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*Original Article*

# Derivation of human health hazard assessment values of 1,2-dichloroethane under the Japan Chemical Substances Control Law

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**ABSTRACT** — 1,2-Dichloroethane, a priority assessment chemical substance under the Japan Chemical Substances Control Law (CSCL), required a detailed human health hazard assessment under Assessment II. We evaluated its general, reproductive, and developmental toxicities, genotoxicity, and carcinogenicity, based on the hazard information provided by domestic and international risk assessment organizations, and the hazard assessment values (HAVs) for oral and inhalation exposure were proposed. For oral exposure, a 78-week gavage carcinogenicity study (US NCI, 1978) with incidence data of hemangiosarcoma in male rats was selected as a significant toxicological endpoint and the lower confidence limit of benchmark dose (BMD) at 10% benchmark response ($BMDL_{10}$) of 9.3 mg/kg/day was obtained as a point of departure (POD). A slope factor of $1.07 \times 10^{-2}$ $(mg/kg/day)^{-1}$ from which a carcinogenic $10^{-5}$ risk of 0.93 µg/kg/day was derived as an oral HAV. For inhalation exposure, a 104-week inhalation exposure carcinogenicity study (Nagano *et al*., 2006) with a $BMDL_{10}$ of 11.5 ppm based on the incidence data of mammary gland tumors (adenocarcinoma + adenoma + fibroadenoma, combined) in female rats was obtained, and the human equivalent $BMDL_{10}$ of 15.7 $mg/m^3$ was calculated. Therefore, a unit risk of $6.40 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ from which a carcinogenic $10^{-5}$ risk of 1.6 $\mu g/m^3$ (0.00039 ppm) was derived as an inhalation HAV.

**Key words:** 1,2-dichloroethane (CAS No, 107-06-2), Chemical Substances Control Law (CSCL), Assessment II for human health effects, Hazard assessment value (HAV)

## INTRODUCTION

In Japan, chemical substances, including existing chemicals, are controlled by the Japan Chemical Substances Control Law (CSCL, 1973) to prevent environmental pollution. In the Risk Assessment II stage, we prepared a draft evaluation report on a target priority assessment chemical substance (PACS) concerning its general, reproductive, and developmental toxicity, genotoxicity, and carcinogenicity, referring mainly to the assessment values obtained from "key studies" published by reliable domestic and international organizations, and determine draft assessment values for oral and inhalation exposures. The prepared draft report and assessment values on human health effects are reviewed for approval by the joint council of the Ministry of Health, Labor, and Welfare (MHLW), Ministry of Economy, Trade, and Industry (METI), and Ministry of the Environment (MOE). The representative hazard assessment values (HAVs) are assigned to the Risk Assessment II stage. The

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risk assessment scheme of chemicals under the CSCL was mentioned in our previous study (Kawashima *et al*., 2022).

1,2-Dichloroethane (CAS No. 107-06-2), known as ethylene dichloride (EDC), was assigned a priority level "high" in the screening assessment in 2010 and designated as a PACS in 2011. Herein, we reported the draft human HAVs of 1,2-dichloroethane for oral and inhalation exposure according to the "Risk Assessment Methodology for Priority Assessment Chemical Substances" (MHLW, METI, and MOE, 2022a) and "Technical Guidelines for Risk Assessment of Priority Assessment Chemical Substances" (MHLW, METI, and MOE, 2022b).

## MATERIALS AND METHODS

1,2-Dichloroethane, a chlorinated hydrocarbon with a molecular formula of $C_2H_4Cl_2$ and a molecular weight of 98.96. It is a clear, colorless liquid with a chloroform-like odor and is highly volatile with a vapor pressure of 8.5 kPa (20°C) and water solubility of 8,690 mg/L (20°C). It is a raw material used to synthesize substances, such as vinyl chloride monomer, ethylenediamine, polyamino resins, and ion exchange resins. It is a film cleaner and solvent in organic synthesis and vitamin extraction (NITE, 2005).

For detailed hazard Assessment II of 1,2-dichloroethane, we collected scientifically reliable information from risk assessment reports published by international, foreign, or domestic risk assessment organizations, according to the "Reliability Assessment of Toxicity Data on Human Health Effects under the CSCL" (METI, 2019). We obtained risk assessment reports published by several organizations, such as the US Agency for Toxic Substances and Disease Registry, the Toxicological Profile draft for public comment (ATSDR draft, 2022), the MHLW Risk Evaluation Draft Report (MHLW draft, 2019), the Dutch Expert Committee on Occupational Safety Advisory report (DECOS, 2019), the EU Scientific Committee on Occupational Exposure Limits (EU SCOEL, 2016), the US Provisional Peer-Reviewed Toxicity Values (EPA PPRTVs, 2006), and the Organization for Economic Co-operation and Development Screening Information Data Set (OECD SIDS, 2002). We evaluated the human health effects of 1,2-dichloroethane and calculated the draft human health HAVs for oral and inhalation exposure, respectively, based on vital toxicity information.

## RESULTS

This section provides an overview of the pharmacokinetics (pharmacodynamics) and hazard information of 1,2-dichloroethane.

### Pharmacokinetics (Pharmacodynamics)

The absorption, distribution, metabolism, and excretion of 1,2-dichloroethane are summarized below. The pathways involved in 1,2-dichloroethane metabolism in humans and animals are shown in Fig. 1.

