Approved: 30 January 2026
DOI: 10.2903/j.efsa.2026.9941

efsa JOURNAL

Statement

Rapid risk assessment on acute reference dose (ARfD) of cereulide in infants and information on acute consumption of infant formulae

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Abstract

EFSA has received a request for a rapid risk assessment for cereulide from the European Commission. Cereulide is a heat-stable emetic toxin produced by *Bacillus cereus*, recently detected in infant formulae from multiple manufacturers. This led to precautionary recalls across several countries. EFSA was requested to determine the acute reference dose (ARfD) for cereulide in infants, based on available toxicological data, and provide information on the acute consumption of infant formulae from EFSA's food consumption database. From the available toxicological data, emesis was considered as the critical endpoint for the acute effects of cereulide. A study in adult Asian house shrews was considered as the most appropriate for benchmark dose (BMD) modelling, and a $BMDL_{10}$ of 4.2 µg/kg body weight (bw), corresponding to a 10% increased risk of emesis, was derived. An ARfD of 0.014 µg/kg bw was then derived by applying a default uncertainty factor (UF) of 100 and an additional UF of 3 for reduced xenobiotic metabolism and renal excretion in infants. Available consumption data confirmed EFSA's previous recommendation to use a high consumption value of 260 mL/kg bw for estimating acute exposure to cereulide via infant formulae. When assessing acute exposure from follow-on formulae, a P95 consumption value of 140 mL/kg bw was considered more appropriate. Cereulide concentrations above 0.054 µg/L and 0.1 µg/L in reconstituted infant formulae and follow-on formulae, respectively, may therefore exceed the derived ARfD.

Keywords: cereulide, acute reference dose, rapid risk assessment, infant formulae, follow-on formulae, BMD

Requestor: European Commission

Question number: EFSA-Q-2026-00071

Correspondence: Ask a Question

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Acknowledgements: In addition to the listed authors, EFSA would like to thank Georgi Chobanov, Federico Cruciani, Nour Elsersawy, Milen Georgiev, Sharon Monti, Eleonora Sarno and Tuuli Tauriainen for the support provided to this output. EFSA also acknowledges European competent authorities that provided information relevant for this output, in particular the Austrian Agency for Health and Food Safety (AGES), the French Agency for Food, Environmental and Occupational Health & Safety (Anses), the German Federal Office of Consumer Protection and Food Safety (BVL), the Belgian Federal Agency for the Safety of the Food Chain (FASFC) and the Food Safety Authority of Ireland (FSAI).

Suggested citation: EFSA (European Food Safety Authority), Eskes, C., Cortiñas-Abrahantes, J., Bottex, B., Dorne, J.L.C.M., Dujardin, B., de Souza, R.F., Horvath, Z., Kouloura, E., Bordajandi, L.R., Rizzi, V., Steinkellner, H., Gilsenan, M. Rapid risk assessment on acute reference dose (ARfD) of cereulide in infants and information on acute consumption of infant formulae. EFSA Journal, 24(1), e9941, https://doi.org/10.2903/j.efsa.2026.9941

ISSN: 1831-4732

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1. Introduction

1.1. Background and Terms of Reference

Recent detections of cereulide, a heat-stable emetic toxin produced by *Bacillus cereus*, in infant formulae from multiple manufacturers have led to precautionary recalls across several EU Member States. Given the heightened vulnerability of infants to this toxin and the absence of regulatory thresholds, it is critical that Member States have a scientifically established acute reference dose (ARfD) for cereulide to inform harmonised risk management decisions.

Pursuant to the standing mandate on rapid risk assessments (RRA) (Ares (2018)4084585), DG SANTE requests EFSA to urgently conduct a RRA to:

1. Determine the ARfD for cereulide in infants, based on available toxicological data, considering infant-specific exposure and sensitivity.
2. Provide information on typical acute consumption quantities per kg body weight and the range of acute consumption quantities per kg body weight of infant formulae from EFSA's food consumption database.

EFSA is requested to finalise its RRA by 30 January 2026 and publish it no later than 2 February 2026.

1.2. Interpretation of Terms of Reference

The present RRA focuses on the acute adverse effects of cereulide and on the consumption of infant formulae and follow-on formulae. As these food categories might be replaced by more specific foods under certain medical conditions, foods for special medical purposes (FSMPs) for infants and young children were also considered.

While it is acknowledged that infant and follow-on formulae may be consumed by infants and young children, it is well known that for these food categories, infants will have the highest consumption in proportion to their body weights. Therefore, the current assessment primarily focusses on infants.

1.3. Additional information

1.3.1. Sources of contamination

Since December 2025, a number of recalls of infant formulae from the market in various countries have been notified by several countries through the EU Rapid Alert System for Food and Feed (RASFF). Contamination with cereulide, a toxin produced by *Bacillus cereus (B. cereus*), was found in infant formulae. The suspected cause of contamination is the presence of cereulide in arachidonic acid used in the infant formulae.

B. cereus is an ubiquitous Gram-positive aerobic or facultative anaerobic bacteria which can produce spores that survive adverse environments (Cui et al., 2019). It is widespread in nature and, due to its highly resistant spores, *B. cereus* is frequently found in various types of food (Arnesen et al., 2008; Dietrich et al., 2021). Some bacterial strains of the *B. cereus*, designated as emetic *B. cereus*, are able to produce cereulide, a heat-stable emetic toxin (BfR, 2020).

Cereulide is frequently found in starchy meals and is difficult to eradicate from the food chain as it is highly resistant to heat, acidity and proteolysis (Vangoitsenhoven et al., 2015; Leong et al., 2023). Cereulide can cause mild food poisoning with emetic symptoms, such as nausea, vomiting, diarrhoea and cramps, also described as "fried rice syndrome" from the first exposure to a fried rice dish contaminated with *B. cereus* (Leong et al., 2023). Cereulide has also been reported to cause acute liver failure and encephalopathy in children after consuming *B. cereus* contaminated foods (Cui et al., 2019).

1.3.2. Chemistry

Cereulide is a 36-membered cyclic dodecadepsipeptide of 1.2 kDa, consisting of three repeats of four amino acid residues [-D-O-Leu-D-Ala-L-O-Val-L-Val-]$_3$. It is highly lipophilic (log $K_{OW} \geq 6$), heat-stable, and is part of the surfactins family of peptides composed of a lipid tail linked to a cyclic oligopeptide (Agata et al., 1994; Arnesen et al., 2008; Cui et al., 2019; Marxen et al., 2015). **Figure 1** depicts the chemical structure of cereulide.

