Food and Chemical Toxicology 62 (2013) 68–75

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

A dietary embryo/fetal developmental toxicity study of arruva, an *R,R*-monatin salt isomer, in Crl:CD(SD) rats

W.A. Brathwaite [a,*], P.L. Casterton [b], A.I. Nikiforov [c], M.O. Rihner [c], E.D. Sloter [d], J.J. Hlywka [e]

[a] *Cargill Limited, Winnipeg, MB, Canada*
[b] *Cargill, Incorporated, Wayzata, MN, USA*
[c] *Toxicology Regulatory Services, Charlottesville, VA, USA*
[d] *WIL Research Laboratories, Ashland, OH, USA*
[e] *Kraft Foods Group, Incorporated, Chicago, IL, USA*

ARTICLE INFO

Article history:
Received 11 June 2013
Accepted 16 August 2013
Available online 23 August 2013

Keywords:
Arruva
R,R-monatin
Oral
Sweetener
Embryotoxicity
Fetal development

ABSTRACT

R,R-Monatin is an intensely sweet substance originally identified in the root bark of *Sclerochiton ilicifolius*. *R,R*-Monatin salt, commonly known as "arruva", has potential for use as a high-potency sweetener food ingredient. Previously, arruva was concluded to present no toxicologically relevant effects to Crl:CD(SD) rats and Crl:CD-1(ICR) mice fed up to 35,000 ppm arruva in the diet for 90 days. In the present study, groups of mated Sprague–Dawley rats (25 Crl:CD(SD) females/group) were exposed continuously to 0 (control), 15,000, 30,000, or 50,000 ppm arruva in the diet during gestation days 6–21. There were no fetal malformations or developmental variations that were attributable to arruva at any exposure level, nor were there any test article-related effects on intrauterine survival. Maternal toxicity, evidenced by lower mean body weights, body weight gains and feed efficiency, was observed at 50,000 ppm. A developmental effect, in the form of lower mean fetal body weight, was noted in the 50,000 ppm group in the presence of maternal toxicity. Therefore, the dietary no-observed-adverse-effect level (NOAEL) for maternal and embryo/fetal developmental toxicity of arruva in pregnant rats during gestation days 6–21 was 30,000 ppm (equivalent to 2564 mg/kg bw/day) based on reductions in maternal and fetal body weights.

1. Introduction

The high-potency sweetener monatin is an amino acid [2-hydroxy-2-(indol-3-ylmethyl)-4-aminoglutaric acid] and was originally identified in the root bark of *Sclerochiton ilicifolius*, a spiny-leaved hardwood shrub native to South Africa (Archibald et al., 1956). There are four possible stereo-isomers (*R,R*-, *S,S*-, *R,S*- and *S,R*-) of monatin (Bassoli et al., 2005); of which the *R,R*-isomer (Fig. 1) is the most sweet, regardless of whether it is the acid or salt form, with a potency estimated to be in excess of 3000 times the sweetness of sucrose (Fry et al., 2012). The name "arruva" is the common/usual name for *R,R*-monatin salt forms and will be used hereafter.

Interest in the technological properties of arruva as a high-potency sweetener compels requisite scrutiny of toxicity and safety (Abraham et al., 2005). Historically, the root bark of *S. ilicifolius* has been used by indigenous South African populations for sweetening foods and medicines with no known reports of adverse reactions (Vahrmeijer, 2010). Until recently, there were no published safety studies conducted with arruva. Arruva was evaluated in 90-day toxicity studies using Crl:CD(SD) rats and Crl:CD-1(ICR) mice fed up to 35,000 ppm arruva in the diet (Hlywka et al., 2011, 2013). The no-observed-adverse-effect level (NOAEL) was determined to be 20,000 ppm in female rats (approximately 1544 mg/kg bw/day) and 35,000 ppm in male rats (approximately 2368 mg/kg bw/day) based on reduced body weight gain in females at the highest dietary concentration. In mice, a NOAEL at the highest dietary concentration of 35,000 ppm was determined in both sexes (approximately 5764 and 8013 mg/kg bw/day for males and females, respectively). These 90-day dietary studies indicated no evidence of systemic toxicity and arruva was concluded to present no toxicologically relevant effects to rats and mice.

As a continuing part of an overall program to evaluate the safety of this sweetener and any potential for general use in food as an ingredient, the present study was undertaken to assess the embryo/fetal developmental toxicity of arruva in Crl:CD(SD) rats. This study was designed and conducted in general accordance with the U.S. Food and Drug Administration (FDA) Redbook 2000 testing guidelines for Reproduction and Development studies (FDA, 2003), and was conducted at WIL Research Laboratories, Ashland, Ohio, USA. The experimental design overview is shown in Fig. 2.

* Corresponding author. Address: Cargill Limited, 300-240 Graham Avenue, Winnipeg, MB R3C 4C5, Canada. Tel.: +1 647 464 8081.
E-mail address: witty_brathwaite@cargill.com (W.A. Brathwaite).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.08.027

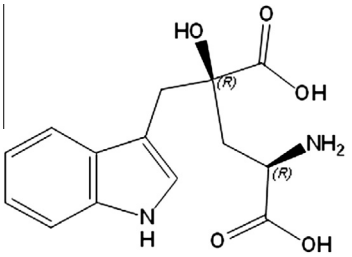

Fig. 1. The *2R,4R*- isomer of 2-hydroxy-2-(indol-3-ylmethyl)-4-aminoglutaric acid.