The human case reports on acute poisoning of workers exposed to 1,2-dichloroethane by inhalation indicate that 1,2-dichloroethane is rapidly absorbed and widely distributed in the human body. Small amounts of metabolites, 2-chloroethanol and monochloroacetic acid, were detected in the blood of human poisoning cases (Nouchi *et al*., 1984). 1,2-Dichloroethane is metabolized by CYP2E1 in the human liver microsomes (Guengerich *et al*., 1991). There have been several death cases 20 hr after inhalation exposure of 1,2-dichloroethane for 30 min (unknown concentration). Occupational exposure by inhalation and dermal exposure resulted in the transfer of 1,2-dichloroethane into breast milk (Urusova, 1953).

The animal experimental results showed that 1,2-dichloroethane is rapidly absorbed by oral and inhalation routes, with the highest blood concentration of 30–44 μg/mL in rats 15 min after an oral dose of 150 mg/kg of 1,2-dichloroethane (Reitz *et al*., 1982). When 25–150 mg/kg of 1,2-dichloroethane was administered orally in rats, there was no correlation between dosage and blood concentration above 50 mg/kg, indicating saturation of absorption in the gastrointestinal tract (Spreafico *et al*., 1980). Upon inhalation, a dose of 150 ppm for 6 hr resulted in the highest blood concentration of 8–10 μg/mL after 1–2 or 2–3 hr (Reitz *et al*., 1980, 1982). Upon oral and inhalation administration, 1,2-dichloroethane was rapidly distributed in the blood, liver, spleen, and brain, with higher distribution in the adipose tissues. The liver saturated the fastest (after 10 min) compared to the other tissues in rats after oral administration of 1,2-dichloroethane. The concentrations in the adipose tissues were saturated after 45–60 min and were approximately five times higher than the blood concentrations (Spreafico *et al*., 1980). Upon inhalation exposure, the concentrations in various tissues of rats exposed to 250 ppm were 20–30 times higher than those exposed to 50 ppm. In the experiments with pregnant rats exposed to 153–1,999 ppm of 1,2-dichloroethane for 5 hr, the maternal blood and fetal concentrations increased in correlation with the dose, indicating that 1,2-dichloroethane crosses the placenta, and the fetal concentration was 0.316 times the maternal blood concentration (Withey *et al*., 1985). A detailed evaluation of metab-

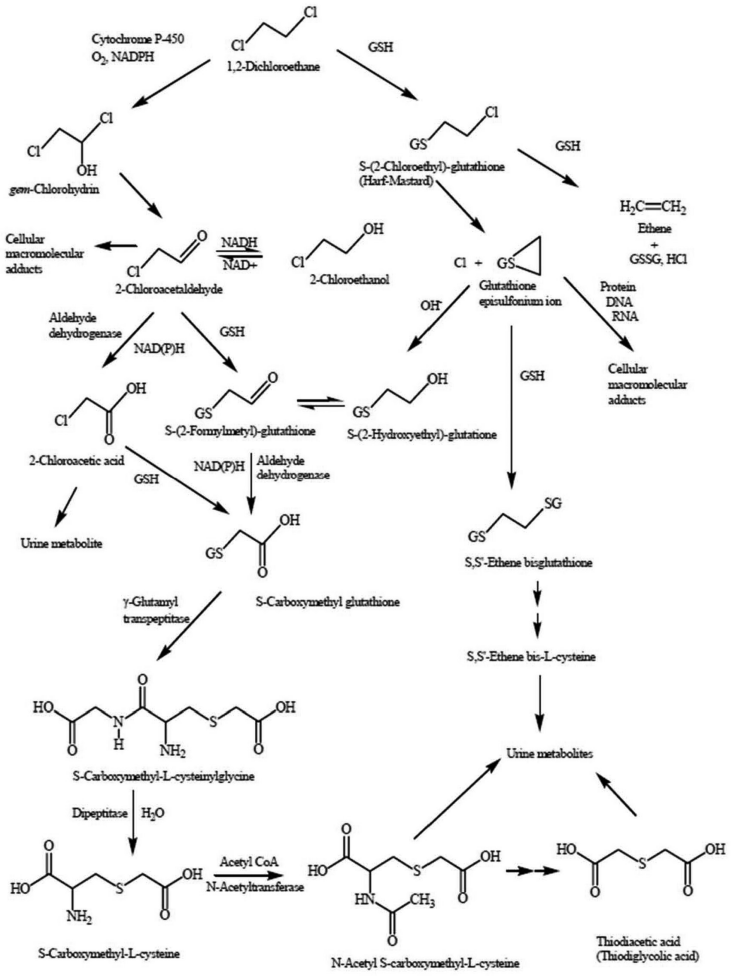


**Fig. 1.** Metabolic pathway of 1,2-dichloroethane (secondary quotation from NITE, 2005: US DHHS, 1999).

olism and excretion of 1,2-dichloroethane demonstrated that diacetic acid sulfide and its sulfate conjugate were found in the urine of rats orally dosed with 150 mg/kg radiolabeled 1,2-dichloroethane. Urinary excretion of radioactivity was 85.7%, and excretion as carbon dioxide in exhaled air was 7.7% (Reitz *et al*., 1980, 1982). In another report, 1,2-dichloroethane was administered orally in mice and rats, and S-carboxymethyl cysteine, diacetic acid sulfide, and chloroacetic acid were found in both animals. Excretion as carbon dioxide in exhaled breath was 18.21% in mice and 8.20% in rats (Nagano *et al*., 1985). There are reports of the detection of diglycolic acid sulfide and thioether. In this experiment, urinary excretion of radioactivity was 62.1% at low doses, whereas it decreased to 7.4% at high doses, suggesting saturation of metabolism or gastrointestinal absorption (Payan *et al*., 1993). Inhalation exposure also showed the same metabolites as oral dose, with diacetic acid sulfide and its sulfate conjugate in the urine after a 6-hr exposure to 150 ppm (Payan *et al*., 1993). Urinary excretion of radioactivity was 84%, and carbon dioxide in the exhaled breath was 7% (Reitz *et al*., 1980, 1982). Another experiment detected diglycolic acid sulfide and its sulfate conjugate, chloroacetic acid, after a 50 ppm exposure (Cheever *et al*., 1990). The measurements of the SH-residue concentrations in the liver after oral and inhalation exposure showed glutathione consumption by 1,2-dichloroethane (Reitz *et al*., 1982). The glutathione conjugate of 1,2-dichloroethane binds DNA and is suggested to cause mutagenicity and carcinogenicity (Cheever *et al*., 1990;

Reitz, 1980, 1982).

