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Subchronic and genetic safety evaluation of a calcium fructoborate in rats

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ABSTRACT

A branded calcium fructoborate product, a nature-identical calcium salt of bis (fructose) ester of boric acid found in plants and a natural source of boron in the human diet and sold under the trade name FruiteX-B® Brand Calcium Fructoborate ("FrxB"), was evaluated in a 90-day dietary toxicity study and two genotoxicity studies. In the 90-day study, four groups of 10 male and 10 female Crl:SD CD® IGS rats were fed diets with FrxB admixtures of 0.56, 1.12, and 1.68% dietary concentration, providing mean overall daily intakes of FrxB in male rats of 385.8, 774.9, and 1161.3 mg/kg bw/day, and 392.1, 784.4, and 1171.1 mg/kg bw/day in female rats. There were no mortalities, no clinical or ophthalmologic signs, body weight, body weight gain, food consumption, food efficiency, Functional Observational Battery (FOB), or Motor Activity (MA) findings associated with the administration of FrxB. There were no adverse changes in hematology, coagulation, clinical chemistry, or urinalysis parameters in male or female rats considered the result of test substance administration. At necropsy, there were no macroscopic, histopathological findings, or organ weight changes deemed related to administration of the test substance. Under the conditions of this study, based on the toxicological endpoints evaluated, the no-observed-adverse-effect level (NOAEL) for FrxB in the diet was 1161.3 and 1171.1 mg/kg bw/day in male and female rats, respectively. Bacterial mutagenicity studies and a micronucleus test using Chinese hamster V79 cells demonstrated no mutagenic or genotoxic potential of the tested brand of calcium fructoborate.

1. Introduction

Claims regarding the role of calcium in the maintenance of bone health are well-known and accepted by regulatory authorities, particularly in the treatment of post-menopausal osteoporosis (FDA, 2008; EFSA, 2010). More recent and less well known is the evidence for the role of the trace mineral boron in support of calcium absorption. Indeed, boron, especially in the form of borates, as an essential nutrient to improve the bone-building capacities of calcium along with the better known co-nutrients magnesium and vitamin D, is now being examined for its other benefits for human health (Price et al., 2012).

Boron is an essential component of fruit and vegetable plants and, consequently, a common constituent of the human diet (Hu et al., 1997; Devirian and Volpe, 2003), with daily intake estimated at approximately 1 mg/day, largely of plant derivation (Rainey et al., 1999). Boron normally exists in the soil as boric acid or borate ion, but both of these forms react readily with a variety of biological molecules to form esters and complexes with numerous mono-, di-, and poly-hydroxy compounds (Woods, 1996). The concentration of sugars, sugar alcohols, and other molecules that may form bis-hydroxy acid-borate complexes has been studied in many plants (Hole, 1996; Bielski, 1982), with the conclusion that virtually no free boron exists that is not complexed with these organic molecules. One widely complexed sugar in nature is fructoboron (Pappin et al., 2012); fructoborate complexes are likely to dominate in plants that contain high concentrations of sorbitol or fructose, such as many pome fruits (Brown and Shelp, 1997). Calcium binding confers stability to organoborate complexes, and such naturally occurring calcium complexes have been identified (Woods, 1996; Kobayashi et al., 1999).

Aside from its role in plant metabolism, boron may well be

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essential in many animal species for its potential influence on biological function, and it is considered a beneficial nutrient for humans (Nielsen, 1996; Hunt, 1998; Hathcock, 2014). Daily intake of boron by humans can vary widely depending on the proportions of various food groups in the diet (Nielsen, 1988). Current dietary levels of boron are estimated at 1–3 mg/kg bw/day in food and an additional 0.2–0.5 mg/day in water for 1 L/day (Nielsen, 1988; Rainey et al., 1999; Meacham et al., 2010). Estimates of safe upper limits range from 9.6 to 20 mg/day (6 mg/day, supplemental, CRN) depending on age and health status, according to regulatory health organizations, including US EPA, Health Canada, 2016, EFSA, WHO, and UK (NAS, 2002; Murray and Schlekat, 2004; Meacham et al., 2010; http://www.hc-sc.gc.ca/dhp-mps/pubs/natur/boron-bore-eng.php#a11). US EPA values between 0.09 and 0.15 mg/kg bw/day, or 5.4–9.0 mg/day for a 60-kg adult human are determined as a benchmark dose based on the no-observed-adverse-effect-level (NOAEL) in dogs and decreased fetal body weight in rats (Allen et al., 1996; US EPA, 2004; Hathcock, 2014). The safe upper limit for calcium is 2500 mg/day for all individuals age 1 and older (NAS, 1999, 2001).

Calcium fructoborate consists of three components: calcium, boron, and fructose (Miljkovic et al., 2009). While the limited investigations to date have not specifically identified calcium fructoborate as occurring naturally, there is a good theoretical basis to believe that this complex does exist in the human food supply (Heimbach, 2002).

