**Table 1.** Aerosol particle count by optical particle counter (OPC) and condensation particle counter (CPC).

| | Date of measurement | 2013/4/29 | 2013/5/1 | 2013/5/3 |
|---|---|---|---|---|
| Equipment | Mass concentration (mg/m³) | 1.25 | 1.25 | 1.38 |
| OPC | Average cpm* (/L) + s.d. | 627,096 + 145,399 | 781,973 + 138,610 | 821,272 + 114,278 |
| | K-value (mg/m³/cpm) | $1.99 \times 10^{-9}$ | $1.60 \times 10^{-9}$ | $1.68 \times 10^{-9}$ |
| CPC | Average cpm (/L) + s.d. | 859,692 + 171,858 | 1,228,545 + 223,371 | 1,317,873 + 217,990 |
| | K-value(mg/m³/cpm) | $1.45 \times 10^{-9}$ | $1.02 \times 10^{-9}$ | $1.05 \times 10^{-9}$ |

*count per minute

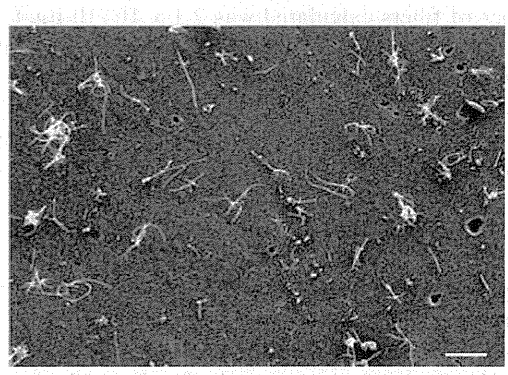

**Fig. 7.** T-CNT aerosol at a concentration of 1 mg/m³ in the main chamber was collected on the Anodisc filter (5 L/min for 3 min). SEM x 1,000. (scale bar is 10 μm)

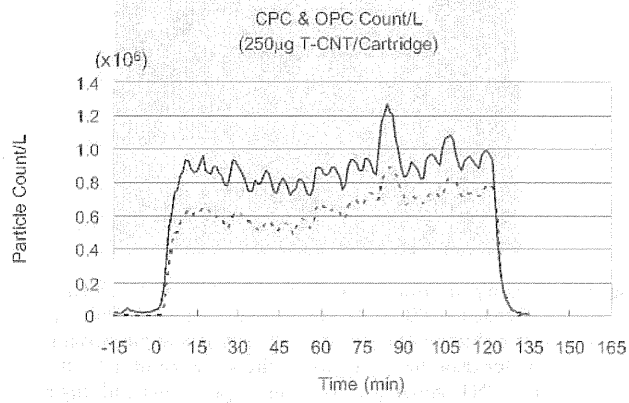


**Fig. 8.** A real time data from condensation particle counter (CPC, solid line) and optical particle counter (OPC, dotted line) from an inhalation chamber injected with T-CNT (250 μg/cartridge) from 0 min to 120 min with an average injection interval of 6 min (for detail see text).

From the amount of weight increase of polytetrafluoroethylene-glass fiber filter after sampling the chamber aerosol, the weight of aerosol per m³ of the chamber air (weight concentration) was calculated as approximately 1.3 mg/m³ (average of three measurements shown in Table 1). At the same time, the particle counts per m³ given by OPC and CPC were recorded (Fig. 8), and the K-value (mg/particle count in m³) was calculated (Table 1).

K-value (mg/m³/cpm), i.e. the weight concentration (mg/m³) divided by OPC or CPC count per minute (cpm) is often used as an indicator of the status of dispersion. Three measurements conducted with a few days' interval showed that not only the K-values itself but also the values used to calculate it were fairly stable over a period of days.

The length distribution of the T-CNT recovered from the lungs of two mice exposed in the whole body inhalation chamber 2 hr a day for 5 days at an average concentration of 1.8 mg/m³ of T-CNT are shown in Fig. 9 along with the data from the spiked lung tissue sample. The average length were 8.4 ± 5.0 μm and 8.3 ± 4.9 μm (Figs. 9a, 9b), comparable to that of the T-CNT in spiked lung tissue sample; 9.5 ± 5.2 μm (Fig. 9c) (width was qualitatively not different, data not shown). The total numbers of the fibers recovered were $5.1 \times 10^6$ and $3.2 \times 10^6$ from the inhaled lungs and $1.6 \times 10^6$ from the spiked lung; the weight of T-CNT deposited in the lung after 2 hr x 5 days of inhalation was roughly calculated as 3 μg/lung.

The fibers recovered from one of the mice were observed with SEM (Fig. 10a). Dispersed single fibers were found and some of which are longer that 20 μm (cf. Fig. 9). It was noted that EDTA and ascorbic acid in the lysis solution were effective in removing the debris from the SEM sample (Fig. 10b).

