III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Toluene (toluol) is a clear, colorless, noncorrosive liquid with a sweet, pungent benzene-like odor. The more important physical properties of toluene are presented in Table X-1. [1,2]

Production of toluene as a by-product of the carbonization of coal was the major source of toluene during the latter part of the 19th century. Although fractional distillation of coal-tar oil accounted for 4% of the toluene production in 1966, the major source (96%) was from petroleum and petrochemical processes including the catalytic reforming reactions. [3] In the United States, the production of toluene [4,5] has increased steadily since 1940 when its production was approximately 31 million gallons; in 1970, production had increased to 694 million gallons.

Approximately 70% of all toluene that is produced is converted into benzene. [5] Another 15% is consumed in the production of chemicals such as toluene diisocyanate, phenol, benzyl and benzoyl derivatives, benzoic acid, toluene sulfonates, nitrotoluenes, vinyl toluene, and saccharin. The remainder is used as a solvent for paints and coatings or as a component of motor and aviation gasolines. [5]

Highly purified toluene (Reagent Grade and Nitration Grade) is presently used for many commercial purposes and contains less than 0.01% benzene as a contaminant [J. D. Hammond, oral communication,

June 1973]. Industrial Grade and 90/120 Grade toluene contain significant quantities of benzene with the 90/120 Grade containing as much as 25%. [1]

Toluene may be encountered as a relatively pure substance or as a constituent of solvent mixtures. When toluene is contained in enclosed systems, potential exposures may occur from transfer of liquid, spillage, or from leaking equipment. Exposures also may occur when toluene is present as a component of paints, thinners, lacquers, and solvents.

Using data obtained from a survey conducted by the U. S. Public Health Service, Bureau of Occupational Safety and Health in 1970, [6] NIOSH estimates that 100,000 persons in the work force could have potential exposure to toluene.

Historical Reports

Early reports on the health effects resulting from exposure to toluene described its toxicity as being similar to that of benzene. [7-9] Certain grades of toluene may contain as much as 25% benzene [1]; thus, its purity must be carefully considered. Because of the benzene content in toluene, conclusions made by the early investigators, [7-9] and even statements in some relatively recent reports, [10,11] have confused the problem of toluene toxicity on the hematopoietic system. Banfer [12] in 1961 reported that commercial toluene in Germany contained up to 15% benzene and other aromatics and

that pure toluene, containing only traces of benzene (up to 0.3%), had been available for only 6 years.

Effects on Humans

(a) Effects on Blood and Hematopoietic Tissue

(1) Bone Marrow

Although studies in experimental animals show rather convincingly that toluene is not myelotoxic, there has been some persistent controversy concerning the effects of toluene on human bone marrow. This is probably due to investigations of groups of industrial workers exposed to toluene derived from coal tar which was contaminated with considerable benzene, frequently as much as 15%. [12] The belief that toluene has myelotoxic properties led to its use in the treatment of leukemia. Francone and Braier [13] in 1954 mentioned the oral administration of toluene for this purpose. They found that leukemia patients tolerated daily doses of 10 g of toluene in olive oil for three weeks without complaints or clinical evidence of side effects. In 1926, however, Hultgren [14] had stated that the methyl derivatives of benzene (toluene, xylene, and mesitylene) should not be used to treat leukemia because they have no effect on the bone marrow. His opinion was based on his research in rabbits.

Parmeggiani and Sassi [15] reported in 1954 on their study of 11 paint and pharmaceutical industry workers who were exposed to toluene vapor ranging from 200 to 800 ppm. Irritation of the conjunctiva and

of the upper respiratory tract mucosa was found in one worker, and nervous excitability in six others. From laboratory investigations and physical examinations, the authors concluded that toluene has no particular action on the bone marrow or on other organs and expressed the opinion that workers can tolerate 300 ppm toluene without hazard to health.

Cieslinska et al [10] in 1969 studied the serum levels of iron and copper and the urinary excretion of porphyrins in 51 female workers with an occupational history of exposure to toluene. These authors interpreted their findings of altered levels of iron or copper in three different groups of these subjects to suggest that toluene, as well as benzene, has a harmful action on the hematopoietic tissue although there were no clinical changes observed in these workers. These investigators emphasized the similarity of the toxic responses evoked by benzene and toluene, but, in actuality, the workers had mixed exposures to these substances.

Capellini and Allessio [16] in 1971 reported results of 17 workers who had been exposed for "several" years to mean atmospheric concentrations of toluene of 125 ppm (range, 80-160 ppm) in a plant manufacturing V-belts for industrial machinery. With regular medical supervision, no changes in the blood picture or liver function were detected in any case. Examinations included hemoglobin values, red cell counts, white cell counts, and platelet counts, all of which were within the same limits as 19 control subjects employed in the same

plant who had never been exposed to toluene vapor inhalation during their occupational activity. Also, blood findings were within normal limits in one 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 central nervous system toxicity [see part (c) of this section].

