Amended Safety Assessment of Toluene as Used in Cosmetics

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ABBREVIATIONS

3β-HSD	3β-hydroxysteroid dehydrogenase
8-OHdG	8-hydroxy-2'-deoxyguanosine
17β-HSD3	17β-hydroxysteroid dehydrogenase
ACTH	adrenocorticotropic hormone
ADME	absorption, distribution, metabolism, and excretion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMP	adenosine monophosphate
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BAL	bronchoalveolar lavage fluid
CaMKIV	calcium/calmodulin-dependent protein kinase
CAS	Chemical Abstracts Service
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CREB1	cyclic adenosine monophosphate responsive element binding protein 1
CRF	corticotropin-releasing-factor
CV	coefficient of variation
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
DTSC	Department of Toxic Substances Control
ECHA	European Chemicals Agency
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
GD	gestation day
GDF9	growth differentiation factor-9
GGT	gamma-glutamyl transaminase
HCIS	Hazardous Chemical Information System
HPA	hypothalamus-pituitary-adrenal
HPT	hypothalamus-pituitary-thyroid
HQ	hazard quotient
HR	hazard ratio
IARC	International Agency for Research on Cancer
IFN	interferon
Ig	immunoglobulin
IGF-1	insulin-like growth factor 1
IL	interleukin
Insl3	insulin-like 3
LC3	light-chain 3
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
ln	natural logarithm
LOAEL	lowest-observed-adverse-effect-level
log K_{ow}	n-octanol/water partition coefficient
MADL	maximum allowable dose level
MI	multiplicative interaction
MOE	margin of exposure
MOS	margin of safety
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MTT	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NHANES	National Health and Nutrition Examination Survey
NMDA	N-methyl-D-aspartate
NOAEC	no-observed-adverse-effect-concentration

NOAEL	no-observed-adverse-effect-level
NR	none reported
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OEL	occupational exposure limits
OR	odds ratio
OVA	ovalbumin
P450c17	cytochrome P450 17α-hydroxylase/c17-20 lyase
P450scc	cytochrome P450 cholesterol side-chain cleavage
Panel	Expert Panel for Cosmetic Ingredient Safety
PCR	polymerase chain reaction
PEL	permissible exposure limit
PGN	peptidoglycan
PND	post-natal day
POD	point of departure
PVN	paraventricular nucleus
REL	recommended exposure limit
RERI	relative excess risk due to interaction
RfC	reference concentration for chronic inhalation exposure
RfD	reference dose for chronic oral exposure
RT-PCR	reverse transcription–polymerase chain reaction
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SED	systemic exposure dose
SKF525A	2-diethylaminoethyl-2,2-diphenylvalerate-HCl
STEL	short-term exposure limit
TG	test guideline
TNF-α	tumor necrosis factor – alpha
TSCA	Toxic Substances Control Act
TSH	thyroid-stimulating hormone
TUNEL	terminal deoxynucleotidyl transferase dUTP nick-end labeling
TWA	time-weighted average
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Toluene, which is reported to function in cosmetics as an antioxidant and solvent. The Panel reviewed the available data to determine the safety of this ingredient. The Panel issued an amended report with a revised conclusion stating Toluene is safe for use in nail products at concentrations up to 20%.

INTRODUCTION

Toluene, which according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*) is reported to function in cosmetics as an antioxidant and a solvent,[1] was previously reviewed by the Panel in a safety assessment that was published in 1987.[2] At that time, the Panel concluded that Toluene is safe as a cosmetic ingredient in the present practices of use and concentration, as stated in that report. The Panel first considered a re-review of this report in March 2005,[3] and the Panel re-affirmed the original conclusion, as published in 2006.[4] Subsequently in 2023, the US Food and Drug Administration (FDA) nominated Toluene for an accelerated re-review; in accord with Cosmetic Ingredient Review (CIR) procedures, the report was re-opened and an amended report was prepared.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted January 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.[1]

Numerous studies were found during this process, many of which were either published prior to 2005, cumulative to the information already provided in this report, or not relevant to cosmetic safety, and were therefore not included herein. However, an appendix containing these references has been provided and can be found at the end of this report.

Excerpts of data from the original 1987 safety assessment are summarized throughout the text of this document, as appropriate, as are excerpts of the original re-review document[3] considered by the Panel in March 2005. These data are identified using *italicized* text. (This information is not included in tables or the Summary section.) For complete and detailed information, the original 1987 report can be accessed on the CIR website (https://www.cir-safety.org/ingredients).

CHEMISTRY

Definition and Structure

Toluene (CAS No. 108-88-3; molecular weight = 92.13 g/mol; log K_{ow} = 2.73), an ubiquitous volatile organic compound, is a homolog of benzene in which one hydrogen atom has been replaced by a methyl group. According to the Dictionary,[1] Toluene is an aromatic compound that conforms to the structure:

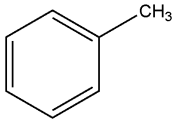

Figure 1. Toluene

Chemical Properties

Toluene is a clear, refractive liquid with an aromatic odor similar to benzene that is both volatile and flammable.[2] *No significant absorption was noted above 300 nm when the ultraviolet absorption spectrum of 300 g/l of Toluene diluted in hexane was measured.*

Toluene is miscible in several organic solvents and has a water solubility of 526 mg/ml.[5] In addition, Toluene has a low molecular weight and is a liquid at room temperature. Chemical properties of Toluene are summarized in Table 1.

Method of Manufacture

Three major sources of Toluene production include petroleum refining processes, as a by-product of styrene production (via the dehydrogenation of ethylbenzene), and as a by-product of coke oven operation (high-temperature carbonization of coal).[2] *Petroleum refining processes to isolate Toluene are either performed via catalytic reforming or pyrolytic cracking. Catalytic reforming involves the catalytic dehydrogenation of selected petroleum fractions, resulting in a mixture of aromatics and paraffins. Toluene is isolated from the reformate via distillation, washing with sulfuric acid, and re-distillation. Toluene can be purified via various extraction and distillation processes (Udex extraction, sulfur dioxide extraction, sulfolane extraction). The grade of Toluene (e.g., pure, commercial, solvent) is defined in terms of boiling ranges.*

Impurities

Commercial Toluene may contain benzene as an impurity.[2] *Toxicological and clinical studies involving Toluene should specify the purity of Toluene used for experimentation to determine if observed effects were caused by Toluene, and not benzene as an impurity.*

According to the Food and Agriculture Organization the United Nations, Toluene should not contain more than 5 mg/ 100 ml non-volatile residues, 0.2% non-aromatic substances, 0.5% benzene, or 2 mg/kg lead.[6] The ingredient should have a purity of no less than 99% and should be negative for hydrogen sulfide and sulfur dioxide. In addition, a European Chemicals Agency (ECHA) dossier on Toluene reported potential impurities of ethylbenzene, *m*-xylene, *o*-xylene, *p*-xylene, and benzene.[7]

Reactivity

Toluene undergoes substitution reactions (halogenation, chloromethylation, nitration, acetylation, benzoylation, mercuration, sulfonation, brosylation, methylation, and isopropylation) on the aliphatic side group (-CH_3) and on the benzene ring at the ortho- and para- positions.[2] *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. Under water chlorination, Toluene may undergo hydrolysis to produce benzaldehyde. In the presence of solvents (e.g., paraffins, naphthenics, and alcoholic hydrocarbons), Toluene may produce azeotropes. Toluene may also undergo photo-oxidation and other photochemical reactions. Toluene is reported to be chemically-stable and unreactive under conditions of use in cosmetic preparations.*

A study was performed to evaluate the pyrolysis products of Toluene. Toluene vapor was passed through nitrogen through a silica tube filled with porcelain chips at 700° C. Reported pyrolysis products included some known or suspected carcinogenic aromatic hydrocarbons (e.g., 1,2-benzathracene, benzene, 3,4-benzofluoranthene).

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of Toluene in cosmetics. Frequency of use data included herein were obtained from the FDA Voluntary Cosmetic Registration Program (VCRP) database and concentration of use data were received in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (Council). It is these values that define the present practices of use and concentration.

No uses were reported for Toluene according to 2023 FDA VCRP data[8] however, according to a concentration of use survey performed in 2023, and updated in 2024, Toluene is used at up to 20% in nail polish and enamel[9] (Table 2). It should be presumed that there is at least one use in every category for which a concentration is reported. In 2002, Toluene was reported to the VCRP to be used in 59 total formulations, at up to 26% in other manicuring preparations (according to 2003 concentration of use survey).[4] All uses and concentrations provided in 2002/2003 were in nail products. Concentration of use data submitted in response to the Council survey in 2023 indicate that Toluene is present in cosmetic formulations other than nail products; however, the companies that submitted this information stated that these values are present in the product as a residual or impurity, and not intentionally added.

According to the California Safe Cosmetics Program Product Database, Toluene is also used in products such as lip glosses at low concentrations.[10] However, it is unknown whether these data are from products that are currently on the market, and it is not known if they are intentional additions of Toluene to cosmetic products (rather than residual amounts; concentrations range from 0.000001 – 0.01%).

Some products containing Toluene may be marketed for use with airbrush delivery systems; however, this information is not available from the VCRP or the Council survey. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the frequency and concentrations of use of this ingredient, and without consumer habits and practices data or particle size data related to this use technology, the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The use of Toluene in cosmetics in the European Union (EU) is restricted to nail products at maximum concentrations of 25%.[11] In addition, according to the EU, caution statements should be used informing users to keep products containing Toluene out of the reach of children. The California Department of Toxic Substance Control (DTSC) requires manufacturers of nail products to certify that their products do not contain more than 100 ppm Toluene;[12] however, DTSC's Safer Consumer Products (SCP) regulatory framework is not based on quantitative risk assessment.[13]

Cosmetic Use Exposure

Nail products, such as those containing Toluene, may be applied several times a week over an extended period of time.[2,14] Areas directly exposed to Toluene include the fingernails, toenails, cuticles, and skin surrounding the nail area. Other areas of the body (e.g., eye region, face) may come in contact with the ingredient prior to the drying of the wet polish. In addition, Toluene may come in contact with the eyes and nasal mucosa during product application due to evaporation.

The amount of Toluene in the breathing zone before, during, and after exposure to nail product application was evaluated in 15 female subjects under simulated-use conditions (conducted following principles of Good Laboratory Practice and Good Clinical Practice).[15] Each subject applied a base coat, two enamel coats, and top coat (all products formulated with 25% Toluene; minimum of 1 min drying time between applications). Sampling began 1 min before application and was continuous throughout the application period until the subject declared her nails to be dry after the 4th application. When nails were dry after top coat application, participants were asked to leave the room until Toluene concentrations returned back to a stable baseline. This procedure was performed 3 times, on 3 consecutive days. Toluene concentrations in the breathing zone were measured using an infrared gas analyzer connected to a recirculation pump with flexible tubing, within a 16 m^3 room that maintained an air flow of about 1.0 changes per hour. The sampling rate was set at 30 l/min. Exposure per subject was calculated by multiplying the mean Toluene concentration by application duration and a pre-calculated minute inhalation constant for women. The mean total of the nail polish applied was 0.5276 g (coefficient of variation (CV)% = 20.60%). The estimated average Toluene exposure amount, which was measured in the breathing zone of all subjects was 0.6 mg on study days 1 (CV% = 33%) and 2 (CV% = 50%), and 0.5 mg (CV% = 40%) on study day 3, with a mean application duration of 15 min.

Analytical air measurements of Toluene content were taken on 178 professional nail technicians who were working with Toluene-containing cosmetic nail products with their clients.[16] The mean Toluene exposure from inhalation was 0.236 ppm, or 0.260 ppm at the 90% upper confidence level. In the same study, the results of air sampling for the customers of the nail technicians were 0.149 ppm (mean) or 0.166 ppm at the 90% upper confidence level.

In 2011, the DTSC measured Toluene concentrations in nail polish products available in the San Francisco Bay Area. Toluene was detected in 83% of nail products that claimed to be Toluene-free at concentrations up to 190,000 ppm.[17] Dermal and inhalation exposure to a salon patron, nail technician, and home user was evaluated. The maximum daily exposure (dermal and inhalation) in salon patrons, nail technicians, and home users was determined to be 2160, 28,200, and 7760 µg/d, respectively.

According to the Scientific Committee on Consumer Products (SCCP), cosmetic nail product application is typically less than 30 min, and although products may come in contact to the keratin of the nail plate, penetration of Toluene through the nail plate is nil or minimal due to the extensive volatilization of the substance.[18,16] In addition, although products come into contact to the skin, this contact is also typically nil or minimal. In 2008, the SCCP concluded that the occasional exposure to Toluene present in nail cosmetics where the exposure may be within the range of 1 – 4 ppm can be considered safe.

Exposure to Toluene as An Impurity in Personal Care Products

While Toluene is an intentionally added cosmetic ingredient in nail enamels, in consideration of total aggregate exposures, it may be worth noting that it has also been reported to be present as an impurity in several products, including hand sanitizers (in amounts of 0.074 – 20,700 ng/g), feminine hygiene products (in amounts of up to 4538 ng/g in feminine sprays and powders), and sunscreen (in amounts of 0.006 – 470 ng/g).[19-21] The mean dermal exposure dose of Toluene was calculated to be 133 ng/kg bw/d (in children) and 94.6 ng/kg bw/d (in adults) in subjects exposed to sunscreens containing Toluene as an impurity, and were calculated to be 14.4 ng/kg bw/d (in children) and 10.3 ng/kg bw/d (in adults) in subjects exposed to hand sanitizers containing Toluene as an impurity. Feminine hygiene products containing Toluene as an impurity were associated with a higher calculated cancer risk (largely due to presence of benzene in products).

Non-Cosmetic

Toluene may be used as an indirect food additive, gasoline additive, ink thinner, non-clinical thermometer liquid, suspension solution for navigation instruments, extraction solvent for plant materials, and as a solvent for many industrial substances (e.g., adhesives).[2] Toluene is also used as a starting material for the production of several chemicals (e.g., benzene), polyurethane resins, detergents, dyes, and drugs.

Several CFR citations have been found regarding the use of Toluene in the food and drug industry. A listing of these CFR citations can be found in Table 3. According to these citations, Toluene may be used as an indirect food additive, a denaturant, and as an ingredient in veterinary pharmaceuticals. Toluene is commonly used in glue and spray paint products; it should be noted that according to Directive 76/769/EEC on certain dangerous substances and preparations, Toluene is banned in glue and spray paint concentrations above 0.1% in products of the general public (in the EU).[16]

TOXICOKINETIC STUDIES

Dermal Penetration/Percutaneous Absorption

In vitro penetration of Toluene through excised rat skin was estimated to be 8.5 nmol/min per cm^2.[2] Blood concentrations of Toluene were determined to be 1.1 and 0.60 µg/ml in guinea pigs dermally exposed to 1 ml Toluene after 0.5 h and 6 h, respectively. The rate of absorption of undiluted Toluene (0.2 ml) through the skin of the hands and forearms of humans was estimated to be 14 - 23 mg/cm^2/h after a 10 - 15 min exposure. When the hands and forearms were immersed for 1 h in an aqueous solution containing 180 - 600 mg Toluene per liter, the rate of absorption was determined to be 0.16 - 0.60 mg/cm^2/h. Study authors estimated that exposure of both hands in a saturated solution of Toluene for 1 h would be equivalent to inhalation exposure to an atmosphere containing 26.6 ppm Toluene for 8 h. Between 2050 and 3370 mg of Toluene was absorbed in volunteers wearing respiratory protection (volunteers emerged hands in pure Toluene for 10 min). 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 to 600 ppm Toluene (masked subjects intermittently exercised during study period to enhance percutaneous absorption).

A physiologically-based pharmacokinetic model was used to evaluate the dermal absorption of Toluene in human subjects.[3] The average dermal permeability coefficient of Toluene was 0.012 ± 0.007 cm/h.

Details of the dermal penetration/percutaneous absorption studies summarized here are found in Table 4. A steady-state flux of 0.00038 g/cm^2/h was determined in an in vitro percutaneous absorption study performed using split-thickness pig skin (skin exposed to undiluted Toluene).[22] Maximum Toluene (tested neat) concentrations of 3.07 ± 0.40 µg/ml (for membranes exposed for 15 min) and 5.38 ± 0.92 µg/ml (for membranes exposed for 240 min) were reported in receptor fluid in an assay using microdialysis membranes inserted into rat abdominal skin.[23] The effect of tape stripping and pre-treatment with topical products (barrier creams) was also evaluated in this study. Neither tape stripping nor topical product usage induced a significant change in dermal penetration of Toluene or o-cresol content. In a study performed in humans evaluating the effect of temperature on dermal absorption, 5 masked (to prevent inhalation exposure) subjects were exposed to Toluene (50 ppm) in inhalation chambers for 4 h.[24] Venous concentrations of Toluene were not statistically different between 25 and 30°C. The maximum mean venous concentration reported in this study was 6.21 ± 0.076 µg/l (at 30° C, measured at 4 h).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Toluene is absorbed by the respiratory tract, gastrointestinal tract, and skin, is rapidly distributed to all tissues, and readily passes through cellular membranes.[2] The amount of Toluene absorbed is proportional to the concentration in inspired air, length of exposure, and pulmonary ventilation. Toluene can be detected in human blood as soon as 10 sec post-exposure. Toluene (100 ppm) absorption via inhalation was determined to be 1.6 mg/min in a study performed in humans. Pulmonary absorption of Toluene in cross-bred dogs within 1 h of exposure to 700, 1500, and 2000 ppm Toluene was determined to be 25, 56, and 74 mg/kg, respectively. Because Toluene is lipophilic, it accumulates in tissues with high fat content. In one study, the half-life of Toluene in human adipose tissue ranged from 0.5 to 2.7 d. Following a 3-h inhalation exposure to 3950 ppm (15 mg/l) Toluene, approximately 626 mg/kg, 420 mg/kg, and 200 mg/kg Toluene reached the liver, brain, and blood of mice, respectively. In a study performed in rats, concentrations of radioactivity following a 10-min exposure to 4600 ppm 4-[^{3}H]Toluene were highest in white adipose tissue, followed in order of decreasing concentration by brown adipose tissue, adrenals, stomach, liver, kidney, brain, blood, and bone marrow. Similar distribution has been reported in rats administered Toluene via oral exposure. Radioactivity present as a volatile compound (likely unchanged Toluene) was observed in the brain and adipose tissue of rats given an intraperitoneal injection of 0.2 mg/ml Toluene. Most of the radioactivity detected in the liver and kidneys was non-volatile. Toluene is predominantly metabolized in the liver. The majority of absorbed Toluene (approximately 84%) metabolizes into benzoic acid, and is excreted as hippuric acid, benzoylglucuronic acid, benzylmercapturic acid, and cresol derivatives. In one study, approximately 16% of absorbed Toluene was expired unchanged through the lungs, whereas 80% was oxidized to benzoic acid and excreted in the urine. Urine of humans exposed to 50 and 800 ppm Toluene for 8 h contained 59% hippuric acid and 41% benzoyl glucuronide. Excretion of these metabolites increased with exposure to the higher concentration of Toluene.

Blood concentrations of Toluene in rats post-oral and inhalation (up to 867 mg/kg or 1000 ppm (6-h exposure)) were compared.[3] The relationship between the two routes of administration were described by the equation: natural logarithm (ln) (oral mg/kg) = 1.27 x ln (inhalation ppm) – 9.22. In a similar study in which rats were exposed to up to 911 mg/kg Toluene (oral) or up to 1145 pp, Toluene (3-h inhalation exposure), the relationship between the two exposure was determined by the equation: ln (oral mg/kg) = -1.44 + 0.95 ln (3 h inhalation ppm).

According to several studies, enzymes responsible for the metabolism of Toluene include CYP2B1, CYP2B2, CYP2B6, CYP2C6, CYP2C8, CYP2C11, CYP1A1, CYP1A2, and CYP2E1.[3] Metabolism of Toluene results in the production of benzyl alcohol, o-cresol, p-cresol, and hippuric acid. A peak blood level of approximately 14 µg/g Toluene occurred 1 h after administration of 0.5 g/kg Toluene to rabbits. In a study evaluating the distribution of Toluene in rat brains, the highest concentration of Toluene was found in the brain stem. The effect of concentration and acute and chronic inhalation exposure to Toluene (up to 0.4 ml; up to 30 d) in rat pups was evaluated. Concentrations of Toluene in the brain, blood, and liver of rats increased with increased exposure levels in rat pups; however, no significant differences were observed in Toluene concentrations in tissues in rats acutely exposed versus chronically exposed. The effect of age, sex, and pregnancy on

cytochrome p450-mediated metabolism was evaluated in rat livers (in vitro exposure up to 5 mM for 10 min). Production of benzyl alcohol increased in a dose-dependent manner in all liver types. Mature females had lower benzyl alcohol production compared to mature males or immature females. Day 21 pregnant rats had lower benzyl alcohol production than day 10 pregnant rats or mature non-pregnant females. Similarly, benzyl alcohol was observed in higher concentrations in males in a different study evaluating Toluene metabolism in rat livers. In studies performed in humans, increased excretion of hippuric acid and o-cresol was apparent in subjects exposed to Toluene via inhalation when exercising, versus at rest. Mean blood and alveolar air concentration of Toluene was determined to be 5.9 nmol/l and 310 nmol/m^3, respectively, in an assay in which subjects were exposed to 50 ppm radiolabeled Toluene for 2 h.

According to studies performed in humans, factors that have an effect on Toluene metabolism and excretion include drugs/other chemicals (e.g., paracetamol, acetylsalicylic acid), ingestion of ethanol, fasting/diet changes, changes in water intake, genetic polymorphisms, and mask-wearing.[3]

Details of the ADME studies summarized here are found in Table 4. Toluene is rapidly absorbed via inhalation, with a total absorption of approximately 50%.[25] When Toluene is orally ingested, the gastrointestinal channel absorbs it almost completely. Toluene that is absorbed into the blood is widely distributed throughout different parts of the body. Reproductive effects may occur following Toluene exposure as it easily passes through the placenta and is secreted into breast milk. It should be noted that Toluene biotransformation may lead to the formation of Toluene epoxides, which may generate reactive oxygen species that can cause oxidative stress and DNA damage.[26]

An assay evaluating pharmacokinetic parameters following Toluene (up to 1 g/kg in corn oil; gavage) administration in rats of different ages (4, 12, and 24 mo) was performed.[27] Blood Toluene concentrations were unaffected by age; however, brain Toluene concentrations were significantly higher in 24-mo-old rats vs. 4 mo-old rats (concentrations 50% higher in 24-mo-old rats). Mean blood concentrations of Toluene in male Norway rats following a 6-h inhalation exposure period were 0.01, 0.33, and 11.84 µg/g, after exposure to 5, 50, and 500 ppm Toluene, respectively (on day 1 of treatment).[28] Mean blood concentrations of Toluene over the 4 collection times (day 1, 5, 10, and 20) were approximately 0.04, 0.35, and 11.62 µg/g, in animals treated with 5, 50, and 500 ppm. Respectively. Pregnant Sprague-Dawley rats exposed to Toluene (8000 or 12,000 ppm; gestation days (GD) 8 – 20; 15, 30, or 45 min whole body exposure) displayed increased Toluene levels in saphenous blood in a concentration- and time-dependent manner.[29] Toluene levels also increased in fetal brains in a concentration-dependent manner, and in placenta and amniotic fluid in a time-dependent manner. The highest mean concentrations of Toluene in maternal saphenous blood, fetal brains, and placenta were determined to be 11, 7.3, and 10.5 ppm, respectively. The effect of temperature on absorption and excretion of Toluene (50 ppm; inhalation exposure) in humans was evaluated in 5 subjects.[24] Results suggested that absorption of Toluene is increased and elimination is decreased in the presence of heat. The maximum venous blood amount of Toluene observed in this study was 0.389 mg/l (measured 2 h into exposure; 30°C).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute dermal median lethal dose (LD_{50}) of Toluene in rabbits was determined to be 14.1 ml/kg.[2] *No deaths were observed in an acute percutaneous assay in which guinea pigs were dosed with 1.732 g/kg Toluene. Acute oral LD_{50}s of Toluene in rats ranged from 2.6 g/kg to 7.53 g/kg. No toxic effects were observed in studies performed in rats given nail products containing 33 - 33.2% Toluene via gavage. Acute inhalation LD_{50}s were determined to be 5320 ppm and 6942 ppm in two studies performed in mice (6 - 7h exposure period). Acute inhalation studies performed in mice, rats, guinea pigs, rabbits, and dogs resulted in adverse effects including mucous membrane irritation, motor incoordination, prostration, changes in respiratory rate, changes in blood serum and enzymes, elevated blood glucose and packed cell volume, decreased body weight, and death (adverse effects reported at concentrations as low as 1250 ppm). Effects varied according to animal species, length of exposure, and concentration of Toluene administered. Mortality was prevalent in several acute subcutaneous, intraperitoneal, and intravenous studies performed in mice, rats, guinea pigs, and rabbits (animals given 0.17 – 8.7 g/kg Toluene).*

Short-Term Toxicity Studies

Progressive symptoms were observed in several species of animals following short-term inhalation of increasingly higher concentrations of Toluene (1 – 12,000 ppm) including irritation of mucous membranes, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death.[2] *In a study in which rats were given Toluene (1 ml/kg/d) via subcutaneous injection for 21 d, adverse effects were observed (e.g., decreased body weight, decrease in erythrocyte and leukocyte counts, focal hepatic necrosis). Similarly, adverse effects (polypnea, necrosis at injection sites, hemorrhagic, hyperemic, and degenerative changes in lungs, kidneys, adrenal glands, and spleen) were observed in guinea pigs given Toluene (0.25 mg/d) subcutaneously for 30 - 70 d. Rabbits treated subcutaneously with Toluene (1 ml/kg/d) for 6 d developed granulopenia and granulocytosis.*

The effect of Toluene on body weight and pathological changes in the heart, lung, stomach, and spleen tissues of New Zealand rabbits (6/group) was evaluated.[30] Animals were exposed to Toluene (1000 mg/l) in an exposure chamber for 8 h/d for 14 d. A control group was left untreated. Body weight in the Toluene-treated groups dropped initially and recovered by day 14 post-exposure. Relative organ tissue weights were similar among control and treated groups. Adverse effects in

organs observed in treated rats include slight fibrotic necrosis in cardiac tissues, lymphocyte infiltrates in lung tissue, congestion with local degenerative changes in lung tissue, lymphocyte infiltrates near the hilum pulmonis, indiscernible emphysema in alveoli, pyknotic cells in gastric pits, swelled gastric glands, congestion between mucosa and submucosa in gastric tissue, and enlarged lymphoid tissue and lymphocyte proliferation in the spleen. These effects were not seen in untreated control animals.