Histologically, the CNTs were found to distribute from bronchial lumen to peripheral alveolar spaces. In the bronchial lumen, the fibers were trapped in the bronchial mucus, either as single fibers or as loose aggregates

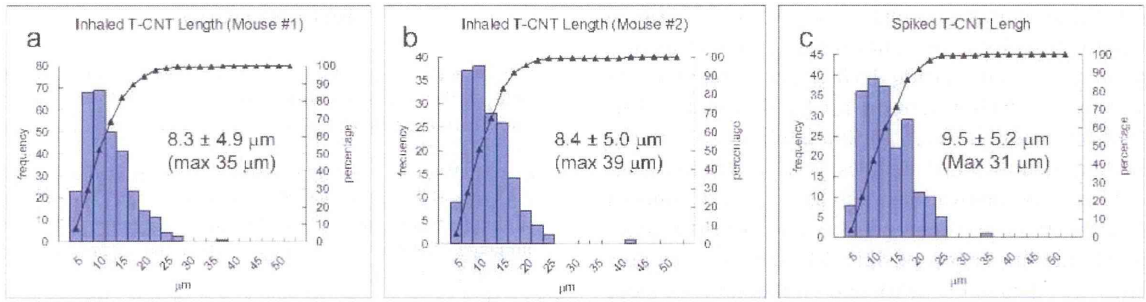


**Fig. 9.** T-CNT recovered from the mouse lung. a, b) Length distribution of T-CNT in the lung of two mice exposed 2 hr a day for 5 days at an average concentration of 1.8 mg/m³ (n = 306 and 166 each, mean ± s.d). c) Length distribution of T-CNT (1 μg) spiked to a non-exposed mouse lung (n = 198, mean ± s.d).

**Fig. 10.** T-CNT recovered from the mouse lung. a) SEM of the sediment of the dissolved lung of a mouse exposed to T-CNT in an inhalation chamber 2 hr a day for 5 days, x 2,000. Long and short single fibers are shown to be inhaled (treated with solution containing EDTA and ascorbic acid). b) SEM of a same sample treated without EDTA and ascorbic. The debris covering the fibers is considered to be iron-based amorphous substances soluble to EDTA, x 2,000. Ascorbic acid was found to be effective in keeping iron ions to be bivalent (ferrous) and soluble. (scale bars are 10 μm)

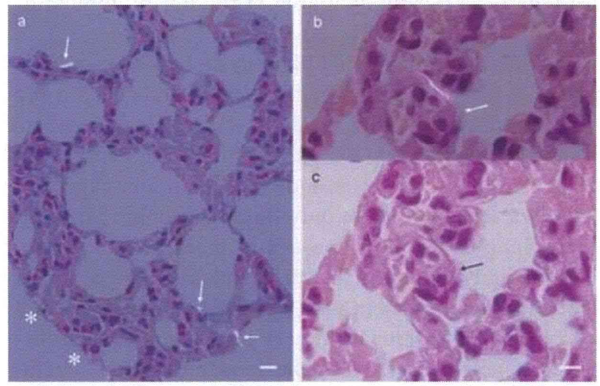


**Fig. 11.** a) A polarized microscopic view of the alveolar region of a lung exposed to 1 mg/m³ of T-CNT for 2 hr a day for 5 days. Arrows indicate single T-CNTs deposited in alveolar spaces phagocytized by alveolar macrophages. Asterisks indicates visceral pleural. (scale bar 10 μm) b,c) Another portion of alveolar region with a tadpole-shaped alveolar macrophage containing single long CNT in its cytoplasm shown in plain and polarized view. The lungs shown here are not inflated with formalin at fixation in order to avoid replacement of the CNTs. (scale bar 5 μm)

without inflammatory or granulomatous response, morphologically interpretable as a view of expectoration by the ciliary movement of the bronchial epithelium. There were no dense aggregates/agglomerates in the lungs so far as examined. In the peripheral alveolar space, single fibers are found phagocytized in alveolar macrophages as shown in Fig. 11. There were only mild inflammatory reactions such as neutrophilic migration against fibers in mucous blanket of the bronchial/bronchiolar segments and fibers in the alveolar space.

## DISCUSSION

The MWCNT treated with the "Taquann" method (T-CNT) consisted of highly dispersed single fibers with marked reduction of aggregates/agglomerates, both in the aerosol and in the resuspended solution. The length and width distribution of the single fibers were not different between the T-CNT and the original U-CNT, indicating that this method is physically mild to the sample and does not shorten the fibers.

The Taquann method consists of two major steps, the

efficient filtration in liquid phase and the idea of critical point drying in order to prevent re-aggregation of the fibers by surface tension during drying. The latter step was inspired by the drying method for SEM samples. TB-sublimation technique used in this study is an alternative method used for SEM samples as well. Our trial-and-error added a few innovations such as gentle kneading of half-frozen TB suspension and a freeze-and-thaw process for a better dispersion (visible differences in fineness of suspension, data not shown), and vibration of the sieve for a faster and better yield of filtrate (approximately 7 fold increase in half the time). This Taquann method does not use high power sonication or other strong mechanical shearing, so that the length distribution of the single fibers did not change. The equipments and reagents used here are mostly available at regular biological or chemical laboratories. The new aerosol generating system by the direct injection of T-CNT had successfully generated highly dispersed aerosol of MWNT-7 and an exposure study confirmed the inhalation of MWNT-7 single fibers in mouse lung down to the peripheral alveolar spaces. In this condition, i.e. five consequtive days of 2 hr exposure, histologically, there were only mild neutrophilic infiltration. A long-term follow up study is underway.

It is highly plausible that the Taquann method can be applied to other types of particles as long as they are not soluble to TB (additional study in preparation). Well-dispersed samples generated by the Taquann method, together with the direct injection and the small scale inhalation chamber system, would facilitate the inhalation toxicity studies more relevant to human exposure not only at the big facilities but also at the small scaled laboratories.

Finally, this dispersion method may also be useful for industries where difficulty in dispersion of nanoparticles was a limiting process in developing new products. For a large scale manufacturing, carbon dioxide critical point drying may be suitable than TB sublimation.

