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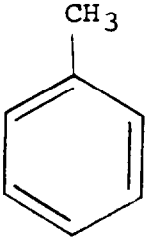
Final Report on the Safety Assessment of Toluene

Toluene has a wide variety of noncosmetic applications. However, the cosmetic use is limited to nail products at concentrations up to 50%. Toluene was practically nontoxic when given orally to rats; acute oral LD_{50} values ranged from 2.6 g/kg to 7.5 g/kg. Results of animal studies indicated that undiluted Toluene is a skin irritant. No skin irritation or sensitization was observed in subjects treated with cosmetic products containing 31–33% Toluene. No phototoxic or photoallergic reactions were noted in subjects treated with 25% or 30% Toluene. The sole cosmetic use of Toluene is in products intended to be applied directly to the nail; therefore, human skin exposure to this ingredient will be minimal under conditions of cosmetic use. On the basis of the available data and the limited user skin exposure from cosmetic products containing Toluene, it is concluded that this ingredient is safe for cosmetic use at the present practices of use and concentration.

CHEMISTRY

Definition and Structure

Toluene (CAS No. 108-88-3) is a homolog of benzene in which one hydrogen atom has been replaced by a methyl group[1]:



Other names for this cosmetic ingredient include: Methacide, Methylbenzene, Methylbenzol, Phenylmethane, Toluol, Antisal 1a, and NCI-C07272.[1-4]

Properties

Toluene is a clear, refractive liquid that has an aromatic odor similar to benzene. It is both volatile and flammable.[1,4-6] Toluene is miscible with water but is immiscible with alcohol, chloroform, ether, acetone, glacial acetic acid, carbon disulfide, ligroin, and benzene.[4,5,7-11]

The ultraviolet absorption spectrum of 300 gm/l of Toluene diluted in hexane was measured. The 300 gm/l concentration corresponded to the highest reported concentration of Toluene used in cosmetics. No significant absorption was noted above 300 nm.[12] Skin photosensitivity reactions of the immunological type occur with wavelengths greater than 320 nm.[13]

Additional chemical and physical data are presented in Table 1.

TABLE 1. Chemical and Physical Data for Toluene

Property	*Value*	*Reference*
Appearance	Colorless liquid	6, 7, 9, 10, 14
Molecular formula	$C_6H_5CH_3$	5, 10, 14
Molecular weight	92.13	1, 7, 9, 11, 14, 15
Boiling point	109–111°C	1, 4, 5, 7–11, 14–18
Melting point	−94.5–−95°C	1, 4, 5, 7, 10, 14, 17, 18
Freezing point	−93.2–−94.991°C	9, 11, 18
Specific gravity	0.8623 (15.6/15.6°C)	1
	0.865 (25/25°C)	8
	0.866	7, 10, 16
	0.866 (20/4°C)	5
	0.867 (20/20°C)	15
	0.87 (25°C)	18
Density	0.861–0.871 (20/20°C)	19
	0.8623 (g/ml at 25°C)	11
	0.866 (20/4°C)	4, 14
	0.869 (20/4°C)	1, 17
	0.871 (13°C)	9
	0.8869 (20/4°C)	18
Vapor density (air = 1)	3.1–3.2	1, 11, 14
Vapor pressure	22 mm Hg at 20°C	6
	22.4 mm Hg at 20°C	15
	28 mm Hg at 25°C	7
	28.4 mm Hg at 25°C	11
	28.7 torr at 25°C	1
	36.7 mm Hg at 30°C	14, 18
Percent in saturated air	3.94 (at 760 mm Hg and 22°C)	1, 11
Liquid viscosity	0.6 cp at 20°C	1
Viscosity (SYS)	<32.6	18
Solubility in water	0.05% (g/100 g water at 20°C)	6
Solubility in distilled water	534.8 ± 4.9 mg/l at 25°C	1, 11
Index of refraction	1.4941 (25°C)	11
	1.4961	17, 18

TABLE 1. (Continued)

Property	*Value*	*Reference*
	1.4967	4
	1.49693 (68°F)	1
	1.497 (20°C)	5
	1.496–1.497	16
Aniline equivalent	15	5
Evaporation rate (n-butylacetate = 1)	2.10	15
Flash point	Closed cup: 40°F (4.4°C)	1, 4–7, 11, 14, 18
Autoignition temperature	896°F	14
	997°F	5
	1025.6°F (552°C)	1
Flammable limits	1.4–6.7%	18
	1.17–7.10% by volume in air	1, 11
Lower explosive limit in air	1.27% by volume	5, 14
	1.3% by volume	6
Upper explosive limit in air	7% by volume	5, 14
	7.1% by volume	6
Residue after evaporation	<0.001%	16
Sulfur compounds (as S)	0.003%	16
Water	0.03%	16
Weight volume conversion in air at 25°C	1 ppm = 3.77 mg/m^3	1, 11, 18
	1 mg/m^3 = 0.265 ppm	1, 11
Specific dispersion	184.40	11
Log octanol–water partition coefficient	2.69	1
Partition coefficient (K_p) in vapor and water	5.14 at 20.06°C	11
Partition coefficient (K_D) in octanol and water	512 ± 22	11
Odor threshold		
Petrol-derived	2.14 ppm	1, 11
Coke-derived	4.68 ppm	1
Critical temperature	320.8°C	11
Critical pressure	40.0 atm	11
Critical density	0.29 g/ml	11
Critical compressibility factor (PV/RT)	0.26	11
Density of saturated air–vapor mixture	1.09 (at 760 mm and 26°C; air = 1)	1
Surface tension	28.53 dynes/cm at 20°C	1
Heat of vaporization	9.115 ± 0.50 Kcal/mol at 25°C	11
Heat of fusion	1.582 Kcal/mol	11
Heat of formation		11
Liquid	2.867 Kcal/mol	
Gas	11.950 Kcal/mol	
Entropy		11
Liquid	52.40 cal/(mol)(°C)	
Gas	76.44 ± 0.3 cal/(mol)(°C)	
Free energy of formation		11
Liquid	27.282 Kcal/mol	
Gas	29.228 Kcal/mol	

Method of Manufacture

Toluene is produced from three major sources: (1) petroleum refining processes, (2) as a byproduct of styrene production, and (3) as a byproduct of coke oven operations.[1]

Petroleum refining processes to isolate Toluene are of two types: catalytic reforming and pyrolytic cracking. Catalytic reforming involves the catalytic dehydrogenation of selected petroleum fractions that are rich in methylcyclohexane and other 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. Toluene is isolated from the reformate by distillation, followed by washing with sulfuric acid and redistillation. Pyrolytic cracking of petroleum yields olefins and pyrolysis gasoline. Toluene is isolated from pyrolysis gasoline by distillation, removal of olefins and diolefins, and redistillation.[1]

The synthesis of styrene by the dehydrogenation of ethylbenzene yields Toluene as a byproduct. The Toluene derived from this method is unsuitable for chemical or solvent use; however, it may be used for gasoline blending or as feed for the manufacture of benzene.[1]

The production of coke by the high-temperature carbonization of coal yields coal tar and crude light oil, both of which contain Toluene. The production of Toluene from distillation of coal tar is minimal; however, some Toluene is isolated from crude light oil.[1]

Toluene may be purified by various extraction and distillation processes (Eldeleauno SO_2 extraction, Udex separation, sulfolane extraction).[16] The various grades of Toluene (pure, commercial, industrial, nitration, solvent, scintillation) are usually defined in terms of boiling ranges.[5]

Impurities

Commercial Toluene may contain benzene as an impurity.[1] Therefore, all toxicological and clinical studies involving Toluene should specify the quality of Toluene used for experimentation. If benzene is present in the Toluene, it should be demonstrated that the observed biological effects are not wholly or partly due to benzene.

Reactivity

Toluene undergoes substitution reactions on the aliphatic side group ($-CH_3$) and on the benzene ring at the ortho and para positions. Such reactions may include halogenation, chloromethylation, nitration, acetylation, benzoylation, mercuration, sulfonation, brosylation, methylation, and isopropylation. These substitution reactions occur at a faster rate with Toluene than with benzene.[1,11]

Toluene is quite stable in air; however, Toluene can be oxidized with air under catalytic conditions to yield benzoic acid. In the presence of heat (or catalyst) and hydrogen, Toluene undergoes dealkylation to produce benzene. In aqueous media under the conditions of water chlorination, Toluene may be chlorinated, followed by subsequent hydrolysis to benzaldehyde. In the presence

of solvents (paraffins, naphthenics, and alcoholic hydrocarbons), Toluene can form azeotropes.[1,11] Toluene also may undergo photooxidation[20] and other photochemical reactions.[1,11] For a more complete description of the types of reactions that Toluene may undergo, the reader is referred to the reviews by the Syracuse Research Corp.[1] and the National Research Council.[11]

Toluene vapor was passed with nitrogen through a silica tube filled with porcelain chips at 700°C. Reported pyrolysis products included some known or suspected carcinogenic aromatic hydrocarbons (Table 2).[11,21]

Toluene is reported to be chemically stable and unreactive under conditions of use in cosmetic preparations.[16]

Analytical Methods

Gas chromatography may be used for the analytical determination of Toluene in blood.[22,23] Methods for the determination of hippuric acid, a major metabolite of Toluene excreted in the urine,* include thin-layer chromatography,[24] high-performance liquid chromatography,[25-27] gas chromatography,[28] and colorimetric methods.[26]

USE

Noncosmetic Use

Noncosmetic applications of Toluene include use as an indirect food additive, gasoline additive, ink thinner, nonclinical thermometer liquid, suspension solution for navigation instruments, extraction solvent for plant materials, and as a solvent for adhesives, rubbers, oils, gums, resins, vinyl organosols, paints, lacquers, and coatings. Toluene also is used as a starting material for the production of benzene, benzaldehyde, benzoic acid, benzoic acid derivatives, benzyl and benzoyl derivatives, saccharin, phenol, caprolactam, explosives (TNT), toluene-diisocyanates, polyurethane resins, detergents (toluene sulfonates), dyes and drugs.[4,5,7,9,18]

Consumer products containing Toluene are listed in Table 3. Indirect food additive uses of Toluene are presented in Table 4.

