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EFFECTS OF FLUORIDE AEROSOL INHALATION ON MICE

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SUMMARY: The effects of fluoride aerosol inhalation on mice were studied using an inhalation chamber. Five-week-old male ICR mice were exposed to airborne fluoride (13.3 mgF/m^3) 4 hr per day for 10, 20 or 30 days. Significant differences in relative lung weight were observed between the exposed groups and the control. No significant changes were found in relative kidney weight and body weight of the exposed mice. Bone fluoride retention and urinary fluoride excretion increased with exposure time.

Keywords: Airborne fluoride, Fluoride aerosol, Fluoride-exposed mice, Fluoride inhalation.

INTRODUCTION

Overexposure to fluoride causes toxicity in animals and humans. Excessive fluoride exposure during the period of tooth development may result in defective tooth formation, and intake of elevated levels of fluoride over prolonged periods of time may result in skeletal fluorosis. Drinking water containing high concentrations of fluoride is the major cause of dental fluorosis and skeletal fluorosis. In some areas of the world, e.g., China and India, there are a large number of people with dental fluorosis and skeletal fluorosis caused by drinking water containing high levels of fluoride.[1-4] Fluoride is also found in a wide range of concentrations in coal, a main fuel energy source for industrial and domestic processes in China. In some districts, especially in rural areas, coal containing high concentrations of fluoride is used for cooking and crop drying. Indoor burning of coal for cooking and crop drying with poor ventilation results in indoor air pollution.[5-7] In 1997, it was reported that there were 16.5 million people with dental fluorosis and 1.08 million people with skeletal fluorosis in endemic areas of coal burning in China.[8] Although effects of occupational fluoride exposure on humans have been reported, there is limited information about the effects of fluoride inhalation on animals and humans because of the difficulties in carrying out exposure experiments.[9-11]

In this study, we exposed mice to airborne fluoride in an inhalation exposure chamber. The potential effects of fluoride inhalation on the animals, including food consumption, body weight, organ weight, urinary fluoride excretion, and fluoride retention in bone, were investigated.

MATERIALS AND METHODS

Animals and Diet: Five-week old, SPR-grade male ICR mice (Clea, Japan Inc., Tokyo) were housed in plastic cages kept at 22±1°**C**, relative humidity of 50±10%, and a light-dark cycle of 12 hr. The animals were divided into 6 groups of 6 each (3 exposure and 3 control groups). Mice were fed an AIN-93M purified diet containing low fluoride (1.0 F mg/kg, diet) and tap water *ad libitum*. The diet (Oriental Yeast Co. Ltd. Tokyo, Japan) contained recommended amounts of vitamins, minerals and other nutrients in α-cornstarch,

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155 g/kg; cornstarch, 466 g/kg; milk casein, 140 g/kg; sugar, 100 g/kg; soybean oil, 40 g/kg; and cellulose, 50 g/kg.

Exposure System: A 0.256-m^3 inhalation exposure chamber was used in the study. The chamber and atomisation system were constructed of stainless steel, tetrafluoroethylene and glass. Fluoride aerosol was generated using an atomiser system (Sibata Co., Tokyo, Japan) by atomising 0.1 M NaF solution followed by dehydrating the aerosol to form submicron particles. The fluoride aerosol concentrations were monitored continuously by a Digital Dust Indicator (Model PCD-1, Sibata Co., Tokyo, Japan) during exposure. The animals were transferred to stainless-steel cages and exposed to the sodium fluoride (NaF) aerosol in the chamber 4 hr per day for 10, 20 or 30 days. After the fluoride exposure, distilled water was provided for 30 min to clean the chamber and animals. The positions of cages within the inhalation chamber were rotated daily. Food and water were not provided to the mice during the 4-hr exposure.

Sampling and Fluoride Analysis: Urine samples of the mice were collected by using metabolism cages. A 0.5-1.0-mL a urine sample from each mouse was mixed with 1.0 mL of total ionic strength adjustment buffer (TISAB III, Orion Research Inc., Boston, USA), and then diluted to 10.0 mL with distilled deionized water.

