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Original Article

Derivation of human health hazard assessment values for toluene under the Japanese Chemical Substances Control Law

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ABSTRACT — Toluene had been designated as a priority assessment chemical substance under the Japanese Chemical Substances Control Law (CSCL), and as a result of prioritization, a detailed human health hazard assessment was conducted under Assessment II. We evaluated its general, reproductive, and developmental toxicities, as well as its genotoxicity and carcinogenicity, based on the hazard information provided by domestic and international risk assessment organizations, and the following hazard assessment values for oral and inhalation exposure are proposed. The hazard assessment value of 0.223 mg/kg/day for oral exposure was calculated from a no-observed-adverse-effect level (NOAEL) of 312 mg/kg/day (equal to an average daily dose of 223 mg/kg/day) based on liver and kidney weight increases in a 13-week oral toxicity study in rats by using an uncertainty factor (UF) of 1,000 (interspecies variation: 10, intraspecies variation: 10, and short test period: 10). The hazard assessment value of 0.1 ppm (0.383 mg/m^3) for inhalation exposure was calculated from a NOAEL of 45 ppm (equal to a continuous exposure level of 10.7 ppm) based on toxic effects on the central nervous system found in epidemiological investigations of occupational exposure by using a UF of 100 (intraspecies variation: 10 and severe effect: 10).

Key words: Toluene (CAS No. 108-88-3), Chemical Substances Control Law (CSCL), Assessment II for human health effects, Hazard assessment value

INTRODUCTION

The Japanese Chemical Substances Control Law (CSCL, enacted in 1973) aims to prevent the pollution of the environment by chemical substances that may be harmful to human health or interfere with the habitat and growth of animals and plants. This law consists of three major parts: 1. prior review of new chemical substances, 2. continuous control measures for chemical substances after they are placed on the market, and 3. regulations and measures according to characteristics such as degradability, accumulation, toxicity, and the residual situation in the environment of chemical substances. Thus, a risk assessment is conducted before and after a substance is released to the market to ensure that appropriate control measures are taken. Since 1973, new chemical substances have to be notified to the government (Ministry of Health, Labour and Welfare [MHLW], Ministry of Economy, Trade and Industry [METI] and Ministry of the Environment [MOE]) for evaluation before their manufacture or import. The 2009 amendment made it mandatory to report all chemical substances with their actual quantities, including existing chemical substances, manufactured or imported above a certain quantity (METI, 2021). At the time, the CSCL was promulgated, about 20,000 chemical substances had already been manufactured or import-

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ed as existing chemical substances. Since then, the Japanese government has been conducting a step-by-step risk assessment of the effects of these chemical substances on human health and ecosystems. Under this risk assessment scheme, a screening evaluation, including hazard and exposure assessments, is initially conducted for existing chemical substances with a production or import volume of 10 tons/year or more. Based on the results of the screening assessment, a prioritization matrix (details in Igarashi *et al*., 2018) is generated, and priority assessment chemical substances requiring a more detailed risk assessment are identified (METI, 2021; MOE, 2014). Substances designated as priority assessment chemical substances are subject to detailed assessment (Assessment II) after prioritization in the risk Assessment I. In the stage of Assessment II, an evaluation draft is prepared for the general toxicity, reproductive toxicity, genotoxicity, and carcinogenicity of the target substance, using assessment values with their respective key studies referred by reliable organizations, to derive draft assessment values for oral and inhalation routes. The most severe derived draft assessment value is selected for the oral and inhalation routes as a representative value for the target substance in the draft evaluation report. Finally, the results of Assessment II on human health effects are reviewed and adopted by the joint council of MHLW, METI and MOE, and the representative hazard assessment values will be applied to Risk Assessment II.

Toluene (CAS No. 108-88-3), an organic compound belonging to the aromatic hydrocarbon family and has the structure of benzene with one hydrogen atom replaced by a methyl group, was assigned to the priority level "high" in the screening assessment of 2010, and designated as a priority assessment chemical substance in 2011. Subsequently, based on the results of the prioritization in Assessment I in 2018, toluene was indicated for a detailed hazard assessment by Assessment II. We report herein careful evaluation results on pharmacokinetics, general toxicity, reproductive and developmental toxicities, genotoxicity, and carcinogenicity of toluene and proposed draft human health hazard assessment values for oral and inhalation exposure.

MATERIALS AND METHODS

Toluene is a clear, colorless liquid that is extremely insoluble in water, but soluble in alcohol and oil, making it widely used as a solvent.