## ACKNOWLEDGMENT

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RESEARCH ARTICLE

# Inhalation carcinogenicity of 1,1,1-trichloroethane in rats and mice

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**Abstract**

Carcinogenicity of 1,1,1-trichloroethane (TCE) was examined by an inhalation exposure of F344 rats and BDF1 mice of both sexes to TCE at 0, 200, 800 or 3200 ppm for 6 h/d, 5 d/week for 104 weeks. In male rats, the incidences of bronchiolo-alveolar adenomas and peritoneal mesotheliomas were significantly increased in the 800 and 3200 ppm-exposed groups, respectively. The incidence of bronchiolo-alveolar adenomas in the 3200 ppm-exposed groups exceeded the range of historical control data in the Japan Bioassay Research Center. In female rats, the tumor incidences were not increased in any organs of the TCE-exposed groups. In male mice, a significant positive trend with dose was shown for incidences of bronchiolo-alveolar carcinomas, combined incidences of bronchiolo-alveolar adenomas/carcinomas and hepatocellular adenomas. The incidence of Harderian gland adenomas was significantly increased in the 3200 ppm-exposed group, and malignant lymphomas of spleen at this highest dose exceeded the range of historical control data. In female mice, the combined incidence of bronchiolo-alveolar adenomas/carcinomas was significantly increased in the 3200 ppm-exposed group, and the incidences of hepatocellular adenomas and combined incidences of hepatocellular adenomas/carcinomas were significantly increased in the 200, 800 and 3200 ppm-exposed groups with dose dependence except the combined incidence of hepatocellular adenomas/carcinomas in the 200 ppm-exposed group. The incidences of bronchiolo-alveolar adenomas in the 3200 ppm-exposed group and combined incidences of hepatocellular adenomas/carcinomas in the 200 ppm-exposed groups exceeded the ranges of historical control data. Thus, this study provided clear evidence of inhalation carcinogenicity for TCE in both rats and mice.

**Keywords**

1,1,1-Trichloroethane, carcinogenicity, inhalation, mouse, rat

**History**

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## Introduction

1,1,1-Trichloroethane (TCE: CAS. 71-55-6) is one of the compounds addressed by the Montreal Protocol, which stipulates that the production and consumption of potentially ozone-depleting substances in the stratosphere are to be phased out. Under the Montreal Protocol, the final phase-out for developed countries for TCE was 1996, with selected exceptions for existing stocks and essential uses; developing countries having until 2015 for their ban to take effect (UNEP, 2003). Subsequently, its atmospheric concentration has declined steadily (Montzka et al., 1996, 1999; Reimann et al., 2005). It was reported that the atmospheric concentration of TCE was 0.025 ppb at Jungfraujoch, Switzerland and at Mach Head, Ireland in 2003 (Reimann et al., 2005). In Japan, the environmental atmospheric concentration of TCE was about 0.02 ppb in Iwate prefecture in 2010 (WMO, 2012).

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TCE was tested for carcinogenicity in rats and mice by the inhalation exposure in a previous study (Quast et al., 1988), which found no evidence of any carcinogenic effects in either species. The International Agency for Research on Cancer (IARC) classified TCE as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) in 1999 (IARC, 1999). The American Conference of Government Industrial Hygienists (ACGIH) also assessed TCE carcinogenicity as A4 (not classifiable as a human carcinogen) in 1996 (ACGIH, 2001) and the Japan Society for Occupational Health (JSOH, 2010) recommended the occupational exposure limit to be 200 ppm based on the central nervous system depressing effects of TCE. Importantly, in the Integrated Risk Information System (IRIS) report, the exposure levels employed in the carcinogenicity tests were too low (U.S. EPA, 2007). The maximum tolerated dose (MTD) was not reached in mice (no adverse effects observed in either sex) and may not have been reached in rats, as the only toxic effects noted were a slight reduction in body weight gain in female rats and slight microscopic hepatic changes in male and female rats exposed to the high concentration of 1500 ppm. Therefore, the possibility of tumors occurring at higher inhalation exposures cannot be ruled out.

With the purpose of providing hazard data for the carcinogenic risk assessment of TCE, the dose–response

relationship between inhalation exposure concentrations of TCE and inhalation carcinogenicity responses was therefore examined here using F344 rats and BDF1 mice of each sex in a 2-year inhalation study.

## Materials and methods

This study was conducted with reference to the Organization for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals 451 "Carcinogenicity Studies" (OECD, 1981a), and in accordance with the OECD Principles of Good Laboratory Practice (OECD, 1981b). The animals were cared for in accordance with the Guideline for Animal Experimentation (Japanese Association for Laboratory Animal Science, 1987). This study was approved by the ethics committee of the Japan Bioassay Research Center (JBRC).

### Chemicals

Analytical-grade TCE (greater than 95% purity, including 1,4-dioxane ranging from 3.34% to 3.50%) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Each lot of the TCE used in this study was analyzed for its purity and stability by gas chromatography and infrared spectrometry before and after its use. Neither decomposition products nor other impurities were detected. No gas chromatographic peak other than TCE was detected in the inhalation exposure chambers.

