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Safety of phenylcapsaicin 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 phenylcapsaicin as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Phenylcapsaicin is a chemically synthesised analogue of capsaicin intended to be marketed in food supplements and in foods for special medical purposes to the general population above the age of 11 years old at a maximum level of 2.5 mg/day. The highest intake of the NF is 2.5 mg/day which corresponds to 36 μg/kg body weight (bw) per day for adults, and 58 μg/kg bw per day for adolescents (10–14 years). The Panel considers that there is no concern with respect to genotoxicity of the NF. The reference point derived based on a 13-week rat study was the lowest of the model averaged $BMDL_{20}$ values of 37.2 mg/kg bw per day in females for increased plasma alanine aminotransferase (ALAT) levels. The Panel concludes that the NF, phenylcapsaicin, is safe under the proposed uses and use levels.

Keywords: phenylcapsaicin, novel food, ingredient, safety

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Summary

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver a scientific opinion on phenylcapsaicin as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The assessment of the safety of this NF, which follows the methodology set out in the EFSA Guidance on the preparation and presentation of an application for authorisation of a NF[1] in the context of Regulation (EU) 2015/2283 and in the Commission Implementing Regulation (EU) 2017/2469, is based on the data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information and additional data identified by the Panel.

The NF which is the subject of the application is phenylcapsaicin (> 98%), a chemically synthesised analogue of capsaicin. The NF is intended to be marketed in food supplements and in foods for special medical purposes to the general population above the age of 11 years old at a maximum level of 2.5 mg/day. The highest intake of the NF is 2.5 mg/day which corresponds to 36 μg/kg body weight (bw) per day for adults (considering an average bodyweight of 70 kg) and 58 μg/kg bw per day for adolescents (10–14 years) (considering an average bodyweight of 43.4 kg).

The information provided on composition, specifications, production process and stability of the NF does not raise safety concerns. The Panel considers that phenylcapsaicin does not have a nutritionally relevant role in the diet and that the consumption of the NF is not nutritionally disadvantageous.

The Panel considers that there is no concern with respect to genotoxicity of the NF. The applicant provided a 90-day study where there were several changes related to effects in the gastrointestinal tract and the liver. The Panel considers both the effects observed as critical effects related to the compound. The reference point (RP) derived based on the critical effects of phenylcapsaicin lowest of the model averaged $BMDL_{20}$ values for females for increased plasma alanine aminotransferase (ALAT) levels was 37.2 mg/kg bw per day (females).

The Panel considers that based on the proposed conditions of use, the margin of exposure between the RP and the maximal exposure to the NF of 1,033 for adults and 643 for adolescents are sufficient.

The Panel concludes that the NF, phenylcapsaicin, is safe under the proposed uses and use levels.

[1] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 7 February 2018, the company aXichem AB, submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place on the European Union (EU) market phenylcapsaicin as a novel food.

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 by carrying out the assessment for phenylcapsaicin as a novel food ingredient.

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's requests for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application: Paulsen et al. (2018).

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.[1] 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 to supporting the safety of the proposed NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. Data claimed to be proprietary by the applicant include: Donath (2016, unpublished), Feng et al. (2012a, unpublished), Feng et al. (2012b, unpublished), Schreib (2015, unpublished), Stiller (2016, unpublished) and Yang and Dong (2015, unpublished). The Panel considers that it could not have reached the conclusion on the safety of the NF under the proposed conditions of use without all these studies provided by the applicant.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications 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 risk that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of phenylcapsaicin with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application is phenylcapsaicin (> 98%), a chemically synthesised analogue of capsaicinoids that occur naturally in plants of the *Capsicum* genus. According to article 3 of the Regulation (EU) 2015/2283, the NF falls under 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. The NF is intended to be marketed in food supplements and in foods for special medical purposes for the general population above the age of 11 years old at a maximum level of 2.5 mg/day.

[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.

3.2. Identity of the NF

The NF is chemically synthesised phenylcapsaicin (> 98%). Phenylcapsaicin has a molecular weight of 337.41 Da, solubility in water of 0.00193 g/L, a partition coefficient (*n*-octanol/water) of 2.34. The pH is 6.12 at 25°C and it has a density of 1.152 g/cm^3 at 20°C. The structure of phenylcapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-7-phenylhept-6-ynamide, $C_{22}H_{23}NO_3$, CAS no: 848127-67-3) is shown in Figure 1. Compared to the naturally occurring capsaicin, phenylcapsaicin presents a phenylethynyl group on the acyl chain.

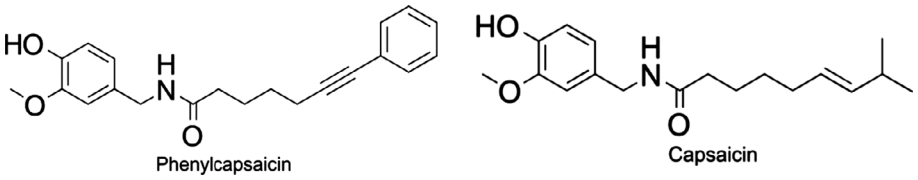

Figure 1: Structure of phenylcapsaicin and the structurally related capsaicin

To characterise the identity of the NF, the applicant provided a series of analyses on phenylcapsaicin including: high-performance liquid chromatography (HPLC), electrospray ionisation mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR), infrared (IR) spectroscopy and ultraviolet (UV) spectroscopy.

3.3. Production process

The NF is produced by a two-step chemical synthesis under controlled conditions. The first step is the production of the acetylenic acid intermediate through the reaction of phenyl acetylene with a carboxylic acid derivative. The second step comprises several reactions of the acetylenic acid intermediate with vanillylamine derivative to produce the final product. Phenylcapsaicin will be formulated with cellulose, fats and/or oil prior to use to a final concentration of 1–1.5%. The applicant provided detailed information on key parameters and critical control points of the production process.

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

3.4. Compositional data

The applicant provided results from batch-to-batch analyses of five different production lots of the NF and the methods used for the analyses (Table 1). In these batches, the NF had a content of phenylcapsaicin > 98%, a water content ≤ 0.2%, a total content of residual solvents < 0.6%, and a content of process-related by-products < 1%. All investigated batches met the specifications (see Section 3.5) regarding microbiological contaminants and heavy metals.