Cosmetic Use

Purpose in Cosmetics

Toluene is used in nitrocellulose nail lacquer† products as a diluent and solvent.[5,15,16] Toluene also is used in nail lacquers to reduce the "blushing phe-

*In the body, Toluene is mainly oxidized to benzoic acid, which after conjugation with glycine is eliminated as hippuric acid in the urine. Although hippuric acid is often used to determine human exposure to Toluene, hippuric acid may be formed from other metabolic processes besides Toluene metabolism.[1,11]

†The term "nail lacquer" is often used to denote nail enamel, nail polish, nail varnish, top coat, and base coat.[15,29,30]

TABLE 2. Pyrolysis Products of Toluene[11,21]

Compound	Weight, % of tar formed	Compound	Weight, % of tar formed
Anthracene	0.009	4,4'-Dimethylbiphenyl	0.99
1,2-Benzanthracene[a]	0.014	Biphenyl	0.27
Benzene[a]	2.54	Fluoranthene	Trace
3,4-Benzofluoranthene[a]	0.002	Fluorene	0.085
10,11-Benzofluoranthene[a]	Trace	Naphthalene	0.042
11,12-Benzofluoranthene[a]	Trace	Phenanthrene	0.12
1,2-Benzofluorene	0.007	Pyrene	Trace
2,3-Benzofluorene	0.017	Stilbene	0.44
1,2-Benzopyrene[a]	0.002	Styrene	0.11
3,4-Benzopyrene[a]	0.002	Toluene	93.5
Chrysene[a]	0.03	*p*-Xylene	0.05
Alkylchrysene	Trace	Resins and losses	0.7
Bibenzyl	1.00		

[a]Suspected carcinogen.[11]

nomenon," which occurs as a result of excessive and rapid evaporation of low boiling solvents.[16]

Product Formulation

Data submitted to the Food and Drug Administration (FDA) in 1984 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Toluene was used in 555 cosmetic products (Table 5). Products formulated with Toluene included nail basecoats and undercoats (32 products), nail polish and enamel (501 products), and "other manicuring preparations" (22 products). Reported concentrations of Toluene in these products ranged from >10–25% (448 products) to >25–50% (107 products).[31]

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators must conform to the format of concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations.[39] Since certain cosmetic ingredients are supplied to the formulator at less than 100% concentration, the concentration reported by the formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. In addition, the fact that data are only submitted within the framework of a concentration range provides opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

TABLE 3. Consumer Products Containing Toluene[1]

Product	*% Toluene content*
China cement, solvent type	20–30
Contact rubber cement	– – –
Microfilm cement, cotton base	27–30
Model cement	Up to 20–25
Plastic cement, polystyrene	24
Shoe cement	– – –
Tire repair, bonding compounds	>80
Paint brush cleaners	– – –
Stain, spot, lipstick, rust removers	– – –
Deicers, fuel antifreeze	30
Fabric dyes	≤60
Indelible inks	– – –
Marking inks	80–90
Stencil inks	40–60
Solvent and thinners	– – –

TABLE 4. Indirect Food Additive Uses for Toluene

Use	*Limitation*	*Reference*
Component of adhesives used in articles intended for packaging, transporting, or holding food	– – –	32
Component of resinous and polymeric coatings for polyolefin films intended for food contact	– – –	33
Component of paper and paperboard in contact with dry food	– – –	34
Component of acrylic and modified plastic acrylics intended for contact with food	– – –	35
Component of cellophane used for food packaging	Residue limit of 0.1% of weight of finished cellophane packaging	36
Component of polysulfide polymer–polyepoxy resins intended for contact with dry food	Use of Toluene limited to that of a solvent	37
Adjuvant substance in the manufacture of foamed plastics intended for food contact	Use of Toluene limited to that of a blowing agent adjuvant in polystyrene at a level not to exceed 0.35% by weight of finished polystyrene foam	38

TABLE 5. Product Formulation Data for Toluene[31]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)	
			>25–50	>10–25
Nail basecoats and undercoats	47	32	18	14
Nail polish and enamel	769	501	74	427
Other manicuring preparations	66	22	15	7
1984 TOTALS		555	107	448

As noted in Table 5, the major use of Toluene is in nail polish and enamel. Most nail polish formulations typically consist of the following constituents[15,29]:

1. A film former (such as nitrocellulose, ethylcellulose, cellulose acetate, cellulose acetate-butyrate, methacrylate polymers, vinyl polymers, or sucrose acetate isobutyrate)
2. Resins to improve gloss and adhesions of the films (such as toluenesulfonamide/formaldehyde resin)
3. Plasticizers to give the film pliability, minimize shrinkage, and soften and plasticize the film former (such as camphor, or dibutyl phthalate)
4. Solvents and diluents to stabilize viscosity and to keep the film former, resin, and plasticizer in a liquid state (such as esters, glycol ethers, nitroparaffins, alcohols, xylene, or toluene)*
5. Thixotropic agents to prevent rapid settling and caking of pigments, and to provide flow properties (clay modified by quaternary ammonium compounds)
6. Coloring substances (such as fluorescent and nonfluorescent dyes, guanine, or inorganic and organic pigments)

A typical nail lacquer might contain 12% nitrocellulose, 5% n-butyl phthalate, 5% aryl sulfonamide-formaldehyde resin, 1–3% camphor, and 1–2% pigment. The solvent may consist of approximately 35% toluene, 40% butyl acetate, 15% ethyl acetate, and 10% ethanol.[40]

Basecoat is formulated in a manner similar to nail polish, but it has a lower nonvolatile content (less nitrocellulose) and a lower viscosity because a thinner film is desirable. It does not contain pigments. Basecoat may contain hydrolyzed gelatin.[29]

*These solvents may cause false-positive irritant reactions if not permitted to evaporate before the nail lacquer is applied under a patch to the skin.[29]

Suggested formulae for various nail products containing Toluene have been described in the literature.[15,41-49]

Exposure to Toluene

Nail products formulated with Toluene can be applied several times a week over a period of many years. The fingernail, the toenail, the nail cuticle, and the skin surrounding the nail are the areas directly exposed to this cosmetic ingredient. Parts of the body that come in contact with the wet nail may also become exposed. Such areas may include the eye region, the face, neck and chest, the retroauricular zone, and the vulva.[29] During application of the cosmetic product to the nail, Toluene may come in contact with eyes and nasal mucosa as a result of evaporation from the formulation.

ABSORPTION

In mammals, Toluene is absorbed by the respiratory tract, skin, and gastrointestinal tract.[11] Since Toluene can readily penetrate many of the body's protective barriers, its absorption is likely passive and dependent on the concentration gradient, so that any physiological characteristic that modifies this gradient would be expected to alter the rate of absorption.[50]

Absorption Through the Lungs

Toluene is readily absorbed through the respiratory tracts of humans and experimental animals.[51-54] The amount of Toluene absorbed (uptake) is proportional to the concentration in inspired air, length of exposure, and pulmonary ventilation (respiratory minute volume).[55-57] Total uptake (absorption) can be estimated as follows:

$$\text{Uptake} = (0.5)(V_e)(C_i)(t)$$

where V_e is the respiratory minute volume in l/minute, C_i is the inspired concentration in mg/l, and t is the length of exposure in minutes.[57,58]

Because of the dependence on respiratory minute volume, the uptake of Toluene by humans is affected by the level of physical activity.[55-57,59-61] Mild to moderate exercise can double or triple the rate of uptake compared to that at rest.[57] An individual's content of adipose tissue generally has little or no effect on the uptake of Toluene (50–150 ppm) during exposures lasting 4 h or less.[55,57]

Toluene can be detected in human aterial blood as soon as 10 seconds after exposure by inhalation. The concentration of the compound in the arterial blood quickly increases during the first 10–15 minutes. After that, the Toluene concentration increases more slowly and reaches a fairly constant concentration after about 25 minutes; during this period, the retention is 75–80%. The rate of retention decreases as the individual approaches a state of equilibrium with respect to absorption, deposition, and excretion of Toluene and its metabolites. After 2–3 h of exposure by inhalation, the rate of retention falls to an almost con-

stant level—40–50%. The average rate of retention over a 5-h period is approximately 50%.[62] Once exposure has ended, Toluene concentration in alveolar airspaces, arterial blood, and venous blood decreases rapidly.[59]

The alveolar concentrations of Toluene in humans have been measured.[63] Results of 40 measurements with three different methods and 16 persons indicated that the average Toluene absorption by inhalation at 100 ppm exposure concentrations was approximately 1.6 mg/minute.

Mature "cross-bred dogs" were exposed by inhalation for 1 h to 700, 1500, and 2000 ppm Toluene. Pulmonary absorption of Toluene within 1 h of exposure was estimated to be 25, 56, and 74 mg/kg, respectively.[64]

Other studies pertaining to the respiratory absorption of Toluene by humans and experimental animals are reviewed in detail by the Syracuse Research Corp.[1]

Skin Absorption

Lung tissues are more permeable to chemicals than is the thicker and more histologically complex dermal tissue.[50] Although Toluene is absorbed less readily through the skin than through the respiratory tract, percutaneous absorption of liquid Toluene can be significant.[1]

Undiluted, liquid Toluene was reported to be absorbed at a rate of 14–23 mg/cm^2 per h through the skin of the forearms and hands when in direct contact with about 0.2 ml (170 mg) of Toluene for 10 or 15 minutes. When the hands and forearms were immersed for 1 h in aqueous solutions containing 180–600 mg of Toluene per liter, the rate of absorption was calculated to be 0.16–0.60 mg/cm^2 per h and increased with a corresponding increase in the concentration of Toluene. Analysis of the applied solutions before and after exposure indicated that appreciable amounts of Toluene were absorbed, ranging from 41 to 100 mg (23.7–57.7%) of the undiluted Toluene applied, and from 52 to 206 mg (27.5–35.9%) in the immersion study. These authors estimated that exposure of both hands to a saturated aqueous solution of Toluene for 1 h would be equivalent to inhalation exposure to an atmosphere containing 26.6 ppm for 8 h.[65,66]

The absorption and excretion kinetics for dermal and inhalation exposures to Toluene has been reported.[67] The investigators found that the maximum Toluene concentration in the blood of subjects who immersed one hand in liquid Toluene for 30 minutes was only 26% of the concentration in blood of subjects who inhaled 100 ppm Toluene vapor for 30 minutes. Toluene was depleted from the blood much more rapidly after termination of the inhalation exposure than after the dermal exposure.

There is significant absorption of Toluene through intact skin of volunteers with respiratory protection who immersed both hands in analytically pure Toluene for 10 minutes.[68] Results of analysis of exhaled air up to 3 h after exposure indicated an exponential decline in exhaled Toluene, ranging from greater than 4 ppm at 20 minutes postexposure to less than 1 ppm after 120 minutes. The authors calculated that between 2050 and 3370 mg of Toluene were absorbed in the 10-minute exposure.

Percutaneous absorption of Toluene vapor from the surrounding air was evaluated by Riihimaki and Pfaffli.[69] Volunteers wearing respiratory protection were exposed to 600 ppm Toluene for 3–5 h. The subjects remained at rest ex-

cept for three exercise periods, each lasting for 10 minutes, which occurred at 0.5, 1.5, and 2.5 h of exposure. The exercise was sufficient to stimulate perspiration and raise the skin temperature slightly, conditions that are thought to enhance percutaneous absorption. The concentration of Toluene in peripheral venous blood, measured at the end of 1, 2, and 3 h of exposure, was constant at approximately 100 μg/l. The observed percutaneous absorption was estimated to be about 0.9% of the amount that would be absorbed from the respiratory tract during a 3.5-h exposure at 600 ppm, assuming that 60% of the inhaled Toluene is retained and 16% of the absorbed dose is exhaled.

Subjects exposed dermally to 1600 mg/m^3 (427 ppm) Toluene for 8 h had no increase in urinary excretion of a metabolite (benzoic acid) of Toluene. It was estimated that absorption of Toluene through the skin would not exceed 5% of absorption through the respiratory tract under the same conditions.[70]

The concentration of Toluene in the blood or guinea pigs was monitored following application to the skin of 1.0 ml of the solvent. At 0.5 and 6 h, blood concentrations of Toluene were 1.1 and 0.60 μg/ml, respectively.[71]

The in vitro penetration of Toluene through excised rat skin was estimated by Tsuruta[72] as 8.50 nmol/minute per cm^2.