Cereulide is synthesised via non-ribosomal peptide synthesis system, encoded by the plasmid mediated cereulide synthetase (*ces*) gene cluster (Makarasen et al., 2009; Cui et al., 2019). Marxen et al. (2015) reported a series of at least 18 cereulide variants identified through mass spectrometric screening and ^{13}C labelling experiments, among which isocereulides A-G were determined for the first time. The data demonstrated a high microheterogeneity in cereulide and showed evidence for a relaxed proof-reading function of the non-ribosomal cereulide peptide synthetase complex giving rise to an enhanced cereulide chemodiversity.

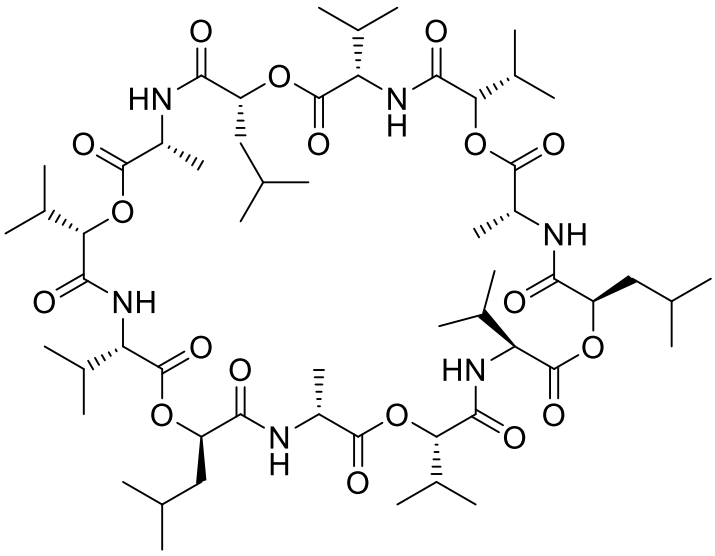

Figure 1. Chemical structure of cereulide.

Cereulide is non-immunogenic and not inactivated by proteolytic enzymes. No enzyme in the human body is known to detoxify cereulide. It is also highly resistant to protease activity (pepsin and trypsin) and is not inactivated during the gastrointestinal passage (Ceuppens et al., 2011, 2012). Cereulide can act as a cation ionophore to disrupt the electrochemical potential gradient on lipid membranes, which may affect mitochondrial function (Makarasen et al., 2009).

1.3.3. Analytical methods

The detection of cereulide is performed either by identifying the toxin itself or the *B. cereus* strains that produce it. There are three types of methodologies reported in the literature, that are often used to detect or quantify cereulide in food samples or human specimens (Arnesen, 2008).

Polymerase Chain Reaction (PCR) screening assays are often used to detect the cereulide producing *B. cereus* strains. These assays target the *ces* gene cluster, which encodes the non-ribosomal peptide synthetase responsible for the biosynthesis of cereulide. While PCR does not measure cereulide itself, it is a robust preliminary tool to identify cereulide producing strains.

A number of **biological assays** have been used to detect cereulide based on its cytotoxic or mitochondrial effects. The HEp-2 cell vacuolation assay is based on the ability of cereulide to cause vacuolation in HEp-2 epithelial cells. The boar sperm biological assay is based on the inhibition of boar sperm motility due to the mitochondria-damaging activity caused by cereulide. The rat liver mitochondria bioassay is developed based on the ability of cereulide to uncouple mitochondrial respiratory activity, this method can provide an estimation of the concentration of cereulide.

The most accurate method for the detection of cereulide is **liquid chromatography tandem mass spectrometry (LC-MS/MS)**, which is used as the reference method for regulatory or clinical interpretation.

An ISO method (18465:2017) provides a standardised LC-MS/MS procedure for the quantification of cereulide in food products (ISO, 2017). The protocol involves extraction of the toxin from food using extraction by acetonitrile, addition of a labelled $^{13}C_6$-cereulide internal standard, with optional extraction for fatty matrices. The MS detection is performed in positive mode, monitoring a specific MRM (multiple-reaction monitoring) transition. An internal-standard calibration curve is used for the quantification. The method has been validated for a number of food matrices (rice, fried rice dish, cream pastry with chocolate, hotdog sausage, mini pancakes, vanilla custard and infant formula) and is considered as fit for purpose (in 't Veld et al., 2019).

Recent findings report that when powder food products intended for infants or young children become contaminated with ingredients containing cereulide, the extraction yields obtained during direct acetonitrile extraction of the powder are considerably low. Prior hydration of the dry matrix has been reported to be an essential step for efficient extraction and to obtain reliable results.[1]

Moreover, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) methods have been developed (Ulrich et al., 2019; Doellinger et al., 2020) which are expected to analyse both the bacteria and the cereulide toxin. However, this method has a limited applicability due to the challenges to obtain an adequate sensitivity for the analysis of cereulide (Koike et al., 2024).

[1] https://favv-afsca.be/sites/default/files/Labo/communicatie/1849808_Analyse_bacillus_cereus_toxinPUB_fr-nl.pdf (Accessed on 30.01.2026)

2. Data and methodologies

2.1. Hazard identification and characterisation

An extensive literature search was conducted on 27 January 2026 with no time restriction, to identify primary research studies, reviews and other information relevant to the present RRA. The bibliographic databases searched were PubMed and Web of Science (all databases). Broad keywords were used to comprehensively capture in vitro and in vivo toxicity studies and human observations, namely only 'cereulide' OR its CAS no. '157232-64-9'. Eligibility criteria for study selection included the availability of an English language abstract.

From the above literature search, a total of 470 studies were identified after removal of duplicates. The titles and abstracts of these studies were screened. Following such screening a total of 87 studies were selected for full text screening. The selection for inclusion or exclusion of the full text of scientific studies was based on consideration of the extent to which the study was relevant to the present rapid risk assessment by applying expert judgement and by taking into consideration the study characteristics (e.g. study design, methodology, endpoint, dosing).

The general principles of the risk assessment process for chemicals in food as described by WHO/IPCS (2020) were applied, which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition, EFSA guidance documents pertinent to risk characterisation were applied.

BMD analyses were carried out following EFSA Guidance (EFSA Scientific Committee, 2022) and the Bayesian BMD Modelling web-app[2] which is available at the EFSA R4EU platform[3] was used.