Subchronic and Chronic Toxicity Studies

No significant test substance-related effects were observed in a 6-mo assay in which rats were given up to 590 mg/kg Toluene per day, via gavage.[2] *No major toxicological effects were observed in chronic inhalation toxicity assays performed in rats exposed to Toluene (up to 1481 ppm). Toxic effects (e.g., nasal/ocular irritation, motor incoordination, lung congestion, liver hemorrhage, death) were observed in dogs exposed to Toluene (2000 – 2660 ppm) via inhalation for 6 mo.*

Studies were performed in mice and rats given Toluene (312 – 5000 mg/kg/d; in corn oil; 13 wk) via gavage.[3] *High doses resulted in death, increases in organ weights (e.g., liver, kidney, heart), dose-dependent necrosis of the brain, and hemorrhage of the urinary bladder.*[3] *Death and increased organ weights were also observed in 13-wk studies in which mice and rats were exposed to Toluene (100 – 3000 ppm) via inhalation. Hyperplasia and erosion of respiratory epithelia was observed in mice and rats exposed to Toluene (up to 1200 ppm) for 2 yr.*

The following study published prior to 2005 has been summarized here in unitalicized text as it is referenced in the margin of safety (MOS) calculation section of this report (this study is not present in the summary section of this report). A 13-wk study was performed in Fischer rats (10/sex/dose) given Toluene (purity: > 99%) in corn oil via gavage in doses of 312, 625, 1250, 2500, or 5000 mg/kg/d.[7] Control animals received the vehicle only. All rats given 5000 mg/kg/d died within the first week of treatment. One female and 8 males in the 2500 mg/kg/d group died before termination of the study. Final mean body weights in males that received 2500 mg/kg/d was 19% lower than vehicle controls. Clinical signs in the high-dosed animals included prostration, hypoactivity, ataxia, piloerection, lacrimation, and excessive salivation. Increased organ weights were observed at doses as low as 625 mg/kg/d; however, increased liver and kidney weights were unaccompanied by histopathological findings at doses of 625 mg/kg/d and lower, and were therefore interpreted as toxicologically non-significant at these doses. No abnormalities were observed upon serum chemistry evaluation or urinalysis. Neuropathological changes in the brain (i.e., neuronal cell necrosis, necrosis/mineralization in granular layer of cerebral cortex) were seen in male and female rats given 1250 or 2500 mg/kg/d. Hemorrhage was apparent in the urinary bladder of males and females in high-dose groups. The no-observed-adverse-effect-level (NOAEL) for repeated dose oral toxicity was considered to be 625 mg/kg/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Teratogenicity and embryotoxicity of Toluene were assessed in hamsters, mice, and rats via various routes of exposure (dermal, oral, inhalation).[2] *Minimal embryotoxic effects were observed in a dermal assay in which Toluene (concentration not stated) was applied to the clipped skin of hamsters on days 7 - 11 of gestation. Toluene was teratogenic at 1.0 ml/kg and embryotoxic at 0.3 ml/kg in mice given Toluene via gavage on days 12 – 15 of gestation. No significant reproductive toxicity was observed in an assay in which Toluene in corn oil (10 ml/kg bw) was given to pregnant mice for 8 d (GD not stated). Rudimentary 14th ribs were observed in pups of females exposed to Toluene (1000 ppm) via inhalation on GD 1 - 17. Adverse effects in fetuses, such as low weight (observed in pregnant mice treated with 133 ppm Toluene on GD 6 - 13) and skeletal abnormalities (observed in pregnant rats treated with 266 -399 ppm Toluene on days 1 - 8 or 9 – 14 of gestation), were observed. Toluene was not teratogenic in fetuses of rats exposed to up to 400 ppm Toluene vapor on days 6 - 15 of gestation or in fetuses of rats exposed to 266 ppm Toluene on days 7 – 14 of gestation.*

An NOAEL for embryotoxicity was determined to be 1.46 µmol/ml in an assay in which embryos were exposed to Toluene.[3] *Toluene (8.67 µg/ml) in a culture medium decreased sperm motility, inhibited in vitro fertilization, and increased preimplantation embryo degeneration. Decreased maternal weight gain and generalized growth retardation of fetuses was observed in assays in which dams were given Toluene via gavage (520 - 650 mg/kg bw; GD 6 - 19; in corn oil). Increased occurrence of pups with low birth weights and adverse effects relating to behavioral tasks in offspring were observed in an assay performed in rats and hamsters given 800 mg/m^3 Toluene via inhalation (6 h/d exposure; rats treated on GD 14 - 20 and hamsters treated on GD 6 - 11). Effects on maternal and fetal parameters were evaluated in well-nourished and malnourished rats given 1.2 g/kg Toluene in corn oil via subcutaneous injection (on GD 8-15 or GD 14 - 20). Adverse effects observed in well-nourished rats include decreased maternal body weight/weight gain, reduced pup weights, and reduced brain weights. Malnourished rats displayed extensive adverse effects (e.g., decreased number of ossification centra, increased fetus deaths, death during labor). No effect was observed on sperm motility of male offspring of pregnant rats exposed to 1200 ppm Toluene via inhalation on GD 7 throughout gestation, and daily after parturition to postnatal day 8. Absolute and relative testes weights were significantly reduced in the pups of Toluene-exposed maternal rats (1800 ppm; GD 7 - 20). A significant increase in the number of apoptotic cells in the cerebellar granule layer of the hippocampus was also observed in this study. Decreases in the volume of the granule cell layer, the hilus, and the commissural-association zone of the hippocampus were observed in rat pups exposed to Toluene via inhalation (100 - 500 ppm; exposed on postnatal days 1 - 28). The neurosomatic development and behavioral effects of Toluene exposure via maternal milk in rat pups was evaluated (lactating rats given injection of 1.2 g/kg Toluene on lactation day 2 to 21 (type of injection not stated)).*

According to the Scientific Committee for Toxicity and Ecotoxicity and the Scientific Committee on Consumer Products, Toluene is considered a reproductive category 3 toxicant (possible risk of harm to unborn child).[16] Similarly, Toluene is classified as hazardous with hazard category 'reproductive toxicity category 1A (may damage fertility or the unborn child)' in the Hazardous Chemical Information System (HCIS) of Safe Work Australia.[31] Toluene is also regulated by the State of California as a developmental toxicant under Proposition 65.[32] Maximum allowable doses of 7000 µg/d (oral) and 13,000 µg/d (inhalation) were established based on protection against adverse developmental effects (if exposure to Toluene from products results in amounts less than these limitations, the product is exempt from Proposition 65 requirements).

Details of the developmental and reproductive toxicity studies summarized here are found in Table 5. Gravid female rats were dosed with 1250 mg/kg Toluene in peanut oil by gavage on days 16 – 19 of gestation, and killed on GD 20.[33] Maternal and reproductive parameters were not affected, and there was no significant difference in the number of fetal external or skeletal malformations between the treatment and the control group. However, a pattern of accelerated development in the upper mid-turn in the treated fetal cochleas was observed.

Gravid female rats (GD 14) and male offspring (post-natal day (PND) 2 or 8) were treated with 5 or 50 ppm Toluene for 5 d via inhalation.[34] Up-regulation of N-methyl-d-aspartate (NMDA) receptor subunits, cyclic adenosine monophosphate (AMP) responsive element binding protein (CREB1), calcium/calmodulin-dependent protein kinase (CaMKIV), and apoptotic-related genes were observed in treated offspring, but not in maternal rats. A no-observed-adverse-effect-concentration (NOAEC) of 600 ppm was determined in male rats and an F1 generation in a study evaluating fertility.[7] Anti-nociception and effects on memory and locomotion were observed in rats subjected to acute, pre-natal, and post-natal exposure to 6000 ppm Toluene (acute exposure: 30 min exposure in 30-d old rats; pre-natal exposure: 2 x/d exposure on GD 8 - 10 in pregnant rats; post-natal exposure: 2x/d exposure on PND 22 - 30 in male F1 pups).[35] In an inhalation study in which mice were exposed to 8000 ppm Toluene for 30 min twice daily via inhalation on GD 7 - 19, neonatal death was significantly increased in the test group compared to controls.[36] In a study in which rats were exposed to Toluene (500 or 1500 ppm) for 6 h/d on days 6 - 20 of gestation and killed on day 21 of gestation, maternal weight gain of the test animals and fetal body weights in the 1500 ppm group were decreased; no other reproductive or developmental effects due to dosing were observed.[37] In another study in which dams were exposed to up to 3000 ppm Toluene via whole-body inhalation for 6 h/d on days 6 – 15 of gestation and killed on day 20 of gestation; the maternal toxicity NOAEL was 750 ppm with a defined maternal and developmental toxicity lowest-observed-adverse-effect-level (LOAEL) of 1500 ppm.[38] There were no effects on reproductive parameters; fetal body weighs were increased in a toxicologically significant manner at 1500 and 3000 ppm.

Several inhalation studies were conducted in rats using short-duration (15 or 30 min; twice daily; various exposure times, including post-natal exposures) high-dose (up to 16,000 ppm) exposures, and effects on post-natal development were evalauted.[39-44] Generally, there were no significant maternal effects, although decreased body weight gains were observed with doses ≥ 8000 ppm Toluene. Fetal malformations were observed at some of the highest doses (> 8000 ppm), and there were indications of impaired cognitive function (at concentrations as low as 1800 ppm).

Female rats were exposed to Toluene (2000 - 8000 ppm) via inhalation, 30 min/d for 28 d, and the effect on of progesterone, estradiol, testosterone, and insulin-like growth factor 1 (IGF-1) levels and ovarian tissue was examined.[45] Progesterone levels (at 4000 and 8000 ppm) and testosterone levels (all dose groups) were statistically significantly increased and IGF1 was significantly decreased (at 8000 ppm); no effect on estradiol was noted. A dose-dependent increase in apoptosis in ovarian tissue was observed. In another study, gravid rats were exposed to 0.09 – 9 ppm Toluene via nasal inhalation for 90 min/d on days 14.5 – 18.5 of gestation; hormone levels (all groups) and messenger RNA (mRNA) levels of steroidogenic enzymes in testicular tissues (control and low-dose group) were measured in male pups.[46] Fetal plasma testosterone concentrations were significantly reduced in males of the 0.9 and 9 ppm, but not the 0.09 ppm, groups. mRNA levels of 3β-hydroxysteroid dehydrogenase (3β-HSD) were significantly reduced after exposure to 0.9 ppm. Effects on immunological biomarkers were also examined in pups following whole-body inhalation exposure of dams to 5 or 50 ppm Toluene for 6 h/d on days 14 – 18 or 19 of gestation, followed by post-natal exposure to groups of pups at various post-natal time frames.[47,48] In one study, total plasma immunoglobulin G2a (IgG2a) levels were statistically significantly increased in the 50 ppm group, and in another study, total IgG1 levels were markedly reduced. Splenic expression of some transcription factors was suppressed.

The following study published prior to 2005 has been included here in unitalicized text as it is referenced in the MOS safety calculation section of this report (this study is not present in the DART table or summary section of this report). The reproductive toxicity of Toluene was assessed in a 2-generation assay using Sprague-Dawley rats (n = 15 – 37/group).[49] Male and female rats from the parental (F0) generation were exposed to Toluene (purity: 99.9%) for 6 h/d, 7 d/wk, for 80 d pre-mating and 15 d of mating at concentrations of 0, 100, 500, and 2000 ppm (i.e., 0, 375, 1875, and 7500 mg/m^3). Pregnant females at all dose levels were exposed from GD 1 - 20 and lactation day 5 - 21. F1 pups designated to produce the F2 generation were treated for 80 d starting immediately post-weaning (lactation day 21) and initially mated at a minimum of 100 d of age. F2 pups were not subjected to inhalation exposure to Toluene. Toluene exposure did not induce adverse effects relating to fertility, reproductive performance, or maternal/pup behavior during the lactation period in males and females of the first generation, but did inhibit growth in F1 and F2 offspring in the 2000 ppm (both sexes treated) and 2000

ppm (females only treated) groups. The study concluded that the Toluene offspring NOAEL is 500 ppm in groups in which maternal animals were exposed, and 2000 ppm for male only treated groups.

GENOTOXICITY STUDIES

Toluene was negative for mutagenicity in a battery of mammalian cell and whole organism test systems.[2] *Microbial assays were also negative for mutagenicity. Single DNA strand breakage in rat hepatocytes evaluated in vitro (exposed to 2.3 mM Toluene) and increased chromosomal aberrations/damaged chromosomes in rats exposed to Toluene via subcutaneous injection (animals exposed to up to 0.8 - 1 g/kg/d for 12 d) and inhalation (animals exposed to 80 - 300 ppm for 15 wk – 4 mo) were apparent.*

Toluene did not induce dominant lethal mutations in an assay performed using male rats given Toluene in corn oil (346 and 692 mg/kg bw/d) via intraperitoneal injection.[3] *Treatments were performed on 5 consecutive days.*

Details of the genotoxicity studies summarized here are found in Table 6. Positive results were observed in in vitro genotoxicity assays including an Ames assay (using *Salmonella typhimurium* strains at up to 50 µl/plate,[50] a comet assay (using human skin disks exposed to up to 100% Toluene vapor),[51] a modified alkaline comet assay (using human lung epithelial carcinoma cells at 0.25 ppmv),[52] a mammalian cell mutagenicity assay using mouse lymphoma cells (up to 500 µg/ml).[53] Conversely, no genotoxicity was observed in other in vitro assays including an Ames assay (using *S. typhimurium* strains at up to 1000 µg/ plate), a chromosomal aberration assay (using Chinese hamster ovary cells at up to 1600 µg/ml), and a sister chromatid exchange (SCE) assay (using Chinese hamster ovary cells at up to 5000 µg/ml).[53] The majority of these in vitro assays were performed with and without metabolic activation. Similarly, mixed results were observed in in vivo genotoxicity assays. Toluene was genotoxic in an alkaline and neutral comet assay using *Drosophila* larvae given up to 100.0 mM Toluene via diet,[54] a comet assay using mice given a 5 or 15 g/kg intraperitoneal injection of Toluene,[26] and a comet assay using mice exposed to Toluene (25 ppm) via inhalation for 4 wk. Negative results were observed in a micronucleus assay using mice exposed to up to 2000 mg/kg Toluene (method of administration not stated)[53] in a bone marrow nucleus assay using mice exposed to 100 ppm Toluene via inhalation for 15 d.[55]

CARCINOGENICITY STUDIES

No tumors were observed in several studies in which Toluene (concentration not stated) was applied topically to mice (applications length ranged from 50 wk to lifetime).[2] *Toluene was not considered to be tumor-promoting in studies in which Toluene (concentration not stated) was applied to the ears following initiation with 7,12-dimethylbenz[a]anthracene. Two of 30 animals developed skin tumors (one squamous cell carcinoma and one squamous cell papilloma) when mice were topically treated with Toluene (1 - 20 µl) for 72 wk. According to one study, tumor promotion in the skin of mice by Toluene is associated with the ability of Toluene to induce epidermal hyperplasia (no other details provided).*

Oral

A carcinogenicity assay was performed in Sprague-Dawley rats (40 - 50/sex/group) given Toluene (purity: 98.34%) in olive oil via gavage for 4 d/wk for 104 wk (2-experiment study).[56] In experiment one, animals were treated with 500 mg/kg bw, and in experiment 2, animals were treated with 800 mg/kg bw (treatment duration and methods same for both experiments). Animals were kept under observation until natural death or 130 wk. Control animals were given the vehicle only. After natural death or killing, animals underwent systemic necropsy, and histopathology was performed. In experiment 1, statistically significantly increased numbers of total malignant tumors (in both males and females), subcutaneous malignant tumors (in males), malignant mammary tumors (in females), and hemolymphoreticular neoplasias (in females) were observed in treated animals versus controls. In experiment 2, a statistically significant increased incidence of total malignant tumors and carcinomas of the oral cavity, lips, and tongue were observed in both male and female rats (compared to controls).

Inhalation

The potential carcinogenicity of inhaled Toluene (purity: > 99%) was evaluated in F344/N rats (60/sex/group) and B6C3F1 mice (60/sex/group).[53] Rats were exposed to Toluene levels of 600 or 1200 ppm, and mice were exposed to Toluene levels of 120, 600, and 1200 ppm (exposure for 6.5 h/d, 5 d/wk; chamber inhalation; unexposed chamber controls). Ten animals per group (except male mice) were removed for evaluation after an exposure period of 15 mo. All other animals were exposed to Toluene for 103 wk. Animals were killed and necropsied following exposure periods. No Toluene-related increases in the number of neoplasms were found in mice or rats during 15-mo or 103-wk studies.

OTHER RELEVANT STUDIES

Cytotoxicity

Cytotoxicity to Toluene in several cell types was observed when evaluated in vitro.[3] *These cells include rat and rabbit pulmonary alveolar macrophages (exposed to 0.02 – 1.0 mM Toluene), glioma (C6) cells (exposed to ≥ 12,000 ppm Toluene), astrocyctes (exposed to 4.7 – 150 µmol/ml Toluene), and mouse fibroblast L929 cells (exposed to 50 – 500 ppm Toluene). In addition to cytotoxicity, in vitro assays revealed certain toxic effects including impaired platelet agglutination, inhibition of*

NMDA currents in oocytes, depression of muscarinic signaling, and inhibition of acetylcholine and γ-aminobutyric acid in human IMR-32 neuroblastoma cells.

The effect of gaseous Toluene on human lung epithelial carcinoma cell line A549 was evaluated in vitro.[52] *Plated cells were exposed to gaseous Toluene (0.25 ppmv; balanced with nitrogen) for 24 h. Control cells were either exposed to synthetic air (80% nitrogen, 20% oxygen) only or left in a carbon dioxide incubator. Cell viability was evaluated by quantifying the amount of lactate dehydrogenase (LDH) released from cells upon damage of the cytoplasmic membrane. Intracellular reduced and oxidized glutathione were also measured in exposed cells using a modified enzymatic recycling method. Differences in amount of released LDH were not statistically different between control groups and the treated group. In addition, effects on glutathione redox status were similar in control and treated groups.*

Effects on Respiratory Tract

Two male subjects exposed to Toluene for 7 - 8 h via inhalation developed transitory mild throat and eye irritation at 200 ppm and lacrimation at 400 ppm.[2] *No other details were provided*.

The effect of long-term, low-level inhalation exposure to Toluene (50 ppm; 6 h/d, 5 d/wk, 6 or 12 wk; whole-body exposure chamber) on airway inflammatory responses was evaluated in female C3H mice (10/group).[57] A control group was exposed to air only. One day after the final Toluene exposure, bronchoalveolar lavage (BAL), spleen, and blood samples were collected. Lungs were also collected for histological analyses. BAL fluid was analyzed for cytokines, chemokines, neurotrophins, and substance P. The total number of inflammatory cells and macrophages significantly increased in both 6- and 12-wk exposed mice compared to controls ($p < 0.05$). The production of interferon-gamma and substance P was significantly decreased in both 6- and 12-wk exposed mice compared to controls ($p < 0.05$). Nerve growth factor was not affected by Toluene treatment. Neurotrophin-3 production in BAL fluid was significantly increased in 12-wk exposed mice only, compared to controls ($p < 0.05$).

The potential for Toluene to elicit microvascular leakage in rat airways was evaluated in male SPF Wistar rats (5/group).[58] Airway microvascular leakage and bronchoconstriction was evaluated in rats treated with 18, 30, 50, 135, or 450 ppm Toluene (control animals exposed to formaldehyde (positive) or clean air (negative), via inhalation, for 10 min. Airway microvascular leakage was also evaluated in rats during 3 consecutive 10-min periods of Toluene inhalation (50 or 135 ppm). Microvascular leakage was evaluated using blue dye injected into the right external jugular vein prior to provocation. The content of the blue dye that extravasated into the tissues was measured as an index of plasma leakage. Toluene exposure induced dye leakage into the trachea and main bronchi in a concentration-dependent manner. Toluene at concentrations of ≥50 and ≥ 30 ppm caused significant responses in the trachea and main bronchi, respectively, which peaked after exposure to 135 ppm for 10 min. Responses were statistically significant compared to the control groups. Further testing revealed that Toluene-induced plasma leakage is predominantly mediated by tachykinins endogenously released from airway sensory nerves.

Effect on Visual Function

Male rats were exposed to Toluene (1000 ppm; 21 h/d; 6 - 11 wk) and functions of the vestibule- and opto-oculomotor systems were tested 1 mo after exposure (via recording of nystagmus induced by vestibular or optokinetic stimuli).[3] *Optokinetic gain in exposed animals were reduced compared to untreated controls. A slight reduction in gain during sinusoidal oscillatory vestibular stimulation was also observed.*

Effect on Lipid Levels

Increased levels of free fatty acids, triglycerides, cholesterol, phospholipids, and blood glucose were observed in rabbits following a single dose of Toluene (0.5 g/kg) in olive oil via gavage.[3] *Significant decreases in basal phospholipid methylation were observed in rats given Toluene (1 g/kg) via intraperitoneal injection ([^{3}H]methionine as methyl donor). These effects were not seen when [3H]adenosylmethionine or S-adenosylmethionine synthetase were used as methyl donors. Toluene produced no change in either phospholipid or cholesterol content of rat pulmonary microsomal membranes when evaluated using thin-layer chromatographic separation.*

Toluene-Induced Endocrine Disruption

Male Wistar rats (n = 9) were treated with Toluene (1500 ppm; 4 h/d; 7 d) via inhalation.[59] Control rats were treated with air in separate chambers. Body weights and adrenal gland weights were evaluated following the last exposure. Microscopic evaluations of the adrenocortical cells, immunohistochemical analysis, analysis of mRNA levels, plasma adrenocorticotropic hormone (ACTH), and serum cortisone levels were evaluated. Body weights had increased significantly less than in controls after Toluene exposure ($p < 0.05$). In addition, a significantly increased adrenal gland weight (left and right combined) and adrenocortical cell size was observed in Toluene-treated rats compared to controls ($p < 0.05$). Hypertrophy of the cortex was observed in the Toluene-exposed group. Immunohistochemical staining revealed aldosterone-positive cells localized within the zona glomerulosa; however, a clear difference between the control and treated groups was not seen. Expansion of the corticosterone-positive area consistent with cortical hypertrophy was observed in the treated group. No obvious difference between control and treated groups were observed in anti-proliferating cell nucleus antigen-immunostaining. Enhancement of the corticotropin-releasing factor expression was seen in the paraventricular nucleus (PVN) of the hypothalamus in treated animals. ACTH concentrations were significantly increased in treated animals

compared to controls ($p < 0.05$), and corticosterone levels were insignificantly elevated. Cytochrome side-chain cleavage mRNA levels in the inhalation group were significantly higher (1.3-fold) compared to the control group.

