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# Safety of calcium fructoborate as a novel food pursuant to Regulation (EU) 2015/2283

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## Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on calcium fructoborate as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF, produced by chemical synthesis, contains a maximum of 2.9% of boron and on average 4.7% calcium and 84.2% fructose. It is intended to be marketed as food supplements targeting the general adult population, excluding pregnant and lactating women, at a maximum level of 220 mg/day (maximum boron intake of 6.4 mg per day). The combined intake of boron from the background diet and the NF is in the range of 9.6–9.9 mg/day (corresponding to up to 0.14 mg/kg body weight (bw) per day given a default bw of 70 kg). This is in the range of the acceptable daily intake (ADI) of 0.16 mg/kg bw per day. Under conditions mimicking the gastrointestinal (GI) environment, the NF is fully hydrolysed and the Panel considered boron toxicity relevant for the safety assessment. The Panel considers that there is no concern with respect to genotoxicity of the NF. The effect induced by the NF in a 13-week rat study is consistent with toxicological findings induced by treatment with boron compounds in animal studies. Epididymides-to-brain weight ratio was identified as the most relevant endpoint and the reference point derived was the lowest model averaged $BMDL_{10}$ value of 529 mg/kg bw per day. This corresponds to 14.8 mg/kg bw per day of boron, which is higher than the critical no observed adverse effect level (NOAEL) (9.6 mg boron/kg bw per day) used for establishing the ADI of 0.16 mg/kg bw per day for boron. The Panel therefore applied the present ADI for boron in the assessment of the NF. The Panel concludes that the NF, calcium fructoborate, is safe under the proposed uses and use levels.



**Keywords:** Calcium fructoborate, novel foods, food supplement, boron, safety

**Requestor:** European Commission

**Question number:** EFSA-Q-2019-00240

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the requestor

On 25 March 2019, the company VDF FutureCeuticals, Inc submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise the placing on the market of Calcium Fructoborate as a novel food.

The novel food is proposed for use in food supplements intended for adult population, excluding food supplements for infants, young children, children, pregnant and lactating women.

The applicant has also requested data protection under Article 26 of the Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on Calcium Fructoborate.

The European Commission asks the European Food Safety Authority to evaluate and inform the Commission as to whether and if so, to what extent, the requirements of Article 26(2)(c) of the Regulation (EU) 2015/2283 are fulfilled in elaborating its opinion on Calcium Fructoborate regarding the proprietary data for which the applicant is requesting data protection.

# 2. Data and methodologies

## 2.1. Data

The safety assessment of this novel food (NF) is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise:

- Detailed Description of the Production Process Section 2.b.1;
- Annex C – Methods of Analysis, Excluding Thermogravimetric Analysis (TGA);
- Annex D – Certificates of Analysis;
- Annex E – Stability;
- Calcium Fructoborate – Particle Size COAs;
- Calcium Fructoborate – Particle Size MOA;
- Attachment – Response 3 – Fructoborate Analysis;
- Attachment – Response 5 – Amino Acid Analysis;
- Attachment – Response 6 – Micro Analysis;
- Attachment – Response 7 – Physiochem Stability;
- Attachment_Clarification_Response – Fructose Stability;
- Annex F – Boron Intake Report;
- Revised background dietary boron intakes response Q9;
- Annex G – Calcium Fructoborate Human Toxicokinetic Study (Unpublished Study report 2018) (sections of the dossier 2.g.3);
- Annex G – Reverse Mutation Assay using Bacteria with Fruitex®B Brand calcium fructoborate (Unpublished Study report 2015a; sections of the dossier 2.i.2);
- Annex G – In vitro Mammalian Cell Micronucleus Assay in Chinese Hamster V79 Cells with Fruitex®B Brand calcium fructoborate (Unpublished Study report 2015b; sections of the dossier 2.i.2);

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

- Annex G – Fruitex®B Brand calcium fructoborate: A 90-Day dietary study in rats (Unpublished Study report 2015c; sections of the dossier 2.i.3);
- Calcium Fructoborate dissociation study (Ca Fructoborate_Response EFSA Q_ADME_06 Apr 2021).

## 2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

# 3. Assessment

## 3.1. Introduction

The NF which is subject to the application is calcium fructoborate. The NF is produced by chemical synthesis and on average contains 2.7% boron, 4.7% calcium and 84.2% fructose.

The NF falls under the category i), i.e. food with a new or intentionally modified molecular structure, where that structure was not used as, or in, a food within the Union before 15 May 1997, as described in Article 3 of Regulation (EU) 2015/2283.

The NF is proposed to be used as an ingredient in food supplements, and the target group is the general adult population, excluding pregnant and lactating women.

## 3.2. Identity of the NF

The NF is calcium fructoborate, a calcium salt tetrahydrate of a bis(fructose) ester of boric acid in the form of a powder, represented by $Ca[(C_6H_{10}O_6)_2B]_2 \cdot 4H_2O$, with a molecular mass of 846 Da. Analytical characterisation of the NF has been performed by thermogravimetric analyses (TGA), Fourier-transform infrared (FT-IR) spectroscopy, liquid and solid state $^{1}H$, $^{13}C$ and $^{11}B$ nuclear magnetic resonance spectroscopy (NMR), in both static and cross-polarisation magic angle spinning (CP-MAS) conditions and elemental analysis for calcium and boron. Data are consistent with those reported in the literature (Dumitru et al., 2010; Rotaru et al., 2010; Edwards et al., 2014).

Synonyms, trade names and abbreviation for the NF are calcium fructoborate tetrahydrate, FruiteX-B®, Uniflex®.

According to the applicant, the NF can be described as calcium bis(β-D-fructopyranose) borate, on the basis that fructose is reported to exist in the crystalline form only as β-D-fructopyranose (Bermúdez et al., 2013). According to the literature (Edwards et al., 2014), the NF, in a commercial form (FruiteX-B®) analysed in a water solution by $^{13}C$ NMR, was shown to be mainly composed of three di-ester forms (relative molar concentration %): α-fructofuranose-B complex (48.4%), β-fructofuranose-B complex (16.5%) and β-fructopyranose-B complex (9%) with the minor constituent being the mono-ester form. Free fructose was also detected (about 25%). The presence of three types of boron-containing molecules was also observed in a water solution of the product by $^{11}B$ NMR: the di-ester (85%), the mono-ester (10%) and free boric acid (5%).