Table 1: Batch-to-batch analyses of five batches of the NF

Parameter (unit)	Batch number				
	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**
Assay (HPLC, %)	98.4	98.34	98.29	98.11	98.17
Moisture (%)	0.07	0.15	0.09	0.2	0.11
Total synthesis related by-products (%)	0.58	0.47	0.44	0.53	0.65
N,*N*-dimethyl formamide (mg/kg)	13	11	12	9	17
Dichloromethane (mg/kg)	19	72	26	4	8
Dimethoxyethane (mg/kg)	2	5	6	3	7
Ethyl acetate (mg/kg)	1900	1900	2002	2100	2008
Other solvents (%)	0.25	0.18	0.15	0.29	0.21

Parameter (unit)	Batch number				
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Microbiological parameters					
Total plate count (CFU/g)	< 10	< 10	< 10	< 10	< 10
Yeast and mould (CFU/g)	< 10	< 10	< 10	< 10	< 10
Coliforms (CFU/g)	< 10	< 10	< 10	< 10	< 10
Escherichia coli (Presence–Absence/10 g)	ND	ND	ND	ND	ND
Salmonella (Presence–Absence/10 g)	ND	ND	ND	ND	ND
Heavy metals					
Lead (mg/kg)	ND	ND	ND	ND	ND
Cadmium (mg/kg)	ND	ND	ND	ND	ND
Mercury (mg/kg)	0.0024	0.006	0.007	0.0038	0.0056
Arsenic (mg/kg)	0.08	0.05	0.11	0.12	0.1

HPLC: high-performance liquid chromatography; CFU: colony forming units; ND: not detected.

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

3.4.1. Stability

The applicant provided two studies on the stability of the NF, where five batches of the NF were stored tightly sealed and in the dark. One study was performed under ambient conditions (25 ± 2°C, 60 ± 3% relative humidity) for up to 24 months, and the other under accelerated conditions (40 ± 2°C; 75 ± 2% relative humidity) up to 6 months.

Upon storage under ambient conditions, there was an average decrease of phenylcapsaicin of 0.29% (0.38–0.22%) after 24 months. Storage under accelerated conditions resulted in phenylcapsaicin decreases of 0.20% (0.31–0.08%) after 3 months and 0.36% (0.49–0.24%) after 6 months.

The Panel considered that the data provided gave sufficient information with respect to the stability of the NF.

3.5. Specifications

The specifications of the NF as provided by the applicant are shown in Table 2.

Table 2: Product specifications for phenylcapsaicin

Parameter	Specification limit	Method
Purity (%)	> 98	HPLC In-house method
Moisture (%)	< 0.5	USP 921
Total synthesis related production by-products (%)	< 1	HPLC In house method
N,*N*-dimethyl formamide (mg/kg)	≤ 880	USP 467
Dichloromethane (mg/kg)	≤ 600	USP 467
Dimethoxyethane (mg/kg)	≤ 100	USP 467
Ethyl acetate (mg/kg)	≤ 5,000	USP 467
Other solvents (%)	≤ 0.5	USP 467
Microbiological parameters		
Total plate count (CFU/g)	< 10	USP 61
Yeast and mould (CFU/g)	< 10	USP 61
Coliforms (CFU/g)	< 10	USP 61
Escherichia coli (Presence–Absence/10 g)	Absence	USP 62
Salmonella (Presence–Absence/10 g)	Absence	USP 62

Parameter	Specification limit	Method
Heavy metals		
Lead (mg/kg)	< 1	USP 231
Cadmium (mg/kg)	< 1	USP 231
Mercury (mg/kg)	< 0.1	USP 231
Arsenic (mg/kg)	< 1	USP 231
Lead (mg/kg)	< 1	USP 231

HPLC: high-performance liquid chromatography; CFU: colony forming units; USP: United States Pharmacopeia.

The Panel considers that the information provided on the specification and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

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

3.6.1. History of use of the source

The NF is a novel compound produced by chemical synthesis.

3.6.2. History of use of the NF

The chemically synthesised phenylcapsaicin has no existing history of use as food in the EU. However, capsaicinoids have a history of use causing the sharply burning taste of the fruits of many varieties of the genus *Capsicum* (e.g. chillies and peppers) (BfR, 2011).

In 2002, the Scientific Committee on Food (SCF) evaluated capsaicin as a flavouring substance or in ingredients with flavouring properties. They concluded that the data available at that time was not sufficient to establish a safe exposure level for capsaicinoids in food. The SCF also gave a rough estimate of the maximum daily intake of capsaicinoids from mild chillies and paprika in Europe of 1.5 mg/day (SCF, 2002).

Risks related to acute exposure to capsaicin were evaluated by the Federal Institute for Risk Assessment (BfR) of Germany in 2011 (BfR, 2011). In their report, the BfR described observations of undesirable effects (such as irritation of mucous membranes, nausea, vomiting and hypertension) in individuals with excessive high consumption; however, the ingested dose of capsaicinoids was unknown in these cases. In this assessment, it was also considered that consumption of one meal containing capsaicin causing hotness which can be tolerated by adults would correspond to an intake of capsaicin up to 5 mg/kg bw (BfR, 2011). The BfR also highlighted that children appeared to be particularly sensitive.

In 2012, EFSA evaluated the safety of the related capsinoid dihydrocapsiate as a NF pursuant to regulation EC 258/97. In that application, chemical synthesised dihydrocapsiate was intended as a NF ingredient for incorporation into foods of various categories at concentration levels varying from 8 to 2,050 mg/kg. Dihydrocapsiate was considered safe under the proposed conditions of use, with the 97.5th percentile intake estimate of 34 mg/day for adults and elderly and calculations based on body weights resulted in the highest intakes being 1.3 mg/kg bw per day for preschool children (EFSA NDA Panel, 2012).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The applicant intends to market the NF to the general population above the age of 11 years.

3.7.2. Proposed uses and use levels

The applicant intends to market the NF as a food supplement and in foods for special medical purposes at levels of 2.5 mg/day. The NF from food supplements and foods for special medical purposes are not meant to be consumed concomitantly.

3.7.3. Anticipated intake of the NF

At the proposed uses and use levels (2.5 mg/day), the intake of the NF could be up to 36 μg/kg bw per day for a 70-kg adult and up to 58 μg/kg bw per day for adolescents (10–14 years) considering an average bodyweight of 43.4 kg (EFSA Scientific Committee, 2012).

3.7.4. Combined intake from the NF and other sources

Phenylcapsaicin is not naturally present in foods, and there will thus not be a combined intake from the NF and other sources.