For the detailed hazard assessment of toluene for the Assessment II, we collected scientifically reliable information on pharmacokinetics, general toxicity, reproductive and developmental toxicities, genotoxicity, and carcinogenicity from risk assessment reports published by international, foreign or domestic risk assessment organizations, according to the guidance (MHLW, METI and MOE, 2018). Finally, we obtained risk assessment reports by several organizations such as U.S. Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Environmental Protection Agency - Integrated Risk Information System (EPA IRIS), European Union Risk Assessment Report (EU RAR), International Agency for Research on Cancer (IARC) and more shown in References as well as original articles of the key studies reported therein. Then, we evaluated the human health effects of toluene, and calculated draft human health hazard assessment values for oral and inhalation exposure, respectively, based on selected key toxicity information.

RESULTS

This section provides an overview of the pharmacokinetics (pharmacodynamics) and hazard information for toluene referenced in this assessment.

Pharmacokinetics (Pharmacodynamics)

The information regarding the absorption, distribution, metabolism, and excretion of toluene is summarized below. The pathways involved in toluene metabolism in humans and animals are shown in Fig. 1.

According to studies on human volunteers, toluene is rapidly absorbed through the respiratory system and more slowly through the digestive system and skin. The main metabolic step of toluene in humans and laboratory animals is side-chain hydroxylation to benzyl alcohol followed by its oxidation to benzoic acid, catalyzed primarily by the cytochrome P-450 enzyme CYP2E1. Most of the benzoic acid is conjugated with glycine to form hippuric acid, but a small amount reacts with UDP-glucuronic acid to form acylglucuronic acid conjugates. In studies on human volunteers and human liver microsomes, minimal amounts (1%–5%) of absorbed toluene are converted to ortho- or para-cresol by CYP1A2, CYP2B6, or CYP2E1 and excreted in urine as sulfate or glucuronide conjugates. Up to about 75%–80% of toluene absorbed by inhalation in humans or animals is found as hippuric acid in urine. The rest of the absorbed toluene is excreted without being metabolized in exhaled air and urine, whereas a small proportion of metabolite conjugates are excreted in the urine. The analysis of kinetic data on toluene levels after inhalation in human blood, exhaled air, adipose tissue, or urine shows that most of the absorbed toluene is rapidly eliminated from the body, whereas a

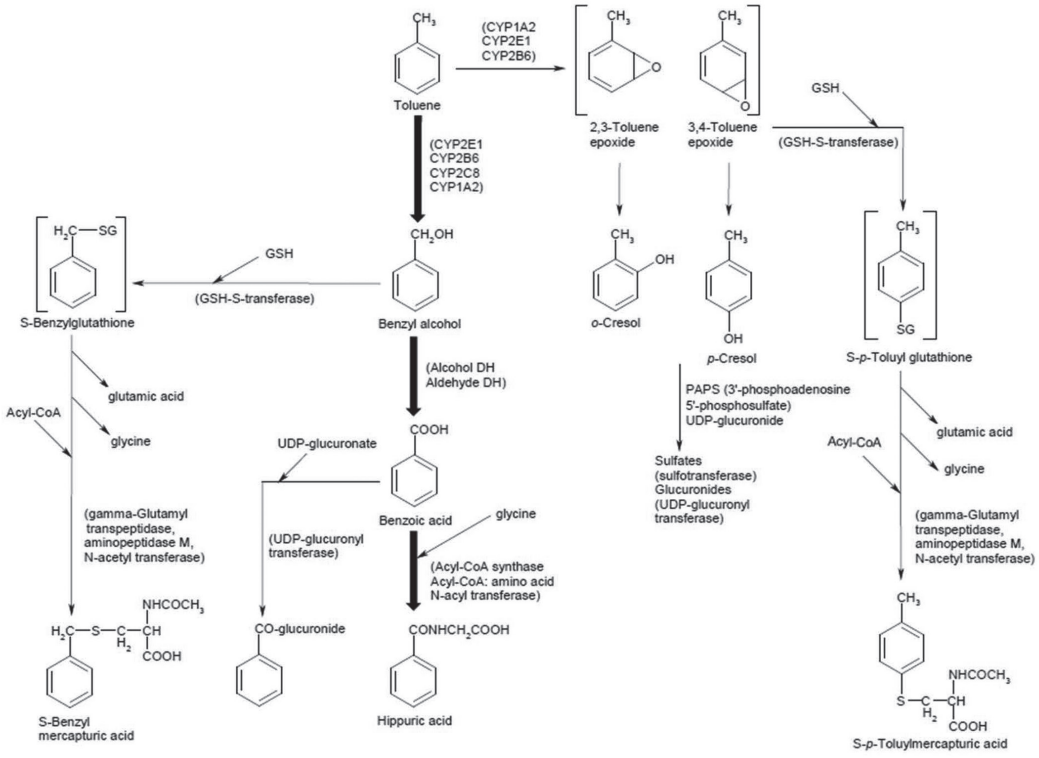

Fig. 1. Toluene metabolism in humans and animals (partially modified from ATSDR (2017)).