Additionally, in a recent paper published in collaboration with the applicant, Xia et al. (2017) described a liquid chromatography (LC) coupled with Q Exactive Orbitrap Mass Spectrometry method developed to identify and quantify the fructoborate ester complex NF (FruiteX-B). The authors concluded that the results were consistent with the previous NMR studies (Edwards et al., 2014) and that the α-fructofuranose-B complex was the dominant product in solution and was analogous to the natural one detected in apricots and raisins.

The NF is produced by chemical synthesis in water and the solution is freeze-dried and ground to a powder.

The Panel noted that the powder resulting from the freeze-drying process is a solid mixture with a composition similar to the one present in solution. The α-fructofuranose-B complex is the predominant species with a percentage of free fructose and boric acid intimately mixed with the complex (no

crystalline borate or boric acid), in agreement with what was observed by Edwards et al. (2014) by solid-state $^{13}C$ and $^{11}B$ NMR.

Following an EFSA request, the applicant agreed that the NF is better represented by the chemical name of the main component: Borate (1-), bis(α-D-fructofuranosato(2)-kappa.O2,kappa.O3)-, calcium, hydrate (2:1:4), (T-4)-. The CAS-No 2247559-29-9 is available only for the β-D-fructofuranose-B isomer. Possible structures for the α- and β-D-fructofuranose-B-complexes are reported in Figure 1.

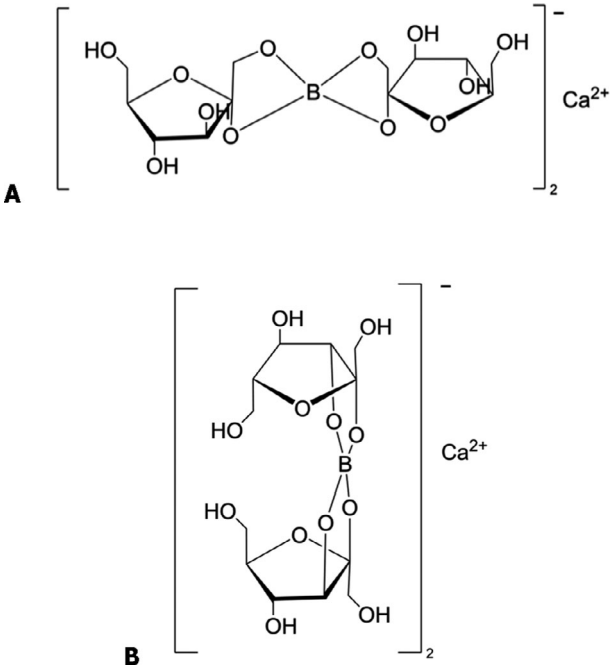


**Figure 1:** Possible chemical structures of calcium α-D-fructofuranose borate (A) and calcium β-D-fructofuranose borate (B) di-esters

## 3.3. Production process

The applicant's facility is certified to meet requirements of the BRC Global Standard for Food Safety, Issue 7: January 2015. This will ensure that the good manufacturing practices are followed and that a Hazard Analysis and Critical Control Point programme is in place and followed (BRC, 2018).

The NF is produced by chemical synthesis whereby fructose is combined with boric acid in water to produce a bis(fructose) ester of boric acid through various heating and mixing processes. Calcium carbonate is then added to produce a solution containing the calcium salt of fructoborate (tetrahydrate). The solution is freeze-dried, ground to produce the final powdered product, and then packaged and stored under representative storage conditions (22 ± 1°C RH 55–60%).

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

## 3.4. Compositional data

The NF consists of calcium fructoborate tetrahydrate.

In order to confirm that the manufacturing process is consistent and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data on chemical and microbiological parameters for a number of different batches of the NF. For each parameter three or four batches were analysed (Table 1).

**Table 1:** Batch-to-batch analysis of NF.

| Parameter (unit) | Batch number | | | | | | | | | | Method of analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| Appearance | White powder | White powder | White powder | White powder | – | – | – | – | – | – | Visual |
| Identification | Conform | Conform | Conform | Conform | – | – | – | – | – | – | FT-IR Spectroscopy |
| Calcium (%) | 4.7 | 4.6 | 4.7 | 4.7 | 5.0 | 4.7 | 4.8 | – | – | – | AOAC 985.01 |
| Boron (%) | 2.7 | 2.5 | 2.6 | 2.6 | 3.1 | 2.7 | 2.7 | – | – | – | AOAC 985.01 |
| Fructose (%) | 83.02 | 84.02 | 81.20 | 81.91 | – | – | – | – | – | – | AOAC 988.12 |
| Total water (%) | 11.6 | 10.2 | 9.5 | 10.3 | – | – | – | – | – | – | Thermogravimetric Analysis |
| Free moisture (%) | 3.51 | 4.12 | 3.99 | 4.07 | – | – | – | – | – | – | AOAC 925.09 and 926.08 |
| Ash (%) | 15.5 | 15.3 | 15.4 | 15.3 | – | – | – | – | – | – | |
| **Contaminants** | | | | | | | | | | | |
| Arsenic (mg/kg) | – | – | – | – | 0.0787 | 0.0736 | 0.0685 | – | – | – | AOAC 993.14M |
| Cadmium (mg/kg) | – | – | – | – | < 0.01 | < 0.01 | < 0.01 | – | – | – | AOAC 993.14M |
| Lead (mg/kg) | – | – | – | – | 0.00696 | 0.00634 | 0.00988 | – | – | – | AOAC 993.14M |
| Mercury (mg/kg) | – | – | – | – | < 0.01 | < 0.01 | < 0.01 | – | – | – | AOAC 993.14M |
| **Microbial** | | | | | | | | | | | |
| Total plate count (CFU/g) | – | – | – | – | – | – | – | < 1,000 | < 1,000 | < 1,000 | AOAC 990.12 |
| Yeast and mould (CFU/g) | – | – | – | – | – | – | – | < 100 | < 100 | < 100 | AOAC 997.02 |
| Coliforms(CFU/g)[9] | – | – | – | – | – | – | – | < 10 | < 10 | < 10 | AOAC 991.14 |
| *Escherichia coli* (CFU/g)[9] | – | – | – | – | – | – | – | < 10 | < 10 | < 10 | AOAC 991.14 |
| *Salmonella* (in 375 g)[10] | – | – | – | – | – | – | – | n.d | n.d. | n.d. | Modified FDA BAM |
| Coagulase-positive *Staphylococcus* (in 1 g)[10] | – | – | – | – | – | – | – | n.d | n.d. | n.d. | Modified FDA BAM |

AOAC: Association of Official Analytical Chemists; BAM: Bacteriological Analytical Manual; CFU: colony forming units FDA: Food and Drug Administration.