The preponderance of the reported clinical evidence indicates that toluene does not possess the myelotoxic properties of benzene. In Browning's experience [17] based on a large number of blood examinations of many persons exposed to toluene, no effect similar to that of benzene on the blood picture had been observed except where the toluene was found to contain some benzene.

(2) Blood Coagulation

An increase in the prothrombin time was found in 191 printers exposed to 170-340 ppm toluene as reported by Pacseri and Emszt [18] in 1970. Only two subjects showed a reduced number of red blood cells. No other hematologic abnormalities were found in these workers. The benzene content of the toluene was not reported.

(3) Phagocytic Activity of Leukocytes

Bansagi [19] found a decreased phagocytic activity of the polymorphonuclear leukocytes of workers exposed to toluene vapor in the printing industry. However, there was no relationship between the decrease of phagocytic activity and the degree of exposure to toluene. Again, the benzene content of the toluene is not known.

(b) Effects on Menstruation

Michon [20] in 1965 reported the effects of aromatic hydrocarbon exposure on the menstrual cycles of 500 women, aged 20 to 40, working in a factory producing leather and rubber shoes. These workers were exposed to a mixture of benzene, toluene, and xylene at unspecified concentrations, but which were claimed to be within permissible limits established at the time in Poland [100 mg/cu m (31 ppm) for benzene, 250 mg/cu m (67 ppm) for toluene, and 250 mg/cu m (58 ppm) for xylene]. The menstrual cycles of these workers were compared with those of 100 women in the same plant not exposed to these hydrocarbons. The author reported that the women exposed to the aromatic hydrocarbon vapors had a prolonged and more intense bleeding than the control group. It seems more likely that the effects noted were related more to exposure to benzene rather than to toluene. The regularity of the menstrual cycle was not affected.

(c) Effects on Central Nervous System

The controlled 8-hour exposures of three human beings twice weekly for a period of three months by von Oettingen et al [21,22] to concentrations of 50 to 800 ppm of toluene are listed as follows:

0 ppm (control) - occasional moderate tiredness explained by lack of physical exercise, unfavorable illumination, and monotonous noise from fans.

50 ppm - no subjective complaints from one subject and drowsiness with very mild headache in the second subject. No after-effects.

100 ppm - moderate fatigue and slight headache on one occasion. No after-effects.

200 ppm - muscular weakness, confusion, paresthesias of the skin, impaired coordination, dilation of pupils, impaired light accommodation, repeated headache, and nausea at the end of exposure. After-effects included fatigue, general confusion, moderate insomnia, and restless sleep.

300 ppm - severe fatigue, headache, muscular weakness, incoordination, slight pallor. After-effects were fatigue, headache, skin paresthesias, and insomnia.

400 ppm - fatigue, mental confusion, headache, skin paresthesias, muscular weakness, dilated pupils. After-effects were fatigue, headache, skin paresthesias, and insomnia.

600 ppm - extreme fatigue, mental confusion, exhilaration, nausea, severe headache, and dizziness after 3 hours exposure. Eight-hour exposures showed incoordination and staggering gait. After-effects included nervousness and some confusion.

800 ppm - severe fatigue, extreme nausea, confusion, lack of self-control, considerable incoordination, and staggering

gait after 3-hours exposure. After-effects included moderate to severe insomnia lasting several days.

It should be noted that the investigators, three in number, were the experimental subjects in these experiments.

Wilson [23] in 1943 reported on the effects of exposure of 100 workers out of a total of 1,000 employees exposed to vapor of commercial toluene who presented themselves to the hospital for examination. The patients were classified into 3 groups: group 1, those patients exposed to toluene vapor from 50 up to 200 ppm; group 2, those persons exposed to vapor from 200 to 500 ppm; and group 3, those workers exposed to vapor from 500 to 1,500 ppm. Exposures were from 6 to 8 hours daily for periods of 1 to 3 weeks.

The following effects were reported at:

50 to 200 ppm (approximately 60% of the patients) - headache, lassitude, and loss of appetite.

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. Complaints were more numerous and more pronounced than at lower exposure levels.

500 to 1,500 ppm (approximately 10% of the patients) - headache, nausea, dizziness, anorexia, palpitation and extreme

weakness. Loss of coordination was pronounced and reaction time was definitely impaired.

The symptoms from 50 to 200 ppm were considered by the author to be due chiefly to "psychogenic" factors rather than to toluene vapor. The author recommended that the vapor concentration of toluene should never exceed 200 ppm. In addition, changes in the blood and bone marrow were noted and exposures to concentrations of toluene over 500 ppm were considered to pose a risk of depression of the bone marrow. The benzene content of the toluene was not reported.

Carpenter et al [24] exposed 2 male subjects to known concentrations of toluene in a 4000-cubic foot room for 7- to 8-hour periods.