small amount accumulated in adipose tissue is gradually eliminated. For example, a three-phase elimination model in occupationally exposed individuals shows half-lives of 9 minutes, 2 hr, and 90 hr for toluene in blood, whereas a single-phase average elimination half-life of 79 hr was found for toluene in adipose tissue. Four-hour inhalation exposure of volunteers to toluene at approximately 50 ppm (192 mg/m^3) reported a biphasic decrease in urinary toluene concentrations with half-lives of 0.88 hr and 12.9 hr (ATSDR, 2017). Studies using laboratory animals showed that absorbed toluene is widely distributed throughout the body, especially in adipose tissue, brain, bone marrow, liver, and kidneys. Absorbed toluene is similarly distributed in pregnant animals and developing fetuses. In addition, toluene readily crosses the placenta, and it has been reported that concentrations in rat fetal blood correspond to approximately 75% of maternal blood concentrations. Toluene is also found in lipid-rich milk (EU RAR, 2003).

Noncarcinogenic effects by oral exposure

There is no information on chronic, and reproduction/development effects of toluene in humans.

Oral administration studies were performed in rats or mice for 13 weeks. In a 13-week gavage study in B6C3F1 mice (NTP, 1990; doses: 0, 312, 625, 1,250, 2,500, and 5,000 mg/kg/day, 5 days/week), a statistically significant increase in the relative liver weight was noted for doses of 1,250 mg/kg and above, but no other toxicity was evidenced.

In a F344/N rat gavage study (NTP, 1990), 10 rats/group/sex were given toluene at doses of 0 (control), 312, 625, 1,250, 2,500, and 5,000 mg/kg/day for 13 weeks (5 days/week). The animals receiving 2,500 mg/kg/day or more showed clinical signs such as prostration, hypoactivity, ataxia, piloerection, lacrimation, excessive salivation, or tremors before the deterioration of their condition that led to the animal death. Indeed, all males and females given 5,000 mg/kg/day died within one week and eight males and one female receiving 2,500 mg/kg/day died. The body weight of the males given 2,500 mg/kg/day was 19% lower than that of the controls at the end of the dosing period, whereas the body weight values of the other exposed groups were similar to those of the controls. The absolute and relative weights of the liver and kidneys were significantly increased for toluene doses of 625 mg/kg/day

Table 1. Absolute and relative liver and kidney weights in a rat 13-week gavage study (NTP, 1990).

	Dose (mg/kg/day)	0 (control)	312	625	1,250	2,500
Males	No. of animals	10	10	10	10	2
	BW at necropsy (g)	315 ± 6.2	328 ± 5.8	329 ± 5.8	321 ± 6.4	238 ± 7.5
	Liver absolute (mg)	10,490 ± 360	11,310 ± 300	11,850 ± 390*	14,400 ± 480**	14,130 ± 1,220*
	relative	33.3 ± 0.81	34.5 ± 0.68	35.9 ± 0.68*	45.0 ± 1.69**	59.4 ± 3.28**
	Kidney absolute (mg)	1,084 ± 14	1,159 ± 34	1,213 ± 39*	1,292 ± 34**	1,227 ± 114*
	relative	3.5 ± 0.06	3.5 ± 0.07	3.7 ± 0.06*	4.0 ± 0.06**	5.1 ± 0.32**
Females	No. of animals	10	10	10	10	9
	BW at necropsy (g)	183 ± 3.2	182 ± 3.5	175 ± 3.8	181 ± 2.7	180 ± 3.4
	Liver absolute (mg)	5,596 ± 112	5,822 ± 177	5,730 ± 225	6,780 ± 162**	8,918 ± 335**
	relative	30.7 ± 0.67	31.9 ± 0.46	32.7 ± 0.87	37.5 ± 0.68**	49.6 ± 1.53**
	Kidney absolute (mg)	686 ± 12	676 ± 19	652 ± 36	733 ± 18*	803 ± 26**
	relative	3.8 ± 0.08	3.7 ± 0.07	3.7 ± 0.17	4.1 ± 0.08**	4.5 ± 0.12**

Values represent means ± standard deviations, *: $p < 0.05$, **: $p < 0.01$ (Dunn's test or Shirley's test compared with controls).
BW, body weight; No, number.

Table 2. Incidence of neuronal necrosis in the brain in a rat 13-week gavage study (NTP, 1990).