The Panel notes that for Batch 5 boron content is above the percentage proposed in specifications. The applicant clarified that batch 5 was produced in 2014 and that batches 1, 2, 3 and 4, produced in 2018, better represent the boron content since during these years the applicant evolved and refined test methods and tightened boron limits of the NF.

Proteins were quantified by the Dumas method to be in the range of 0.22–0.41% and were attributed by the applicant to nitrogen impurities in water utilised in the manufacturing process. Fat was measured using acid hydrolysis and extraction of the NF in ether and was detected in the range of 0.3–0.6%. However, the applicant excludes the presence of fat and ascribes these results to lack of specificity of the method.

The applicant did not provide the actual values of the microbiological parameters but stated that the microbiological values of all of the analysed samples do not exceed the given specification limits.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

### 3.4.1. Stability

The applicant performed tests to determine the physicochemical stability of the NF on 3 batches stored at 22 (± 1°C) and under dry (55–60% RH) conditions. Data reported a stability up to 42 months.

The applicant analysed the NF by solid-state $^{13}C$ and $^{11}B$ NMR. The resulting spectra remained unchanged after storage, indicating that the NF was stable when stored under standard conditions for up to 57 months. Total fructose was analysed in nine batches (three 7 months old, three 3 years old

and three 5 years old). The fructose content was between 81 and 85% in all batches. The results show that the composition of the NF is stable when stored under warehouse conditions (22 ± 1°C, RH 55–60%).

Stability of the NF complex was tested also for one batch under accelerated conditions (50°C for up to 6 h or at 70°C for 18 h). No changes of the solid-state NF were detected by $^{13}$C-and $^{11}$B-NMR.

The applicant also provided information on the microbiological stability of the NF after 42 months under ambient conditions defined as temperature of 22 ± 1°C and in dry environment (55–60%) in three batches. No biological contaminants of concern have been detected.

The Panel considers that the data provided sufficient information with respect to the stability of the NF and considers the recommended shelf-life of 36 months (unopened under cool and dry conditions) proposed by the applicant adequate.

## 3.5. Specifications

The specifications of the NF are indicated in Table 2.

**Table 2:** Specifications of the NF

| Parameters | Specification |
|---|---|
| **Identity, purity and composition** | |
| Appearance | White powder |
| Free moisture | < 5.0% |
| Calcium | ≥ 4.5% |
| Boron | 2.5–2.9% |
| Fructose | > 80% |
| **Heavy metals** | |
| Arsenic | ≤ 1 mg/kg |
| **Microbiological parameters** | |
| Total plate count | ≤ 1,000 CFU/g |
| Yeast and mould | ≤ 100 CFU/g |
| Coliforms | ≤ 10 CFU/g |
| *Escherichia coli* | < 10 CFU/g |
| *Salmonella* | Not detected in 25 g |
| Coagulase-positive *Staphylococcus* | Not detected in 1 g |

CFU: colony forming units.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

## 3.6. History of use of the NF and/or of its source

Fructoborates are naturally occurring in fruits, vegetables, certain nuts and legumes, and are naturally absorbed by animal cells (Mogoşanu et al., 2016).

Boric acid is a permitted form of boron for use in the manufacture of food supplements as set forth in Commission Regulation (EC) No 1170/2009.

Calcium carbonate is a permitted form of calcium for use in the manufacture of food supplements as set forth in Regulation (EC) No 1925/2006.

Crystalline fructose is used as a nutritive sweetener in foods and beverages.

The applicant states that the NF has been marketed and consumed as food supplement (216 mg/day) in U.S. and Canada for more than 17 years.

## 3.7. Proposed uses and use levels and anticipated intake

### 3.7.1. Target population

The target population proposed by the applicant is the general adult population. Pregnant and lactating women, infants, children or adolescents are excluded considering that few data are available regarding the safety of NF in these population groups.

### 3.7.2. Proposed uses and use levels

The applicant intends to market the NF for use in food supplements, at a maximum dose of 220 mg/day, which corresponds to 3.1 mg/kg bw per day for adults.[3]

The NF is not intended to replace other foods in the diet.

Under the condition of use, and considering that typically the NF contains 2.7% boron (with a maximum of 2.9%), 4.7% calcium and 84.2% fructose, the NF at a maximum dose of 220 mg/day will provide an average intake of 6 mg boron (maximum intake 6.4 mg boron), 10 mg calcium and 185 mg fructose per day.

### 3.7.3. Combined intake from the NF and other sources (only the max value of exposure)

According to the scientific opinion on the tolerable upper intake level (UL) for boron (EFSA NDA Panel, 2004), data on dietary intake of boron were limited since, at the time the opinion was adopted, it was not included in the food composition databases used for national dietary surveys. The background intake of boron was estimated from analysis of samples from the 1994 Total Diet Study using a consumption data base from the 1986/87 Dietary and Nutritional Survey of British Adults (MAFF, 1997) and resulted in a mean intake from food of 1.5 mg/day and a 97.5 percentile of 2.6 mg/day and a mean intake from water of 0.2–0.6 mg/day.

The estimated combined intake levels from the background diet (from food and water) and 6.4 mg boron from the NF food supplements correspond to a maximum of 9.6 mg boron/day.

It should be noted that the applicant provided an estimation of the background dietary exposure of boron considering more recent consumption data than in the UL opinion by EFSA, using mean/median occurrence values of boron from the literature, and including alcoholic beverages (contrary to the approach of MAFF (1997), cited in the UL in EFSA NDA Panel (2004 opinion). This estimation resulted in a 95th percentile intake of 3.47 mg/day in adults from the background diet (from food and beverages). Using the background exposure estimated by the applicant and the maximum intake of boron from the NF the combined exposure to boron corresponds to 9.87 mg/day.

### 3.7.4. Estimate of exposure to undesirable substances

The applicant claims that no undesirable substances are present in the NF.

## 3.8. Absorption, distribution, metabolism and excretion (ADME)

The NF is comprised of calcium, fructose and boron and it is expected that under gastrointestinal conditions, the low pH in the stomach and/or non-specific enzymes may catalyse the hydrolysis of the ester bonds in the fructofuranose borate to form fructose and boric acid which are readily absorbed.

The applicant provided evidence of dissociation of the NF in the gastrointestinal (GI) system by submitting additional $^{11}$B-NMR data. The results show that calcium fructoborate, starting from pH 6.86 is gradually releasing boric acid and the dissociation is complete at pH 4.

