Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food and Chemical Toxicology 43 (2005) 1619–1625
www.elsevier.com/locate/foodchemtox

Mutagenicity of chromium picolinate and its components in *Salmonella typhimurium* and L5178Y mouse lymphoma cells

Paul Whittaker [a,*], Richard H.C. San [b], Jane J. Clarke [b], Harold E. Seifried [c], Virginia C. Dunkel [a]

[a] *Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD 20740-3835, United States*
[b] *BioReliance, Rockville, Maryland, United States*
[c] *National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States*

Received 28 February 2005; accepted 10 May 2005

Abstract

Chromium picolinate is one of the most commonly used chromium dietary supplements available in the United States, and it has been marketed to consumers for use in weight loss, increasing muscle mass, and lowering serum cholesterol. Chromium picolinate is a synthetic compound that provides a bioavailable form of Cr(III) that is absorbed better than dietary chromium. However, there are several reports that it can have adverse effects. In order to study the mechanism of observed cellular toxicity and mutagenicity, chromium picolinate and its component compounds, chromium (III) chloride and picolinic acid, were evaluated in *Salmonella typhimurium* and L5178Y mouse lymphoma cells. Neither chromium picolinate nor chromium chloride induced a mutagenic response in *S. typhimurium*. However, in the L5178Y mouse lymphoma mutation assay, chromium picolinate induced mutagenic responses without and with the addition of S9.
Published by Elsevier Ltd.

Keywords: Mutagenicity; Chromium; Chromium picolinate; *Salmonella typhimurium*; Mouse lymphoma

1. Introduction

Trivalent chromium (Cr(III)) is an essential nutrient for maintaining normal carbohydrate and lipid metabolism and is required by the body in very small quantities. Recent studies suggest that a low molecular weight chromium-binding substance may amplify insulin receptor tyrosine kinase activity in response to insulin (Davis and Vincent, 1997; Vincent, 1999). An Adequate Intake (AI) was set based on estimated mean intakes; the AI for ages 19–50 years is 25 μg/day for women and 35 μg/day for men (Food and Nutrition Board, Institute of Medicine, 2002). As recommended by the Food and Nutrition Board of the National Research Council, the best assurance of adequate and safe chromium intake is consumption of a varied diet balanced with other essential nutrients (Food and Nutrition Board, Institute of Medicine, 2002).

It is difficult to determine dietary chromium intakes because chromium content in food can be altered during food processing (Food and Nutrition Board, Institute of Medicine, 2002).

Chromium picolinate is a popular dietary supplement and advocates have claimed a variety of beneficial health effects such as weight loss, lowering of serum cholesterol, and increased muscle mass. However, chromium

Abbreviations: FDA, US Food and Drug Administration; NCI, National Cancer Institute; AI, Adequate Intake; GRAS, Generally Recognized As Safe; RDI, Reference Daily Intake; TK, Thymidine kinase; MMS, Methyl methanesulfonate; DMBA, 7,12-Dimethylbenzanthracene; TFT, Trifluorothymidine.

* Corresponding author. Tel.: +1 301 436 1797; fax: +1 301 436 2665.
E-mail address: paul.whittaker@cfsan.fda.gov (P. Whittaker).

0278-6915/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.fct.2005.05.003

picolinate is not approved as a food additive nor listed as Generally Recognized as Safe (GRAS) by the FDA. The FDA has established a Reference Daily Intake (RDI) for chromium of 120 μg (21 CFR 101.9, 2004) to assist consumers in understanding the nutritional significance of the levels of this nutrient in the context of the total daily diet.

To study the mechanism of observed cellular toxicity and mutagenicity, chromium picolinate and its component compounds, chromium (III) chloride and picolinic acid, were evaluated in *Salmonella typhimurium* and L5178Y mouse lymphoma cells.

2. Materials and methods

2.1. Chemicals

The manufacturer/supplier, Chemical Abstracts Service numbers, purity and formula of each compound are listed in Table 1. The compounds were acquired by the FDA, NCI and the NCI Chemical Carcinogen References Standards Repository (Midwest Research Institute [MRI], Kansas City, MO) and supplied as coded samples to the contract laboratory (BioReliance, Rockville, MD). Stock solutions of each compound were prepared in the appropriate solvent immediately prior to use. The final concentration of solvent was 10% water or 1% dimethyl sulfoxide (DMSO).

2.2. Salmonella/mammalian-microsome mutagenicity assay

The experimental materials, methods, and procedures as explained below are based on those described by Ames et al. (1975).

2.3. Salmonella typhimurium strains

S. typhimurium histidine auxotrophs TA98, TA100, TA1535, and TA1537 were obtained from Dr. Bruce Ames, University of California, Berkeley. Cultures were grown overnight in Oxoid nutrient broth No. 2 and were removed from incubation when they reached a density of $1–2 \times 10^9$ cells/ml. On the day of use, all tester strain cultures were checked for genetic integrity as recommended by Ames et al. (1975).

Table 1
Chemical formulas for compounds

Chemical name	CAS number	Formula	Purity (%)	Source
Chromium chloride	10060-12-5	$CrCl_3 \cdot 6H_2O$	99	Sigma-Aldrich
Picolinic acid	98-98-6	$C_6H_5NO_2$	99	Sigma-Aldrich
Chromium picolinate	14639-25-9	$C_{18}H_{12}CrN_3O_6$	99	USDA (Dr. Richard A. Anderson)

2.4. S9 preparation

Liver S9 homogenate was prepared from male Sprague–Dawley rats and Syrian golden hamsters that had been injected with Aroclor 1254 at 500 mg/kg body weight. The postmitochondrial (microsomal) enzyme fractions were prepared as described by Ames et al. (1975). The components of the S9 mix were 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 100 mM sodium phosphate (pH 7.4), and the appropriate S9 homogenate at a concentration of 0.1 ml/ml of mix. For each plate receiving microsomal enzymes, 0.5 ml S9 mix was added.

2.5. Plate incorporation methodology

In the mutagenicity assay, the plate incorporation method, as originally described by Ames et al. (1975), was used. For testing in the absence of S9 mix, 100 μl of the tester strain and 50 μl of solvent or test chemical were added to 2.5 ml of molten selective top agar at 45 ± 2 °C. When S9 was used, 0.5 ml of S9 mix, 50 μl of tester strain, and 50 μl of solvent or test chemical were added to 2.0 ml of molten selective top agar at 45 ± 2 °C. After vortexing, the mixture was overlaid onto the surface of 25 ml of minimal bottom agar. After the overlay had solidified, the plates were incubated for 48 h at 37 ± 2 °C. Five doses of test chemical, together with the appropriate concurrent solvent and positive controls, were tested in triplicate on each tester strain without metabolic activation and also with activation by induced rat and hamster liver S9 preparations.

The doses to be tested in the mutagenicity assay were selected based on the levels of cytotoxicity observed in a preliminary dose range-finding study using strain TA100. Ten dose levels of the chemical, one plate per dose, were tested both in the presence and absence of induced hamster S9. If no toxicity was observed, a total maximum dose of 10 mg of test chemical per plate was used. The criteria used to evaluate a test were as follows: for a test article to be considered positive, it must induce at least a doubling (TA98 and TA100) in the mean number of revertants per plate of at least one tester strain. This increase in the mean revertants per plate must be accompanied by a dose response to increasing concentrations of the test chemical. If the study shows a dose–response but with a less than threefold increase on TA1535 or TA1537, the response must be confirmed in a repeat experiment.