Dose (mg/kg/day)	0 (control)	312	625	1,250	2,500	5,000
Males	0/10	0/10	0/10	6/10**	8/10** (8/10 dead[#])	0/10 (all dead[$])
Females	0/10	0/10	0/10	0/10	7/10** (1/10 dead[$])	0/10 (all dead[$])

[#]: 8 males receiving 2,500 mg/kg/day toluene died at 3, 3, 6, 7, 7, 8, 8, and 10 weeks.
[$]: 1 female given 2,500 mg/kg/day toluene and all animals receiving 5,000 mg/kg/day died within 1 week of dosing.
**: $p < 0.01$ (Fisher exact test compared with controls).

or more in males and 1,250 mg/kg/day or more in females (Table 1). Neuronal necrosis in the rat brain was observed in the dentate gyrus and Ammon's horn of the hippocampus for toluene concentrations of 1,250 mg/kg/day or more in males and 2,500 mg/kg/day in females (Table 2). Increased liver and kidney weights were considered a possible adaptive response to toluene metabolism. However, the levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, and creatinine, which are indicators of liver and kidney damage, were not measured, and we could not conclude those as adaptive response, and therefore treated them as a sign of toxicity.

Based on these results, we estimated that the no-observed-adverse-effect level (NOAEL) for no increase of the liver and kidney weights was 312 mg/kg/day, and the NOAEL for no toxic effects in the central nervous system (CNS) was 625 mg/kg/day.

Information regarding toluene reproductive toxicity after oral exposure is lacking, but. data on its developmental toxicity are available s. In a study on ICR/SIM mice receiving toluene by gavage from gestation days 8 to 12 (Seidenberg *et al*., 1986), no fetal toxicity or teratogenicity was observed for doses of up to 1,800 mg/kg/day. In a gavage study in ICR mice at 6–13 days of gestation (Hardin *et al*., 1987), no fetal toxicity or teratogenicity was observed for doses of 3,000 mg/kg/day or lower. Toluene administration at 520 mg/kg/day by gavage in SD rats at gestation days 6–19 (Gospe *et al*., 1994) prevented maternal and fetal body weight gain but no other abnormalities, including malformations, were observed. In a study, in which toluene was given in drinking water (at a dose equivalent to 72 mg/kg/day) to Nya mice during gestation, lactation, and up to 55 days after weaning (Kostas and Hotchin, 1981), no dose-dependent changes were observed in pups' growth, survival, developmental indices, or performances in the rotating rod test. Because no teratogenicity or developmental disorders were reported after oral exposure of pregnant animals to toluene, we concluded that toluene has no developmental toxicity.

Noncarcinogenic effects by inhalation exposure

Epidemiological investigations of occupational exposure by inhalation have shown that toluene mainly affects the CNS (neurobehavioral and audiovisual effects) and have suggested an increased rate of spontaneous abortions. In animals, in addition to the effects on CNS, inhibition of weight gain and effects on the kidney and nasal

Table 3. Occupational studies reported by Seeber *et al*. and Schäper *et al*. (ATSDR, 2017).

Study author, date	Neurological endpoint(s) evaluated	NOAEL (ppm)
Seeber *et al*. (2004, 2005)	Subjective symptoms, symbol digit substitution, switching attention, and memory span (initial study 2004; follow-up analysis of the same data in 2005)	45
Schäper *et al*. (2003, 2008)	Audiometry (two reports of the same study)	45
Schäper *et al*. (2004)	Color vision (Lanthony desaturated panel D-15d, Ishihara plates)	43

cavity have been reported.

Regarding the CNS effects in humans, Foo *et al*. (1990) conducted eight neurobehavioral tests on 30 female workers who were working with adhesives containing toluene in an electronics assembly plant in Singapore. Female workers exposed to toluene during an eight-hour workday (88 ppm [332 mg/m^3], average work duration: 5.7 years) showed no clinical symptoms. However, their performances in six tests including hand elaboration, visual function, and verbal memory tests, were significantly inferior to those of 30 female workers from the low exposure control group (exposed to 13 ppm [50 mg/m^3], average work duration: 2.5 years). Thus, Foo *et al*. reported that the lowest-observed-adverse-effect level (LOAEL) of toluene on neurobehavioral functions was 88 ppm (332 mg/m^3).