The applicant provided a human single dose study (Unpublished study report, 2018). Five human subjects (1 female and 4 males) received 216 mg of the NF. The total amount of boron measured in blood serum at baseline and after 60, 120 and 180 min from the administration was 56, 160, 140 and 127 ng/mL, respectively. EFSA notes that sampling earlier than 60 min and data on excretion were not provided.

Considering that the boron present in the NF is systemically available and given the NF is composed on average of 2.7% boron, 4.7% calcium and 84.2% fructose, the applicant concluded that calcium and fructose of the NF will also be systemically available and provided information on ADME for each component individually.

Regarding ADME of calcium and fructose the applicant referred to the respective assessments from the NDA Panel (EFSA NDA Panel, 2010, 2015).

The ADME of boron is well known and reviewed in several scientific publications (WHO, 1998; Devirian and Volpe, 2003; EVM, 2003; EFSA NDA Panel, 2004; EPA, 2004; EFSA ANS Panel, 2013). "*. . .Boron as borate is readily, and almost completely absorbed (> 90%) from the human gut. The mechanism has not been defined. Essentially, 100% of boron ingested in the range 0.4-3 mg/day is excreted in faeces and urine and there is no evidence of boron accumulation (Hunt et al., 1997).*

[3] Based on default body weight of 70 Kg for adults (EFSA Scientific committee, 2012).

*Supplementation with 10 mg of boron/day resulted in the recovery of 84% of the dose in the urine (Samman et al., 1998). . . .The primary route of elimination is by glomerular filtration (Dourson et al., 1998; Murray, 1998) and > 90% of the administered dose is excreted via urine, regardless of the route of exposure or administration. The 3- to 4- fold higher clearance in rats compared to humans arises from the higher glomerular filtration rate in rats. In humans, excretion is relatively rapid, with a half-life of elimination of 24 h or less (Nielsen, 1986, 1988; Litovitz et al., 1988)*" (EFSA NDA Panel, 2004).

Boron as boric acid has been shown to be evenly distributed in the body fluids via passive diffusion. It has been reported that borates are not metabolised by biological systems since the boron-oxygen bound is very stable (Emsley, 1989). Boron after oral exposure is rapidly distributed in all tissues. Levels of boron are similar to plasma levels in soft tissues, with the exception of adipose tissue for which the levels are lower. The highest levels of boron are found in bone, "which possibly represents a second kinetic compartment, as elimination kinetics for bone differ from those of soft tissue and body fluids" (EFSA ANS Panel, 2013).

## 3.9. Nutritional information

The NF consists of calcium, fructose and boron, which are naturally occurring in foods. The contribution of the NF for adults at the proposed level of use for calcium (10 mg/day) is small in comparison to intake with background diet (average and P95 intakes in adults in European countries have been estimated to range between 690 and 1,122 and 1,151 and 2,188 mg per day, respectively) (EFSA NDA Panel, 2015), DRVs (set at 1,000 mg/day for individuals aged 18–24 years and at 950 mg/day for those aged 25 years and above) (EFSA NDA Panel, 2015) or the UL (2,500 mg/day for adults) (EFSA NDA Panel, 2012). Similarly, the contribution of fructose (185 mg/day) is negligible when compared to observed intakes from background diet (mean intake of 16 g/day of free fructose, and if fructose is added in sucrose, up to 150 g/day in high consumers) (EFSA NDA Panel, 2005).

The essentiality of boron has not been established. On the other hand, based on adverse effects observed in rats, a UL of 0.16 mg/kg bw per day, corresponding to a UL of 10 mg per day in adults considering a default bw at that time of 60 kg, was established by EFSA (NDA Panel, 2004). An acceptable daily intake (ADI) for boron of 0.16 mg/kg bw per day was also established (EFSA ANS Panel, 2013). Taking into account the present default bw for adults of 70 kg (EFSA Scientific Committee, 2012), the acceptable daily intake for boron amounts to 11.2 mg boron/day. The highest estimated P95th intake of boron from background diet in adults was 3.47 mg/day. A high intake of boron from the background diet added to the intake via the NF results in boron exposure in the range of 9.6–9.9 mg/day as estimated by EFSA and the applicant, respectively (see 3.7.3). This is in the range of, but not exceeding, the UL of 11.2 mg boron/day.

The NF is not intended to replace any other foods and does not contain any antinutritional factors.

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

## 3.10. Toxicological information

The list of toxicological studies, which were provided and claimed proprietary by the applicant, is reported in Table 3. All the studies have also been published in 2016 (Marone et al., 2016). The studies were conducted with the NF.

**Table 3:** Toxicological studies submitted by the applicant

| Reference | Type of study | Test system | Dose |
|---|---|---|---|
| Unpublished study report (2015a) | Bacterial reverse mutation test (GLP, OECD TG 471) | *Salmonella* Typhimurium TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA | Up to 5,000 μg/plate (absence and presence of S9 mix) |
| Unpublished study report (2015b) | *In vitro* mammalian cell micronucleus test (GLP, OECD TG 487) | Chinese Hamster V79 | 2,000 μg/mL (absence and presence of metabolic activation) |
| Unpublished study report (2015c) | 90-day repeated dose oral dietary toxicity study (OECD TG 408, limit test) | CD® IGS® Sprague–Dawley rats | Dietary levels of 400, 800 and 1,200 mg/kg bw per day |

GLP: Good Laboratory Practice; OECD: Organisation for Economic Co-operation and Development; bw: body weight.

### 3.10.1. Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test (unpublished study report, 2015a; Marone et al., 2016) and an *in vitro* mammalian micronucleus test (unpublished study report, 2015b; Marone et al., 2016). These studies were conducted in compliance with the Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998) and in accordance with the OECD test guidelines No 471 and 487, respectively.

The bacterial reverse mutation test was performed with the NF with histidine-dependent auxotrophic mutants of *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and a tryptophan-dependent mutant of *Escherichia coli* WP2 uvrA. A preliminary experiment followed by two main experiments was conducted. Based on the findings of the preliminary test two experiments were performed using the following doses: 31.6, 100, 316, 1,000, 2,500 and 5,000 μg/plate for the five strains in presence and absence of S9. The first experiment was performed according to the plate incorporation method, while the second was performed according to the pre-incubation method. No precipitation of test material and no cytotoxicity were detected in all the test conditions. The NF did not induce a biologically relevant increase in the mean of revertant colonies compared to the negative control (solvent) in all strains tested, both in the absence and presence of metabolic activation.