Gastrointestinal Absorption

Absorption of Toluene from the gastrointestinal tract is nearly complete.[1,62] In studies with rabbits, 76% of an oral dose of Toluene was excreted in the urine as hippuric acid, whereas 18% of the oral dose was expired through the lungs unchanged.[73]

In rats, the concentration of radioactivity in the blood reached a maximum 2 h after gastric intubation of 100 μl 4-^{3}H-Toluene in peanut oil, whereas maximum concentrations of Toluene in blood were reached 15–30 minutes after exposure by inhalation. Although absorption from the lungs following inhalation was more rapid, the relative radioactivities in various tissues were about equal after oral and inhalation exposure.[74]

METABOLISM

Toluene is metabolized in humans and animals by the pathways outlined in Figure 1. The major site for the metabolism of Toluene in both animals and humans is the liver, where the compound undergoes sidechain oxidation to benzoic acid. Some metabolism in other tissues also may occur.[50] Most of the benzoic acid is subsequently conjugated with glycine and excreted in the urine as hippuric acid, although a large amount of conjugation with glucuronic acid also occurs, resulting in urinary excretion of benzoylglucuronic acid.[75] Oxidation of the alkyl sidechain to carboxylic acid is typically a rapid and spontaneous sequence of reactions that is catalyzed by a microsomal NADH-dependent enzyme system in tissues with a high redox potential (i.e., liver and kidneys).[76] The formation of benzyl alcohol involves an NADH-dependent alcohol hydratase or monooxygenase (oxidase). The intermediate then is rapidly converted to benzaldehyde by an NAD-dependent alcohol dehydrogenase. The aldehyde is

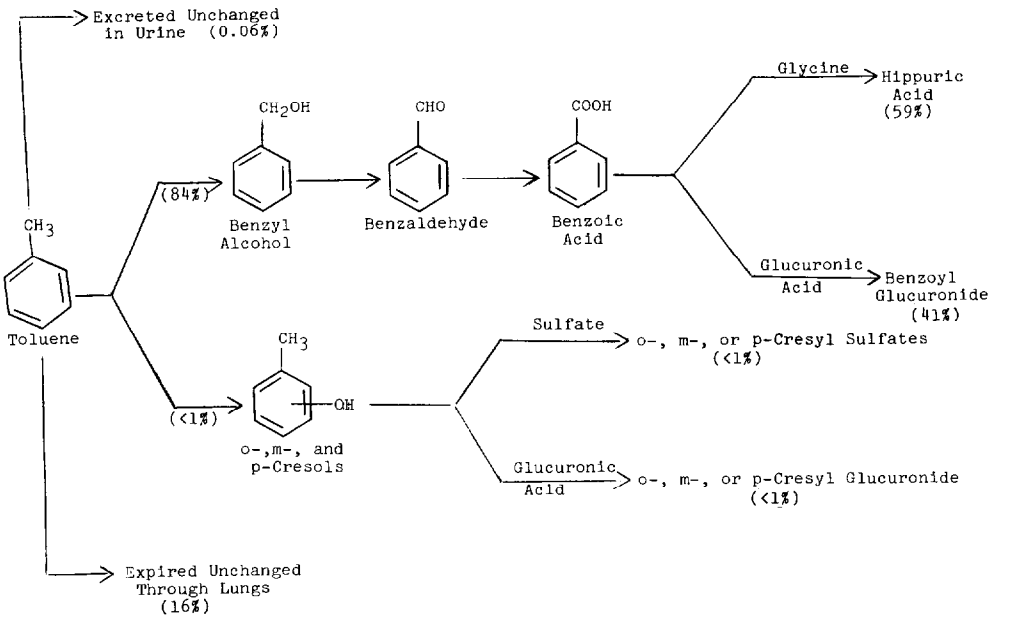


FIG. 1. Metabolism of Toluene in mammals.[50,77]

then oxidized to benzoic acid by means of an NAD-dependent aldehyde dehydrogenase.[50]

Minor amounts of Toluene undergo ring hydroxylation, probably via arene oxide intermediates, to form *o*-, *m*-, and *p*-cresol, which are excreted in the urine as sulfate or glucuronide conjugates.[78,79] The sidechain-oxidized compounds are rapidly conjugated with glucuronic acid or glycine to render them chemically inert.

DISTRIBUTION AND STORAGE

Upon entering the body, Toluene is rapidly distributed to all tissues by the circulatory system. Toluene is lipophilic, readily passes through cellular membranes, and accumulates primarily in those tissues with a high fat content. Toluene can be measured in many body tissues during and immediately after exposure. However, after exposure ends, the compound rapidly dissipates from tissues with a low fat content.[50] The quantity of Toluene absorbed by a tissue depends on the partition coefficient (tissue/blood), on the duration of exposure, and on the rate of metabolism. The rate of its absorption depends on the perfusion of the tissue and on the concentration gradient.[62]

As indicated by its partition coefficient, Toluene is highly soluble in lipid and sparingly soluble in water (Table 6). Hence, it is likely to associate with the lipid and lipoprotein components of the plasma—primarily those of the chylomicrons.[1,62] As measured in rabbits, the tissue/blood partition coefficients for fatty tissues are high (113 for adipose tissue and 35 for bone marrow); for other tissues, they range from 1 to 3. The solubility of Toluene in human blood, as indi-

TABLE 6. Partition Coefficient for Toluene at 37°C[1]

	Partition coefficient	*Reference*
Fluid/air or material/air		
Water	2.23	51
Oil, olive	492	
Blood, human	15.6	
Fat, human, peritoneal	1296	
Oil, olive	1380	54
Lard	1270	
Blood, human	15.6	
Blood, human	14.64	52, 53
Blood, rabbit	10.41	
Plasma, rabbit	16.99	
Tissue/blood (rabbit)		
Liver	2.58	52, 53
Kidney	1.54	
Brain	3.06	
Lung	1.92	
Heart	2.10	
Muscle, femoral	1.18	
Bone marrow, red[a]	35.43	
Fat, retroperitoneal	113.16	

[a]20% fat by volume.

cated by its partition coefficient (blood/air) is 14.64–15.6 (Table 6).

Table 7 presents the volumes and perfusion of four tissue groups in relation to the distribution coefficient (rabbit tissue), biological half-time, and distribution volumes, the distribution volume being:

$$V_{dist} = V \times g$$

and the biological half-time being:

$$t_{1/2} = V_{dist} \times (\ln 2)/V$$

The distribution volume indicates the amount of Toluene (expressed in millimoles) that can accumulate in the tissue at a blood concentration of 1 nmol/l. The largest quantity of Toluene accumulates in fatty tissue, with retention being positively dependent on an individual's amount of fat.[62] Carlsson and Ljungquist[60] estimated that the half-life of Toluene in human adipose tissue ranged from 0.5 to 2.7 days.

Results of animal studies indicate a relatively high distribution of Toluene on the stomach wall in the case of inhalation (partition coefficient stomach/blood = 4–5). No data are available on Toluene passing through the placenta.[62]

Mice were exposed to 3950 ppm Toluene (15 mg/l) for 3 h in an inhalation chamber. Concentrations of the compound reached 626 mg/kg in liver, 420 mg/kg in brain, and 200 mg/kg in blood by the end of the exposure.[80,81]

TABLE 7. Volumes and Perfusion of the Four Tissue Groups, Their Partition Coefficients, Biological Half-times and Distribution Volumes for Toluene[62]

Parameter	Symbol (unit)	Tissue group			
		Vessel rich[a]	Muscle[b]	Fat[c]	Vessel poor[d]
Volume in a 70-kg person	V (l)	6.0	33.0	14.5	12.5
Percentage of the minute volume of the heart to tissue group	%	75.0	18.1	5.4	1.5
Perfusion in a 70-kg person at a heart-minute volume of 7 l/minute	V (l/minute)	5.25	1.27	0.38	1.10
Tissue/blood distribution coefficient (rabbit)	λ	2.3	1.6	74.3	1.9
Tissue/blood distribution coefficient (human)	λ	---	---	81-83	---
Biological half-time	$t_{1/2}$ (h)	2 minutes	0.5	77	2.8
Distribution volume	V_{dist} (l)	14	53	1,189	23

[a]Vessel rich group—brain, heart, liver, intestines, kidneys, and endocrine glands.
[b]Muscle group—muscles and skin.
[c]Fat group—fatty tissue and yellow bone marrow.
[d]Vessel poor group—bones, connective tissue, lung tissue.

Rats were exposed by inhalation to 550 ppm methyl-^{14}C-Toluene for 1 h. Immediately after exposure, the amount of radioactivity in white adipose tissue was more than twice the amount in any other organ and more than six times that in brain tissues. Radioactivity in the fatty tissue continued to increase after the exposure had ended and was slightly higher 1 h after exposure than immediately after exposure. All other tissues examined had lower concentrations 1 h after the end of the exposure. Six hours after exposure, radioactivity had decreased to almost zero in the brain and adrenal tissues but was still measurable in the liver, kidneys, and fatty tissue.[59] It was suggested that the radioactivity in the kidneys and liver 6 h postexposure was likely due to Toluene metabolites and excretion products and not to the parent molecule.[50]

Pyykko et al.[74] exposed rats by inhalation to 4600 ppm 4-^{3}H-Toluene for 10 minutes. The concentration of radioactivity reached a maximum in most tissues within 15–30 minutes; the concentration in white adipose tissue reached a maximum 1 h after exposure. The highest concentration of radioactivity was found in white adipose tissue, followed in order of decreasing concentrations by brown adipose tissue, adrenal, stomach, liver, kidney, brain, blood, and bone marrow. Loss of radioactivity from adipose tissue and bone marrow occurred more slowly than the loss from other tissues.

Distribution of Toluene in tissues following oral exposure is similar to that for inhalation exposure. In rats administered 4-^{3}H-Toluene in a single gastric intubation, maximum radioactivity was reached 2–3 h in all tissues except white adipose tissue, where the maximum occurred 5 h after exposure.[74]

In mice given a single intraperitoneal injection of 0.20 mg/kg of Toluene, almost all of the radioactivity in the adipose tissues was a volatile compound that was probably unchanged Toluene. Approximately 70% of the radioactivity in the brain within 8 minutes after injection was present as a volatile material (again probably unchanged Toluene), whereas most of the radioactivity detected in the liver (64%) and kidneys (78%) was nonvolatile.[82]

A male teenager who died from sniffing glue had the following concentrations of Toluene in his tissues: heart blood, 11 mg/kg; liver, 47 mg/kg; brain, 44 mg/kg; kidneys, 39 mg/kg.[83,84]

It has been suggested that the dissipation of Toluene (radioactivity) from tissues appears to be related directly to the amount of vascularization and perfusion of the tissue as well as the presence of enzyme systems needed to metabolize the parent compound. These factors, along with the high partition coefficient for the compound, provide an explanation for the relatively fast uptake but slow release of Toluene in adipose tissue.[50]

EXCRETION

The major portion of inhaled or ingested Toluene is eliminated within 12 h of the end of exposure as free Toluene in expired air (9–18%) and as the metabolite hippuric acid in the urine (60–75%). Two percent or less of absorbed Toluene appears in the urine as benzylmercapturic acid and as cresol derivatives (glucuronides and sulfates). Metabolism of Toluene to benzylmercapturic acid suggests the formation of reactive intermediates that potentially could bind to tissue macromolecules,[1] but no such binding has been demonstrated. Small quantities of free Toluene and benzyl alcohol are excreted in the urine and feces.[62]

Srbova and Teisinger[85] reported that following inhalation exposure of humans to Toluene, approximately 16% of the absorbed compound was exhaled unchanged through the lungs, whereas 80% was oxidized to benzoic acid and excreted in the urine. A small amount (0.6%) of absorbed Toluene was excreted in the urine unchanged. Von Oettingen et al.[86] exposed humans by inhalation to 50 and 800 ppm Toluene for 8 h. Urinary metabolites consisted of approximately 59% hippuric acid and 41% benzoyl glucuronide. Urinary excretion of metabolites increased with the concentration of Toluene and was essentially complete within 14 h. In other studies, small amounts of free Toluene were detected in the expired air of humans exposed dermally to either 200 mg of liquid Toluene[65] or 600 ppm Toluene vapor for 3.5 h.[69] In the latter investigation, samples of exhaled air had detectable quantities of Toluene for at least 4 h after the exposure ended, but no Toluene was detected in samples 20 h after exposure. Respiratory protection was used in this study to preclude inhalation of the vapor.[69]