Seeber *et al*. (Seeber *et al*., 2004, 2005; Schäper *et al*., 2003, 2004, 2008) reported the occupational history and toluene exposure of German photo-printing employees (14 plants, average employment duration: 13.4 years), and found time-weighted average (TWA) levels of 45 and 10 ppm (Schäper *et al*., 2003, 2008), 45 and 9 ppm (Seeber *et al*., 2004, 2005), or 43 and 9 ppm (Schäper *et al*., 2004) for the high exposure (printers, n = 106–181) and the low-exposure (end processing, n = 86–152) groups, respectively. The comparison of their neurological performances (e.g., auditory, color vision, and brain cognitive functions)revealed no significant differences between the high- and low-exposure groups. Therefore, we determined NOAEL for neurological effects of 45 ppm (Table 3).

Ng *et al*. (1992a) investigated the rate of spontaneous abortions in female workers handling toluene-containing adhesives (mean exposure: 88 ppm, range: 50–150 ppm, 55 workers) at an electronics assembly plant in Singapore, with female workers from other departments (13 ppm, range 0–25 ppm, 31 workers) as internal controls and women (n = 190) who underwent medical examinations before and after childbirth as external controls. The spontaneous abortion rate (12.4%) of toluene-exposed female workers (average working years: 10.0 years, total number of pregnancies: 105) was significantly higher than that of the internal controls (2.9%, average working years: 9.7 years, the total number of pregnancies: 68) and external controls (4.5%, total number of pregnancies: 444). Thus, Ng *et al*. (1992a) reported a LOAEL for increased spontaneous abortion rates of 88 ppm.

In a two-generation reproductive developmental toxicity study using SD rats (Roberts *et al*., 2003), parents, and offspring were exposed to toluene through whole-body inhalation. The body weight gain and skeletal variations were inhibited in fetuses and newborns after exposure to 2,000 ppm. There were no effects on fertility or behavior of pups during lactation in any of the exposure groups. In a study, in which male and female SD rats were exposed to toluene by whole-body inhalation from before mating to seven days of gestation (Ono *et al*., 1996), a statistically significant decrease in the epididymis weight and sperm count was observed in males exposed to 2,000 ppm. Repression of the female body weight gain and a statistically significant increased fetal mortality were also observed, but there were no changes in the females' mating behavior or fertility. In a study on pregnant rats exposed to toluene by inhalation during the organogenesis period, a dose of 2,000 ppm prevented body weight gain in females and induced growth retardation of the pups; however, the external, internal, and skeletal examination of the fetuses and the behavior analyses of the offspring revealed no abnormalities. Thus, inhalation exposure to toluene does not affect fertility or fetal organogenesis; however, a high exposure prevents maternal body weight gain, impairs testicular spermatogenesis, and delays fetal and neonatal growth. These effects are believed to be caused by nutritional disorders. A study on eight New Zealand rabbits exposed to toluene by inhalation during gestation days 7 to 20 (Ungváry and Tatrai, 1985) reported the death of two females, embryo resorption in two females, and abortion in four females. Because the doses were high enough to cause death, this study was not considered in determining the relationship between toluene exposure and abortion.

Mutagenicity (genotoxicity)

Chromosomal aberrations in peripheral lymphocytes obtained from the blood of humans after occupational exposure to toluene were found in six out of 10 studies, whereas the four others reported no anomalies (Pelclova *et al*., 1990, 2000; Schmid *et al*., 1985; Bauchinger *et*

al., 1982; Nise *et al*., 1991; Funes-Cravioto *et al*., 1977). Negative results for the micronucleus test were reported in three out of six studies and positive results were obtained in the three other studies (Gonzalez-Yebra *et al*., 2009; Pitarque *et al*., 2002; Nise *et al*., 1991). No sign of DNA damage was found in eight out of 14 studies, whereas six studies reported DNA damage after toluene exposure (Moro *et al*., 2012; Heuser *et al*., 2005, 2007; Hammer *et al*., 1998; Priya *et al*., 2015; Bauchinger *et al*., 1982). However, in all the aforementioned studies describing positive effects, a possible coexposure to other drugs or smoking could not be excluded. Therefore, we concluded that there was no obvious evidence of genotoxicity in humans.

In vitro genotoxicity studies obtained negative results regarding the effect of toluene in bacterial reversion mutation (Connor *et al*., 1985), micronucleus (Zarani *et al*., 1999), and the sister chromatid exchange tests (Gerner-Smidt and Friedrich, 1978).

Toluene oral or inhalation administration had no effects on chromosome aberration (Gad-El-Karim *et al*., 1984), micronucleus (Bird *et al*., 2010), and DNA damage (Martinez-Alfaro *et al*., 2010), and in the dominant lethal tests in rats (Brusick and Mazursky, 1981).

In summary, considering that toluene had no effects in many *in vitro* and *in vivo* studies and that there was no solid evidence of genotoxicity in human *ex vivo* studies, we concluded that toluene is not genotoxic to humans.