The following responses were reported at:

200 ppm - transitory, mild throat and eye irritation and slight exhilaration.

400 ppm - mild eye irritation, lacrimation, lassitude, nausea, and hilarity.

600 ppm - lassitude, hilarity, verbosity, boisterousness. After termination of the exposure, anorexia, and listlessness.

800 ppm - metallic taste, transitory headaches, extreme lassitude, areas of dimmed vision (scotomata), verbosity, "inebriation", and slight nausea.

Capellini and Alessio [16] in 1971 reported complaints from one worker employed 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. The worker had irritation of the conjunctiva and an occasional feeling of stupor during work, and often reported insomnia and nervousness. No findings of central nervous system changes were reported in 17 other workers in another department [see part (a) (1) of this section] who had mean exposures over several years to 125 ppm of toluene with extremes of 80 ppm and 160 ppm.

Longley et al [25] in 1967 described an episode of acute toluene exposure involving 29 men. Toluene concentrations were estimated to have ranged from 10,000 ppm at waist level to 30,000 ppm at floor level. The effects at these concentrations for unspecified exposure periods were dizziness, "drunkenness", collapse, and loss of consciousness. They recovered spontaneously after removal from the contaminated atmosphere without any after-effects, two months after the acute exposure.

A case of habituation to toluene extending over a ten-year period was reported by Satran and Dodson. [26] Acute intoxications were characterized by headache, "inappropriate" speech, and brief episodes of memory loss. Despite the long period of toluene exposure, which caused many episodes of loss of consciousness, none of the clinical or laboratory studies indicated systemic pathological abnormalities. Electroencephalographic abnormalities were found, consisting of excessive episodic slow activity and occasional sharp,

nonfocal discharges. These findings were regarded as being consistent with diffuse encephalopathy but the features of the record were nonspecific.

The first case in man of permanent encephalopathy from repeated, prolonged exposure to toluene vapor was reported by Knox and Nelson [27] in 1966. A 33-year old man purchased a gallon of pure toluene from a paint store every four to six weeks for 14 years to satisfy his addiction to toluene vapor. The inhalation technique consisted of several breaths of toluene vapor taken by mouth from a soaked rag until he noted reddening of vision and had lightheadedness. A typical day started with inhalation of several breaths of toluene vapor at the bedside soon after awakening. This went on all day at frequent intervals. He carried a small vial of toluene and a rag in his pocket so that he could take a whiff in public without arousing any suspicion. The result of this bizarre addiction was permanent cerebral atrophy. The clinical signs were ataxia, tremulousness, emotional lability, marked snout reflex (distorted nostrils on subjection to sniff test), and positive Babinski toe reflex on the right side. The brain damage was confirmed by electroencephalography and pneumoencephalography. This same individual was the subject of a paper published by Grabski in 1961 [28] when he reported cerebellar degeneration after six years of toluene vapor inhalation.

Gusev [29] in 1965 reported his USSR study on the relationship of inhalation of toluene vapor with changes in the

electroencephalogram of human subjects. Toluene concentrations of 1 mg/cu m (0.27 ppm) caused a distinct, statistically significant intensification of the electric potentials from the left temporal-occipital leads in all four subjects tested. A concentration of 0.6 mg/cu m (0.16 ppm) of toluene was subliminal with respect to its effect on the electrical activity of the cerebral cortex and imperceptible to the subjects with respect to odor. The author recommended this concentration as the maximum permissible one-time concentration of toluene. Further investigations are necessary to validate these findings.

Toluene has been found in the atmosphere of nuclear submarines at a concentration of 0.18 ppm, according to Chiantella et al [30] in 1966. The toluene originated from the paint solvents, mineral spirits, and diesel fuel used in the submarine.

Kowal-Gierczak et al [11] in 1969 reported on changes in the production of serum glycoproteins, seromucoid, and haptoglobulins in 53 women exposed during the workday to toluene vapor at average workroom concentrations of 0.25 mg/liter (67 ppm). In all the women the production of at least one of these substances was abnormal, the most frequent of which was shown by the glycoproteins (expressed by the concentration of sialic acid) followed by seromucoid and haptoglobulin levels. No clinical or laboratory evidence of liver changes or altered liver function was observed by the authors. They speculated that glycoprotein changes (expressed by the changes in the

concentration of blood sialic acid) might have reflected early changes in liver function. Exact environmental levels of toluene were not reported.