The fluoride aerosol concentrations in the chamber were monitored by a PCD-1 digital dust indicator during exposure and sampled by use of a newly developed Andersen type AND sampler (Sibata Co., Tokyo, Japan) with 19 and 35 mm T60A20 filters (Pallflex Prod. Corp., Putnam, Conn., USA) for 10-30 min at a flow rate of 2.5 L/min.[12] Before sampling, the filters were washed twice with distilled deionized water for 10 min with an ultrasonic cleaner to decrease background fluoride levels, and then dried at 50° **C**. The sampled filter was cut into pieces and placed in a mixture made up of 1.0 mL of TISAB III solution and 9.0 mL of deionized distilled water.

Fluoride content in the diet was determined by a micro diffusion method. In this procedure, 0.1−1.0 g of powdered sample was accurately weighed in a plastic petri dish (Falcon, Becton Dickinson, New Jersey, USA). Then, 2.0 mL of distilled deionized water was added. Vaseline was applied to the inside rim of the petri dish cover, and 50 µL of 0.05 N NaOH solution was placed in five separate drops on the inside cover of petri dish. 1.0 mL of 1.5 M H_2SO_4-HMDS (hexamethyldisiloxane, $C_6H_{18}OSi_2$) saturated solution was then added through a hole of the cover. The hole was immediately sealed with Vaseline. After diffusion overnight at room temperature, 25 µL of 0.15 M acetic acid was added to the NaOH drops on the petri dish lid. The NaOH drops were collected with pipette, and deionized distilled water was added to give a final volume of 100 µL. The fluoride concentration in each sample was determined with a fluoride electrode (see below).

After exposure for 10, 20 and 30 days, the animals were weighed, anesthetized by intraperitoneal injection of sodium pentobarbital and sacrificed by severing the main abdominal artery. The lung, liver and kidney were removed, washed with 0.9% NaCl solution, weighed immediately, and stored at -40°**C.**

The leg bones were collected, and the muscles were removed and incubated at 37°C in Papain solution (1:350 w/v) for 24 hr. The bones were washed with distilled water, dried at 105°C for 2 hr, heated at 300°C for 3 hr, and then at 600°C for 6 hr in an electric furnace (Model TMF-1100, Tokyo-Rika Co., Tokyo, Japan). The fat-free bones were ground into powder, and 0.02 g of the powder was accurately weighed into a test tube, followed by the addition of 5.0 mL of 0.25 M HCl solution. The mixture was stored overnight at room temperature. About 0.2 mL of 0.05% bromophenol blue solution was added, the pH of the solution was adjusted to 5.2-5.6 with 0.25 M NaOH solution. Finally, 2.0 mL TISAB III solution was added and the sample was diluted to 20.0 mL with deionized distilled water.

Fluoride concentrations in all samples were determined with a model 9609BN combination fluoride ion selective electrode and a 720A pH/ISE Meter (Orion Research, Boston, USA). Freeze-dried urine 2671a (U.S. Department of Commerce, National Institute of Standards and Technology, MD, USA) was used as standard reference material for fluoride analysis. The results are shown in Table 1.

TABLE 1. Fluoride concentration in standard reference material (mg/L)

SRM-2671a	Determined values	Certified values
Low level	0.56 ± 0.02 (0.53 - 0.58)	0.55 ± 0.03
Elevated level	5.5 ± 0.1 (5.3 - 5.6)	5.7 ± 0.3

Values are means ± SD, n=9.

Powdered bone samples were digested with 1 mL of a mixture of 60% HNO_3 and 61% $HClO_4$ (3:1, v/v) in a Pyrex test tube and heated at 50°C for 2 hr, and then at 105°C overnight with a Teflon-ball. The sample was then heated at 140° C for 6 hr without the Teflon-ball, and at 180° C for 2 hr. The digest was diluted to 5.0 mL with deionized distilled water and the Ca, Zn, Mg, Fe and Cu concentrations of the digested samples were determined by inductively coupled plasma atomic emission spectrometer (ICP-AES) (Model Spectro Flame-EOP, Spectro Analytical Instruments, Germany).

Statistical Analysis: The results were analyzed for significant differences between groups using Student's t test. Differences with $p < 0.05$ were considered significant.

RESULTS

Fluoride concentrations in distilled water, CE-2 commercial diet, and AIN-93M purified diet (Clea Co., Tokyo, Japan) were 0.007±0.001 mg/L, 57.3±6.7 mg/kg, and 1.0 mg/kg, respectively.