Bone Mass Toxicity

The bone mineral density and content of the femoral neck of male Swiss albino mice (10/group; 1 control group untreated) exposed to Toluene (300 ppm; purity: 99.9%; full-body inhalation chamber; 6 h/d) for 8 wk was evaluated via X-ray absorptiometry.[60] Bone mineral density and bone mineral content were determined to be 0.008 ± 0.005 g/cm^2 and 0.011 ± 0.006 g in the treated group, respectively and 0.190 ± 0.007 g/cm^2 and 0.020 ± 0.009 g in control animals, respectively. Bone mineral density and bone mineral content were significantly lower in treated versus control groups ($p < 0.05$).

Ototoxicity

Ototoxicity was observed in several studies using rats exposed to Toluene via inhalation (1000 - 8000 ppm; 8 d – 13 wk exposure).[3] Ototoxicity in these studies were measured via brainstem audiometry, auditory sensitivity, neurologic testing, flash evoked potential test, cortical flicker fusion test, auditory brainstem response to clicks test, auditory brainstem response to tone-pips at 10 kHz and 30 kHz test, somatosensory-evoked potentials test, caudal nerve action potentials to single and paired stimuli tests, morphological investigations of the cochlea, electrocochleographic testing, and electrophysiological testing. Permanent hearing loss was observed in a study in which guinea pigs were given 1000 ppm Toluene via inhalation (5-d exposure). Toluene did not produce ototoxic effects when evaluated in chinchillas given 2000 ppm Toluene via inhalation (10-d exposure) and a 95 A-weighted decibels 500 Hz band noise (auditory brainstem response evaluated).

The effect of Toluene exposure on the hearing of male albino guinea pigs (5 - 7/group) was evaluated.[61] Guinea pigs were evaluated following Toluene exposure alone, or along with glutathione-depletion (induced via a low protein diet), and/or cytochrome p450 inhibition (induced via 2-diethylaminoethyl-2,2-diphenylvalerate-HCl (SKF525A) injection). Guinea pigs were exposed to Toluene vapor (1750 ppm, 6 h/d, 5 d/wk; whole-body exposure) for 4 non-consecutive weeks (animals were treated every other week to allow for recovery). Control animals were left unexposed. To inhibit cytochrome P450, some groups of animals were given a 50 mg/kg subcutaneous injection of SKF525A on the Mondays of treatment weeks. Auditory function was evaluated via electrocochleography and histological analysis. A statistically significant Toluene-induced hearing loss was provoked in cytochrome p450-inhibited guinea pigs on a normal diet, and in cytochrome p-450 inhibited guinea pigs on a low protein diet. Disrupted stria vascularis and spiral fibers in the apical coil of the cochlea were observed in animals with hearing loss. Hearing loss was similar among unexposed controls and guinea pigs treated with Toluene alone or in those treated with Toluene plus a low protein diet.

Toluene Abuse

Studies have been found in the literature indicating the toxic effects of Toluene following inhalation abuse (e.g., glue/spray paint sniffing).[3] Some of these effects include impaired mentation, memory, motor strength, gait, and neuropsychological function, auditory/visual disturbances, paresis, atrophy of various areas of the brain (e.g., cerebellum, brain stem), cardiovascular collapse, ventricular dilation, metabolic acidosis, renal injury, white matter changes, and death. Adverse effects in infants exposed to Toluene in utero due to maternal abuse have also been reported. These effects include growth impairment, developmental delays, hyperchloremic acidosis, mild language/speech impairment, dysmorphic physical features, microcephaly, and death.

Several reports of adverse effects following Toluene abuse (via inhalation) have been found in the literature.[62-71] These adverse effects include neurological symptoms (e.g., slurred speech, slowed response, confusion, uncontrolled laughing, disorientation, memory loss), tiredness, headache, blurred vision, hallucinations, tremors, dyspnea, convulsions, vomiting, renal, cardiac, and hepatic abnormalities/injury, adrenal dysfunction, metabolic alterations, hypokalemia, leukoencephalopathy, growth impairments, and death. Autopsies of fatal cases of Toluene ingestion revealed traumatic brain injury, hemorrhages, internal organ congestion, and hemorrhagic pulmonary edema.

Neurotoxicity

Many studies were found in the literature involving the effect of Toluene (administered via inhalation (50 - 3000 ppm; 2 h – 80 wk exposure)) on brain proteins and chemicals in rats.[3] These effects include a reduction of affinity and increase in density of the β-adrenergic antagonist [^{3}H]dihydroalprenolol binding sites in the frontoparietal cortex, increase of $^{45}Ca^{2+}$ uptake into unstimulated synaptosomes, inhibitory effects on acetylcholinesterase, adenosine triphosphatase, and magnesium activated adenosine triphosphatase, increase in activities of neurotransmitter-synthesizing enzymes, reduction in [^{3}H]neurotensin binding, increased binding of [^{3}H]etorphine and [^{125}I]vasoactive intestinal polypeptide, increase and decrease on activity of Ca^{2+}/Mg^{2+} adenosine triphosphatase (dependent on exposure time), increase in neuron-specific γ-enolase and glial marker proteins, increase in the number and intensity of tyrosine hydroxylase-immunoreactive fibers and terminals in the forebrain, depletion of striatal 3,4-dihydroxyphenylacetic acid, decreased glial fibrillary acidic protein in the thalamus, decreased acetylcholine release in the striatum, increased gamma-aminobutyric acid in the cerebellum, increased dopamine levels in the striatum, increase in tyrosine and tryptophan hydroxylation in certain catecholaminergic cell groups, and a decrease in the biosynthesis rate of 5-hydroxytryptophan in the ventro-median-hypothalamus. In a study performed in mice given Toluene (up to 405 mg/l in drinking water; 28 d exposure), an increase in endogenous levels of biogenic amine transmitters (norepinephrine, dopamine, and serotonin) was observed. Formation of reactive oxygen species within cortical synapses were observed in rats given 1 g/kg Toluene via intraperitoneal injection (researchers concluded that a metabolite of

Toluene (possibly benzaldehyde) was responsible for this effect). Elevated levels of reactive oxygen species were also observed in mitochondrial fractions of the lung, liver, and kidney tissue, and in crude synaptosomal fractions from the cerebellum, hippocampus, and striatum of rats given 1.5 g/kg Toluene in corn oil via intraperitoneal injection.

Effects on the sleep-wake cycle were observed in several studies in which rats were treated with Toluene (80 - 2730 ppm; 2 h – 3 wk exposure) via inhalation.[3] *Decreased spatial memory, increased locomotor activity and rearing behaviors, and reduced balance were observed in rats given 80 ppm Toluene for 4 wk. The test substance did not have an effect on dopamine agonist binding to dopamine receptors in this study. Increased levels of extracellular dopamine in the prefrontal cortex were observed in rats treated with 3000 ppm Toluene for 40 min. These effects were not observed in the nucleus accumbens. No neurobehavioral or gross pathological changes were observed 6 mo after rats were treated with up to1500 ppm (6 mo of treatment) Toluene. In addition, no neuronal loss was noted in this study. However, a statistically significant neuron loss (in the hippocampus) of 16% was observed in rats treated with Toluene (1500 ppm; 6 mo of treatment), compared to untreated controls. In an in vitro assay using guinea pig hippocampal slices, Toluene (0.2 ng/ml – 20 µg/ml) had both excitatory and inhibitory biphasic effects on neurotransmission. Toluene treatment (1.3 ml/kg/d; intraperitoneal injection; 4 d) reduced immunostaining of neuropeptide Y (an appetite stimulant) in the paraventricular nucleus and increased neuropeptide Y staining in the arcuate nucleus. Increased immunostaining of galanin (appetite stimulant) was also observed in both the paraventricular and arcuate nuclei.*

Several neurotoxic and behavioral effects were observed in studies performed in mice and rats treated with Toluene. These effects include decreased shock avoidance, central nervous system dysfunction, effect on gait, impaired operant/ regulating behavior, decreased rearing activity, increased narcosis, and decreased spatial memory/learning abilities (the majority of these studies were performed via inhalation at concentrations ranging from 105 - 8000 ppm). Intraperitoneal studies performed in rats suggest that Toluene may affect developing brains via the alteration in the function of NMDA and γ-aminobutyric acid$_A$ receptors (at 1 g/kg), and may increase seizure susceptibility (at 500 mg/kg).

Details of the neurotoxicity/behavioral toxicity studies summarized below are found in Table 7.

Numerous neurotoxicity and behavioral studies performed in animals were found in the literature, and several adverse effects caused by Toluene were noted. These effects include hypothalamus-pituitary adrenal (HPA) and hypothalamus-pituitary-thyroid (HPT) axes dysfunction, up- and down-regulation of the expression of NMDA receptor subunits, poor memory retention, poor spatial and learning performance, impaired reversal learning, white matter abnormalities, changes in locomotor activity, motor incoordination, impaired swimming ability, anti-nociception, changes in anxiety levels, decreased brain chemicals (e.g., dopamine, nerve growth factor), histopathological abnormalities of the brain, and decreased hippocampal neurogenesis.[36,34,72-75,62,76,77,35,41,78,19,79-81] The majority of these studies were performed in animals exposed to Toluene via inhalation at concentrations ranging from 5 – 16,000 ppm. Altered neuroplasticity was observed in an assay performed in 17 human subjects exposed to Toluene (peak of 200 ppm) via inhalation.[82] Exposure did not have an effect on cortico-spinal ability, intracortical inhibition, or learning.

Effect of Toluene on Oxidative Stress Markers in the Brain

The effect of Toluene (purity: 99.5%) on oxidative stress markers in the brain was evaluated in an acute (treatment with 0 or 1019 ± 14 ppm Toluene for 6 h) and subchronic (treatment with 0, 10 ± 1.4, 97 ± 7, or 995 ± 43 ppm Toluene; 6 h/d; 5 d/wk; 13 wk) inhalation studies using male Long-Evans rats (6/group).[83] Brains were dissected for evaluation within 30 min following exposure in the acute study, and 18 h after the last exposure in the subchronic study. Brain regions (frontal cortex, hippocampus, cerebellum, and striatum) were evaluated for oxidative stress parameters (total aconitase, protein carbonyls, glutathione synthetase, γ-glutamylcysteine synthetase, superoxide dismutase, total antioxidants, nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase-1, and nicotinamide adenine dinucleotide (NADH) ubiquinone reductase activities). A significant increase of NADH ubiquinone reductase, γ-glutamylcysteine synthetase, and glutathione reductase in the cerebellum and total antioxidants in the frontal cortex ($p < 0.05$) was observed in Toluene-treated animals in the acute assay. In the subchronic assay, dose-dependent increases in NADPH quinone oxidoreductase-1 activity were observed in the cerebellum. A similar increase was observed in NADH ubiquinone reductase activity in the frontal cortex, hippocampus, and cerebellum. Adverse effects of Toluene exposure on superoxide dismutase and total antioxidants were most prevalent in the striatum compared to other brain regions. Total aconitase activity was inhibited in the striatum and cerebellum, increased in the hippocampus, and unchanged in the frontal cortex. Protein carbonyls increased significantly in both the frontal cortex and cerebellum.

c-Fos in the Brain

c-Fos immunoreactivity in the brain following whole-body exposure to 5000 ppm Toluene vapor for 0, 5, 10, or 30 min was examined using groups of 7 - 8 male Sprague-Dawley rats.[84] Quantitative analyses revealed increases in c-Fos immunoreactivity in about one-third of the brain structures examined, with most of these structures significantly activated only after prolonged Toluene exposure. The majority of brain structures activated by Toluene were found in the forebrain and midbrain, with particularly pronounced activation in nuclei implicated in the processing of rewarding, emotional, and olfactory stimuli, and those controlling motor output.

Immunotoxicity

Immunotoxicity (impaired function of splenocytes and adversely affected interleukin-2 (IL2) synthesis) was observed in male mice given 405 mg/l Toluene in drinking water for 4 wk.[3] These effects, however, were not observed at lower dose levels (up to 80 mg/l). Spleens of 10 mice were observed in an assay in which animals were treated with 600 mg/kg Toluene in vegetable oil (method of administration not stated). The test substance was observed to stimulate splenic mast cell populations and inhibit other amino-containing structures 6 h post-dose.

To investigate the effect of Toluene inhalation on immune responses to ovalbumin (OVA), groups of 6 C3H/HeN mice were exposed to 0, 5, 50, or 500 ppm of Toluene for 6 h/d, 5 d/wk for 3 or 6 wk.[85] The allergic mouse groups were immunized with OVA intraperitoneally on days 0 and 7 and as an aerosol on days 21 and 23. One day after the final exposure, mice were killed and BAL fluid and lung, spleen and blood samples were obtained. Real time polymerase chain reaction (PCR) was used for analysis of expression levels of mRNA in the lung, fluorescence activated cell sorter analysis was used to examine splenic cell immunophenotypes, and enzyme-linked immunosorbent assay (ELISA) was used to analyze lung samples and determine plasma Ig levels.

Allergic mice exposed to Toluene for 3 wk did not exhibit any changes in their plasma, lung, or spleen samples. The lungs of mice exposed to 50 ppm Toluene for 6 wk (with and without OVA) were examined microscopically. As compared to controls, slight hyperplasia was observed in the bronchial epithelial cells of the 50 ppm non-allergic group; there was no accumulation of macrophages and neutrophils observed. In the allergic 50 ppm group, an increase in the hypertrophy and hyperplasia of the epithelial cells and mucus secretion in the lung was observed; no marked accumulation of inflammatory cells in the alveoli was noted. Histological changes and increased amounts of fibronectin were observed in the lungs of 50 ppm allergic mouse group. Exposure to Toluene for 6 wk did not increase the number of inflammatory cells in BAL fluid of non-allergic mice; however, the number of BAL cells was increased in the 50 (but not 500) ppm allergic mouse group. Exposure to 500 ppm significantly increased the expressions of transcription factors STAT3, STAT4 and STAT5a mRNAs in the spleens of non-allergic mice. In the allergic mouse group, the expressions of splenic STAT3, STAT4, STAT5a, STAT6, GATA3 and Foxp3 mRNAs were significantly enhanced following exposure to 50 ppm Toluene for 6 wk, but the expression of T-bet mRNA was not increased. Regarding the Th1/Th2 balance, the expressions of IL-4 and IL-12 mRNAs were enhanced in the spleens of the 50 ppm allergic mouse group. Splenic immunophenotypes were not affected in any of the mice exposed for 6 wk. Total IgG1 antibody production in the plasma was significantly increased in the 50 ppm allergic mouse group, but not the other groups; IgE and IgG2a levels were not affected. The majority of these effects were not observed in a statistically significant manner at the lowest concentration tested.

The pathological effects of inhaled Toluene (0 (control), 1000, 2000, or 4000 ppm; purity ≥ 99.5%) exposure to the lung and brains of male Swiss-Webster mice (n = 68 (number of animals per group not stated); PND 28) were evaluated.[86] Animals received a single 30-min exposure in a static vapor exposure chamber. Lung and brain tissue were extracted 24-h post-exposure, and histology and immunochemistry were evaluated. Morphological abnormalities of the lung tissue (e.g., irregular cellular architecture) were observed in the 2000 and 4000 ppm groups. Markers of immune system activity and cellular proliferation in the lung revealed no significant differences between control and treated groups. Animals treated with 2000 ppm Toluene showed significantly increased astrogliosis in the striatum compared to controls ($p < 0.05$). This effect was also seen in animals treated with 4000 ppm; however, this was statistically insignificant ($p = 0.08$).

Effect of Toluene Inhalation on Olfactory Parameters

Female OF-1 mice were exposed to 1000 ppm Toluene via inhalation (full-body exposure in chamber) for 5 h/d, 5 d/wk, for 4 wk, and evaluated for olfactory changes each week during treatment and for each week for 1 mo. after treatment.[87] Structural modifications (density of cells and thickness of olfactory epithelium) were observed soon after the start of exposure. The number of cells did not change at the beginning of exposure (week 1 and 2), decreased markedly later (week 3 and 4), increased significantly the first week of the recovery period and stayed stable during the following weeks. A decrease in the thickness of neuroepithelium was observed at week 1, followed by an increase at week 2 and 3.

Hepatotoxicity

Hepatotoxic effects were evaluated in male Wistar albino rats (8/group) given a single dose of 6 ml/kg Toluene (use of vehicle not stated; purity: 99.5%) via gavage (control group given physiological saline).[88] Intracardiac blood samples were taken 3 h after administration and evaluated for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values. Liver tissues were excised and immunohistochemical and histopathological assessments were performed. In addition, a terminal transferase dUTP nick end labeling (TUNEL) assay was performed to determine if apoptosis was apparent in liver tissues. AST ($p = 0.026$) and ALT ($p = 0.005$) levels were statistically significantly increased compared to control groups. Slight degeneration of hepatocyte and mononuclear cell infiltration in liver tissue sections, and a high immunoreactivity for Bax and caspase-3 protein was observed in treated animals. Apoptotic cell numbers were statistically increased in the treated group compared to the control group ($p = 0.000$).

The hepatotoxic effects of noise and Toluene were evaluated in male New Zealand White rabbits (6/group).[89] Animals were exposed to Toluene (1000 ± 50 ppm (inhalation)) or noise (100 ± 5 dB) alone, or in combination. Treatment occurred for 8 h/d for 14 d. Blood samples were taken at 5 different times (immediately before exposure, immediately after exposure, and 3, 7 and 14d post-exposure). Serum levels of AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transferase

(GGT), superoxide dismutase, antioxidant -capacity, malondialdehyde, and glutathione peroxidase were determined. Histological analyses were also performed. Exposure to Toluene alone resulted in decreased levels of ALP, ALT, GGT, and superoxide dismutase, and increased levels of AST, catalase, and malondialdehyde. Exposure to both Toluene and noise resulted in increases in AST, ALT, GGT, malonaldehyde, total antioxidant capacity, and superoxide dismutase. Exposure to both Toluene and noise also resulted in a decrease in catalase and ALP levels. Histopathology revealed minor cell swelling, minor hepatic lipidosis, and eosinic cytoplasms in animals treated with Toluene only. Significant swelling and damage of the liver tissue were observed following exposure to Toluene and noise simultaneously.

Toluene-Induced Cardiotoxicity

Acute cardiotoxicity was evaluated in male Wistar albino rats (10/group) given 6 ml/kg Toluene (purity: 99.5%) via gavage (control animals given serum; composition of serum not stated).[90] Blood pressure, heart rate, blood samples, heart tissues, and serum troponin T levels were evaluated. Heart tissue sections were also evaluated for caspase-3-immunoreactivtiy and apoptosis in a TUNEL assay. Blood pressure and heart rate was significantly lower ($p < 0.05$), and troponin T levels were significantly increased ($p = 0.01$) in the treated versus control group. Heart tissue sections of Toluene-treated rats showed congestion and edema. Higher TUNEL positivity ($p < 0.01$) and immunoreactivity for caspase-3 protein were observed in the treated group compared to control group. In a different study, heart rate and blood pressure were evaluated in male Long-Evans rats given Toluene (0.4, 0.8, and 1.2 g/kg).[91] Toluene doses of 0.8 and 1.2 g/kg resulted in tachycardia and raised blood pressure.

Combined Effect of Toluene Exposure and Allergic Stimulation on Genotoxicity

The effect of allergic stimulation on genotoxicity in the brains of Toluene-exposed male Balb/c mice (n = 6/group) was evaluated.[26] Mice were exposed to Toluene (25 ppm) or air control for 6 h/d for 4 wk in a nose-only exposure chamber. To detect whether allergic conditions affect Toluene exposure, mice were immunized with a control (saline) or OVA on days 0, 14, 21, and 28, approximately 1 h before Toluene exposure. Aluminum hydroxide (2 mg) was also intraperitoneally administered on days 0 and 14. Mice were challenged with nebulized OVA as a booster on days 21 and 28 during the exposure period and killed following the last exposure. A Comet assay was performed to evaluate DNA damage in different regions after the brain and in leukocytes immediately after sacrifice. A significant increase of IgG1 in immunized mice subjected to OVA sensitization and challenge were observed. Significant DNA damage was observed in the hippocampus and leukocytes of OVA-immunized mice following Toluene exposure, compared to controls ($p < 0.05$). Results of the Comet assay performed in this study on mice chronically treated with Toluene and unexposed to OVA can be found in the Genotoxicity section of this report.

In Vitro Skin Viability Following Exposure to Toluene Vapor

Human skin disks (n = 3/group) were obtained and positioned in a glass vial. Toluene (0.01 – 100%; in corn oil) were pipetted at the bottom of the vial, avoiding direct contact with the skin, and vials were sealed off and incubated for 8 h.[51] Control skin samples were incubated with corn oil only. Skin viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In vitro skin exposures to Toluene resulted in statistically-significantly reduced cell viability, at all tested concentrations, in a dose-dependent manner, compared to controls.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Undiluted Toluene produced slight to moderate skin irritation in rabbits when evaluated in 4 different assays (single patch applications; majority under occlusive conditions).[2] Slight to moderate skin irritation and slight skin necrosis was observed in a study in which rabbits were exposed to 10 - 20 applications of undiluted Toluene over a 2 - 4 wk period (level of occlusion not stated). Minimal to moderate erythema was observed in rabbits after a single occlusive patch of a nail basecoat containing 33.2%. No irritation was observed in a single insult occlusive patch test performed in humans using the same basecoat. No irritation was observed in a 3-patch study (semi-occlusive conditions) in which rabbits were exposed to a nail polish containing 33% Toluene. This test substance was non-irritating and non-sensitizing in assays performed in humans (under occlusive conditions). Similarly, a nail polish containing 31.23% Toluene was not considered to be cumulatively irritating or sensitizing in assays performed in humans under occlusive conditions. No phototoxic or photoallergic reactions were observed in assays performed in humans using nail products formulated with 25 and 30% Toluene.

OCULAR IRRITATION STUDIES

Ocular irritation following exposure to undiluted Toluene without rinsing, and exposure to undiluted Toluene with rinsing was evaluated in rabbits.[2] Toluene was considered an irritant in non-rinsed eyes (irritation index = 22.67/110),and was considered a slight irritant in rinsed eyes (irritation index = 13.33/110). Slight irritation of the conjunctival membrane was observed after application of 2 drops of undiluted Toluene to rabbits. Severe ocular irritation was observed in a range-finding assay in which rabbit eyes were treated with a single dose of Toluene in propylene glycol, water, and/or deodorized kerosene (concentration of Toluene reported to be higher than 15%). A nail polish containing 33% Toluene was considered mild eye irritant in an assay performed in rabbits.

CLINICAL STUDIES

Case Reports

Non-Occupational

A 22-yr-old man experience extensive chemical burns, acute renal failure, and disseminated intravascular coagulation, that eventually led to death, after spilling a sealer containing 65% Toluene on his clothing.[3] A 60-yr-old man who consistently worked with glue containing 90% Toluene was admitted to the hospital with astenia and weight loss of 6 mo duration. Abnormalities upon diagnostic testing were found (e.g., cortical atrophy). Symptoms subsided following suspension of work.

A 65-yr-old male farmer with previously diagnosed arterial hypertension presented to the hospital following accidental consumption of approximately 150 ml of an organic solvent.[92] Initial symptoms post-consumption included tiredness, confusion, weakness, drunken-like actions, and gastrointestinal symptom. The patient reported severe chest pains approximately 40 min after ingestion. Toxicological analyses of the patient's blood indicated the presence of Toluene and xylene isomers. Elevated markers of myocardial necrosis were apparent. An echocardiogram scan showed hypokinesia within the apical segments of the lateral posterior, anterior, and inferior walls, with an ejection fraction of 38%. Angiography revealed a muscular bridge causing a 30-50% stenosis in the middle of the circumflex branch of the left coronary artery. Symptoms subsided during 4 d of hospitalization, and the patient was discharged.

A 31-yr-old woman with a history of anxiety and depression presented to the hospital with acute onset of generalized weakness and lower back and abdominal pain.[93] The patient reported worsening of the symptoms over the course of 6 d. Laboratory testing revealed hypokalemia, metabolic acidosis, and renal tubular acidosis, which were treated with oral and intravenous potassium and sodium citrate-citric acid. The patient's anion gap closed and bicarbonate level normalized upon treatment; however, the hypokalemia persisted. The patient denied paint or glue sniffing, but stated that she was exposed to paint, which was deemed the cause of symptoms. The patient's symptoms resolved during 2 d of hospitalization. A few weeks post-discharge, a Toluene test sent to an external laboratory during the patient's hospital stay reported a Toluene blood level of 4.12 mg/l.

