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Evaluation of toxicity to triclosan in rats following 28 days of exposure to aerosol inhalation

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ABSTRACT

The present study was conducted to investigate the potential subchronic toxicity of triclosan (TCS) in rats following 28 days of exposure by repeated inhalation. Four groups of six rats of each sex were exposed to TCS-containing aerosols by nose-only inhalation of 0, 0.04, 0.13, or 0.40 mg/L for 6 h/day, 5 days/week over a 28-day period. During the study period, clinical signs, mortality, body weight, food consumption, ophthalmoscopy, hematology, serum biochemistry, gross pathology, organ weights, and histopathology were examined. At 0.40 mg/L, rats of both sexes exhibited an increase in the incidence of postdosing salivation and a decrease in body weight. Histopathological alterations were found in the nasal septum and larynx. There were no treatment-related effects in rats of either sex at ⩽0.13 mg/L. Under the present experimental conditions, the target organs in rats were determined to be the nasal cavity and larynx. The no-observed-adverse-effect concentration in rats was determined to be 0.13 mg/L.

1. Introduction

Triclosan (TCS; 2,4,4′-trichloro-2′-hydroxydiphenyl ether; CAS No. 3380-34-5) is a nonionic, broad-spectrum antimicrobial agent that has been used as an active ingredient in cosmetic and personal care products, including soaps, face and skin cleansers, mouthwash, toothpastes, and clothing for several decades (Bhargava and Leonard, 1996; Tan et al., 2002; Dayan, 2007). This chemical is effective against certain types of fungi and various types of bacteria by blocking the active site of the enoyl-acyl carrier protein reductase (ENR), which is an essential enzyme for fatty acid biosynthesis in fungi and bacteria (McMurry et al., 1998; Heath et al., 2000). Because humans do not possess this ENR and TCS has low acute toxicity, the use of TCS has not been highly regulated, and it is accepted as well tolerated and safe (Bhargava and Leonard, 1996; Kwon et al., 2013).

Exposure to TCS may occur through ingestion or dermal contact with this chemical through the use of or exposure to various TCS-containing products. TCS is also used in household, veterinary, and industrial cleaning products, and in textile and plastic manufacturing processes. Estimated annual use of TCS is more than 300 tons in the United States and 350 tons in Europe (Halden and Paull, 2005). TCS is found in the majority of more than 700 antibacterial and personal care products as an active ingredient in the range 0.1–0.3% (w/w) (Schweizer, 2001; Sabaliunas et al., 2003; Dann and Hontela, 2011). Occupational exposure to TCS may occur through inhalation or dermal contact at workplaces where TCS is produced or used. Industrial workers may be exposed to TCS at high concentrations during its manufacture or use (HSDB, 2004). Recently, spray-type air fresheners and deodorants that contain TCS have been increasingly used for odor removal indoors, suggesting that people may be repeatedly exposed to TCS by inhalation. Because of the widespread applications of TCS, human exposure to TCS has steadily increased, and this may have a severe impact on health (Bhargava and Leonard, 1996; Jones et al., 2000; Calafat et al., 2008).

The most common adverse effects of TCS exposure in humans and animals are skin irritation and sensitization, and eye and respiratory tract irritation (EPA, 2008; Bhutani and Jacob, 2009; Rodricks et al., 2010). TCS toxicology in laboratory animals has demonstrated that TCS has a low acute oral (LD_{50} > 5000 mg/kg in rats) and dermal (LD_{50} > 9300 mg/kg in rabbits) toxicity, at least in these animals (Lyman and Furia, 1969; Ullmann, 1980; NTP, 2008). A study of local irritation showed that a single instillation of 0.1 g TCS into rabbit eyes caused a moderate corneal and conjunctival irritation for 7 days (Ullmann, 1980). According to a

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90-day repeated oral dose toxicity study, oral exposure to TCS caused suppression in body weight and food consumption in rats; the no-observed-adverse-effect level as 65 and 82 mg/kg/day for males and females, respectively (Goldsmith and Craig, 1983). By contrast, TCS was found to have relatively high toxicity in a repeated inhalation toxicity study in rats. More than 50% of the rats died after a single 2 h exposure to 1.3 mg/L; the LC_{50} value for rats was <1.3 mg/L for either sex (Leutkemeier et al., 1974). In addition, TCS has toxic effects, including causing a decrease in cell viability with morphological changes in L2 rat epithelial lung cells, and acute inflammatory and permeability effects in the lung after a single intratracheal instillation of 1 mg/kg (Kwon et al., 2013). Despite the widespread use of TCS, there is a lack of information regarding its repeated-dose toxicity after inhalation.

The aim of this study was to determine the potential subchronic toxicity of TCS in Sprague–Dawley (SD) rats by nasal inhalation exposure. This study was conducted in compliance with the Good Laboratory Practice (GLP) Regulations and Test Guideline 412 of the Organization for Economic Co-operation and Development (OECD, 2009).

