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Subchronic toxicity study of yttrium nitrate by 90-day repeated oral exposure in rats

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ABSTRACT

Concerns regarding the adverse effects of long-term exposure to low levels of rare earth elements (REEs) from foods on human health have arisen in recent years. Nevertheless, no official acceptable daily intake (ADI) has yet been proposed for either total REEs or individual REE. In accordance with the Organization for Economic Co-operation and Development (OECD) testing guideline, the present study was undertaken to evaluate the subchronic toxicity of yttrium, a representative heavy REE with higher contaminated level in foods in China, to achieve a no observed adverse effect level (NOAEL) which is a critical basis for the establishment of an ADI. Yttrium nitrate was orally administered to rats at doses of 0, 10, 30 and 90 mg/kg/day for 90 days followed by a recovery period of 4 weeks. The following toxicity indices were measured: mortality, clinical signs, daily food consumption and weekly body weight; urinalysis, hematology, blood coagulation, clinical biochemistry and histopathology at the end of administration and recovery periods. No toxicologically significant changes were found in any yttrium-treated group as compared to the concurrent control group. Under the present experimental condition, the NOAEL in rats was thus set at 90 mg/kg for yttrium nitrate, *i.e.* 29.1 mg/kg for yttrium.

1. Introduction

The rare earth elements (REEs) are a group of metals which are comprised of lanthanum, 14 lanthanides, yttrium (Y) and scandium. Due to their unique physical and chemical properties, REEs have been widely utilized in industrial and medical fields in the last decades (Du and Graedel, 2011; USEPA, 2012). The growing increases in REEs-related industrial activities imply human occupational exposure to REEs. Human environmental exposure to REEs has also been found in the populations residing in REEs mining areas in China (Peng et al., 2003; Tong et al., 2004; Zhu et al., 2005; Wei et al., 2013). REEs have also been widely used in agricultural and zootechnical fields as fertilizers for crops and as feed additives for livestock, poultry and aquaculture in China (Hu et al., 2002; Pang et al., 2002; He et al., 2010). Established and growing evidence points to REEs-related marine, fresh water and soil pollution and accumulation. As a result, different levels of REEs from agricultural products of mining areas (Li et al., 2013; Jin et al., 2015; Zhuang et al., 2017) or non-mining areas (Li et al., 2012; Jiang et al., 2012; Song et al., 2016) have been detected, thereby increasing their accumulations in human body when ingested through food chain.

REEs have been reported to exert miscellaneous toxicities to animals and humans via different exposure routines (Reviewed by Seishiro and Suzuki, 1996; Rim et al., 2013; Pagano et al., 2015). Concerns regarding the adverse effects of long-term exposure to low levels of REEs from foods on human health have arisen, as they can accumulate in blood, brain and bone after entering into human body (Seishiro and Suzuki, 1996). However, the existing

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toxicological studies are yet confined to few light REEs, and scanty toxicological data are available for other REEs, especially heavy REEs. More importantly, no official acceptable daily intake (ADI) value, the amount of a chemical or food contaminant that can be ingested daily over a lifetime without appreciable health risk, has yet been proposed for either total REEs or individual REE.

Yttrium (Y) is a heavy REE, and has also been widely used in industrial, medical and agricultural fields. In China, relative high contaminated levels of yttrium in foods have been detected (Jiang et al., 2012; Song et al., 2016). Therefore, the potential for adverse human health effects from oral exposure to yttrium, especially through long-term consumption of yttrium-contaminated foods, warrants greater attention. Nevertheless, there are only few oral exposure toxicological studies for yttrium. Schroeder and Mitchener (1971) previously reported that the growth of mice was depressed when they were given 5 ppm of Y^{3+} in drinking water, and the longevity was increased in Y^{3+}-fed mice. Rats were fed with water dissolved different levels of Y^{3+} for 6 months, and it was shown that low level (0.534 mg/L) of Y^{3+} might improve the functions of learning and memory in rats, but high level (5340 mg/L) of Y^{3+} could restrain the learning-memory functions and growth-development in rats (Wu et al., 2006). In the recent toxicological investigations performed in accordance with the guideline issued by the Organization of Economic Co-operation and Development (OECD) (OECD TG 426), the effects of yttrium on the neurobehavior and cognitive ability of rat offspring have been assessed. Dams were orally exposed to 0, 5, 15, or 45 mg/kg daily of yttrium nitrate from gestation day (GD) 6 to postnatal day (PND) 21, and their offspring were given the same doses until PND 63. The results revealed that oral exposure of rats to yttrium nitrate in doses up to 45 mg/kg daily had no adverse effects on their neurobehavioral development and cognitive ability (Li et al., 2015a,b).