### Noncarcinogenic effects by oral exposure

There is no information on humans' general, reproductive, and developmental toxicity of 1,2-dichloroethane. Two oral repeated dose toxicity studies and three reproductive developmental studies are available for animal studies.

In a 90-day oral toxicity study in rats (Daniel *et al*., 1994; a key study designated by the Food Safety Commission of Japan, 2008; NITE, 2005), 1,2-dichloroethane was administered to SD rats (10/sex/dose) by gavage at doses of 37.5, 75, and 150 mg/kg/day (solvent: corn oil) for 90 days. As a result, an increase in the relative weight of the kidney, liver, and brain and a decrease in hemoglobin and hematocrit values were detected at 75 mg/kg/day and above. Based on the results, the no observed adverse effect level (NOAEL) was determined to be 37.5 mg/kg/day. In a 13-week drinking water dose toxicity study (US NTP, 1991; a key study designated by ATSDR draft, 2022; US EPA PPRTVs, 2010; MOE, 2003) using F344/N rat (10/sex/dose), 1,2-dichloroethane at 0, 500, 1,000, 2,000, 4,000, and 8,000 ppm (equivalent to 0, 58, 102, 182, 320, and 601 mg/kg/day) was dosed for 13 weeks. As a result, both sexes observed an increase in the absolute and/or relative kidney weights at 500 ppm and above. In females, slight or mild tubular regeneration in the kidney was also observed dose-dependently. Based on the result, the lowest observed adverse effect level (LOAEL) was 58 mg/kg/day.

Regarding reproductive and developmental toxicity of 1,2-dichloroethane, a 2-year dietary exposure and mating study in rats (Alumot *et al*., 1976), a two-generation drinking water reproduction study in mice (Lane *et al*., 1982), and an oral developmental study in rats (Payan *et al*., 1995) have been performed. These studies showed no toxic effects on the reproductive and developmental parameters, including external, visceral, and skeletal examinations.

### Noncarcinogenic effects by inhalation exposure

Regarding humans, the following three investigations have been reported, but no information on the effects on reproduction and development is available. Regarding animals, two repeated dose toxicity studies and four reproductive and developmental toxicity studies are available.

Kozik (1957, a key investigation designated by US EPA PPRTVs, 2010) reported a study on workers in a Russian airplane factory handling adhesives containing 1,2-dichloroethane as a solvent. The concentrations of 1,2-dichloroethane in various working environments ranged from 5–40 ppm. Of the 83 workers examined from 1951–1955, 19 had liver and bile duct diseases, 13 had neurological symptoms, 11 had autonomic imbalance, 10 had hyperthyroidism or goiter, and 5 had symptoms of asthenia. In a complex visual-motor reaction test, workers exposed to 1,2-dichloroethane showed more errors than the control group. Brzozowski *et al*. (1954) reported symptoms in workers exposed to 10–200 ppm of 1,2-dichloroethane in a Polish oil refinery. Six of the 42 workers complained of anorexia, abnormal white blood cell counts, dizziness, nausea, vomiting, and loss of appetite. Rosenbaum (1947) researched 100 workers exposed to up to 25 ppm of 1,2-dichloroethane for 6 months to 5 years in a Russian industry from 1934–1945. All workers showed no changes in blood and organ function tests, but some reported autonomic nervous disorder, diffuse erythroderma, muscle tension, bradycardia, hyperhidrosis, fatigue, irritability, and insomnia.

Regarding animals, in a 12-month inhalation toxicity study (Spreafico *et al*., 1980; a key study designated by US EPA PPRTVs, 2010; NITE, 2005; MOE, 2006), SD rats (8–10/sex/dose) were exposed to 1,2-dichloroethane vapors at 0, 5, 10, 50, and 150 ppm (0, 20.6, 41.1, 205.5, and 616.5 mg/m$^3$). At 50 ppm and above, an increase in ALT and uric acid, a decrease in cholesterol in both sexes, and an increase in γ-GTP in females were observed. At 150 ppm, an increase in glucose was observed in both sexes. Based on the results, the NOAEL was determined to be 10 ppm (41.1 mg/m$^3$). In a 2-year inhalation study (Cheever *et al*., 1990; a key study designated by MOE, 2006), SD rats (50/sex) were exposed to 1,2-dichloroethane vapors at 50 ppm (200 mg/m$^3$) for 7 hr a day, 5 days a week, for 2 years. Therefore, there were no effects on survival, body weight gain, organ weight, and histopathological examination. Furthermore, the NOAEL was 50 ppm (200 mg/m$^3$).

A reproductive and developmental toxicity study in rats (Rao *et al*., 1980), prenatal developmental toxicity study in rats (Rao *et al*., 1980; Payan *et al*., 1995), and developmental toxicity study in rabbits (Dow Chemical Company, 1979) have been performed. These studies showed no toxic effects on the reproductive and developmental toxicity parameters, including external, visceral, and skeletal examinations.