(c) Effects on Skin

(1) Local Effects

Toluene is an excellent fat solvent. Repeated or prolonged skin contact with liquid toluene will remove the natural lipids from the skin, causing drying, fissuring, and dermatitis. [31]

(2) Percutaneous Absorption

Dutkiewicz and Tyras [32] in 1968 reported the rate of absorption of liquid toluene and aqueous solutions of toluene through the skin of the hand and forearm of nine human volunteers. The quantity of toluene absorbed was the difference between the volume applied to the skin and the volume remaining 10 or 15 minutes after contact with the skin. For liquid toluene, the amounts absorbed ranged from 41 to 100 mg and the rates of absorption ranged from 14 to 23 mg/sq cm/hour. The concentration of toluene in the aqueous solutions used ranged from 189 to 607 mg/liter. The amounts of toluene absorbed percutaneously ranged from 52 to 206 mg, which corresponded to absorption rates of 160 to 600 µg/sq cm/hour, respectively. The quantity of toluene absorbed increased with increasing concentration of the hydrocarbon in the aqueous solution. The investigators used a measured quantity of toluene put in a watchglass, which was placed on the arm. After 10 minutes, they

washed the arm and measured chemically the residual toluene. The difference was attributed to percutaneous absorption. The authors believed that skin absorption from contact with liquid toluene should be taken into account in the evaluation of toluene exposure.

Piotrowski [33] in 1967 reported the skin absorption of toluene vapor in three male subjects exposed unclothed in a chamber to a toluene vapor concentration of 1,600 mg/cu m (427 ppm) for 8 hrs. The subjects were protected from inhalation of the toluene vapor by breathing uncontaminated air from outside the chamber through a respirator. Analysis of the urine samples collected at the end of the exposure period showed no increase in the excretion level of benzoic acid. The author concluded that "one can assume that the possibility of toluene vapor absorption through the skin will not exceed 5% of the amount absorbed in the same period of time through the respiratory tract." Gerarde [31] in 1960 stated that liquid toluene is poorly absorbed through the intact skin so that systemic intoxication by percutaneous absorption is highly improbable.

(d) Effects on the Eye

McLaughlin [34] in 1946 reported that two workers accidentally splashed with toluene suffered transient disturbances of the eyes, consisting of corneal damage and conjunctival irritation. Complete recovery resulted within 48 hours with no loss of vision.

Grant [35] reported on another worker splashed with a solution of stearic acid in toluene who experienced only transient epithelial

injury. He felt immediate, severe, burning pain and had involuntary blepharospasm. Although the eyes were not irrigated until 4-5 minutes after the accident, only moderate conjunctival hyperemia and corneal epithelial edema resulted, with complete return to normal in 2 days.

Carpenter et al [24] in 1944 reported mild eye irritation in volunteers exposed for 8 hours to 200 ppm toluene vapor.

A burning sensation in the eyes of one worker exposed to an average of 250 ppm toluene vapor (range, 210-300 ppm) was reported in 1971 by Capellini and Alessio. [16] The length of the exposure was not stated.

(e) Effects on Kidneys, Liver, and Lungs

O'Brien et al [36] in 1971 described a case of hepatorenal damage from chronic toluene vapor exposure in a 19-year-old male glue sniffer. Toluene caused serious but apparently reversible injury to the kidneys and liver after three years of glue sniffing. The principal component of the inhaled solvent was toluene (80% v/v), while other constituents were not mentioned. During the patient's hospitalization following a severe episode of toluene exposure, the concentration of toluene in the serum was found to be 180 ppm.

Greenburg et al [37] in 1942 found enlarged livers in 13 out of 61 painters (21%) exposed to toluene concentrations ranging from 100 to 1,100 ppm. Careful breathing zone sampling was performed and environmental levels were subgrouped into increments of approximately 100 ppm; however, only the number of workers exposed at each level of

toluene was presented and no comparison could be made between the incidence of liver enlargement and the degree of toluene exposure. The hepatomegaly was 3 times the frequency observed in the control group of 430 fur workers having no exposure to toluene.

Epidemiologic Studies

Banfer [12] reported in 1961 on his study of the effect of toluene containing 0.3% benzene on the peripheral blood elements (RBC, Hgb, WBC, and granulocytes) of 889 rotogravure printers and helpers employed for more than three years and compared the findings with those from 478 nonexposed subjects in the industry. Studies were made at 6-month intervals (3 months by law for workers under 18 years of age) for "several" years. The only environmental air levels reported consisted of samples taken on a single day from 5 different places in the machine room. Three samples showed the toluene concentration below 200 ppm, one value reached 200 ppm, and the fifth sample indicated 400 ppm. No effects on the formed elements of the blood were seen which were different from the controls. Also, 6 sternal biopsies were reported and no pathological changes of the bone marrow were found.

In 1942 von Oettingen et al [21,22] reported on the results of the exposure of three human subjects repeated 15 times over a three-month period to concentrations of toluene ranging from 50 to 800 ppm.

No abnormal changes were found in the peripheral blood leukocyte count.