2.2. Dietary consumption data

The EFSA Comprehensive European Food Consumption Database (EFSA Comprehensive Database) provides a compilation of existing national information on food consumption at individual level and was first built in 2010 (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in its EFSA Guidance (EFSA, 2011). The most recent version of the database, updated and published in June 2025, includes 14 dietary surveys carried out across EU Member States covering infants.[4] Together, the surveys provide dietary data for 5,059 participants and 15,541 consumption days.

Data on the consumption of infant formulae and follow-on formulae, both solid and liquid forms, were extracted from the Comprehensive Database. Solid (powder) formulae were converted to liquid forms (ready for feeding), by using a dilution factor of 8 as recommended based on information extracted from the Global New Products Database and on factors previously used by EFSA (EFSA, 2018).

Acute food consumption was estimated by selecting the survey days on which infant formulae or follow-on formulae were consumed and calculating the total consumption per kilogram of body weight for each type of formulae within a single day. In accordance with the EFSA Guidance on the risk assessment of substances present in food intended for

[2] https://zenodo.org/record/7334435#.Y5osYXbMLD4

[3] https://r4eu.efsa.europa.eu

[4] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

infants below 16 weeks of age (EFSA Scientific Committee, 2017), the group of infants was stratified as follows: i) infants below 16 weeks of age; and ii) infants from ≥ 16 weeks to < 12 months of age. The resulting consumption distribution was then used to calculate the mean and the 95th percentile for each type of formulae, survey and age group.

3. Assessment

3.1. Hazard identification and characterisation

3.1.1 Hazard identification

3.1.1.1. Adverse effects in experimental animals

In Agata et al. (1995), groups of 5 male adult Asian house shrews (*Suncus murinus*) were given single oral doses of 4, 8, 16 and 32 µg cereulide/kg bw. Emesis was monitored for 120 min. While at the lowest dose no animals vomited, at 8 µg/kg bw one, at 16 µg/kg bw three and at 32 µg cereulide/kg bw five out of five animals vomited. It is noted that in this study a negative control group was not used, the group size was rather small, only male animals were tested, the observation period after dosing is short, and no other parameters were reported. In addition, an experiment with intraperitoneal (i.p.) application of cereulide is reported in the publication, but as this route of exposure is considered not relevant for the present assessment, the results are not further discussed here.

In Shinagawa et al. (1995), a group of 3 Rhesus monkeys (sex not specified) received a single intragastric (i.g.) dose of 70 µg of cereulide per animal, and all animals showed emesis between 2 and 4 h after treatment. The weight of the animals was not reported and therefore a dose per kg bw cannot be established. In addition, only a single dose was tested and therefore the results cannot be used for a dose-response assessment. This publication also contains results from single i.p. and intravenous (i.v.) exposure of cereulide to mice but as this route of exposure is not relevant for the present assessment these are not further discussed here.

In Shinagawa et al. (1996), groups of Asian house shrews (*Suncus murinus*) were given single oral doses of 0, 10, 20 and 40 µg cereulide (corresponding to 0, 200, 400 and 800 µg cereulide/kg bw, assuming a weight of 50 g per animal which is the average weight from the weight range reported by the authors (50-70 g for males and 35-45 g for females)). In the control and low dose groups 0 out of 2 animals had emesis, while in the mid dose group one out of 3, and in the high dose group 3 out of 3 animals showed emesis. It is noted that the sex of the animals was not reported and that the very small group size renders any dose-response assessment very uncertain. Therefore, these results were not considered for dose-response analysis. In this publication also, experiments with i.v. and i.p. exposure of mice and i.p. exposure in Asian house shrews are reported, but since these routes of exposure are not relevant for the present assessment the results are not further discussed here.

In Yokoyama et al. (1999), male BALB/c mice were i.p. injected with single doses of cereulide. The route of exposure is considered not relevant for the present assessment, thus the results are not discussed further in the present assessment.

Bauer et al. (2018) reported results from four different experiments with Large White piglets. Groups of piglets (4 treated, 1 control each) received single oral doses (given with milk) of 10, 30 and 150 µg cereulide/kg bw in the three acute trials, or daily 10 µg cereulide/kg bw over a period of 7 days ("chronic" trial). In the acute trials, no effect on

blood cell composition or biochemical markers were seen, while creatine kinase increased at both doses of 30 and 150 µg/kg bw. In the chronic trial, creatinine kinase increased and blood cell populations remained stable. A series of individual observations of neurological effects such as tremor, seizures and convulsions (often most pronounced in the high-dose group) were reported from the acute studies. Because detailed analyses are mainly presented from the high dose acute group and the study consists of four individual single dose group experiments, the study was not considered for dose-response assessment.

In Lin et al. (2021), groups of male BALB/c mice received one i.g. daily dose of 50 µg cereulide/kg bw for 28 days. Repeated exposure to cereulide induced intestinal inflammation, decreased food intake, changes in gut microbiota and suppression in serotonin synthesis. Since the results (control + one dose experiment) cannot be used for dose-response assessments, these are not discussed further in the present assessment

Li et al. (2021) administered via gavage doses of 0, 10, 50 and 200 µg cereulide/kg bw to groups of 6 specific pathogen-free male BALB/c mice (sex not specified) for 28 days. The authors reported that at 50 and 200 µg cereulide/kg bw oxidative stress and abnormal expression of inflammatory factors were observed. The authors also reported reduced body weight at these doses. At the highest dose, reduced relative liver and increased relative kidney and spleen weights were reported. Tentative dose-response analyses with the data on bw and relative organ changes (data not shown) were performed by EFSA for this assessment. However, the data on bw changes have not been presented in numerical form and had to be extrapolated from a graph with low resolution and the number of animals in the experiments was unclear: together these factors made the analysis uncertain. Reliable dose-response analyses could not be obtained for the decreased relative liver weights and the increased relative kidney weights, leaving the increased relative spleen weights seen at high dose only as a single finding with unclear biological relevance. Therefore, the results have not been considered for dose-response assessment.

Taking into account all available information, the results on emesis from the oral study of Agata et al. (1995), despite the limitations in the study design, were considered as the most appropriate for deriving an ARfD for cereulide.

3.1.1.2. Observations in humans

In the literature, observations in humans were limited to case reports, case series and outbreak reports, where cereulide producing *B. cereus* caused a number of adverse effects ranging from mild symptoms in the gastrointestinal tract, such as nausea and vomiting, to fulminant liver failure, with rare need for transplantation. Cereulide poisoning is characterised by the usually rapid onset of gastrointestinal effects, including mainly nausea and vomiting and often these cases are not reported. More severe intoxication cases involve acute liver failure, massive rhabdomyolysis, acute kidney injury and/or encephalopathy. Rare cases of deaths have been reported. Multiple emetic *B. cereus* outbreaks affected children and adults were primarily linked to foods based on rice, pasta, dairy, and ready-made meals. **Table 1** describes the case reports of cereulide poisoning, providing information on the contaminated food and details on the detection methods and concentration of cereulide, when available.