The Panel notes that phenylcapsaicin acts via the transient receptor potential vanilloid subfamily 1 (TRPV1) receptor as other capsaicinoids do. The intended intake of phenylcapsaicin of 2.5 mg/day may add to the intake of capsaicinoids from foods if consumed simultaneously. The estimated maximum daily intake of capsaicinoids from mild chillies and paprika in Europe is 1.5 mg/day (SCF, 2002). This is low compared to the 5 mg/kg bw which may be consumed by an adult with one meal (BfR, 2011).

3.7.5. Estimate of exposure to undesirable substances

With the proposed conditions of use and specifications of the residual process-related by-products, the Panel considers the exposure to undesirable substances does not raise safety concerns.

3.7.6. Precautions and restrictions of use

No additional precautions of use were addressed by the applicant.

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

The applicant provided two *in vivo* ADME studies performed in parallel, one with phenylcapsaicin and one with capsaicin (Feng et al., 2012a,b). The studies were neither performed according to OECD test guideline 417 nor compliant with good laboratory practice (GLP). Male Sprague–Dawley (SD) rats received a single oral dose of 50 mg/kg of the ^{14}C radiolabelled test material. Bile, plasma, blood and tissue samples were collected at 0.5, 2 and 24 h post-dosing, and urine and faeces samples were collected up to 7 days post dosing. Radioactivity was determined in blood, plasma, liver, brain, stomach, small intestine, kidney, spleen, lung, heart, testes, skeletal muscle and fat. Metabolites were identified in plasma, urine and faeces. In addition, radioactivity and metabolites in bile were determined in bile duct-cannulated rats up to 24 h after oral application (Appendix A).

Both, phenylcapsaicin and capsaicin were rapidly absorbed at similar rates reaching maximum levels in the blood 0.5 h post-dosing. The radioactivity was distributed to various tissues, and was most abundant in the small intestine, stomach and liver within 0.5 h post-dosing. An increase in radioactivity was found in fat from 0.5 to 24 h post-dosing.

Numerous metabolites were identified. The dominant metabolic pathway for capsaicin was glucuronidation, whereas it was oxygenation and glucuronidation for phenylcapsaicin. Of the labelled ^{14}C-phenylcapsaicin and ^{14}C-capsaicin, 72% and 47% were identified in the bile within 24 h post-oral dose to rats, with approximately 2% and 0.4% as the intact parent compound.

The compounds were excreted through both urine and faeces with a majority occurring in the first 24 h after oral dosing. A higher amount of radioactivity was excreted in the faeces after application of phenylcapsaicin compared to capsaicin. The recovery rates of phenylcapsaicin (54.1%) and capsaicin (47.6%) in excreta and carcass were similar within 7 days post dosing. The lack of full recovery in faeces, urine and carcass was attributed by the authors of the study to transfer of ^{14}C from the radiolabelled carbonyl group to acetyl coenzyme after cleavage of the amide bond. This could lead to incorporation into endogenous substances, e.g. in fat or to biotransformation to $^{14}CO_2$ and other ^{14}C labelled volatile organic compounds that are expired. Radioactivity in expired air was, however, not measured.

Overall, the Panel notes that phenylcapsaicin derived radioactivity was rapidly absorbed, widely distributed in various tissues, metabolised by the liver, excreted into the bile and eliminated mainly via faeces.

3.9. Nutritional information

The Panel considers that phenylcapsaicin does not have a nutritionally relevant role in the diet and is not nutritionally disadvantageous under the proposed conditions of use.

3.10. Toxicological information

The applicant provided a bacterial reverse mutation test, an *in vitro* mammalian micronucleus test and a subchronic toxicity study. After a request from the Panel, the applicant also provided an unpublished study (Yang and Dong, 2015) cited by Paulsen et al. (2018).

3.10.1. Genotoxicity

In a bacterial reverse mutation test compliant with OECD 471 and GLP with *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102, with and without metabolic activation (Schreib, 2015 unpublished; Paulsen et al., 2018), phenylcapsaicin was non-mutagenic (Appendix B). In an *in vitro* mammalian cell micronucleus test with human lymphocytes compliant with OECD 487 and GLP with and without metabolic activation (Donath, 2016 unpublished; Paulsen et al., 2018), phenylcapsaicin was not clastogenic or aneugenic (Appendix B). The Panel considers that taking into account the results of the tests performed, there are no concerns regarding genotoxicity of the NF.

3.10.2. Subchronic toxicity

The applicant provided a subchronic 90-day repeat-dose oral toxicity study in compliance with OECD TG 408 and GLP in which groups of Wistar rats (15/sex per group in control and high-dose group and 10/sex per group in mid- and low-dose groups) were administered the NF by gavage at dose levels of 0, 30, 100 or 250 mg/kg bw per day (Stiller, 2016; unpublished; Paulsen et al., 2018).

In this study, several targets systems were affected after administration of phenylcapsaicin to rats (Appendix C). Effects were observed in the gastrointestinal tract, such as salivation and diarrhoea, inflammation in the glandular stomach, forestomach and caecum, which can be attributed to the irritating properties of the compound. The haematological effects (decreases in erythrocytes and reticulocytes) may be secondary to bleeding and sustained inflammation in the gastrointestinal tract. Reduced spleen weights, depletion of lymphocytes in spleen and lymph nodes as well as reduced cellularity in the bone marrow point to the haematopoetic system as another target. Furthermore, phenylcapsaicin affects the liver as can be concluded from increases in liver enzyme levels (alanine aminotransferase (ALAT) and alkaline phosphatase (AP), increases in prothrombin time, decreased levels of glucose, cholesterol, bilirubin and bile acids, increased liver weights and effects in histopathological examinations, which included diffuse hepatocellular hypertrophy, periportal vacuolation, eosinophilic cytoplasmic inclusions and focal necrosis.

Most effects were dose related, and not reversed in the recovery group or even more pronounced (e.g. white blood cell (WBC) counts, cholesterol). In contrast, for some liver enzymes (aspartate aminotransferase (ASAT), ALAT and AP), levels in the male recovery group were even lower than in the controls. Male rats were generally more susceptible than female rats.

The authors of the study considered 100 mg/kg bw per day as the no observable adverse effect level (NOAEL) for systemic toxicity based on degenerative changes in the liver and 30 mg/kg bw per day as the NOAEL for local effects due to irritating effects in the stomach.