A commonly used test to determine occupational exposure to Toluene is based on the excretion of hippuric acid and/or o-cresol in the urine.[87–92]

TOXICOLOGY

Acute Oral Toxicity

Reported acute oral LD^{50} values for Toluene in rats range from 2.6 g/kg to 7.53 g/kg (Table 8), making this solvent "practically nontoxic" according to the classification system of Hodge and Sterner.[93]

In two separate studies, nail products containing Toluene were evaluated for acute toxicity. In the first evaluation, a nail basecoat containing 33.2% Toluene was administered by stomach tube to five female, albino rats. Animals were observed for 7 days following the single 15.0 g/kg dose. No deaths were reported, and all rats had normal weight gains.[99] In the second study, a nail polish formulated with 33% Toluene was given by gavage to 10 Sprague-Dawley rats (5 males, 5 females). No deaths or "toxic effects" were observed following the single 5 ml/kg dose.[100]

Acute Effects from Intraperitoneal Injection

Mortality is produced in rats and mice by a single injection of Toluene in the dose range of 0.8 to 1.7 g/kg.[101-103]

Koga and Ohmiya[82] estimated an IP LD_{50} for Toluene in male mice of 1.15 g/kg; respiratory failure was the primary cause of death in these animals. An IP LD_{50} of 1.64 g/kg was reported for female mice by Ikeda and Ohtsuji.[104]

In rats, a single IP Toluene dose of 0.65 g/kg produced apathy, whereas 1.5–1.7 g/kg produced death from respiratory failure.[103] A single 1.7 g/kg dose also was lethal to rats, mice,[102] and guinea pigs.[105]

Savolainen[106] observed that the concentration of radioactivity in the CNS was highest in the cerebrum following IP injection of methyl ^{14}C-Toluene; radioactivity was not detected in the CNS 24 h after the single exposure.

Acute Effects from Intravenous Injection

Intravenous injection of 0.2 ml Toluene/kg (0.17 g/kg) produced 100% mortality in 15 rabbits.[107]

TABLE 8. Acute Oral Toxicity

Animal tested	*Oral LD_{50}*	*Reference*
Rats	7.53 g/kg	94, 95
Wistar adult rats	7.0 g/kg	96
Sprague-Dawley rats	5.58 g/kg	97
Sprague-Dawley rats		98
14-day old	2.6 g/kg[a]	
Young adult	5.5 g/kg[a]	
Mature adult	6.4 g/kg[a]	

[a]Analytical grade Toluene.

Acute and Subchronic Effects from Subcutaneous Injection

In acute studies, a single subcutaneous injection of 1.1–1.25 g/kg and 4.3–8.7 g/kg Toluene produced mortality in rats and mice, respectively.[102,103] Braier[107] reported that a single 4 ml Toluene/kg dose injected into rabbits produced marked transient granulopenia within 24 h and marked granulocytosis and ensuing death in all animals by the end of the second day. A small area of induration was seen at the injection site.

The subchronic effects of Toluene in rats, guinea pigs, and rabbits were also evaluated. Toluene was administered to rats by subcutaneous injection at a dose of 1 ml/kg for 21 days. Treated animals had slight induration at the injection site, decreased body weights, transient decrease in erythrocyte and leukocyte counts, hyperplasia of bone marrow, moderate hyperplasia of Malpighian corpuscles in the spleen, marked pigmentation of the spleen, focal necrosis of the liver, and slight cloudy swelling in the kidneys. No lesions of the heart, testes, or lungs were noted.[103]

Guinea pigs were given Toluene by subcutaneous injection at a dose of 0.25 ml/day for 30–70 days. Necrosis developed at the injection site. Polypnea and convulsions occurred during the last days of survival (survival period: 30–70 days). Hemorrhagic, hyperemic, and degenerative changes in lungs, kidneys, adrenal glands, liver, and spleen also were noted.[108]

Subcutaneous injection of 1 ml/kg of Toluene for 6 days produced transient granulopenia and granulocytosis in rabbits; no change in the bone marrow was observed.[107]

Dermal Toxicity

The acute dermal LD_{50} of Toluene (single dose) in rabbits was 14.1 ml/kg.[94,95] In another study, a percutaneous dose of 1.732 g/kg failed to kill any guinea pigs.[105]

Increased local capillary permeability in rabbits[109] and hemoglobinuria in rats[110] were observed following application of Toluene to the skin. Application to the skin of 1 ml of Toluene for 16 h produced karyopyknosis, karyolysis, spongiosis, perinuclear edema, and cellular infiltration in the dermis; no hepatic or renal damage was noted.[111] Reduced weight gain was noted for 1–3 weeks in guinea pigs treated percutaneously with 2.0 ml of Toluene. However, body weights were comparable to those of control animals at week 4.[105]

Skin Irritation

Undiluted Toluene produced slight to moderate skin irritation in rabbits when tested by four different procedures. Slight skin necrosis also was observed in one study in which Toluene was repeatedly applied to rabbit skin over a 2–4-week period (Table 9).

Two nail products were evaluated in separate studies for skin irritation. In the first evaluation, a nail basecoat (0.5 ml) containing 33.2% Toluene was applied under an occlusive patch to the clipped skin of each of nine albino rabbits. After 24 h, the patches were removed and the treated sites graded for erythema and edema. Five of the nine rabbits developed skin reactions following the single

TABLE 9. Skin Irritation

Material tested	*Animals tested*	*Method*	*Results*	*Reference*
Undiluted Toluene	6 male albino rabbits	*Journal Officiel de la Republique Francaise*[112,113]: 0.5 ml applied under occlusive dressing to clipped skin (intact and abraded) for 23 h	Moderate skin irritation (PII = 3.25/8.0)	117
Undiluted Toluene	6 male albino rabbits	Association Francaise de Normalisation[114]: 0.5 ml applied under occlusive dressing to clipped skin (intact and abraded) for 4 h	Moderate skin irritation (PII = 3.42/8.0)	117
Undiluted Toluene	3 albino rabbits	Organisation for Economic Cooperation and Development[115]: 0.5 ml applied under occlusive and semiocclusive dressing to intact clipped skin for 4 h	Slight skin irritation (occlusive dressing: PII = 2.94/8.0) Slight skin irritation (semiocclusive dressing: PII = 2.13/8.0)	117
Undiluted Toluene	Unspecified number of albino rabbits	Adams et al.[116]: 10–20 applications were repeatedly made to ear and shaved abdomen over a 2–4-week period	Slight to moderate skin irritation and slight skin necrosis	96

exposure. Of these five, three rabbits had minimal erythema and two had moderate erythema. The remaining four animals had no skin irritation.[118] It was not reported whether the product was applied with or without solvents.

In the second study, a nail polish containing 33% Toluene was applied "dry" under a semiocclusive patch (open) to the clipped, intact skin of six female, albino rabbits. Applications of the product (0.5 ml) were made every other day for a total of three exposures. Skin reactions were evaluated both 24 and 48 h after each application. No skin irritation was observed.[119]

Ocular Irritation

Results of studies with rabbits indicate that Toluene is an ocular irritant. However, the results have varied as to the degree of irritation produced. These studies are summarized below and in Table 10.

The ocular irritation potential of undiluted Toluene was assessed in male albino rabbits. A single 0.1 ml dose of the test material was instilled into one eye of each of 12 rabbits; the untreated eye served as control. Treated and control eyes were either given no rinse (6 rabbits) or were rinsed with a solution of boric acid, sodium borate, sodium chloride, and phenylmercury borate 30 seconds after instillation (6 rabbits). Lesions of the conjunctiva, iris, and cornea were scored over a 7-day observation period by means of the numerical system of Kay and Calandra.[120] For rabbits given no rinse, the highest irritation index at any one evaluation was 22.67 (max = 110), indicating that Toluene was an "irritant." For

TABLE 10. Ocular Irritation

Material tested	*Animals tested*	*Method*	*Results*	*Reference*
Undiluted Toluene	12 albino rabbits	Single application to one eye of each rabbit; eye received either no rinse (6 rabbits) or a borate solution rinse (6 rabbits)	No rinse: "irritant" With rinse: "slight irritant"	121
Undiluted Toluene	Unspecified number of albino rabbits	2 drops instilled into right eye with no further treatment; eyes observed up to 7 days thereafter	Slight irritation of conjunctival membrane; no corneal injury	96
"Excess of 15%" Toluene in propylene glycol, water, and/or deodorized kerosene	5 albino rabbits	Single application of 0.005 ml to cornea; no water rinse given	Severe ocular irritation	94, 122

rabbits given an eye rinse, the highest irritation index was 13.33, indicating that the test substance was a "slight irritant".[121]

Slight irritation of conjunctival membranes was observed after application of 2 drops of undiluted Toluene to the right eye of an unspecified number of albino rabbits. No corneal injury was noted.[96]

In a range-finding study with rabbits, a single 0.005 ml dose of Toluene produced severe ocular irritation when instilled into the cornea at concentrations in excess of 15%. Vehicles used included propylene glycol, water, and deodorized kerosene. A total of five eyes were treated; the treated eyes received no water rinse.[122] Similar results were reported by Smyth et al.[94]

A nail polish formulated with 33% Toluene was evaluated for ocular irritation. The product was instilled in a single 0.1 ml dose into the conjunctival sac of one eye of each of nine albino rabbits. The untreated eyes served as the control. Three of the nine female rabbits received no water rinse after treatment, a second group of three rabbits received a water rinse in the treated eye 4 seconds after exposure, and a third group of three received a water rinse in the treated eye 2 seconds after product instillation. In the "no rinse group," all three animals developed erythema of the conjunctivae, which cleared by day 9. One of these rabbits also developed chemosis of the conjunctiva, which cleared by day 6. In the "4 second group," one of three rabbits had erythema and chemosis, which cleared by day 7. No rabbits in the "2 second group" developed irritation. The investigator concluded that the nail polish was a "mild eye irritant."[123]

Acute Inhalation Toxicity

In studies with mice, acute inhalation LD_{50} values for Toluene of 5320 ppm (<0.1% benzene) and 6942 ppm (99.5% purity) were estimated by Svirbely et al.[124] and Bonnet et al.,[125] respectively. The exposure period was 6–7 h.

Inhalation of 4000 ppm technical grade Toluene for 4 h produced death in one of six rats.[94,95] Inhalation of 4000 ppm Toluene (purified by distillation) for 4 h was lethal to two of three guinea pigs within a few days; the third animal was severely prostrated.[126] Inhalation of 55,000 ppm was lethal to six rabbits within 24–62 minutes.[127] Von Oettingin et al.[128] reported that inhalation of 850 ppm Toluene (0.01% benzene) for 1 h by six dogs produced an increase in respiratory rate and a decrease in respiratory volume.

RD_{50} values for Toluene of 5300 ppm[129] and 3373 ppm[130] were estimated for Swiss mice. The RD_{50} is the concentration necessary to depress the respiratory rate by 50%.

Male Sprague-Dawley rats were exposed by inhalation to 0 or 2000 ppm Toluene for 48 h. Compared to nontreated controls, the treated rats had impaired psychomotor performance, elevated blood glucose, elevated serum alanine aminotransferase and aspartate aminotransferase, increased packed cell volume, and decreased body weight.[131]

Inhalation of 4000 ppm Toluene (99.9% pure) for 3 h by male ICR mice had no effect on blood lactate dehydrogenase activity. However, a significant increase in serum glutamic-oxaloacetic transaminase activity was observed 24 h after the single exposure.[132]

Mucous membrane irritation and incoordination were noted in rats exposed by inhalation to 1250 ppm Toluene for 18–20 h.[103]

Subchronic Inhalation Toxicity

Progressive symptoms typically observed in experimental animals after subchronic inhalation exposure to increasingly higher concentrations of Toluene include irritation of the mucous membrane, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death.[1] Results of selected subchronic inhalation studies are summarized in Table 11.

