	A.M.	6.8	11.4	3.0	13.1	70	30	9.2	3.9
	5	P.M.								
Second	20	P.M.	--	--	4.6	--	74	26	--	--
Exposure period:										
First	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	--	--	5.4	--	78	22	--	--
Second	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	--	--	4.6	--	82	18	--	--
Third	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	--	--	4.0	--	75	25	--	--
Fourth	20	A.M.	6.5	12.3	4.4	12.2	71	29	8.7	3.5
	20	P.M.	6.3	11.5	3.9	14.5	66	34	9.6	4.9
Fifth	20	A.M.	6.5	11.1	4.8	12.5	71	29	8.9	3.6
	20	P.M.	5.9	10.6	6.2	14.5	65	35	9.4	5.1
2 Weeks After	10	A.M.	7.2	15.0	5.2	11.0	75	25	8.3	2.7
Exposure	10	P.M.	6.8	13.6	5.0	12.3	68	32	8.4	3.9
2500 ppm										
Preexposure period:										
First	10	A.M.	6.8	12.3	4.0	11.0	77	23	8.5	2.5
	10	P.M.								
Second	20	A.M.	6.6	12.1	4.2	13.4	73	27	9.8	3.6
Exposure period:										
First	20	A.M.	6.5	11.6	6.6	16.6	67	33	11.1	5.5
	20	P.M.	6.0	10.4	7.7	12.1	51	49	6.2	5.9
Second	20	A.M.	6.5	11.5	4.6	15.4	70	30	10.8	4.6
	20	P.M.	6.6	10.9	5.2	11.3	55	45	6.2	5.1
Third	20	A.M.	6.4	11.5	4.8	15.9	69	31	11.0	4.9
	20	P.M.	6.5	11.2	4.2	11.3	56	44	6.3	5.0
Fourth	20	A.M.	6.5	11.8	5.6	14.0	64	36	9.0	5.0
	20	P.M.	6.4	11.0	6.1	12.0	55	45	6.6	5.4
Fifth	20	A.M.	6.0	10.5	5.8	13.3	67	33	8.9	4.4
	20	P.M.	6.5	10.8	5.3	9.9	53	47	5.3	4.6
2 Weeks After	10	A.M.	7.2	14.4	4.5	15.8	73	27	11.5	4.3
Exposure	10	P.M.	5.6	11.7	4.9	11.1	63	37	7.0	4.1

Male Wistar rats administered a daily subcutaneous dose of 1.0 cc/kg body weight for 14 days had a normal leucocyte count, thymus and spleen weight, femoral marrow nucleated cell count, and femoral marrow nucleic acid content (Gerarde, 1956).

Wolf et al. (1956) could find no effect on femoral bone marrow, spleen, bone marrow counts, or hematological parameters in female Wistar rats orally dosed with concentrations of 94.4% pure toluene of up to 590 mg/kg/day for 24 weeks. Neither did exposure of Fischer 344 rats for 24 months (6 hours/day, 5 days/week) to 30, 100, or 300 ppm 99.98% pure toluene have any hematological effects (Table 12-3). There were no changes in the bone marrow or spleen (CIIT, 1980).

Speck and Moeschlin (1968) noted that subcutaneous injection of 300 mg/kg or 700 mg/kg pure toluene administered daily to rabbits for 6 and 9 weeks, respectively, had no myelotoxic effects. There were no changes in DNA-synthesis of bone marrow cells as measured by incorporation of ^{3}H-methylthymidine or in peripheral blood elements (leucocytes, thrombocytes, reticulocytes, or erythrocytes).

In a study made by Braier (1973), subcutaneous injection of 862 mg/kg body weight daily for 6 days produced a moderate depression of granulocytes during the first 2 days of treatment. This was followed by a sharp rise in granulocytes by the end of 6 days, a rise which was twice that of the pretreatment level. No significant change was noted in the bone marrow. In contrast, subcutaneous injection of benzene at the same dosage elicited a progressive decrease in granulocyte count throughout the period of treatment.

The effects of toluene and benzene on the incorporation of ^{59}Fe in erythrocytes were studied by Andrews et al. (1977). While benzene inhibited the incorporation of ^{59}Fe, toluene did not.

The studies suggesting a myelotoxic effect include Horiguchi and Inoue (1977) who exposed groups of 6 male mice to toluene vapor at concentrations of 1, 10, 100, and 1000 ppm for 6 hours daily over a period of 20 days and found that the two highest doses decreased red cell count. Concentrations of 10 ppm and above decreased thrombocyte count. All groups showed an increase in white cell count midway in the study, followed by recovery except in the 100 ppm group. Slight hypoplasia of the bone marrow was noted at the highest dose.

Taheuchi (1969) observed a transient increase in ceucocytes in 6 Donryu strain rats exposed to 2000 ppm 99.9% pure toluene containing less than 0.2 ppm benzene in the course of 8 hour daily exposures for 32 workers as well as a transient decrease of eosinophile counts upon exposure to 200, 100, or 2000 ppm toluene under the same regimes (see Table 12-7). After 32 weeks of toluene exposure all groups including an unexposed control group were subjected to 39 8-hour daily exposures to benzene prior to histopathological examination after sacrifice. Adrenal weight to body weight was depressed significantly in all groups which had been exposed to toluene. Histologically the zona glomerulosa of the adrenal cortex of toluene exposed rats was thicker while the zxona fasciculata and zona reticularis were reduced. The authors suggested that toluene affected the hypothalama-pituitary-adrenal system. While that hypothesis is tenable since the rats exposed to toluene differed from unexposed controls, all grops exposed and unexposed to toluene were also exposed to benzene, therefore, this conclusion can only be regarded as sensitive. An alotract of a lates paper (Taheuchi et al., 1972 cited in CA79:28056e), which was not available for review, noted that exposure of male rats to toluene for 8 hours daily for 4 weeks increased adrenal weight and eosinophil counts and decreased corticosteroid concentration after 1 week.

Topical application of 10 g/kg toluene to rats 4 hours daily for 4 months had no effect on maturation of erythroblasts in the bone marrow, but an increase of plasmic and lymphoid reticular cells in the marrow indicated an impairment of leucopoiesis. A lower dosage of 1 g/kg toluene daily had no effect (Yushkevich and Malysheva, 1975).

Chromosomal damage in the bone marrow and leucocytosis was noted in rats that had been exposed to inhalation of 112 ppm of toluene, 4 hours daily, for 4 months. Recovery from leucocytosis occurred one month after termination of exposure, but the chromosomal damage was unchanged. On the other hand, inhalation of a combination of toluene and benzene produced chromosomal aberrations, which were approximately equal to the sum of aberrations induced by single administration of the solvents. Whereas benzene caused leukocytopenia, the mixture caused leukocytosis (Dobrokhotov and Enikeev, 1977).

In the studies of Matsumoto et al., (1971) exposure of Donryu male rats to inhalation of 2000 ppm toluene vapor 8 hours/day, 6 days/week for 43 weeks decreased the ratios of thymus weight to body weight and spleen weight to body weight.

Although the evidence tends to weigh more heavily toward the absence of a myelotoxic effect from toluene exposure in animals, the suggestion made in NRC (1980) that the positive findings may indicate subtle unrecognized hematopoietic responses is sound. For example, the effect of toluene on hematocrit and mean corpuscular hemoglobin concentration in female Fischer rats and not in male rats is of interest in view of the observation of Hirokawa (1955) where there appears to be a higher susceptibility of the female rabbit to benzene. In that study the pattern of decrease of erythrocytes, hemoglobin content, while blood cells, increase of mean corpuscular volume, decrease of mean corpuscular hemoglobin concentration in the female was simulated in the estradiol propionate treated orchidectomized male.

There was no increase of erythrocyte fragility seen in 6 rats that inhaled 20,000 ppm "toluene concentrate" for 45 minutes (Carpenter et al., 1976b). A slight increase in coagulation time was noted in rabbit blood by Fabre et al. (1955) and in rats by von Oettigen et al. (1942b).

12.4.2 Cardiovascular Effects

Several animal studies have shown that massive doses cause a number of electrocardiographic changes. In addition, a sensitization of the heart to low oxygen levels was observed.

Inhalation of glue fumes containing toluene for 1 minute significantly slowed sinoartrial heart rate of 8 ICR mice and slightly lengthened the P-R interval. Subjecting the animals to 5 minutes of asphyxia after inhalation of the glue fumes produced a 2:1 atrioventricular block in all animals within an average of 42 seconds of asphyxia. In contrast after 24 5-minute periods of asphyxia the sinoatrial heart rate rose the P-R internal did not lengthen, and atrioventicular (AV) block did not occur in 12 mice (Taylor and Harris, 1970).

In acute inhalation of toluene atrial fibrillation, bradiarrhythmia, and asystole, along with respiratory paralysis occurred. Injection subcutaneously of 2 doses of 0.1 ml/100 g body weight daily for 6 weeks elicited repolarization disorders, atrial fibrillation, and in some of the rats, ventricular extrasystoles (Moravai et al., 1976).

Intravenous injection of 0.01 mgm/kgm epinephrine into dogs following inhalation of toluene vapors (concentration did length of exposure varied, but unspecified) elicited ventricular fibrillation (Chenoweth, 1946). This observation is of interest because the "sudden death" syndrome following "glue sniffing" in humans might possibly be explained by an increased secretion of epinephrine which could cause fibrillation of the heart as a result of the combined effect of the two compounds.

Intravenous injection of 0.05 mg/100 g body weight of toluene into rats reduced arterial blood pressure; however, injection of the same dosage by the intraperitoneal or subcutaneous route had no effect on blood pressure (Moravai et al., 1976). No effect on blood pressure was seen in the chronic inhalation studies of von Oettingen et al. (1942b) where dogs were exposed to inhalation of 200 to 600 ppm toluene several times weekly for several months. In this study there was no effect observed on circulation, heart rate, venous pressure, spinal pressure, respiratory rate, minute volume, or respiratory volume.