The Panel noted that some effects were statistically significant already at the lowest dose of 30 mg/kg bw per day and therefore evaluated these effects. Upon request by EFSA, the applicant provided a benchmark dose (BMD) assessment and some considerations on the evaluation of the effects. The BMD assessment followed the EFSA Guidance on the BMD approach (EFSA Scientific Committee, 2017), using the EFSA web-tool for BMD analysis, which is based on PROAST software (Appendix D). In addition, EFSA performed a BMD analysis of the effects on bodyweight and the erosions and ulcers in the stomach applying the same methodology.

A dose-dependent decrease in bodyweight was observed in rats of both sexes, with no significant changes in the food consumption observed in the rats. This can be considered an adverse effect, and the lowest of the model averaged $BMDL_{05}$ for body weight is 77.4 mg/kg bw per day (males).

The clinical symptom 'moving of the bedding' was observed in both sexes, which can be interpreted as a sign of discomfort. This finding also occurred in the same intensity in the recovery group. Similarly, salivation was also observed in the recovery group, indicating that in addition to irritating

effects of the NF, the expectation of treatment *per se* likely triggers responses in the animals treated with the test material. The effects were therefore not used for deriving a reference point.

One female rat developed at 30 mg/kg bw per day slight (grade 1) erosion/ulcer of the glandular stomach. At higher dose levels, the number of animals affected and severity of effects increased, and also other effects in the stomach occurred. Therefore, this finding reflects first signs of irritation in the stomach. These effects are also relevant for humans, as for capsaicin an increase in discharge of parietal cells, pepsin secretion and potassium exfoliation (desquamation) of stomach cells was found after ingestion of 1.5 g capsaicin dissolved in 100 mL water, the NOAEL was 0.5 g in this study (Myers et al., 1987). In the BMD analysis of the findings on erosions and ulcers in the stomach, the lowest of the model averaged $BMDL_{10}$ was 38.7 mg/kg bw per day (females).

For other endpoints of liver toxicity, such as hepatocellular hypertrophy and periportal vacuolation as well as relative liver weight there was a clear dose response and the BMD analyses resulted in BMD values with narrow BMD confidence intervals. The lower confidence limit of the benchmark dose (BMDL) values for these data are in close agreement with each other; the lowest of the model averaged $BMDL_{05}$ values for relative liver weights is 55.0 mg/kg bw per day (females) and the lowest of the model averaged $BMDL_{10}$ values for hepatocellular hypertrophy is 56.4 mg/kg bw per day (males). Cholesterol levels were reduced by 30% in male rats and 19% in female rats, (statistically significant in males) at the low dose of 30 mg/kg bw per day. There was a reduction of total bilirubin levels (20% in male rats, 22% in female rats, statistically significant in female rats). The Panel notes that decreased bilirubin levels indicate enzyme induction in the liver and may be considered as a non-adverse effect. At higher dose levels, there are additional effects on the liver, i.e. at 100 mg/kg bw per day, there is an increase in liver weight, hypertrophy and vacuolation in liver cells. The increase in prothrombin time at this dose level may also point to liver toxicity. Thus, there is a dose-related continuum of effects in the liver. There was a statistically significant dose-related increase in plasma ALAT levels in females at the highest dose. An increase in ALAT concentrations in the blood can be considered an adverse effect. The benchmark response (BMR) of 20% was suggested by the EFSA Scientific Committee et al. (2017) for liver enzymes. The lowest of the model averaged $BMDL_{20}$ values for ALAT was 37.2 mg/kg bw per day (Females), and was used as the reference point (RP) for the NF.

3.10.3. Human studies

No human studies were available assessing the safety of the NF.

3.10.4. Other studies

Contact of the skin or the mucous membranes with capsaicin causes a sensation of heat, burning and hyperaemia. This effect is mediated by binding to 'transient receptor potential vanilloid subfamily 1' (TRPV1) in specific nerve fibres (C-fibres) that transport calcium into the cells and subsequent release of various mediators, e.g. Substance P.

To investigate, whether phenylcapsaicin acts similarly as capsaicin, an *in vitro* study was performed by the applicant (Yang and Dong, 2015) and provided upon request from EFSA. In this study, the uptake of fluorescent calcium via the TRPV1 channel was investigated in HEK293 cells stably expressing the human TRPV1 channel. The EC_{50} (half maximal effective concentration) of phenylcapsaicin for stimulating Ca uptake was 57.8 nM, while for capsaicin it was 26.4 nM, indicating that phenylcapsaicin was about half as active as capsaicin.

The Panel notes that this study indicates that phenylcapsaicin acts as a capsaicin analogue.

3.11. Allergenicity

The Panel considers that the NF is a well-defined mixture with a high content of phenylcapsaicin > 98% produced from non-protein chemical sources and that it is not expected to have allergenic potential.

4. Discussion

The NF which is the subject of the application is phenylcapsaicin (> 98%) a chemically synthesised analogue of capsaicin. The highest intake of the NF is 2.5 mg/day which corresponds to 36 μg/kg bw per day for adults (considering an average bodyweight of 70 kg) and 58 μg/kg bw per day for adolescents (10–14 years) (considering an average bodyweight of 43.4 kg).

The information provided on composition, specifications, production process and stability of the NF does not raise safety concerns. The Panel considers that phenylcapsaicin does not have a nutritionally relevant role in the diet and 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 there were several changes related to effects in the gastrointestinal tract and the liver. The Panel considers both the effects observed as critical effects related to the compound. The RP derived based on the critical effects of phenylcapsaicin lowest of the model averaged $BMDL_{20}$ values for the increase in plasma ALAT levels was 37.2 mg/kg bw per day (females).

The Panel considers that based on the proposed conditions of use, the margin of exposure between the RP and the maximal exposure to the NF of 1,033 for adults and 643 for adolescents are sufficient.

5. Conclusions

The Panel concludes that the NF is safe under the proposed uses and use levels.

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, Donath (2016, unpublished), Feng et al. (2012a, unpublished), Feng et al. (2012b, unpublished), Schreib (2015, unpublished), Stiller (2016, unpublished) and Yang and Dong (2015, unpublished).

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 phenylcapsaicin. Ref. Ares(2018)4394500, dated 27/08/2018.
2) On 28 August 2018, EFSA received a valid application from the European Commission on phenylcapsaicin as NF, which was submitted by aXichem AB, and the scientific evaluation procedure started.
3) On 20 November 2018, 7 February 2019 and 11 February 2019 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 29 January 2019 and twice on 11 February 2019 additional information was provided by the applicant and the scientific evaluation was restarted.
5) During its meeting on 15 05 2019, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of phenylcapsaicin as a NF pursuant to Regulation (EU) 2015/2283.