Chronic Oral and Inhalation Toxicity

Results of four studies indicated no major toxicological effects in rats following chronic oral or inhalation exposure to Toluene. However, toxic effects were observed in a fifth study in which dogs were exposed by inhalation to $\geq$2000 ppm Toluene for 6 months. These studies are discussed below.

The chronic oral toxicity of Toluene was assessed by Wolf et al.[96] Three groups of 10 female Wistar rats were given an olive oil solution of Toluene emulsified with a 5–10% aqueous solution of acacia by oral intubation. Toluene doses were either 118, 354, or 590 mg/kg per day 5 days a week for 6 months (193 days). The total volume of the test solution administered daily was never greater than 2–3 ml. A group of 20 rats served as controls and were given doses of 2.5 ml of olive oil emulsified in the acacia solution. No adverse effects were noted with respect to general appearance, behavior, growth, body and organ weights, blood urea nitrogen, total erythrocyte and leukocyte counts, differential leukocyte counts, or hemoglobin concentration. At necropsy and microscopic examination, no treatment-related changes were found in lungs, heart, liver, kid-

ney, spleen, testes, adrenals, pancreas, and femoral bone marrow. No deaths were reported.

The chronic toxicity of Toluene (>99.98%) by inhalation was assessed in Fischer-344 rats. Four groups of 240 animals (120 males and 120 females per group) were exposed for 6 hours/day, 5 days/week, for up to 24 months to Toluene concentrations of 0, 30, 100, or 300 ppm. The calculated time-weighted average concentrations for the 24 months of exposure were 0.0, 30.1, 99.7, and 299.0 ppm, respectively. Randomly selected rats were killed after 6, 12, or 18 months to determine progression of toxic effects; all remaining animals were killed for study after 24 months. Males in the Toluene treatment groups were heavier than control males throughout the study. However, there was no dose–response relationship demonstrable within the treatment groups. The 300 ppm group had increased mean corpuscular hemoglobin concentration. Female rats exposed to 100 and 300 ppm for 24 months had reduced packed cell volumes. Other hematological parameters were not significantly different from controls. There were no significant differences among control and treated groups with respect to clinical appearance, blood chemistries (BUN, SGPT, SAP), urinalysis (pH, specific gravity, microscopic and biochemical constituents), absolute organ weights (brain, heart, kidneys, liver, lungs, gonads), or gross and microscopic lesions. The authors concluded that Toluene caused no chronic toxicity or oncogenicity at the concentrations tested. A partial list of the large battery of tissues and organs examined included brain, pituitary, heart, lungs, esophagus, adrenals, lymph nodes, kidney, bladder, ovaries, stomach, thymus, skin, mammary gland, bone marrow, nasal turbinate, eyes, and testes.[138] The National Toxicology Program has determined that this study was inadequate for carcinogenicity evaluation. The several factors that preclude a definite conclusion of noncarcinogenicity are outlined by the Syracuse Research Corp.[1]

Groups of 15 Sprague-Dawley rats of each sex were exposed by inhalation to Toluene concentrations of 1, 100, or 1481 ppm Toluene 6 h per day, 5 days per week for 26 weeks. Initially, the high-dose group was exposed to 2000 ppm, but the dose was lowered to 1500 ppm after seven exposures because CNS depression was apparent. A battery of blood and clinical chemistry tests (BUN, SGPT, SAP, glucose), urinalysis, and neurohistological examination of tissue were performed. The only treatment-related effects observed were an increased incidence of dry rales and staining of the anogenital fur in the high-dose treatment group. Significant changes in the values obtained for tests of blood and urine were not found, with the exception of a dose-related decrease in blood glucose values and a dose-related increase in SGPT activities in female rats. Body weights were significantly greater in the high-dose male rats than in the control rats, but this was not considered a toxic effect. Treatment-related neurohistopathological changes were not found.[139]

Exposure of 24 OFA rats to 1000 ppm Toluene by inhalation for 6 h a day, 5 days a week for 6 months produced no treatment-related effects. No differences were observed between treated and nontreated control rats with respect to body weight gain, hematological parameters (RBC and WBC counts, hemoglobin, mean corpuscular volume, packed cell volume, sedimentation rate), and tissue histology (lungs, liver, spleen, kidneys, gonads, and other unspecified "principal" organs).[140]

TABLE 11. Subchronic Inhalation of Toluene

Animal	*Toluene tested*	*Results*	*Reference*
Male Sprague-Dawley rat	1000 ppm 8 h/d × 7 d × 13 wk	Retarded weight gain during exposure; however, weight gain similar to controls by end of experiment. Hematocrit values, psychomotor performance, blood glucose, serum ALAT, and serum ASAT also similar to controls	131
Rat	1600 ppm 18 to 20 h/d × 3 d	Mild twitching; drop in body temperature; death. Histology: severe cloudy swelling of kidneys; no effect on liver, heart, or testes	103
Rat	3184 ppm 4 h/d × 30 d	Increased activities of SGOT, SGPT, and increased concentrations of β-lipoproteins. Decreased activities of catalase, peroxidase, and decreased concentrations of glutathione and total cholesterol	133
Rat	2500 ppm or 5000 ppm 7 h/d × 5 d × 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; reduced leukocyte count after each exposure; pulmonary lesions; casts in renal tubules in all rats within 2 wk of exposure	128
Rat	5 d/wk × 15 wk	Cytochrome P-450, ethoxycoumarin o-deethylase increased; UDP glucuronsyltransferase increased only at end of exposure	128
Male Sprague-Dawley rat	7 consecutive cycles daily, 5 d/wk × 8 wk: each cycle, 10 min of 12,000 ppm followed by 20 min toluene-free recovery interval	Depression of body weight gain; increased SGOT, serum LDH activities; no effect on BUN levels. Depression of kidney, brain, and lung weights. No lesions of brain, lung, liver, heart, or kidney; no indication of hepatic lipid vacuolation	132
CFY rat (both sexes)	265 ppm 6 h/d × 5 d/wk for 1, 3 or 6 mo	Bromsulphthalein retention decreased; cytochrome P-450 increased independent of period of exposure; SGOT and SGPT activities unaffected	134
CFY rat (both sexes)	929 ppm 8 h/d × 5 d/wk for 1 wk, 6 wk, or 6 mo	Cytochrome P-450 increased independent of exposure period; no effect on SGOT or SGPT; aniline hydroxylase and aminopyrine N-demethylase activity increased; cytochrome b_5 concentrations increased. Dilation of cisternae of rough endoplasmic reticulum; increase of autophagous bodies, which was dose and time dependent; retarded growth of females but not males; glycogen content decreased	134
Male CFY rat	398, 796, 1592 ppm 8 h/d × 5 d/wk × 4 wk	Cytochrome P-450 increased with dose	134
Rat, guinea pig, dog, monkey	107 ppm continuously for 90 d, or 1085 ppm 8 h/d, 5 d/wk × 6 wk	No effect on leukocytes, hemoglobin, or packed cell volume. No lesions of liver, kidney, lungs, spleen or heart; no effect on brain or spinal cord of dogs and monkeys	135

Male ICR mice	7 consecutive cycles daily, 5 d/wk × 8 wk: each cycle, 10 min of 12,000 ppm followed by 20 min toluene-free recovery interval	Depression of body weight gain; no effect on serum LDH; decreased BUN concentrations; SGOT activities increased (not significantly). Depression of kidney, brain and lung weights. Histology: no lesions of brain, lung, liver, heart or kidneys; no indication of *hepatic lipid vacuolation*	132
Male ICR mice	4000 ppm for 3 h/d × 1, 3, or 5 d	SGOT activities increased after 1 and 3 days of treatment; no effect 24 h after 5 d	132
Male ICR mice	4000 ppm for 3 h/d × 5 d/wk × 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, or lung weights. SGOT activity increased after 4 wk of exposure and 2 wk postexposure, but not after 2 wk or 8 wk of exposure; no change in BUN. No lesions of heart, lung, kidney, brain or liver	132
Mice	1, 10, 100, or 1000 ppm 6 h/d × 20 d	No effect on body weight; 1 and 10 ppm produced increase in RBC count on 10th day, recovery on day 20; 100 ppm and 1000 ppm produced decrease of RBC count; all doses produced increase (40–70%) of WBC count on day 10, recovery for all doses except 1000 ppm; 10 ppm to 1000 ppm produced slight decrease in density of bone marrow cells and in megakaryocytes and red cell elements; 1000 ppm produced slight hypoplasia of red cell elements, slight to moderate disturbance in maturity of neutrophils and thrombocytes, moderate increase of reticulocytes. No lesions in brain, lung, liver, spleen, or kidney	136
Guinea pig	1250 ppm 4 h/d × 6 d/wk (18 exposures)	*Prostration, marked liver and renal degeneration, marked pulmonary* inflammation	126
Guinea pig	1000 ppm 4 h/d × 6 d/wk (35 exposures)	Slight toxic degeneration in liver and kidney	126
Dogs (2 experimental, 1 control)	2000 ppm 8 h/d × 6 d/wk × 4 mo, and then 2660 ppm 8 h/d, 6 d/wk × 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	137
Dogs	200, 400, or 600 ppm: three 8 h exposures for 1 wk then five 7 h exposures for 1 wk and finally 850 ppm for 1 h	No effect on circulation or spinal pressure; increase of respiratory rate, small increase of minute volume, decrease of respiratory volume	128
Dogs	400 ppm 7 h/d × 5 d	Moderate temporary lymphocytosis	128

h, hour; d, day; wk, week; SGOT, serum glutamic oxalacetic transaminase; SPGT, serum glutamic pyruvic transaminase; ALAT, serum alanine aminotransferase; ASAT, serum aspartate aminotransferase; WBC, white blood cell; RBC, red blood cell; UDP, uridine 5′-phosphate; BUN, blood urea nitrogen; mo, *month.*

Two dogs were exposed 8 h a day, 6 days a week for 4 months to 2000 ppm Toluene. Following this 4-month exposure, the Toluene concentration was increased to 2660 ppm for 8 h a day, 6 days a week for an additional 2 months (6 months total exposure). Slight nasal and ocular irritation occurred at the lower concentration, and motor incoordination that preceded paralysis of the extremities occurred in the terminal phase. Death occurred on days 179 and 180, respectively. There was no effect on gain in body weight, on the bone marrow, or on the adrenal, thyroid, or pituitary glands. Congestion in the lungs, hemorrhage in the liver, a decrease of lymphoid follicles, and hemosiderosis in the spleen were observed. Glomeruli of the kidneys were hyperemic, and albumin was found in the urine.[137]

Genotoxicity

Toluene was negative for mutagenicity in a battery of microbial, mammalian cell, and whole organism test systems. However, there were several reports of increased chromosome aberrations in the bone marrow of rats exposed by inhalation or by subcutaneous injection to Toluene,[141-143] as well as reports of increased sister-chromatid exchanges and chromosome aberrations in the lymphocytes of workers chronically exposed to Toluene.[144,145] These studies are summarized below.

No mutagenicity was observed when Toluene was tested in the Ames *Salmonella* assay with strains TA1535, TA1537, TA1538, TA98, and TA100 or in the *Escherichia coli* WP2 reversion to trp^+ prototrophy assay (Table 12).[146-150] These reverse mutation assays were all performed in the presence and absence of Aroclor 1254-induced rat liver hemogenate (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.[150] Snow et al.[150] conducted plate incorporation assays in sealed plastic bags and chambers as well as vapor exposures in desiccators to prevent excessive evaporation.