### Mutagenicity (genotoxicity)

A human ex vivo study (Cheng *et al*., 2000) reported that sister chromatid exchange (SCE) frequency using lymphocytes obtained from 51 men working in two vinyl chloride monomer manufacturing plants in Taiwan was

determined, and increased SCE frequency was associated with 1,2-dichloroethane exposure (approximately 1 ppm) but not with vinyl chloride monomer exposure.

*In vitro* genotoxicity study on 1,2-dichloroethane showed positive results with or without S9 in most bacterial reverse mutation tests (Barber *et al*., 1981; Brem *et al*., 1974; Rannug *et al*., 1978), and its glutathione conjugate showed strong mutagenic activity (Rannug *et al*., 1978). In tests with *E. coli,* a positive result was obtained without S9 (Brem *et al*., 1974). The prophage induction test showed a weak positive reaction with S9 (DeMarini *et al*., 1992). All the three gene mutation tests with human and Chinese hamster ovary cells showed positive results. Two human cell-based tests in AHH-1 cells, which have stronger glutathione S-transferase activity, showed dose-dependent positive results with higher mutation induction (Tan *et al*., 1981; Crespi *et al*., 1985; Ferreri *et al*., 1983). Although no clear dose correlation has been observed in studies with human lymphocytes, positivity has been reported in the absence of S9 in micronucleus test and comet assay *in vitro* (Tafazoli *et al*., 1998). Also, an unscheduled DNA synthesis test in the presence of S9 was positive (Perocco *et al*., 1981). Regarding DNA binding ability, the *in vitro* experiments detected DNA adducts upon incubation with DNA, and the amount of such adducts increased with the addition of liver microsomes or cytoplasmic soluble fraction (Arfellini *et al*., 1984).

*In vivo* genotoxicity studies showed positive results in micronuclei, chromosomal aberrations in the bone marrow, and DNA damage (comet) assays using rat blood treated with a single intraperitoneal dose of 1,2-dichloroethane (Lone *et al*., 2009). Also, after a single intraperitoneal dose to Swiss mice, the SCE frequency increased in the bone marrow (Giri and Que Hee, 1988). Studies demonstrating intraperitoneal dosing in mice or oral dosing in transgenic mice showed no significant increase in micronuclei in the bone marrow or peripheral blood (Sasaki *et al*., 1994; King *et al*., 1979; Armstrong *et al*., 1993). In addition, mutation assays in the liver and testis after single oral or multiple intraperitoneal injections into *lacZ* transgenic mice were negative in both organs (Hachiya *et al*., 2000). The comet assay examined the stomach, liver, kidney, bladder, lung, brain, and bone marrow, and resulted in the detection of DNA damage in all those organs (Sasaki *et al*., 1998). In a DNA damage study in mice, single-strand DNA breaks were observed in the liver after oral or intraperitoneal dose, while inhalation exposure studies showed negative results (Storer *et al*., 1983, 1984, and 1985). Several mouse and rat studies investigated DNA binding ability; all the tests were positive, with particularly high binding in the liver and kidneys, but only slight binding was observed in the lungs. (Banerjee, 1988; Arfellini *et al*., 1984; Baertsch *et al*., 1991).

The cell-transformation tests with BALB/c-3T3 cells exposed to vapors in a closed system showed negative results (Arthur D. Little, Inc., 1983), while the tests with fetal hamster cells inoculated with SA7 virus exposed to the same vapors showed accelerated transformation (Hatch *et al*., 1983). Cell transformation was observed in tests with mouse C3H/10T1/2 cells (Schultz *et al*., 1992).

These results make it difficult to conclude the mutagenicity (genotoxicity) of 1,2-dichloroethane since *in vivo* tests showed negative and positive results. However, we considered it appropriate to categorize 1,2-dichloroethane as a mutagenic (genotoxic) substance since most *in vitro* mutagenicity tests showed positive results.

### Carcinogenic effects by oral exposure

IARC (1999) posted a report on human epidemiological investigations; however, due to the possibility of mixed exposure with other chemicals, this cannot be included in the assessment.

In a rat oral gavage carcinogenicity study (US NCI, 1978; a key study designated by Food Safety Commission, 2008; MOE, 2004; WHO GDWQ, 2003; MHLW, 2003; WHO CICAD, 1998; CEPA, 1994; EPA IRIS, 1987), Osbone-Mendel rats (50/sex/dose, except for 20/sex in control) were treated with 1,2-dichloroethane at 0 (solvent: corn oil), 47, and 95 mg/kg/day (time weighted average [TWA] dose, corrected doses of 5 days/week dosing) for 78 weeks, followed by observation of recovery for 32 weeks. Therefore, all the high-dose males and females died by week 23 or 15 of the post-dose observation period, respectively. In males, a significant increase in squamous cell carcinoma in the fore stomach in the high-dose group and hemangiosarcoma in the spleen, liver, adrenal gland, pancreas, stomach, and abdominal cavity in the low and high-dose groups were observed. In females, mammary adenocarcinoma was significantly increased in the high-dose group (Table 1).