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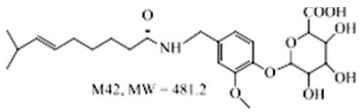
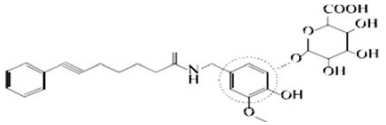
Abbreviations

ADME	absorption, distribution, metabolism and excretion
ALAT	alanine aminotransferase
AP	alkaline phosphatase
ASAT	aspartate aminotransferase
BfR	German Federal Institute for Risk Assessment
BMD	benchmark dose
BMDL	lower confidence limit of the benchmark dose
BMDU	upper confidence limit of the benchmark dose
BMR	benchmark response
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
EC_{50}	half maximal effective concentration
ESI-MS	electrospray ionisation mass spectrometry
GLP	good laboratory practice
HPLC	high-performance liquid chromatography
IR	infrared
NOAEL	no observable adverse effect level
NF	novel food
NMR	nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
RP	Reference point
TRPV1	transient receptor potential vanilloid subfamily 1
USP	United States Pharmacopeia
UV	ultraviolet
WBC	white blood cell count

Appendix A – Comparisons of ADME properties in rats between capsaicin and phenylcapsaicin

Table A.1: Results of ADME in rats of capsaicin and phenylcapsaicin modified from Feng et al. (2012a,b)

	Capsaicin	**Phenylcapsaicin**
Dose level	50 mg/kg including 100 p,Ci/kg	50 mg/kg including 100 p,Ci/kg
Cumulative radioactive dose in urine within 7 days (%)	15.26	3.25
Cumulative radioactive dose in faeces within 7 days (%)	32.26	50.86
Recovery in urine and faeces within 7 days	47.61	54.11
Radioactivity in carcass after 7 days	No data	4.2%
Cumulative radioactive dose in bile (%) within 24 h	47.06	72.29
Percent parent in bile of the dose (%) within 4 h	1.99	0.36
T_{max} in tissues and blood, plasma	0.5 h	0.5 h
Tissues with highest radioactivity after 0.5 h (% of dose)	Small intestine 8.5% Stomach 3.9% Liver > 0.7%*	Small intestine 14.3% Stomach 1.6% Liver > 1.8%*
Total number of metabolites	50	27
Metabolic pathway	Hydrolysis, deamination, hydrogenation, dehydrogenation, oxygenation, conjugation with glutathione, demethylation, glucuronidation and sulfation	Hydrolysis, deamination, dehydrogenation, glucuronidation, oxygenation, conjugation with glutathione, demethylation and sulfation
Major metabolic pathway and metabolites	M42 (31.8% of the radioactive dose in bile), glucuronidation	M18 (61.97% of the radioactive dose in bile), oxygenation and glucuronidation
		
Blood to plasma ratio (0.5–24 h)	0.61–1.17	0.55–0.88

ADME: absorption, distribution, metabolism and excretion.
Note: Tissues collected: liver, brain, stomach, small intestine, kidney, spleen, lung, heart, testes, skeletal muscle and fat.
*: Only piece of liver was cut and used as a sample.

Appendix B – Genotoxicity studies of Phenylcapsaicin

Table B1: Results of Schreib (2015) unpublished full study report and Donath (2016) unpublished full study report

Study	Bacterial reverse mutation test assay	In vitro mammalian Cell micronucleus assay
Title of the study report	Schreib (2015), unpublished full study report (confidential) Paulsen et al. (2018), published report	Donath, 2016;. unpublished full study report (confidential) Paulsen et al., 2018 published report
Method	OECD 471	OECD 487
Tested system	*Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537	Human lymphocytes
Test material	NF Batch: 20141201 (98.8% phenylcapsaicin)	NF Batch: 20141201 (98.8% phenylcapsaicin)
Dose/ concentration	Two independent experiments (plate incorporation (experiment 1) and pre-incubation test (experiment 2)). Eight concentrations of phenylcapsaicin were used for each bacterial strain in triplicate with and without the presence of metabolic activation system (S9 mix). 3.16, 10.0, 31.6, 100, 316, 1,000, 2,500 and 5,000 μg/plate	Dose selected based on the results of the cytotoxicity measurements and the precipitation study of the substance. Short-term exposure (incubated for 4 h) – With metabolic activation (S9 mix) at concentrations 50, 100, 130 and 140 μg/mL – Without metabolic activation (S9 mix) at concentrations of 50,100, 120 and 130 μg/mL Long-term exposure (exposed to the NF for 44 h) – Absence of metabolic activation (S9 mix) at concentrations of 10, 15 and 20 μg/mL
Authors conclusions	No increases in revertant colony numbers of any of the strains tested occurred in the presence or absence of metabolic activation in both experiments. There was precipitation of the test item in all strains at a concentration of 5,000 μg/plate. Toxicity was observed at concentrations of 316 μg/plate and above in the absence of S9. In the presence of S9 at 1,000 μg/plate and above, toxicity was observed in the preincubation test with S9, and 2,500 μg/plate and above in the plate incorporation test	There was no statistically significant enhancement of micronucleated cells observed in the experiments. Phenylcapsaicin was not clastogenic or aneugenic in the *in vitro* mammalian cell micronucleus test. Cytostasis was close to the limit of 55 ± 5% in all experiments, 58, 57 and 60% at highest dose tested

Appendix C – Results of oral dose 90-day study

Table C.1: Summary of the 90-day study by Stiller (2016) and the significant findings in the study

Title of the study report	90-day repeated-dose oral toxicity study in Wistar rats with phenylcapsaicin including a 28-day recovery period (Stiller, 2016)
Tested system	Groups of 6-week-old adult Wistar rats
Test material	The NF Batch: 20141201 (98.8% phenylcapsaicin)
Dose/concentration (route of administration)	The NF (suspended in polyethylene glycol 400) was prepared daily and administered to the rats by oral gavage at doses levels of: Control (15 animals/sex): 0 (vehicle-only) Low dose (10 animals/sex): 30 mg/kg bw per day Mid dose (10 animals/sex): 100 mg/kg bw per day High dose (10 animals/sex): 250 mg/kg bw per day
Statistical analysis	Results of the body weight, food consumption, parameters of haematology, blood coagulation and clinical biochemistry and absolute and relative organ weights were analysed using one-way ANOVA and a post hoc Dunnett's test Statistical comparisons of data acquired during the recovery period were performed with a Student's t-test
Method	OECD 408