In the same year Greenburg et al [37] studied a group of 61 workers who had been exposed to toluene and to no other toxic volatile solvents, so far as was known, for periods extending from 2 weeks to 5 years. The reported atmospheric concentrations ranged from 100 ppm to 1,100 ppm in increments of approximately 100 ppm (see Effects on Humans (e) above). As previously stated, comparisons could not be made between the observed toxic effects and the degree of toluene exposure. Although there was no record of severe illness, Greenburg et al found evidences of mild intoxication, enlarged livers, macrocytosis, mild depression of the erythrocyte level, absolute lymphocytosis, and elevation of the hemoglobin level and the mean corpuscular hemoglobin concentration. These investigators concluded that early chronic toluene intoxication in man is "best evidenced by hepatomegaly" (enlargement of the liver) "and macrocytosis" (enlarged red blood cells).

Forni et al [38] in 1971 investigated the changes in the chromosomes of peripheral blood lymphocytes in rotogravure plant workers exposed to toluene concentrations of 200 ppm throughout most of the work shift and to concentrations well above 200 ppm for very short periods. The group of workers exposed only to toluene for periods of 3 to 15 years showed a somewhat higher rate (0.8%) of unstable chromosome changes and of calculated breaks (0.83%) compared

with the controls (0.61 and 0.67%, respectively) but the differences were not statistically significant. The authors concluded that chronic inhalation of toluene vapor at concentrations in the order of 200 ppm did not significantly affect the rate of chromosome changes in peripheral blood lymphocytes but cautioned that it would not be appropriate to conclude from this study that prolonged exposure to toluene concentrations of about 200 ppm lacks toxic effects on chromosomes. The comparisons were made with a group of controls whose frequencies of chromosome changes were somewhat dispersed, suggesting to the authors that a different individual susceptibility to chromosome damage might exist from unknown environmental agents.

Animal Toxicity

Because of the close chemical similarities which exist between toluene and benzene, early animal investigations emphasized the comparative toxicity of these two hydrocarbons. [7,8,39-44] In general, toluene was considered to be more toxic than benzene in the production of narcosis. In 1903, Chassevant and Garnier [39] reported toluene to be more toxic in guinea pigs than benzene when the toluene was administered in single doses. The effects of toluene were reported to resemble those of benzene poisoning but were more delayed in onset. Lehmann [40] found that at equal atmospheric concentrations the order of increasing narcosis was benzene, xylene, and toluene. In contrast, Rambousek [42] in 1913 considered toluene to be less toxic

than benzene in dogs, cats, and rabbits. Toluene produced narcosis more slowly and recovery was not as rapid as with benzene. Also, convulsions or spasms were not observed in animals dosed with toluene.

Toluene has also been reported to exert toxic effects on the blood and blood-forming organs. [7-9] Selling [7] in 1911 reported that toluene produced an initial destruction of the white blood cells, but, compared with benzene, its action was feeble. The bone marrow readily compensated for any destructive effect on the blood cells. Ferguson et al [8] in 1933 concluded from their animal experiments and a review of the literature that the actions of benzene and toluene on the blood were very similar.

It is interesting that the toxicity of toluene was recognized as early as 1903 [39]; however, animal data from the earlier studies is of doubtful validity because prior to the 1940's in the United States, and even up to the mid-1950's in some other countries, the possibility of separate toxic effects for toluene and benzene was generally not recognized. Toluene was considered to possess myelotoxic properties similar to benzene, the difference being only one of degree. As previously pointed out (see Historical Reports), toluene frequently contained benzene in significant quantities [12,23] but its presence was seldom mentioned and attempts were rarely made to identify the benzene either qualitatively or quantitatively. Twenty-five years ago, commercial grades of toluene contained up to 15% benzene. [45] Thus, in any evaluation of reported myelotoxicity for toluene, the

benzene content, if known, is an important factor for consideration. More recent animal studies discussed below [46-49] have reported the lack of toluene toxicity on the blood and bone marrow.

(a) Inhalation

Batchelor [9] in 1927 reported exposing rats to toluene vapor ranging from 620 to 1,600 ppm daily for total exposure times of 18 to 120 hours. The reported effects indicate the toluene probably contained benzene but any quantity is unknown. With exposures to 1,600 ppm, the animals first developed instability and incoordination with evidence of mucous membrane irritation and light narcosis. By the third day, a mild twitching became evident, a general hypertonicity of the body musculature developed, the body temperature fell as much as 7 C; the animals became weak and died. With concentrations of 1,250 ppm, slight instability and incoordination appeared with signs of mucous membrane irritation. At concentrations of 1,100 ppm and 620 ppm the animals showed no signs of toxicity. In a little under half of the cases, even at the lowest concentration of 620 ppm, increases of from 4 to 13% appeared in the red cell count, and in five of the cases a reduction of 28% to 56% was found in the white cell count. With these findings, definite evidence of hyperplasia in the bone marrow was noted in the majority of cases.

In 1928, Smyth and Smyth [50] reported that guinea pigs were severely prostrated, but no deaths resulted after 18 daily 4-hour exposures to 1,250 ppm of toluene purified by repeated distillation to

produce a benzene-free product. Daily 4-hour exposures to 4,000 ppm caused fatalities in the exposed animals. Exposures to 1,000 ppm for 35 days resulted in no untoward effects. No mention was made of any blood changes.