Table 1. Case reports on cereulide poisoning.

Subject (Age & Sex)	Year & Country	Symptoms	Exposure Information	Cause of Poisoning & Cereulide Measurement	Study
48-year-old female	**2023, Switzerland**	Emesis, diarrhoea, chills, abdominal cramps; later severe liver dysfunction, **rhabdomyolysis, acute liver failure**	Rice salad containing ham & salmon, stored improperly at 8°C for 3 days	WGS confirmed ***ces***, *nhe*, *sph* genes; **no cereulide quantification** performed	Chatelanat et al. (2024)
202 affected people of mixed ages	**2021, India**	Vomiting; abdominal pain; few of them had also diarrhoea	Fried rice served at a public gathering, cooked the previous day and kept at room temperature	***ces*** gene detected by PCR; **no cereulide quantification** performed	Saikia et al. (2024)
19-year-old female	Year unspecified, Country unspecified	1-week fever, cough, diarrhoea, nausea/vomiting; **acute fulminant hepatitis**	Exposure not linked to food	No cereulide measurement; diagnosis based on stool positive for *B. cereus*	Korani et al. (2024, meeting abstract)
11-year-old girl (severe case) and 13-year-old sister (mild case)	**2022, France**	Rapid vomiting, abdominal pain; **liver failure, pancreatitis, rhabdomyolysis, acute kidney injury** (multi-organ failure); cerebral edema and **hepatic encephalopathy**	Leftover lasagna stored in faulty refrigerator during heatwave (40°C); 11-year-old ate an entire platter; 13-year-old ate a few bites	Cereulide and nonhemolytic enterotoxin detected in stool; method not reported, reference to the HEp-2 cells vacuolation assay	Thery et al. (2022)
25-year-old male; four ~22-year-old female coworkers	**2021, Austria**	All: Nausea/vomiting, diarrhoea; 25-year-old male: **acute liver failure, acute kidney injury**, mild ARDS, **hepatic encephalopathy**	Fried rice balls made from rice stored 3 days at 8–9°C; 25-year-old male ate twice whereas the rest only once	*Bacillus cereus* (8 x 10^6 cfu/g foodstuff); **cereulide quantified:** 37 ± 11 µg/g in food; 7 ± 1 ng/mL serum; 67 ± 7 ng/mL urine (SIDA-LC-MS/MS)	Schreiber et al. (2022)
13-month-old male	**2015, Germany**	Vomiting, abdominal pain, and increasing somnolence; **fulminant liver failure, rhabdomyolysis** and **kidney failure** (multi-organ failure) → **liver transplant**	2-day-old rice meal stored in refrigerator	Cereulide-producing *B. cereus* detected in rice grain via PCR + cytotoxicity assay on HEp-2 cells; **no cereulide quantification** performed	Tschiedel et al. (2015)
20 children aged 10–18 months	**2012, Belgium**	Profuse vomiting within 30 min; no diarrhoea, no fever	Mashed rice-cucumber-chicory meal; rice cooked day before & cooled improperly	**Cereulide measured:** 3.1–4.2 µg/g in food; 0.350 µg/g in vomit (LC-MS/MS)	Delbrassinne et al. (2015)

Subject (Age & Sex)	Year & Country	Symptoms	Exposure Information	Cause of Poisoning & Cereulide Measurement	Study
Mixed ages (children & adults; multiple outbreaks)	**2007–2013, Germany**	Vomiting within 0.5–6 h	Various meals: pasta, cheese, rice dishes, poultry, potatoes	**Cereulide quantified** in selected samples: e.g., 2 µg/g cheese, 1 µg/g rice, 0.3 µg/g chickpeas	Messelhäusser et al. (2014)
46 cases: children (2–6 years) + 3 adults	**2007, Germany**	Vomiting, abdominal pain; median incubation 4 h	Rice pudding served warm from multiple pots; inadequate cleaning; electricity interruption during heating	No cereulide detected in the only vomit sample; *ces* negative isolate	Wambo et al. (2011)
20-year-old male	**2008, Belgium**	Headache, abdominal pain, nausea; profuse vomiting for several hours; watery diarrhoea; **sudden death** ~10 h after the meal	Leftover spaghetti with tomato sauce, prepared 5 days earlier, kept at room temperature, reheated in microwave	Emetic *B. cereus* isolated; pasta 9.5×10^7 CFU/g; **cereulide quantified** at 14.8 µg/g (LC–MS/MS)	Naranjo et al. (2011)
11-year-old male	**2010, Japan**	Abdominal cramps and vomiting 30 min after meal → drowsiness, convulsions, severe consciousness disturbance; **liver failure severe brain edema/ encephalopathy** and systemic organ damage	Fried rice, prepared the day before and refrigerated	*B. cereus* cultured from gastric fluid, stool, and fried rice; cereulide produced by isolates (PFGE confirmed food–patient match); **no cereulide quantification** performed	Ichikawa et al. (2010)
1-year-old boy 2-year-old girl 26-year-old mother	**2008, Japan**	Vomit, onset 30 min post meal 1-year-old boy: **encephalopathy, death**	Reheated fried rice	Cereulide detected in serum in both children	Shiota et al. (2010)
Outbreak: 37 exposed; 24 symptomatic	**2005, Korea**	Vomiting, headache, abdominal pain; onset 1–2 h post-meal	Fried rice at a cafeteria; others ate plain cooked rice (no symptoms)	Emetic *B. cereus* detected in vomit; *ces* positive by PCR; cereulide detected by LC–MS (qualitative; **no concentration reported**)	Kim et al. (2010)
9-year-old female (plus an adult friend with mild illness)	**2008, Switzerland**	Severe emesis, prolonged seizures; shock; **fulminant hepatitis**; renal and pancreatic insufficiency	Reheated pasta with sauce, prepared ~48 h earlier	*B. cereus* isolated from the pasta; strain *nhe* and *ces* positive by PCR (genotypic confirmation of cereulide-producing strain); **no cereulide quantification** performed	Pósfay-Barbe et al. (2008)