Parameter	Sex	Control 0 mg/kg bw per day	Low dose 30 mg/kg bw per day	Mid dose 100 mg/kg bw per day	High dose 250 mg/kg bw per day	Control 0 mg/kg bw per day + Recovery	High dose 250 mg/kg bw per day + Recovery
Clinical observations							
Animals with signs of toxicity (frequency)	M	55.7	117.3	165.9**	203.5**	NA	198.2**
	F	55.1	103.5	164.7**	205.1**	NA	191.2**
Diarrhoea (frequency)	M	55.2	60.7	57.9	67.3**	NA	65.4**
	F	55.1	52.5	54.6	62.0**	NA	66.6**
Salivation (frequency)	M	0	4.8	42.3**	59.9**	NA	60.0**
	F	0.0	6.1	45.1**	58.2**	NA	58.6**
Piloerection (frequency)	M	0	0	0	0.9	NA	0.4**
	F	0.0	0.0	0.1	3.5	NA	0.0
Musculoskeletal system wasp waist (frequency)	M	0	0	0	0	NA	0.4**
	F	0.0	0.0	0.1	1.6	NA	0.0
Spontaneous Activity Reduced (frequency)	M	0	0	0	3.9**	NA	4.0**
	F	0.0	0.0	0.1	2.5**	NA	4.0**
Moving the bedding (frequency)	M	0	51.8	65.7**	68.9**	NA	68.0**
	F	0	44.8*	64.4**	68.0**	NA	62.0*

Parameter	Sex	Control 0 mg/kg bw per day	Low dose 30 mg/kg bw per day	Mid dose 100 mg/kg bw per day	High dose 250 mg/kg bw per day	Control 0 mg/kg bw per day + Recovery	High dose 250 mg/kg bw per day + Recovery
Bodyweight							
Day 91 (g)	M	356.53	351.30	347.60	322.80	–	329.80
	F	209.33	206.22	205.44	194.56	–	210.50
Day 119 (g)	M	–	–	–	–	379,00	366.25
	F	–	–	–	–	210,00	207.25
Haematology							
RBC (10^{12}/L)	M	9.15	9.14	8.86	8.72*	9.27	8.71
	F	8.26	8.00	8.08	7.58	7.99	7.83
WBC (10^{9}/L)	M	4.23	4.80	3.40	3.69	4.81	2.90*
	F	3.34	2.90	2.17	3.64	2.05	1.38
MCV (fL)	M	52.36	52.35	52.73	54.03*	52.22	54.45
	F	55.08	53.49	54.39	54.94	54.82	55.33
Monocytes (%)	M	2.35	1.89	2.75	2.22	3.44	1.53
	F	2.08	1.89	2.57	3.79*	2.52	2.80
Reticulocytes (%)	M	1.99	1.70	1.46	1.07**	1.35	1.70*
	F	2.57	2.37	1.88	1.60	1.58	1.34
Prothrombin Time (seconds)	M	22.30	23.68	25.76**	24.75*	22.92	23.43
	F	24.09	25.80	25.74	24.83	23.88	25.83*
Clinical biochemistry							
Total bilirubin (μmol/L)	M	2.21	1.77	1.39**	1.78	2.32	1.13**
	F	2.64	2.07*	1.99*	2.11	2.36	1.90
Total bile acids (μmol/L)	M	69.52	45.49	34.59*	34.13*	48.55	25.58
	F	66.06	54.36	55.41	67.15	39.78	31.90
Total cholesterol (mmol/L)	M	1.74	1.25*	1.02**	1.08*	1.66	0.65***
	F	1.27	1.03	1.08	1.33	1.08	1.32
Glucose (mmol/L)	M	7.32	6.66	4.77*	4.53*	11.93	5.16**
	F	5.22	4.85	4.50	6.48	6.71	7.30
Alanine aminotransferase (U/L)	M	35.54	31.23	32.58	44.59	39.30	15.48***
	F	34.58	26.48	35.90	59.83*	25.84	38.03
Aspartate aminotransferase (U/L)	M	106.80	88.50	91.62	91.60	90.80	29.63**

Parameter	Sex	Control 0 mg/kg bw per day	Low dose 30 mg/kg bw per day	Mid dose 100 mg/kg bw per day	High dose 250 mg/kg bw per day	Control 0 mg/kg bw per day + Recovery	High dose 250 mg/kg bw per day + Recovery
	F	86.93	79.90	85.34	101.85	59.30	85.13
Alkaline phosphatase (U/L)	M	103.15	112.62	113.50	123.31	115.31	33.10**
	F	54.68	72.13	74.84	100.48*	57.27	67.46
Urea (mmol/L)	M	9.06	9.49	9.38	9.58	5.26	2.23***
	F	10.23	10.01	9.82	9.58	5.26	4.75
Sodium (mmol/L)	M	131.00	125.50	121.20	125.70	125.60	57.67**
	F	128.70	133.44	130.44	141.38	97.00	113.75
Potassium (mmol/L)	M	4.13	3.71	3.71	3.89	3.78	1.60***
	F	3.91	3.55	3.43	4.11	2.45	2.97
Organ weights							
Liver (mean weight (g))	M	8.5285	8.7392	8.8405	11.3357****	9.3246	9.2301
	F	5.3444	5.4581	5.9997	7.2987****	5.5875	6.0377
Liver weight (relative to body weight %)	M	2.4232	2.4856	2.5398	3.5107	2.5543	2.8617
	F	2.6058	2.6478	2.9255	3.7866	2.5819	2.8658
Spleen (mean weight (g))	M	0.7841	0.7419	0.6560	0.6200****	0.6453	0.7266
	F	0.5240	0.5003	0.5230	0.4525****	0.5042	0.4993
Histopathology							
Liver							
Hepatocellular hypertrophy, diffuse (incidence/mean severity)	M	0	0	3/1.0	9/1.0	0	0
	F	0	0	0	7/1.0	0	0
Vacuolation (fatty change), periportal (incidence/mean severity)	M	0	0	1/1.0	4/1.5	0	0
	F	2/1.0	1/1.0	0	3/1.7	1/1.0	1/1.0
Eosinophilic cytoplasmic inclusions, periportal hepatocytes (incidence/mean severity)	M	0	0	0	1/1.0	0	0
	F	0	0	0	0	0	0
Focal necrosis (incidence/mean severity)	M	0	0	0	0	0	0
	F	0	0	0	1/1.0	0	0