12.4.3 Gonadal Effects

Matsumoto et al. (1971) found that Donyru strain male rats exposed to inhalation of 100 or 200 ppm toluene vapor 8 hours/day, 6 days/week for one year produced no change in erythrocyte and leucocyte counts, and no change in seven total protein or cholinesterase activity. However at the higher dose degeneration of germinal cells of the testes was found in four of 12 animals while normal germinal epithelium was found in controls. Testicular weight was lower than controls at both dose levels. There was a trend toward a decrease of testicular to body weight ratio.

12.5 Summary

The most pronounced effect of toluene in animal studies is on the central nervous system. Acute exposure to high levels of toluene has been linked with depression of the central nervous system. A level of approximately 1000 ppm toluene vapor appears to have little or no effect on gross observations of this parameter. While a dose related response of instability, incoordination and mild narcosis was observed in rats exposed daily to toluene vapor at concentrations of 1250 and 1600 ppm. No effects was noted at 1100 ppm (Batchelor, 1927). Inhalations of 1000 ppm toluene vapor for 4 hours did not increase rearing reactions (standing on hind legs) in rats (Takeuchi and Hisanaga, 1977). Operant behavior

(conditioned avoidance response) was unaffected at 1000 ppm of vapor in the studies of Shigeta et al. (1978) and at 800 ppm in the studies of Krivanck and Mullin (1978). Neither did inhalation of 1000 ppm for 6 hours/day, 5 days/week for 13 weeks produce observable behavioral effects in rats in the pilot study for the chornic CIIT report (CIIT, 1980). Smyth and Smyth (1928) noted that daily inhalation of 1250 ppm for 4 hours each day for 18 days produced narcosis in guinea pigs while no effect was noted at 1000 ppm during a longer period of exposure. Fabre et al. (1955) noted that exposure to 2000 ppm toluene for 8 hours daily 6 days weekly for 4 months produced only slight nasal and ocular irritation after a transient initial hyperactivity in one of two dogs. No behavioral effects were found in rats and dogs after inhalation of 980 ppm "toluene concentrate" (450 ppm toluene) for 6 hours daily for 13 weeks.

However, use of more sensitive lmethods of detection have revealed an effect in single behavioral parameters and the central nervous system at lower levels. EEG changes were seen in rats after inhalation of 1000 ppm (Fodov et al., 11973; Takeuchi and Hisanaga, 1977). A deficit was noted in unconditioned reflexes and simple behavior at 800 ppm for 4 hours in rats (Krivanck and Mullin, 1978), in multiple response schedule at 574 ppm in rats (Colotla and Bautista, 1979); in wheel-turning in rats at 1 ppm (Houguchi and Inoue, 1977). Neuromodulator content in the hypothealamus was affected at 500 ppm (Anderson et al., 1980).

Early studies suggested a myelotoxic effect by toluene. However, several studies done since the early 1940's using toluene of greater purity have indicated an absence of injurious effect on blood-forming organs by toluene in rats and dogs (von Oettingen et al., 11942; Gerarde, 1959; Wolfe et al., 1956; Fabre et al., 1955; Jenkins et al., 1970; Carpenter et al., 1976b, CIIT, 1980). Nonetheless there is no unanimity on this point. Leukocytosis impaired leukoporesis and chromosomal damage in the bone marrow have been observed in some

studies (Houguchi and Inoue, 1977; Dohokhotor and Enichiev, 1977; Lyapkalo, 1973; Yushkench and Malsheva, 1975).

Inhalation of concentrations of up to 1085 ppm toluene for 6 weeks or 300 ppm for 24 months, and ingestion of 590 mg toluene/kg body weight for 61 months produced no liver damage (Svirbely et al., 1944; Carpenter et al., 1976b; Jenkins et al., 1970; CIIT, 1980; Wolf et al., 1956). Exceptions were the studies of von Oettingen et al. (1942) where inhalation of 600 ppm toluene caused increase of weight and volume in the liver of rats; the studies of Fabre et al. (1955) in dogs were hemorrhagic livers were found at Ungavny et al., 1976 where 0.05 or 0.1 ml/100 g toluene injected intraperitoneally produced histological changes in the liver.

However, in a more recent study by Ungvary et al. (1980) where male CFY rats were exposed to daily inhalation of 265 ppm or 929 ppm analytical grade toluene and female rats were exposed to lower doses only five times a week up to 6 months no abnormal histological changes were found in the liver although growth was inhibited at the higher concentration in males and at the lower dose in females. Subchronic exposure to inhalation of toluene had no specific hepatoxic effect, although signs of adaption compensation were observed.

Renal changes consisting of casts in collecting tubules of rats were observed in the studies of von Oettingen et al. (1942b) after exposure to inhalation of 600 ppm. Hyperemic renal glomeruli and albuminuria were seen in 2 dogs after inhalation of toluene vapors at concentrations of 2000 ppm followed by 2660 ppm for 4 and 2 months, respectively (Fabre et al., 1955). Slight renal degeneration was observed in guinea pigs (Smyth and Smyth, 1928; Sessa, 1948). No renal damage was found after repeated inhalation of 1085 ppm toluene for 6 weeks in rats, guinea pigs, dogs, or monkeys, up to 300 ppm for 24 months in rats or ingestion of 590 mg toluene/kg body weight for 6 months in rats (Jenkins et al., 1970; CIIT, 1980; Wolf et al., 1956).

Irritation effects were noted in the respiratory tract in dogs, guinea pigs, and rats (Browning, 1965; Gerarde, 1960; Fabre et al., 1955; von Oettingen et al., 1942b; Smyth and Smyth, 1928; Sessa, 1948). Sensitization of the heart after inhalation of toluene was observed in mice, rats, and dogs (Taylor and Harris, 1970; Nowai et al., 1976; Chenoweth, 1946).

The acute oral toxicity (LD50) of toluene is in the range of 6.0 to 7.5 g/kg in rats (Kimura et al., 1971; Smyth et al., 1976b; Withey and Hall, 1975; Wolf et al., 1956). Exposure to toluene by the dermal route revealed in LD50 of 14.1 mg/kg in the rabbit (Smyth et al., 1969). Slight to moderate irritation of the rabbit and guinea pig skin was observed after acute and subacute application of toluene (Kronen et al., 1979; Wolf et al., 1956) while application to the rabbit cornea caused slight to moderate irritation (Wolf et al., 1956; Smyth et al., 1965; Carpenter and Smyth, 1946).

The LC50 for mice is in the range of 5500 to 7000 ppm of vapor for an exposure period of 6 to 7 hours (Svirbely et al., 1943; Bonnet et al., 1979). An LC50 of 8800 ppm of "toluene concentrate" for 4 hours (4,038 ppm toluene) was observed in rats (Carpenter et al., 1976b). In guinea pigs exposure inhalation to 4000 ppm for 4 hours caused death in 2 of 3 animals (Smyth and Smyth, 1928).

Subchronic treatment of rats (von Oettingen et al., 1942b) and rats, guinea pigs, dogs, and monkeys (Jenkins et al., 1970; Smyth and Smyth, 1928) reveal that exposure to inhalation levels of 200 and 1085 ppm, respectively, do not hae a deleterious effect on hematology and organ pathology with the exception of the study of Hougenchi and Inonu (1977) in mice which showed changes in blood elements at levels as low as 10 ppm. Toluene levels of 590 mg/kg/day administered orally for six months were tolerated by rats with no adverse effects (Wolf et al., 1956).

The only chronic study was the study performed for CIIT (1980) in rats exposed for 24 months to inhalation of toluene at levels up to 300 ppm. No effect on hematology, clinical chemistry, body weight or histopathology were noted except for two hematologic parameters in the females. Females exposed to 100 or 300 ppm showed reduced hematocrit levels and mean corpuscular hemoglobin concentration was increased at 300 ppm concentrations of toluene.

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 readily absorbed 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 Masshelein (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 = $C_i - C_e \dot{V}_e$ 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/minute).

Nomiyama and Nomiyama (1974a) measured the pulmonary retention ($\frac{C_i - C_e}{C_i} 100$) 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 Masshelein (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 (50 watts) exercise. 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/minute) 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 Masshelein (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, one slightly overweight, and 3 obese subjects (Carlsson and Lindqvist, 1977).

Ovrum and coworkers (1978), monitoring 4 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' 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; 9.6-20.2 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 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

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]

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]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. (Adapted from Carlsson and Lindqvist, 1977)

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 Masshelein, 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 8 hours of exposure were roughly proportional to the concentrations of toluene (200 to 800 ppm) in the atmosphere of the exposure chamber. Veulemans and Masshelein (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 minute/l x uptake rate (mg/minute).

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 Masshelein, 1978b).

Absorption through the respiratory tract has been less extensively studied 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 concentra

tion 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 readioactivity when removed from the chamber at the end of the exposure (Bergman, 1979). This value is a rought 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 71,000 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/hour. 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 µg/cm^2/hour 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 blood of subjects who immersed one hand in liquid toluene for 30 minutes was only 22% of the maximum concentration (790 µg/l) in blood of subjects who inhaled 100 ppm toluene vapor for 2 hours. Blood was collected from the cubital vein of the (unexposed) arm at invervals during and after exposure. Sato and Nakajima (1978) suggested that some of the toluene that penetrates the stratum corneum may, rather than entering the systemic circulation, be subsequently given off into the air. Toluene does appear to pass from the skin into the bloodstream relatively slowly 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 three 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/minute 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 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 µ 14-^{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).

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,

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.

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 10 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' exposure to 4600 ppm 4-^{3}H-toluene. The concentration in white adipose tissue reached a maximum 1 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 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 4 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 8 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 Masshelein (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 Masshelein, 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.