### Animals

F344/DuCrj (SPF) rats and Crj: BDF1 (SPF) mice of both sexes were obtained at 4 weeks of age from Charles River Japan, Inc. (Kanagawa, Japan). The animals were quarantined and acclimated for 2 weeks, and then divided by stratified randomization into four body weight-matched groups, each comprising 50 rats and 50 mice of both sexes. The animals were housed individually in stainless-steel wire hanging cages (125 mm [W] × 216 mm [D] × 176 mm [H] for rats and 95 mm [W] × 116 mm [D] × 120 mm [H] for mice) in stainless steel inhalation exposure chambers from Sibata Scientific Technology Ltd. (Saitama, Japan) maintained at a temperature of 23 °C ± 2 °C and at a relative humidity of 55% ± 10%. The chamber for rats and mice consists of three parts, one rectangular parallelepiped and two quadrangular pyramid. The sizes of rectangular parallelepiped are 3.00 m [W] × 1.80 m [D] × 1.00 m [H] and quadrangular pyramid are 2.16 m [W] × 1.30 m [D] × 1.00 m [H] for rats. The sizes of rectangular parallelepiped are 1.50 m [W] × 1.80 m [D] × 1.24 m [H] and quadrangular pyramid are 1.08 m [W] × 1.30 m [D] × 0.93 m [H] for mice. Fluorescent lighting was controlled automatically to give a 12-h light/dark cycle. All rats and mice had free access to sterilized water and γ-irradiation-sterilized commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan). The body weights measured immediately before the first exposure to TCE or clean air were 122–144 g for male rats, 95–112 g for female rats, 20.2–25.4 g for male mice and 15.9–20.4 g for female mice.

### Experimental design

Groups of 50 male and 50 female rats and mice were exposed to airflow containing TCE vapor at target concentrations of 200, 800 or 3200 ppm (v/v) for 6 h/d, 5 d/week and for 104 weeks. Fifty rats and 50 mice of both sexes, serving as respective controls, were handled in the same manner as the TCE-exposed groups, but exposed to clean air in the inhalation exposure chambers. The highest concentration of 3200 ppm was selected based on body weight decrement and other toxicity data from a 13-week inhalation exposure study conducted at the JBRC. Male rats exhibited decreased body weight during a period of 13-week exposure to 4400 ppm TCE, but 13-week exposure to 3000 ppm TCE did not cause body weight decrement or overt toxicity. Male and female mice died during a 13-week period of exposure to 10 000 ppm, and one out of 10 female mice died during a period of 13-week exposure to 6700 ppm, but 13-week exposure of female mice to 4400 and 3000 ppm TCE did not cause clear body weight decrement or overt toxicity. Therefore, it was decided that the highest exposure concentration in the present carcinogenicity test should be 3200 ppm in rats and mice.

### Exposure to TCE

Airflow containing TCE vapor at target concentrations of 200, 800 or 3200 ppm was prepared by a vaporization technique. Saturated vapor–air mixtures were generated by bubbling clean air through TCE liquid in a temperature-regulated glass flask (40 °C), and by cooling through a thermostatted condenser at 18 °C. Airflow containing the saturated vapor was diluted with clean air, and then warmed to 40 °C in a thermostatted circulator which served to stabilize the vapor concentration by complete gasification of TCE. The flow rate of the vapor–air mixtures was regulated with a flow meter, and after further dilution with humidity- and temperature-controlled clean air in a spiraling line mixer, they were supplied to inhalation exposure chambers. Four inhalation exposure chambers of 7600 L in volume for rats and four of 3700 L for mice were used in this study. The air change rate in the exposure chamber was 6 ± 1 air changes/h. Each exposure chamber accommodated 100 individual cages for 50 males and 50 females of either rats or mice. Chamber concentrations of TCE were monitored by gas chromatography for every 15 min, and maintained constant at 200.4 ± 4.1 (mean ± SD), 796.6 ± 8.8 and 3181.1 ± 34.1 ppm for the exposure of rats and at 200.6 ± 3.3, 800.6 ± 8.7 and 3204.3 ± 24.0 ppm for the exposure of mice throughout the 2-year exposure period.

### Clinical observations and analysis, and pathological examination

The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured once a week for the first 14 weeks, and every 4 weeks thereafter. Urinary parameters were measured in the last week of the 2-year exposure period with Ames Reagent Strips (Multistix for rats and Uro-Labstix for mice, Siemens

Healthcare Diagnostics Inc., Tarrytown, NY). Blood was collected for blood biochemistry from abdominal aorta under anesthesia after overnight fasting at the end of the 2-year exposure period. The blood samples were analyzed with an automatic analyzer (Hitachi 705, Hitachi, Ltd., Ibaraki, Japan) and a flame photometer (Hitachi 750, Hitachi, Ltd., Ibaraki, Japan) for blood biochemistry.

All rats and mice underwent complete necropsy, when organs were removed, weighed and examined for macroscopic lesions. All organs and tissues indicated in the OECD test guideline (OECD, 1981a) and the entire respiratory tract, including the nasal cavity, pharynx and larynx, were sampled for histopathology in all the animals. The organs and tissues were fixed in 10% neutral buffered formalin, routinely processed for embedding in paraffin, and 3 μm-thick sections were prepared and stained with hematoxylin and eosin (H & E).

### Statistics and data analysis

Incidences of neoplastic lesions were analyzed for any dose–response relationship indicated by a significant positive trend by Peto's test (Peto et al.,1980) and for significant differences from the clean air-exposed group by Fisher's exact test. Incidences of non-neoplastic lesions and urinary parameters were analyzed by Chi-square test. Survival curves were plotted according to the method of Kaplan–Meier (1958), and the log-rank test (Peto et al., 1977) and Fisher's exact test were used to test a statistically significant difference in survival rate between any TCE-exposed rat or mouse group of either sex and the relevant clean air-exposed group. Body weights, organ weights and blood biochemical parameters were analyzed by Dunnett's test. All tests were two-tailed test except for the Peto's test and Fisher's exact test. In all cases, a $p$ value of 0.05 was used as the level of significance.

## Results

### Rat study

*Survival, body weights, food consumption and clinical analyses*

There were no significant differences in the survival rates between any of the TCE-exposed groups and the controls in both male and female (Figure 1).