2. Materials and methods

2.1. Animals and environmental conditions

Specific pathogen-free SD rats of each sex (6 weeks of age) were obtained Orient Bio Inc. (Seoul, Republic of Korea). The rats were acclimated for 1 week before starting the experiments. The rats were housed in a room maintained at 19–26 °C and 50 ± 10% relative humidity with artificial lighting from 08:00 to 20:00 and 13–18 air changes per hour. The rats were housed singly in stainless-steel wire mesh cages (255 mm × 465 mm × 200 mm high) and allowed access to sterilized tap water and commercial rodent chow (LabDiet 5002; PMI Nutrition, USA) ad libitum. The experiments were conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all methods used in this study were approved by the Animal Care and Use Committee of Korea Institute of Toxicology (1208-0266).

2.2. Test chemical and exposure

TCS (purity: 99.7%) was purchased as a single lot (BCBG0479V) from Sigma–Aldrich (Milwaukee, WI, USA) and consisted of mostly cylinder-shaped particles as seen under scanning electron microscopy (Fig. 1). The TCS was weighed and then dissolved in corn oil at 0.21 g/ml (21.3% w/v). This solution was aerosolized using a mist generator (Sibata, Tokyo, Japan) with 8–14 L/min of airflow. The aerosol generating system was directly connected to the nasal inhalation exposure chambers (Sibata, Tokyo, Japan) and TCS aerosols were diluted with clean air and supplied to the chambers based on minute volume (Fig. 2). Exposure was for 6 h/day, 5 days/week for 4 weeks (total exposure: 20 sessions, 120 h) according to the applicable guideline 412 (OECD, 2009). During the exposure, food and water were not supplied. Control rats were exposed to the corn oil vehicle in the inhalation chamber for the same period. TCS aerosols were sampled at least 3 times during every 6 h exposure period through a sampling line using glass fiber filters. The glass fiber filters were subsequently weighed on an electronic balance. Particle size distribution in the TCS and vehicle aerosols was measured using a small-size cascade impactor (Minimoudi M135, MSP, MN, USA). Temperature, relative humidity, pressure, and air ventilation in the chambers were recorded using an environmental controller (Shibata, Tokyo, Japan).

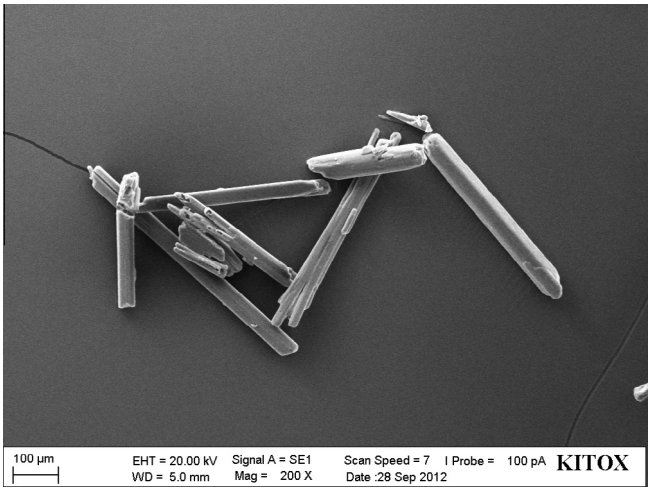

Fig. 1. Characterization of TCS. TCS is over 99% pure and is available in the solid form as a white to off-white crystalline powder with a barely detectable aromatic odor. It is consisted of mostly cylinder shaped particles under scanning electron microscopy.

2.3. Experimental groups

Before testing, the rats were evaluated through clinical observations and body weight determinations during a quarantine period to assure freedom from potentially confounding variables. Twenty-four male and twenty-four female rats were randomly assigned to four experimental groups: three treatment groups receiving 0.04, 0.13, and 0.40 mg/L concentrations of TCS and a vehicle control group exposed to corn oil. Each group consisted of 6 rats of each sex.

2.4. Dosage selection

The experimental concentrations were selected according to our earlier feasibility study. In brief, TCS is a sticky powder, and it is difficult to generate a dust. We dissolved TCS in various solvents such as 2–80% ethyl alcohol solution, a mixture of Tween 80 (0.5%) and ethyl alcohol (5%) in distilled water, and corn oil. TCS was not soluble below 50% ethyl alcohol or in the mixture of Tween 80 and ethyl alcohol. However, it dissolved well in corn oil. Therefore, corn oil was selected as the vehicle and a high concentration was selected to achieve the maximum concentration attainable. We found that 0.40 mg/L is a maximum attainable concentration using our mist generator. In this study, therefore, concentrations of 0.40, 0.13, and 0.04 mg/L were selected as high, medium, and low concentrations, respectively, using a scaling factor of ×3.

2.5. Clinical observations and mortality

Clinical signs of toxicity, morbidity, and mortality were observed and recorded before, during, and after exposure. Any evidence of exposure-related effects was recorded using Path/Tox System software (version 4.2.2; Xybion Medical Systems, NJ, USA).

2.6. Body weight and food consumption

Body weight was measured at the beginning of exposure, twice weekly during the exposure, and before necropsy. Food consumption was measured once a week during the exposure. The weight of food was measured before it was added to each cage, and the remaining food was measured on the next day to calculate the difference, which was regarded as the daily food consumption (g/rat/day).