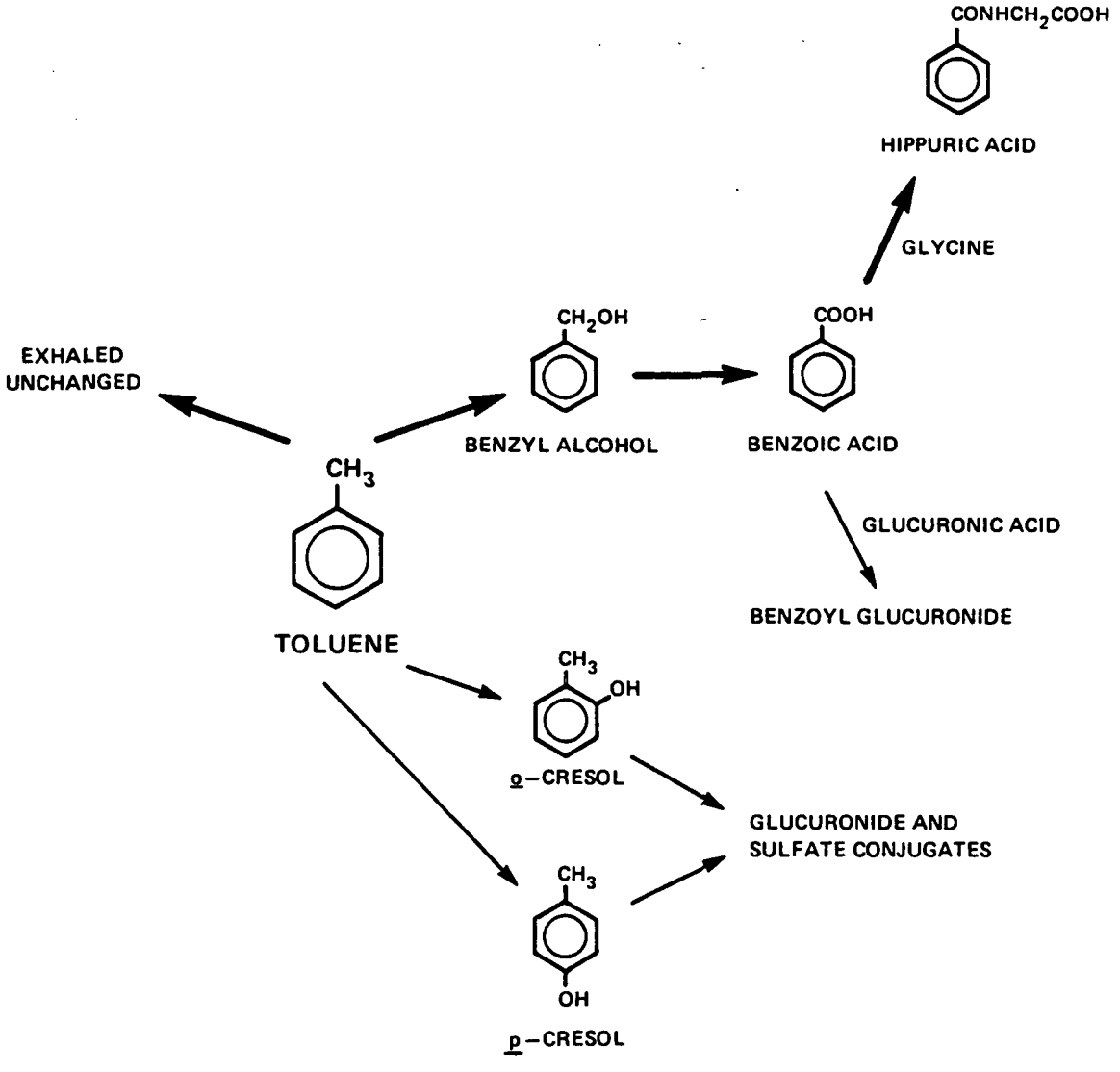

Figure 13-1. Metabolism of Toluene in Humans and Animals (Adapted from Laham, 1970)

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 Masshelein, 1979). The maximum rate of hippuric acid formation from benzoic acid was reported by Amsel and Levy (1969) to be about 190 μmol/minute, 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/minute) or 11 mmol/m^3 (270 ppm) during heavy work (pulmonary ventilation of 30 l/minute).

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 4 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 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, 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 2 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 (0.4-1.0% 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. Approximately 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 did not, however, 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 Masshelein, 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 (t1/2) for the decay of toluene in peripheral venous blood were 0.355 min^{-1} (t1/2 = 1.95 minutes), 0.0197 min^{-1} (t1/2 = 35.2 minutes), and 0.00339 min^{-1} (t1/2 = 204 minutes). Rate coefficients and half lives for the decay of toluene in

alveolar air were 0.437 min^{-1} (t1/2 = 1.59 minutes), 0.0262 min^{-1} (t1/2 = 26.5 minutes), and 0.00313 min^{-1} (t1/2 = 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 coefficient 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 Masshelein (1978b). Astrand et al. (1972) have reported that peripheral venous concentrations declined more gradually than did arterial concentrations.

Veulemans and Masshelein (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 Masshelein (1978a) did not begin monitoring expired air as soon after exposure ended and Nomiyama and Nomiyama (1978b) sampled expired air infrequently during the period used by Sato et al. (1974b) to determine the first 2 exponential phases. Rate coefficients for the rapid and slow phases were calculated by Veulemans and Masshelein (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} (t1/2 = 8.16 minutes) for men and 3.22 h^{-1} (t1/2 = 12.9 minutes) for women; the rate constant for the slow phase was 0.335 h^{-1} (t1/2 = 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 Masshelein (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 Masshelein (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 4 hours after the end of exposure than had been observed for the related lung clearances during exposure. This variability could partially be explained 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 4 hours of desaturation. Additionally, subjects who had been exposed to toluene while exercising expired less of the absorbed amount during the first 4 hours of desaturation than did subjects who had been exposed while resting (Veulemans and Masshelein, 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 Masshelein, 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 Masshelein, 1979; Nomiyama and Nomiyama, 1978). Hippuric acid excretion (mg/hour) declined fairly rapidly after cessation of about 4 hours' 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 Masshelein (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 Masshelein, 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 factors (body weight, body fat, cardiorespiratory parameters) (Veulemans and Masshelein, 1979). Exercise during exposure increased the rate of excretion of hippuric acid (Veulemans and Masshelein, 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/h for diuresis of greater than 30 ml/h (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 one 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 at about the end of 3 hours exposure, excretion increased again during the afternoon's 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 $\pm$ 0.10, 2.55 $\pm$ 0.55, and 5.99 $\pm$ 1.20 mg/ml (mean $\pm$ 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.

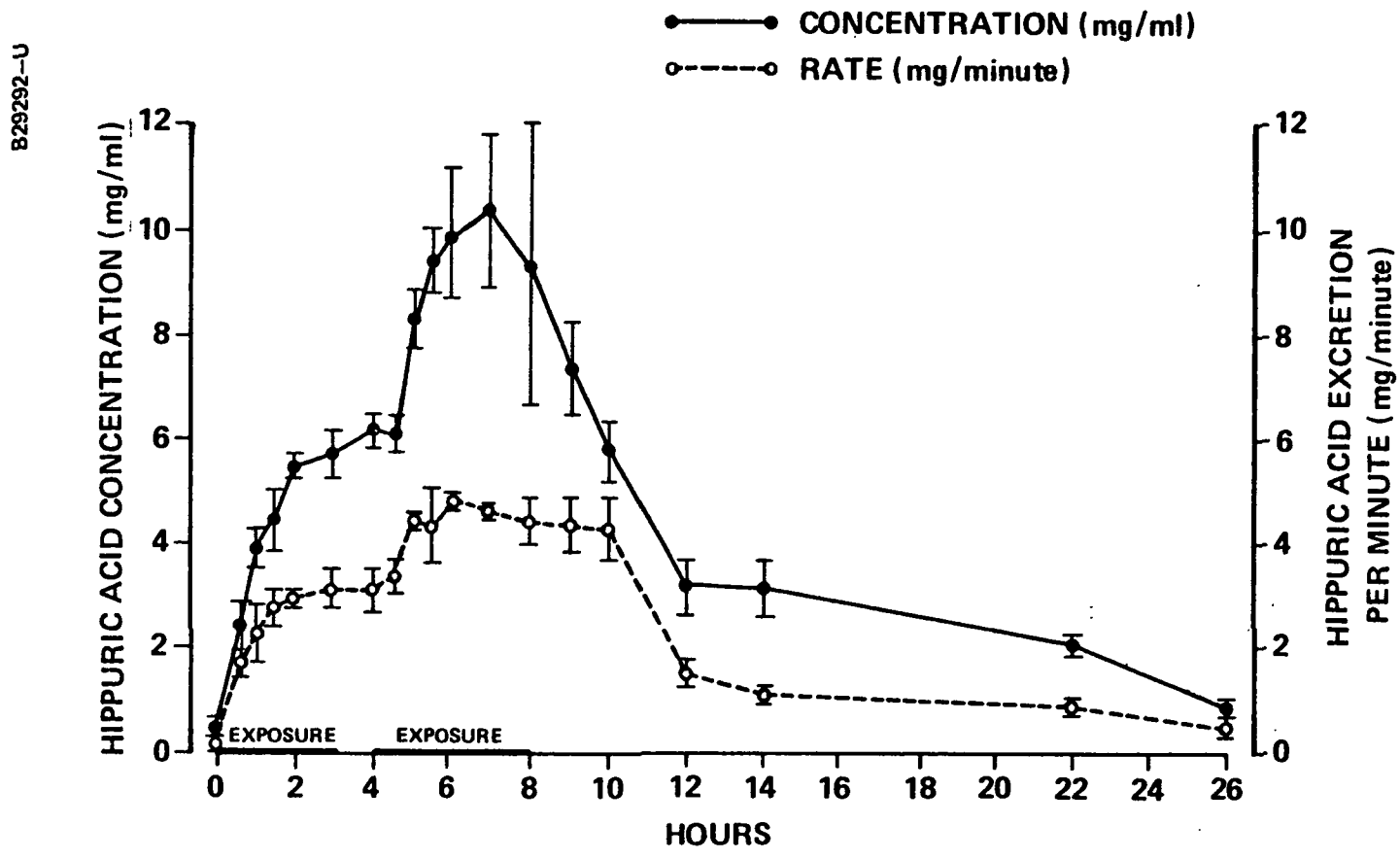

Figure 13-2. Urinary Concentrations and Excretion Rates of Hippuric Acid in Volunteers Exposed to Toluene (Volunteers were exposed to 196 ppm toluene for 3 hours in the morning and for 4 hours in the afternoon with one hour's break in between. Points are means ± SEM.) (Ogata et al., 1970)

Spot urine samples collected from workers after at least 3 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 fiduccial 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 which 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 doseing (El Masrs 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 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.