In male rats, the terminal survival rates of 0 (control), 200, 800 and 3200 ppm-exposed groups were 68%, 72%, 72% and 56%. Tumor deaths started to occur at the 72nd week with a peritoneal mesothelioma in the 3200 ppm-exposed group. Microscopic examination of rats dying before the end of the 2-year exposure period revealed that 10 males died of peritoneal mesotheliomas in the 3200 ppm-exposed male group. Therefore, the decreased survival rate in the 3200 ppm-exposed group were attributed to the increased number of neoplasm-related deaths, although statistical significance was not observed. Growth rates of TCE-exposed groups were not significantly decreased as compared with the controls (Figure 2). The terminal body weight of 3200 ppm-exposed male rats was 96% of that of the controls. The body weights of 0, 200, 800 and 3200 ppm-exposed groups at the end of the 2-year exposure period were $429 \pm 45$, $438 \pm 23$, $442 \pm 36$ and $410 \pm 33$ g.

In female rats, the terminal survival rates of 0 (control), 200, 800 and 3200 ppm-exposed groups were 76%, 76%, 84% and 76%. Growth rates of TCE-exposed groups were not

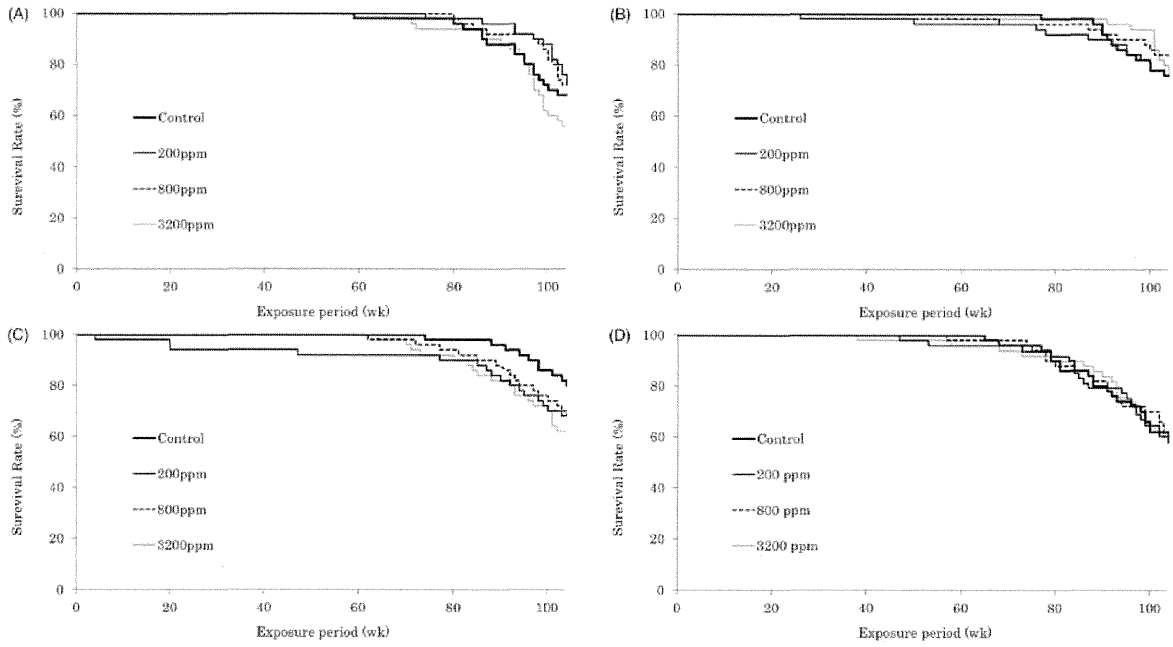


Figure 1. Survival curves of both rats and mice exposed to TCE vapor at 200, 800 and 3200 ppm and clean air as the control for 2-year. (A) Male rats. (B) Female rats. (C) Male mice. (D) Female mice.