Carcinogenic effects by oral exposure

No information regarding the carcinogenicity of human oral exposure to toluene was found.

Toluene was administered to SD rats (40–50 animals/sex) by gavage at doses of 0, 500, and 800 mg/kg/day for 4 days/week in a 104-week oral gavage study (Maltoni *et al*., 1997). Mammary tumors, lymphoma, and leukemia were observed in females receiving 500 mg/kg/day. Head tumors, lymphoma, and leukemia were found in males exposed to 800 mg/kg/day, and lymphoma and leukemia in females given 800 mg/kg/day. However, this study was deemed not suitable for risk assessment because the tumor incidence was not dose-dependent and, the tumor data reporting did not meet the guideline requirements.

Carcinogenic effects by inhalation exposure

In a gravure printing plant in West Germany, a cohort investigation of cancer and mortality incidences was conducted on 6,830 men (duration of employment: at least one year) (Wiebelt and Becker, 1999). The cohort was divided into three groups: the printing process group (exposure concentrations: less than 200 ppm from 1960 to 1985 and less than 100 ppm after 1985), the preparation group (less than 30 ppm), and the final processing group (less than 30 ppm). The correlations between the groups and the total number of cancers or the number of cancers in specific tissues were investigated. No increase in the number of cancers was found. In contrast, a greater incidence of lung cancer and colorectal cancer was observed in 7,814 men and women (toluene exposure concentrations: 10–72 ppm) working in a U.S. shoe manufacturing plant (Walker *et al*., 1993). However, the association between toluene exposure and cancer was not clear due to the potential effects of smoking and coexposure to other chemicals (2-butanone, acetone, and/or hexane). In Sweden, a greater risk of mortality and morbidity from airway, gastric, and colorectal cancers was found in 1,020 individuals (exposure concentrations: 450 ppm until the 1940s and 30 ppm until the mid-1980s) who worked in the printing industry for more than three months (Svensson *et al*., 1990). However, when the investigation was restricted to workers who had been exposed for more than five years, no association between the increased relative risk and cumulative toluene dose was found. Thus, there is no clear indication of a link between toluene exposure and increased cancer incidence in humans.

An inhalation study (Gibson and Hardisty, 1983) in F344 rats, in which 120 animals/group/sex were exposed to toluene at 0, 30, 100, and 300 ppm by whole-body inhalation for two years (6 hr/day, 5 days/week) revealed no non-neoplastic changes nor greater tumorigenesis attributable to toluene exposure. In addition, a two-year carcinogenicity study of whole-body inhalation exposure (6.5 hr/day, 5 days/week) to toluene at doses of 0, 600, and 1,200 ppm in F344/N rat (50 animals/group/sex) and 0, 120, 600, and 1,200 ppm in B6C3F1 mice (60 males/group, 50 females/group) was conducted (NTP, 1990). Inflammation of the nasal mucosa, erosion of the olfactory epithelium, and metaplasia of the olfactory epithelium were observed in rats. In mice, the body weight of females exposed to 1,200 ppm toluene was slightly lower than that of the controls. However, toluene did not cause an increase in tumors in rats or mice.

Toxicological mechanisms

Toluene is rapidly absorbed and widely distributed throughout the body, particularly in lipids and adipose tissue, brain, bone marrow, liver, and kidneys. Toluene absorbed into the body is metabolized and excreted by the liver and kidneys, but in animals exposed to high doses orally, it is believed to induce an increase in the organ weight due to the load placed on these organs. Inhalation of toluene by animals may cause histopathological chang-

es (e.g., inflammation of the nasal mucosa, erosion of the olfactory epithelium, metaplasia of olfactory epithelium) due to the irritation of the nasal cavity. The toxicity of a short-term and high-level exposure to toluene has been reported in various organs and systems in addition to the CNS. However, investigations mostly focused on the toxicity mechanisms in the CNS, as CNS toxicity seriously affects human health. Since toluene has a high lipid affinity, its toxicity mechanisms include 1) effects on cell lipid bilayers and cell membrane functional proteins, 2) direct damage to brain tissues via lipid damage, oxidative stress, and apoptosis, 3) effects on synthesis, release, and degradation of neurotransmitters, 4) effects on receptor binding, hypothalamic–pituitary–adrenal axis disruption, and 5) neuroinflammation (ATSDR, 2017). Although the mechanisms involved are not completely understood, the increased rate of neurological disorders, decreased performance on neurobehavioral tests, and impaired hearing and vision induced by medium- to long-term exposure to toluene may be attributed to repetitive interactions of toluene with membrane proteins and phospholipids in neurons.