Table 13-3. Toluene Concentrations in Air and Peripheral Venous Blood (Konietzko et al., 1980)[a]

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

[a]Means and range of eight workers are given in parentheses.

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/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 Masshelein, 1978a).

The uptake of toluene by humans was about 50% of the amount inspired (Veulemans and Masshelein, 1978a; Carlsson and Lindqvist, 1977, Ovrum et al., 1978). Total uptake (absorption) can be approximated as follows: Uptake = 0.5 $\dot{V}e$ Ci t, where $\dot{V}e$ is the respiratory minute volume in l/min, Ci is the inspired concentration in mg/l, and t is the length of exposure in minutes (Ovrum et al., 1978; Veulemans and Masshelein, 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 Masshelein, 1978a; Carlsson and Lindqvist, 1977). A subject's content of adipose tissue had little or no effect on the uptake of toluene during exposure lasting 4 hours or less (Veulemans and Masshelein, 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 Masshelein, 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 22% of the maximum concentration in peripheral venous blood of subjects who inhaled 100 ppm toluene vapor for 2 hours (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 Masshelein, 1979; Ogata et al., 1970; El Masri et al., 1956). Much of the remaining toluene (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 Masshelein, 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 Masshelein 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 quantification of exposure or uptake from urinary hippuric acid concentration or excretion rates unreliable (Immamura and Ikeda, 1973; Veulemans et al., 1979; Veulemans and Masshelein, 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.

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 1 year following termination of exposure, but survival was only 35% (7/20). Doak et al. (1976) applied estimated toluene volumes of 0.05-0.1 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 16-20 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 Bacterial DNA Damage/Repair Assays

The ability of toluene to induce DNA damage has been evaluated in two studied by comparing its differential toxicity to wild-type and DNA repair-deficient bacteria (Fluck *et al.*, 1976; Mortelmans and Riccio, 1980). Two species have been tested with negative results: *Escherichia coli* W3110 and p3478 (*polA*$^+$ and *polA*$^-$, respectively) and *Salmonella typhimurium* SL4525 (*rfa*) and SL4700 (*rfa*) (*rec*$^+$ and *rec*$^-$, respectively). In the first study, Fluck *et al.* (1976) applied 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)

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	

NR = not reported. [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.

study growth inhibition was also found to be comparable with both the wild-type and repair-deficient strains of the E. coli and Salmonella typhimurium when sterile filter discs inoculated with 0.001-0.01 µl toluene were placed in the centers of culture plates; these assays were performed both with and without metabolic activation. In quantitative growth inhibition tests, Mortelmans and Riccio (1980) again found that toluene (0.001-0.01 µl/plate) was not differentially toxic to either the DNA repair-sufficient or repair-deficient strains of the E. coli or Salmonella typhimurium. In these 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 and recorded (instead of measuring the diameter of the zone of growth inhibition).

14.2.2 Mutagenesis in Microorganisms

Reverse mutation testing of toluene was negative in Salmonella typhimurium tester 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), Escherichia coli WP2 (Mortelmans and Riccio, 1980), and Saccharomyces cerevisiae D7 (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 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

Table 14-2. Microbial Mutagenicity Assays

Table 14-2. Microbial Mutagenicity Assays

Test	Indicator Strains	Metabolic Activation[a]	Dose	Application	Response	Reference
Reverse Mutation						
Salmonella typhimurium	Footnote b	±	0.001-5.0µl/plate	Plate incorporation	--	Litton Bionetics, Inc., 1978a
		±	0.004-0.031%[c]	Liquid suspension	--	
Salmonella typhimurium	Footnote b	±	0.01-10µl/plate	Plate incorporation	--	Mortelmans and Riccio, 1980
Salmonella typhimurium	Footnote b	±	5 µl/plate	Plate incorporation	--	Nestmann *et al.*, 1980
Salmonella typhimurium	Footnote b	±	0.115-2.3 µl/plate	Plate incorporation	--	Bos *et al.*, 1981
Salmonella typhimurium	TA98, TA100	±[d]	0.3 µl-100 µl/plate	Plate incorporation[e]	--	Snow *et al.*, 1981
		±[d]	11-3764 ppm	Vapor exposure[f]	--	
Escherichia coli	WP2	±	0.01-10 µl/plate	Plate incorporation	--	Mortelmans and Riccio, 1980
Saccharomyces cerevisiae	D7	±	0.001-0.5%[g]	Liquid suspension	--	Mortelmans and Riccio, 1980
Mitotic Crossing-Over						
Saccharomyces cerevisiae	D7	±	0.001-5.0%[g]	Liquid suspension	--	Mortelmans and Riccio, 1980
Mitotic Gene Conversion						
Saccharomyces cerevisiae	D4	±	0.001-5.0µl/plate	Plate incorporation	--	Litton Bionetics, Inc., 1978a
		±	0.138-1.1%[c]	Liquid suspension	--	
Saccharomyces cerevisiae	D7	±	0.001-5.0%[g]	Liquid suspension	--	Mortelmans and Riccio, 1980

[a] Aroclor 1254-induced rat liver homogenate S-9 fraction.

[b] Strains TA98, Ta100, TA1535, TA1537, and TA1538 tested.

[c] 50% mortality at the highest dose.

[d] The toluene was tested with both Aroclor-induced S-9 and toluene-induced S-9.

[e] 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.

[f] 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.

[g] 100% mortality at 0.1% and 0.5%.

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 for its ability to induce mitotic crossing-over in the yeast Saccharomyces cerevisiae D7 (Mortelmans and Riccio, 1980) and mitotic gene conversion S. cerevisiae D4 and D7 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980). Toluene did not elicit a positive response in any of these tests (Table 14-2).

14.2.3 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 0.05-0.30 µl/ml, with and without mouse liver S-9 activation.

14.2.4 Cytogenetic Test Systems

14.2.4.1 Micronucleus Test

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 LD50 for male mice (Koga and Ohmiya, 1978).

14.2.4.2 Chromosomal Aberrations

Two reports from the Russian literature have concluded that toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection (Dobrokhotov, 1972; Lyapkalo, 1973). In an analysis of 720 metaphasal disks 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 metaphase aberrations. Sixty-six percent of the induced aberrations were chromatid breaks, 24% were chromatid fractures, 7% were chromosome fractures, and 3% involved multiple aberrations. The frequency of spontaneous aberrations in 600 metaphasal marrow disks 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 benzene was injected daily for 12 days, the damage was approximately additive (33.33% aberrations). The significance of the positive elastogenic effects attributed to toluene are difficult to assess, however, because the purity of the sample employed was not stated, and because the distinction between chromatid breaks and gaps 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 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 inhalation, the frequency of chromosome damage was still elevated. A total of 96 rats were used in this study, but the number of rats sacrificed 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 two different experiments. In one 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 triethylene melamine (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

Table 14-3. Rat Bone Marrow Cell Aberrations Following Intraperitoneal Injection of Toluene (Litton Bionetics, Inc., 1978a)

Treatment[a]	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[d]
					Structural[c]	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)[b]	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,2ac,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]The toluene used was 99.96 wt. % pure (ethylbenzene, 0.03%; p-xylene, <0.01%; m-xylene, <0.01%; sulfur, 0.4 ppm) (Fowle, 1981).

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

[c]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.

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

chromosomal aberrations in cultured human lymphocytes. Benzene and xylene at the same 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 131-532 ppm benzene for 2-7 years and subsequently to toluene in the general range of 200-400 ppm for 14 years; 24 of the workers were exposed only to toluene for 7-15 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 two different rotoprinting factories who had a history of exposure to pure toluene (benzene concentration, $\geq$0.05%; average benzene concentration, 0.006%) at 8-hour time-weighted average (TWA) concentrations of 7-112 ppm. The average age of the

Table 14-4. Frequency of Unstable and Stable Chromosome Changes and Chromosome Counts in Subjects Exposed to Benzene or Toluene or Both (Forni et al., 1971)

Expsoure Subjects	No. of Cases	Age Range	Total Cells Counted	% Cells		% Cells With Chromosome Number		
				C_u[a]	C_s[b]	<46	46	>46 (Polyploid)
Benzene (+ toluene)	10	36-54	964	1.66(1.87)[c,d,e]	0.62[d,e]	13.1	86.0	0.9(0.52)
Toluene	24	29-60	2,400	0.80(0.83)[c]	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]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.

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

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

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

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

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 1.5-26 years and air concentrations of toluene showed TWA values of 100-200 ppm, with occasional rises up to 500-700 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 2-10 years prior to 1950, and subsequently to toluene as stated above, are included for comparison.

14.2.4.3 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 and no metabolic activation

Table 14-5. Effect of Occupational Toluene Exposure and Smoking on Chromosomal Aberrations and Sister Chromatid Exchanges (Maki-Paakkanen et al., 1980)

Occupational Toluene Exposure (yr)	No. of Subjects	Mean Age (yr)	Cells Analyzed[a]	Cells with Chromosomal Aberrations (%)				Sister Chromatid Exchanges (SCEs)	
				Gaps Excluded			Gaps Included		
				Chromatid Type	Chromosome Type	Total	Total	Cells Analyzed[b]	Mean per Subject per Cell[c]
Total Worker (14.6-yr average exposure)	32	34.2[d]	--	1.0	0.5	1.5	2.5	--	8.5
Total Control	15	34.2[d]	--	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[e]	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

Abbreviation: yr = year

[a]100 cells analyzed per individual.

[b]30 cells analyzed per individual.

[c]Calculated from individual means.

[d]Mean value.

[e]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.