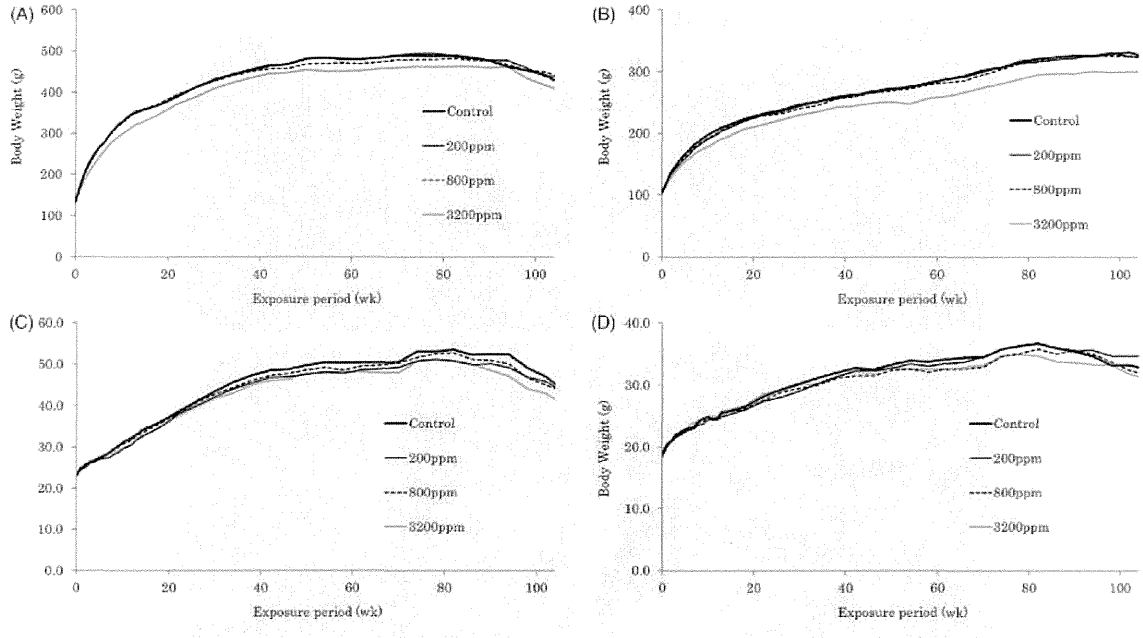


Figure 2. Body weight changes of both rats and mice exposed to TCE vapor at 200, 800 and 3200 ppm and clean air as the control for 2-year. (A) Male rats. (B) Female rats. (C) Male mice. (D) Female mice.

Table 1. Numbers of rats exposed to 1,1,1-trichloroethane by inhalation for 2-year.

| Groups | Control | 200 ppm | 800 ppm | 3200 ppm | Peto's | JBRC historical control data | |
|---|---|---|---|---|---|---|---|
| Number of animals | 50 | 50 | 50 | 50 | test | Incidence[a] | Min–Max[b] |
| Male | | | | | | | |
| Lung | | | | | | | |
| Bronchiolo-alveolar adenoma | 0 (0.0%) | 1 (2.0%) | 7 (14.0%)# | **4** (8.0%) | † | 16/649 (2.5%) | 0/50-3/50 (0-6%) |
| Peritoneum | | | | | | | |
| Mesothelioma | 1 (2.0%) | 2 (4.0%) | 1 (2.0%) | **16** (32.0%)## | †† | 17/649 (2.6%) | 0/50-4/50 (0-8%) |
| Female | | | | | | | |
| Lung | | | | | | | |
| Bronchiolo-alveolar adenoma | 1 (2.0%) | 1 (2.0%) | 2 (4.0%) | 0 (0.0%) | | 13/649 (2.0%) | 0/50-3/50 (0-6%) |
| Peritoneum | | | | | | | |
| Mesothelioma | 1 (2.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | | 1/649 (0.2%) | 0/50-1/50 (0-2%) |

# and ## Significantly different from the control group at $p \leq 0.05$ and $p \leq 0.01$ by Fisher's exact test, respectively.
† and †† Significantly different at $p \leq 0.05$ and $p \leq 0.01$ by Peto's test, respectively.
[a]Number of animals bearing tumor/number of animals examined in the 13 historical inhalation studies.
[b]Number of animals bearing tumor/number of animals examined in a single historical study.
The bold and underlined values indicate the tumor incidences exceeding the maximum tumor incidence in the JBRC historical control data.

significantly decreased as compared with the controls (Figure 2). The terminal body weight of the 3200 ppm-exposed group was 91% of that of the controls. The body weights of 0, 200, 800 and 3200 ppm-exposed groups at the end of 2-year exposure period were 328 ± 28, 324 ± 37, 325 ± 30 and 298 ± 53 g.

In both male and female rats, food consumption was not suppressed in any TCE-exposed groups as compared with the respective controls. No exposure-related changes in any hematological, blood biochemical or urinary parameter were found in any TCE-exposed groups.

*Pathology*

In female rats, the relative lung, kidney and brain, weights of the 3200 ppm TCE-exposed group were significantly increased as compared with those of the controls, although in male rats, organ weights were not significantly increased.

Macroscopic examination at necropsy revealed that incidences of peritoneal nodules and brown-colored ascites were significantly increased in male rats exposed to 3200 ppm TCE. Table 1 presents incidences of selected tumors in rats exposed by inhalation to TCE or clean air (the controls) for 2-year, with reference to the tumor incidences in the JBRC historical control data.

In male rats, a significant positive trend was shown for incidences of bronchiolo-alveolar adenomas in the lung and mesotheliomas in the peritoneum by Peto's test. The Fisher's exact test demonstrated that the bronchiolo-alveolar adenomas were significantly increased in the 800 ppm-exposed rats and the incidence of bronchiolo-alveolar adenomas in the