It has been hypothesized that neurological effects are caused by altered activities of enzymes involved in neurotransmitter synthesis and degradation, leading to changes in neurotransmitter levels and binding of neurotransmitters to membrane receptors in certain regions of the brain (ATSDR, 2017). For example, the levels of neurotransmitters such as glutamate, taurine, dopamine, norepinephrine, serotonin, and acetylcholine are increased in various brain regions of rats that inhaled toluene (Rea *et al*., 1984; Aikawa *et al*., 1997), and toluene may inhibit glutamate binding to the N-methyl-d-aspartate (NMDA) receptor leading to the impaired coordination and memory observed after toluene exposure (Lo *et al*., 2009).

Phenobarbital treatment of rats shortens the recovery time from a coma induced by a single intraperitoneal dose of toluene (Ikeda and Otsuji, 1971). In addition, inhibition of toluene metabolism by ethanol pretreatment promotes toluene-induced hearing impairment (Campo *et al*., 1998). These data suggest that toluene itself, and not its metabolites, is the substance affecting the CNS (EPA IRIS, 2005).

In addition to the effects of toluene on the CNS, the analysis of reproductive and developmental toxicity in animals showed that inhalation of high concentrations, which inhibited maternal weight gain, caused developmental impairments in fetuses and newborns such as low body weight, delayed physical development, and learning disabilities (ATSDR, 2017). The possible effects on the neuroendocrine system and the decreased weight of the epididymis and sperm count in rats observed after high exposure to toluene (Ono *et al*., 1996) have been attributed to oxidative stress-induced apoptosis. However, because of the limited amount of data and the lack of discrimination with other factors' effects, no clear mechanisms of action have been identified (ATSDR, 2017).

DISCUSSION

As described previously, toluene does not exhibit obvious mutagenicity (genotoxicity), and no reliable data regarding its carcinogenicity have been obtained for both the oral and inhalation routes. Therefore, we decided to derive hazard assessment values based on the non-carcinogenic effects induced by oral and inhalation exposure to toluene.

Derivation of hazard assessment value for oral exposure

Since there were no reports on long-term oral exposure or reproductive toxicity, we selected the 13-week oral administration study of toluene in rats (NTP, 1990) as the key study for the hazard assessment of oral exposure because…and calculated the hazard assessment values for each endpoint: effects on the liver and kidney weights or the CNS.

When liver and kidney weight increases were used as endpoints, the NOAEL for no weight increases was estimated to be 312 mg/kg/day (5 doses per week), and an average daily dose of 223 mg/kg/day was calculated. An oral hazard assessment value of 0.223 mg/kg/day was obtained for toluene by dividing the average daily dose by a UF of 1,000 (interspecies variation: 10, intraspecies variations: 10, and short test periods: 10).

When the endpoint was the effect on the CNS (neuronal necrosis in the brain for doses of 1,250 mg/kg/day or more), the NOAEL was estimated to be 625 mg/kg/day (5 times per week) and an average daily dose of 446 mg/kg/day was calculated. A hazard assessment value of 0.446 mg/kg/day was obtained by dividing the average daily dose by a UF of 1,000 (interspecies variation: 10, intraspecies variations: 10, and severe effect on the CNS: 10). We did not apply the UF for a short study period, because the neuronal necrosis in the brain was only confirmed for exposure to high doses of toluene and was not induced by long-term exposure to low doses (Gibson and Hardisty, 1983; NTP, 1990).

Finally, we selected 0.223 mg/kg/day as the oral hazard assessment value for toluene among the assessment values obtained for both systemic and CNS effects endpoints, as this value is more safety-conscious (Table 4).

Table 4. Summary of hazard assessment values for toluene.

Exposure route	Hazard assessment value	Basis data and derivation method
Oral	0.223 mg/kg/day[(a)]	A 13-week oral administration study in rats (NTP, 1990) was selected as the key study. The NOAEL without liver and kidney weight increases was estimated to be 312 mg/kg/day (5 doses per week). The derived oral hazard assessment value was 0.223 mg/kg/day, which was obtained by dividing the average daily equivalent NOAEL of 223 mg/kg/day by a UF of 1,000 (interspecies variability: 10, intraspecies variability: 10, and short study period: 10).
Inhalation	0.1 ppm[(b)] (0.383 mg/m^3) (Equivalent to a daily intake of 0.153 mg/kg/day[(c)])	Epidemiological investigations of occupational exposure to toluene (Seeber *et al*., 2004, 2005; Schäper *et al*., 2003, 2004, 2008) were selected as key studies. The NOAEL for no neurological effects (auditory, color vision, and brain cognitive functions) was estimated to be 45 ppm. The derived inhalation hazard assessment value was 0.1 ppm (0.383 mg/m^3), which was obtained by dividing the average continuous exposure equivalent NOAEL of 10.7 ppm by a UF of 100 (intraspecies variation: 10 and severe effect: 10).