In mice oral gavage carcinogenicity study (US NCI, 1978; a key study designated by MOE, 2004; WHO CICAD, 1998; CEPA, 1994), B6C3F1 mice (50/sex/dose, except for 20/sex in control) were treated with 1,2-dichloroethane. The males were administered 0, 97, and 195 mg/kg/day (TWA doses, corrected doses of 5 days/week dosing), and females were administered 0, 149, and 299 mg/kg/day (TWA doses) for 78 weeks and observed for recovery for 13 weeks. As a result, a significant increase in mortality occurred in the high-dose group (20%, 31%,

**Table 1.** Number of tumor-bearing animals in rat oral carcinogenicity study (US NCI, 1978)

| mg/kg/day | 0 Pooled vehicle control [a)] | 0 Matched vehicle control [b)] | 47 (TWA dose) | 95 (TWA dose) | Trend test | |
|---|---|---|---|---|---|---|
| Male | | | | | | |
| Forestomach: squamous cell carcinoma | 0/60 | 0/20 | 3/50 | 9/50[##] * | (↑↑) | ↑↑ |
| Hemangiosarcoma | 1/60 | 0/20 | 9/50[##] * | 7/50[#] | (↑) | - |
| Female | | | | | | |
| Mammary gland: adenocarcinoma | 1/59 | 0/20 | 1/50 | 18/50[##] ** | (↑↑) | ↑↑ |

a) A control group in another compound study conducted at the same time, kept in the same animal room and receiving the same solvent and evaluated by the same pathologist.
b) Solvent control group in this study
[#]: $p \leq 0.05$, [##]: $p \leq 0.01$ Fisher exact test with the pooled vehicle control
*: $p \leq 0.05$, **: $p \leq 0.01$ Fisher exact test with the matched vehicle control
(↑): $p \leq 0.05$, (↑↑): $p \leq 0.01$ Cochran-Armitage test with the pooled vehicle control
↑: $p \leq 0.05$, ↑↑: $p \leq 0.01$ Cochran-Armitage test with the matched vehicle control

**Table 2.** Number of tumor-bearing animals in the mouse oral carcinogenicity study (US NCI, 1978)

| mg/kg/day | 0 Pooled vehicle control [a)] | 0 Matched vehicle control [b)] | Male 97 Female 149 (TWA dose) | Male 195 Female 299 (TWA dose) | Trend test | |
|---|---|---|---|---|---|---|
| Male | | | | | | |
| Lung: alveolar/bronchiolar adenoma | 0/59 | 0/19 | 1/47 | 15/48[##] ** | (↑↑) | ↑↑ |
| Liver: hepatocellular carcinoma | 4/59 | 1/19 | 6/47 | 12/48[##] | (↑↑) | ↑ |
| Female | | | | | | |
| Mammary gland: adenocarcinoma | 0/60 | 0/20 | 9/50[##] * | 7/48[##] | (↑↑) | - |
| Uterus: endometrial stromal sarcoma | 0/60 | 0/20 | 5/49[#] | 5/47[#] | (↑) | - |
| Lung: alveolar/bronchiolar adenoma | 2/60 | 1/20 | 7/50[#] | 15/48[##] * | (↑↑) | ↑↑ |

a) A control group in another compound study conducted at the same time, kept in the same animal room and receiving the same solvent and evaluated by the same pathologist.
b) Solvent control group in this study
[#]: $p \leq 0.05$, [##]: $p \leq 0.01$ Fisher exact test with the pooled vehicle control,
*: $p \leq 0.05$, **: $p \leq 0.01$ Fisher exact test with the matched vehicle control,
(↑): $p \leq 0.05$, (↑↑): $p \leq 0.01$ Cochran-Armitage test with the pooled vehicle control,
↑: $p \leq 0.05$, ↑↑: $p \leq 0.01$ Cochran-Armitage test with the matched vehicle control

and 72% for control, low, and high doses, respectively) in females. In males, the incidence of alveolar/bronchial adenoma and hepatocellular carcinoma in the high-dose group, and in females, mammary adenocarcinoma, endometrial stromal sarcoma, and alveolar/bronchial adenoma in the low and high-dose groups increased significantly (Table 2).

## Carcinogenic effects by inhalation exposure

There were four human epidemiological investigations, Hogstedt *et al*. (1979), Austin (1983), Benson *et al*. (1993), and Olsen *et al*. (1997). However, due to the possibility of mixed exposure with other chemicals, these cannot be included in the assessment.

Nagano *et al*. (2006) reported the following studies in rats and mice regarding animal carcinogenicity. In the rat study (a key study designated by MOE, 2006; EU SCOEL, 2016), F344 rats (50 animals/sex/group) were exposed to 1,2-dichloroethane vapors at concentrations of 0, 10, 40, and 160 ppm (whole-body exposure: 0, 40, 160, and 640 mg/m$^3$) for 6 hr per day, 5 days per week for 104 weeks. Therefore, a statistically significant increase in the following tumors was observed at 160 ppm: fibroadenoma in the mammary glands in males and fibroma of the

**Table 3.** Number of tumor-bearing animals in rat inhalation carcinogenicity study (Nagano *et al*., 2006)

| Sex | Tumor | | Exposure concentration (ppm) | | | | | Historical data | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 10 | 40 | 160 | Peto's test | Incidence [a] (%) | Minimum – Maximum [b] |
| Male | Mamary gland: Fibroadenoma | | 0/50 | 0/50 | 1/50 | 5/50* | ↑↑ | 13/749 (1.7) | 0/50 – 3/50 |
| | Subcutis: Fibroma | | 6/50 | 9/50 | 12/50 | 15/50 | ↑ | 55/749 (7.3) | 1/50 – 10/50 |
| | Peritoneum: Mesothelioma | | 1/50 | 1/50 | 1/50 | 5/50 | ↑ | 16/479 (2.1) | 0/50 – 4/50 |
| Female | Subcutis: Fibroma | | 0/50 | 0/50 | 1/50 | 5/50* | ↑↑ | 8/747 (1.1) | 0/50 – 4/50 |
| | Mammary gland: | Adenoma | 3/50 | 5/50 | 5/50 | 11/50* | ↑↑ | 28/747 (3.7) | 0/50 – 9/50 |
| | | Fibroadenoma | 4/50 | 1/50 | 6/50 | 13/50* | ↑↑ | 76/747 (10.2) | 0/50 – 8/50 |
| | | Adenocarcinoma | 1/50 | 2/50 | 0/50 | 5/50 | ↑ | 5/474 (0.7) | 0/50 – 2/50 |
| | Combined adenoma, fibroadenoma and adenocarcinoma | | 8/50 | 8/50 | 11/50 | 25/50** | ↑↑ | 104/747 (13.9) | 2/50 – 10/50 |