Toluene was tested with and without metabolic activation in *S. cerevisiae* for the (1) induction of reversions to isoleucine independence in strain D7,[147] (2) induction of mitotic gene conversion to tryptophan independence in strains D4 and D7,[146] and (3) induction of mitotic crossing-over at the ade2 locus in strain D7.[147] The compound did not produce any positive mutagenic response in any of these assays (Table 12).

The photochemical formation of mutagens from various aromatic compounds was studied by Suzuki et al.[151] An aqueous solution containing Toluene and an aqueous nitrate solution containing Toluene were both irradiated with a 100 W high pressure mercury lamp (250–577 nm). The reaction mixtures were then evaluated for mutagenicity in a modified Ames assay, using *S. typhimurium* (TA98) in both the presence and absence of liver microsomal fraction (S-9) of PCB-induced rats. A positive mutagenic response was observed in bacteria exposed to photolytic products of the Toluene/nitrate solution. Mutagenic responses were greater in those assays in which there was an absence of metabolic activiation, as compared to those assays in which S-9 activation was present. No mutagenicity was observed in bacteria exposed to photolytic products in the nitrate-free solution.

TABLE 12. Microbial Mutagenicity Assays

Type of assay	*Strain*	*Metabolic activation*[a]	*Toluene dose*	*Application*	*Mutagenic response*	*Reference*
Reverse mutation						
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.001 to 5.0 μl/plate	Plate incorporation	Negative	146
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.004 to 0.031%[b]	Liquid suspension	Negative	146
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.01 to 10 μl/plate	Plate incorporation	Negative	147
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	5 μl/plate	Plate incorporation	Negative	148
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.115–2.3 μl/plate	Plate incorporation	Negative	149
S. typhimurium	TA98, 100	Yes and no[c]	0.3 to 100 μl/plate	Plate incorporation[d]	Negative	150
		Yes and no[c]	11 to 3764 ppm	Vapor exposure[e]	Negative	
E. coli	WP2	Yes and no	0.01 to 10 μl/plate	Plate incorporation	Negative	147
S. cerevisiae	D7	Yes and no	0.001–0.5%[f]	Liquid suspension	Negative	147
Mitotic gene conversion						
S. cerevisiae	D4	Yes and no	0.001–5.0 μl/plate	Plate incorporation	Negative	146
		Yes and no	0.138–1.1%[b]	Liquid suspension	Negative	
S. cerevisiae	D7	Yes and no	0.001–5.0%[f]	Liquid suspension	Negative	147
Mitotic crossing-over						
S. cerevisiae	D7	Yes and no	0.001–5.0%[f]	Liquid suspension	Negative	147

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

Inhibition of growth and induction of DNA damage by Toluene were evaluated in two studies by comparing differential toxicity to wild-type and DNA repair deficient bacteria. Two species were tested with and without metabolic activator with negative results: *E. coli* (W3110 pol A^+ and p3478 pol A^-) and *S. typhimurium* (SL4525 rfa rec^+ and SL4700 rfa rec^-).[147,152]

Breaks in single DNA strands were observed by Sina et al.[153] in rat hepatocytes exposed in vitro for 3 h to 2.3 mM Toluene. Toluene vapor was a potent mitostat in studies with intact grasshopper embryos (*Melanophus sanguinipes*). Arrested metaphases of the exposed embryos had a c-mitotic appearance and highly contracted and scattered chromosomes.[154] Roots of *Vicia faba* seedling exposed to Toluene developed chromosomal alterations and a longer than normal mitotic cycle.[155]

Drosophila melanogaster males were fed 500 or 1000 ppm Toluene for 24 h. No significant increase in recessive lethals was noted in the total of 3281 X-chromosomes examined.[156]

The ability of Toluene to induce dominant lethal mutations in sperm cells was evaluated by Litton Bionetics.[157] CD-1 mice were exposed by inhalation to 100 or 400 ppm of the compound 6 h per day, 5 days per week for 8 weeks. No increase in pre- or postimplantation loss of embryos or reduction in fertility of treated males was observed.

Toluene failed to induce specific locus forward mutation in the L5178, thymidine kinase mouse lymphoma cell assay. The compound was evaluated at concentrations of 0.05–0.30 μl/ml, with and without mouse liver S-9 activation.[146]

In the micronucleus test, Toluene doses of 250, 500, and 1000 mg/kg were given by IP administration to groups of 32 Swiss male mice. No increase was observed in micronucleated polychromatophilic erythrocytes of the bone marrow.[158] In a second micronucleus test, Toluene induced no clastogenic activity when administered in two oral doses of either 860 or 1720 mg/kg to male and female CD-1 mice.[159]

Two reports concluded that Toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection.[142,143] In an analysis of 720 metaphase cells from the bone marrow of five rats that had been subcutaneously injected with 0.8 g/kg per day Toluene for 12 days, Dobrokhotov[142] found 78 (13%) with chromosomal aberrations. Sixty-six percent of the aberrations were chromatid breaks, 24% were chromatid "fractures," 7% were chromosome "fractures," and 3% involved multiple aberrations. The frequency of spontaneous aberrations in 600 marrow metaphase cells from five control rats injected with vegetable oil averaged 4.16% (65.8% were breaks and 32.4% were chromatid aberrations; no "fractures" or multiple lesions were recorded). The significance of the positive clastogenic effects attributed to Toluene in this study is difficult to assess, since the purity of the sample employed was not stated and because the distinction between chromatid breaks and fractures was unclear.

Lyapkalo[143] administered 1 g/kg per day Toluene to six rats by subcutaneous injection for 12 days. Treatment with Toluene resulted in chromosome aberrations in 11.6% of the bone marrow cells examined (84 aberrant metaphases/724 cells) compared with 3.9% (40/1033) in olive oil injected controls. The types of aberrations that were observed consisted of "gaps" (60.5%), chro-

matid breaks (38.4%), and isochromatid breaks (1.2%). The purity of the Toluene used in this study was not stated.

Dobrokhotov and Enikeev[141] reported that rats exposed to 80 ppm (610 mg/m^3) Toluene by inhalation 4 h daily for 4 months had damaged metaphase chromosomes in 21.6% of the bone marrow cells analyzed. The percentage of metaphase cells with damaged chromosomes in bone marrow cells from air-exposed control rats was 4.0%. The number of cells evaluated and the purity of the Toluene were not specified.

In contrast to the aforementioned cytogenetic studies, Litton Bionetics[160] found that IP injection of Toluene into Charles River rats did not induce bone marrow chromosomal aberrations. The compound was injected at doses of 22, 71, and 214 mg/kg in two different experiments. In one study, five rats were killed at 6, 24, and 48 h following injection of each dose; in a second study, five rats were treated daily at each dose for 5 days, and the rats were killed 6 h after injection of the last dose. Approximately 50 cells per animal were evaluated for damage. Dimethyl sulphoxide (DMSO, the solvent vehicle) administered IP at 0.65 ml/rat was used as the negative control, and triethylenemelamine (TEM) in saline at 0.3 mg/kg was used as the positive control.

Male Wistar rats exposed by inhalation to Toluene (300 ppm, 6 h/day, 5 days/week for 15 weeks) did not have more chromosome aberrations in the bone marrow cells than nonexposed control animals. The frequency of sister chromatid exchanges (SCEs) was analyzed in cultured bone marrow cells of the exposed animals only after 11, 13, and 15 weeks of exposure. There was a statistically significant increase of SCEs in rats exposed for 11 and 13 weeks, but the frequency was in the control range after 15 weeks of Toluene exposure.[156]

Evans and Mitchell[161] reported that Toluene did not alter SCE frequencies in cultured Chinese hamster ovary (CHO) cells. In this study, CHO cells without rat liver S-9 homogenate were exposed to 0.0025–0.04% Toluene for 21.4 h, and CHO cells with S-9 homogenate were exposed to 0.0125–0.21% for 2 h.

In vitro exposure to Toluene at concentrations of 1.52 mg/ml, 15.2 μg/ml and 152 μg/ml had no effect on the number of sister-chromatid exchanges (SCEs) or number of structural chromosomal aberrations in cultured human lymphocytes. However, cytotoxicity was observed at the highest dose.[162] The data from this study cannot be adequately evaluated, since the purity of the Toluene was not indicated, no positive control experiments were performed, no metabolic activation system was employed, and the type of scoring system for chromosome damage was not specified.

Lymphocytes from 32 rotogravure workers with occupational exposure to Toluene were studied for chromosome aberrations and SCEs. The frequencies of these did not differ from the corresponding values of 15 unexposed control subjects. However, a significant increase of SCEs was observed in smoking subjects, both occupationally exposed and unexposed.[156]

Peripheral blood lymphocytes of 34 workers from a rotogravure printing plant and of 34 matched controls from outside the plant were compared for chromosomal aberrations. Ten of the workers were exposed daily to benzene (131–532 ppm) for 2–7 years and subsequently to Toluene (200–400 ppm) for 14 years. Twenty-four workers were exposed only to Toluene for 7–15 years. No significant differences were found between the Toluene and control groups in

frequencies of stable and unstable chromosome aberrations or in chromosome counts. Approximately 100 metaphase cells from each subject or control were scored. The proportion of chromosome changes was significantly higher statistically in the benzene/toluene group compared with controls and in the benzene/toluene group relative to the Toluene group.[163]

Maki-Paakkanen et al.[164] reported no evidence of clastogenicity in cultured peripheral blood lymphocytes from 32 workers from two different rotoprinting factories who had a history of exposure to Toluene (benzene concentrations, ≤0.05%) at 8-h, time-weighted average concentrations of 7–112 ppm. The average age of the workers was 34.2 years, and the average length of employment was 14.6 years. Results of analyses indicated that the frequencies of chromosome aberrations and sister-chromatid exchanges were not significantly different from those of 15 unexposed workers. Similarly, no significant deviations were observed in the frequencies of aberrations in relation to duration of exposure.

Bauchinger et al.[144,145] performed cytogenic analyses on peripheral lymphocytes from 20 male rotogravure plant workers exposed for ≥16 years to Toluene (<0.3% benzene). A group of 24 workers from the same plant, but without exposure to Toluene, served as controls. Toluene concentrations in the workroom air ranged from 200 to 300 ppm. Small amounts of liquid Toluene also were used by the workers to wash the hands. The measured concentrations of Toluene in the blood were reportedly between 0.001 and 0.01%. There was no exposure to other chemicals. As compared with the 24 nonexposed controls, exposed workers had a significantly greater number of chromatid breaks, chromatid exchanges, and chromatid gaps. The number of SCEs also was significantly increased in smoking and nonsmoking Toluene-exposed workers compared with the corresponding control group. The authors suggested that Toluene or its metabolite may induce a weak clastogenic effect in human lymphocytes in vivo.

Carcinogenicity

A 2-year inhalation study with rats and mice is being conducted by the National Toxicology Program at Research Triangle Park. The investigation is currently in the tissue evaluation phase, and no publication date has been established.[165] No neoplasms were observed in mice given a subcutaneous exposure to Toluene. Results of skin painting studies in mice were negative for carcinogenicity. These studies are summarized below.