The NF was also evaluated for its potential to induce micronuclei (MN) in Chinese Hamster V79 cells. A main experiment was performed in duplicate and the concentrations were selected on the basis of a preliminary cytotoxicity test where toxicity was not detected while precipitation was observed starting from 1,000 μg/mL in presence of S9. The cells were incubated with the NF at concentrations up to 2,000 μg/mL for either 4 or 24 h in the absence of metabolic activation and at concentrations up to 1,000 μg/mL for 4 h in the presence of metabolic activation. No precipitation of the text material was observed and cytotoxicity of 30% was detected at the highest doses. The NF did not induce a statistically significant increase in the micronucleated cell frequency in Chinese Hamster V79 cells.

Based on the results of these studies, the Panel considers that there are no concerns regarding genotoxicity of the NF.

### 3.10.2. Subchronic toxicity

The applicant has provided a subchronic 90-day toxicity study in CD® IGS® Sprague–Dawley rats compliant with OECD guidance 408 (unpublished report 2015c; Marone et al., 2016). The study was not performed in full compliance with GLP standards in a GLP-compliant facility. Animals were treated with the NF via diet and dietary concentrations were adjusted to achieve target doses of 0, 400, 800 and 1,200 mg/kg bw per day. Based on the mean overall daily dietary intake (Days 0-91), this corresponded to a NF intake of 385.8, 774.9 and 1,161.3 mg/kg bw per day for male rats and to 392.1, 784.4 and 1,171.1 mg/kg bw per day for female rats. Based on the test substance content of 2.8% boron the values corresponded to 10.8, 21.7 and 32.4 mg boron/kg bw per day in male and 11, 22 and 33 mg boron/kg bw per day in female rats.

No treatment-related effects were observed during ophthalmoscopic examination, clinical observation, functional observational battery (FOB) and motor activity. Body weight, body weight gain, food consumption and food efficiency were not affected by the treatment.

The changes observed in haematological parameters were: decrease of erythrocytes count (RBC) and haematocrit (HCT) in male mid and high dose, decreased haemoglobin concentration (HGB) in male high dose, decreased mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in females at high dose, decrease in absolute eosinophils (AEOS) in females at mid and high doses, increase in absolute reticulocytes (ARET) in males at high dose. These effects were dose-dependent and treatment-related but individual effects were observed only in one sex. A non-dose-dependent increase in prothrombin time was observed in females at all doses as well as a decrease in activated partial thromboplastin time in females at mid dose.

Clinical chemistry analysis showed a decrease of sorbitol dehydrogenase (SDH) in males at mid and high doses and in females at high dose. A dose-related decrease was observed for cholesterol in both males and females while decrease of serum alanine aminotransferase (ALT), triglycerides (TRIG) and potassium (K) was observed in males at high dose. These changes were considered treatment related.

Urinalysis changed statistically significantly only in males. At mid and high doses urinary volume was increased but not in a dose-dependent manner, and specific gravity (SG) and urobilinogen (URO) were decreased while pH was increased at high dose.

In males, the liver-to-body weight and liver-to-brain weight ratios were significantly decreased in the middle dose group but not at the highest dose. The epididymides-to-brain weight ratio showed a dose-related decrease and was significantly decreased (–12.0%) in the highest dose group.

In female rats, the adrenal-to-body weight ratio increased with dose and was significantly increased (+25.1%) in the high dose group. Also, the uterus-oviducts-to-body weight (58.5%) and uterus-to-brain weight (+45.6%) ratios were significantly higher in the high dose group, but dose-responses were less evident.

Gross pathology revealed a dose-dependent increase in number of uteri presented as fluid filled (control 2/10, low dose 4/10, mid dose 5/10 and high dose 8/10). Microscopically these findings corresponded to luminar dilatation of the uteri and oviducts secondary to the oestrus phase of the oestrus cycle.

A single incidence of unilateral renal tubular carcinoma was observed in the left kidney of one male rat in the high dose group. This was not considered to be treatment-related since no other neoplastic or pre-neoplastic changes were observed in any animal.

The effects induced by the NF are consistent with toxicological findings induced in studies with boric acid and disodium tetraborate in mice, rats, pigs and dogs, and effect levels related to boron are comparable with those of the NF (EFSA NDA Panel 2004; EFSA ANS Panel 2013).

The Panel selected the epididymides-to-brain weight ratio as the most relevant endpoint for benchmark dose modelling (Annex A). A benchmark response (BMR) of 10% was chosen due to the variability in the control animals. The model averaged $BMDL_{10}$ value for decreased epididymides-to-brain weight ratio was 529 mg/kg bw per day, which corresponds to 14.8 mg/kg bw per day of boron.

### 3.10.3. Reproductive and developmental toxicity

The Panel notes that no studies on reproductive and developmental toxicity were provided by the applicant.

The adverse effects of boron on reproduction and development are exhaustively discussed in the EFSA Opinion related to the UL of boron (EFSA NDA Panel, 2004) and the subsequent Scientific Opinion on the re-evaluation of boric acid (E 284) and sodium tetraborate (borax) (E 285) as food additives (EFSA ANS Panel, 2013). The no observed adverse effect level (NOAEL) for reproductive toxicity in rats was reported as 17.5 mg boron/kg bw per day (Weir and Fisher, 1972; EFSA NDA Panel, 2004).

Boric acid is also a well-described teratogen, consistently producing visceral and skeletal malformations as well as decreases in foetal body weight across species. For developmental toxicity in rats, a NOAEL of 9.6 mg/kg bw per day expressed as boron was identified by Price et al. (1996). This study was pivotal in the establishment of both the UL for boron and the group ADI for boric acid and sodium tetraborate (EFSA ANS Panel, 2013).

### 3.10.4. Human data

Human studies have been performed using the NF under the trade name FruiteX B® (Table 4). All these studies have been designed to evaluate efficacy endpoints, however, when safety parameters have been measured, they remained in the normal range. No adverse events have been reported.

## 3.11. Allergenicity

Considering the food production process and that, if present proteins would be present only in trace amounts in the NF, the Panel considers that the risk of allergic reactions to the NF is low.

