Food and Chemical Toxicology 47 (2009) 729–733

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Chronic toxicity and carcinogenicity studies of chromium picolinate monohydrate administered in feed to F344/N rats and B6C3F1 mice for 2 years

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ARTICLE INFO

Article history:
Received 14 August 2008
Accepted 4 January 2009

Keywords:
Trivalent chromium
Diet
Supplement
Body weight
Preputial gland
National Toxicology Program

ABSTRACT

Trivalent chromium (Cr(III)) has been proposed to be an essential element, which may increase sensitivity to insulin and thus participate in carbohydrate and lipid metabolism. Humans ingest Cr(III) both as a natural dietary constituent and in dietary supplements taken for weight loss and antidiabetic effects. Chromium picolinate (CP), a widely used supplement, contains Cr(III) chelated with three molecules of picolinic acid and was formulated in an attempt to improve the absorption of Cr(III). In order to examine the potential for CP to induce chronic toxicity and carcinogenicity, the NTP conducted studies of the monohydrate form (CPM) in groups of 50 male and female F344/N rats and B6C3F1 mice exposed in feed to concentrations of 0, 2000, 10,000 or 50,000 ppm for 2 years; exposure concentrations were selected following review of the data from NTP 3-month toxicity studies. Exposure to CPM did not induce biologically significant changes in survival, body weight, feed consumption, or non-neoplastic lesions in rats or mice. In male rats, a statistically significant increase in the incidence of preputial gland adenoma at 10,000 ppm was considered an equivocal finding. CPM was not carcinogenic to female rats or to male or female mice.

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1. Introduction

Trivalent chromium (Cr(III)) has been proposed to be an essential element, which may increase sensitivity to insulin and thus participate in carbohydrate and lipid metabolism (Anderson, 1989). The mechanism involves increased insulin binding through increasing the number of insulin receptors and increasing insulin receptor phosphorylation when the chromium is bound to a low molecular weight chromium binding substance (LMWCr; also referred to as chromodulin) and insulin is present (Anderson, 1998). In the blood, Cr(III) is bound to and transported to tissues by transferrin, a process regulated, at least in part, by insulin (Clodfelder et al., 2001). Cr(III) deficiency may contribute to glucose intolerance and diabetes mellitus (Type 2); however, the essentiality of Cr III and the ability of Cr III to increase insulin sensitivity have been questioned (Stearns, 2000; Stallings and Vincent, 2006). The most recent dietary guideline for adequate Cr(III) ingestion is 20–45 μg, set by the Institute of Medicine in 2001 (IOM, 2001). Typical serving sizes of a variety of foods and beverages, including broccoli, grape juice, whole wheat English muffins, mashed potatoes, dried garlic, dried basil, beef cubes, orange juice, turkey breast, whole wheat bread, red wine, unpeeled apple, banana, and green beans, provide 1–13 μg Cr(III) (NIH, 2007).

Cellular uptake of Cr(III) by cells is thought to occur by diffusion or phagocytosis, which results in very low absorption and excretion primarily in the feces. However, absorbed Cr(III) is widely distributed to tissues (Hepburn and Vincent, 2003; Anderson et al., 1996). Chromium picolinate (CP), which contains Cr(III) chelated with three molecules of picolinic acid (Evans and Pouchnik, 1993), was formulated in an attempt to increase the absorption of Cr(III) over non-chelated forms, such as chromium chloride. CP is widely used as a dietary supplement, primarily because of claims of increased metabolic (weight reducing) and antidiabetic effects. Cr(III)-containing supplements have become very popular, generating estimated annual sales in the hundreds of millions of dollars in the mid to late 1990s (Federal Trade commission, 1996; Mirasol, 2000); these supplements are available over the counter as pills, chewing gums, sports drinks, and nutrition bars (Vincent, 2001), either alone or in combination with other supplements. Numerous clinical studies have been conducted with daily doses of CP containing 200–1000 μg Cr(III) (Cefalu and Hu, 2004; Komorowski et al., 2008) and in one study modeling human exposure to CP, a dose containing 600 μg Cr(III) was chosen (Stearns et al., 1995a).