Parameter	Sex	Control 0 mg/kg bw per day	Low dose 30 mg/kg bw per day	Mid dose 100 mg/kg bw per day	High dose 250 mg/kg bw per day	Control 0 mg/kg bw per day + Recovery	High dose 250 mg/kg bw per day + Recovery
Stomach							
Forestomach							
Hyperkeratosis, diffuse (incidence/mean severity)	M	0	0	0	10/1.5	0	0
	F	0	0	0	8/1.3	0	0
Squamous hyperplasia (incidence/mean severity)	M	0	0	0	10/1.0	0	0
	F	0	0	0	8/1.0	0	0
Oedema, submucosa (incidence/mean severity)	M	0	0	0	3/2.0	0	0
	F	0	0	0	3/1.0	0	0
Inflammation, submucosa (incidence/mean severity)	M	0	0	0	0	0	0
	F	0	0	0	1/1.0	0	0
Glandular stomach							
Erosion/Ulcer (incidence/mean severity)	M	0	0	1/1.0	3/1.7	0	1/1.0
	F	0	1/1.0	1/2.0	5/2.4	0	1/1.0
Inflammation (incidence/mean severity)	M	0	0	1/1.0	3/1.7	0	1/1.0
	F	0	0	2/1.0	7/1.6	0	0
Cecum							
Congestion, lamina propria (incidence/mean severity)	M	0	0	2/1.0	8/1.0	0	0
	F	0	0	0	2/1.0	0	0
Increased basophilia, surface epithelial cells (incidence/mean severity)	M	0	0	3/1.0	9/1.0	0	0
	F	0	0	0	5/1.0	0	0
Increased apoptosis, surface epithelial cells (incidence/mean severity)	M	0	0	0	2/1.0	0	0
	F	0	0	0	0	0	0
Others							
Bone marrow (sternum) – decreased cellularity (decreased cell density) (incidence/mean severity)	M	0	0	0	5/2.0	0	1/1.0
	F	0	0	0	6/1.8	0	1/1.0
Spleen – lymphoid depletion (incidence/mean severity)	M	0	0	NE	0	NE	NE
	F	0	0	0	1/1.0	0	0
Thymus – atrophy (incidence/mean severity)	M	9/1.2	10/1.3	9/1.2	10/1.7	5/1.2	5/1.0
	F	10/1.3	9/1.4	9.1.4	8/2.3	5/1.4	4/1.5

Parameter	Sex	Control 0 mg/kg bw per day	Low dose 30 mg/kg bw per day	Mid dose 100 mg/kg bw per day	High dose 250 mg/kg bw per day	Control 0 mg/kg bw per day + Recovery	High dose 250 mg/kg bw per day + Recovery
Lymph node – axillary – lymphoid depletion (incidence/mean severity)	M	0	NE	NE	0	NE	NE
	F	0	0	0	1/1.0	0	0
Lymph node – mesenteric – lymphoid depletion (incidence/mean severity)	M	0	NE	NE	0	NE	NE
	F	0	0	0	1/1.0	0	0
Adrenal glands – cortical hypertrophy, diffuse (incidence/mean severity)	M	0	0	0	2/1.1	0	0
	F	0	0	0	5/1.0	0	0

bw: body weight; ANOVA: analysis of variance; RBC: red blood cell; WBC: white blood cell; MCV: mean corpuscular volume; C: control group; F: female; HD: high-dose group; LD: low-dose group; M: male; MD: mid-dose group; NA: not applicable; NE: not examined, U: enzyme activity.
Values for the HD recovery group are compared to values for the C recovery group, when such data are available. otherwise, values are compared to values for the C group.
*: significantly different from control ($p < 0.05$).
**: significantly different from control ($p < 0.01$).
***: significantly different from control ($p < 0.001$).
****: significantly different from control (p = not reported).

Appendix D – Benchmark dose modelling reports

Benchmark dose modelling of continuous data

Data Description

The endpoint analysed was plasma alanine aminotransferase levels, summary data for the endpoint is found in Table D.1. The BMD analysis was performed using individual data, and therefore slightly different results may be obtained using the summary data in the analysis.

Table D.1: Summary data of Alanine aminotransferase (Stiller, 2016)

Dose	ALAT (U/L)	SD	n	Sex
C	34.58	10.99	10	F
LD	26.48	8.32	8	F
MD	35.90	10.63	9	F
HD	59.83	35.89	8	F
C	35.54	8.88	10	M
LD	31.23	9.07	10	M
MD	32.58	7.27	10	M
HD	44.59	19.78	10	M

Software used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 66.35, for the underlying calculations.

Selection of the BMR

The benchmark response (BMR) used is a 20% change in mean response compared to the controls. The benchmark dose (BMD) is the dose corresponding with the BMR of interest.

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

Results

Fitted Models

Table D.3: List of fitted BMD models

Model	Converged	Loglik	npar	AIC
full model	Yes	−32.32	9	82.64
full-v	Yes	−31.43	10	82.86
null model	Yes	−41.22	2	86.44
null model-a	Yes	−41.20	3	88.40
Expon. m3-	Yes	−35.58	4	79.16
Expon. m3-a	Yes	−35.54	5	81.08
Expon. m3-b	Yes	−34.42	5	78.84
Expon. m3-ab	Yes	−34.16	6	80.32
Expon. m5-	Yes	−35.50	5	81.00
Expon. m5-a	Yes	−35.46	6	82.92
Expon. m5-b	Yes	−34.33	6	80.66
Expon. m5-ab	Yes	−33.86	7	81.72
Hill m3-	Yes	−35.58	4	79.16
Hill m3-a	Yes	−35.54	5	81.08
Hill m3-b	Yes	−34.42	5	78.84
Hill m3-ab	Yes	−34.16	6	80.32
Hill m5-	Yes	−35.50	5	81.00
Hill m5-a	Yes	−35.47	6	82.94
Hill m5-b	Yes	−34.33	6	80.66
Hill m5-ab	Yes	−33.86	7	81.72
Inv.Expon. m3-	Yes	−35.53	4	79.06
Inv.Expon. m3-a	Yes	−35.49	5	80.98
Inv.Expon. m3-b	Yes	−34.35	5	78.70
Inv.Expon. m3-ab	Yes	−34.03	6	80.06
Inv.Expon. m5-	Yes	−35.49	5	80.98
Inv.Expon. m5-a	Yes	−35.46	6	82.92
Inv.Expon. m5-b	Yes	−34.31	6	80.62
Inv.Expon. m5-ab	Yes	−33.84	7	81.68
LN m3-	Yes	−35.55	4	79.10
LN m3-a	Yes	−35.51	5	81.02
LN m3-b	Yes	−34.38	5	78.76
LN m3-ab	Yes	−34.09	6	80.18
LN m5-	Yes	−35.49	5	80.98
LN m5-a	Yes	−35.46	6	82.92
LN m5-b	Yes	−34.31	6	80.62
LN m5-ab	Yes	−33.84	7	81.68