In 1943 Svirbely et al [44] reported the acute toxicity of toluene vapor in mice. The toluene contained not more than 0.01% benzene. They found the minimum lethal concentration (MLC) to be 20 mg/liter (5,300 ppm) for an 8-hour exposure. They concluded that toluene has a greater acute toxicity and stronger narcotic action than benzene by inhalation and by other routes of administration. The principal pathological findings were pulmonary irritation, renal irritation, and evidence of cellular damage in the spleen. No evidence of blood damage was found. The authors stated that the short duration of exposures and early sacrificing of animals probably prevented the appearance of pathological changes, if any were to appear.

In 1955, Fabre et al [46] reported on two dogs exposed 8 hours/day, 6 days/week for 4 months to 7.5 mg/liter (2,000 ppm) of toluene vapor then to 10 mg/liter (2,660 ppm) for 2 additional months. During the last 2 months of the exposures, the animals manifested signs of central nervous system intoxication, incoordination, and paralysis of the hind legs. No hematological abnormalities (blood or bone marrow) were found in these animals. Microscopic examination of

the lungs, liver, kidney, heart, and spleen showed congestive changes. The toluene used was analyzed and contained less than 0.1% benzene.

Takeuchi [47] in 1969 described results of rats exposed to 200, 1,000, and 2,000 ppm toluene vapor 8 hours/day for 32 weeks. At the end of the exposure period no significant changes were found in body weight, leukocyte count, erythrocyte count, eosinophil count, and hemoglobin levels of the exposed animals as compared with the controls. The toluene used was analyzed at 99.9% purity with less than 0.2 ppm of benzene being present in the 2,000 ppm toluene concentration.

Taylor and Harris [51] in 1970 studied effects in mice exposed for 10 minutes to unspecified high concentrations of toluene-containing glue and toluene vapor and found evidence of cardiotoxicity. This was manifested as a slowing of the sinoatrial rate and prolongation of the P-R interval of the electrocardiogram. Neither the total composition of the glue nor the purity of the toluene was specified.

Furnas and Hine [52] in 1958 reported the effects of exposure to 5000, 10,000, and 20,000 ppm of chemically pure toluene vapor on the electroencephalogram (EEG) of rats having cortically implanted electrodes. The investigators failed to detect any abnormal EEG changes at 5,000 ppm for 20 minutes or 10,000 ppm for 40 minutes. At 20,000 ppm for an unspecified period of time, they found a spikelike

EEG activity which was assumed to be a manifestation of a convulsant effect.

(b) Subcutaneous Administration

In 1956, Gerarde [48] reported the effects on the blood, thymus, spleen, and bone marrow of albino rats of repeated subcutaneous doses of chemically pure (analyzed) toluene and other alkylbenzenes in 1.0 mg/kg doses. No abnormalities were found in the leukocyte count of the peripheral blood or in the total number of nucleated cells in the femoral bone marrow or the weight of the thymus glands or spleen of the animals dosed with toluene.

Speck and Moeschlin [49] in 1968 investigated the influence of "pure" toluene and xylene injected subcutaneously in rabbits on the synthesis of deoxyribonucleic acid (DNA) in bone marrow cells and the resulting peripheral blood cell count. No depression of bone marrow function was found as measured by the uptake of tritium-labeled thymidine. No decrease in the number of peripheral blood elements or variation in the differential counts was found. The doses of toluene administered were 300 mg/kg/day for 6 weeks or 700 mg/kg/day for up to 9 weeks. Rats given the same amounts of benzene developed aplastic anemia and autoradiography of the bone marrow revealed marked inhibition of DNA synthesis. The authors stated that their results "present a substantial argument for the lack of myelotoxicity of toluene and xylene."

(c) Effects on the Eye

Wolf et al [53] instilled 0.1 ml of undiluted toluene directly onto the right eye of rabbits. A barely perceptible irritation of the conjunctival membranes was noted within 1 to 4 hours in 3 of the 6 animals tested at 24, 48, and 72 hours after treatment. Examination of the cornea with sodium fluorescein solution revealed no evidence of even superficial necrosis in any of the treated eyes.

(d) Metabolism

In man and rabbits, about 20% of absorbed toluene is excreted unchanged by the lungs while about 80% is converted to benzoic acid and excreted in the urine as hippuric acid, the glycine conjugate. Bakke and Scheline [54] reported in 1970 that about 0.4 - 1.1% of the dose of toluene is hydroxylated to ortho- and para-cresol. Furthermore, small amounts of benzyl alcohol were detected in the hydrolyzed urine extracts. This suggested that benzyl alcohol may be formed as an intermediate step in the production of benzoic acid. Gerarde [31] found an increased urinary excretion of organic sulfate after dosing rats with large amounts of toluene subcutaneously. This indicated that an additional metabolic pathway was used to detoxify toluene if the concentrations in the blood were elevated.