[(a)]: 0.223 mg/kg/day = 312 mg/kg/day × 5/7 days / UF 1,000.
[(b)]: 0.1 ppm (0.383 mg/m^3) = 45 ppm × 5/7 days x 8/24 hr / UF 100 (1 ppm = 3.831 mg/m^3).
[(c)]: 0.153 mg/kg/day = 0.383 mg/m^3 × 20 m^3/day / 50 kg.

Derivation of hazard assessment value for inhalation exposure

We calculated the hazard assessment value for toluene inhalation exposure from epidemiological investigations of human occupational exposure showing effects on the CNS and an increase in the spontaneous abortion rate, rather than calculating it from animal experimental studies showing different effects than those observed in humans.

Regarding the increase in the spontaneous abortion rate, we considered at first deriving the inhalation hazard assessment value from the study of Ng *et al*. (1992a), in which the LOAEL for no effect on the spontaneous abortion rate was 88 ppm. However, the U.S. Agency for Toxic Substances and Disease Registry (ATSDR, 2017) noted that this report (Ng *et al*., 1992a) was based on a questionnaire survey that may be inaccurate and that the spontaneous abortion rates in the internal and external control groups were lower than those commonly reported, making it unreliable and requiring further data. Furthermore, we were unable to confirm the level of toluene exposure, although the authors (Ng *et al*., 1992a) indicated that an average exposure of 88 ppm (range 50–150 ppm) was previously reported by Foo *et al*. (1988, 1990) and Ng *et al*. (1992b). Therefore, we did not use the report by Ng *et al*. (1992a) to derive the hazard assessment value, although the possibility of an increased spontaneous abortion as a toxic effect of toluene cannot be fully ruled out.

To derive the hazard assessment value using the effects of toluene on the CNS as an endpoint, we selected and reviewed the following two investigations of occupational toluene exposure, which incorporate a wide range of test items such as neurobehavioral and audiovisual effects. 1) Foo *et al*. (1990) reported a LOAEL of 88 ppm because the performances of the exposed group (88 ppm) were significantly inferior to those of the control group (13 ppm) in six out of eight neurobehavioral function tests, including manual dexterity, visual function, and verbal memory tests. 2) Seeber *et al*. (2004, 2005) and Schäper *et al*. (2003, 2004, 2008) reported a NOAEL of 45 ppm because there was no significant difference in the effects of toluene on the CNS (effects on auditory, color vision, and brain cognitive functions) between the exposed group (45 ppm) and the control group (9 ppm). We decided that the investigations (Seeber *et al*., 2004, 2005; Schäper *et al*., 2003, 2004, 2008), from which a NOAEL could be obtained are appropriate key studies to derive the hazard values. The NOAEL for no neurological effects (auditory, color vision, and brain cognitive functions) was evaluated to be 45 ppm, and a continuous exposure value of 10.7 ppm was calculated. An inhalation hazard assessment value of 0.1 ppm (0.383 mg/m^3) was obtained for toluene by dividing the average continuous exposure by a UF of 100 (intraspecies variation: 10 and severe effect: 10). This value corresponds to a daily human intake of 0.153 mg/kg/day assuming a human body weight of 50 kg and a respiratory volume of 20 m^3/day (Table 4). The obtained inhalation hazard assessment value of 0.1 ppm (0.383 mg/m^3) for toluene is 1/500 to 1/1,500 of the toluene exposure of female workers (mean 88 ppm, range 50 to 150 ppm) reported by Ng *et al*. (1992a). Therefore, we considered that a safety margin was secured to prevent the increase in the incidence of spontaneous abortions.

We conducted the herein described detailed assessment of human health effects of toluene for Assessment II under the CSCL and suggested the draft hazard assessment values of 0.223 mg/kg/day for oral exposure and 0.1 ppm for inhalation exposure, respectively.

Additional Information

Regarding the present hazard assessment values, the joint meeting of the MHLW, METI, and MOE adopted these draft values in September 2021 and the Risk Assessment (Primary) Evaluation II (Human Health Effects) of toluene was conducted in January 2022. In this Risk Assessment, it was concluded that if the current handling and emission conditions are kept unchanged, toluene will not pose concerns to human health (METI, 2022).

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Conflict of interest---- The authors declare that there is no conflict of interest.

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