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Evaluation of subchronic inhalation toxicity of methylcyclopentane in rats

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ABSTRACT

The aim of this study was to verify subchronic inhalation toxicity of methylcyclopentane (CAS No. 96-37-7) in Sprague-Dawley rats. Four groups of 10 rats of each gender were exposed to methylcyclopentane vapor by whole-body inhalation at concentrations of 0, 290, 1300, or 5870 ppm for 6 h per day, 5 days/week over a 13-week period. During the study period, clinical signs, mortality, body weight, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, gross pathology, organ weights, and histopathology were examined. Exposure-related clinical signs (salivation and rubbing) were observed in both genders of the 5870 ppm group. There was an increase in liver weight for both genders but the kidney weight was only higher in females than controls. However, no toxicologically significant changes were observed in body weight, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, necropsy findings, or histopathology in any of the treatment groups. Under the present experimental conditions, the target organs were determined to be kidney and liver in rats. The no-observed-adverse-effect concentration was considered to be 1300 ppm/6 h/day in rats.

1. Introduction

Methylcyclopentane (MCP; CAS No. 96-37-7) is a flammable, colorless liquid with a sweet odor. It is an organic compound with the chemical formula $CH_3C_5H_9$ and a vapor pressure of 137.5 mmHg at 25 °C. It is a substantial component of commercial hexane and other mixed hexane products (Ono et al., 1981; Yang et al., 2006). MCP is widely used in organic synthesis as an extractive solvent and as an azeotropic distillation agent. In addition, this chemical has been found in air and marine water samples, suggesting the possibility of its widespread dispersal or emission from natural sources (Daughtrey et al., 1999; HSDB, 2011). Therefore, it is important that the potential human health risks are assessed and that occupational exposure is managed accordingly.

Limited data are available documenting the potential toxicity of MCP in humans and experimental animals (Malbergier, 1990; Daughtrey et al., 1999). Humans are mainly exposed to MCP in its liquid or vapor form in the workplace. Severe exposure to MCP vapor causes dizziness, nausea, vomiting, loss of consciousness, and collapse. If MCP is aspirated, it causes severe lung irritation, which rapidly develops into pulmonary edema, and central nervous system excitement followed by depression. Exposure to the liquid form of MCP causes eye and mild skin irritation if it is allowed to remain in contact. According to the American Conference of Governmental Industrial Hygienists, the threshold limit value-time weighted average and short-term exposure limit for MCP have been recommended as 400 ppm and 750 ppm, respectively. Otherwise, there is no Occupational Safety and Health Administration permissible exposure limit for MCP or an Environmental Protection Agency acute exposure guideline level. Ono et al. (1981) conducted an 8-week repeated oral dose study of MCP that resulted in distinct functional peripheral nerve impairment in rats. In another study, rats that were exposed to C6 isomers (MCP consists of 24.6% C6 isomers) for 22 h/day, 7 days/week for 6 months at a concentration of 500 ppm did not show any treatment-related evidence of neurotoxic effects (Egan et al., 1980). However, these studies are unsuitable to identify the subchronic toxicity of MCP because the authors used a commercial source of hexane or the animals were exposed to MCP orally rather than the more appropriate exposure by inhalation. Thus, no studies have been published on the repeated inhalation toxicity of MCP performed in compliance with the current regulatory guidelines.

Therefore, we conducted this study to characterize the potential subchronic inhalation toxicity of MCP via whole-body exposure in

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Sprague-Dawley (SD) rats. This study was designed and conducted under Test Guideline 413, "Subchronic Inhalation Toxicity: 90-Day Study" from the Organization for Economic Cooperation and Development (OECD, 2009) and followed modern Good Laboratory Practice Regulations.

2. Materials and methods

2.1. Animal husbandry and maintenance

Specific pathogen-free SD rats were used. Fifty animals of each gender (7 weeks of age) were purchased from Orient Bio Inc. (Seoul, Republic of Korea). The rats were acclimated for 1 week before starting the experiments. The animals were housed in a room maintained at a temperature of 19–26 °C and a relative humidity of 50 ± 10% with artificial lighting from 08:00 to 20:00 and 13 to 18 air changes per hour. The animals were housed singly in stainless steel wire mesh cages and allowed access to sterilized tap water and commercial rodent chow (PMI Nutrition, St. Louis, MO, USA) *ad libitum*. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all methods used were approved by the Animal Care and Use Committee of Korea Institute of Toxicology.

2.2. Test chemical and exposure

MCP (88.5% MCP, 6.6% n-hexane, 4.5% c-hexane, and 0.4% others) was purchased as a single lot (Lot 100304) from Suheung Co. (Seoul, Republic of Korea). Whole body exposure chambers and a gas generator (HCT Co., Seoul, Republic of Korea) were used to expose the rats to MCP. Airflow containing MCP vapor at the target concentration was prepared using a vaporization technique. The saturated vapor-air mixture was generated by bubbling clean air through MCP liquid in a temperature-regulated glass flask (25 °C) and then cooling it by passing it through a chiller at 20 °C. The air containing the saturated vapor was diluted with clean air and supplied to an inhalation exposure chamber. The MCP concentration in the chamber during exposure was measured using gas chromatography (Shimadzu, Tokyo, Japan) and controlled using a flow meter (Fig. 1). The test animals were exposed to 290, 1300, or 5870 ppm MCP or fresh air for 6 h/day, 5 days/week for 13 weeks. Inhalation exposure was carried out from 10:00 to 16:00 in a stainless steel chamber (1.5 m^3). The experimental design was based on the usual working schedule of workers as well as the major exposure route for the test chemical.