Ikeda and Ohtsuji [55] reported in 1971 that following treatment of rats with phenobarbital, there was an increase in the rate of disappearance of toluene from the blood, a reduced sensitivity of the central nervous system, and a shortened sleeping time after injection

of toluene. These phenomena were explained by an enhanced hepatic metabolism induced by phenobarbital.

Abou-el-Makarem and co-workers [56] noted in 1967 that toluene metabolites are poorly excreted in the bile of rats. Less than 2% of a dose of toluene was found in the bile 24 hours after dosing.

Smith et al [57] reported in 1954 that about 18% of an oral dose of toluene was eliminated unchanged in the expired air.

Van Rees [58] in 1967 reported the influence of toluene on the metabolism of benzene in rats by measuring urinary phenol excretion after dosing the animals with toluene and benzene simultaneously. He found that toluene diminished the amount of phenol excreted during the first 8 hours after the administration of benzene. It appears that toluene inhibits the metabolism of benzene when the two compounds are administered simultaneously.

In summary, animal experiments indicate the main toxic effects of toluene to be upon the central nervous system. In general, daily 4- to 8-hour exposures of up to about 1,000 ppm of toluene produce little or no effect in different species of animals. At concentrations from 1,000 to about 2,000 ppm, the effects vary from those of instability, incoordination, and light narcosis to tremors, muscular hypertonicity, and general weakness. At high exposure levels, prostration and death occur. Regarding toluene effects on the blood and blood-forming organs by either inhalation or parenteral administration, purified toluene has been shown to produce no blood

abnormalities nor alteration of bone marrow function in various animal species, even at dose levels which produce marked central nervous system effects. When instilled onto the eye, undiluted toluene produces irritation of the conjunctiva; the effects are transient and no reports of corneal damage in animals have been found. Approximately 80% of absorbed toluene is metabolized to benzoic acid, conjugated, and excreted in the urine as hippuric acid. About 20% of absorbed toluene is excreted unchanged by the lungs.

Correlation of Exposure and Effect

In evaluating the effects of toluene exposures, care must be taken to assess the purity of the compound used in a given study. Benzene is a common contaminant of toluene [1,12,17] and, considering the unique effects of benzene on the hematopoietic system, investigators have frequently attributed effects to toluene which more correctly reflect the myelotoxic property of the benzene contaminant. [8,9]

A critical evaluation of the reports of experimental and occupational inhalation exposures to toluene has shown that the only documented exposures of human subjects to essentially pure toluene were those reported by von Oettingen et al. [21,22] These investigations used toluene which, on spectrophotometric analysis, was shown to contain not more than 0.01% benzene. This same high purity

source of toluene was used by Svirbely et al [44] in their studies of the toxicity of toluene in experimental animals.

The study of von Oettingen et al [21,22] involving the controlled 8-hour exposures of subjects to purified toluene produced mild fatigue, muscular weakness, impaired coordination, moderate dilation of the pupils, and paresthesias of the skin at the 200 ppm level. These same symptoms were intensified at 300 ppm whereas mental confusion was also noted as a result of exposure at 400 ppm. The narcotic effects became more severe at higher exposure levels. At 50 and 100 ppm, only mild to moderate fatigue and drowsiness were experienced by all 3 of the subjects toward the end of exposure periods. This same degree of tiredness was reported during exposure to a zero concentration of toluene. Observed variations in the pulse rate, diastolic blood pressure, and pulse pressure, respiratory rate and minute volume were within control limits. Thus, a 100 ppm concentration of toluene or below constituted a level of exposure which did not produce deleterious effects, whereas exposure to 200 ppm evoked the initial effects of narcosis. The experimental findings reported by von Oettingen et al [21,22] were supported by those of Wilson [23] in that exposures to concentrations less than 200 ppm were considered to be due chiefly to factors other than the toluene vapor. Exposures to toluene concentrations of 200 ppm and higher showed impairment of coordination and reaction time and momentary loss of

memory. Wilson [23] believed that the vapor concentration of toluene should never exceed 200 ppm.

In considering most reports of occupational exposures to toluene, a lack of information has been apparent about either the purity of the toluene or the accurate atmospheric concentrations of toluene with other solvent vapors at work sites. The following studies, [12,15,16,18] as relatively recent investigations, indicate the absence of the myelotoxic effects ascribed by earlier investigators to toluene. [8,9,23]

Parmeggiani and Sassi [15] in 1954 reported on their study of 11 paint and pharmaceutical workers exposed to atmospheric concentrations of toluene ranging from 200 to 800 ppm. From the results of their clinical study they concluded that toluene had no particular action on the bone marrow or on the other organs. The purity of the toluene was not reported.