Table 14-6. Chromosome Aberrations in Rotoprinting Factory Workers (Funes-Cravioto et al., 1977)

	Group[a]		
	Control	Toluene	Benzene/Toluene
No. of Subjects	49	14	8
Age (year)			
Range	0.16-63	23-54	54-65
Mean	24.4	37.2	61.3
No. of Cells Analyzed			
Total	5000	1,400	800
Abnormal			
Total	217	108	76
Frequency range (%)	0-20	2-15	4-17
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)	0-22	2-17	6-17
Mean (per 100 cells)	5.1	8.9	11.9

[a]Exposure details provided in accompanying text.

system was employed. Twenty-six cells/dose were scored for SCEs andcytotoxicity 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%-0.04% toluene for 21.4 hours, and CHO cells with activation were exposed to 0.0125%-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 7-112 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 (Subsection 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 2-10 yers and subsequently to toluene; exposure to benzene and toluene ranged from 2-26 years. TWA concentrations of toluene generally ranged from 100-200 ppm (occasionally to 500-700 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 in 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 12-15 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 on days 6-15, and a significant reduction in fetal weight was measured in the 0.5 and 1.0 ml/kg groups. After exposure to toluene on days 6-15 at 1.0 ml/kg, a statistically significant increase in the incidence of cleft palate was noted which reportedly did not appear to be due merely to a general retardation in growth rate; however, when toluene, at a level of 1.0 ml/kg, was administered on days 12-15 decreased maternal weight gain was the only effect observed. Maternal toxicity was not seen after exposure to toluene on days 6-15 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 339 ppm also had an effect on material 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 9-14, 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 1-8 (399 ppm) and during the entire period of pregnancy (days 1-21) 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 1-8 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 for 24 hours/day on days 7-14 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 9-14 of gestation. Tatrai et al. (1980) concluded that the exposures to 266 ppm toluene were not teratogenic (no external, internal, or skeletal malformations 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

Table 14-7. Teratogenicity Evaluation of Toluene in CFY Rats and CFLP Mice (Hudak and Ungvary, 1978)

	Rats					Mice		
	Air Inhalation	Toluene		Air Inhalation	Toluene	Air Inhalation	Toluene	
	Days 1-21 8 h/d	266 ppm Days 1-21 8 h/d	399 ppm Days 1-8 24 h/d	Days 9-14 24 h/d	399 ppm Days 9-14 24 h/d	Days 6-13 24 h/d	133 ppm Days 6-13 24 h/d	399 ppm Days 6-13 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[a] (%)	46.6	44.1	44.0	46.9	41.8	--	--	
No. live fetuses	111	133	95	348	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[b] (%)	7.2	16	46**	6.9	17.3	6.5	27.6**	--
External malformations[c]	0	0	0	0	0	0	0	--
No. fetuses dissected[d]	54	64	49	179	110	64	58	0
Internal malformations[e]								
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[f]	0	17**	7**	11	24**	3	1	--

Table 14-7. Teratogenicity Evaluation of Toluene in CFY Rats and CFLP Mice (Hudak and Ungvary, 1978) (Cont.)

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

Abbreviations: h = hour; d = day.
*P < 0.01 (t-test); ** P < 0.05 (Mann Whitney U Test); *** P < 0.01 (Mann Whitney U Test)

[a]Percent of starting body weight.

[b]Percent of living fetuses weighting < 3.3 g (rats) or 0.9 g (mice).

[c]Agnathia, brachimelia, missing tail.

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

[e]Thymus hypolasia also looked for.

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

[g]Fissura sterni and agnathia also looked for.

Table 14-8. Teratogenic Effects of Exposure to Toluene, Benzene, and a Combination of Toluene and Benzene in CFY Rats (Tatrai et al., 1980)

Inhalation on days 7-14 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. Teratogenic Effects of Exposure to Toluene, Benzene, and a Combination of Toluene and Benzene in CFY Rats (Tatrai et al., 1980) (Cont.)

Inhalation on days 7-14 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	--	

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

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 ppm or 400 ppm toluene vapor for 6 hours/day on days 6-15 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 related 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 treted 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 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

Table 14-9. Teratogenicity and Reproductive Performance Evaluation in Rats Exposed to Toluene (Litton Bionetics, Inc., 1978b)

	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[a]
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[b]	108(51/57)	105(47/58)	104(51/53)
Number of fetuses examined for skeletal changes[c]	212	221	224
Number of fetuses with normal skeletal examinations	139	150	158
Fetuses with commonly encountered skeletal changes[d,e]	67(20)	62(20)	58(20)
Fetuses with unusual skeletal variations[e,f]	6(4)	9(4)	8(6)

[a]The increase in total resorptions at this dose was attributed to the total resorption of the litter of one particular female.

[b]Numbers of male/females examined in parentheses.

[c]Four specimens from one litter were not examined (missing).

[d]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.

[e]Number of litters in parenthesis.

[f]These were generally cases of more severe and extensive retarded ossification.

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 LD50" 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 5-100 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 three 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 Effects in Humans

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, imflammatory, or degenerative lesions in mice relative to unexposed controls. 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 in 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), and was negative in the micronucleus test (Kirkhart, 1980). 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 100-200 ppm toluene for

1-16 years, but it is probably 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 6-15 of gestation (Nawrot and Staples, 1979). The effect reportedly did not appear to be due merely to a 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 micro somal enzymes by phenobarbital prior to administration of the mixture alleviated the suppression.

Andrews et al. (1977) coadministered 440 mg/kg 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 suprimposable 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 (Sata 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 coadministered 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 1 man. Forty-one millimoles benzoic acid or 7.4 mmol methylbenzoic acid was ingested singly or in combination by 1 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 1 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 5 different dose levels revealed a departure from an additive model. They concluded that the effect of coadministration 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 $\mu W/cm^2$, 380-700 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 20-50%. 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.

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

Table 16-1. Concentrations of Toluene in Stoppered Flasks (Dunstan et al., 1975)

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

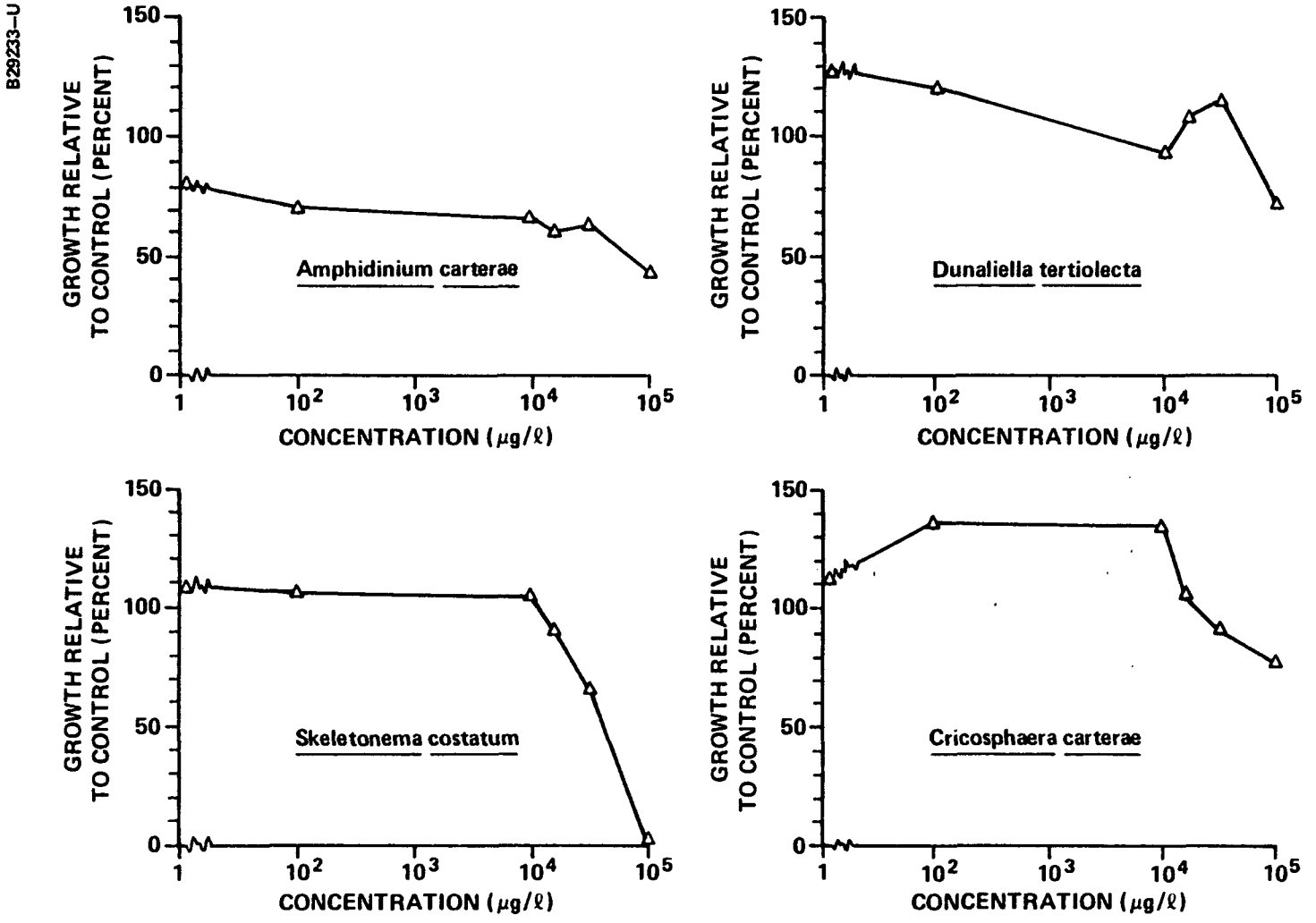

Figure 16-1. Phytoplankton Growth in Various Concentrations of Toluene (Organisms were grown in stoppered flasks. Growth, measured by cell numbers and in vivo chlorophyll, was determined on the 2nd and 3rd days of logarithmic growth. Concentrations of low molecular weight hydrocarbons are in theoretical values.) (Dunstan et al., 1975)

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 1 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/minute 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 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 kind and extent of injury were recorded after 1 month to allow for a recovery period. Temperature of the plants was held at 25°C.