*: $p \leq 0.05$, **: $p \leq 0.01$ Fisher's exact test, ↑: $\leq 0.05$, ↑↑: $\leq 0.01$ Peto's test

a) Number of tumors/number of animals tested in 15 inhalation carcinogenicity tests and their percentages

b) Minimum and maximum number of animals with tumors/number of animals tested in one inhalation carcinogenicity study

Underline: Number of animals that exceeded the maximum value of background data of the study site

**Table 4.** Number of tumor-bearing animals in the mouse inhalation carcinogenicity study (Nagano *et al*., 2006)

| Sex | Tumor | Exposure concentration (ppm) | | | | | Historical data | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 10 | 30 | 90 | Peto's test | Incidence[a] (%) | Minimum – Maximum[b] |
| Male | Liver: Hemangiosarcoma | 0/50 | 4/49 | 6/50* | 5/50* | - | 27/748 (3.6) | 0/50 – 5/50 |
| Female | Mammary gland: Adenocarcinoma | 1/49 | 2/50 | 1/50 | 6/50 | ↑↑ | 20/749 (2.7) | 0/50 – 4/50 |
| | Lung: Combined bronchioloalveolar adenoma and bronchioloalveolar carcinoma | 5/49 | 1/50 | 4/50 | 11/50 | ↑↑ | 49/749 (6.5) | 0/50 – 6/50 |
| | Liver: Hepatocellular adenoma | 1/49 | 1/50 | 1/50 | 6/50 | ↑↑ | 33/749 (4.4) | 1/50 – 4/50 |
| | Uterus: Endometrial stromal polyp | 2/49 | 0/50 | 1/50 | 6/50 | ↑↑ | 26/748 (3.5) | 0/50 – 4/50 |
| | Lymph node: Malignant lymphoma | 6/49 | 17/50** | 22/50** | 12/50 | - | 241/749 (28.6) | 2/50 – 23/50 |

*: $p \leq 0.05$, **: $p \leq 0.01$ Fisher's exact test, ↑: $\leq 0.05$, ↑↑: $\leq 0.01$ Peto's test

a) Number of tumors/number of animals tested and their percentages in 15 inhalation carcinogenicity studies

b) Minimum and maximum number of animals with tumors/number of animals tested in one inhalation carcinogenicity study

Underline: Number of animals exceeding the maximum value of background data of the study site

subcutaneous tissue, and adenoma and fibroadenoma of the mammary glands in females. The incidence of tumors observed at 160 ppm, fibroma of the subcutaneous tissues in males at 40 ppm and above, and mammary tumors (adenocarcinoma + adenoma + fibroadenoma) in females at 40 ppm and above exceeded the maximum values in the background data in the laboratory (Table 3).

In the mouse study (a key study designated by DECOS, 2019), BDF1 mice (50 animals/sex/group) were exposed to 1,2-dichloroethane vapors at doses of 0, 10, 30, and 90 ppm (whole-body exposure: 0, 40, 120, and 360 mg/m$^3$) for 6 hr per day, 5 days per week for 104 weeks. As a result, compared to the background data in a laboratory, mammary adenocarcinoma, bronchioloalveolar adenoma/carcinoma, hepatocellular adenoma, and endometrial stromal polyps in the uterus were significantly increased at 90 ppm in females. The incidence of angiosarcoma in the liver at 30 ppm and above in males and malignant lymphoma at 10 and 30 ppm in females was significantly increased when compared to the control, but the incidence of these two tumors was not significant by trend tests; the latter being within the range of background data (Table 4).

## Toxicological mechanisms

The physical properties of 1,2-dichloroethane, such as

its high lipophilicity, vapor pressure, and serum/air partition coefficient, suggested rapid absorption via the alveolar membrane of the lungs and gastrointestinal mucosa (ATSDR draft, 2022). Once in the body, 1,2-dichloroethane is distributed to several organs and tissues, accumulating more in the lipophilic tissues. The absorbed 1,2-dichloroethane produces reactive intermediates covalently bound to cellular macromolecules in metabolism studies in rats and mice (Fabricant *et al*., 1980; Jean *et al*., 1989). The biotransformation of 1,2-dichloroethane is highly dependent on the amount of glutathione in the liver and is linear at lower doses. However, at high doses, the cytochrome P-450 (CYP) enzyme becomes saturated, resulting in a disproportionate increase in glutathione conjugate. At remarkably high doses, the GSH pathway becomes saturated. Thus, as the CYP-mediated biotransformation process saturates with increasing exposure, higher levels of 1,2-dichloroethane circulate throughout the body. Instead, of being detoxified and removed, many glutathione-bound reactive intermediates are formed, and toxicity is enhanced (D'Souza *et al*., 1987; Reitz *et al*., 1982). Glutathione-S-transferases, which catalyze the conjugation of biological substances with glutathione, are present in the liver, kidney, intestine, testis, adrenal gland, and lung and are mainly found in high levels (>95%) in the cytoplasm of their parenchymal cells (Parkinson, 1996). The putative glutathione-dependent metabolites, such as S-(2-chloroethyl)glutathione and S-(2-chloroethyl)-L-cysteine, is spontaneously converted to form electrophilic episulfonium ions that bind to cellular macromolecules and are believed to be the most abundant glutathione-dependent metabolites (Peterson *et al*., 1988). Rapid depletion of hepatocyte glutathione and binding of S-(2-chloroethyl)glutathione and S-(2-chloroethyl)-L-cysteine to liver DNA and protein have been demonstrated *in vitro* (Jean and Reed, 1989). Similarly, the renal cortex has been reported to contain highly active glutathione S-transferases capable of catalyzing the initial conjugation reaction (Lock, 1989), and S-(2-chloroethyl)glutathione conjugates and S-(2-chloroethyl)-L-cysteine conjugates have nephrotoxic effects in rats. Because cytochrome P450s (mainly CYP2E1), which catalyze competitive metabolic reactions, have relatively low activity in the kidney, the metabolism of 1,2-dichloroethane in the kidney is shifted to the production of toxic metabolites, thus making the toxic effects of 1,2-dichloroethane more likely to occur in the kidneys. 1,2-Dichloroethane promotes lipid peroxidation in the rat hepatocytes (Sano *et al*., 1990), arterial endothelial cells, and aortic smooth muscle cells (Tse *et al*., 1990) *in vitro*. This may be related to autopsy findings in acute occupational exposure fatalities, which showed tissue damage in the liver, kidney, and heart (Nouchi *et al*., 1984).

