Food and Chemical Toxicology 75 (2015) 50–57

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Safety assessment of dietary bamboo charcoal powder: A 90-day subchronic oral toxicity and mutagenicity studies

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ARTICLE INFO

Article history:
Received 22 August 2014
Accepted 4 November 2014
Available online 13 November 2014

Keywords:
Bamboo charcoal powder
90-day subchronic oral toxicity
Ames test
Micronucleus test
Comet assay

ABSTRACT

Vegetable carbon has been used as food additive in EU (E153) and China for many years; however, no experimental data have been available on its dietary safety. This study was designed to evaluate the subchronic toxicity and genotoxicity of bamboo charcoal powder (BCP). In the study of subchronic oral toxicity, BCP was administered orally at doses of 2.81, 5.62, and 11.24 g/kg BW for 90 days to SD rats. Additional satellite groups from the control group and high dose group were observed for a 28-day recovery period. At the end of the treatment and recovery periods, animals were sacrificed, and their organs were weighed and blood samples were collected. The toxicological endpoints observed included clinical signs, food consumption, body and organ weights, hematological and biochemical parameters, macroscopic and microscopic examinations. The results showed no significant differences between the BCP treated groups and control group. The genotoxicity of BCP was assessed with the Salmonella typhimurium mutagenicity assay (Ames test) and a combination of comet assay and mammalian erythrocyte micronucleus protocol. The results did not reveal any genotoxicity of BCP. Based on our study, the no-observed-adverse-effect level (NOAEL) for BCP is 11.24 g/kg BW/day.
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1. Introduction

Bamboo charcoal powder (BCP), a vegetable carbon, is a natural black powder made from perennial bamboo. It has an atomic weight of 12.01 g/M. Production procedures of BCP provided by the supplier include high temperature carbonization of bamboo wood in a rotary kiln, ultra-fine grinding by zirconia grinding media in a roller mill, separating the smaller particles from the larger ones by cyclone, purified by hydrochloric acid washing, neutralized, dried, and irradiation sterilized by ^{60}Co. It is a porous, tasteless and odorless material, which may contain minor amounts of nitrogen, hydrogen and oxygen and cannot be dissolved in water and organic solvents (JECFA, 2006).

Besides its widespread current use as a therapeutic agent, there has been increasing interest in vegetable carbon, and BCP in particular, as a food ingredient and pigment in recent decades. Many new foods containing BCP have emerged, such as charcoal cake, charcoal bread, charcoal cookies, charcoal desserts, charcoal ice cream, charcoal candy, and charcoal peanuts (Fig. 1). In particular, charcoal peanuts are popular snack food which sells well on Taobao shop, the largest online retail platform in China. BCP is also used extensively in Japan, South Korea, and China (including Taiwan) as a food ingredient or food pigment additive with purported health benefits. In China, vegetable carbon black (Chinese national standards number: 08.138) is a legal food additive approved by the Ministry of Health of the People's Republic of China. According to the Hygienic Standards for Uses of Food Additives (Ministry of Health of P.R.China, 2011), it is approved for use as a pigment in beverages, candy, rice products, wheat flour products, cookies and biscuits.

Vegetable carbon (also vegetable black), derived from plant materials, is authorized as a food additive in the EU (EINECS number: 231-153-3) in all foodstuffs with a few exceptions in which the use of food colors is specifically prohibited or restricted (Commission Directive 94/36/EC, 1994), and no specific CAS Registry Number is given for vegetable carbon. Although vegetable carbon has been evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970, 1977 and 1987 (JECFA, 1971, 1978, 1987), the

Contribution:
Jia Zhenchao took full responsibility for the research. Jia Zhenchao performed the study design and the experiment, and wrote the initial draft of the manuscript; Zhong Yuting, Yan Jiuming, Lu Yedan assisted to do experiments; Song Yang and Chen Jinyao performed the revision and checked the integrity of data presented; Zhang Lishi reviewed and revised the drafts. All authors read and approved the final manuscript.

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http://dx.doi.org/10.1016/j.fct.2014.11.002
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Fig. 1. Image of BCP food.