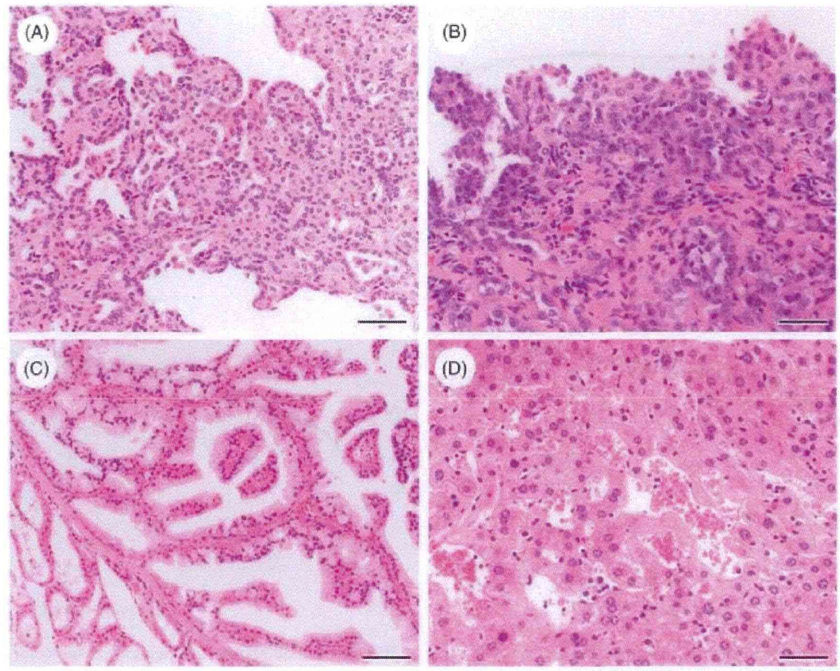


Figure 3. (A) A bronchiolo-alveolar adenoma in the lung of a male rat exposed to 3200 ppm TCE for 2-year. Bar indicates 100 μm. H&E stain. (B) A peritoneal mesothelioma in a male rat exposed to 3200 ppm TCE for 2-year. Bar indicates 50 μm. H&E stain. (C) Harderian gland adenoma of a male mouse exposed to 3200 ppm TCE for 2-year. Bar indicates 100 μm. H&E stain. (D) Hepatocellular adenoma in the liver of a female mouse exposed to 3200 ppm TCE for 2-year. Bar indicates 250 μm. H&E stain.

800 and 3200 ppm-exposed groups exceeded the respective maximum tumor incidences of the historical control data of JBRC, although the incidence in the 3200 ppm-exposed group was less than that in the 800 ppm-exposed rats. Histopathologically, the bronchiolo-alveolar adenomas were distinct masses with glandular patterns of tumor cells (Figure 3A). Those tumors compressed surrounding alveolar spaces. Peritoneal mesotheliomas were significantly increased in the 3200 ppm-exposed male rats, and their incidence exceeded the maximum tumor incidence in the historical control data. The mesotheliomas involved the surface of the peritoneal cavity, especially the scrotal sac, and were composed of one to several layers of the neoplastic mesothelial cells covering pedunculated fibrovascular stalks (Figure 3B). In female rats, incidences of tumors were not increased in the TCE-exposed groups.

Exposure-related, neoplastic lesions other than those described in Table 1 were not observed in either male or female rats. As a non-neoplastic lesion, eosinophilic change of the olfactory epithelium in the nasal cavity and cortical hyperplasia in the adrenals were observed at slightly increased severity (nasal cavity) and incidence (adrenal) in the females exposed to 3200 ppm.

## Mouse study

### *Survival, body weights, food consumption and clinical analyses*

The survival rates of the TCE-exposed groups and controls are shown in Figure 1.

In males, slightly decreased survival of the 3200 ppm-exposed group was observed. However, there was no significant difference in the survival rate at any time point of the 2-year exposure period between the 200 and 800 ppm-exposed groups and the controls. The terminal survival rates of 0 (control), 200, 800 and 3200 ppm-exposed groups were 80%, 68%, 68% and 62%. Growth rates of TCE-exposed groups were not significantly decreased as compared with the controls (Figure 2). The body weights of the 0, 200, 800 and 3200 ppm-exposed groups at the end of the 2-year exposure period were $45.2 \pm 6.4$, $44.6 \pm 7.5$, $44.3 \pm 6.6$ and $41.6 \pm 6.2$ g.

In female mice, there were no significant differences in the survival rates between any TCE-exposed group and the controls. The terminal survival rates of 0 (control), 200, 800 and 3200 ppm-exposed group were 58%, 58%, 58% and 59%. Growth rates of TCE-exposed groups were not significantly decreased as compared with the controls (Figure 2). The body weights of 0, 200, 800 and 3200 ppm-exposed groups at the end of 2-year exposure period were $32.9 \pm 4.8$, $34.7 \pm 3.5$, $32.0 \pm 4.2$ and $31.4 \pm 3.2$ g.

In male and female mice, food consumption was not suppressed in any TCE-exposed group of either sex as compared with the respective controls. Dose-related changes in hematological or blood chemical parameters measured at the end of the 2-year exposure period were not observed in any TCE-exposed groups (data not shown), although statistical significance was found sporadically for some of those parameters. Urinalysis at the last week of the 2-year exposure period demonstrated increased ketone bodies in the 3200 ppm-exposed male mice.

Table 2. Numbers of tumors in male mice exposed to 1,1,1-trichloroethane by inhalation for 2-year.

| Groups<br>Number of animals | Control<br>50 | 200 ppm<br>50 | 800 ppm<br>50 | 3200 ppm<br>50 | Peto's test | JBRC historical control data<br>Incidence[a] | <br>Min–Max[b] |
|---|---|---|---|---|---|---|---|
| Lung | | | | | | | |
| Bronchiolo-alveolar adenoma | 4 (8.0%) | 8 (16.0%) | 4 (8.0%) | 1 (2.0%) | | 46/598 (7.7%) | 2/50–9/50 (4.0–18.0%) |
| Bronchiolo-alveolar carcinoma | 3 (6.0%) | 5 (10.0%) | 6 (12.0%) | 10 (20.0%) | ∣↑ | 67/598 (11.2%) | 1/50–11/50 (2.0–22.0%) |
| Combined bronchiolo-alveolar adenoma/carcinoma | 7 (14.0%) | 13 (26.0%) | 10 (20.0%) | 11 (22.0%) | ↑ | 113/598 (18.9%) | 3/50–14/50 (6.0–28.0%) |
| Liver | | | | | | | |
| Hepatocellular adenoma | 10 (20.0%) | 8 (16.0%) | 12 (24.0%) | 15 (30.0%) | ↑ | 101/598 (16.9%) | 2/50–15/50 (4.0–30.0%) |
| Hepatocellular carcinoma | 14 (28.0%) | 12 (24.0%) | 10 (20.0%) | 15 (30.0%) | | 148/598 (24.7%) | 1/50–18/50 (2.0–36.0%) |
| Combined hepatocellular adenoma/carcinoma | 23 (46.0%) | 19 (38.0%) | 21 (42.0%) | 26 (52.0%) | | 225/598 (37.6%) | 4/50–30/50 (8.0–60.0%) |
| Spleen | | | | | | | |
| Malignant lymphoma | 3 (6.0%) | 4 (8.0%) | 3 (6.0%) | **9** (18.0%) | ∣↑ | 24/597 (4.0%) | 1/50–4/50 (2.0–8.0%) |
| Harderian gland | | | | | | | |
| Adenoma | 1 (2.0%) | 4 (8.0%) | 4 (8.0%) | **8** (16.0%)# | ∣↑ | 30/598 (5.0%) | 1/50–5/50 (2.0–10.0%) |