**Table 4:** Summary of human studies

| Reference | Study design | Study population | Duration of study | Doses route of administration | Safety-related parameters investigated |
|---|---|---|---|---|---|
| Scorei et al. (2011) | Randomised double-blind, placebo-controlled study | 60 participants (aged 59–68 years, 43 females, 17 males) with knee primary osteoarthritis 15 per group | 15 days | 0, 57, 113, or 226 mg per day, in 2 divided doses (2 × 1 capsule) | Lipid (triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol) and inflammation markers (C-reactive protein, erythrocyte sedimentation rate, fibrinogen) were assessed in venous blood of fasted subjects at study begin and after 15 days of treatment (efficacy parameters).<br>No adverse effects on blood lipids or inflammation markers<br>No adverse events reported. |
| Reyes-Izquierdo et al. (2012) | Double-blind, placebo-controlled, parallel study | 20 participants (aged > 44–< 65 years, mean BMI of 28 kg/m$^2$) with minor knee osteoarthritis 10 (4 males and 6 females) per group | 14 days | 0 or 216 mg per day, in 2 divided doses (2 × 1) capsule) | Laboratory chemistry in blood of fasted subjects (prior treatment and at day 7 and 14): serum glucose, blood urea nitrogen, creatine, AST, ALT, LDH, GGTP, total bilirubin, alkaline phosphatase, total proteins, albumin, globulin, uric acid, calcium, phosphorus, iron, sodium, potassium, chlorine, $CO_2$, triglyceride, total cholesterol, HDL and LDL.<br>Blood chemistry remained within normal range in both groups.<br>No adverse events reported. |
| Militaru et al. (2013) | Randomised, double-blind, placebo-controlled, parallel study | 116 subjects (mean age of 65 years; BMI 24–27 kg/m$^2$, 71 males and 45 females) with stable angina pectoris 29 per group | 60 days | Placebo: no intervention<br>Group 1: 112 mg calcium fructoborate per day (as one capsule)<br>Group 2: 112 mg calcium fructoborate and 20 mg resveratrol per day (as one capsule)<br>Group 3: 20 mg resveratrol per day (as one capsule) | Inflammation biomarkers (hs-CRP), left ventricular function markers (N-terminal prohormone of brain natriuretic peptide) and lipid markers (total cholesterol, LDL cholesterol, HDL-cholesterol, triacylglycerols). Statistically significantly decreased hs-CRP, total cholesterol, LDL cholesterol and triacylglycerol; increased HDL cholesterol compared to baseline (all treatment groups; parameters measured in blood after 30 and 60 days of administration). |

| Reference | Study design | Study population | Duration of study | Doses route of administration | Safety-related parameters investigated |
|---|---|---|---|---|---|
| Reyes-Izquierdo et al. (2014) | Randomised, double-blind, placebo-controlled, parallel study | 92 healthy adults (mean age 49.2 years, mean BMI 26.6 kg/m$^2$) with self-reported knee discomfort completed the study<br>30–32 (initially 16 males and 16 females) per group | 14 days | Placebo: 160 mg fructose and 30 mg silica oxide per day<br>Group 1: 220 mg calcium fructoborate, 1,500 mg glucosamine and 400 mg chondroitin sulfate per day<br>Group 2: 1,500 mg glucosamine and 400 mg chondroitin sulfate per day in 2 divided doses (2 × 2 capsules, placebo 2 × 1 capsule) | Laboratory chemistry in blood of fasted subjects (prior treatment and at day 7 and 14): key electrolytes, enzymes, lipids and glucose.<br>No significant changes in blood chemistry parameters and no indication of unusual effects in any group. |
| Pietrzkowski et al. (2014) | Randomised, double-blind, placebo-controlled, parallel study | 60 healthy adults (aged 35–65 years, mean BMI of 26.7 kg/m$^2$) with self-reported knee discomfort 30 (15 males and 15 females) per group | 14 days | 0 or 220 mg per day, in 2 divided doses (2 × 1 capsule) | No treatment-related adverse events were reported |
| Rogoveanu et al. (2015) | Randomised, double-blind, placebo-controlled, parallel study | 78 healthy adults (aged 40 to 60 years, BMI 24–27 kg/m$^2$)<br>26 (10–13 males and 13–16 females) per group | 30 days | Placebo: 80 mg fructose divided in 2 doses.<br>Treatment with 56 or 112 mg calcium fructoborate per day, divided in 2 doses (2 × 1 capsule) | Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, hs-CRP, homocysteine, IL-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1)<br>Total cholesterol, LDL cholesterol, triglycerides, Il-6 and MCP-1 were statistically significantly reduced in both calcium fructoborate groups compared to placebo at study end;<br>Hs-CRP and IL-1β were statistically significantly lower in high dose group versus placebo; HDL cholesterol was statistically significantly increased in low dose group versus placebo. No adverse effects were reported. |

AST: aspartate aminotransferase; BMI: body mass index; GGTP: gamma-glutamyl transpeptidase; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; IL-1β: interleukin 1 beta; IL-6: interleukin 6; LDL: low-density lipoprotein; LDH: lactate dehydrogenase; MCP-1: monocyte chemoattractant protein-1.

# 4. Discussion

The NF which is the subject of the application is calcium fructoborate (containing boron ranging from 2.5% to 2.9%, 4.7% calcium and 84.2% fructose).

The NF is proposed for use in food supplements for the adult population, excluding pregnant and lactating women. The highest intake of the NF is 220 mg/day which corresponds (considering a body weight of 70 kg) to 3.14 mg/kg bw per day for adults. Under the condition of use, the NF will provide a maximum intake of 6.4 mg boron, 10 mg calcium and 185 mg fructose per day (corresponding to 0.0914 mg/kg bw per day of boron, 0.14 mg/kg bw per day of calcium and 2.64 mg/kg bw per day of fructose).

The information provided on composition, specifications, production process and stability of the NF does not raise safety concerns.

ADME data show that the NF dissociates in the GI tract into calcium, fructose and boron. These components are readily absorbed.

The Panel notes that essentiality of boron has not been established. The combined intake of boron from the NF and the background diet ranges from 9.6 to 9.9 mg/day (corresponding to 0.14 mg/kg bw per day) and may be close to the UL of 11.2 mg boron/day and ADI of 0.16 mg/kg bw per day for boron. The Panel considers that the consumption of the NF is not nutritionally disadvantageous under the proposed conditions of use.

The Panel considers that there is no concern with respect to genotoxicity of the NF.