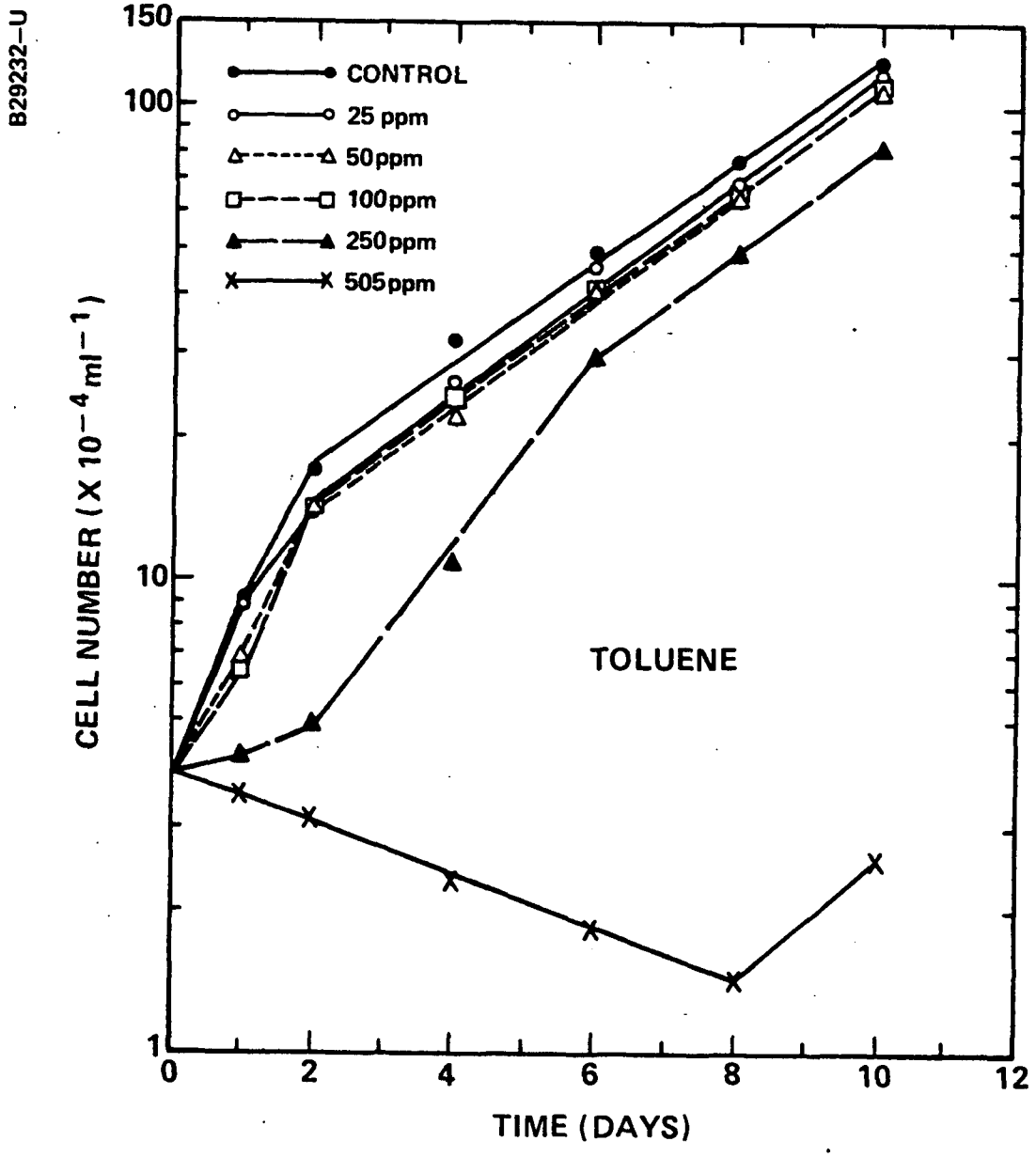

Figure 16-2. Growth of Chlorella vulgaris in Medium Containing Toluene (Data plotted are the average of three replicates. Lines of best fit were determined using regression coefficients. Numbers represent initial hydrocarbon concentration on a parts per million basis. The arrow on the ordinate indicates starting cell concentration.) (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 EC50 (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

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

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 6.4-12.0 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 mg/l to 6.4 mg/l, the percentage of injury to barley after a 2-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.

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

16-3. Toxic Effects of Toluene Vapor on Carrots, Tomatoes, and Barley (Currier, 1951)

Material	Concentration	Percent Injury[a] 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

Abbreviations: h = hour; ND = not determined.

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

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 8 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 2 days of exposure and remained relatively constant except for a temporary decline on day 6. The average tissue concentration during the exposure period was 1.5 ppm. The calculated bioconcen

tration factor (BCF) is 1.25 (which is equivalent to 1.5 ppm in tissue/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 2 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 8 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 4 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 1 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 4 hours in all groups. The BCF values at 8 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 1 hour after the depuration phase began at all exposure concentrations. The depuration time by which no ^{14}C-toluene was detectable in tissue was 1 hour in the mussels exposed to 0.05 µl ^{14}C-toluene/kg, 4 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 8 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 8 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 (1 hour versus 4 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 4 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 4 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 effluents 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 1 mullet with an offensive odor con

tained 5 ppm toluene. Additional experiments showed that toluene was accumulated by caged eels kept for 10 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, 4 eels were exposed in seawater to which a mixed solution of benzene, toluene, and xylenes was added daily for 5 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 10 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 10 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 10 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.

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 (50-100,000 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_6rt 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 1 m deep has been reported to be between about 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 ± 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 of toluene to sediments and suspended particles may interfere with volatilization and may influence the availability of toluene to aquatic organisms.

Most of the reported aquatic toxicity studies with toluene have used a static exposure technique. In most cases, the LC50 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 LC50 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 LC50 values at more than one exposure period showed that LC50 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 (Subsection 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 are most closely related to the effects that may occur in nature during accidental spills, because toluene concentrations rapidly decrease in both situations. Flow-through tests provide some insight into the expected effects of chronic release of toluene into the aquatic environment, as might occur in areas receiving refinery or petrochemical effluents.

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 pure toluene is unlikely to be serious because toluene is so rapidly lost through volatilization. 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; however, toluene spills are likely to cause acute mortality of aquatic species if the spillage occurs in areas of shallow water and restricted water flow, such as in certain portions of estuaries, lakes, and streams. Toluene is acutely toxic to many aquatic species of 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 received refinery and petrochemical effluents, the effects of such low-level chronic pollution 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. 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 1 hour of exposure to 61-65 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 17-18°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 7.5-8.5, methyl orange alkalinity < 100 ppm, temperature 17-22°C). For these toxicity tests, 10 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 LC50 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 3.8-6.4 cm and 1-2 g for the first 3 species and 1.9-2.5 cm and 0.1-0.2 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 LC50 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 LC50 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 LC50 for fathead minnows in soft water was not significantly different from the 96-hour LC50 for the same species in hard water. Comparison of the 95% confidence limits of the 96-hour LC50 values in soft water for the 4 species indicated that the LC50 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 LC50 values significantly lower than goldfish and guppies. The 96-hour LC50 was not significantly different from the 24-hour LC50 for any of the species tested in soft water.

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. supposedly Tests were conducted under identical conditions.	
Mosquitofish (Gambusia affinis)	17-22	SU	1340	1260	---	1180	560	ppm	Tests were conducted in aerated turbid pond water.	Wallen et al., 1957
Goldfish (Carassius auratus)	20±1	SM	58	---	---	---	---	mg/l	Test was conducted in tap water (pH 7.8)	Bridie et al., 1979
Goldfish (Carassius auratus)	25	SU	57.7 (48.9-68.8)	57.7 (48.9-68.8)	---	57.7 (48.9-68.8)	---	mg/l	Test was conducted in soft water.	Pickering and Henderson, 1966
Goldfish (Carassius auratus)	17-19	FM	41.6 (32.0-71.7)	27.6 (21.6-36.0)	25.3 (20.1-31.9)	22.80 (17.1-30.0)	---	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 LC50 (and 95% confidence interval) was 14.6 (10.7-20.0) ppm.	Brenniman et al., 1976
Fathead minnow (Pimephales promelas)	25	SU	46.3 (37.0-59.4)	46.3 (37.0-59.4)	---	34.3 (22.8-45.9)	---	mg/l	Tests were conducted in soft water.	Pickering and Henderson, 1966
Fathead minnow (Pimephales promelas)	25	SU	56.0 (44.7-67.1)	56.0 (46.7-67.1)	---	42.3 (33.5-53.5)	---	mg/l	Tests were conducted in hard water.	
Bluegill sunfish (Lepomis macrochirus)	25	SU	24.0 (18.9-30.5)	24.0 (18.9-30.5)	---	24.0 (18.9-30.5)	---	mg/l	Tests were conducted in hard water.	Pickering and Henderson, 1966

Table 17-1. Acute Toxicity of Toluene to Fish and Aquatic Invertebrates (Cont.)

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
Bluegill sunfish (Lepomis macrochirus)	NR	SU	16.6 (15.0-19.1)	13.3 (11.6-14.8)	12.7 (11.5-14.5)	12.7 (11.5-14.5)	10.0	ppm	Only these data cited in U.S. EPA, 1980.	U.S. EPA, 1978
Guppies (Poecilia reticulata)	25	SU	62.8 (55.0-73.7)	61.0 (52.8-71.9)	---	59.3 (50.9-70.3)	---	mg/l	Tests were conducted in hard water.	Pickering and Henderson, 1966
Zebrafish (Brachydanio rerio)	20±1	FU	---	25-27	---	---	---	mg/l	Tests were conducted in closed aquaria with dechlorinated hard tap water at a flow rate of 6 l/h.	Slooff, 1978 Slooff, 1979
Medaka (Oryzias latipes)	25±2	SU	80 (mean=80)	20-135 (mean=63)		23-110 (mean=54)	≤16	mg/l	Range and mean of LC50 values for different stage embryos	Stoss and Haines, 1979
Medaka (Oryzias latipes)	25±2	SU	44	36		32	---	mg/l	LC50 values for fry. The 168-h LC50 was 23 mg/l.	Stoss and Haines, 1979
Coho salmon fry (Oncorhynchus kisutch)	---	FM	---	---	---	9.36	---	µl/l	Unparasitized	Moles, 1980
	---	FM	---	---	---	3.08	---	µl/l	Parasitized	Moles, 1980
MARINE										
Coho salmon (Oncorhynchus kisutch)	8	SU	---	22.4	22.4	22.4	10	ppm	Tests were conducted in artificial saltwater (pH 8.1, 30°/oo salinity).	Morrow et al., 1975
Pink salmon fry (Oncorhynchus kisutch)	12	SM	5.4 (4.4-6.5)	---	---	---	---	ppm	Tests were conducted according to methods of Korn et al., 1979.	Thomas and Rice, 1979
Pink salmon (Oncorhynchus kisutch)	4	SM	---	---	---	6.41 (5.73-7.18)	---	µl/l	Tests were conducted with salmon fry acclimated to 28°/oo seawater at different temperatures.	Korn et al., 1979
	8	SM	---	---	---	7.63 (6.86-8.48)	---			
	12	SM	---	---	---	8.09 (7.45-8.78)	---			

Table 17-1. Acute Toxicity of Toluene to Fish and Aquatic Invertebrates (Cont.)