A man in his 40s was found dead in his home shortly after spraying a wood coating varnish in a sealed off room (sized 4 x 3 x 3 m).[94] Moderate congestion and edema of the lungs and minimal steatosis of the hepatic cells were found upon autopsy/histological examination. Toxicological analyses revealed Toluene in the blood, and the death was diagnosed as acute Toluene intoxication.

A 29-yr-old woman with a history of Raynaud's syndrome, alcohol excess (reported as abstinent for months), and pancreatitis presented to the emergency department with gastrointestinal symptoms, headache, lethargy, and confusion.[95] The patients' Glasgow Coma Score was 12/15. Mild hepatorenal injury was evident upon biochemistry. The patient developed hypokalemia, hypernatremia, and hypophosphatemia, requiring management. Collateral history taken from the patient's mother revealed that the patient had been making jewelry for 2 mo using epoxy glue (containing Toluene), in a small unventilated room in her home. After 24 h, the patient's neurological state was improved and biochemistry returned to baseline.

Occupational

Koilonychia and hapolonychia of the fingernails were observed in 6 of 16 cabinet workers percutaneously exposed to a thinner mixture containing 30% Toluene, 30% xylene, and 40% methyl alcohol.[2] The majority of these workers had an average exposure time of about 2 yr.

No complaints of respiratory tract irritation were reported for volunteers or workers exposed to Toluene at concentrations of 800 - 1500 ppm for 8 h. Moderate conjunctival irritation and corneal damage was noted in 3 workers who were accidentally splashed in the eyes with Toluene.

A male construction worker displayed a slow reaction time, headache, nausea, and vomiting following application of an adhesive tape with a strong smell to pipes in non-ventilated underground apartments for 5 d.[96] The patient was working 10 h/d, and did not use personal protective equipment. Imagining revealed subcortical and periventricular white matter lesions plus involvement of bilateral cerebellar dentate nuclei. Benzoic acid was found in the blood and urine of the patient upon toxicology screening, and Toluene was detected in the adhesive tape. The patient was diagnosed with toxic leukoencephalopathy induced by Toluene and recovered with 6 mo of intensive care.

A 38-yr-old presented to the hospital with complaints of chronic headache and nausea.[97] Imaging revealed a T2-hyperintense cerebral white matter lesion in the left frontoparietal lobe with loss of gray-white matter differentiation, accompanied by faint T2-hyperintense lesions in the corpus splenium and right periventricular white matter. The patient was treated for possible encephalitis. Symptoms subsided; however, magnetic resonance imagining (MRI) findings remained the same. No neurological deterioration was observed during follow-up. Later, hospital personnel discovered that the lacquer thinner (composed of about 60% Toluene) was regularly used at the patient's workplace (patient reported 5 yr history at the company, and retired 3 mo prior to hospital visit). Toluene exposure was determined to likely be the cause of the patient's symptoms and MRI findings.

Occupational Toxicity/Epidemiology/Case Reports

Several occupational toxicity studies were performed comparing the prevalence of genotoxicity in printing factory workers exposed to Toluene compared to unexposed individuals.[2] In some studies, the number of chromosomal aberrations and SCEs were similar among Toluene-exposed workers versus unexposed individuals (approximate exposure: 7 - 400 ppm; average time of employment: 7 - 15 yr). However, a significantly greater number of chromatid breaks, chromatid exchanges, chromatid gaps, and SCEs were observed in subjects occupationally exposed to Toluene (approximate exposure: 200 – 300 ppm; average time of employment: ≥ 16 yr) compared to unexposed controls.

Concentrations of Toluene in the blood of workers occupationally exposed to Toluene were reported to be up to 94 mg/l.[3] Blood concentrations of humans not occupationally exposed to Toluene and those who were occupationally exposed to Toluene were compared. The mean blood concentration of those occupationally exposed to Toluene was significantly higher (2785 ± 3756 ng/l) than those not exposed to Toluene occupationally (829 ± 1175 ng/l). The maximum amount of expired Toluene in the breath of Toluene-exposed workers was determined to be 4 ± 0.8 µg/l after 22 ± 10 min of exposure. Gasoline workers exposed to 60.3 and 527 ppb Toluene in their personal air space had exhaled breath concentrations of 4.3 to 41.8 ppb Toluene. The maximum amount of o-cresol and hippuric acid in the urine of workers occupationally exposed to Toluene was determined to be 2.8 µg/ml and 3.02 mg/ml respectively. The release of Toluene from adipose tissue was significantly lower when compared to elimination from blood in humans occupationally exposed to Toluene.

Several adverse effects were observed in studies on workers occupationally exposed to Toluene. These effects include changes in liver enzymes, color vision impairment, organic brain syndrome, mild chronic encephalopathy, memory loss, unstable mood, inhibition of psychomotor skills and manual dexterity, impaired cognitive functioning, headaches, eye irritation, effects on auditory and visual pathways, and a reduction in reproductive hormones.

SCE frequencies were compared in printing workers exposed to Toluene and an unexposed control group. SCE rates were higher in the exposed group versus the unexposed group. Increased risk of mortality from cancers of the bone, connective tissue, and lung were observed in a cohort study performed in workers occupationally exposed to Toluene. In a different cohort study, cancer of the respiratory tract was the only cancer type with increased incidence in workers exposed to Toluene versus unexposed controlled subjects.

Many studies regarding occupational toxicity to Toluene were found in the literature. Details of these studies are found in Table 8. Reported adverse effects relating to occupational exposure to Toluene include an increased risk for reproductive disorders and birth defects, impaired gonad function/decreased sperm activity/quality, cardiotoxicity (e.g., decreased maximum heart rate), impaired learning/memory, genotoxicity, increased lipid peroxidation, increased liver enzymes, increased risk of cancer, increased risk of metabolic syndrome, increased oxidative stress levels, dry skin/itching, dry eyes, and increased general health risks.[98-112] No Toluene-related neuropsychological effects were observed in a cross-sectional study performed in furniture workers exposed to Toluene.[113113]

Occupational Exposure Limits

Occupational exposure limits have been placed by several organizations. A listing of these organizations, along with their regulations/recommendations are found in Table 9.

RISK ASSESSMENT

Toxicity Values and Minimal Risk Levels of Toluene

A reference concentration for chronic inhalation exposure (RfC; an estimate of an inhalation exposure, for a given duration, to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime), was determined by the US Environmental Protection Agency (EPA).[114] This estimate was based on animal and human studies available in the literature and a calculated adjusted inhalation NOAEL of 46 mg/m^3. The RfC was determined to be 5 mg/m^3. A reference dose for chronic oral exposure (RfD) was evaluated via similar methods and determined to be 0.08 mg/kg/d. In addition, repeated-dose toxicity data (inhalation) from ECHA indicate Toluene did not cause adverse effects in the rat following inhalation exposure to 300 ppm for up to 24 mo (6 h/d, 5 d/wk).[7] The NOAEC for chronic systemic or local toxicity in this study was 300 ppm (1131 mg/m^3). Human data (reported under Epidemiological studies) demonstrate no evidence that long-term exposure to Toluene (98 mg/m^3 for 21 yr) adversely affects human neurological or cognitive function. Minimal risk levels (defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (non-carcinogenic) over a specified duration of exposure) determined by the Agency for Toxic Substances and Disease Registry (ATSDR) were determined to be 2 ppm for acute (≤ 14 d) exposure and 1 ppm for chronic (≥ 365 d) inhalation exposure.[115] In addition, oral acute and chronic minimal risk levels were determined to be 0.8 and 0.2 mg/kg/d, respectively.

MOE/MOS Calculation of Toluene in Nail Products

Margin of exposure (MOE) is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US EPA and the European Commission Scientific Committee on Consumer Safety (SCCS) guidelines. An MOE value greater than 100 has traditionally been considered an indication of safety. The basis for this MOE value of

100 comes from two multiplication factors: a 10-fold factor for extrapolating data from test animals to human beings (interspecies extrapolation), and an additional 10-fold for differences among the human population (intraspecies extrapolation). Notably, the MOE value is sometimes referred to as the MOS despite the parameters being definitionally different.

In order to thoroughly assess the safety of Toluene in nail products, a tiered approach was followed in the calculation of an MOE. Tier 1 investigates exposure to Toluene via nail product use based on the most conservative parameters (100% systemic absorption via inhalation, dermal, incidental ingestion, and possible nail plate penetration exposure). Tier 2 presents a more realistic assessment of exposure, taking into account the amount of Toluene that is likely evaporated during the drying process as well as the skin area surrounding the nails, assuming 21.5% of the total content of Toluene would contribute to a systemic dose (inhalation plus dermal exposure). The third tier considers measurement of Toluene in the breathing zone of female subjects following exposure to nail polish products under simulated-use conditions[15] (Approximately 0.445% Toluene (0.6 mg) was detected in the breathing zone of women subjects in one application (one application is equivalent to 4 coats applied to 10 nails); other details of this assessment can be found in the Cosmetic Use Exposure section of this report).

Parameters that are the same among each calculation include the number of applications per day (1 application/d)[116] the weight of the adult (60 kg)[117] and the maximum use concentration of Toluene in nail products (20%).[118] In addition, an NOAEL of 625 mg/kg bw/d (from a 13-wk oral study in rats, for neuropathological toxicity) was used for each calculation (details can be found in the subchronic and chronic toxicity studies section of this report).[7]

Tier 1

Toluene may readily volatilize into air; the primary exposure route to Toluene from nail products, as stated by the California DTSC, is through vapor inhalation.[12] Additionally, exposure to Toluene may also occur through skin contact with nail products as well as via accidental hand-to-mouth exposure. Depending on the applied concentration, some chemical substances in nail products may penetrate the nail plate and reach the tissue under the nail, particularly when the nail is thin, soft, or damaged.

The amount of the nail polish applied in each application may vary depending on different use habits and practices. The in silico tool VERMEER Cosmolife estimates that the amount of nail polish use per application for 10 nails is 300 mg/kg bw/d.[119] In an unpublished study submitted by the Council in 1991,[15] it was found that the average application amount of nail polish applied was 527.6 mg (with a coefficient of variation of 20.60%); one application was defined as applying four coats (one base coat, two enamel coats, and a top coat) on 10 nails. (This results in approximately 1055.2 mg for application to 20 nails, based on a conservative exposure assumption.)

In a first tier approach with conservative exposure parameters, the SED is calculated based on an assumption that 100% of the total content of Toluene will contribute to a systemic dose, and an applied total amount of 1055.2 mg product across 20 nails.[116] The daily SED $_{\text{inhalation + dermal + incidental ingestion + possible nail plate penetration}}$ of Toluene resulting from nail products was calculated accordingly:

$$\frac{1\ application\ per\ day\ x\ 1055.2\ mg\ product\ x\ 20\%\ (maximum\ use\ concentration)\ x\ 100\%\ (Toluene\ content\ contributing\ to\ systemic\ dose)}{60\ kg\ (adult\ weight)} = 3.517\ mg/kg\ bw/d$$

The MOE is then calculated as follows:

$$\frac{625\ mg/kg\ bw/d\ (NOAEL)}{3.517\ mg/kg\ bw/d\ (SED)} = 177$$

Tier 2

In the second tier, a more realistic exposure assessment was carried out to refine the exposure model.

In a safety assessment of formaldehyde applied in nail hardeners, the Danish Environmental Protection Agency determined formaldehyde in nail hardeners may reach the skin surrounding the nail and evaporate during the drying process of the nail hardener.[116] Additionally, it was presumed that formaldehyde reacting on the nail plates will not contribute to the systemic dose as there is no specific data available regarding this potential contribution. The daily SED of formaldehyde is calculated based the following model parameters:

- It is estimated that about 25% of the total content of formaldehyde in the product will evaporate during the drying process. Furthermore, it is assumed that formaldehyde is released in the close area around the person (1 m^3), and half of formaldehyde is absorbed via inhalation or dermal absorption, while the remaining 75% will coat the nail plate and the skin surrounding the nail.
- The nails surface area (10 nails) amounts to a maximum 40 cm^2; the skin surrounding the nails amount to 4 cm^2, which corresponds to about 9% of the total area of nail and skin. Hence, it is presumed that 9% of the nail hardeners applied will coat the skin around the nail, potentially contributing to the systemic dose.

- Consequently, when calculating the SED, only the portion covering the skin surrounding the nail (9%) and half of the portion that evaporates during the drying process (12.5% of the total product content) are included. In total, 21.5% (9% + 12.5%) of the formaldehyde content in the product will contribute to the systemic dose.

As Toluene is less volatile than formaldehyde (formaldehyde has a higher vapor pressure than Toluene at room temperature), a similar risk assessment was conducted for Toluene as used in nail polish and enamel products, based on the following exposure parameters and assumptions:

- It is assumed that about 25% of the total content of Toluene in nail products will evaporate during the drying process, and half of Toluene is absorbed via inhalation or dermal absorption.
- It is presumed that around 9% of the applied nail polish and enamel products will cover the skin around the nail.
- In total, 21.5% (9% +12.5%) of the total content of Toluene will contribute to a systemic dose.
- It is assumed that a negligible amount of Toluene can penetrate the nail.
- Applied amount of 1055.2 mg product across 20 nails[116]

Daily SED $_{\text{inhalation + dermal}}$ of Toluene resulted from nail product use:

$$\frac{1\ application\ per\ day\ x\ 1055.2\ mg\ product\ x\ 20\%\ (maximum\ use\ concentration)\ x\ 21.5\%\ (Toluene\ content\ contributing\ to\ systemic\ dose)}{60\ kg\ (adult\ weight)} = 0.756\ mg/kg\ bw/d$$

MOE calculation:

$$\frac{625\ mg/kg\ bw/d\ (NOAEL)}{0.756\ mg/kg\ bw/d\ (SED)} = 826$$

It should be noted that in the second-tier approach, 12.5% of the total content of Toluene in nail polish products is assumed to be inhalable per application per day, which equals to 26.4 mg [= 1055.2 mg (daily applied amount of nail polish) × 20% (maximum use concentration) × 12.5% (inhalable fraction)]. In comparison, a study indicated that personal inhalation exposures to Toluene during the application of nail lacquers in residences ranged from approximately 1.03 to 2.82 mg/person/d (the mean Toluene levels measured in the breathing zone during the nail lacquer application ranged from 0.85 to 2.4 ppm).[115,120] Therefore, the assumption that 12.5% of the total Toluene content in nail polish products is inhaled remains a conservative estimation.

Tier 3

In the third tier, the measurement of Toluene exposure in the breathing zone of women under simulated-use conditions has been considered.

According to an unpublished study, the amount of Toluene in the breathing zone before, during, and after exposure to nail product application was evaluated in 15 female subjects under simulated-use conditions: each subject applied 4 coats of nail polish product per application (each coat formulated with 25% Toluene) within a 16 m^3 room that maintained an air flow of about 1.0 changes per hour.[15] The mean total of the nail polish applied was 0.5276 g (CV% = 20.60%). The estimated average Toluene exposure amount, which was measured in three applications in the breathing zone of all subjects, was 0.6 mg (CV% = 33%, the mean Toluene levels measured in the breathing zone during the nail lacquer application ranged from 1 to 4 ppm) and the grand mean application duration was 15 min. Therefore, only 0.6 mg Toluene was detected in the human breathing zone during application of nail polish products. Considering the Toluene concentration in nail polish products of this study is 25%, it indicated that around 0.455% of Toluene [0.455% = 0.6 mg ÷ (527.6 mg ×25%) ×100%] was available for inhalation. The following parameters were used for this MOE derivation:

- Approximately 9% of the applied nail polish and enamel products will cover the skin around the nail.
- 0.455% of Toluene is available in the breathing zone under simulated-use conditions
- In total, 9.455% (9% + 0.455%) of the total content of Toluene will contribute to a systemic dose.
- Applied amount of product: 1.0552 g (or 1055.2 mg) for 20 nails

$$\frac{1\ application\ per\ day\ x\ 1055.2\ mg\ product\ x\ 20\%\ (maximum\ use\ concentration)\ x\ 9.455\%\ (Toluene\ conteent\ contributing\ to\ systemic\ dose)}{60\ kg\ (adult\ weight)} = 0.333\ mg/kg\ bw/d$$

MOE calculation:

$$\frac{625\ mg/kg\ bw/d\ (NOAEL)}{0.333\ mg/kg\ bw/d(SED)} = 1880$$

Values of MOE calculations for all three tiers are greater than 100. This figure threshold is generally considered to be protective. The standard MOS value of 100 is derived from multiplying two factors: a 10-fold factor accounts for the extrapolating data from test animals to human beings (interspecies extrapolation), and an additional 10-fold for accommodating differences among the human population (intra-species extrapolation).[117]

The DTSC's findings indicate that the maximum daily exposure (dermal and inhalation) for individuals using nail products at home was 7760 µg/d,[17] which equals to 129.3 ug/kg bw/d (or 0.129 mg/kg bw/d), based on the assumption that an adult weighs 60 kg. Therefore, the systemic exposure level of 0.333 mg/kg bw/d, as noted in Tier 3, still represents a conservative estimate.

MOS calculation based on the US EPA RfC for neurologic effects

The US EPA established an RfC of 5 mg/m³, starting from an adjusted NOAEL of 46 mg/m³ derived from data on human occupational exposure.[114] This adjustment shifted the exposure levels from an 8-h time-weighted average (TWA) used in occupational settings to a comprehensive 24-h exposure scenario. The assessment focused on neurological effects, based on evidence from various occupational studies. To account for differences in human variability, the EPA incorporated an uncertainty factor of 10.

In the conversion of RfC value from inhalation to oral exposure, it is assumed that oral absorption is equivalent to inhalation absorption, i.e., an absorption ratio of 100% absorption for both inhalation and oral routes represents a conservative estimate, compensating for the uncertainties associated with the differences in toxicokinetics/pharmacokinetics between these two routes of exposure. Other relevant exposure parameters used for the extrapolation are listed below:

- Human body weight: 60 kg
- Human inhalation volume: 10 l/min[121]
- Human exposed to Toluene for 24 h/d (adjusted from occupational exposure to 24-h/d exposure), equivalent to 1440 min/d

Therefore, the extrapolated oral point of departure (POD) for neurologic effects can be derived based on the following equation:

Extrapolated oral POD = 5 mg/m^3 × 10 l/min × 1440 min × 0.001 (unit conversion of l to m^3) ÷ 60 kg (human bw) = 1.2 mg/kg bw/d.

MOS = extrapolated oral POD/human exposure (SED in Tier 3) = 1.2 mg/kg bw/d ÷ 0.333 mg/kg bw/d = 3.6

In this calculation, the RfC is based on human occupational data and already incorporates an uncertainty factor of 10 to account for human variation. The RfC is a dose generally considered safe, and because it has already been adjusted with uncertainty factor, a MOS ≥1 is acceptable.[122]

Considering that the EPA's RfC of 5 mg/m^3 adjusts the human occupational TWA value from an 8-h exposure to a 24-h/7-d exposure and applies an additional uncertainty factor to account for potentially susceptible subpopulations, this approach inherently incorporates a high degree of conservatism obviating the need for Tier 1 and 2 analysis.

MOE calculation based on the NOAEL for DART

A developmental NOAEL was chosen via the evaluation of two studies (details of these studies can be found in the DART section of this report). In one study, the LOAEL for both maternal and developmental toxicity was established at 1500 ppm. For the extrapolation from LOAEL to NOAEL, an uncertainty factor of 3 is applied, as the study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) and US EPA Good Laboratory Practices Standard. Consequently, the NOAEL is calculated as 1500 ppm divided by 3, resulting in 500 ppm.

In a different two-generation study investigating the effect of Toluene on reproduction,[49] the offspring NOAEL was determined to be 500 ppm. Therefore, based on the findings from both studies, 500 ppm (1875 mg/m^3) was selected as the NOAEL for the DART endpoint.

In the conversion of NOAEL value from inhalation to oral exposure, it is assumed that the absorption rate for oral exposure is equivalent to that for inhalation. This represents a conservative estimate, as it compensates for the uncertainties associated with the differences in toxicokinetics and pharmacokinetics between these two routes of exposure. The relevant exposure parameters used for the extrapolation are listed below:

- rat body weight: 0.3 kg[123]
- at inhalation volume: 0.24 l/min[123]
- Rats were exposed to Toluene for 6 h/d,[49,38] equivalent to 360 min/d

Therefore, the extrapolated oral NOAEL from inhalation study for DART can be derived based on the following equation:

Extrapolated NOAEL = 1875 mg/m^3 (500 ppm) × 0.24 l/min × 360 min × 0.001 (unit conversion of l to m^3) ÷ 0.3 kg (human bw) = 540 mg/kg bw/d.

MOE calculation for DART in Tier 1:

$$\frac{540\ mg/kg\ bw/d\ (NOAEL)}{3.517\ mg/kg\ bw/d\ (SED)} = 153$$

MOE calculation for DART in Tier 2:

$$\frac{540\ mg/kg\ bw/d\ (NOAEL)}{0.756\ mg/kg\ bw/d\ (SED)} = 714$$

MOE calculation for DART in Tier 3:

$$\frac{540\ mg/kg\ bw/d\ (NOAEL)}{0.333\ mg/kg\ bw/d(SED)} = 1621$$

When applying the NOAEL for the DART endpoint in the MOE calculation, the results are 153, 714, and 1621 for Tier 1, Tier 2 and Tier 3, respectively.

EPIDEMIOLOGICAL STUDIES (NON-OCCUPATIONAL)

A cross-sectional study including 3011 US adults from the National Health and Nutrition Examination Survey (NHANES) was performed to evaluate the association of urinary exposure biomarkers of volatile organic compounds (including Toluene) with liver injury biomarkers and risk of non-alcoholic fatty liver disease.[124] NHANES surveys were released every 2 yr and evaluated from 2011 - 2016. Throughout this period, urinary volatile organic compound metabolites were measured. The presence of the Toluene metabolite *N*-acetyl-*S*-(benzyl)-L-cysteine in the urine was associated with increased AST, GGT, ALP, albumin, AST/ALT ratio, and Hepamet fibrosis scores.

The association between exposure to certain chemicals (including Toluene) in ambient air during pregnancy and cases of acute lymphoblastic leukemia and acute myeloid leukemia was evaluated in a case-control study.[125] A total of 69 cases of acute lymphoblastic leukemia (2994 controls) and 46 cases of acute myeloid leukemia (19,209 controls) were ascertained from the California Cancer Registry records of children (< 6 yr of age) between the years of 1990 and 2007. Information on chemical exposures was taken from community air monitors (monitors collected 24-h air samples every 12 d). Exposure to Toluene during third trimester was associated with an increased risk for acute lymphoblastic lymphoma (adjusted odds ratio (OR): 1.22; 95% confidence interval (CI) 0.90, 1.65)) and acute myeloid lymphoma (adjusted OR: 1.50; 95% CI 1.04, 2.16). In addition, an increased risk of acute lymphoblastic lymphoma (adjusted OR: 1.19; 95% CI 0.70, 2.02) and acute myeloid lymphoma (adjusted OR: 2.02; 95% CI 1.03, 3.94) was positively associated to exposure to Toluene during the child's first year.

SUMMARY

Toluene is reported to function in cosmetics as an antioxidant and a solvent. Toluene was previously reviewed by the Panel in a safety assessment published in 1987. At that time, the Panel concluded that Toluene is safe as used in the present practices of use and concentration as stated in that report. This conclusion was reconsidered at the March 2005 Panel meeting and the conclusion was re-affirmed, as published in 2006. In 2023, members of the US FDA nominated Toluene for an accelerated re-review, and thus, according to CIR procedures, this report has been re-opened for evaluation.

No uses were reported according to 2023 FDA VCRP survey data; however, 2023 concentration of use data report that Toluene is used at up to 20% in nail polish and enamel. In 2002, Toluene was reported be used in 59 total formulations at up to 26% in other manicuring preparations (according to 2003 concentration of use survey).

In the EU, the use of Toluene in cosmetics is restricted to nail products at a maximum concentration of 25%. In addition, the EU requires caution statements informing users to keep Toluene-containing products away from children.

The amount of Toluene in the breathing zone before, during, and after exposure to nail product application was evaluated in 15 female subjects. The average Toluene exposure amount ranged from 0.5 – 0.6 mg, with a mean application duration of 15 min. The mean Toluene exposure via inhalation was 0.236 ppm in an assay in which analytical air measurements were taken on 178 professional nail technicians. In a different study, the maximum daily exposure (via dermal and inhalation) in nail salon patrons, nail technicians, and home users were reported to be 2160, 28,200, and 7760 µg/d, respectively.

Toluene has been reported to be an impurity in several products including hand sanitizers, feminine hygiene products, and sunscreens. Feminine hygiene products containing Toluene as an impurity were associated with a higher calculated cancer risk (largely due to presence of benzene in products).