Abbreviations: Cr(III), trivalent chromium; NTP, National Toxicology Program; CP, chromium picolinate; CPM, chromium picolinate monohydrate; LMWCr, low molecular weight chromium binding substance.

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0278-6915/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.fct.2009.01.006

Thus, it is likely that human exposure through consumption of supplements is in this range. Although one study suggested that the absorption of Cr following Cr(III) exposure is not enhanced by picolinic acid (Olin et al., 1994), another study showed higher Cr tissue concentrations following exposure to CP compared to chromium chloride (Anderson et al., 1996). The NTP conducted absorption, distribution, metabolism, and excretion studies of [14C]-chromium picolinate monohydrate (CPM) in mice and rats to differentiate the metabolic fates of chromium and [14C]-picolinic acid (NTP, 2008). The patterns of urinary and fecal excretion of chromium and picolinic acid suggest that most of the picolinic acid is not bound to chromium during absorption and that picolinic acid is more readily absorbed than chromium. These findings are consistent with previous studies (Hepburn and Vincent, 2002, 2003).

Neither CP (Anderson et al., 1997) nor other Cr(III) compounds (Ivankovic and Preussmann, 1975; Anderson et al., 1997; MacKenzie et al., 1958; Schroeder et al., 1964, 1965) have displayed evidence of toxicity following oral exposure. No studies examining the carcinogenic potential of chromium picolinate in animals or humans have been reported; however, previous carcinogenicity studies of other Cr(III) compounds in rodents following oral exposure were negative (Ivankovic and Preussmann, 1975; Schroeder et al., 1964, 1965).

Although Cr(III) has been shown to be genotoxic in acellular test systems that permit direct contact with DNA (Snow and Xu, 1991; Snow, 1994; Bridgewater et al., 1994), Cr(III) compounds, including CP, often give negative or conflicting results in standard *in vivo* and *in vitro* genetic toxicity assays. This disparity appears to result from the very low cellular uptake of Cr(III). CP is not mutagenic in the Ames assay (Whittaker et al., 2005; NTP, 2008), but some laboratories have reported increases in gene mutations or chromosomal aberrations in cultured mammalian cells treated with chromium picolinate (Stearns et al., 1995b, 2002; Whittaker et al., 2005). In contrast, other laboratories have reported no increases in these lesions in similar studies with CP (Gudi et al., 2005; Slesinski et al., 2005). Results of more recent *in vitro* and *in vivo* genotoxicity studies with CP were also negative. These studies included assessment of micronucleated erythrocytes in mice, DNA damage in mice, and DNA damage in cultured mammalian cells after exposure to CP (Andersson et al., 2007). Other Cr(III) salts have also given negative results in a number of *in vitro* and *in vivo* assays (Zeiger et al., 1992; Amrani et al., 1999; Witt et al., 2000; Whittaker et al., 2005).

Because there is widespread human exposure to CP in dietary supplements as well as a limited body of evidence suggesting that CP is genotoxic, the NTP conducted toxicity and carcinogenicity studies of CPM, in male and female F344/N rats and B6C3F1 mice. The route chosen for these studies was dosed feed, because humans are exposed in the diet and in supplements. CPM was selected for testing because it was commercially available. Three month studies were first conducted to characterize the subchronic toxicity of CPM and aid in the selection of doses for 2-year studies (NTP, 2008; Rhodes et al., 2005). Rats and mice were exposed to 0, 240, 800, 2000, 10,000 or 50,000 ppm. The highest exposure concentration of 50,000 ppm (5%) used in these studies is considered to be a limit dose in feed studies because a higher concentration of chemical is thought to alter the nutritional content of the diet. Exposure did not result in toxicity to rats or mice, as evidenced by the lack of biologically significant changes in survival, mean body weight and body weight gain, feed consumption, hematology, clinical chemistry, organ weights, histopathology and sperm morphology and vaginal cytology. A small increase in the frequency of micronucleated erythrocytes, which resulted in a significant positive trend in female mice exposed to CPM for 3 months was judged to be an uncertain finding. There was no evidence of an increase in micronucleus frequency in male mice. Based on these results, the three highest exposure concentrations (2000, 10,000 and 50,000 ppm) were selected by the NTP for testing in the 2-year studies. The objective of the present report is to present the major findings from the NTP 2-year toxicity and carcinogenicity studies of CPM.