Although it is difficult to conclude from *in vivo* mutagenicity tests, we treated 1,2-dichloroethane as a mutagenic (genotoxic) substance since it has shown positive results in most *in vitro* mutagenicity tests.

Regarding carcinogenic mechanisms, a 28-day inhalation study in rats following 1,2-dichloroethane exposure (LeBaron *et al*., 2021) reported negative results in the comet assay and DNA adduct tests in mammary glands and liver, and no genotoxic effects or specific DNA adducts associated with carcinogenesis in the mammary tissue were detected. However, the compound was positive for DNA binding ability *in vitro* (Cheever *et al*., 1990; Reitz, 1980, 1982) and *in vivo* studies (Banerjee, 1988; Arfellini *et al*., 1984; Baertsch *et al*., 1991). Therefore, DNA adducts formation and other factors may be involved in carcinogenicity in animals. Since there is no evidence that the carcinogenicity mechanism is due to non-genotoxicity, it is reasonable to consider 1,2-dichloroethane as a genotoxic carcinogen with no threshold level.

The analog 1,2-dibromoethane is activated by the same mechanism described above and is a strong carcinogen. DNA adducts in the liver and kidneys of rats after the intraperitoneal dose of either of the compounds at a dose of 5 mg/kg showed that 1,2-dichloroethane led to 10–50 times lower DNA adduct formation than 1,2-dibromoethane (Watanabe *et al*., 2007). Therefore, the *in vivo* genotoxic induction effects of 1,2-dichloroethane may be weaker than that of 1,2-dibromoethane.

## DISCUSSION

A review of toxicity data of 1,2-dichloroethane in humans and experimental animals showed that general toxicity targets included the liver, kidney, and central nervous system. Regarding reproductive and developmental toxicity, there were no apparent toxic effects of 1,2-dichloroethane. Mutagenicity studies showed positive results in *in vitro* systems, although it is difficult to draw clear conclusions from *in vivo* systems. Carcinogenicity studies of 1,2-dichloroethane in rats and mice for oral and inhalation routes illustrated its carcinogenic potential. Based on these test results and the discussion of the mechanism of action presented in the previous section, 1,2-dichloroethane has been designated a genotoxic carcinogen with no threshold level.

### Derivation of HAV for oral exposure

We selected animal studies with dose-dependent tox-

icity profiles for the oral route and identified NOAELs to evaluate hazard assessments. (1) For non-carcinogenic effects, a rat 90-day oral gavage study (Daniel *et al*., 1994) was selected, and NOAEL was 37.5 mg/kg/day, a dose that did not show any changes in the kidney and liver relative weights, hemoglobin levels, or hematocrit levels. The NOAEL of 37.5 mg/kg/day was divided by the uncertainty factors (UF) 1,000 (species differences: 10, individual differences: 10, and study periods: 10), and a temporary HAV of 37.5 μg/kg/day was derived. (2) A rat oral gavage carcinogenicity study (US NCI, 1978) was selected for carcinogenic effects because the incidence of angiosarcoma (spleen, liver, adrenal gland, pancreas, stomach, and peritoneum) in males increased from the lower dose. According to the "Technical Guidelines for Risk Assessment of Priority Assessment Chemical Substances" (MHLW, METI, and MOE, 2022b), if there is no carcinogenic threshold, the BMD method is applied in principle. The $BMDL_{10}$ is obtained from the carcinogenicity test data and extrapolated linearly from the POD to obtain the slope factor. The slope factor derives the $10^{-5}$ risk level as HAV. In this study, BMD analysis was performed with the BMDS (Version 3.1.2) software published by the US EPA using the above incidence data of angiosarcoma. The analysis methodology, including model selection, followed the method presented in the "Guidance on the Application of the Benchmark Dose Method" (NIHS accessed on Dec. 15, 2022). Therefore, the lower confidence limit of BMD at 10% benchmark response ($BMDL_{10}$) of 9.3 mg/kg/day was obtained as a POD. A slope factor of $1.07 \times 10^{-2}$ $(mg/kg/day)^{-1}$ was obtained by dividing 0.1 (10% response) by the POD, and a temporary HAV, a carcinogenic $10^{-5}$ risk of 0.93 μg/kg/day, was obtained by dividing $10^{-5}$ by the slope factor. Of these calculated assessment values shown in (1) non-carcinogenic and (2) carcinogenic effects, the smaller value of 0.93 μg/kg/day was selected as the oral HAV for 1,2-dichloroethane (Table 5).