As well-known, for a chemical or food additive or contaminant, the no observed adverse effect level (NOAEL) which is primarily derived from the repeated dose toxicity studies in rodents, is a critical basis for the establishment of an ADI, a very critical value for food safety risk assessment. However, the general toxicity of yttrium following long-term repeated oral exposure has not been scientifically assessed in compliance with the international testing guidelines and regulatory requirements. Accordingly, no NOAEL or ADI value for yttrium has yet been developed to date. Therefore, it is extremely necessary to reveal the long-term toxicological effects of yttrium via oral exposure by using current international test guidelines. In this research, according to the OECD guidelines for the Testing of Chemicals "Repeated Dose 90-Day Oral Toxicity Study in Rodents" (OECD Test No. 408) and Good Laboratory Practice (GLP), the subchronic toxicity of yttrium to rats was evaluated and the NOAEL was estimated, which can provide scientific data for the food safety risk assessment of yttrium and REEs.

Our present subchronic toxicity study reveals that repeated oral exposure of rats to yttrium nitrate in doses up to 90 mg/kg daily for 90 days exerts no adverse effects on rats, and the NOAEL is set at 90 mg/kg for yttrium nitrate, *i.e.* 29.1 mg/kg for yttrium. Accordingly, the ADI value for yttrium was estimated to be 145.5 μg/kg/day by using an uncertainty factor of 200. Based on the current contaminated levels of yttrium in foods and average dietary intake of yttrium for adults in China, this estimated ADI value indicates the daily intake of yttrium from foods is relative acceptable and safe for adults.

2. Materials and methods

2.1. Test substance

Yttrium nitrate stock solution with the concentration of 218.28 mg/mL was provided by China National Center for Food Safety Risk Assessment (Beijing, China). When used, yttrium nitrate stock solution was diluted to the indicated dosing concentrations with distilled water and was adjusted to pH 6.0 by using 1 mol/L sodium hydroxide.

2.2. Animals and dosing

Sprague-Dawley (SD) rats (50–70 g) were obtained from the Laboratory Animal Center of Academy of Military Medical Sciences (Beijing China). The rats were housed at Evaluation and Research Center for Toxicology, Institute of Disease Control and Prevention, PLA (Beijing China). After 6 days of acclimatization and quarantine, the healthy rats were randomly assigned into control group or one of 3 yttrium dosing groups according to body weights separated by sex, with 15 rats per sex per group. All rats were housed five per sex in standard PVC cages (*i.e.* 3 cages per sex per group) in a ventilated room (10–20 air exchanges/hr) with controlled illumination (12 h light/dark cycle), temperature (20°C–25 °C) and humidity (40%–70%). Feed (standard rodent diet) and tap water were provided *ad libitum*. Rats in 3 yttrium exposure groups were given yttrium nitrate solution by oral gavage at a daily dose of 10, 30, and 90 mg/kg body weight, respectively. Control rats received equivalent volumes of vehicle of distilled water only. The application volume for all groups was 1 mL/100 g body weight. The animals were treated with the test substance or vehicle once daily for a period of 90 days followed by a recovery period of 4 weeks. Ten rats (2 cages) per sex of each group were subjected to necropsy one day after the last administration (the end of treatment period), and the remaining 5 rats (1 cage) per sex of each group were subjected to necropsy 28 days after the last administration (the end of recovery period).

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Institute of Disease Control and Prevention, PLA (approval No.: 2013-021), and were in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The protocol was generally based on the OECD guidelines for the Testing of Chemicals "Repeated Dose 90-day Oral Toxicity Study in Rodents" (OECD Test No. 408) (OECD, 1998). And the study was conducted at Evaluation and Research Center for Toxicology, Institute of Disease Control and Prevention, PLA (Beijing China), a Good Laboratory Practice (GLP)-certified laboratory, and was in strict accordance with the GLP principles of China Food and Drug Administration and Ministry of Environmental Protection of China.

2.3. Observations and examinations

The following parameters/indices were determined: mortality, daily clinical observation, daily food consumption, weekly body weight; urinalysis, hematology, blood coagulation, clinical biochemistry and histopathologic examination at the end of administration and recovery periods. Animals were fasted overnight before blood collection.

2.3.1. Clinical observation and mortality

All animals were checked daily for any clinically abnormal signs. A check for moribund and dead animals was made once daily. If animals were in a moribund state, they were sacrificed and necropsied.

2.3.2. Body weight and food consumption

Body weights were recorded on the first day of administration, weekly thereafter and prior to sacrifice.

The 24 h feed intake pattern on rats was measured weekly for each cage. Briefly, at weekly interval, the supplied amount of food