2. Materials and methods

2.1. Chemical and dose formulations

CPM CAS No. 27882-76-4) used in the 2-year studies was a combination of chemical obtained from TCI America (Portland, OR) and from Sigma–Aldrich (St. Louis, MO). The chemical, a reddish-purple crystalline powder, was identified as CPM by infrared and proton nuclear magnetic resonance spectroscopy, X-ray diffraction, and electrospray ionization-mass spectrometry. The presence of approximately 1 mole of water per formula unit was confirmed by Karl Fischer titration and weight loss on drying assays. Purity was determined by elemental analyses, proton-induced X-ray emission spectroscopy, inductively coupled plasma-atomic emission spectroscopy, high-performance liquid chromatography with ultraviolet–visible detection, UV detection, or ICP-mass spectrometric detection. The overall purity of the chemical was determined to be greater than 95%.

CPM was stable as a bulk chemical for at least 2 weeks when stored in sealed amber glass containers at temperatures up to 60 °C, as determined by inductively coupled plasma-atomic emission spectroscopy and high-performance liquid chromatography with ultraviolet–visible detection. To ensure stability, the bulk CPM was stored at room temperature, protected from light, in sealed plastic buckets. No degradation was detected during the 2-year studies.

The dose formulations were prepared monthly by mixing CPM with feed. Homogeneity and stability of the dose formulations were assessed by high-performance liquid chromatography with ultraviolet–visible detection. Homogeneity of the 50,000 ppm dose formulation was confirmed. The stability of this formulation was confirmed for at least 42 days at room temperature when stored in double-thick sealed plastic bags, protected from light. Periodic analysis confirmed that all 167 dose formulations for rats and all 99 for mice were within 10% of the target concentrations.

2.2. Animals and animal maintenance

The studies were conducted at Southern Research Institute (Birmingham, AL). Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms (Germantown, NY). Animals were quarantined for 12 days prior to the initiation of the studies, and were approximately 5–6 weeks old at the beginning of the studies. Study animals were distributed randomly into groups of approximately equal initial mean body weights and identified by tail tattoo. Rats and mice were housed 1 (male mice), 3 (male rats) or 5 (female rats and mice) to a cage. The animal room was maintained at a temperature of 72 ± 3 °F, a relative humidity of 50% ± 15%, a 12 h light-dark cycle, and 10 air changes per hour. Irradiated NTP-2000 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA) was available *ad libitum* and changed weekly. Tap water was available *ad libitum* via an automatic watering system. Animals were killed by asphyxiation with CO_2.

Animal use was in accordance with the United States Public Health Service policy on humane care and use of laboratory animals and the Guide for the Care and Use of Laboratory Animals. These studies were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations (21CFR, Part 58).

2.3. Study design

Groups of 50 male and 50 female rats and mice were fed diets containing 0, 2000, 10,000, or 50,000 ppm CPM for 105 weeks. All animals were observed twice daily. Animals were weighed initially, once a week for the first 13 weeks, once a month thereafter, and at the end of the studies. Feed consumption was measured weekly for the first 13 weeks of the study and monthly thereafter. Clinical findings were recorded monthly. Complete necropsies and microscopic examinations were performed on all core study rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (eyes were initially fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g. adrenal gland, kidney, ovary), samples from each organ were examined. Additional details regarding the pathology data generation, quality assurance review, and NTP pathology working group are available elsewhere (NTP, 2008). Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985).

2.4. Statistical methods

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's

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