Subject (Age & Sex)	Year & Country	Symptoms	Exposure Information	Cause of Poisoning & Cereulide Measurement	Study
Five children (7 - 14 years)	**2003, Belgium**	All: vomiting, elevated liver enzymes; 7-year-old girl: pulmonary haemorrhage; **fulminant liver failure, death** ~13 h after the meal	Reheated pasta salad, prepared ~3 days earlier	*B. cereus* counts in pasta salad: 10^7–10^8 CFU/g; stain *ces* positive by PCR in isolates from food and vomit; **no cereulide quantification** performed	Dierick et al. (2005)
17-year-old male Adult father	Year unspecified**, Switzerland**	Nausea, vomiting; 17-year-old male: **fulminant liver failure**, **rhabdomyolysis, acute renal failure, brain edema, death**	Reheated pasta with pesto, prepared ~4 days earlier	Cereulide detected in pan residue and human specimens by HEp-2 cell assay	Mahler et al. (1997)

Abbreviations: ARDS: Acute Respiratory Distress Syndrome; *ces*: cereulide synthetase**;** CFU: Colony Forming Unit; HEp2: Human Epithelial type 2; *nhe*: nonhemolytic enterotoxin; PFGE: Pulsed-Field Gel Electrophoresis, PCR: Polymerase Chain Reaction; SIDA-LC-MS/MS: Stable Isotope Dilution Assay-Liquid Chromatography-tandem Mass Spectrometry; *sph*: sphingomyelinase; WGS: Whole Genome Sequencing.

Overall, the human data did not provide sufficient information for dose-response analysis. However, the human case studies show that nausea and vomiting are the most common and frequently reported early effects of cereulide poisoning supporting emesis seen in shrews upon acute exposure as the critical endpoint for the hazard characterisation of cereulide.

3.1.1.3. In vitro toxicity studies

The in vitro studies identified in the literature search addressed the effects of cereulide on different cell types, including intestinal cells, liver cells, pancreas cells, sperm cells and other cell types as described here after.

Regarding intestinal cells, Beisl et al. (2021) showed cereulide (10 and 100 ng/mL, 24 h treatment) to alter the barrier function of differentiated human Caco-2 cells cultured in a transwell system, by increasing the transepithelial electrical resistance and increasing the expression of the tight junction protein claudin-4. In another study, Beisl et al. (2020) showed statistically significant cytotoxicity of cereulide in Caco-2 cells (measured by Neutral Red assay with 0.1, 1, 10, 25, 50 and 100 ng/mL) observed at concentrations equal or higher than 10 ng/mL after 24 h and 48 h treatment, and 2.5 ng/mL after 72 h treatment. No cytotoxicity was observed at any concentration following 5 h treatment. Following 5 h treatment with 25 ng/mL cereulide, Interleukin-1β (IL-1β) relative gene transcription and IL-8 secretion significantly decreased, whilst tumour necrosis factor (TNF)-alpha relative gene transcription significantly increased, suggesting that cereulide influences the immune response of differentiated human intestinal Caco-2 cells. Decleer et al. (2018) showed mitochondrial impairment in Caco-2 cells exposed to cereulide, reflected by a reduction of maximum cell respiration. Rajković et al. (2014) showed a statistically significant decrease of mitochondrial activity (MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay) and protein content (sulforhodamine B assay), following a three-day treatment (0.062, 0.125, 0.25, 0.5, 1, 2 and 4 ng/mL) with cereulide concentrations equal or higher than 1 and 0.125 ng/mL, respectively. Cell counts showed cells were released from the differentiated monolayer at 0.5 ng/mL of cereulide.

Additionally, 2 ng/mL of cereulide increased the lactate presence in the cell culture medium. Proteomic data showed that 1 ng/mL cereulide led to a significant decrease in energy managing and H_2O_2 detoxification proteins and to an increase in cell death markers. These findings suggest that low doses of cereulide alter the metabolism of differentiated Caco-2 cells. Finally, Jääskeläinen et al. (2003) showed that the lowest effective concentration producing visible mitochondrial membrane potential effects in Caco-2 cells exposed for 5–20 min at 37°C, was 10–20 ng/mL suspension (2-5 x 10^6 cells/mL).

Regarding liver cells, Beisl et al. (2022) showed statistically significant cytotoxicity of cereulide in HepG2 cells (measured by Neutral Red assay with 0.1, 1, 2.5, 5, 10 and 50 ng/mL) observed at concentrations equal or higher than 2.5 ng/mL after 5 h treatment, and 1 ng/mL after 24 h treatment. The authors suggested that cereulide induces autophagy via the LC3 pathway via which damaged mitochondria might be eliminated. Li et al. (2021) showed that cereulide induces reactive oxygen species (ROS) accumulation, which caused endoplasmic reticulum (ER) stress, leading to apoptosis in HepaRG hepatic cells. Similar effects were observed with the human embryonic kidney HEK-293 cells. Decleer et al. (2018) showed mitochondrial impairment in HepG2 cells exposed to cereulide, reflected by a reduction of maximum cell respiration.

Two studies investigated the effects of cereulide on pancreas cells. Hoornstra et al. (2013), investigated among others, the effects of cereulide on a mouse pancreatic insulinoma cell line (MIN6). Upon exposure to cereulide, the pancreatic islets (MIN6) disintegrated into pyknotic cells, followed by necrosis, at concentrations (10 to 20 ng/mL) two log units below that required for other tested somatic human, porcine, or murine cell death. The authors showed a decreased relaxed permeability to propidium iodide in all exposed cells following: a 20 min treatment with concentrations higher than 14 ng/mL cereulide, a 8 h treatment with 13 ng/mL cereulide, and a 24 h treatment with doses equal or lower than 7 ng/mL cereulide. In addition, loss of mitochondrial membrane potential was observed in all exposed cells following: a 20-min treatment with 14 ng/mL cereulide, an 8-h treatment with 1 ng/mL cereulide, and a 24-h treatment with 2 ng/mL cereulide. Virtanen et al. (2008) investigated the effects of cereulide on fetal porcine Langerhans islets in culture. Exposure to 1 ng/mL cereulide caused necrotic cell death of the islet cells impairing their insulin content within 2 days.