# Significantly different from the control group at $p \leq 0.05$ by Fisher's exact test, respectively.
↑ and ∣↑ Significantly different at $p \leq 0.05$ and $p \leq 0.01$ by Peto's test, respectively.
[a]Number of animals bearing tumor/number of animals examined in the 12 historical inhalation studies.
[b]Number of animals bearing tumor/number of animals examined in a single historical study.
The bold and underlined values indicate the tumor incidences exceeding the maximum tumor incidence in the JBRC historical control data.

Table 3. Numbers of tumors in female mice exposed to 1,1,1-trichloroethane by inhalation for 2-year.

| Groups<br>Number of animals | Control<br>50 | 200 ppm<br>48 | 800 ppm<br>50 | 3200 ppm<br>49 | Peto's test | JBRC historical control data<br>Incidence[a] | <br>Min–Max[b] |
|---|---|---|---|---|---|---|---|
| Lung | | | | | | | |
| Bronchiolo-alveolar adenoma | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | **5** (10.2%) | ∣↑ | 23/599 (3.8%) | 0/50–5/50 (0.0–10.0%) |
| Bronchiolo-alveolar carcinoma | 1 (2.0%) | **3** (6.3%) | 1 (2.0%) | 2 (4.1%) | | 17/599 (2.8%) | 0/50–3/50 (0.0–6.0%) |
| Combined bronchiolo-alveolar adenoma/carcinoma | 1 (2.0%) | 3 (6.3%) | 1 (2.0%) | **7** (14.3%)# | ∣↑ | 40/599 (6.7%) | 1/50–6/50 (2.0–12.0%) |
| Liver | | | | | | | |
| Hepatocellular adenoma | 2 (4.0%) | **9** (18.8%)# | **14** (28.0%)## | **19** (38.8%)## | ∣↑ | 29/599 (4.8%) | 1/50–5/50 (2.0–10.0%) |
| Hepatocellular carcinoma | 2 (4.0%) | 1 (2.1%) | 2 (4.0%) | 1 (2.0%) | | 12/599 (2.0%) | 0/50–2/50 (0.0–4.0%) |
| Combined hepatocellular adenoma/carcinoma | 4 (8.0%) | **10** (20.8%) | **16** (32.0%)# | **20** (40.8%)## | ∣↑ | 40/599 (6.7%) | 1/50–6/50 (2.0–12.0%) |
| Spleen | | | | | | | |
| Malignant lymphoma | 4 (8.0%) | 4 (8.3%) | 5 (10.0%) | 3 (6.1%) | | 33/599 (5.5%) | 1/50–7/50 (0.0–14.0%) |
| Harderian gland | | | | | | | |
| Adenoma | 3 (6.0%) | 4 (8.3%) | 1 (2.0%) | 2 (4.1%) | | 20/599 (3.3%) | 1/50–6/50 (0.0–12.0%) |

# and ## Significantly different from the control group at $p \leq 0.05$ and $p \leq 0.01$ by Fisher's exact test, respectively.
↑ and ∣↑ Significantly different at $p \leq 0.05$ and $p \leq 0.01$ by Peto's test, respectively.
[a]Number of animals bearing tumor/number of animals examined in the 12 historical inhalation studies.
[b]Number of animals bearing tumor/number of animals examined in a single historical study.
The bold and underlined values indicate the tumor incidences exceeding the maximum tumor incidence in the JBRC historical control data.

### *Pathology*

There were no significant differences in weights of various organs between any TCE-exposed groups and the respective male and female controls.

Macroscopic examination at necropsy revealed that the incidences of nodules in the liver were increased in an exposure concentration-related manner in female mice. These nodules in the liver were histopathologically diagnosed as hepatocellular adenomas or carcinomas. Tables 2 and 3 present selected tumor incidences in mice of both sexes exposed to TCE or clean (the control) air for 2-year, with reference to the tumor incidences of the JBRC historical control data.

In male mice, a significant positive trend was shown for the incidences of bronchiolo-alveolar carcinomas and combined incidences of bronchiolo-alveolar adenomas and carcinomas (adenomas/carcinomas) in the lung, hepatocellular adenomas, malignant lymphoma of spleen and Harderian gland adenoma by Peto's test. Malignant lymphomas of spleen were not significantly increased in any of the TCE-exposed groups, although the incidence in the 3200 ppm-exposed group exceeded the maximum tumor incidence in the historical control data. Harderian gland adenomas, composed of cuboidal to tall columnar cells with papillary growth and abundant, foamy, pale cytoplasm (Figure 3C), were significantly increased in the 3200 ppm-exposed mice by Fisher's exact test and the incidence exceeded the maximum tumor incidence in the historical control data. Exposure-related neoplastic lesions other than those listed in Table 2 were not observed.

In female mice, a significant positive trend was shown for incidences of bronchiolo-alveolar adenomas, and combined