Conduct of this study complied with FDA Good Laboratory Practice Regulations (FDA, 1987) and Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (OECD, 2003).

2. Materials and methods

2.1. Test article

Enzymatically-sourced arruva [sodium/potassium *2R,4R*-2-amino-4-carboxy-4-hydroxy-5-(3-indolyl) pentanoate] (96.5% UV purity; 5.9% moisture) used in this embryotoxicity/fetal development study was supplied by Cargill, Incorporated. The basal diet, PMI Nutrition International LLC Certified Rodent LabDiet® 5002 (meal), was used as the control diet and in the preparation of the arruva salt/dietary admixes (test diet).

2.2. Preparation of diets

The test diets were prepared weekly as weight/weight (arruva/diet) mixtures with basal diet, without any further correction for purity. Diets were stored at room temperature and protected from light.

2.3. Analysis of arruva in rat diet

Arruva in rodent diet at concentrations of 5000–50,000 ppm were previously determined to be stable at ambient temperatures for up to 10 days (data not shown). Samples collected from the dietary admixes prepared for the first and second weeks were analyzed for concentration and homogeneity at all inclusion levels (15,000–50,000 ppm). Control group diet samples were also collected to verify the absence of arruva. All analyses were performed by a previously validated high performance liquid chromatographic tandem mass spectrometry (HPLC/MS/MS) method.

2.4. Test animals

One hundred twenty-five sexually mature, virgin female Crl:CD(SD) rats approximately 70 days of age were received in good health from Charles River Laboratories, Inc., Raleigh, North Carolina and acclimated for 13 days. Individual body weights were recorded and detailed physical examinations were performed during the acclimation period to ensure the use of healthy animals. All animals were individually housed in clean, stainless steel, wire-mesh cages suspended above cageboard except when rats were paired for mating in the home cage of the male. Cages were kept at a room temperature of 22 ± 3 °C with a relative humidity of approximately 50 ± 20% and a 12 h light/12 h dark photoperiod. Reverse osmosis-treated drinking water delivered by an automatic watering system was provided *ad libitum* throughout the study period. Basal diet was provided *ad libitum* throughout the acclimation period. Animals were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals" (NRC, 1996).

At the conclusion of the acclimation period, all available females were weighed and examined in detail for physical abnormalities. Those judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 g) were placed in a cage with a resident male from the same strain and source for breeding. The selected females were approximately 12 weeks old when paired for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. These rats were maintained under similar laboratory conditions as the females. Positive evidence of mating was confirmed by the presence of a vaginal plug or the presence of sperm in a vaginal lavage. Mating pairs were examined daily. The day on which evidence of mating was identified was termed "gestation day 0" and the animals were separated.

2.5. Assignment of animals to treatment groups

Group assignments were generated based on body weight stratification in a block design, using a validated computerized randomization procedure, on gestation day 0. Bred females were randomly assigned to a group, with each group consisting of 25 females. Individual body weights at randomization were within ±20% of the mean. Body weight values ranged from 222 to 282 g (mean = 248 ± 12.7) on gestation day 0. Replacement animals were arbitrarily assigned based on body weight. Two females with physical abnormalities in the 50,000 ppm group were replaced prior to the start of treatment.

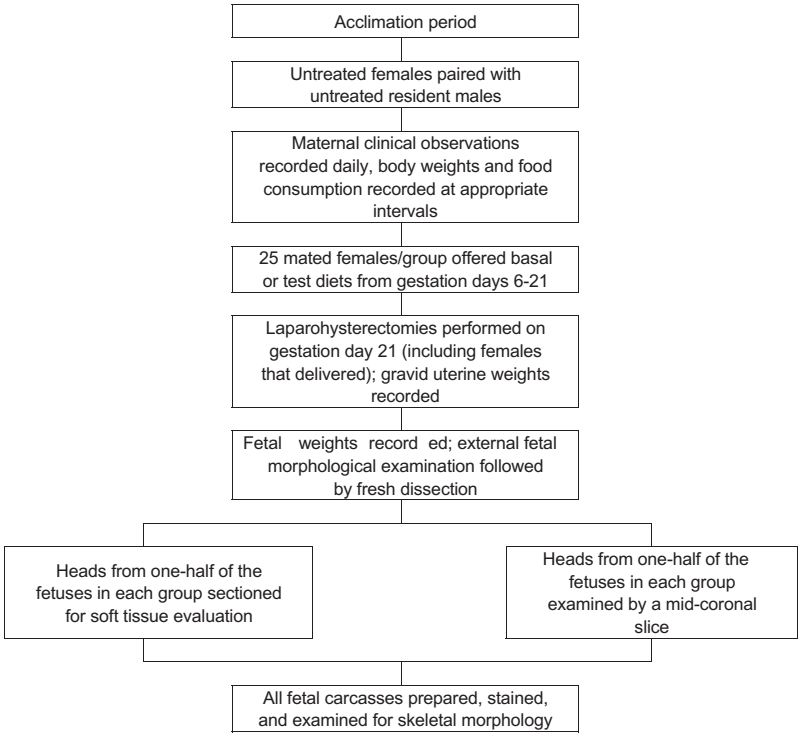

Fig. 2. Experimental design overview of a dietary embryo/fetal developmental toxicity study with pregnant female Crl:CD(SD) rats fed arruva during gestation days 6–21.