The applicant provided a 90-day study where several changes related to haematology and urinalysis parameters and effects on weight of epididymides, adrenals and uterus-oviducts were detected. The Panel considered the effects and effect levels based on boron content to be consistent with those of other boron compounds. The most sensitive endpoint in the 90-day study was analysed with the BMD approach using model averaging, and a $BMDL_{10}$ of 529 mg/kg bw per day was derived for epididymides-to-brain ratio. The calculated $BMDL_{10}$ from the most relevant endpoint in the 90-day study with the NF corresponds to 14.8 mg/kg bw per day of boron, which is higher than the critical NOAEL of 9.6 mg/kg bw per day for developmental toxicity of boron used by NDA and ANS Panel for establishing the UL of boron of 11.2 mg per day and the ADI of 0.16 mg/kg bw per day (11.2 mg per day at a bw of 70 kg) (EFSA NDA Panel, 2004; EFSA ANS Panel, 2013).

Taking into account that the 90-day study conducted with the NF provided a higher reference point than the critical NOAEL that has been used to derive the ADI for boron, and that the NF is hydrolysed completely in the GI tract, the Panel applied the ADI for boron also in the assessment of the NF.

Considering that the combined intake of boron from the NF and the background diet (0.14 mg/kg bw per day) does not exceed the ADI of 0.16 mg/kg bw per day, the intake of the NF of 220 mg/day in adults, containing up to 6.4 mg boron, does not raise safety concerns.

# 5. Conclusions

The Panel concludes that the NF, calcium fructoborate, is safe for the adult population, excluding pregnant and lactating women, at intake levels up to 220 mg/day (3.14 mg/kg bw per day).

## 5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant:

- Annexes to the dossier which relate to the Risk Assessment of the identity, the manufacturing process, composition, stability and analytical methods (see Section 2.1).
- Genotoxicity studies: Bacterial Reverse Mutation Test; In Vitro Mammalian Cell Micronucleus Test in Chinese Hamster Ovary Cells.
- Human Toxicokinetic Study, Calcium Fructoborate dissociation study and Subchronic Toxicity 90-Day Feeding Study in Rats.

## 6. Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of calcium fructoborate (Ares(2019)4412347 - 10/07/2019; amendments of the letter (Ares(2020)2401290 - 06/05/2020 and Ref. Ares (2020)7347734 - 04/12/2020)).
2) On 10/07/2019, a valid application on calcium fructoborate, which was submitted by VDF FutureCeuticals, Inc., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/0998) and the scientific evaluation procedure was initiated.
3) On 19/12/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 05/08/2020, 08/02/2021, 29/03/2021 and 19/04/2021, EFSA requested the applicant to provide clarifications on the information provided.
5) On 17/07/2020, 19/01/2021, 12/03/2021, 06/04/2021 and 27/04/2021 additional information were provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
6) During its meeting on 25/05/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of Calcium Fructoborate as a novel food pursuant to Regulation (EU) 2015/2283.

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## Abbreviations

| | |
|---|---|
| ADI | acceptable daily intake acceptable daily intake |
| ADME | absorption, distribution, metabolism and excretion |
| AEOS | absolute eosinophils |
| ALT | alanine aminotransferase |
| ANS | Panel on Food Additives and Nutrient Sources added to Food |
| AOAC | Association of Official Analytical Chemists |
| ARET | absolute reticulocytes |
| AST | aspartate aminotransferase |
| BAM | Bacteriological Analytical Manual |
| BMD | benchmark dose |
| BMDL | lower confidence limit of the benchmark dose |
| BMDU | upper confidence limit of the benchmark dose |
| BMI | body mass index |
| BMR | benchmark response |
| BRC | British Retail Consortium |
| bw | body weight |
| CFU | colony forming units |
| CP-MAS | cross-polarisation magic angle spinning |
| DRVs | dietary reference values |
| FDA | Food and Drug Administration |
| FOB | functional observational battery |
| FT-IR | Fourier-transform infrared spectroscopy |
| GGTP | gamma-glutamyl transpeptidase |
| GI | gastrointestinal |
| GLP | Good Laboratory Practice |
| HCT | haematocrit |
| HDL | high-density lipoprotein |
| HGB | haemoglobin |
| Hs-CRP | high-sensitivity C-reactive protein |
| IL-1β | interleukin 1 beta |
| IL-6 | interleukin 6 |
| LC | liquid chromatography |
| LDL | low-density lipoprotein |
| MAFF | Ministry of Agriculture, Fisheries and Food |
| MCH | mean corpuscular haemoglobin |
| MCP-1 | monocyte chemoattractant protein-1 |
| MCV | mean corpuscular volume |
| MN | micronuclei |
| NDA | Panel on Nutrition Novel Foods and Food allergens |
| NF | novel food |
| NMR | nuclear magnetic resonance spectroscopy |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| RH | relative humidity |
| RBC | erythrocytes count |
| SDH | sorbitol dehydrogenase |
| SG | specific gravity |
| TG | Test Guidelines |
| TGA | thermogravimetric analysis |
| TRIG | triglycerides |

| | |
|---|---|
| UL | Tolerable Upper Intake Level |
| URO | urobilinogen |
| US | United States |
| WHO | World Health Organization |

# Annex A – Benchmark dose modelling report

## Benchmark dose modelling of continuous data

### Data Description

The endpoint analysed was epididymides to brain weight ratio. Summary data for the endpoint are given in Table A.1. The BMD analysis was performed using mean data.

**Table A.1:** Summary data of epididymides to brain weight ratio (Unpublished study report, 2015c)

| Dose | Mean | SD | n | Sex |
|---|---|---|---|---|
| 0 | 0.6597 | 0.0505 | 10 | M |
| 385.8 | 0.6499 | 0.0487 | 10 | M |
| 774.9 | 0.6197 | 0.0587 | 10 | M |
| 1161.3 | 0.5805 | 0.0821 | 10 | M |

SD: standard deviation; n: number of animals.

### Software used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 69.0, for the underlying calculations.

### Selection of the BMR

The BMR (benchmark response) used is a 10% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported as BMDL and the upper bound as BMDU.

## Results

### Fitted Models

**Table A.2:** List of fitted BMD models

| Model | Converged | Loglik | npar | AIC |
|---|---|---|---|---|
| **Full model** | Yes | 37.36 | 5 | −64.72 |
| **Null model** | Yes | 32.01 | 2 | −60.02 |
| **Expon. m3-** | Yes | 37.36 | 4 | −66.72 |
| **Expon. m5-** | Yes | 37.36 | 5 | −64.72 |
| **Hill m3-** | Yes | 37.36 | 4 | −66.72 |
| **Hill m5-** | Yes | 37.36 | 5 | −64.72 |
| **Inv.Expon. m3-** | Yes | 37.36 | 4 | −66.72 |
| **Inv.Expon. m5-** | Yes | 37.36 | 5 | −64.72 |
| **LN m3-** | Yes | 37.36 | 4 | −66.72 |
| **LN m5-** | Yes | 37.36 | 5 | −64.72 |

AIC: Akaike information criterion; Loglik: log likelihood; npar: number of parameters.