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
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 (240-420)	310 (240-420)	---	---	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 (21.36-21.68)	---	---	---	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
Bay shrimp (Crago franciscorum)	16	SM	12 (10-13)	---	---	4.3 (3.1-5.8)	---	µl/l	Tests were conducted with 25°/oo salinity seawater.	Benville and Korn, 1977
Shrimp (Eualus spp.)	4	SM	---	---	---	21.4 (19.5-23.5)	---	µl/l	---	Korn et al., 1979
	8	SM	---	---	---	20.2 (17.9-22.8)		µl/l	---	Korn et al., 1979
	12	SM	---	---	---	14.7 (13.1-16.6)		µl/l	---	Korn et al., 1979

Table 17-1. Acute Toxicity of Toluene to Fish and Aquatic Invertebrates (Cont.)

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
Grass shrimp (Pacaemonetes pugio)	20	SM	20.2 (16.3-22.5)	---	---	---	---	mg/l	Adults at 15°/oo salinity.	Potera, 1975
	20	SM	17.2 (14.9-19.4)	---	---	---	---	mg/l	Adults at 25°/oo salinity.	Potera, 1975
	10	SM	37.6 (35.0-40.3)	---	---	---	---	mg/l	Adults at 15°/oo salinity.	Potera, 1975
	10	SM	38.1 (36.1-39.6)	---	---	---	---	mg/l	Adults at 25°/oo salinity.	Potera, 1975
Grass shrimp (Pacaemonetes pugio)	20	SM	30.6 (21.3-44.5)	---	---	---	---	mg/l	Larvae at 15°/oo salinity.	Potera, 1975
	20	SM	25.8 (18.8-34.6)	---	---	---	---	mg/l	Larvae at 25°/oo salinity.	Potera, 1975
Grass shrimp (Palaemonetes pugio)	NR	SU	---	---	---	9.5	---	mg/l	---	Neff et al., 1976
Mysid shrimp (Mysidopsis bahia)	NR	SU	64.8 (50.9-82.5)	56.3 (43.0-70.8)	56.3 (43.0-70.8)	56.3 (43.0-70.8)	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 (Nitocra spinipes)	20	SM	24.2 (19.8-30.2)	---	---	---	---	mg/l	15°/oo salinity.	Potera, 1975
	20	SM	74.2 (52.0-100.5)	---	---	---	---	mg/l	25°/oo salinity.	Potera, 1975
Pacific oyster (Crassostrea gigas)	20-21.5	SU	---	1050	---	---	---	mg/l	Larvae.	Legore, 1974

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

Static acute LC50 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 LC50 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 2.3-4.3 g) than Pickering and Henderson (1966) to determine the static 24-hour LC50. 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 LC50, 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 LC50 for goldfish reported by Pickering and Henderson (1966).

Much larger goldfish (length, 13-20 cm; weight, 20-80 g) were used by Brenniman et al. (1976) to determine the acute toxicity of toluene under flow-through exposure conditions. The LC50 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 ± 0.3; temperature 17-19°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 ≥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 LC50 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 LC50 was significantly lower than the 24-hour LC50. These LC50 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 LC50 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.

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, 5-7 cm) per concentration in tapwater (pH 7-8, hardness 268 ± 54 mg/l) at 20 ± 1°C. The 48-hour LC0 (0% mortality), LC50, and LC100 (100% mortality) values determined at each laboratory were as follows:

	48-Hour Lethal Concentration Values (mg/l)		
	LC0	LC50	LC100
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 LC50 of toluene to zebrafish (Brachydanio rerio) was 25-27 mg/l. This test was conducted under flow-through (6 l/hour) 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 LC50, 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 LC50 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 10 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 deter mine the sensitivity of different stages of embryo development, tests were begun with eggs of various age's after fertilization. Tests with fry were all begun within 24 hours after hatching. Nominal initial toluene concentrations were

used for calculation of LC50 values. The LC50 values for embryos varied with length of exposure and the age at time of introduction. The mean 24-, 48-, and 96-hour LC50 values for all ages of embryos were 80, 63, and 54 mg/l. The range of LC50 values was 20 to 135 mg/l at 48 hours and 23 to 110 mg/l at 96 hours (Stoss, personal communication). Early ($\leq$3.5 hours old) and late ($\geq$192 hours old) embryos had significantly lower LC50 values at each exposure period than embryos of intermediate age at time of introduction. The 24-, 48-, 96-, and 168-hour LC50 values for fry were 44, 36, 32, and 23 mg/l, respectively (Stoss, personal communication). These values were lower than the mean embryo LC50 values for the same exposure period; however, fry LC50 values were greater than the LC50 values for the susceptible early and late stage embryos and lower than most of the LC50 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 ($\leq$1 g fish/l water) to give nominal concen trations 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 mortality 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 LC50 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 LC50 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 (6-8°C; 26-28 °/oo salinity). Groups of fry were then acclimated to 4, 8, or 12°C for determination of the 96-hour LC50 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 LC50

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 LC50 values did not overlap, indicating that temperature affected the toxicity of toluene. There was no significant difference between 24- and 96-hour LC50 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 LC50 of toluene with somewhat larger (1-2 g, 4.5-5.5 cm) pink salmon fry at 12°C in seawater. The 24-hour LC50 (and 95% confidence interval) was 5.4 (4.4-6.5) ppm, which is significantly different from the 96-hour LC50 value of 8.1 ppm (7.5-8.8) 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 LC50 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 LC50 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 LC50 by probit analysis. Initial exposure concentrations were determined by gas-liquid chromatography. The 24-hour LC50 (± 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 LC50 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 LC50 values (and 95% confidence intervals), based on initial nominal

concentrations, were both 310 (240-420) mg/l. The "no discernible effect concentration" was 28 mg/l. This LC50 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 LC50 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 30-50 newly hatched brine shrimp were exposed to 5 toluene concentrations in 100 ml seawater. The estimated 24-hour LC50, 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 LC50, determined in natural seawater (25 °/oo salinity) at 16°C, was 12 µl/l(ppm). The 96-hour LC50 for this species (4.3 µl/l) was significantly lower than the 24-hour LC50 (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 26-28 °/oo salinity seawater for 4 days and then exposed in groups of 10-15 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 LC50 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 LC50 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 LC50 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 LC50 of toluene to the grass shrimp, Palaemonetes pugio. The 24-hour LC50 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 LC50 values between adults and larvae at the same salinity and temperature, or between adults tested at the same temperature but at different salinities. The LC50 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 LC50 for the copepod, Nitocra spinipes, at a temperature of 20°C and at salinities of either 15 °/oo or 25 °/oo. The 24-hour LC50 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 LC50 value.

Neff et al. (1976) also determined the static 96-hour LC50 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 LC50 values reported by Potera (1975).

Caldwell et al. (1976) determined the 48- and 96-hour LC50 of toluene to larval stages of the dungeness crab (Cancer magister) under flow-through exposure conditions. The 48- and 96-hour LC50 values were 170 and 28 mg/l, respectively.

Static acute LC50 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 LC50 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 LC50 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 (25.3-30.8 °/oo salinity) at 20-21.5°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 2 hours after beginning exposure. The Na^+ concentration returned to the control level by 3 hours. Blood K^+ decreased after 2 hours but was still elevated at 4 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 (Section 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 LC50 (5.38 ppm), the authors exposed fry to several toluene concentrations, expressed as percentages of the LC50. Significant increases in opercular breathing rate at 12°C occurred at exposure concentrations of 94% and 69% of the LC50, but not at 45% or 30% of the LC50. The breathing rate remained elevated throughout the 15-hour exposure period only at 94% of the LC50, at which concentration 6 of 23 fish died. The breathing rate at a toluene exposure concentration of 69% of the LC50 reached a maximum at 3 hours and returned to control level by 15 hours. Additional experiments showed that exposures to 71% of the LC50 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 LC50, 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 3-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 8-day old embryos of the Japanese medaka, Oryzias medaka. The basal (unexposed) rate was determined for each of 3 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 5-minute intervals for 40 minutes. The average rate before exposure was 0 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 Subsection 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 3.2 ppm, but not at 7.7 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 LC50 for this species was between 277 and 485 ppm (Subsection 17.3.1.2). The application factor between acute and sub-chronic toxicity was between 36 and 152.

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 (0.1-0.5 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 (Subsection 15.3). Maximum ^3H-toluene counts were equal in fed and unfed larvae, but were reached

more quickly (1 hour versus 4 hours) by the fed animals. The ^{3}H-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 1 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 1 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 1 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 6 hours lowered the toxicity to the same extent as 3 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 (ET50, 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 (33.5-34.5 °/oo salinity seawater at 8-10°C) was changed every 2 days. The interpolated or extrapolated ET50 values were as follows:

Toluene Concentration (ppm)	ET50 (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 1 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 8 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.

18. HUMAN RISK ASSESSMENT

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 (6-9 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:

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 Safty 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 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 $\leq$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 Section 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, 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. Although acute and subchronic inhalation studies on experimental animals are available, the uncertainties inherent in extrapolating from experimental mammals to human populations outweigh the benefits of the controlled nature of these studies.