In an assay evaluating the effect of age on Toluene distribution in rats (4, 12, and 24 mo; exposed to up to 1 g/kg Toluene in corn oil; gavage), blood Toluene concentrations were unaffected by age; however, concentrations of Toluene in the brain were significantly higher in 24-mo-old rats vs. 4-mo-old rats. Mean blood concentrations of Toluene in rats following a 6-h inhalation exposure period were 0.01, 0.33, and 11.84 µg/g, after exposure to 5, 50, and 500 ppm Toluene, respectively (on day 1 of study). Toluene concentrations increased in a time- and dose-dependent manner in pregnant rats exposed to Toluene via inhalation (800 or 12,000 ppm; GD 8 - 20; 15 - 45 min exposure). Toluene levels also increased in fetal brains in a concentration-dependent manner, and in placenta and amniotic fluid in a time-dependent manner. An assay was performed in humans (exposed to 50 ppm Toluene via inhalation) evaluating the effect of temperature on absorption and excretion of Toluene. Increased heat increased absorption of Toluene and decreased elimination. A similar study was performed evaluating the effect of heat on the percutaneous absorption of Toluene (exposure via Toluene vapor; masked subjects). In this assay, the presence of heat did not affect percutaneous absorption levels. A steady-state flux of 0.00038 $g/cm^2/h$ was determined in an in vitro percutaneous absorption study performed using split-thickness pig skin (skin exposed to undiluted Toluene). Maximum Toluene concentrations of 3.07 ± 0.40 µg/ml (for membranes exposed for 15 min) 5.38 ± 0.92 µg/ml (for membranes exposed for 240 min) were reported in an microdialysis assay performed in rats.

The effect of Toluene on body weight and pathological changes in organs was observed in rabbits exposed to 1000 mg/l oluene via inhalation for 14 d (8 h/d). Body weights in the Toluene-treated group initially dropped, but recovered. Organ tissue weights were similar among control and treated groups; however, abnormalities were noted in the heart, lung, stomach and spleen tissues.

Gravid female rats were dosed with 1250 mg/kg Toluene in peanut oil by gavage on days 16 – 19 of gestation and killed on GD 20. Maternal and reproductive parameters were not affected, and there was no significant difference in the number of fetal external or skeletal malformations between the treatment and the control group. However, a pattern of accelerated development in the upper mid-turn in the treated fetal cochleas was observed. Up-regulation of NDMA subunits, CREB1, CaMKIV, and apoptotic–related genes were observed in animals treated with up to 50 ppm Toluene for 5 d on PND 2 or 8. A NOAEC of 600 ppm was determined in male rats and an F1 generation in a study evaluating fertility. Anti-nociception, and effects on memory and locomotion were observed in rats subjected to a pre-natal and post-natal exposure to Toluene (6000 ppm). In an inhalation study in which mice were exposed to 8000 ppm Toluene for 30 min twice daily via inhalation on GD 7 - 19, neonatal death was significantly increased in the test group compared to controls. In a study using rats, the animals were exposed to Toluene (500 or 1500 ppm) for 6 h/d on GD 6 - 20 and killed on GD 21. Maternal weight gain of the test animals and fetal body weights in the 1500 ppm group were decreased; no other reproductive or developmental effects due to dosing were observed. In another study in which dams were exposed to up to 3000 ppm Toluene via whole-body inhalation for 6 h/d on GD 6 – 15 and killed on GD 20; the maternal toxicity NOAEL was 750 ppm with a defined maternal and developmental toxicity LOAEL of 1500 ppm. There were no effects on reproductive parameters; fetal body weights were increased in a toxicologically significant manner at 1500 and 3000 ppm.

Several inhalation studies were conducted in rats using short-duration (15 or 30 min; twice daily; various exposure times, including post-natal exposures) or high-dose (up to 16,000 ppm) exposures, and effects on post-natal development were evaluated. Generally, there were no significant maternal effects, although decreased body weight gains were observed with doses ≥ 8000 ppm Toluene. Fetal malformations were observed at some of the highest doses (> 8000 ppm), and there were indications of impaired cognitive function.

Female rats were exposed to Toluene (2000 - 8000 ppm) via inhalation, 30 min/d for 28 d, and the effect on several hormone levels and ovarian tissue was examined. Progesterone levels (at 4000 and 8000 ppm) and testosterone levels (all dose groups) were statistically significantly increased and IGF-1 was statistically significantly decreased (at 8000 ppm); no effect on estradiol was noted. A dose-dependent increase in apoptosis in ovarian tissue was observed. In another study, gravid rats were exposed to 0.09 – 9 ppm Toluene via nasal inhalation for 90 min/d on GD 14.5 – 18.5; hormone levels (all groups) and mRNA levels of steroidogenic enzymes in testicular tissues (control and low-dose group) were measured in male pups. Fetal plasma testosterone concentrations were significantly reduced in males of the 0.9 and 9 ppm, but not the 0.09 ppm, groups. mRNA levels of 3β-HSD were significantly reduced after exposure to 0.9 ppm. Effects on immunological biomarkers were also examined in pups following whole-body inhalation exposure of dams to 5 or 50 ppm Toluene for 6 h/d on GD 14 – 18 or 19, followed by post-natal exposure to groups of pups at various post-natal time frames. In one study, total plasma IgG2a levels were statistically significantly increased in the 50 ppm group, and in another study, total IgG1 levels were markedly reduced. Splenic expression of some transcription factors was suppressed.

Positive results were observed in in vitro genotoxicity assays including an Ames assay (using *Salmonella typhimurium* strains at up to 50 µl/plate), a comet assay (using human skin disks exposed to up to 100% Toluene vapor), a modified alkaline comet assay (using human lung epithelial carcinoma cells at 0.25 ppmv), a mammalian cell mutagenicity assay using mouse lymphoma cells (up to 500 µg/ml). Conversely, no genotoxicity was observed in other in vitro assays including an Ames assay (using *S. typhimurium* strains at up to 1000 µg/plate), a chromosomal aberration assay (using Chinese hamster ovary cells at up to 1600 µg/ml), and an SCE assay (using Chinese hamster ovary cells at up to 5000 µg/ml). Similarly, mixed results were observed in in vivo genotoxicity assays. Toluene was genotoxic in an alkaline and neutral comet assay using *Drosophila* larvae given up to 100.0 mM Toluene via diet, a comet assay using mice given a 5 or 15 g/kg intra-peritoneal injection of Toluene, and a comet assay using mice exposed to Toluene (25 ppm) via inhalation for 4 wk. Negative

results were observed in a micronucleus assay using mice exposed to up to 2000 mg/kg Toluene (method of administration not stated) and in a bone marrow nucleus assay using mice exposed to 100 ppm Toluene via inhalation for 15 d.

In a carcinogenicity assay performed in rats given Toluene (500 or 800 mg/kg bw; in olive oil) via gavage for 2 yr, increased numbers of total malignant tumors and carcinomas were observed in treated rats versus controls. Conversely, no test substance-related increases in the number of neoplasms were observed in a 2-yr study in which rats and mice were exposed to Toluene (up to 12,000 ppm) via inhalation.

A significant increase in the number of inflammatory cells and significant decrease in the production of interferon-gamma and substance P was observed in treated mice versus controls in an assay in which mice were treated with low-levels of Toluene (50 ppm) for 6 or 12 wk. Significantly increased neurotrophin-3 production was also observed in exposed mice.

Abnormalities in adrenal glands (increased weight) and adrenocortical size were observed in rats treated with Toluene (1500 ppm) via inhalation for 7 d. Adrenocortical hypertrophy and significant increases in ACTH serum concentrations were also observed in treated animals.

The effect of Toluene (300 ppm; 8 wk; inhalation exposure) on bone mass toxicity was examined in male mice. Bone mineral density and bone mineral content were significantly lower in treated versus control groups.

Hearing loss was evaluated in guinea pigs exposed to Toluene alone, or along with a low protein diet, and/or cytochrome p450 inhibition. A statistically significant Toluene-induced hearing loss was provoked in cytochrome p450-inhibited guinea pigs on a normal diet, and in cytochrome p-450 inhibited guinea pigs on a low protein diet. Hearing loss was similar among unexposed controls and guinea pigs treated with Toluene alone, or those treated with Toluene plus a low protein diet.

Several Toluene abuse cases have been found in the literature. These cases report a myriad of symptoms following abuse including neurological, gastrointestinal, cardiac, hepatic, pulmonary, and adrenal abnormalities and dysfunction.

According to neurotoxicity and behavioral studies performed in animals, exposure to Toluene can result in HPA/HPT axes dysfunction, up- and down-regulation of the expression of NMDA subunits, memory, learning, and motor impairments, decreased hippocampal neurogenesis, and alteration of brain chemicals. Altered neuroplasticity was observed in 17 human subjects exposed to Toluene (peak of 200 ppm) via inhalation.

Acute and subchronic assays were performed in rats evaluating the effect of Toluene (up to 1019 ppm (6 h treatment) in acute studies and up to 995 ppm (6 h/d treatments) in 13-wk studies) on oxidative stress markers in the brain. Increased oxidative stress was apparent in both acute and 13-wk assays.

The effect of Toluene (up to 4000 ppm; single 30 min exposure) on lung and brain tissue inflammation was evaluated in mice. Immune system activity and cellular proliferation were similar among control and treated groups. However, treated animals displayed morphological abnormalities and increased astrogliosis in the striatum. c-Fos immunoreactivity following exposure up to 5000 ppm Toluene for up to 30 min was increased in about one-third of the brain structures examined, and the majority of brain structures activated by Toluene were found in the forebrain and midbrain.

The effect of Toluene inhalation on immune responses were investigated using groups of 6 C3H/HeN mice exposed to up to 500 ppm of Toluene for 6 h/d, 5 d/wk, for 3 or 6 wk. Low levels of Toluene exposure (50 ppm) in mice immunized with OVA might dysregulate immune responses to OVA via the activation of transcription factors. The number of BAL cells and plasma total IgG1 antibody production were increased in the 50-ppm allergic group.

The potential for Toluene (up to 450 ppm for 10 min or up to 135 for 3, 10-min sessions) to elicit microvascular leakage was evaluated in rat airways. Toluene exposure induced dye leakage into the trachea and main bronchi in a concentration-dependent manner.

Olfactory changes were assessed in female mice exposed to 1000 ppm Toluene (5 h/d) for 4 wk. Changes in density and thickness of olfactory epithelium and neuroepithelium were observed during treatment.

Hepatotoxic effects were evaluated in male rats given a single oral dose of Toluene (6 ml/kg) via gavage. Increased AST and ALT numbers, degeneration of hepatocyte and mononuclear infiltration, high immunoreactivity, and increased apoptosis was observed in treated animals. Hepatotoxicity was also evaluated in rabbits exposed to noise (100 dB) and Toluene (up to 1000 ppm) combined or separately. Treatment occurred for 14 d. Histopathology revealed minor cell swelling, minor hepatic lipidosis, and eosinic cytoplasms in animals treated with Toluene only. Significant swelling and damage of the liver tissue were observed following exposure to Toluene and noise simultaneously.

Decreased blood pressure and heart rate, and increased troponin T levels were observed in rats given 6 ml/kg Toluene via gavage (single dose). Also observed in treated animals were cardiac congestion and edema. In a different study evaluating cardiotoxicity, Toluene (up to 1.2 g/kg; method of administration not stated) resulted in tachycardia and raised blood pressure.

The effect of allergic stimulation on genotoxicity in the brains of Toluene-exposed mice (25 ppm for 6 h/d for 4 wk) was evaluated. Significant DNA damage was observed in the hippocampus and leukocytes of ovalbumin-immunized mice following Toluene exposure, compared to controls.

The in vitro skin viability of human skin disks exposed to Toluene vapor (up to 100%; in corn oil) was evaluated. In vitro skin exposures to Toluene resulted in statistically-significantly reduced cell viability, at all tested concentrations, in a dose-dependent manner, compared to controls.

A 65-yr-old man presented to the emergency department with severe chest pain 40 min after accidental ingestion of an organic solvent. Cardiotoxicity was apparent upon testing and the patient's blood indicated the presence of Toluene and xylene isomers. Laboratory testing in a 31-yr-old woman with weakness and back/abdominal pain revealed metabolic acidosis and persistent hypokalemia. The patient reported exposure to paint. The patient's Toluene level in the blood was reported to be 4.12 mg/l. A man in his 40s was found dead in his home with apparent toxicity observed in the lungs and liver shortly after spraying a wood coating varnish in a sealed off room. Toxicological analyses revealed Toluene in the blood, and the death was diagnosed as acute Toluene intoxication. A 29-yr-old woman reported to the emergency department with gastrointestinal symptoms, headache, lethargy, and confusion; laboratory analysis revealed electrolyte abnormalities. These symptoms were determined to be due to continuous exposure to epoxy glue containing Toluene (used in small unventilated room).

White matter lesions, headache, and nausea were reported in 2 case reports. Symptoms were reported to be due to occupational exposure to Toluene.

Many occupational toxicity and epidemiological assays were found in the literature. Exposure to Toluene occupationally is associated with reproductive disorders, cardiotoxicity, neurological disorders, genotoxicity, hepatotoxicity, increased risk of cancer and metabolic disorders, increased oxidative stress levels, and eye and skin irritation. No Toluene-related neuropsychological effects were observed in a cross-sectional study performed in furniture workers exposed to Toluene. In a 4-yr study evaluating color perception in individuals occupationally exposed to Toluene, no significant association between adverse effects relating to color vision perception and Toluene exposure were observed.

A reference concentration for chronic inhalation exposure and a reference dose for chronic oral exposure of 5 mg/m^3 and 0.08 mg/kg/d, respectively, were determined by the US EPA. Minimal risk levels of 2 ppm, 1 ppm, 0.8 mg/kg/d, and 0.2 mg/kg/d were determined by the ATSDR for acute inhalation, chronic inhalation, acute oral, and chronic oral exposures, respectively.

Three MOE calculations were performed according to different levels of exposure and different toxicological endpoints. The lowest MOE value based on neuropathological and DART endpoints were determined to be 177 and 153, respectively. All calculated MOE values were above 100, and were thus considered to be protective.

The association between Toluene exposure (measured as urinary biomarkers) and non-alcoholic fatty liver disease was evaluated in a cross-sectional study using 3011 US adults. The presence of the Toluene metabolite *N*-acetyl-*S*-(benzyl)-L-cysteine in the urine was associated with increased aspartate aminotransferase, GGT, ALP, albumin, AST/ALT ratio, and Hepamet fibrosis scores.

The association between exposure to chemicals (including Toluene) in ambient air during pregnancy and cases of acute lymphoblastic leukemia and acute myeloid leukemia was evaluated in a case-control study. Exposure to Toluene during third trimester and during a child's first year of life were associated with a higher risk of both acute lymphoblastic leukemia and acute myeloid leukemia.

DISCUSSION

This assessment reviews the safety of Toluene as used in cosmetic formulations, in accordance with the product categories and concentrations of use identified in the Use section and Use table. Toluene is reported to function as an antioxidant and solvent in nail products. The Panel reviewed the available data and determined that Toluene is safe for use in nail products at concentrations up to 20%..

The safety of this ingredient in nail products was supported by a lack of irritation and sensitization in human assays and conservative MOS/MOE calculations (based on both neuropathological and DART endpoints) yielding values above 100. The Panel noted the potential for Toluene to result in reproductive and endocrine toxicity; however, this concern was mitigated as these effects were observed at high concentrations not relevant to cosmetic exposure.

The Panel also noted regulations from the California Department of Toxic Substances Control (DTSC) mandating that manufacturers of nail products certify that their products do not contain more than 100 ppm Toluene. After review of the data for each endpoint, the Panel could not come to the conclusion that Toluene should not exceed 100 ppm in nail products, and instead determined that Toluene is safe in nail products at the current maximum use concentration of 20%.

In addition, the Panel noted data regarding Toluene exposure levels following the use of nail products by nail technicians in the workplace. While this information is included in the report, MOE calculations were not calculated based on occupational exposure, as exposure in the workplace is not the purview of the Panel; instead, government organizations

such as the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) oversee and advise on occupational safety.

Lastly, the Panel expressed concern regarding impurities (e.g., benzene) that may be present in products containing Toluene. The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Toluene is safe for use in nail products at concentrations up to 20%.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	liquid	2
Odor	sweet, pungent, benzene-like	7
Color	colorless	2
Molecular Weight (g/mol)	92.13	2
Density (g/ml @ 20 ºC)	0.861 - 0.871	2
Viscosity (cp @ 20 ºC)	0.6	2
Vapor Pressure (mmHg @ 25ºC)	28.4	7
Vapor Density (mmHg)	3.14	7
Melting Point (ºC)	-94.9	7
Boiling Point (ºC)	110.6	7
Water Solubility (mg/ml @ 25ºC)	0.59	114
log K_{ow} (@ ºC)	2.73	126
Index of Refraction	1.4961	2
Flash Point (ºC)	4.4	114

Table 2. Frequency (2023/2002) and concentration (2024/2003) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[8]	**2002**[4]	**2023**[118]	**2003**[4]
Totals*	**NR**	**59**	**0.000001 – 20**	**20 – 26**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*NR*	*57*	*0.000001 – 20*	*20 – 26*
Rinse-Off	*NR*	*2*	*0.000001 – 0.01*[a]	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray or Aerosol	NR	NR	0.000001 – 0.000002[a]	NR
Incidental Inhalation-Powder	NR	NR	0.000001[a,b]	NR
Dermal Contact	NR	NR	0.000001 – 0.000004[a]	NR
Deodorant (underarm)	NR	NR	0.000001 – 0.000002[a]	NR
Hair - Non-Coloring	NR	NR	0.000001[a]	NR
Hair-Coloring	NR	NR	0.01[a]	NR
Nail	NR	59	10 – 20	20 – 26
Mucous Membrane	NR	NR	0.000001 – 0.000004[a]	NR
Baby Products	NR	NR	0.000001[a]	NR
as reported by product category				
Baby Products				
Baby Lotions/Oils/Creams	NR	NR	0.000001[a,b]	NR
Hair Preparations (non-coloring)				
Hair Conditioner	NR	NR	0.000001[a]	NR
Shampoos (non-coloring)	NR	NR	0.000001[a]	NR
Hair Coloring Preparations				
Hair Tints	NR	NR	0.01[a]	NR
Manicuring Preparations (Nail)				
Basecoats and Undercoats	NR	21	NR	NR
Nail Extenders	NR	NR	10	NR
Nail Polish and Enamel	NR	23	20	20 – 25
Nail Polish and Enamel Removers	NR	2	NR	NR
Other Manicuring Preparations	NR	13	NR	26
Personal Cleanliness Products				
Bath Soaps and Detergents	NR	NR	0.000001 – 0.000004[a]	NR
Deodorants (underarm)	NR	NR	not spray: 0.000001 – 0.000002[a] aerosol: 0.000001 – 0.000002[a]	NR

NR – not reported

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] Companies reporting these values indicate that this is not intentionally added Toluene, it is in the product as a residual amount or impurity

[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 3. CFR citations on Toluene

Citation	Details
21CFR1310.02; 21 CFR1310.04; 21CFR1313.15; 21CFR1313.24	List 2 chemical; controlled import and export by Drug Enforcement Administration
21CFR175.105	Indirect food additive; ingredient may be used as in adhesives for use in food handling preparations (e.g., packaging) according to certain conditions
21CFR175.320	Indirect food additive; ingredient may be used in resinous and polymeric coatings for use on food-contact surfaces according to certain conditions
21CFR176.180	Indirect food additive; ingredient may be used as component of paper and paperboard in contact with dry food according to certain conditions
21CFR177.1010	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (acrylic and modified acrylic plastics, semi-rigid and rigid) according to certain conditions
21CFR177.1200	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (cellophane) according to certain conditions
21CFR177.1580	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (polycarbonate resins) according to certain conditions
21CFR177.1650	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (polysulfide polymer-polyepoxy resins) according to certain conditions
21CFR178.3010	Indirect food additive; ingredient may be used as adjuvant in the manufacture of foamed plastics intended for use in contact with food according to certain conditions
21CFR520.580	Oral dosage animal drugs; ingredient used in combination with dichlorophene as de-worming treatment in animals under certain limitations; single dose of 120 mg Toluene per pound body weight or divided dose of 120 mg Toluene per 5 pounds body weight, daily, for 6 d
27CFR21.132; 27CFR21.151	Denaturant authorized for denatured spirits
16CFR1700.14	Solvents for paint of other similar surface-coating material containing 10% or more by weight of Toluene requires special packaging to protect children from injury and illness

Table 4. Dermal penetration/percutaneous absorption and ADME studies on Toluene

Parameter Measured	Test System or Species	No./Group	Vehicle/Dose	Protocol	Results	References
DERMAL PENETRATION/PERCUTANEOUS ABSORPTION						
IN VITRO						
Percutaneous absorption	Split-thickness pig skin	6	100% (Toluene purity: ≥ 99.5%)	Six jacketed static Franz cells (orifice diameter 9 mm, corresponding to skin exposure area of 0.64 cm^2) with mounted split-thickness pig skin used to evaluated percutaneous absorption; at start of experiment, donor compartment filled with test material (1 ml; neat) and capped with glass stopper; experiments ran for 4 - 9 h; aliquots of receptor fluid sampled at predefined times; steady-state flux, permeability coefficient, and lag time calculated	Steady-state flux: 0.00038 $g/cm^2/h$ Permeability coefficient: 0.00044 cm/h Lag time: 27.4 min	22
Animal						
Dermal penetration	Male Wistar rats	82 rats; 3 dialysis membranes/rat	100%; 200 µl	Three microdialysis membranes (3000 kDa) were inserted intradermally at a length of 2 cm into the abdominal skin of anesthetized rats; distance between membranes was less than 1 mm; micropipette tips were inserted in the membranes and connected to a microdialysis pump; membranes perfused with albumin solution 5% at 10 µl/min; a skin area of 1 x 0.6 cm^2 above the membranes exposed to Toluene for 15 or 240 min; dialysate sampled at 20-min intervals; effects of tape stripping and pretreatment with topical products (barrier creams) also assessed in 5 - 8 samples at each evaluation period	Maximum Toluene concentrations were reached 60 min after exposure (3.07 ± 0.40 µg/ml in samples exposed for 15 min; 5.38 ± 0.92 µg/ml in samples exposed for 240 min); in 15 min exposure experiments, dermal Toluene concentrations reached baseline values after 240 min; in 240 min exposure experiments, a plateau of approximately 6 µg/ml was reached after 60 min; after 15-min and 240-min exposures, the *o*-cresol content was 8.04 ± 1.0 and 12.7 ± 1.4 µg, respectively; neither tape stripping or barrier creams usage induced a significant change on dermal Toluene penetration or *o*-cresol content	23

Table 4. Dermal penetration/percutaneous absorption and ADME studies on Toluene

Parameter Measured	Test System or Species	No./Group	Vehicle/Dose	Protocol	Results	References
			Human			
Percutaneous absorption	Humans	5 subjects	50 ppm	Subjects placed in inhalation chambers (approximately 18.1 m^3) and exposed to Toluene for 4 h; subject wearing masks led to pumps that fed clean air, preventing exposure via inhalation; concentrations continuously monitored with infrared spectrophotometer every 5 - 7 min; exposure were held at 3 different temperatures: 21, 25, and 30 °C; exposures at different temperatures spaced by a at least 1 wk to minimize cumulative exposure.	Dermal exposure to Toluene did not result in statistically significant differences in venous concentrations between 25 and 30°C (technical problems towards the end of exposure prevented accurate readings at 21°C). Mean venous concentrations of Toluene (µg/l): At 21°C 0-h exposure: 0.66 ± 0.32 2-h exposure: 6.05 ± 0.94 4-h exposure: -* 30 min post-exposure: -* At 25°C 0-h exposure: 0.73 ± 0.11 2-h exposure: 5.30 ± 0.35 4-h exposure: 6.19 ± 1.05 30 min post-exposure: 4.16 ± 0.57 At 30°C 0-h exposure: 0.13 ± 0.04 2-h exposure: 4.89 ± 0.72 4-h exposure: 6.21 ± 0.076 30 min post-exposure: 3.00 ± 0.41 *reading not reported as they may be inaccurate due to technical difficulties	24
			ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION			
			ORAL			
			Animal			
Distribution	Male brown Norway rats (4, 12, or 24 mo.old)	6 - 12/group	corn oil; 0, 0.3, 0.65, and 1 g/kg	Animals of different ages treated with test substance via gavage and killed after 45 min or 4 h post-dose; pharmacokinetic parameters including blood and brain Toluene concentrations measured	Brain Toluene concentration was significantly elevated in 24 mo old rats at 4 h after dosing with either 0.3 or 1 g/kg (concentrations were approximately 50% higher in 24-mo-old rats versus 4-mo-old rats). Blood Toluene concentrations were unaffected by age. In animals treated with 1 g/kg, 45 min post-dose, the maximum amount of Toluene in the blood and brain were approximately 35 and 90 mg/l in, respectively in 24 mo old rats (similar results observed in 12 mo old rats). Four h post-dose, in animals treated with 1 g/kg, the maximum amount of Toluene in the blood and brain were approximately 40 (in 4 mo rats) and 80 mg/ml (in 24 mo old rats).	27