### Estimated Model Parameters

**Table A.3:** Estimated model parameters for the BMD models

| Model | EXP | HILL | INVEXP | LOGN |
|---|---|---|---|---|
| **Estimate for var-** | 0.009042 | 0.009042 | 0.009041 | 0.009041 |
| **Estimate for a-** | 0.6583 | 0.6583 | 0.6576 | 0.6579 |
| **Estimate for CED-** | 1013 | 1013 | 1010 | 1012 |
| **Estimate for d-** | 1.877 | 1.879 | 0.3003 | 0.5901 |

### Weights for Model Averaging

**Table A.4:** Estimated model parameters for the BMD models

| EXP | HILL | INVEXP | LOGN |
|---|---|---|---|
| 0.25 | 0.25 | 0.25 | 0.25 |

### Final BMD Values

**Table A.5:** Final BMD values for epididymides to brain weight ratio

| Endpoints | Subgroup | BMDL | BMDU |
|---|---|---|---|
| Mean | All | 529 | 1400 |

Confidence intervals for the BMD are based on 200 bootstrap data sets.

## Visualisation

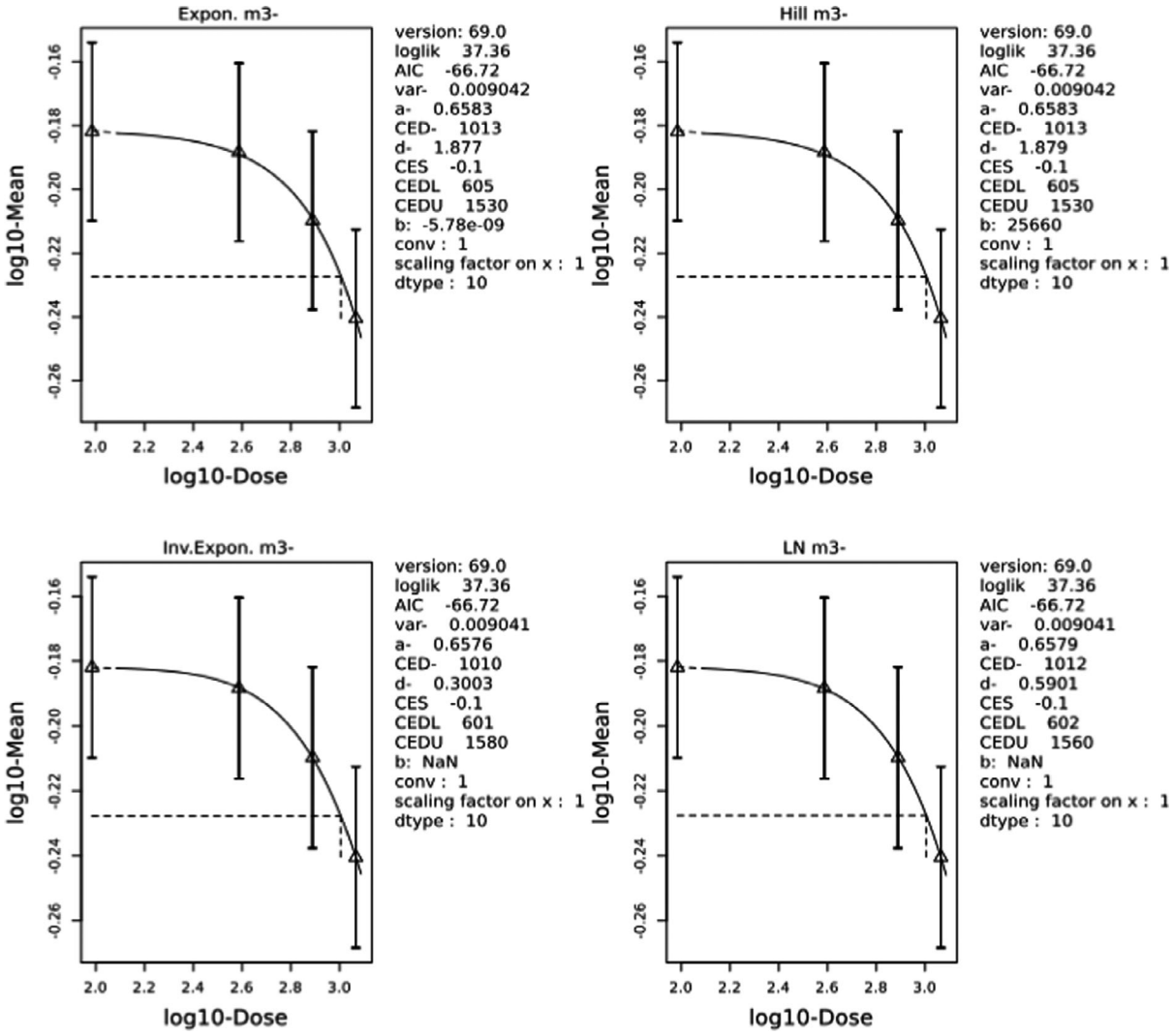


**Figure A.1:** Visualisation of the individual BMD model curves

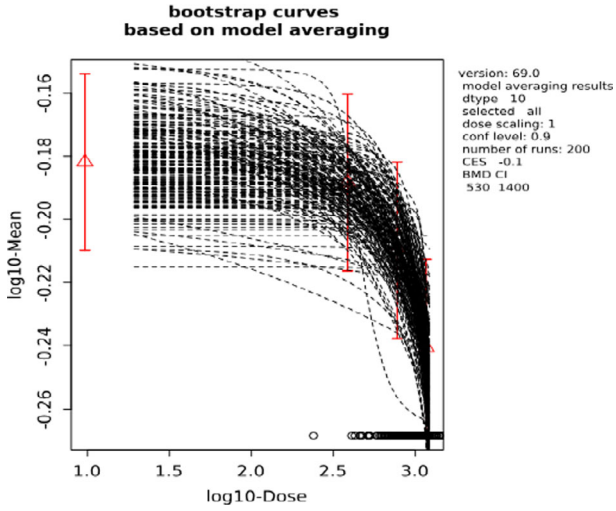


**Figure A.2:** Visualisation of bootstrap curves based on BMD model averaging