Toluene suspended in an aqueous gel was applied to the surface of a filter disc. The filter disc was then implanted subcutaneously into the dorsolumbar region of 10 male and 10 female Alderley Park Swiss mice. Each filter disc contained 0.02 mmol of Toluene. Three months after implantation, the surviving mice were killed, and the implant site tissue was removed for histopathological evaluation. No tumors were observed.[166]

Toluene was used as a vehicle control in a study in which benzo(a)pyrene was tested for carcinogenicity. Toluene was applied to the shaved interscapular skin of 20 SWR, 17 C3HeB, and 17 A/He female mice three times a week for life. Mice were 10–14 weeks old on the initial exposure. No skin tumors developed in the Toluene-only treated mice.[167]

Benzo(a)pyrene (20 nmol) or 15,16-dihydro-11-methylcyclopent(a)phenan-

thren-17-one (20 nmol) in a vehicle of 10 μl of Toluene/croton oil (99:1 v/v) was applied twice weekly to the shaved dorsal skin of T.O. mice. The number of exposed mice with skin tumors at 75 weeks was 14/20 and 19/20, respectively. No skin tumors were observed at 75 weeks in the 20 mice topically treated twice weekly with the Toluene/croton oil vehicle.[168]

Groups of Swiss male mice were treated on the ears with either (1) 1.5% DMBA in mineral oil (one exposure), (2) 1.5% DMBA in mineral oil (one exposure) followed after 1 week by two weekly exposures of Toluene for 20 weeks, (3) Toluene twice a week for 20 weeks, or (4) DMBA in mineral oil (one exposure) followed by biweekly applications of mineral oil. Of the 23 surviving mice treated once with DMBA, 1 tumor was observed at 20 weeks. Of the 35 surviving mice treated with DMBA and Toluene, 7 tumors were observed. None of the 14 surviving mice treated with Toluene developed tumors. The negative control group (DMBA and mineral oil) had 8 tumors in 53 survivors at 20 weeks.[169]

Frei and Kinsley[170] examined the promoting effect of Toluene in Swiss mice following initiation with 7,12-dimethylbenz[a]anthracene (DMBA). The ears of the mice were topically treated once with 0.1 ml of 1.5% DMBA in mineral oil. One week after DMBA initiation, mineral oil or Toluene was applied twice a week for 20 weeks. Eleven of 35 mice developed tumors (6 permanent, 5 regressing) following exposure to DMBA and Toluene, whereas, 8 of 53 negative control animals (DMBA and mineral oil) developed tumors (all permanent). Fourteen mice topically treated for 20 weeks with Toluene alone (no DMBA initiation) developed 2 tumors (1 permanent, 1 regressing).

Doak et al.[171] applied Toluene (0.05–0.1 ml) to the backs of CF_1, C_3H, and CB_9H mice twice weekly for 56 weeks. For each strain, approximately 50 mice (25 male, 25 female) were tested. No difference was observed between treated and control mice with respect to frequency of skin or systemic tumors. It was not clear in this study if the Toluene was applied under an occlusive dressing or if it was allowed to evaporate.

Toulene was applied twice a week for 50 weeks to the clipped dorsal skin of 20 TO albino mice. Animals were observed for a period of 1 year after treatment. A second group of 20 mice served as untreated controls. No skin tumors developed in either group; however, survival was only 35% (7 of 20) in the treatment group.[172]

No skin tumors were observed in skin painting studies in which 50 mg of Toluene was applied twice a week for 80 weeks to 50 male C3H/HeJ mice[173] or in which Toluene was applied twice weekly for 50 weeks to 10 male and 10 female TO albino mice.[174]

Lijinsky and Garcia[175] used Toluene as a vehicle control in the carcinogenicity testing of various polynuclear hydrocarbons. Toluene (1–20 μl) was applied twice a week for 72 weeks to the clipped interscapular skin of 30 Swiss female mice. Two mice developed skin tumors. One animal developed squamous cell carcinoma, and one developed squamous cell papilloma. The "average latent period" of the first tumor was 58 weeks. Twenty-four and fifteen mice survived to 60 and 80 weeks, respectively.

In a brief abstract, Frei and Ritchie[176] reported that tumor promotion in the skin of mice by Toluene and other "irritant solutions" was associated with the ability of these agents to induce epidermal hyperplasia. No other details were specified.

Teratogenicity and Embryotoxicity

The teratogenicity and embryotoxicity of Toluene were assessed in hamsters, mice, and rats by various routes of exposure (skin, oral, inhalation).

Overman[177] reported that Toluene produced "minimal embryotoxic effects" in hamsters following applications to clipped skin. Applications were made every day for 2 h between days 7 and 11 of gestation. Animals were killed at day 15 of gestation. A decrease in fetal size and weight and an increase in the incidence of prenatal death were noted. Observed malformations included fetal hemorrhage and gastroschisis. No malformations appeared in the control groups. The dose and vehicle were not specified.

Toluene was administered to CD-1 mice by gavage from days 6 through 15 of gestation at 0.3, 0.5, and 1.0 ml/kg body weight per dose. The compound was also given by gavage from days 12 through 15 of gestation at 1.0 ml/kg per dose. The vehicle used was cottonseed oil (0.5% of maternal body weight/dose). Maternal toxicity was not observed after exposure to Toluene (days 6–15) at any dose. However, an increase in embryonic deaths at all doses and a reduction in fetal weight in the 0.5 and 1.0 mg/kg groups was noted. An increased incidence of cleft palate was observed after exposure to 1.0 ml/kg on days 6–15 of gestation. The same dose given on days 12–15 of gestation produced decreased maternal weight gain. The authors concluded that Toluene was teratogenic at 1.0 ml/kg and embryotoxic at 0.3 ml/kg.[178]

Toluene was dissolved in corn oil and administered by gavage for 8 days to pregnant CD-1 mice. The daily oral dose was 10 ml/kg body weight, which corresponded to 2350 mg of Toluene per kg of body weight. No differences between test and control groups were observed in terms of number of maternal deaths, mean maternal weight, number of animals producing litters, incidence of resorbed fetuses, litter size, number of live or dead fetuses, and mean litter weight. The investigators concluded that Toluene caused no significant reproductive toxicity.[179]

Female ICR mice were exposed by inhalation to 100 or 1000 ppm Toluene 6 h a day from the first to seventeenth day of gestation. No differences were observed between control and treated groups with respect to litter size, incidence of resorbed fetuses, number of implantation sites, number of live or dead fetuses, fetal body weight, external malformations, eye or ear opening, weaning, and incidence of body hair. The incidence of skeletal abnormalities was similar between treated and control groups, with the exception of extra 14th ribs and rudimentary 14th ribs in the 1000 ppm dose group. The authors stated that the high incidence of 14th ribs "suggested the possible teratogenicity of Toluene."[180,181]

Hudak and Ungvary[182] studied the teratogenic and embryotoxic effects of Toluene in CFY rats and CFLP mice. The mice were exposed by inhalation to 500 mg/m^3 (133 ppm) Toluene for 24 h/day from days 6 to 13 of pregnancy. Rats were exposed to Toluene in one of three dosage regimens: (1) 1500 mg/m^3 (399 ppm) for 24 h/day from day 9 to 14 of pregnancy, (2) 1500 mg/kg (399 ppm) for 24 h/day from day 1 to 8 of pregnancy, or (3) 1000 mg/m^3 (266 ppm) for 8 h/day from day 1 to 21 of pregnancy. Exposure of mice to 133 ppm Toluene was associated with decreased average fetal weights and an increased incidence of weight-retarded fetuses. Irregular sternebrae and extra ribs were observed in the fetuses of rats treated with 399 ppm Toluene on days 9–14 of pregnancy. Re-

tarded skeletal growth and decreased weights were noted in fetuses of rats exposed on days 1–8 of pregnancy to 399 ppm Toluene. Retarded skeletal development was also observed in the fetuses of rats treated with Toluene at 266 ppm.

Pregnant CFY rats were exposed by inhalation to benzene (400 mg/m^3), Toluene (1000 mg/m^3), or a combination of the two solvents from day 7 to day 14 of gestation. Exposure to benzene or benzene plus Toluene was associated with decreased fetal weight, whereas exposure to Toluene or benzene plus Toluene was associated with increased incidence of extra fetal ribs. Exposure to benzene, Toluene, and benzene plus Toluene caused skeletal retardation in the fetuses but did not produce increases in the rates of external, internal, or skeletal malformations.[183]

In other studies with CFY rats, Toluene potentiated the maternal and embryonic toxicity of acetylsalicylic acid.[184]

No evidence of teratogenicity was observed in the 20-day old fetuses of Charles River rats that were exposed to 100 or 400 ppm Toluene vapor for 6 h/day on days 6–15 of gestation. At microscopic examination, no unusual incidence of visceral or skeletal abnormalities was observed. Unusual skeletal variations were observed in a small but comparable number of fetuses from both the exposed and control groups, but these changes were in most cases attributed to retarded bone ossification and were not considered to be malformations as such. No maternal deaths occurred during the study, and the sex ratio of the offspring did not differ significantly between the treated and control groups.[160]

Groups of 20 CFY rats were exposed to 266 ppm (1000 mg/m^3) Toluene, 125 ppm (400 mg/m^3) benzene, or a combination of these concentrations of Toluene and benzene vapor for 24 h/day on days 7–14 of gestation. A group of 22 rats inhaling air served as controls. Fetuses were examined on day 21 of pregnancy. Continuous exposure to 266 ppm Toluene was 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. Also, it was reported that the incidence of extra ribs was higher in the group exposed to Toluene in combination with benzene than in the groups exposed to Toluene alone. Maternal loss, maternal weight gain, number of litters, mean implantation/dam, placental weight, fetal loss, and fetal weight loss were not significantly affected by the Toluene exposures. Exposure to 125 ppm benzene did cause decreases in maternal weight gain, placental weight, and fetal weight, but these effects appeared to be inhibited by concurrent exposure to 266 ppm Toluene. Further, it was reported that postinhalation fetal loss (the number of dead and resorbed fetuses relative to the number of total implantation sites) 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.[185]

CLINICAL ASSESSMENT OF SAFETY

Effects on Skin and Nails

Toluene's degreasing action removes natural lipids of the skin, which in turn may cause dryness, fissures, and contact dermatitis.[186]

Koilonychia and hapalonychia of the fingernails were observed in 6 of 16

cabinet workers exposed percutaneously to a thinner mixture containing 30% Toluene, 30% xylene, and 40% methyl alcohol. Most of the affected workers had an average exposure of 2 years.[187]

No skin irritation was observed when 20 subjects were exposed in a single insult occlusive patch test to a nail basecoat containing 33.2% Toluene.[188] The length of the exposure period was not reported.