18.2.1 Effects of Single Exposures

The effects on humans of single exposures to toluene for periods of up to 8 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; Anderson and Kaada, 1953; 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 (7-8 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 aftereffects 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 ppm 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 5 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 300-800 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 0.35-0.79 ppm and 0.40-0.85 ppm, respectively. May (1966) reports a mimimum 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:

10,000-30,000 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.
300-800 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.
100-300 ppm	:	Detectable signs of incoordination may be expected during exposure periods up to 8 hours.
200 ppm	:	Mild throat and eye irritation.
50-100 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.

One of the more striking features of the data on the subchronic and chronic effects of toluene exposure on humans is the failure of increased periods of intermittent exposures to cause clearly increasingly severe effects. Although the utility of the available studies for estimating firm dose-response relation ships is somewhat limited by the failure to define precisely levels and durations of exposure, problems of sample sizes, the potential role of other toxic agents in eliciting the reported effects, and some apparent inconsistencies among the available studies, the weight of the evidence suggests that the types of effects seen and the levels at which these effects are seen are relatively independent of the duration of exposure.

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: 50-200 ppm, 200-500 ppm, and 500-1500 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). 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 100-1100 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 evidence of liver 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 200-400 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 studdy 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 200-800 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 (210-300) 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, however, 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 (80-160) 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, however, 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 15-200 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 compli-

cated by the absence of quality assurance throughout the study and the use of an inparropriate strain of rats for study of myelotoxicity. 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.

18.1.3 Acceptable Daily Intake (ADI) Based on Inhalation Exposure

Given the uncertainties detailed above in the data on the effects of long-term toluene exposure on humans and experimental animals, the reported NOELs in both humans and experimental animals must be regarded with caution in attempting to estimate an ADI for intermittent (occupational) or continuous (environmental) exposures.

The American Conference of Governmental Industrial Hygienists (ACGIH) (1979) has set the Threshold Limit Value (TLV) for toluene at 100 ppm which is the same as the NIOSH criteria and OSHA has adopted a standard of 200 ppm; however, both the acute data on humans provided by von Oettingen and coworkers (1942a; 1942b) as well as the suggestive, if equivocal, data on occupational exposures near or below 100 ppm (Matsushita et al., 1975; Hanninen et al., 1976; Seppalainen et al., 1978) suggest that these values have little, if any, margin of safety. Nonetheless, given the reported human NOELs above 100 ppm (Suhr, 1975; Capellini and Alessio, 1971) and the continuous subchronic exposure NOEL for experimental animals at 107 ppm (Jenkins et al., 1970), the TLV can be used, albeit somewhat arbitrarily, as an equivocal NOEL for humans in deriving an ADI. Because of the uncertainty of this value, a safety or uncertainty factor should be applied following the guidelines of the National Academy of Sciences (NAS, 1977) as recently expanded by the U.S. EPA (1980c). The use of these safety

factors for deriving acceptable limits of exposure to air pollutants has recently been proposed by Kim (1981) and Su and Wurzel (1981).

Based on all of the available toxicity information, reasonable arguments could be made for using uncertainty factors ranging from 5 to nearly 100. An uncertainty factor of 5 would put the presumed safe occupational exposure level at 20 ppm, only 10 ppm below the lowest reported observed-effect level (i.e., 30 ppm: Hanninen et al., 1976; Seppalainen et al., 1978). The uncertainty factor of 5 could be defended because the 30-ppm effect level also involved exposure to several other known toxic agents. A safety factor of 100 would give considerable weight to the reported human effects below 100 ppm and to the fact that the carcinogenic and teratogenic potential of toluene has not been adequately investigated (Section 18.4). Although the uncertainty factor of 100 would certainly be protective of CNS impairment or other toxic effects, it could easily be challenged as overly conservative. The weight of the evidence suggests that an uncertainty factor of 10 would be protective for most individuals and is consistent with the general approach for applying uncertainty factors recommended by the National Academy of Sciences (1977).

Using an uncertainty factor of 10, the ADI for humans based on inhalation data could be estimated at 2.69 mg/kg body weight, using a modification of the Stokinger and Woodward (1958) approach where:

$$ADI = \frac{TLV \times BR \times AC}{UF \times BW}$$

TLV = Threshold Limit Value, 100 ppm = 377 mg/m^3

BR = Cubic meters of air breathed per workday = 10 m^3

UF = Uncertainty Factor

BW = Human Body Weight = 70 kg

AC = Absorption Coefficient = 0.50

Here, the absorption coefficient of 0.5 was taken as the approximate mid-range of retention values reported by Ovrum and coworkers (1978) and Carlsson and Lindqvist (1977). As detailed in Section 13, the absorption coefficient is not a true pharmacokinetic parameter and varies with period of exposure and level of activity. The absorption coefficient is used here only to obtain a reasonable approximation of the ADI.

As discussed in the beginning of Section 18.2.2, the ADI derived above is applicable to intermittent occupational exposures that are assumed to occur 5 days per week. Spreading the ADI over a 7-day per week exposure yields an ADI of 1.92 mg/kg/day. Assuming that humans breathe a total of 24 m^3 per day, an equivalent ambient air level can be estimated to be 2.98 ppm or 11.2 mg/m^3 (100 ppm/10 x 5/7 x 10/24). Because toluene is rapidly absorbed and rapidly eliminated on inhalation exposures, this simplistic derivation of a "safe" ambient air level should be regarded with skepticism and is at best a crude approximation. Given the known pharmacokinetic patterns of toluene and its apparent lack of cumulative toxicity, a safe ambient air level may be substantially higher. Conversely, given the paucity of actual data on continuous exposures, an upward adjustment of this "safe" ambient air level does not seem prudent.

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 LD50s 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. An ADI could be derived from this study by averaging the five-day dose over a several day week and using an uncertainty factor as discussed above. Given the scant data available on oral exposures, the uncertainty of route-to-route as well as species-to-species conversions, and the potential teratogenic effects of toluene (Section 18.5.3), a conservative uncertainty factor of 1000 seems appropriate. This is identical to the approach taken by the U.S. EPA in deriving an ambient water quality criterion for toluene. Because the estimate is based on a free-standing NOEL, the resulting ADI of 0.42 mg/kg or 29.5 mg for a 70 kg human may be more protective than predictive of a toxic threshold (U.S. EPA, 1980c).

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.

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).

Although the above data are not adequate for assessing the potential carcinogenicity of toluene with great assurance, they are also inadequate for supporting carcinogenicity as a valid biologic endpoint in quantitative risk assessment.

18.4.2 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

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

Micronucleus Test
mouse

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 (200-400 ppm--Forni *et al.*, 1971; 7-112 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 100-200 ppm for 1-16 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 6-15 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 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 1-8) and mice (399 ppm, 24 hours/day, days 6-13). 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 1-21; 266 ppm, 24 hours/day, days 7-14) or mice (133 ppm, 24 hours/day, days 6-13). 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 6-15 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, from which the ADI is derived. As discussed in Section 18.3, this was one consideraton in recommending an uncertainty factor of 1000. Because teratogenic effects were not noted at the two lower dose levels in a study by Nawrot and Staples (1979), a more conservative approach does not seem justified. Although this approach may be protective, it is not predictive of levels of human exposure that might pose a teratogenic or embryotoxic hazard. One possible approach to a predictive teratogenic/embryotoxic exposure is to again use the cubed root of the body weight ratios for interspecies conversion (U.S. EPA, 1980c, Freireich et al., 1966; Rall, 1969) (see Section 18.3). Assuming a body weight for mice of 0.035 kg and a human female body weight of 55 kg, the dose that might be expected to induce a teratogenic effect in humans is 74.5 mg/kg (866 mg/kg x (55 kg x 0.035 kg)$^{1/3}$) or a total daily dose of about 4100 mg (74.5 mg/kg x 55 kg). As discussed in the following section, this is much higher than current levels of human exposure from environmental sources. Although this suggests a substantial margin of safety, quantitative methods for high-to-low dose or species-to-species extrapolation for teratogenic chemicals have not yet been validated. Consequently, the above approach should be considered speculative, at best, and perhaps superficial.

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 6-13, 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 6-15 of gestation, or in the Tatrai et al. (1980) study when rats were continuously exposed to 266 ppm toluene on days 7-14. 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. Nonetheless, the derived "safe" level for occupational exposure of 10 ppm seems protective in view of the negative results of the Litton and Tatrai et al. (1980) studies. The derived "safe" level for ambient air, 2.98 ppm, is about 45 times below the lowest effect level on continuous exposure noted by Hudak and Ungvary (1978). Since the effect noted was low fetal weight rather than skeletal growth retardation or anomalies, the margin of safety seems adequate, although it would be desirable to have a no-effect level for embryotoxic effects on continuous exposures.

18.5 CURRENT POTENTIAL HAZARDS TO HUMANS

The following ADIs have been estimated for humans:

Inhalation:	2.69 mg/kg 10 ppm (37.5 mg/m^3) occupational air 2.98 ppm (11.2 mg/m^3) ambient air
Oral:	0.42 mg/kg
Dermal:	none--probably not highly toxic

As detailed in Section 10 (Tables 10-2, 10-3, and 10-4), the only group at possible high risk are workers who are exposed to toluene at or near the TLV. The small or nonexistent margin of safety associated with this TLV has been discussed in Section 18.1.3.

For non-occupational exposures, the worst-case total daily dose from Table 10-3 is about 15.5 mg/day or 0.22 mg/kg/day (15.5 x 70), which is not corrected for incomplete retention of inhaled toluene. Correcting this estimate by using an inhalation absorption coefficient of 0.5, the estimated worst-case

daily dose is 0.11 mg/kg. Thus, compared to the most conservative ADI (0.42 mg/kg), there is a margin of safety of about four between the ADI and the current worst-case levels of exposure. This analysis suggests that ambient exposure to toluene does not currently present a human health hazard given the known toxic effects of this compound. Although this is reassuring, uncertainties over the carcinogenic and teratogenic effects of toluene should be a matter of concern and future research. In addition, dysmenorrhea in female workers (Matsushita et al., 1975), degeneration of germinal epithelium in the testes of rats (Matsushita et al., 1971), and increased follicle-stimulating hormone (FSH) levels in rats (Andersson et al., 1980) have been associated with toluene exposure and suggest that the reproductive effects of this compound should also be considered in formulating research needs.

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