Table 4. Dermal penetration/percutaneous absorption and ADME studies on Toluene

Parameter Measured	Test System or Species	No./Group	Vehicle/Dose	Protocol	Results	References
INHALATION						
Animal						
Absorption and Excretion	Male brown Norway rats	8 - 12/group	5, 50, 500 ppm; Toluene purity: ≥ 99.75	Animals exposed to Toluene via inhalation in 200 l glass/stainless steel inhalation chambers; exposure duration of 6 h/d, 5 d/wk for 4 wk; blood was taken on days 1, 5, 10, and 20 and urine samples were taken 3 days before the study, and study days 1, 5, 10, and 20	Mean blood concentrations on day 1 of animals treated with 5, 50, and 500 ppm Toluene were approximately 0.01, 0.33, and 11.84 µg/g, respectively. Mean blood concentrations of Toluene over the 4 collection times were approximately 0.04, 0.35, and 11.62 µg/g, in animals treated with 5, 50 and 500 ppm, respectively. The amount of *o*-cresol excreted in the urine directly correlated to Toluene blood concentrations. The relationship between the amount of *o*-cresol (nmol) excreted and Toluene blood concentrations was described by the equation: ln(*o*-cresol) = 5.204 + 0.553 x ln(Toluene blood concentration) (r = 0.9795).	28
Distribution	Pregnant Sprague-Dawley rats	8 - 15/group	8000 or 12,000 ppm	Pregnant rats were exposed to Toluene via inhalation (full-body chamber exposure) for 15, 30, or 45 min/ exposure. Exposures occurred twice a day from GD 8 through GD 20. Immediately after the second exposure on GD 8, GD14, and GD20, blood was drawn. Animals were killed after blood draw on GD 20, and maternal tissue specimens, placenta, amniotic fluid, and fetal brain were collected for evaluation.	Maternal saphenous blood Toluene levels increased in a concentration- and time-dependent manner; the highest mean maternal saphenous blood Toluene concentration (approximately 11 ppm Toluene) was observed in animals treated with 12,000 ppm Toluene on GD14 for 45 min/ exposure. Maternal cerebellum, heart, kidney, and liver appeared to be saturated after 30 min on GD 20, suggesting extensive distribution. Toluene levels increased in fetal brains in a concentration-dependent manner, and in placenta and amniotic fluid in a time-dependent manner. The highest mean concentrations of Toluene in the placenta and fetal brain were approximately 10.5 ppm (in rats treated with 12,000 ppm Toluene; 30 min exposures) and 7.3 ppm (in rats treated with with 8000 ppm Toluene; 45 min exposures). Concentrations of Toluene in amniotic fluid were very low (less than^{120}5 ppm).	29
Human						
Absorption and Excretion	Human	5 subjects	50 ppm	Subjects placed in inhalation chambers (approximately 18.1 m^3) with 4-h exposure to Toluene; concentrations continuously monitored with infrared spectrophotometer every 5 - 7 min; exposure were held at 3 different temperatures: 21, 25, and 30 °C exposures at different temperatures spaced by a at least 1 wk to minimize cumulative exposure; blood, urine, and exhaled air evaluated before, during, and after each exposure	-Mean venous blood amounts of Toluene measured 2 h into exposure -0.374 mg/l at 21°C -0.362 mg/l at 25 °C -0.389 mg/l at 30 °C -Mean exhaled air amounts of Toluene 1.5 h into exposure: -0.036 mg/l at 21°C -0.037 mg/l at 25 °C -0.042 mg/l at 30 °C -Mean amount of Toluene in urine after 4-h exposure: -10.22 µg/l at 21°C -10.23 µg/l at 25 °C -7.57 µg/l at 30 °C -Mean amount of *o*-cresol in urine after 4-h exposure -39.21 µg at 21°C -21.17 µg at 25 °C -26.78 µg at 30 °C Results suggest increased absorption and a decreased elimination of Toluene in the presence of heat	24

GD = gestation day

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			ORAL		
Peanut Oil	Gravid female Sprague-Dawley rats; 8/group	0 or 1250 mg/kg (equiv. to 3 h inhalation of 8000 ppm); Toluene purity: 99.8%	Dams were dosed by gavage on days 16 – 19 of gestation (the time period for cochlear development) and killed on GD 20. The uterine horns and the ovaries were removed and the number of implantation sites, resorptions, and live and dead fetuses, and number of corpora lutea were determined. Two to three fetuses per litter were collected specifically for the TUNEL assay to determine apoptosis.	All maternal parameters evaluated, including weight gain, liver and kidney weights, placental weight, implantations, resorptions, pre-implantation loss, corpora lutea, and number of live fetuses, were comparable between the test and the control group. No differences were observed microscopically in the liver; however, it was noted that 75% of kidney sections in treated dams had evidence of renal pathology. Fetal weights were lower (not statistically significant) in the test group. The fetal/placental weight ratio was statistically-significantly increased in the test animals. There was no significant difference in the number of fetal external or skeletal malformations between the treatment and the control group. However, fetuses of the treatment group had an increased frequency and severity of enlarged renal pelvises. A pattern of accelerated development in the upper mid-turn in the treated fetal cochleas was observed; the researchers stated that this accelerated development suggests that Toluene may induce excessive cell death resulting in premature maturation of the cochlea. Four control and 4 treated embryos were appropriate for use in the TUNEL assay. Apoptosis was present in the mid and apical turns of treated but not control samples; results were similar in the other sections of the cochlea,	33
			INHALATION		
Air	Experiment 1: 18 Gravid C3H/HeJ mice; PND 2; PND 8 male offspring (6/group) Experiment 2: 10 Gravid C3H/HeJ mice; PND 49 male offspring (number of animals not stated)	0, 5, or 50 ppm	Experiment 1: Dams (GD 14) and male offspring (PND 2 or PND 8) were exposed for 6 h/d for 5 d. Exposures were made in stainless streel glass chambers. On PND 21,expression levels of NMDA receptor subunits, cyclic AMP responsive binding element binding protein, CREB1, CaMKIV, and apoptotic related genes (Bax, Bcl). In addition, mRNAs in the hippocampus estimated, immunohistochemical analyses, general developmental toxicity analysis performed. Experiment 1: Dams (GD 13) and male offspring (PND 49) treated as stated above and evaluated for body and organ weight changes	NMDA receptor subunit NR1, NR2A, and NR2B mRNAs were increased significantly in the hippocampus of PND 21 male mice after exposure to Toluene at 5 or 50 ppm during PND 8-12 ($p < 0.05$). NR2B mRNA was also increased significantly in the hippocampus of PND 21 male mice after exposure 50 ppm exposure during PND 2-6 ($p < 0.05$). CaMKIV mRNAs were up-regulated in PND 21 male mice exposed to 50 ppm Toluene during PND 2-6 and PND 8-12, but not during GD 14-18 ($p < 0.05$). CaMKIV mRNA was also up-regulated in the hippocampus of PND 21 male mice exposed to 5 ppm during PND 8-12, but not during PND 2-6 or GD 14-18 ($p < 0.05$). Similar patterns of up-regulation of CREB1 mRNAs were observed in the hippocampus of PND 21 male mice. Almost all memory function-related gene mRNAs and pro-apoptotic and anti-apoptotic ratio increased significantly in mice exposed to 5 or 50 ppm Toluene on PND 8-12. Mice exposed on GD 14-18 showed no significant change. Increased active caspase-3 immunoreactive cells were found in hippocampal C1 area of male mice exposed to 5 ppm Toluene on PND 8-12. Body weight was significantly reduced in 5 ppm Toluene-exposed PND 49 mice compared to controls ($p < 0.05$). Relative organ weights of the brain, thymus, liver, lung, and testis were not significantly different between groups. Kidney weight was reduced significantly in treated PND 49 mice compared to controls ($p < 0.01$).	34

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Sprague-Dawley rats (15/sex/dose)	0, 600, or 2000 ppm; Toluene purity: 98%	Rats were exposed to Toluene vapor at 600 or 2000 ppm for 6 h/d. Females were exposed from 14 d prior to mating until GD 7. Males were exposed for a total of 90 d (including 60 d pre-mating and during mating. Effects on fertility were evaluated.	No abnormalities were observed in mating behavior, fertility, or fetus mortality. The number of dams with dead fetuses was marginally increased in the 2000 ppm group. A significant decrease in epididymides weight and sperm count was observed in males exposed to 2000 ppm compared to controls. The NOAEC for parental males and the F1 generation was determined to be[7]600 ppm.	7
Air	Gravid Wistar rats (number of animals used not stated; prenatal exposure); male Wistar pups (25/group; post-natal exposure); 30-d old Wistar rats (10/group (sex not stated); acute exposure)	0 or 6000 ppm; Toluene purity: 99.8%	Acute exposure: 30-d old Wistar rats were exposed to 6000 ppm or air via inhalation (30 min exposure; static chamber exposure) Prenatal exposure: Pregnant Wistar rats exposed to Toluene (6000 ppm) or air from GD 8-20 (30 min exposures, 2x/d, static chamber exposure); number of animals used not stated Postnatal exposure: Male pups (from pregnant Wistar rats exposed during prenatal exposure; 25/group) weaned on PND 21 and re-exposed to Toluene (6000 ppm) or air via inhalation, 2x/d, from PND 22-30; with this design 4 treatment groups were established: -prenatal air + postnatal air (A/A) -prenatal air + postnatal Toluene (A/T) -prenatal Toluene + postnatal air (T/A) -prenatal Toluene + postnatal Toluene (T/T) Evaluations performed: behavioral (anxiety (burying behavior), nociception (hot-plate test), long-term and short-term memory (step-through inhibitory avoidance task and object recognition test) and locomotor activity); all evaluations occurred after last exposure	Acute Toluene exposure significantly decreased burying behavior compared to controls. Chronic exposure to Toluene during adolescence, alone (A/T), or in combination with prenatal Toluene treatment (T/T) also decreased burying behavior. The T/T group received the highest number of electrical shocks in burying behavior test. The acute Toluene-treated group also produced a significant increase of shocks compared to the control group. Anti-nociception observed in the acute exposure Toluene group, and in the A/T and T/T groups during the hot-plate test. All Toluene treatments results in impaired short-term memory in the object recognition test; however, only post-natal exposure impaired long-term memory in the passive-avoidance test. Acute exposure to Toluene resulted in significant increase in locomotion compared to control rats. Groups A/T and T/T displayed significantly augmented locomotor activity	35
Air	Gravid Swiss-Webster mice; 14 test and 13 controls	0 or 8000 ppm	Dams were exposed for 30 min twice daily via inhalation on GD 7 until parturition (GD 19). Exposures were made in sealed 29-l cylindrical glass jars with acrylic lids equipped with injection ports; Toluene was injected onto filter paper under the lid and volatilized via a fan. Pups were examined for morphological anomalies on PND 1, and litter observations were made. (Litters of 4 pups or less were not used.) Maternal and fetal parameters were examined throughout lactation until weaning (PND 21). After weaning, a maximum of 4 pups from each group were placed in each cage and left undisturbed until PND 42; at that time, they were placed in individual cages for 1 wk prior to determining food consumption, and then the pups were weighed every week until PND 90. Dams were killed at the end of lactation, and blood was collected for measurement of serum corticosterone and prolactin levels. RNA was extracted from the hypothalamic PVN.	Maternal body weight gains decreased throughout dosing; at GD 19, body weight gain was 16.7% less in test dams as compared to controls. Food intake was not affected by dosing. The number of live litters was comparable between groups, and there were no statistically significant differences among groups in gestational length, number of live pups, or sex ratio on PND 1. Neonatal death was significantly increased in the test group compared to controls. As compared to controls, pups of the test group had statistically significantly lower body weight on PND 21, but not on the other days of lactation. No effect on body weights were observed after weaning. Dosing did not have a significant effect on serum corticosterone, thyrotropin releasing-hormone mRNA expression, or prolactin levels of dams.	36

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid Sprague-Dawley rats; 15 low-dose and controls; 16 high-dose	0, 500, or 1500 ppm; Toluene purity: 99.7%	Performed according to US EPA (TSCA) 40CFR798.4350. Dams were exposed for 6 h/d using a 200 l glass/stainless-steel inhalation chambers on days 6- 20 of gestation. The dams were killed on day 21 of gestation, the uterus was removed, and various maternal and fetal parameters were assessed.	Maternal weight gain and corrected weight gain were statistically significantly decreased in test animals compared to controls. Fetal body weights were statistically significantly decreased in the 1500 ppm group as compared to controls. No adverse effects on the average number of implantations and of live fetuses or the incidence of non-live implants and resorptions were observed. There was no significant change in the occurrence of any individual external, visceral and skeletal variations. The total number with fetal variations was statistically significantly decreased in both exposure groups; this was attributed to biological variations and was not considered toxicologically significant.	37
Air	Gravid Sprague-Dawley rats; 12/group	0, 500, 1000, 2000, 3500 and 5000 ppm; Toluene purity: 99.9%	In a range-finding study, dams were exposed via inhalation (whole-body) for 6 h/d on days 6 – 15 of gestation. All animals were observed daily for toxicological effects and mortality and killed on GD 20. A Caesarean section was performed, and all fetuses were examined and weighed.	Dose-responsive maternal and developmental toxicity at 2000 ppm and greater, including decreases in maternal and fetal body weights and post-implantation loss, was observed. Narcosis was observed in animals at 3500 and 5000 ppm. One 5000 ppm female died of unknown cause following the first exposure on GD 6.	38
Air	Gravid Sprague-Dawley rats; 25/group	0, 250, 750, 1500 and 3000 ppm; Toluene purity: 99.9%	Dams were exposed via whole-body inhalation for 6 h/d on days 6 – 15 of gestation and were observed twice daily (before and after exposure) for signs of toxicity, changes in general appearance and mortality. A Caesarean section was performed on all animals on day 20 of gestation, and various maternal and fetal parameters were assessed.	From dose initiation until study termination, clinical signs of toxicity included ataxia and hyper-responsivity in dams of the 1500 dose group and ataxia, hyper-responsivity, increased water intake, and decreased food consumption in dams of the 3000 ppm dose group. Decreased maternal body weight gain was observed during the exposure period only for dams of the 1500 ppm group and during exposure until GD 20 for dams of the 3000 ppm group. No adverse effects on implantation, number and viability of fetuses, or fetal sex distribution were observed. Compared to controls, mean fetal weight was statistically significantly reduced in the 250, 1500, and 3000 ppm groups; the researchers stated that extensive statistical analysis indicated there was no toxicologically significant dose-related effect on fetal body weight at or below 750 ppm. Mean litter weight was decreased in the 3000 ppm group. Instances of reduced or unossified skeletal elements occurred in the 1500 and 3000 ppm groups. Low incidences (≤ 2.5%) of various malformations occurred in the 250, 1500, and 3000 ppm groups; since there was no increase in the incidence of specific or total malformations with increased exposure, these were not considered test article-related. The maternal toxicity NOAEL was 750 ppm with a defined maternal and developmental toxicity LOAEL of 1500 ppm.	38

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid female Mol:WIST; 16/group	0 or 1800 ppm	Dams were exposed via whole-body inhalation for 6 h/d on days 7 – 20 of gestation and were observed daily following exposure. Maternal and fetal weights and fetal parameters were assessed following delivery, and developmental and neurobehavioral effects were studied during lactation and until study termination at wk 14. Litters were not culled, but litters with less than 4 pups were not included in the post-natal evaluations. On PND 10, 1 male pup/litter with a body weight just below males with median body weight was removed from litters with at least 6 pups and used for other studies. After weaning on PND 21, 1 male and 1 female from each litter having the median body weight were kept for further behavioral testing. Females that had not given birth and all the dams were killed on PND 8 and 31, respectively, and examined for macroscopical changes and the number of uterine implantation sites.	No clinical signs of toxicity were observed in the dams during the exposure period. The number of dams not pregnant, neonatal deaths, or the sex distribution were comparable among the test and control groups. No pups with external malformations were observed in any of the groups. The litter size and the number of implantations were higher and the incidence of post-implantation loss lower in the exposed group compared to the control group, but the differences were not statistically significant. Body weights of pups from the test group were statistically significantly lower than controls until PND 10. Neurobehavioral evaluation of the pups revealed no effects on motor function (rotarod), activity level (open field), acoustic startle, and prepulse inhibition. Measurements of hearing function using auditory brain stem response revealed small effects in male pups for the test group. Performance in a Morris water maze during initial learning gave some indications of impaired cognitive functions which was confirmed during further testing, especially in reversal and new learning. Effects on cognitive functions seemed most marked in female offspring.	39
Air	Gravid female Sprague-Dawley rats; 21 test animals/group, 16 controls	0, 8000, or 12,000 ppm	Dams were exposed for 15 min twice daily via inhalation on days 8 – 20 of gestation; daily exposures were 2 h apart. Exposures were made in sealed 36-l cylindrical glass jars with acrylic lids equipped with injection ports; Toluene was injected onto filter paper under the lid and volatilized via a fan. After delivery, litters were examined and culled to 10 on PND 1, and pups were examined daily until weaning. Litters were weaned on PND 21, at which time the dams were killed and the uterine horns excised. The teratogenic impact on prenatal and early neonatal growth, perinatal outcome and neurobehavioral development of offspring was assessed.	Three rats in each dose group were not gravid; all pregnant rats gave birth to live litters. One dam of the high-dose group died on GD 17. Six dams (1 control, 3 from the 8000 ppm group, and 2 from 12,000 ppm group) had litters with $\leq$ 6 pups; these litters were used for reporting birth outcome data, but not used in analyzing neonatal development. There were no statistically significant differences between treated groups and controls in maternal weights or weight gains during gestation, percent live births, mean number of implant sites litter size, or sex ratio on PND 1. Five and 9 of the dams of the 8000 and 12,000 ppm groups, respectively, had malformed, "runted," or dead pups, as compared to 2 dams in the control group. The differences between the control and the 12,000 ppm groups were statistically significant for the percent of affected litters (12.50% vs. 52.94%, respectively), as well as the percent of affected pups/litter (0.55% vs. 2.79%, respectively). Marginal differences ($p = 0.051$) were observed for this parameter between the 8000 and the 12,000 ppm groups in the number of affected pups/litter (0.294 vs. 0.778, respectively) and the percent affected pups/litter (2.12% vs. 2.79%, respectively). Pup body weights in the 12,000 ppm group were statistically significantly less than those in the control and the 8000 ppm groups on PND 1; body weights of pups from the treated groups recovered by PND 16, and there were no statistically significant differences by PND 21. No pups from the 0 or 8000 ppm and 3 pups of the 12,000 group died during the study. A significant effect was observed for test animals in the negative geotaxis test. There was no significant effect of exposure on surface righting time, forelimb grip strength, or hanging strength.	40

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid Sprague-Dawley rats; 18 low-dose; 21 high-dose; 17 controls	0, 8000, or 12,000 ppm	Dosing as above, with 15 min exposure twice daily. The pups were weighed and examined on PND 1 for obvious physical abnormalities. Pups with any noticeable physical abnormalities or deemed "runts" were weighed and culled. All litters were then culled pseudo-randomly to 10 (5 males and 5 females, when possible) from the remaining pups. Two pups from each litter were selected on PND 28; spatial learning and memory were assessed in a Morris water maze.	In the 12,000 ppm group, maternal weight gain on GD 20 and overall litter weight on PND 1 were statistically significantly less than controls. There was no effect on litter size. There were no significant differences in litter mean body weighs at PND 28. There was no difference between test and control groups in acquisition initially in the Morris water maze. However, pups of the 12,000 ppm group displayed performance deficits during a probe trial and in reversal learning on PND 44.	41
Air	Gravid female Sprague-Dawley rats; 10/group	0, 8000, 12,000, or 16,000 ppm	Dosing as above, with 15 min exposure twice daily. Two studies were performed on offspring– one examining metabolic rate and body composition post-weaning and the other examining weight gain in response to consumption of 3 different diets.	Litter weights showed a statistically significant linear decrease as a function of dose. There were no significant differences among the groups at PND 30.	42
	offspring (males and females); 12 test offspring/group, 11 controls		Post-weaning offspring (~30 d old) were placed in metabolic cages for 3 h. The rates of oxygen consumption and carbon dioxide output were measured. The offspring were killed after metabolic testing and fat analysis was performed.	Pups of the 16,000 ppm had statistically lower energy expenditures than control pups and those of the 12,000 ppm group; pups of the 8000 ppm group had lower energy expenditures than the controls, but the difference was not statistically significant. Respiratory quotients were higher in the test groups, but the differences were not statistically significant. Pups in the 8000 and 16,000 ppm groups had significantly greater percentage of body fat as well as total body fat than the other groups.	
	3 male and 3 female offspring/group		Starting on PND 72, rats were exposed sequentially to 3 different diets (regular chow for 16 d, purified diet for 10 d, purified high-fat diet for 18 d).	There were trends for an effect of dose on food intake during chow and during high-fat diet consumption, with rats in the 12,000 ppm group consuming more than the 0 ppm group on both diets.	
Air	Gravid female Sprague-Dawley rats; 23 low-dose and 21/group for the control and high-dose	0, 8000, or 12,000 ppm	Dosing of dams as above, except exposure was for 30 min twice daily. Pups were assessed from PND 4 to PND 21, and teratogenic impact on prenatal and early neonatal growth, perinatal outcome, and pre-weaning neurobehavioral development of offspring was assessed.	Maternal weight gains were statistically significantly decreased in both test groups as compared to controls on GD 20. There were no significant differences in the mean overall weight of pups among the litters on PND 1; however, on PND 21, mean overall weight of pups of the treated groups were lower than controls. Litter size was similar between all groups. In the 12,000 ppm group, 20 pups in 15 different litters had malformations, including missing digits, missing limbs, and missing eyes (unilateral anophthalmia). The combined cumulative frequency of malformed, "runted," or dead pups in the control, 8000, and 12,000 ppm groups was 15.8, 33.33, and 63.16% of litters, respectively; the rate of incidence was statistically significantly increased in the 12,000 ppm group compared to controls, but the 8000 ppm group was not significantly different from either of these groups. No significant delays were observed in reaching maturational milestones.	43

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid female Sprague-Dawley rats; 8/group	0, 8000, 12,000, or 16,000 ppm Toluene purity: ≥ 99.5%	Dosing as above, with 30 min exposure twice daily. Gross dysmorphology, skeletal defects, and soft tissue anomalies were evaluated in fetal rats.	Aside from a sedative effect and immobility initially following exposure, no abnormal behavior was observed in dams of the treated groups. Maternal weight gains were decreased in all test groups as compared to controls on GD 20. Statistically significant differences were observed in many parameters in treated rats when compared to controls. Reduced growth, including decreases in placental weight, fetal weight, and crown-rump length, was observed in all test groups. A significant increase in gross physical malformations, such as short or missing digits (most common anomaly observed) and missing limbs, was observed in all test groups. There was a significant increase in skeletal defects, including misshapen scapula, missing and supernumerary vertebrae and ribs, and fused digits, in all test groups. Ossification of the extremities was significantly reduced at all dose levels. An increase in soft tissue anomalies was also observed at all dose levels, and there was a dose-dependent increase in the number of anomalies, including cardiac defects (most common soft tissue anomaly), microcardia or cardiomegaly, microgastria or gastromegaly, caudally displaced abdominal organs, displaced or ectopic testes, and hypoplastic or distended bladder were observed in all test groups; 100% of the litters of the 16,000 ppm group had soft tissue anomalies.	44
			EFFECT ON HORMONE LEVELS		
Air	female Wistar rats/10 group	0, 2000, 4000, or 8000 ppm	Animals were exposed via inhalation, 30 min/d, for 28 d. (Details of exposure methodology were not provided.) The animals were killed at study termination, blood samples were obtained, and the ovaries were removed and weighed. A portion of the ovaries were prepared for microscopic examination, immunostaining, and TUNEL assay, and a second portion was collected and stabilized in RNA later and stored for molecular assays. Levels of progesterone, estradiol, testosterone, and IGF-1 were measured using ELISA.	Statistically significant changes as compared to controls included increased body weights in the 2000 ppm group, decreased ovarian weights in the 4000 and 8000 ppm dose groups, a decreased number of growing follicles in the 8000 ppm dose group, and an increase in the number of abnormal ovarian follicles in all treated groups (the highest number was found in the 4000 ppm group). A statistically significant increase was observed in progesterone levels in the 4000 and 8000 ppm dose groups and in testosterone levels of all dose groups; no effect on estradiol was noted. IGF-1 was significantly decreased in the high-dose group. Compared to controls, mRNA levels of the Insl3, Cyp19, ccnd1, Igf-1, Actb, GDF-9 and Atg5 genes were statistically significantly decreased in all dose groups, and the expression of the Cyp17a, Lhr, Esr2 and Lc3 genes at 4000 and 8000 ppm and the Esr1 gene in the 2000 ppm-group were statistically significantly increased. Expression of the GDF protein was significantly decreased, and of LC3 was significantly increased, in the ovaries of rats in all dose groups when compared to controls. A dose-dependent increase in apoptosis in ovarian tissue was observed; in test animals, this increase was statistically significantly different in the 4000 and 8000 ppm groups.	45
Air	Gravid female Long-Wistar rats/4 group	0.09, 0.9, or 9 ppm	Animals were exposed via nasal inhalation for 90 min/d on days 14.5 – 18.5 of gestation to determine the effect of Toluene on the synthesis and secretion of testosterone in fetal rats. Plasma testosterone levels (3–5 fetuses/sex/litter) were measured using ELISA. mRNA levels of steroidogenic enzymes in testicular tissues from control and 0.9 ppm fetuses (3 litters/group with 2–5 male fetuses/litter) were measured using real-time PCR methods.	No statistically significant effects on maternal body weight gain or total number of fetuses were observed. Fetal plasma testosterone concentrations were significantly reduced in males of the 0.9 and 9 ppm, but not the 0.09 ppm, groups. mRNA levels of 3β-HSD were significantly reduced after exposure to 0.9 ppm; mRNA levels of P450scc, P450c17, 17β-HSD3, and Insl3were not significantly altered in the 0.9 ppm group. The 3β-HSD-immunoreactive area in the interstitial region of fetal testes was significantly reduced in the 0.9 and 9 ppm groups, but not in the 0.09 ppm group.	46