A nail polish containing 31.23% Toluene was assessed for its cumulative skin irritation potential. Applications of the product (0.3 ml) were made under closed patches everyday for 21 consecutive days to the skin of the back of 10 subjects. Contact periods were for 23 h, and applications of the product were made to the same site. The composite total score for the 10 subjects treated with nail polish was 16 out of a maximum possible score of 630, indicating minimal irritation with "essentially no evidence of cumulative irritation." Composite scores of 7/630 and 569/630 were reported for baby oil (negative control) and deodorant (positive control), respectively, indicating "no evidence of cumulative irritation" and "strong potential for cumulative irritation."[189]

A repeated insult patch test was used to evaluate the skin irritation and sensitization potential of a nail polish containing 33% Toluene. Occlusive "dry patches" containing the test material were applied to the upper back of 148 subjects (59 males, 89 females) every Monday, Wednesday, and Friday over 3 consecutive weeks for a total of nine induction applications. Following a 2-week nontreatment period, two consecutive 48-h challenge patches were applied to a site adjacent to the original induction site. No skin reactions were observed.[190]

The Maximization Test described by Kligman[191] and Kligman and Epstein[192] was used to assess the sensitization potential of a nail polish containing 31.23% Toluene. The product (0.3 ml) was applied under an occlusive dressing to the forearm of 25 subjects for five 48-h periods. Since the product contained volatile ingredients, the product was applied to the patch and allowed to air-dry prior to application. Throughout the induction phase, the test sites were pretreated with 24-h patches containing 1.5% sodium lauryl sulfate in aqueous solution. Following a 10-day nontreatment period, a challenge patch of the nail polish was applied under occlusion for 48 h to a previously untreated site. The challenge site was pretreated for 1 h with a 10.0% aqueous solution of sodium lauryl sulfate. Evaluations were made 48 and 72 h after treatment. No reactions were observed during the induction or challenge phases.[193]

In separate studies, two nail products were evaluated for phototoxicity and photoallergenicity. One product contained 30% Toluene and was evaluated on 28 subjects.[194] The second product was formulated with 25% Toluene and was assessed on 30 subjects.[195] In each instance, the light source was a xenon Arc Solar Simulator (150W), which was filtered to produce a continuous emission spectrum in the UVA and UVB region (290–400 nm). Prior to testing, each panelist's "minimal erythemal dose" was determined according to the procedures outlined in the Federal Register.[196] For the induction phase, each product (0.1 ml) was applied to the skin of the back under an occlusive patch. Twenty-four hours later, the patch was removed, and the sites were irradiated with three times the individuals' minimal erythemal dose using the full xenon lamp spectrum. Forty-eight hours later, the sites were evaluated. The procedure of product exposure and light exposure was repeated twice weekly for a total of six induction exposures. Following a 10-day nontreatment period, the product was applied under an occlusive patch to a previously untreated site adjacent to the in-

duction site. Twenty-four hours later, the challenge patch was removed, and the sites were irradiated for 3 minutes with a filtered (Schott WG345 filter) light source. Challenge sites were evaluated 15 minutes and 24, 48, and 72 h after UV exposure. Control sites were subjected to the same induction and challenge procedures, with the exception that control sites were not subject to irradiation. No phototoxic or photoallergic reactions were observed to the two nail products formulated with 30% and 25% Toluene.[194,195]

Respiratory Tract and Ocular Irritation

Two male subjects exposed to Toluene for 7–8 h developed transitory mild throat and eye irritation at 200 ppm and lacrimation at 400 ppm.[127] No complaints of respiratory tract irritation were reported by volunteers or workers exposed to Toluene concentrations of 800–1500 ppm for 8 h.[86,128,197]

Transient epithelial injury consisting of moderate conjunctival irritation and corneal damage was noted in three workers who were accidentally splashed in the eyes with Toluene. Complete recovery generally occurred within 48 h.[198]

Effects on Cardiovascular Function

Toluene has been implicated in a number of sudden deaths due to glue or solvent sniffing. In a study of 110 cases of sudden, unexpected death in solvent abusers, Bass[199] reported that the deaths were not due to suffocation secondary to intoxication but were due to a direct effect of the solvent itself. Toluene, benzene, gasoline, trichloroethane, and fluorocarbon propellants were individually implicated as causing sudden cardiovascular collapse. Severe cardiac arrhythmia resulting from light anesthesia was proposed as the most likely explanation for the cause of sudden death. Several authors have suggested that sniffing volatile hydrocarbons may cause sensitization of the myocardium to epinephrine.[199–201]

Ogata et al.[202] found an apparent decrease in the pulse rate of 23 volunteers who were exposed to 200 ppm Toluene for periods of 3 h or 7 h, but no effect was observed in those exposed to 100 ppm concentration. 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 rate or electrocardiogram of 15 other subjects during either rest or light exercise.[55] In other studies, experimental exposure to Toluene at concentration of 100–700 ppm for 20 minutes[203] or 50–800 ppm for 8 h[86,128] did not produce any definite effects on heart rate or blood pressure. Suhr[204] observed that the pulse rate and blood pressure 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.

Occupational Exposure Limits

The American Conference of Governmental Industrial Hygienists[205] has adopted for toluene a "threshold limit value-time-weighted average" (TLV-TWA) and a "threshold limit value-short-term exposure limit" (TLV-STEL) of 100 ppm (375 mg/m^3) and 150 ppm (560 mg/m^3), respectively. The TLV-TWA is defined as

the airborne concentration for a normal 8-h workday and a 40-h workweek to which nearly all workers may be repeatedly exposed without adverse effect. The TLV-STEL is the maximal airborne concentration to which workers can be exposed for a period up to 15 minutes without causing irritation, chronic or irreversible tissue change, or necrosis. The threshold limit values are used as guides in the control of health hazards and are not intended to be used to differentiate between safe and unsafe concentrations.

SUMMARY

Toluene is a clear liquid that is insoluble in water. It is produced from either petroleum refining processes, as a byproduct of styrene production, or as a byproduct of coke oven operations. Commercial Toluene may contain benzene as an impurity; however, no data were available regarding the impurity content of cosmetic grade Toluene. Under experimental conditions, Toluene undergoes substitution reactions on the aliphatic side group ($-CH_3$) and on the benzene ring at the ortho and para positions. Under conditions of cosmetic use, Toluene is considered stable and unreactive.

Toluene has a wide variety of noncosmetic applications, including use as an indirect food additive, gasoline additive, solvent, and thinner (Tables 3 and 4). Cosmetic applications include use in nail products as a diluent and solvent. Cosmetic manufacturers participating in the voluntary cosmetic product registration program with the Food and Drug Administration reported that Toluene was used in 1984 in 555 nail and manicuring products. Reported concentrations of Toluene in these products ranged from >10–25% (448 products) to >25–50% (107 products) (Table 5).

The nail, the nail cuticle, and the skin surrounding the nail are the areas directly exposed to cosmetic formulations containing Toluene. Areas of the body that come in contact with the "wet" nail may also become exposed. During application of nail products, Toluene may come in contact with eyes and nasal mucosa as a result of evaporation from the formulation.

In mammals, Toluene is absorbed by the respiratory tract, gastrointestinal tract, and skin. Significant absorption may occur through the intact human skin. In one study, the rate of absorption of undiluted Toluene through the skin of the hands and forearms of humans was estimated at 14–23 mg/cm² per hour.

Although Toluene is likely metabolized to some extent in most mammalian tissues, the major site for metabolism is in the liver. Most of the absorbed Toluene (approximately 84%) undergoes sidechain oxidation to benzoic acid. Benzoic acid is subsequently conjugated with glycine and excreted in the urine as hippuric acid, although a large amount of conjugation with glucuronic acid occurs, resulting in urinary excretion of benzoylglucuronic acid. Small amounts of absorbed Toluene appear in the urine as benzylmercapturic acid and cresol derivatives. Approximately 16% of the absorbed Toluene is expired unchanged through the lungs (Fig. 1).

Toluene is lipophilic and accumulates primarily in those tissues with a high fat content. In one study, the half-life of Toluene in human adipose tissue ranged from 0.5 to 2.7 days.

Toluene was practically nontoxic when given orally to rats; reported acute

oral LD_{50} values ranged from 2.6 g/kg to 7.53 g/kg (Table 8). The acute dermal LD_{50} in rabbits was 14.1 ml/kg. A single IP injection of 0.8–1.7 g/kg was lethal to rats, mice, and guinea pigs.

A single subcutaneous injection of 1.1–1.25 g/kg and 4.3–8.7 g/kg was lethal to rats and mice, respectively. Granulopenia, followed by granulocytosis and eventual death, was noted in rabbits given Toluene as a single, subcutaneous dose of 4 ml/kg. In subchronic studies, rats given Toluene by subcutaneous injection at a dose of 1 ml/kg for 21 days had induration at the injection site, a decrease in body weight, a decrease in erythrocyte and leukocyte counts, hyperplasia of the bone marrow and spleen, focal hepatic necrosis, and nephrosis. Guinea pigs administered Toluene at a subcutaneous dose of 0.25 mg/day for 30–70 days developed polypnea, convulsions, necrosis at the injection site, as well as hemorrhagic, hyperemic, and degenerative changes in lungs, kidneys, adrenal glands, and spleen. Rabbits treated with Toluene at a subcutaneous dose of 1 ml/kg per day for 6 days developed granulopenia and granulocytosis.

Undiluted Toluene produced slight to moderate skin irritation in rabbits when tested by four different procedures. Skin necrosis was slight in one study in which Toluene was repeatedly applied to the skin of rabbits over a 2–4-week period (Table 9). Results of studies with rabbits indicate that undiluted Toluene is an ocular irritant (Table 10).

Acute inhalation LD_{50} values for Toluene in mice were 5320 ppm and 6942 ppm; the exposure period in these two studies were 6–7 h. Effects observed in mice, rats, guinea pigs, rabbits, and dogs after acute inhalation of Toluene included mucous membrane irritation, motor incoordination, prostration, changes in respiratory rate, changes in serum and blood enzyme activities, elevated blood glucose and packed cell volume, decreased body weight, and death. These effects varied according to animal studied, length of Toluene exposure, and Toluene concentration.

Progressive symptoms observed in experimental animals following subchronic inhalation to increasingly higher concentrations of Toluene included irritation of the mucous membranes, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death (Table 11).

No significant treatment-related effects were observed in four studies in which rats were given chronic oral doses or chronic inhalation exposures to Toluene. Parameters examined in those studies generally included appearance, behavior, growth, body and organ weights, blood chemistry, urinalysis, gross and microscopic lesions, and mortality.

Toluene was negative for mutagenicity in a battery of mammalian cell and whole organism test systems. Results of microbial assays also were negative for mutagenicity (Table 12). There were several reports of increased chromosome aberrations in the bone marrow of rats exposed by inhalation or by subcutaneous injection to Toluene, as well as reports of increased sister-chromatid exchanges and chromosome aberrations in the lymphocytes of workers chronically exposed to Toluene.

A 2-year inhalation study with rats and mice is being conducted by the National Toxicology Program at Research Triangle Park. Results of the investigation have not yet been published. No neoplasms were observed in mice given a 3-month subcutaneous exposure to Toluene. With the exception of one report, results of numerous skin painting studies in mice were negative for carcinogenic-

ity. The teratogenicity and embryotoxicity of Toluene were assessed in hamsters, mice, and rats by various routes of exposure (skin, oral, inhalation). Results of these studies were mixed.

Clinical data were limited to five studies involving cosmetic products. No skin irritation or sensitization was observed in subjects treated with cosmetic products containing 31–33% Toluene. No phototoxic or photoallergic reactions were noted in subjects treated with 25 or 30% Toluene.

Throat irritation, eye irritation, and/or lacrimation were noted in two subjects exposed to airborne concentrations of 200 and 400 ppm Toluene. Transient irritation of the conjunctiva and transient injury of the cornea were observed in several workers accidentally exposed in the eyes to Toluene.

Toluene has been implicated in a number of sudden deaths due to glue or solvent sniffing. Several reports also suggested that sniffing volatile hydrocarbons may cause sensitization of the myocardium to epinephrine.

DISCUSSION

No data were available to the CIR Expert Panel regarding the impurities found in cosmetic grade Toluene. One possible impurity, benzene, is a carcinogen. Therefore, cosmetic products formulated with Toluene should be benzene-free.

Two studies concluded that Toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection.[142,143] The significance of positive clastogenic effects attributed to Toluene in these studies is difficult to assess, since the purity of the test samples was not reported. More definitive carcinogenic studies were available. In eight studies, Toluene did not induce cancer. In one study, 1 of 30 mice developed skin cancer; however, there were no untreated controls for comparison.

Results of animal studies indicated that undiluted Toluene is a skin irritant. Thus, there is a potential for Toluene to cause skin irritation in humans. However, the sole cosmetic use of Toluene is in products intended to be applied directly to the nail. Therefore, human skin exposure to this ingredient will be minimal under conditions of cosmetic use.

CONCLUSION

On the basis of the available data presented in this report, the CIR Expert Panel concludes that Toluene is safe as a cosmetic ingredient in the present practices of use and concentration.

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