A number of studies suggest the use of a sperm bioassay to detect cereulide. Andersson et al. (1998) showed a 50% effective loss of motility of boar spermatozoa upon 24 h exposure to 0.5 ng of purified cereulide per mL of extended boar semen. In another study, Andersson et al. (2004), showed loss of sperm motility after 5 min exposure at 0.3 +/- 0.1 ng of purified cereulide (per assay 5.4 x 10^6 sperm cells in 0.2 mL). Jääskeläinen et al. (2003) showed visible mitochondrial membrane potential effects in boar spermatozoa treated for 5–20 min at 37°C, at 2 ng/mL cereulide (for 27 × 10^6 cells/mL), whereas bull sperms were 100 times less sensitive to cereulide than boar sperms, with an effective concentration of 100–1000 ng/mL suspension (27 × 10^6 cells/mL). The authors also showed that boar sperm cells were equally sensitive to cereulide as compared to other investigated human cell lines (i.e. HeLa, Caco-2, Calu-3 and Paju) regarding the loss of mitochondrial membrane potential. Finally, Hoornstra et al. (2013), showed loss of mitochondrial membrane potential of porcine spermatozoa treated for 20 min and 24 h to 7 and 1 ng/mL of purified cereulide. Furthermore, the authors mention that purified cereulide induced an efflux of K^+ from porcine sperm cells, representing ca. 10% depletion of the cellular stores within 10 min.

In a study comparing the effects of cereulide on different cell types, Hoornstra et al. (2013), compared the effects of cereulide on the mitochondrial membrane potential and on the permeability to propidium iodide on different cell types including human peripheral blood mononuclear cells (PBMC), keratinocytes (HaCaT), kidney cells (PK-15), fibroblast (L-292) as well as on porcine spermatozoa and insulinoma MIN6 cells as described above. Andersson et al. (2007) investigated the effects of *B. cereus* extracts, which contained measured amounts of cereulide, on HepG2 liver cell line, Hepa-1 hepatoma cell line, Hep-2 epithelioma cell line, boar spermatozoon, *S.* Typhimurium and *V. fischeri*. However, it is unclear whether the *B. cereus* extracts contained other potential toxins. Teplova et al. (2004) showed that human neural crest-derived tumor cell line Paju were more sensitive to cereulide effects on their mitochondrial membrane potential, when cells were undifferentiated as compared to when cells were differentiated. Finally, Jääskeläinen et al. (2003), compared the lowest cereulide concentration producing visible effects on the mitochondrial membrane potential of four human cell lines (Caco-2, Paju, HeLa (cervical cancer) and Calu-3 (lung)) as well as boar and bull spermatozoa. The authors concluded that human cells and boar sperm were equally sensitive to cereulide, while boar sperms were 100 times more sensitive to cereulide than bull sperms.

3.1.1.4. Mode of action

As a highly stable and liphophilic cyclic dodecadepsipeptide toxin, cereulide is highly resistant to heat, pH changes as well as proteolysis. These characteristics allow the structure of the toxin to resist cooking and digestive enzymes. Cereulide is absorbed into the blood across the intestinal tract and distributed throughout the systemic circulation as a liphophilic compound. A toxicokinetic study available in rabbits after 5 µg i.v. injection reports that the maximum plasma concentration is achieved in 2.6 h with an elimination half-life of 10.2 h and a primary route of excretion in the faeces with significant amounts measured within 48 h (Cui et al., 2016). As described above, it has been reported to cause acute failure of a number of organs and systems including liver, brain, pancreas, kidney, testis, necrosis of colon mucosa and intestinal flora and immune system (Rajković et al., 2014; Yang et al., 2023).

On an acute basis, cereulide binds to 5-HT3 receptors in the stomach and the small intestine resulting in the suppression of mitochondrial activity via the fatty oxidation pathway, stimulation of the afferent vagus nerves which sends immediate signals to the vomiting centre in the medulla oblongata triggering the emetic effects (0.5-6h) (Yang et al., 2023). Recently, Huo et al. (2024) constructed a single-nucleus transcriptomic atlas of neurons from the nucleus of the solitary tract involved in brain defensive responses from toxins and highlighted that cereulide can directly modulate vagal sensory neurons inducing retching-like behaviour as involuntary chest and stomach spasms without producing vomit.

At the cellular level, cereulide acts primarily as a potent mitochondrial toxin disrupting its membrane potential and as a potassium ionophore. Such effects lead to cell damage and apoptosis, in many cell types particularly neurons, gastrointestinal cells, hepatocytes, immune and pancreatic cells (see above). In immune cells, inhibition of cytotoxicity on bacteria and cytokine production of natural killer cells, causes swelling of mitochondrial leading to apoptosis which has also been shown in pancreatic beta cells and hepatocytes (Paananen et al., 2002; Vangoitsenhoven et al., 2014, 2015; Yang et al., 2023). Beta cells are particularly sensitive since at low doses mitochondrial activity and insulin secretion is impaired leading to cell death, and these are both key traits in the pathophysiology of diabetes (Vangoitsenhoven et al., 2015). Fulminant hepatitis has been reported in humans and results from inhibition of mitochondrial oxidation of long-chain fatty acids resulting in

microvesicular steatosis (Mahler et al., 1997). Decleer et al. (2018) observed mitochondrial dysfunction after 10 days of 0.25 nM exposure to cereulide in both Caco-2 and HepG2 cells and reflected through reduction of maximal cell respiration, reduced ATP production and proton leaks. At 0.50 nM cereulide, mitochondrial respiration was almost completely shut down, especially in HepG2 cells (Decleer et al., 2018). Mitochondrial toxicity and potassium ionophoric properties have been shown to cause swelling, uncoupling oxidative phosphorylation forming highly selective adducts with K+ as shown in rat mitochondria and neurons (Teplova et al., 2006; Saris et al., 2009; Mikkola et al., 1999).

Overall, cereulide acts as a cation ionophore disrupting the electrochemical potential gradient on lipid membranes, which impairs mitochondrial function. The main mode of action of cereulide involves binding to the 5-HT3 receptor in the stomach and small intestine, impairing mitochondrial activity and stimulating the vagus nerve leading to emesis. At the cellular level, cereulide impairs mitochondrial activity through its potassium ionophoric properties and inhibits the fatty acid oxidation pathway leading to cell death in a number of cell types including hepatocytes, gastrointestinal cells, Beta pancreatic cells, neurons and natural killer cells. These effects can lead to fulminant liver failure and encephalopathy in children after consuming *B. cereus* contaminated foods as reported in **Section 3.1.1.2** on observations in humans.

3.1.2 Hazard characterisation

3.1.2.1. Previous hazard characterisation

A previous hazard characterisation of cereulide available in the public domain has been performed by the Dutch National Institute for Public Health and the Environment (RIVM) by Wijnands et al. (2013), which used the Agata et al. (1995) study discussed above under adverse effects in experimental animals (Section **3.1.1.** on Hazard identification). The RIVM assessment used BMD modelling for the quantal dose-response data in the adult Asian house shrews (*Suncus murinus*) using the PROAST software and EFSA's earliest guidance on benchmark dose modelling using a frequentist approach (EFSA, 2009). A benchmark response of 10% for the quantal data reporting frequency of emesis in the shrews was used and resulted in a $BMDL_{10}$ of 3 µg/kg bw. An ARfD of 0.03 µg/kg bw for humans was then derived applying the UF 100 accounting for interspecies differences (10) and human variability (10). It is noted that this value for the ARfD was not specifically derived for cereulide present in food for infants below 16 weeks.