### Derivation of HAV for inhalation exposure

We selected the following investigations and studies for determining the HAVs for the inhalation route. (1) In occupational investigations, factory workers in a Russian airplane factory handling adhesives containing 1,2-dichloroethane (Kozik, 1957) with a TWA of 15 ppm (62 $mg/m^3$), at which effects on the nervous system and hepatotoxicity were observed, was selected as the LOAEL. The continuous exposure equivalent to LOAEL of 14.8 $mg/m^3$ (62 $mg/m^3 \times 8/24$ hr × 5/7 days) was obtained. This equivalent LOAEL of 14.8 $mg/m^3$ was divided by the uncertainty factors (UFs) 1,000 (individual differences: 10, LOAEL use: 10, and insufficient information on exposure period: 10) to obtain a temporary HAV of 14.8 $\mu g/m^3$ (0.0036 ppm). (2) For examining the non-carcinogenic effects, a 12-month repeated inhalation toxicity study (Spreafico *et al*., 1980) was selected. The NOAEL was 41.1 $mg/m^3$, with no change in ALT, uric acid levels, and cholesterol levels. A continuous exposure equivalent NOAEL was 8.56 $mg/m^3$ (41.1 $mg/m^3 \times 7$ hr/day × 5 days/week), and the human equivalent NOAEL was 16 $mg/m^3$ (=8.56 $mg/m^3 \times (0.26/20) \times (50/0.35)$ [Rat body weight: 0.35 kg, rat respiratory volume: 0.26 $m^3$/day, human body weight: 50 kg, and human respiratory volume: 20 $m^3$/day]). The human equivalent NOAEL of 16 $mg/m^3$ was derived by UF 100 (10 for individual differences and 10 for interspecies differences) to derive a temporary HAV of 160 $\mu g/m^3$ (0.038 ppm). (3) For evaluating carcinogenic effects, a 104-week inhalation exposure carcinogenicity study in rats (Nagano *et al*., 2006) was selected, in which a clear dose-response relationship in the incidence of the female mammary tumor was observed. Since all mammary gland tumors (adenoma, fibroadenoma, and adenocarcinoma) are considered to have the same cells of origin and similar developmental mechanisms, and since benign tumors can transform into malignant tumors, the combined number of all types of mammary glands was applied for the BMD analysis as described above. As a result, a $BMDL_{10}$ of 11.5 ppm (47.3 $mg/m^3$) based on the incidence data of mammary gland tumors (adenocarcinoma + adenoma + fibroadenoma, combined) in female rats in this 104-week inhalation exposure carcinogenicity study (Nagano *et al*., 2006) was obtained. The continuous exposure equivalent of $BMDL_{10}$ was 8.45 $mg/m^3$ (47.3 $mg/m^3 \times 6$ hr/day × 5 days/week), and the human equivalent dose of $BMDL_{10}$ was 15.7 $mg/m^3$ (=8.45 $mg/m^3 \times (0.26/20) \times (50/0.35)$ was calculated. A unit risk of $6.40 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ and a carcinogenic $10^{-5}$ risk of 1.6 $\mu g/m^3$ (0.00039 ppm) were derived as a temporary HAV. Of the calculated values shown in (1) occupational investigations through (3) carcinogenic examinations of this section, the smallest value of 1.6 $\mu g/m^3$ (0.00039 ppm) from (3) the evaluation of carcinogenic effects, was finally selected as the inhalation HAV for 1,2-dichloroethane. This value corresponds to a daily intake of 0.64 μg/kg/day (=1.6 × 20 × 1.0/50) [human respiratory volume: 20 $m^3$/day, absorption rate: 1.0, and body weight: 50 kg] (Table 5).

We conducted a detailed Assessment II of the human health effects of 1,2-dichloroethane under the CSCL. We suggested the draft HAVs of 0.93 μg/kg/day for oral exposure and 1.6 $\mu g/m^3$ (0.00039 ppm) for inhalation exposure, respectively.

**Table 5.** Summary of hazard assessment values for 1,2-dichloroethane

| Route of exposure | Hazard assessment value | Basis data and derivation method |
|---|---|---|
| Oral | 0.93 μg/kg/day | From the results of the rat 78-week oral gavage carcinogenicity study (US NCI 1978), male rat angiosarcoma was selected and $BMDL_{10}$ 9.3 mg/kg/day obtained from the BMD analysis. A slope factor of $1.07 \times 10^{-2}$ $(mg/kg/day)^{-1}$ and a carcinogenic risk $10^{-5}$ of 0.93 μg/kg/day were derived. |
| Inhalation | 1.6 $\mu g/m^3$ (0.00039 ppm) (equivalent to a daily intake of 0.64 μg/kg/day) | From the results of a 104-week rat inhalation exposure carcinogenicity study (Nagano *et al*. 2006), the human equivalent dose of $BMDL_{10}$ 15.7 $mg/m^3$ was obtained from $BMDL_{10}$ 11.5 ppm obtained from $BMDL_{10}$ analysis by selecting female rat mammary gland tumors (adenocarcinoma + adenoma + fibroadenoma combined). As a result, a unit risk of $6.40 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ and a carcinogenic risk $10^{-5}$ value of 1.6 $\mu g/m^3$ (0.00039 ppm) were derived. |

## Additional Information

A joint meeting with the MHLW, METI, and MOE was held in September 2022 at the Risk Assessment (Primary) Evaluation II (Human Health Effects) Conference for 1,2-dichloroethane, where the proposed HAVs were adopted. When the predicted environmental concentrations were calculated, the locations where the concentrations exceeded the HAVs were identified. Therefore, it was determined that collecting actual measurement data through environmental monitoring must be considered (METI, 2022).

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

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