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			EFFECT ON IMMUNOLOGICAL BIOMARKERS		
Air	Gravid female BALB/c mice; 6/group	0, 5, or 50 ppm	Gravid females were exposed via whole-body inhalation for 6 h/d on GD 14 to parturition (GD 19), with or without aerosolized PGN (200 µg/10 ml; GD 14, 17, and 19 via nebulizer). Pups of the Toluene- and PGN-exposed groups were dosed with PGN (100 µg; PND 7, 10, 13, 16, and 19, i.p.). Dams were killed the day following the final inhalation exposure, and spleen, lung, and blood samples were collected. Plasma total IgE, IgG1, and IgG2 antibodies and cytokine levels within the lung were measuring using ELISA, and splenic mRNA expression levels were measured using real-time PCR. Th1/Th2 balance was determined at 3 wk via ELISA and RT-PCR methods.	There were no statistically significant differences in body weights of pups from dams exposed to Toluene, with or without PGN, as compared to controls. Spleen weights at PND 21 were not affected by Toluene alone, but were increased significantly with PGN (all groups). Total plasma IgG2a levels were statistically significantly increased in the 50 ppm (alone) group, as compared to both the 0 and 5 ppm groups. Total IgE and IgG1 were not affected by exposure to Toluene alone; PGN did have some effects. Splenic expression of transcription factors T-bet, GATA-3, and Foxp3 mRNAs was statistically significantly suppressed in pups from groups given 5 or 50 ppm Toluene without, but not with, PGN. Splenic IL-4, IL-12, and IFN-γ mRNAs were not significantly affected by Toluene, with or without PGN; however, IL-12 showed a dose-dependent decrease in all groups. Cytokines in the lungs were not affected by dosing.	47
Air	Experiment 1: gravid female C3H/HeN mice; 2/group male pups (PND 2); 6/group male pups (PND 8); 6/group	0, 5, or 50 ppm Toluene purity: 99.8%	Gravid females were exposed via whole-body inhalation for 6 h/d on days 14 -18 of gestation; 3 male pups/dam (GD 14 - 18 group) were used for assessment at PND 21. Each of the groups of male pups were exposed for 5 consecutive days (i.e., PND 2 – 6 or PND 8 – 12) and assessed on PND 21. Immunological biomarkers in the blood and spleen of the pups were examined by ELISA, real-time RT-PCR, flow cytometry, and histological analysis.	On PND 21, body weight and weight of the thymus, left lung, and spleen were similar between the GD 14 - 18 and PND 2 – 6 exposure groups and control groups, but the weights of the thymus and spleen were decreased in the PND 8 – 12, 5 ppm group. Plasma IgE levels were similar for all test and control groups. Plasma total IgG1 levels were markedly reduced in all groups of pups exposed to 5 ppm during all developmental stages, as well as the GD 14 -18 and PND 8 -12, 50 ppm groups. IgG2a levels were not changed in the pups exposed during gestation only, were statistically significantly decreased in the PND 2 – 6, 5 ppm group, and were significantly increased in the PND 8 – 12, 5 ppm group. Splenic T-lymphocyte subsets were suppressed in the PND 8 – 12, 50 ppm group, and IL-12 mRNA, T-bet mRNA, and Foxp3 were suppressed in all PND 2 – 6 and 8 – 12 test groups. There was marked activation of extramedullary hematopoiesis in the spleen in the PND 8 – 12, 50 ppm group.	48
Air	Experiment 2: male offspring of 10 untreated C3H/HeN mice; 6/group	0 or 50 ppm Toluene purity: 99.8%	Pups were exposed for 6 h/d on PND 8 – 12 and killed on PND 42. The effects on plasma antibody levels, splenic lymphocyte subsets, and splenic expression of cytokines and transcription factors were evaluated.	As compared to controls, statistically significant decreases were observed in plasma total IgG2a, splenic CD19+ B lymphocyte subset, CD4+ lymphocyte subset, and T-bet mRNA. The CD3+ lymphocyte subset was increased.	

Abbreviations: 3β-HSD = 3β-hydroxysteroid dehydrogenase; 17β-HSD3 =17β-hydroxysteroid dehydrogenase; CaMKIV = calcium/calmodulin-dependent protein kinase IV; CREB1 = cyclic adenosine monophosphate responsive element binding protein 1; ELISA = enzyme-linked immunosorbent assay; EPA = Environmental Protection Agency; GD = gestation day; GDF9 = growth differentiation factor-9; Ig = immunoglobulin; IGF-1 = insulin-like growth factor 1; IL = interleukin; Insl3 = insulin-like 3; IFN = interferon; LC3 = light chain 3; LOAEL = lowest-observed-adverse-effect-level; NMDA = N-methyl-D-aspartate; NOAEC = no-observed-adverse-effect-concentration; NOAEL = no-observed-adverse-effect-level; cytochrome P450c17 = P450 17α-hydroxylase/c17-20 lyase; P450scc = cytochrome P450 cholesterol side-chain cleavage; PCR = polymerase chain reaction; PGN = peptidoglycan; PND = post-natal day; PVN = paraventricular nucleus; RT-PCR = reverse transcription–polymerase chain reaction; TSCA = Toxic Substances Control Act; TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling; US = United States

Table 6. Genotoxicity studies on Toluene

Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VITRO		
DMSO	0.5, 2.5, 5, 25, and 50 µl/plate Toluene purity: 95%	*S. typhimurium* strains TA97, TA98, TA100, and TA102	-Ames assay; performed with and without metabolic activation -OECD TG 471 -Negative controls: blank and DMSO; use of positive control not stated	Genotoxic; positive results observed on the TA97, TA98, and TA102 strains without S9; after adding S9, Toluene caused further mutagenicity on the TA 100 strain; controls gave expected results; positive results observed at concentrations as low as 2.5 µl/plate	50
DMSO	10, 33.3, 100, 333.3, and 1000 µg/plate	*S. typhimurium* strain TA 98, TA100, TA 1535, and TA 1537	-Ames assay performed with and without metabolic activation -Negative control: DMSO -Positive control: sodium azide, 2-aminoanthracene, 2-aminoacridine, 4-nitro-*o*-phenylenediamine	Non-genotoxic; controls gave expected results	53
Corn oil	0.01 – 100%	Human skin disks	-Comet assay -Skin disks obtained from 3 different subjects exposed to Toluene vapor (8 h incubation) -Control skin disks were exposed to the vehicle only	Genotoxic at concentrations of 10,000 and higher; dose-dependent responses observed; controls gave expected results	51
Nitrogen	0.25 ppmv	Human lung epithelial carcinoma cell line A549	-Modified alkaline comet assay -Cells incubated with test substance (gaseous Toluene) for 3 and 24 h -Comet evaluation made immediately after exposure, and after 3 and 24 h -Negative control exposures with synthetic air -Positive control: hydrogen peroxide	Genotoxic; DNA damage observed during first 3 h of exposure; effect repaired within 24 h; controls gave expected results	52
DMSO	Without metabolic activation (trial 1): 31.25, 62.5, 125, 250, and 500 µg/ml Without metabolic activation (trial 2): 50, 100, 200, and 300 µg/ml Without metabolic activation (trial 3): 150, 175, 200, 225, 250, and 75 µg/ml With metabolic activation (trial 4): 6.25, 12.5, 25, 50, 100, 200 µg/ml With metabolic activation (trial 5): 125, 150, 175, 200, 225, and 250 µg/ml	Mouse lymphoma L5178Y TK+/- cells	-Mammalian cell mutagenicity assay -Cells incubated with test substance for 4 h -3 trials performed without metabolic activation, 2 trials performed with metabolic activation -Negative control: DMSO -Positive control: methyl methane sulfonate	Genotoxic; genotoxicity observed at high concentrations with and without metabolic activation; controls gave expected results	53
DMSO	50, 160, 500, and 1600 µg/ml	Chinese hamster ovary cells	-Chromosomal aberration assay performed with and without metabolic activation -Negative control: DMSO -Positive control: mitomycin C	Non-genotoxic; controls gave expected results	53
DMSO	50, 160, 500, 1600, and 5000 µg/ml	Chinese hamster ovary cells	-SCE assay performed with and without metabolic activation -Negative control: DMSO -Positive control: mitomycin C	Non-genotoxic; controls gave expected results	53

Table 6. Genotoxicity studies on Toluene

Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VIVO		
NR	1.0, 10.0, 50.0, and 100.0 mM Toluene purity: 99.5%	Third instar larvae of wild type *Drosophila melanogaster* (Oregon R^+) (15/group)	-Larvae exposed to test substance via diet for 12, 24, and 48 h and evaluated for genotoxicity; DNA damage evaluated in gut cells of larvae -Alkaline and neutral Comet assay -Positive control: ethyl methanesulfonate and γ-irradiation -Negative control: extract from larvae exposed to control food	Genotoxic; significant increase in DNA migration after 24 and 48 h observed, when compared to control, in both alkaline and neutral Comet assays ($p < 0.01$); controls gave expected results	54
Corn oil	500, 1000, or 2000 mg/kg (Toluene purity; technical grade)	B6C3F1 mice (sex and number of animals not stated)	-Micronucleus assay; exposure via gavage -Negative control: corn oil -Positive control: dimethylbenzanthracene	Non-genotoxic; controls gave expected results	53
NR	5 or 15 g/kg	Male Balb/c mice (9/group)	-Comet assay -Mice intraperitoneally injected with Toluene and killed after 2 h -DNA damage evaluated in different regions of brain, hepatocytes, and leukocytes -Negative control: corn oil -Use of positive control not stated	Genotoxic; acute exposure to Toluene induced significant levels of DNA damage in the hippocampus, cerebellum, and cortex, in a dose-dependent manner, compared to control ($p < 0.05$); no significant level of DNA damage in hepatocytes of leukocytes; controls gave expected results	26
NR	25 ppm	Male Balb/c mice (6/group)	-Comet assay -Animals nose-exposed to test substance or filtered air (negative control) for 4 wk (6 h/d, 5 d/wk) -One day following final Toluene inhalation, mice killed for collection of blood and brain samples -DNA damage evaluated in different regions of brain and leukocytes -Use of positive control not stated	Genotoxic; significant levels of DNA damage in the hippocampus, cerebellum, and cortex, compared to controls ($p < 0.05$); no significant DNA damage in leukocytes; controls gave expected results	26
NR	100 ppm Toluene purity: > 99%	Male CD-1 mice (10/group)	-Bone marrow micronucleus assay -Animals exposed via inhalation (whole-body inhalation chambers) for 6 h/d for 15 d for a total of 8 exposures (exposures occurred on study days 1, 2, 5, 6, 7, 9, 12, 13, and 15) -Control animals exposed to air only -Use of positive control not stated -Animals killed 18 h after last exposure and bone marrow cells collected	Non-genotoxic; Percent of erythrocyte micronuclei and polychromatic erythrocytes not statistically-significantly different than control group; controls gave expected results	55

DMSO = dimethyl sulfoxide; NR = not reported; OECD = Organisation for Economic Co-operation and Development; SCE = sister chromatid exchange; TG = Test Guidelines

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
	ANIMAL		
	Oral		
White matter changes, immunohistochemical parameters	-F344/N rats (6/sex/group) exposed to Toluene in olive oil via oral gavage at 0, 500, or 800 mg/kg, 4 d/wk, for 104 wk -Animals killed within days of final Toluene exposure -Brains of animals exposed via inhalation were sliced at 3 coronal levels (frontal horn of lateral ventricle, anterior hippocampus, and mid cerebellum) and evaluated -In brains of animals exposed orally, immunohistochemical staining was used to detect reactive astroglial and microglial changes, neuron populations, and cytochrome p450 upregulation	-No abnormalities in the neocortex, hippocampus, brainstem, or cerebellum observed -No white-matter abnormalities were observed in orally-treated rats -A mild widespread increase in reactive microglia was detected in female rats given Toluene via gavage at 800 mg/kg; however, no significant differences were detected in neurons or astrocytes -No evidence of myelin degeneration in control or treated groups -Immunohistochemical studies did not reveal any major abnormalities in comparison to controls	73
	Inhalation		
Anxiety/withdrawal	-Male Swiss Webster mice (n = 260; number per group not stated) -Animals exposed to either 5000 ppm Toluene vapor or air for 30 min or 24 h -Mice tested in a battery of 4 behavioral tasks reflective of anxiety either immediately after or 24 or 72 h after exposure	-Mice exposed to Toluene for 30 min showed decreases in anxiety-like behaviors, whereas mice abstinent from Toluene for 24 h after a 24-h exposure displayed increases in anxiety-like behaviors -Anxiety-like behaviors not observed 72 h post-exposure	19
HPA and HPT axes	-Male adolescent Wistar rats (9/group) -Animals exposed to Toluene (4000 or 8000 ppm; purity: 99.8%) via inhalation (30 min; 2x/d; 5 d/wk; 2 wk); control animals treated with air -HPA and HTP axes function evaluated via measurement of CRF release, CRF mRNA levels, ACTH, and corticosterone serum levels -Bloods samples collected 30 min after last exposure; brain tissues collected	-Both concentrations of Toluene significantly reduced CRF mRNA transcription in the PVN ($p < 0.001$), compared to controls -Toluene produced a concentration-dependent increase in ACTH at both concentrations ($p < 0.001$) and an increase in serum corticosterone levels at 8000 ppm ($p < 0.001$), compared to controls -Toluene significantly decreased pro-TRH mRNA levels in the hypothalamic PVN at both concentrations ($p < 0.05$) -Non-statistically significant decrease in serum TSH levels observed after exposure to 8000 ppm Toluene -8000 ppm significantly increased T_3 serum concentrations compared to control ($p = 0.001$); T_4 levels similar in control and treated groups	36
White matter changes, immunohistochemical parameters	-F344/N rats and B6C3F1 mice exposed to 0 or 1200 ppm Toluene in an inhalation chamber for 6.5 h/d, 5 d/wk, for 60 wk (n = 10/sex/group) or 103 wk (n = 50/sex/group) -Animals killed within days of final Toluene exposure -Brains of animals exposed via inhalation were sliced at 3 coronal levels (frontal horn of lateral ventricle, anterior hippocampus, and mid cerebellum) and evaluated -In brains of animals exposed orally, immunohistochemical staining was used to detect reactive astroglial and microglial changes, neuron populations, and cytochrome p450 upregulation	-No abnormalities in the neocortex, hippocampus, brainstem, or cerebellum observed -Focal artefactual vacuolation was evident in the white matter of approximately 10% of control and treated mice; the white-matter of exposed rats and mice was otherwise normally arranged with normal cell density -No evidence of myelin degeneration in control or treated groups -Immunohistochemical studies did not reveal any major abnormalities in comparison to controls	70
Learning and memory function	-Male C3H/HeJmice (number of animals not stated) exposed to 5 ppm Toluene via inhalation during PND 8 - 12, 2 control groups (0 ppm group and day-of room control group) -All exposures occurred for 6 h/d -On PND 49, animals allowed to swim freely during four 60-s trials to adapt to water; the next day, mice subjected to water maze task performed on 7 consecutive days (6 d for acquisition/training and 1 d for reversal phase to test memory retention -On 7^{th} day, after completion of reversal phase, animals subjected to a visible platform test to examine visual acuity and sensorimotor activity; escape latency evaluated	-Poor spatial and learning performance observed in treated mice -Significant prolongation of mean escape latency in the control (0 ppm; $p < 0.01$) group and the Toluene-exposed group ($p < 0.05$) on 5^{th} and 6^{th} day compared with corresponding room control -On 7^{th} day, when reversal phase was performed, a significant prolongation of the mean escape latency in the Toluene-exposed group was observed compared to room control group ($p < 0.05$)	34
Locomotor activity	-Adolescent (PND 28) and adult (PND 90) male Sprague-Dawley rats (n = 8/group) exposed to Toluene (0, 8000, or 16,000 ppm; purity: ≥ 99.5%) via inhalation over 12 d (different exposure types per day: 2 15-min exposures separated by 120 min intervals; 2 15-min exposures separated by 30 min interval; 6 5-min exposures with 30-min intervals separating exposures) -Locomotor activity quantified during Toluene exposures and for 30 min following completion of the final daily Toluene exposure	-Compared to adults, adolescents displayed greater locomotor activity on the first day and generally greater increases in activity over days than adults during Toluene exposure -Adults displayed greater locomotor activity compared to adolescents in the recovery period following exposure -Age group differences were clearest following the pattern of brief (5-min) repeated binge exposures	74

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
Behavioral effects, motor function, memory, and visual function	-Male Long-Evans rats (20/group) -Toluene vapor inhalation (0, 10, 100, or 1000 ppm; purity: 99.5%; 6 h/d, 5 d/wk, 4 wk) -10 rats from each group selected for behavioral assessments (motor activity, anxiety-related behavior, learning and performance of signal detection test) -10 rats from each group used for neurophysiological assessments of visual function	-No significant differences in motor activity, maze performance, lever-press frequencies, or visual discrimination in control and treated groups -Toluene treatment at the highest concentration reduced accuracy of signal detection at the end of training; further analysis of this effect revealed a greater influence of attentional impairment than visual or motor dysfunction	76
Behavioral effects, cognitive, and motor function	-Male Long-Evans rats (n = 248 total; number per group not stated) -Toluene vapor inhalation (0, 10, 100, or 1000 ppm; purity: 99.5%; 6 h/d, 5 d/wk, 13 wk) -10 rats from each group evaluated for motor activity, anxiety, visual discrimination, and visual signal detection behavior -10 rats from each group selected for fear conditioning	-No significant differences in motor activity, trace fear conditioning, maze performance, or visual discrimination between control and treated groups -All rats exposed to Toluene acquired the lever-press response later than controls	62
Behavioral effects, cognitive, and motor function	-Long-Evans rats (6 - 10/group) -Rats exposed to Toluene vapor (5000 ppm) or control conditions for 30 min -Animals subjected to water maze task, swimming/ visible platform tasks (measuring sensory-motor abilities and stamina), and trials evaluating the performance of a well-learned task, reversal learning, and long-term recall	-Immediately after Toluene exposure, rats were initially severely impaired in their swimming ability and in their ability to learn and perform a visible platform task; swimming behavior mostly returned to normal about 20 min after exposure, although cognitive impairments were still evident -Rats without Toluene exposure showed normal spatial recall; Toluene-exposed rats displayed severely impaired reversal learning	77
Cognitive function and locomotor activity	-12 male adolescent Wistar rats (PND 35 - 40)/group and 12 adult male Wistar rats/group -Animals treated with 2000 ppm Toluene, 5 min/d, 40 d; control groups (of each age group) treated with air only -Adolescent rats and adult rats either evaluated 24 h last treatment or 90 d after last treatment -Locomotor activity, habituation to environment, object exploration/habituation to object, spatial novelty, and object change evaluated	-Adolescent animals showed recognition memory impairment 24 h after last exposure, which normalized by day 90 post-exposure -Adult animals also showed recognition memory impairment that was still evident 90 d post-exposure -Significant decreases in locomotor activity observed in adults tested both 24 h and 90 d post-exposure, compared to controls ($p < 0.05$) -Habituation indices significantly higher in adolescent animals compared to adult animals at both 24 h and 90 d post-exposure	81
Spatial learning and memory	-12 male adolescent Wistar rats (PND 35 - 40)/group and 12 adult male Wistar rats/group -Animals treated with 2000 ppm Toluene, 5 min/d, 40 d; control groups (of each age group) treated with air; groups based on when animals assessed post-treatment -Adolescent rats and adult rats either evaluated 24 h last treatment or 90 d after last treatment -Spatial learning and memory evaluated using water maze test	-Adolescent rats treated with Toluene showed a decrease in time and distance traveled to find hidden platform 24 h after last exposure -Adult rats treated with Toluene showed a decrease in acquisition time and distance traveled 90 d after last exposure	78
Intraperitoneal Injection			
NMDA receptors and memory retention	-Female C3H/HeN mice (10/group) -Mice administered Toluene (300 mg/kg) in olive oil via intraperitoneal injection; control mice injected with olive oil only -10 mice treated with Toluene 60 min before test phase (and after training phase) -Novel object recognition test performed -Hippocampus collected 24 h after injection; total RNA isolated from hippocampal samples -NMDA receptor subunit expression (18S, NR1, NR2B) evaluated	-Toluene-injected mice did not prefer novel objects and showed poor discrimination between novel and familiar objects (novel object exploration time was increased significantly in control mice $p < 0.01$, but not in Toluene-treated mice) -Toluene-treated mice showed decreased expression of NMDA receptor subunit NR2B mRNA in the hippocampus ($p < 0.05$); effect not observed for oth[69] subunits	72
Hippocampal neurogenesis	-Male C57BL/6 mice (n = 240 total; number per group not stated) -Mice given intraperitoneal injection of Toluene in corn oil (500 mg/kg); control mice given corn oil only -Changes in hippocampal neurogenesis evaluated using 2 immunohistochemical markers for neurogenesis: Ki-67 and doublecortin -Depression and memory tasks also evaluated after treatment to assess hippocampal neurogenesis-related behavioral dysfunction	-The number of Ki-67- and doublecortin-positive cells in the dentate gyrus of adult hippocampi declined acutely between 0 and 24 h post-treatment, and increased gradually from 2-8 d post-treatment -Ki-67 and doublecortin immunoreactivity decreased in a dose-dependent manner -Treated mice showed significant depression-like behavior and memory deficits compared to untreated controls	80