3.1.2.2. Consideration of critical effects, dose-response modelling and derivation of an ARfD

In this rapid risk assessment, EFSA reviewed the available toxicological data in animals, human observations, in vitro evidence and the mode of action of cereulide (see **Section 3.1.1** on Hazard identification). Overall, EFSA concludes that the dose-response data from the *in vivo* toxicological data is relatively scarce, and that, in the current state of knowledge, the Agata et al. (1995) study is still the most appropriate dataset for BMD modelling. It should be highlighted however that the Agata et al. (1995) study did not include information about negative control. The BMD modelling was performed using the Bayesian approach recommended in the EFSA guidance on BMD modelling (EFSA Scientific Committee, 2022) considering extrapolation of the average model to estimate background probability of emesis. A BMR of 10% was used as an increased odds in emesis from the background probability and resulted in a $BMDL_{10}$ of 4.2 µg/kg bw. For more details of the EFSA BMD modelling of cereulide see **Appendix 1**.

Considering the limited number of animals in each dose group (n = 5), and the absence of control group, it is clear that extrapolation below the lowest dose considered in the experiment is subject to uncertainty, with a default prior for the background probability of emesis ranging from 0 to 0.81. Acknowledging that, in the absence of exposure to cereulide, the probability of emesis would be closer to zero rather than to 81%, a second analysis was performed using an informative prior distribution for the background parameter. Consequently, the resulting $BMDL_{10}$ was reduced by 27% to 3.08 µg/kg bw (vs. 4.2 µg/kg bw). This difference is considered minor when compared to the conservative uncertainty factors applied to the $BMDL_{10}$ to derive the ARfD in this context.

An ARfD of 0.014 µg/kg bw was derived by applying the UF of 100 to account for interspecies differences (10) and human variability (10), and the additional UF of 3 recommended in the EFSA guidance on infants below 16 weeks of age to allow for differences in xenobiotic metabolism and renal excretion between adults and infants. The rationale for the additional UF of 3 is also recommended as a relevant and conservative approach when the metabolic or renal excretion pathway is unknown (EFSA Scientific Committee, 2017).

3.2. Acute dietary consumption

3.2.1. Infant formulae

Acute consumption estimates for infant formulae, obtained from the EFSA Comprehensive Database (see **Section 2.2**), are reported in **Table 2**.

Table 2. Acute consumption of infant formulae (liquid)[(a)] in infants from the EFSA Comprehensive Database.

Country	Survey	Total days	Consumption days	Mean [(b)] (mL/kg bw)	P95 [(c)] (mL/kg bw)
Infants 0-4 months					
Bulgaria	NUTRICHILD	553	276	148	265
Croatia	NIPNAD-2017-2021	34	16	94	.
Cyprus	CY 2014-2017-LOT1	285	182	148	262
Estonia	DIET-2014-EST-C	22	6	81	.
France	INCA3	95	69	178	264
Italy	IV SCAI CHILD 2017-2020	16	8	156	.
Latvia	LATVIA_2014	130	31	149	.
All countries, all surveys		**1177**	**592**	**150**	**264**
Infants 4-12 months					
Bulgaria	NUTRICHILD	1167	477	68	157
Croatia	NIPNAD-2017-2021	610	127	83	133
Cyprus	CY 2014-2017-LOT1	510	181	72	142
Denmark	IAT 2006-07	5771	3396	39	89
Estonia	DIET-2014-EST-C	991	115	59	125
Finland	DIPP 2001-2009	1500	838	57	106
France	INCA3	101	22	114	.
Germany	VELS	927	136	33	76
Italy	IV SCAI CHILD 2017-2020	289	21	81	.

Country	Survey	Total days	Consumption days	Mean (b) (mL/kg bw)	P95 (c) (mL/kg bw)
Latvia	LATVIA_2014	293	100	63	151
Poland	EU-MENU PL LOT1	564	28	84	.
Portugal	IAN-AF 2015-2016	491	81	68	168
Slovenia	SI.MENU-2018	578	189	48	107
Spain	ENALIA	572	23	33	.
All countries, all surveys		**14364**	**5734**	**48**	**109**

(a): Infant formula powder transformed into liquid by means of a ratio of 1:8.
(b): Mean estimates based on dietary surveys/population groups with less than 5 consumers may not represent the population group and are thus not included in this table.
(c): 95th percentile estimates based on dietary surveys/population groups with less than 59 observations may not be statistically robust and were not included in this table (Meeker et al., 2017).

The Comprehensive Database includes a limited number of surveys covering infants younger than 4 months, because this age group is particularly difficult to capture in dietary surveys due to practical challenges related to their recruitment and participation. In line with these constraints, the EFSA EU Menu Guidance recommends initiating data collection from infants aged 3 months onwards (EFSA, 2014).

The acute P95 consumption estimates for infants below 16 weeks of age derived from the Comprehensive Database are aligned the high consumption value of 260 mL/kg bw per day recommended by the EFSA Scientific Committee for dietary exposure assessment of infants below 16 weeks of age via infant formulae (EFSA Scientific Committee, 2017). Despite the fact that this value was derived for repeated exposure, the EFSA Scientific Committee noted that, for very young infants, feeding practices are highly regular and consumption volumes show limited intra-individual variability. This consumption value would therefore also cover acute toxicity and consider potential periods of high sensitivity for other toxicity endpoints.

For the 4–12-month age group, acute consumption estimates expressed per kilogram of body weight are generally lower than those observed for infants younger than 16 weeks. These lower estimates are partly attributable to the significant increase in body weight that occurs between 4 and 12 months. In addition, over this period infants progressively shift from an infant formulae-based (or human milk-based) diet to a more diversified diet. As complementary foods are introduced, daily consumption volumes of infant formulae become more variable and generally tend to decrease as infants approach 12 months of age.

It is therefore concluded that, for consumption of infant formulae, infants below 4 months of age represent the most critical age group, as they have the highest infant formulae intake relative to body weight and may be exclusively fed on infant formulae. Therefore, the high consumption value of 260 mL/kg bw per day proposed by EFSA's Scientific Committee is considered the most appropriate for estimating acute exposure to cereulide via infant formulae.