Scientific Committee on Food (SCF) in 1977 and 1983 (SCF, 1977, 1984), and Food Additives and Nutrient Sources added to Food (ANS) in 2012 (EFSA, 2012), all of the Committees did not establish an acceptable daily intake (ADI) because the absence of animal toxicological data. In view of its use as a traditional therapeutic agent and a safe consumption history, the SCF always recommended the maintenance of vegetable carbon as a food additive after comprehensive reviewing. Although consumed as a legal food additive in many countries, vegetable carbon is not listed as a permitted food color in the USA (Code of Federal Regulations, 1988). Vegetable carbons have been approved for human food use on an assumption of safety in the absence of available toxicological data in animals; it is imperative to evaluate its dietary safety in vivo.

In our previous studies, including acute toxicity and 28-day repeated dosing oral toxicity in SD rats, it was indicated that the median lethal dose (LD_{50}) of BCP for both male and female rats is more than 11.24 g/kg BW/day and the NOAEL is 11.24 g/kg BW/day (J. Zhenchao, unpublished results). Based on our previous studies, a 90-day subchronic oral toxicity study and a battery of genotoxicity tests were carried out to further evaluate the safety of BCP.

2. Materials and methods

2.1. Chemicals and reagents

2.1.1. The origin and characteristics of the BCP

BCP used in the experiment was purchased from Shanghai Hainuo Charcoal Limited Company (Shanghai, China). It is a commercial food-grade product, with full name of nano bamboo charcoal power (batch number: HN-130810). The manufactured procedures comply with the General Hygienic Regulation for Food Production (Ministry of Health of P.R.China, 2013). The detailed characteristics of BCP provided by the supplier are listed in Table 1.

2.1.2. Other chemicals and reagents

Ethyl methanesulfonate (EMS), 4-nitroquinoline 1-oxide (4-NQO), sodium azide (NaN_3), mitomycin C (MMC), 2-aminofluorene (2-AF), and 1,8-dihydroxyanthraquinone (Dan), dimethylsulfoxide (DMSO), Giemsa stain, low-melting agarose (LMA) and Triton X-100 were bought from sigma (USA). Normal-melting agarose (NMA), EDTA and Tris were bought from Amreco (USA). NaOH, NaCl and DMSO were products from Nanjing Biotech. Co. Ltd (China). PBS was bought from Hyclone (USA). S9 was obtained from Sichuan Center for Disease Control and Prevention.

2.2. Animals

Male and female Sprague-Dawley (SD) rats and Kunming mice of Specific Pathogen Free (SPF) grade were purchased from Dashuo Laboratory Animal Reproduction Center (Chengdu, China) (Certificate No. SCXK2013-24). Five weeks old rats weighing 88.2 ± 9.5 g (male) and 71.1 ± 7.9 g (female) when first arrived were used for the 90-day subchronic oral toxicity study. The mouse at 6 weeks of age and weighing 19.6 ± 1.1 g (male) and 18.8 ± 1.0 g (female) when first arrived were used for the combination of comet assay and mammalian erythrocyte micronucleus protocol. Every five animals by sex in the same group were assigned randomly and housed socially in one cage; both the rats and mice were housed the same way. The cages are of appropriately size (460 × 300 × 160 mm for rats and 325 × 245 × 157 mm for mice), plastic, with regular ventilation in an environmentally controlled room with 12 h light/12 h dark conditions. The temperature was 22 ± 2 °C with relative humidity of 55 ± 10%. Each animal was assigned a unique identification number. Cage padding was replaced every three days. Food and water were provided ad libitum. The rats and mice were fed with a standard rodent maintenance diet purchased from the Dashuo Laboratory Animal Reproduction Center. Following a 7-day quarantine and

Table 1
The characteristics of BCP.

Item	BCP
Color	Black
Taste	Tasteless
Odor	Odorless
Status	Powder
Purity (%)	95.5
Moisture (%)	1.10
Ash (%)	2.10
pH	9.7
As (mg/kg)	0.40
Pb (mg/kg)	0.59
Hg (mg/kg)	0.002
Ge (mg/kg)	0.077
Polyaromatic hydrocarbons	Certificated[a]
Alkali soluble matter	Certificated[a]
Apparent density and porosity (g/mL)	0.38

[a] The certificated detection method was according to JECFA (JECFA, 2006).

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