Estimated Model Parameters

Table D.4: Estimated model parameters for the BMD models

	EXP	HILL	INVEXP	LOGN
Estimate for var-	0.1466	0.1466	0.1463	0.1465
Estimate for a-	30.67	30.66	30.62	30.64
Estimate for CED-F	149.7	149.5	136.2	141.8
Estimate for CED-M	210.9	210.8	206.4	208.2
Estimate for d-	2.112	2.115	0.3659	0.6916

Weights for Model Averaging

Table D.5: Weights for the models used in the BMD model averaging

EXP	HILL	INVEXP	LOGN
0.24	0.24	0.26	0.25

Final BMD Values

Table D.6: Final BMD values for plasma alanine aminotransferase (ALAT) levels

Endpoint	Subgroup	BMDL	BMDU
ALAT	F	37.2	235
ALAT	M	86.6	593

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

Visualisation

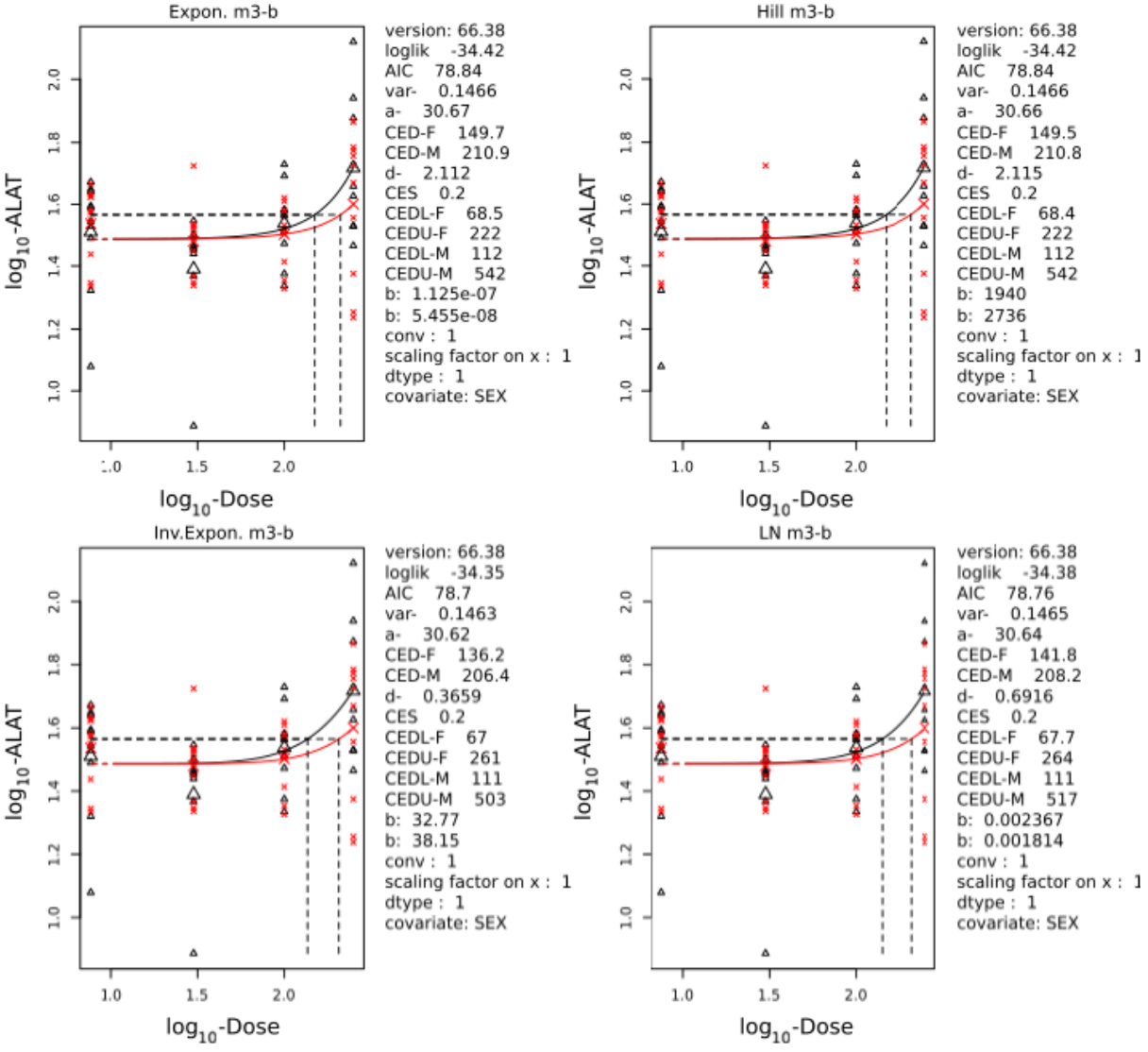


Figure D.1: Visualisation of the individual BMD model curves

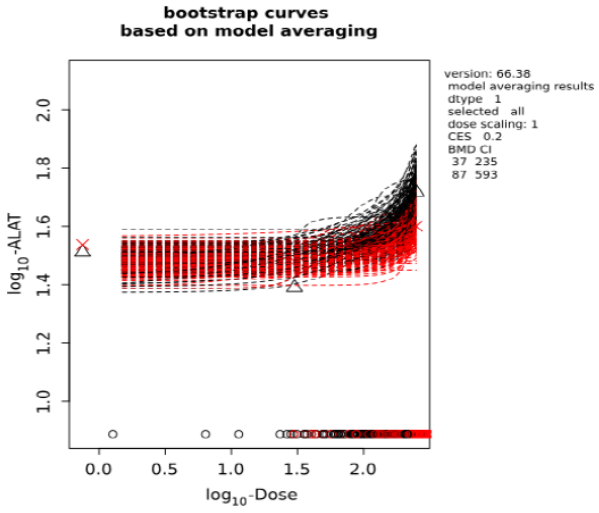


Figure D.2: Visualisation of bootstrap curves based on BMD model averaging