As boron's mechanism of action in human health becomes better elucidated, particularly for its proposed enhancement of calcium and magnesium retention in osteoporosis prevention and treatment (Nielsen et al., 1987; Nielsen, 1990; Hunt et al., 1994, 1997), and its possible influence on steroid hormone metabolism (Lee et al., 1978; Nielsen et al., 1987; Samman et al., 1998; Miljkovic et al., 2004), it has a historically compelling physiologic role in the maintenance of joint health (Travers et al., 1990; Newnham, 1994; Gaby, 1999), reduction in the risk of prostate cancer (Zhang et al., 2001; Gallardo-Williams et al., 2004), enrichment of omega-3 fatty acids in bone health (Nielsen and Stoecker, 2009), and improvement of cognitive function (Penland, 1994, 1998). The enhanced fructo-boron-calcium complex has been clinically tested for efficacy as a cancer therapy and anti-tumor support (Scorei and Popa, 2010; Scorei, 2011), and for its antioxidant (Scorei et al., 2005) and anti-inflammatory (Scorei et al., 2011; Scorei and Rotaru, 2011; Reyes-Iquierdo et al., 2012; Militaru et al., 2013; Pietrzkowski et al., 2014; Rogoveanu et al., 2015) capabilities.

Nonetheless, despite its suggested health benefits, limited safety information is available for the calcium-boron or calcium fructoborate complex as a food ingredient. Orally administered boron is readily absorbed through the gastrointestinal tract (mechanism undefined) and is rapidly excreted by the kidney unchanged (90% recovery) in first order pharmacokinetics with a $t^{½}$ of 1 day (Jansen et al., 1984; Moseman, 1994; Samman et al., 1998). Boron is not metabolized in humans or rodents. Male dogs and male rodents appear to be sensitive to boron and high levels of exposure results in testicular atrophy, inhibition of spermatogenesis, and sterility (17.5 mg/kg bw/day in rodents) (Weir and Fisher, 1972; Lee et al., 1978; NTP, 1987; Chapin and Ku, 1994; EPA, 2004; EC, 2010). Fertility and fetal weight reductions, as well as increases in embryolethality and fetal skeletal abnormalities in rodents and rabbits with boron exposure are well documented (EFSA, 2004; EPA, 2004; EC, 2010), with a NOAEL of 9.6 mg boron/kg bw/day in the developing fetus, based on decreased fetal body weight (Price et al., 1996). Boron's carcinogenicity and reproductive effects are inconclusive and unobserved in humans (EPA, 2004; Duydu et al., 2011; Julla, 2014). Nonetheless, boron is confirmed to accumulate in bone (4.3–17.9 ppm) in humans and animals but not in soft tissues (0.02–0.75 ppm), including that of the testis or reproductive tissues (Ku et al., 1991; Treinen and Chapin, 1991; Moseman, 1994). Concentrations of boron in bone remained elevated (3-fold higher) well after plasma concentrations returned to normal (32 weeks vs. 4 days, respectively) (Chapin et al., 1997). During a preliminary preclinical pharmacokinetic study to determine the disposition of boron in plasma and urine of male rats after single-dose administration of calcium fructoborate (containing 2.8% boron) or boron citrate via oral gavage, no toxicity was noted. The highest dose of calcium fructoborate administered was 37.5 mg/kg bw, equivalent to 2250 mg calcium fructoborate (63.0 mg boron) for a 60-kg human (Vyrex, 1998).

Coordinate with recommended regulatory guidelines for Generally Recognized as Safe (GRAS) status as a dietary supplement, the safety of FruiteX-B® Brand Calcium Fructoborate (FrxB) was investigated in a subchronic toxicity study with rats and in *in vitro* genotoxicity studies. The dietary admixture levels for the oral toxicity study were selected based on the intended use of FrxB, which is anticipated to result in a daily intake of approximately 216 mg, or 3.6 mg/kg bw for a 60-kg individual. The lowest level of dietary admixture, 0.56%, was chosen to produce an intake of approximately 400 mg FrxB/kg bw/day, providing approximately a 100-fold safety factor over the anticipated human exposure. The middle and high admixture levels were chosen as 2× and 3× the low level.

2. Materials and methods

2.1. Materials

The test substance, derived from a natural food-form plant mineral carbohydrate complex of fructo-boron, was manufactured and supplied by VDF FutureCeuticals Inc., Momence, IL, and designated as FruiteX-B® Brand Calcium Fructoborate (FrxB). Analysis of the test substance according to manufacturer's specification is shown in Table 1.

FrxB, formed by the reaction of boric acid with fructose and calcium carbonate, is the calcium salt of a bis(fructose) ester of boric acid with a chemical formula of $Ca[(C_6O_6H_{10})^2B]_2$. As a naturally occurring constituent of fresh fruits and vegetables and marketed as a dietary supplement, fructoborate comprises two fructose molecules complexed to a single boron atom, with a molecular weight of approximately 367 Da. During commercial production, liquid- and solid-state 1^H, 13^C, and 11^B NMR has been employed to identify and quantify the detailed chemical structure, thermal stability, and adulteration of FrxB (Edwards et al., 2014; Penn et al., 1997).

2.2. Toxicity studies – methods

The repeated-dose subchronic feeding toxicity study work undertaken by the testing laboratory, Eurofins/Product Safety Labs (Dayton, NJ), was in accordance with the most recent Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). In support of safety, the study was performed in compliance with the OECD Guidelines for the Testing of Chemicals, Section 4, No. 408, "Repeated Dose 90-day Oral Toxicity Study in Rodents," adopted 21 September 1998, and U.S. FDA Guidelines "Toxicological Principles for the Safety Assessment of Food Ingredients" [Redbook 2000], IV.C.4a. "Subchronic Toxicity Studies with Rodents," 2003. Studies of genotoxicity were conducted in conformance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM(98)17 OECD, Paris, 1998) and U.S. FDA Good Laboratory Practices (21 CFR 58, 1987). Genotoxicity studies conducted at Bioservice Scientific Laboratories (BSL) GmbH in Planegg, Germany, were in compliance