In 1961 Banfer [12] stated that sufficient quantities of toluene containing up to 0.3% benzene had been available in Germany for industrial uses for only about 6 years. He made reference to a 1954 statement of Humperdinck in a trade union report that in Germany so-called purified toluene contained 15% benzene and 10% xylene. In the study of rotogravure printers and helpers exposed for more than 3 years to the vapors arising from printing inks containing toluene but no benzene detectable by chemical analysis of the inks and thinners, extensive blood studies and some bone marrow tests were performed

which failed to indicate any significant changes. Only 3 out of 889 blood tests (0.33%) were found with total white cell counts of less than 4,000/cu mm as compared with 1 out of 155 control subjects (0.64%) from other departments in the plant. The absolute lymphocyte count never exceeded 5,000 in either the printers or in the controls. The absolute number of granulocytes was not observed below a lower limit of 2,000 in any case. During the first 6 months of the study (in 1957), 5 sternal punctates from printers with white cell counts of less than 5,000 were evaluated at two hospital medical clinics and no pathological bone marrow changes were detected in any case. There were no evidences of damage to any of the blood cell elements of the printers and helpers throughout this study. Analysis of the atmosphere was limited to samples collected at 5 sites in the machine room on a particular day; the atmospheric toluene concentration was unspecified but below 200 ppm in 3 of the samples, at 200 ppm in the fourth, and 400 ppm in the fifth sample. Benzene and xylene were not detected in any of these samples by infrared spectrophotometry.

The finding of liver enlargement in painters exposed to toluene concentrations reported by Greenburg et al [37] in 1942 was considered important because liver enlargement had not been previously described. Neither clinical nor laboratory evidence of hepatic disease could be correlated with the hepatomegaly. The possibility was suggested that the liver enlargement might be compensatory in character rather than an indication of hepatic disease; however, the data were considered

insufficient to answer the question. Because of the incidence of enlarged livers and elevated mean corpuscular volume of the blood noted from the study, these 2 indexes were suggested to most likely reveal the early presence or absence of toluene toxicity. In addition to the elevated mean corpuscular volume, findings included mild depression of the erythrocyte level, elevation of the hemoglobin value, and lymphocytosis. Although the study had eliminated painters having known prior benzene exposure, the blood findings were so consistent with that of benzene poisoning that benzene contamination of the toluene vehicle in the paints cannot be overruled. Also, the liver enlargement could have been due to paint ingredients other than toluene. Volatile components such as ethyl alcohol, ethyl acetate, butyl alcohol, and petroleum naphtha were present in quantity in the lacquers, dopes, and brush washes used.

From the study of Pacseri and Emszt [18] in 1970, a decrease in the prothrombin level was reported but no other hematologic abnormalities were noted in printers exposed to atmospheric toluene concentrations ranging from 170 to 340 ppm.

Capellini and Alessio [16] in 1971 reported no changes in Hgb values, RBC, WBC, and platelet counts, or changes in liver function of workers exposed for several years to toluene vapor which ranged from 80 ppm to 300 ppm although findings of central nervous system toxicity were found from exposures to concentrations of 210 to 300 ppm. The benzene content of the toluene was not reported.

From the data of Gerarde [31] and Piotrowski and Tyras [33] it may be concluded that systemic intoxication by percutaneous absorption of toluene in the vapor phase is improbable. If skin contact with liquid toluene is experienced, probably by immersion of hands and arms, sufficient quantitites might be absorbed such that the percutaneous route may be important. [32]

Animal toxicity studies by von Oettingen et al [21,22] on the effects of inhaled toluene indicated that exposures up to 600 ppm produced increased hippuric acid excretion (up to 27%) in dogs and rats. At concentrations above 1,000 ppm, slight but statistically insignificant decreases in total leukocyte counts were reported in rats and guinea pigs after 30 exposures for 8 hours/day, 5 days/week. At 2,500 ppm, rats showed muscular incoordination, and complete narcosis at 5,000 ppm for 2 to 3 hours.

Furnas and Hine [52] reported in 1958 on the neurotoxicity of toluene to rats whose initial exposures to 5,000 ppm proved to be ineffective in producing central nervous system changes. Exposures were increased to 10,000 ppm for 20 minutes and then to 20,000 ppm for 1 hour. At the highest level, there was decreased mobility but no quivering or twitching and no hyperresponse to auditory stimuli. The source of the toluene was a chemically pure product provided by one of the petroleum companies.

Gerarde [48] in 1956 reported no abnormalities in the leukocyte count of the peripheral blood, in the total number of nucleated cells

in the femoral bone marrow, or in the weight of the thymus glands and spleens of rats given daily subcutaneous injections of 1 ml/kg of toluene in olive oil for 2 weeks. It was concluded that the attachment of an alkyl group to the aromatic ring, as in toluene, resulted in a loss of myelotoxicity which is characteristic of benzene.