2.3. Preparation of the calibration curve

The linearity of the calibration curve was examined at five different concentrations. The calibration concentrations were approximately 264, 572, 2904, 5720, and 7216 ppm. Standard MCP gas (0.88%; Nano Gas Co., Seoul, Republic of Korea) was used to prepare the calibration curve. It was diluted with clean air using a gas-tight syringe (SGE Analytical Science, Sydney, Australia) and Tedlar bags (Top Trading ENG, Seoul, Republic of Korea) as calibration standards. The calibration standard samples were prepared from an average of three measurements. The calculated response factor (RF; RF = peak area/concentration) for each standard must be within ±10% of the mean RF for acceptance and the coefficient of correlation (R^2) should be >0.99. The conditions used for detecting MCP by gas chromatography were as follows: detector, flame ionization detector; column, silicon DC-200 20% Chromosorb W (AW-DMCS); column temperature, 35 °C; detector temperature, 150 °C; injector temperature, 150 °C; H_2 gas pressure, 60 kPa; air pressure, 60 kPa; carrier gas, N_2; and injection volume, 1 mL of gas sample.

2.4. Conditions in the inhalation chamber

The environment in the chamber was maintained at a temperature of 24–26 °C and a relative humidity of 44–50% with 12–15 air changes/hour. The MCP concentration in the chamber was measured using a calibration curve. MCP chamber concentration was monitored using gas chromatography every 7 min during the exposure period. The means for each 6 h exposure day MCP concentrations were compiled and the grand mean and standard deviation of those means were calculated (n = 65–66).

2.5. Experimental groups and selection of concentrations

Forty healthy animals of each gender (8 weeks of age) were randomly divided into the following four groups: control (0 mg/L) group, low-dose group (1.00 mg/L, 290 ppm), middle-dose group (4.47 mg/L, 1300 ppm), and high-dose group (20.21 mg/L, 5870 ppm). The control group was exposed to filtered air under the same conditions as the exposed groups. During the exposure period, water was supplied by an automatic water supply system. The concentrations of 290, 1300, and 5870 ppm were selected according to a previous study (Ono et al., 1982) and the Globally Harmonized System of Classification and Labeling of Chemicals categorization.

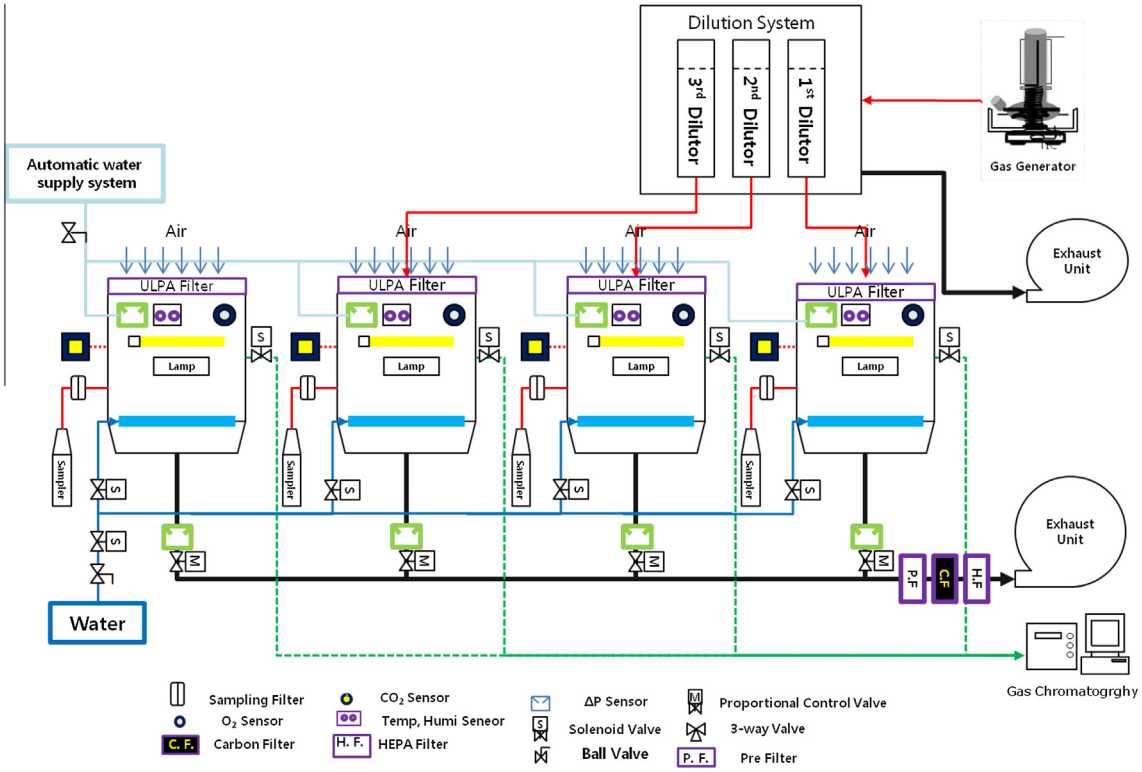

Fig. 1. Schematic diagram of the MCP exposure system. The concentration in each inhalation chamber was controlled by mixing MCP vapor and ultra-low penetration air (ULPA)-filtered air. The vapor concentrations were monitored by gas chromatography using a flame ionization detector-equipped chromatograph and a silicone DC-200 column with 20% Chromosorb.