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
Locomotor activity, motor coordination, passive avoidance learning	-Male Sprague-Dawley rats (6/group) -Intraperitoneal injection of Toluene in corn oil (250, 500, or 750 mg/kg; purity: 99.8%) -Locomotor activity observed, rotarod and step-through inhibitory avoidance tests performed (Toluene administered either 30 min prior to training, immediately after training, or 30 min prior to memory retention session in step-through inhibitory avoidance test)	-Toluene produced a dose-dependent increase in locomotor activity ($p < 0.01$) -Toluene at doses of 500 and 750 mg/kg produced significant motor incoordination -Toluene administered 30 min prior to training did not affect initial step-through latency during training session, but it dose-dependently reduced the latency to step-through during memory retention test session ($p < 0.001$) -When Toluene was given either 30 min before memory retention test or immediately after training session, no significant effect on the latency to step-through during the test session was observed -Further testing revealed that NMDA receptor blockade and dopamine neurotransmission may be responsible for these effects	75
Histopathological, immunohistochemical, and biochemical effects	-Male New Zealand white rabbits (10/group) -Toluene-treated group given single dose of 876 mg/kg via intraperitoneal injection; control group left untreated; Toluene purity: 99.9% -Blood samples collected 5 h post-administration, and animals euthanized -Brain tissue (prefrontal cortex, hippocampus, hypothalamus, substantia nigra, and entorhinal cortex) evaluated after euthanasia for biochemical parameters (nerve growth factor, tumor necrosis factor-alpha (TNF-α)), dopamine, and glial fibrillary acidic protein levels), histopathological parameters, and immunohistochemical parameters (Bax C3 immunoreactivity)	-Statistically significant increased TNF-α levels in Toluene-treated rats compared to controls -Statistically significant decreased levels of dopamine (secreted from substantia nigra), nerve growth factor (developed from hippocampal neurons), and glial fibrillary acidic protein levels (secreted from astrocyte cells) compared to controls -Areas of focal vacuolar degeneration, gliosis, and perivascular demyelination, many pyknotic cells, and necrosis observed in Toluene-treated animals -Distinct excessive expansions of blood vessels, severe degeneration of cell structure, and dispersed cell borders observed in Toluene-treated animals -Abnormalities of the nuclei structure of oligodendrocyte cells and damage in sequential neurons of hippocampus observed in Toluene-treated animals -Cytoplasm of the cortex cell showed serious immune reactivity in Toluene-treated group (effect not observed in control group)	79
HUMAN			
Inhalation			
Cortical excitability, neuroplasticity, and motor learning	-Placebo-controlled, randomized, crossover study -17 healthy subjects -Subjects participated in inhalation sessions of Toluene (single peak of 200 ppm) exposure and one under placebo (clean air) exposure (at least 1 wk in between sessions to avoid interference) -Whole-body exposure in a ventilated 28 m^3 exposure laboratory -Toluene concentration in the air of the chamber was exponentially increased from 0 to 200 ppm over a period of 25 min; after reaching the plateau of 200 ppm, this concentration was kept constant for 10 min; subsequently, the jet nebulizer was turned off and within the next 20 min, Toluene concentration decreased to 0 ppm; approximately 1 h later, a second 200 peak was generated -During both peaks, participants performed light physical exercise -Subjects assessed with different transcranial magnetic stimulation measurements, motor thresholds, short-latency intracortical inhibition and intracortical facilitation, and short-interval afferent inhibition before and after clean air or Toluene exposure -Long-term potentiation-like neuroplasticity induced by anodal transcranial direct stimulation over the motor cortex evaluated, and subjects performed serial reaction time task	-Toluene abolished plasticity induced by anodal transcranial direct stimulation, attenuated intracortical facilitation, and increased inhibition in the short-latency afferent inhibition measure -Cortico-spinal excitability and intracortical inhibition not effected by Toluene exposure -Toluene exposure did not alter performance of the motor learning task (serial reaction time task)	82

ACTH = adrenocorticotropin hormone; AMP = adenosine monophosphate; CRF = corticotropin-releasing-factor; HPA = hypothalamus-pituitary-adrenal; HPT = hypothalamus-pituitary-thyroid; NMDA = N-methyl-D-aspartate; PND = post-natal day; PVN = paraventricular nucleus; TNF-α = tumor necrosis factor – alpha; TSH = thyroid-stimulating hormone

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	Reproductive Toxicity		
Study evaluating relationship between reproductive disorders and birth defects in offspring of male painters with exposure to organic solvents	-Random samples of painters and carpenters drawn from workers affiliated with the Dutch Trade Union for Construction Workers -Reproductive outcomes, occupational exposures, and lifestyle habit data collected via self-administered questionnaires -Subjects: 381 male painters (who fathered pregnancies) exposed to organic solvents in paints, thinners and cleansers and 300 carpenters (who fathered pregnancies) with little or no exposure to solvents -OR with 95% CI calculated by univariate and multiple logistic regression analysis -Quantitative exposure estimates of male painters at 3 mo prior to conception also evaluated	-Quantitative exposure estimates at 3 mo prior to pregnancy - low (0.17 - 0.38 mg/m^3); intermediate (0.38 - 1.02 mg/m^3); high (1.03 - 4.66 mg/m^3) -Adjusted ORs (95% CI) in painters exposed to Toluene at different exposure levels: -prolonged prothrombin time: -low exposure: 1.2 (0.5 - 2.5) -intermediate exposure: 1.1 (0.5 - 2.2) -high exposure: 1.1 (0.5 - 2.7) -spontaneous abortion: -low exposure: 1.3 (0.5 - 3.4) -intermediate exposure: 0.4 (0.0 - 3.2) -high exposure: none -preterm birth: 1.0 -low exposure: 1.6 (0.7 - 3.9) -intermediate exposure: 1.5 (0.7 - 3.2) -high exposure: 0.8 (0.3 - 2.0) -low birth weight: -low exposure: 1.5 (0.5 - 4.3) -intermediate exposure: 1.6 (0.7 - 3.8) -high exposure: 1.9 (0.8 - 4.7) -birth defects – all: -low exposure: 2.1 (0.7 - 5.9) -intermediate exposure: 3.0 (1.3 - 7.0) -high exposure: 2.2 (0.8 - 6.0) -congenital malformations: -low exposure: 6.8 (1.3 - 35.9) -intermediate exposure: 3.9 (0.6 - 24.5) -high exposure: 8.9 (0.8 - 95.9) -functional developmental disorders: -low exposure: 0.4 (0.0 - 3.2) -intermediate exposure: 2.9 (1.1 - 7.7) -high exposure: 1.5 (0.5 - 4.6)	98
Study evaluating the effect of occupational exposure to solvents (including Toluene) in shoe making, spray painting, or paint manufacturing, on semen and function of accessory gonads	-24 exposed male subjects working in either the shoemaking, spray painting, or paint manufacturing industry in Zhejiang, China (exposed for at least 1 yr) -37 age- and occupationally-matched non-exposed controls with similar physical activity used as controls -Subjects interviewed about reproductive history, tobacco/alcohol use, and occupational and medical histories -Mean concentrations of benzene, Toluene, xylene in work airborne were 103.34 (0 - 7070.3), 42.73 (0 - 435.8), 8.21 (0 - 133.1) mg/m^3, respectively -Blood and semen samples collected from each subject -Semen analysis: liquefaction time, semen pH value, sperm concentration, total sperm count, percentage vitality, sperm activity, acrosin activity, seminal fructose, seminal GGT activity, LDH C4 activity	-Toluene detected in blood and semen of exposed workers only; not detected in control group (Toluene found in blood and semen in ranges of 0.30 - 17.17 and 0.11 - 0.40 µmol/l, respectively) -Statistically significant decreased levels of sperm activity, acrosin activity, and GGT activity, and LDH C4 activity were observed in exposed workers compared to controls *It should be noted that these results refer to workers who were potentially exposed to multiple solvents, and not Toluene alone	99
Study evaluating the effect of occupational exposure to hydrocarbons (including Toluene) in rubber factory workers on semen quality	-48 exposed male workers in production area of rubber factory in Mexico City (exposed for ≥ 2 yr) -42 unexposed male controls from administrative offices of same company -Medical history, reproductive history, exposure to other gonadotoxic agents evaluated -Environmental concentration evaluation of aromatic hydrocarbons in the workplace performed -Weekly semen samples collected from each participant for 3 wk; spermatobioscopies performed -Samples evaluated for liquefaction, volume, pH, agglutination, viscosity, non-specific aggregation, sperm count, motility, and white blood cells	-Exposed workers in contact with a mixture of hydrocarbons that contained: 220.7 - 234 mg/m^3 ethylbenzene, 31.9 – 47.8 mg/m^3 benzene, 189.7 – 212.5 mg/m^3 Toluene, and 47 – 56.4 mg/m^3 xylene -Number of subjects with normozoospermia greater in unexposed group (76%) compared to exposed group (17%) -Statistically significant decreased sperm viscosity, liquefaction, sperm count, sperm motility, and proportion of sperm with normal morphology were observed in exposed workers compared to controls *It should be noted that these results refer to workers who were potentially exposed to multiple solvents, and not Toluene alone	100

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	Cardiotoxicity		
Study evaluating the effect of occupational exposure of Toluene in furniture polishing industry workers on cardiac rhythm	-40 male workers in polishing industry with more than 3 mo. exposure to solvents including Toluene evaluated -38 control subjects working in other fields; matched by age, sex, smoking habits, and living accommodations -12-lead surface electrocardiogram and 24-h Holter recordings performed to determine QRS duration, PR duration, P wave dispersion, corrected QT dispersion, and heart rate variability parameters -Hippuric acid levels studied in urine samples taken at end of shift to reflect Toluene exposure level in exposed group	-Mean hippuric acid levels in exposed group: 1141.0 ± 851.7 mg/l; mean exposure time of 198.3 ± 150.0 -Maximum heart rate significantly lower in Toluene-exposed group compared to controls (130.5 ± 15.1 vs 138.6 ± 16.0) -Corrected low frequency and corrected low frequency/corrected high frequency significantly lower in Toluene exposed group vs. control (43.6 ± 7.2 vs 50.7 ± 10.5 and 1.4 ± 0.4 vs 2.2 ± 1.0, respectively) -Mean corrected high frequency, root-mean square successive difference, and standard deviation of all 5-min normal-to-normal interval mean values statistically-significantly higher in Toluene-exposed groups -Long-term exposure to Toluene did not cause increase in arrhythmia frequency, but deteriorated cardiac function markers; long-term exposure caused significant decreased in sympathetic activity and increase in vagal activity markers	101
	Neurotoxicity		
Cross-sectional study evaluating the neuropsychological effects of exposure to Toluene in workers in furniture enterprises	-Exposed group occupationally exposed to Toluene via painting and varnishing furniture in Karabaglar, Izmir, Turkey (n = 122 males) -Non-exposed male group engaged in other aspects of production (n = 88) -Individuals completed questionnaires (involving neuropsychological/neurological symptoms, exposure history, demographics) -Blood samples taken from individuals during the middle of the week within 2 - 4 h of completing work shift -All workers in exposed group were regularly exposed to solvents for 8 h/d	-Statistically significant difference in blood Toluene levels observed in exposed group vs. non-exposed group (levels were 6.95 times higher in exposed group) -No differences were observed in the average neurological and psychological symptoms between exposed and non-exposed groups	113
Longitudinal follow-up study evaluating the potential delayed central nervous system effects due to long-term occupational exposure to solvents (including Toluene) in rotogravure printers	-12 male rotogravure printers; 19 control male (refinery or carriage shop repair workers) subjects -Rotogravure printers mainly exposed to Toluene; past mean Toluene exposure estimated to be approximately 1500 mg/m^3 during the 1950s and early 1960s -By the end of the printers' working life (mid 1980s), mean levels of Toluene were approximately 43 and 157 mg/m^3, at 2 printing shops -Subjects evaluated 20 yr after occupational exposure, applying neuropsychological tests, symptoms and social interaction questionnaires, medical examinations, and exposure assessments	-More pronounced deterioration over time in printers than in referents in cognitive functioning affecting reasoning and associative learning -Printers performed significantly worse than referents in verbal memory and sustained attention at follow-up; dose-effect relationship noted for reasoning -Slightly higher depression score noted for printers vs. referents	102

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	Genotoxicity		
Study evaluating the potential genotoxic effects of Toluene in industrial painters occupationally exposed to Toluene	-34 male industrial painters from Rio Grande do Sul, Brazil, occupationally exposed to paints containing Toluene; 27 control male subjects with no history of occupational exposure -Subjects filled out questionnaires about general health, lifestyle, and time of occupational exposure -Urine, blood, and buccal cell samples obtained at end of work shift on last day of work week (evaluated for Toluene metabolites, creatinine, cotinine, malondialdehyde and protein carbonyl levels, ischemia-modified albumin, albumin, and hepatic enzymes) -Comet assay performed using whole blood; damage index calculated -Micronucleus assay performed using buccal cells	-Mean amount of blood Toluene, hippuric acid, and *o*-cresol levels in painters: 0.07 ± 0.01 mg/l, 0.56 ± 0.10 g/g creatinine, 0.04 ± 0.01 mg/l, respectively -Blood Toluene and *o*-cresol were not found in controls; however, a mean amount of hippuric acid of 0.41 ± 0.06 g/g creatinine was observed in controls -Damage index of controls determined via Comet assay: 39.4 ± 2.5 -Damage index of painters determined via Comet assay: 60.4 ± 3.6 ($p < 0.001$ compared with controls) -In the micronucleus assay, the frequency of abnormal cells did not show significant difference between painters and the control group ($p > 0.05$) -Malondialdehyde, the lipid peroxidation biomarker, was significantly higher in painters compared to controls ($p < 0.001$) -Painters showed higher ischemia-modified albumin concentrations ($p < 0.05$) and decreased albumin levels ($p < 0.001$) compared to controls -Statistically significant increased liver enzyme levels were observed in painters compared to controls	103
Study evaluating the potential cytogenic damage in offset printing works	-14 exposed printing workers in Turkey (sex not stated) -12 unexposed male controls -Mean duration of employment: 10.36 yr; 45 h/wk -Industrial thinner used by offset printing workers contain about 65% Toluene; workers also exposed to other compounds such as cobalt and hydroquinone -Blood samples taken from all participants and SCE, chromosomal aberration, and micronuclei assays performed	-SCE, chromosomal aberration, and micronuclei frequency was significantly higher in exposed individuals compared to controls ($p < 0.001$) *It should be noted that these results refer to workers who were potentially exposed to multiple solvents, and not Toluene alone	104
	Carcinogenicity		
Case-control study assessing relationship between solvent exposure and acute myeloid leukemia	-Study comprised 15,332 incident cases (male and female) of acute myeloid leukemia diagnosed in Finland, Norway, Sweden, and Iceland from 1961 - 2005 and 76,600 controls matched by year of birth, sex, and country -Occupational records linked with Nordic Occupational Cancer Study job exposure matrix to estimate quantitative values for 26 occupational exposure factors -HR with 95% CI estimated using conditional logistic regression models -Cases of Toluene with exposures ≤ 42.4 (1954 controls), 42.4-61 (1602 controls), and > 612 (400 controls) ppm/yr were 424, 296, and 76, respectively	HR levels for high levels of Toluene (HR 1.35, 95% CI 0.74 - 2.46) were slightly elevated; p-value: 0.49	105
Case-control study assessing relationship between Toluene exposure and lung cancer	-1236 cases of lung cancer (male and female) identified from 18 hospitals in Montreal, Canada between 1996 and 2001; 1512 population-based controls matched on age and sex -Subjects interviews on lifestyle behaviors, sociodemographic characteristics, and job histories (including equipment/chemicals used during job, safety measures, and duration of job) -Team of chemists and industrial hygienists assessed exposure to 294 occupational agents (including Toluene) -Multivariate logistic regression used to estimate ORs and 95% CIs	Lung cancer was associated with exposure to Toluene (OR = 1.31; 95% CI 0.99 – 1.84)	106

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
Case-control study assessing relationship of maternal occupational exposure to solvents during pregnancy and childhood acute lymphoblastic leukemia	-790 cases of childhood acute lymphoblastic leukemia; 790 controls based; all cases diagnosed in Quebec, Canada between 1980 and 2000 -Maternal occupational exposure to solvents (alkanes, aliphatic alcohols, chlorinated alkanes, aliphatic ketones, mononuclear aromatic hydrocarbons, and aliphatic esters) before and during pregnancy; home exposure to solvents also evaluated; exposure measured using exposure coding -Conditional logistic regression to estimated ORs and 95% CIs -Number of leukemia cases based on exposure to solvents 2 yr before pregnancy up to birth and number of leukemia cases based on exposure to solvents during pregnancy evaluated -Number of leukemia cases based on exposure level (no exposure, some exposure, greater exposure) to solvents 2 yr before pregnancy up to birth also evaluated	2 yr before pregnancy up to birth OR (95% CI): 1.88 (1.01 - 3.47); during pregnancy OR (95% CI): 2.25 (1.02 - 4.95) (increased risk); however, no indication of increased risk with increased level of exposure	107
Metabolic Syndrome			
Cohort study evaluating the association between occupational exposure to potential hazards (including Toluene) and metabolic syndrome	-Retrospective cohort based on 31,615 health examinees (1182 of which exposed to Toluene; male and female) in Republic of Korea from 2012 - 2021 -Demographic and behavior-related risk factors treated as confounding factors -Time-dependent Cox regression analysis used to calculate HRs; 95% CI -Adjusting for confounders (age, sex, smoking, alcohol intake, exercise frequency, and family history) -HR >1 suggests increased risk -HRs also calculated for combined exposure to Toluene and night shift (n = 157), Toluene and xylene (n = 380), Toluene with noise (n = 154), Toluene with styrene (n = 92), Toluene with copper (n = 120), and Toluene with antimony (n = 8) -If RERI > 0, the two substances had an additive effect, if MI >1, the two substances had a multiplicative effect	-Unadjusted and adjusted HRs for Toluene: 1.80 and 1.42, respectively -Toluene exposure plus night shift: -HR: 2.43 -RERI: 0.58 -MI: 1.20 -Toluene exposure plus noise exposure HR -HR: 1.65 -RERI: 0.08 -MI: 1.01 -Toluene exposure plus styrene exposure HR -HR: 1.91 -RERI: 0.42 -MI: 1.24 -Toluene exposure plus copper exposure HR -HR: 1.83 -RERI: -0.45 -MI: 0.70 -Toluene exposure plus antimony exposure HR -HR: 1.94 -RERI: -0.31 -MI: 0.75	108
Color Vision Impairment			
Follow-up study evaluating the potential effects of occupational exposure to Toluene on color vision	-4 yr study on color perception performed in individuals occupationally exposed to Toluene (< 50 ppm) in rotogravure printing in Germany (sex not stated) -Color vision measured 3 times throughout study period (162 participants completed all 3 examinations) -Study design based on 2 factors for stratification: intensity of exposure (high: printing division; low: end-processing division) and duration of exposure (short versus long) -Current individual Toluene exposure measured twice per year -Work history taken via questionnaire -Color discrimination abilities evaluated using a de-saturated panel test; screenings for low near vision acuity, red-green discrimination deficiencies, and contrast sensitivity also performed; color confusion index calculated -Multiple regressions performed	-Average exposure level for Toluene in breathing zone was 25.7 ± 21.0 ppm in printing area (n = 93) and 3.5 ± 3.6 ppm in the end-processing area (n = 69) -Mean exposure durations were 23 ± 6 yr for "long exposure" and 7 ± 2 yr for "short exposure" -Repeated analysis and multiple regressions did not reveal significant effects of Toluene on color vision perception with respect to intensity or duration of exposure	127

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	General/Other		
Study evaluating occupational health risks of Toluene, in the automobile repair industry	-Concentrations of benzene, Toluene, and xylenes monitored at 140 operating positions in 2018 (Beijing, China) -Long-term exposure concentration range of Toluene: 0.1 – 49.7 mg/m^3 -Short-term exposure concentration range of Toluene: 0.1 – 98.7 mg/m^3 -Occupational health risks evaluated using Singaporean model -Risk rating evaluated based on hazard rating (mainly determined based on the carcinogenicity classification published by IARC, exposure level/frequency, weekly exposure, average working hours per week, reduction factor, and permissible exposure level -Risk rating based on scale of 1 - 5	Risk ratings for Toluene for individuals exposed short-term and long-term to paint mixing and paint spraying ranged from 2 - 3 (low-medium risk)	109
Study evaluating the health risks/effects of different cooking methods (steaming, frying, and grilling) due to exposure to aromatic hydrocarbons (including Toluene)	-Chefs from Nanjing, China answered questionnaire to provide personal information and exposure time to kitchens (sex not stated) -Morning urine, saliva, and oral epithelial cell samples collected from 6 chefs of each style of cooking (steaming, frying, and grilling) -Internal and external exposure evaluated (air concentrations of Toluene, metabolites of Toluene (S-benzylmercapturic acid) quantified in urine -Chefs wore monitoring equipment measure real-time respiratory rate, systolic blood pressure, diastolic blood pressure, and ratio between forced expiratory volume within 1 s, and forced vital capacity -Malondialdehyde and 8-OHdG concentrations in urine and saliva used as indicators of systemic oxidative stress -Samples and cardiopulmonary indicators collected and evaluated during each of the 4 seasons of 2017	-Air concentrations of Toluene in frying kitchens, grilling kitchens, and steaming kitchens were 226 ± 122.2 $\mu g/m^3$, 122.1 ± 107.0 $\mu g/m^3$, and 87.58 ± 87.42 $\mu g/m^3$, respectively -Mean urinary concentrations of S-benzylmercapturic acid in chefs in frying kitchens, grilling kitchens, and steaming kitchens were 3.31 ± 3.21, 1.56 ± 2.12, and 1.41 ± 1.19 µmol/mol creatinine, respectively -Toluene in cooking pollution had no effect on blood pressure or lung function -Toluene associated with increased oxidative stress levels	110
Study evaluating the health risk of exposure to Toluene in the automobile industry	-Study performed in 115 automobile workers (sex not stated) in Iran in 2021 -Vapors gathered during work hours using adsorbent tube of activated coconut charcoal; values collected for 6 different types of shops: press, body, paint, pre-delivery inspection, assembly, and material storage -Non-cancer health risk evaluated by determining the HQ; HQ calculated by dividing the exposure concentration of Toluene by the maximum acceptable daily concentration of exposure (5 mg/m^3) -HQ ≤ 1 shows the absence of non-carcinogenic health effects; HQ > 1 represents the presence of non-carcinogenic health effects	-Mean concentrations of Toluene in the breathing zone of workers in press, body, paint, pre-delivery inspection, assembly, and material storage shops: 0.0790, 0.3883, 0.1240, 32.3923, 1.8572, and 0.0441 ppm, respectively -Mean HQ value in non-cancer risk assessments were higher than the acceptable limit for Toluene in the shop of pre-delivery inspection (7.832) -Mean HQ value of all shops combined: 1.396	111
Study evaluating the effect of long-term exposure to volatile organic compounds (including Toluene) and incidence of carcinogenic and non-carcinogenic adverse health effects	-53 beauty technicians (male and female) from Seoul, Korea recruited – these individuals delivered one or more of the following services: hair drying, hair dressing, hair coloring, haircut, epilation, nail art, facial shaving, facial steam, makeup, eyelash extensions, and waxing -Questionnaire given to assess exposure factors (total years as technician, frequency of work per week, duration in salon per day); interviews also performed -Indoor air samples collected during regular business hours over 6 business days -Personal air sampling performed using passive sampler worn during 8-h shift -HQs evaluated for non-carcinogenic assessment; HQ > 1 indicates adverse non-carcinogenic concern	-Mean indoor air concentration of Toluene: 14.99 $\mu g/m^3$ -Median personal exposure concentration: 17 $\mu g/m^3$ -Non-carcinogenic HQ mean: 0.003 -Main adverse effects reported in technicians include dry skin, skin stinging/itching, and dry eyes – technicians who experienced adverse health effects had significantly higher concentrations of acetone, benzaldehyde, and Toluene than those who did not experience adverse health effects ($p < 0.05$)	112

8-OHdG = 8-hydroxy-2'-deoxyguanosine ; CI = confidence interval; GGT = gamma-glutamyl transaminase; HQ = hazard quotient; HR = hazard ratio; IARC = International Agency for Research on Cancer; LDH = lactate dehydrogenase; MI = multiplicative interaction; OR = odds ratio; RERI = relative excess risk due to interaction; SCE = sister chromatid exchange

Table 9. Exposure level regulations/recommendations of Toluene by various organizations

Organization	Regulation	Reference
Occupational Safety and Health Administration	-PEL: 200 ppm 8-h TWA; ceiling: 300 ppm; acceptable peak over ceiling: 500 ppm over 10 min -PEL in shipyard employment: 200 ppm TWA [29CFR1915.1000]	128
California Occupational Safety and Health Administration	-PEL: 10 ppm; ceiling: 500 ppm; STEL: 150 ppm	129
The National Institute for Occupational Safety and Health	-REL: 100 ppm TWA; STEL: 150 ppm	130
California Office of Environmental Health Hazard Assessment	-MADL – inhalation: 13,000 µg/d* -MADL – oral: 7000 µg/d* -REL: 5000 $\mu g/m^3$ (acute); 830 $\mu g/m^3$ (8-h); 420 $\mu g/m^3$ (chronic)	131,32
American Conference of Governmental Industrial Hygienists	-Threshold limit value: 20 ppm	132
European Union Scientific Committee on Occupational Exposure Limits	-OEL: 50 ppm 8-h TWA; 15-min STEL: 100 ppm	133
Lower Olefins and Aromatics Racial and Ethnic Minority Acceleration Consortium for Health Equity working group	-OEL: 20 ppm 8-h TWA; 150-min STEL: 100 ppm; and skin notation to indicate that the dermal absorption of liquid Toluene can substantially contribute to body burden	133

MADL = maximum allowable dose level; OEL = occupational exposure limit; PEL = permissible exposure limit; REL = recommended exposure limit; STEL = short-term exposure limit; TWA = time-weighted average
*exposures at which Proposition 65 warning labels are required

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Data Appendix

Toxicokinetics/Dermal Penetration

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