3.2.2. Follow-on formulae

There were not sufficient data to reliably estimate the consumption of follow-on formulae in the 0–4-month age group, because only 23 consumption days were reported in the Comprehensive Database. This limited data availability is consistent with the expectation that follow-on formulae are generally consumed after 6 months of age and some of the

records reported in this younger age group may also result from misreporting of infant formulae.

Acute consumption estimates for follow-on formulae by infants between 4 and 12 months (see **Table 3)** are comparable to those for infant formulae in the same age group. These results align with the growth-related changes and the dietary transition already outlined for infant formulae (see **Section 3.2.1**).

Table 3. Acute consumption of follow-on formulae (liquid)[(a)] in infants from the EFSA Comprehensive Database

Country	Survey	Total days	Consumption days	Mean [(b)] (mL/kg bw)	P95 [(c)] (mL/kg bw)
Bulgaria	NUTRICHILD	1167	8	64	.
Croatia	NIPNAD-2017-2021	610	133	59	115
Cyprus	CY 2014-2017-LOT1	510	226	60	126
Denmark	IAT 2006-07	5771	1196	29	61
Estonia	DIET-2014-EST-C	991	224	53	107
Finland	DIPP 2001-2009	1500	33	91	.
France	INCA3	101	59	61	130
Germany	VELS	927	434	37	76
Italy	IV SCAI CHILD 2017-2020	289	131	45	87
Poland	EU-MENU PL LOT1	564	244	71	142
Portugal	IAN-AF 2015-2016	491	249	48	105
Slovenia	SI.MENU-2018	578	30	43	.
Spain	ENALIA	572	475	50	90
All countries, all surveys		**14364**	**3442**	**44**	**98**

(a): Infant formula powder transformed into liquid by means of a ratio of 1:8.
(b): Mean estimates based on dietary surveys/population groups with less than 5 consumers may not represent the population group and are thus not included in this table.
(c): 95th percentile estimates based on dietary surveys/population groups with less than 59 observations may not be statistically robust and were not included in this table (Meeker et al., 2017).

Overall, considering the 95th percentile estimate for the Polish survey, it is concluded that a high consumption value of 140 mL/kg bw is appropriate for estimating acute exposure to cereulide via follow-on formulae.

3.2.3. Food for special medical purposes for infants and young children (FSMP)

Consumption data for FSMPs are not available in the EFSA Comprehensive Database. Exposure assessments in other regulatory domains assume that the amounts of FSMPs consumed by infants are comparable to those of similar foods (i.e. infant and follow-on formulae) consumed by the general infant population (EFSA ANS Panel, 2017). On this basis, the same high consumption value of 260 mL/kg bw is recommended for use in the acute exposure assessment for FSMPs intended for infants between 0 to 4 months and 140 mL/kg bw for FSMPs intended for infants between 4 to 12 months.

3.3. Final considerations

Due to uncertainty regarding extraction of cereulide from powder formulae with the current extraction protocols, these final considerations focus on liquid reconstituted formulae. By

calculating the ratio between the ARfD and high consumption values derived in this rapid risk assessment, cereulide concentrations in final reconstituted (liquid) infant formulae above 0.054 µg/L may exceed the derived ARfD. For reconstituted follow-on formulae (liquid), cereulide concentrations above 0.1 µg/L may exceed the derived ARfD.

4. Conclusions

- An extensive literature search of toxicological data for cereulide in animals and humans as well as in vitro evidence and a review of previous assessments provided a basis to identify emesis as the critical effect.
- BMD modelling of the data from the critical study resulted in a $BMDL_{10}$ of 4.2 µg/kg bw for increased risk of emesis.
- An ARfD of 0.014 µg/kg bw was established by applying the default UF of 100 and an additional UF of 3 to account for differences in xenobiotic metabolism and renal excretion between adults and infants.
- A high consumption value of 260 mL/kg body weight is considered the most appropriate for estimating acute exposure to cereulide via infant formulae (liquid). This is consistent with the previous recommendation of EFSA to assess dietary exposure of infants below 16 weeks of age.
- When assessing acute exposure from follow-on formulae (liquid), which are typically not consumed by infants below 16 weeks of age, a P95 consumption value of 140 mL/kg body weight is considered more realistic.
- Cereulide concentrations above 0.054 µg/L and 0.1 µg/L in reconstituted infant formulae and follow-on formulae, respectively, may exceed the derived ARfD.
- Due to the lack of consumption data for FSMPs, it is assumed their intake is comparable to formulae. When the FSMP is intended for infants below 16 weeks of age, the high consumption value proposed for infant formulae is recommended. When the FSMP is intended for infants between 4–12 months, the value derived for follow-on formulae may be considered instead.

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6. Abbreviations

ARfD	acute reference dose
B. cereus	*Bacillus cereus*
BMD	benchmark dose
BMDL	benchmark dose lower credible limit

BMR	benchmark response
bw	body weight
ces	cereulide synthetase
EFSA	European Food Safety Authority
ER	endoplasmic reticulum
FSMP	food for special medical purposes
i.g.	intragastric
i.p.	intraperitoneal
i.v.	intravenous
IL	interleukin
LC-MS/MS	liquid chromatography tandem mass spectrometry
MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MRM	multiple-reaction monitoring
MTT	3-(4,5-dimethylthiazolyl-2)-2,5-Diphenyltetrazolium Bromide
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
RASFF	EU Rapid Alert System for Food and Feed
RIVM	Dutch National Institute for Public Health and the Environment
ROS	reactive oxygen species
RRA	rapid risk assessment
SIDA-LC-MS/MS	stable isotope dilution assay-LC-MS/MS
TNF	tumour necrosis factor
UF	uncertainty factor

Appendix 1: Benchmark Dose Modelling report

The Appendix 1 provides a comprehensive overview of the benchmark dose (BMD) analysis carried out for the critical study, employing the Bayesian BMD model averaging technique. The Bayesian BMD analysis was conducted in accordance with the EFSA BMD guidance (EFSA Scientific Committee, 2022).

A. Data Description

Data used were incidences of emesis in Asian house shrews seen upon oral acute exposure (Agata et al., 1995). For details on the study see **Section 3.3.1.1** on Adverse effects in experimental animals.

Dose in µg/kg bw	Number of animals showing emesis	Number of animals per group
4	0	5
8	1	5
16	3	5
32	5	5

B. Software Used

Results are obtained using the EFSA web-tool for Bayesian BMD analysis, which uses the R-package [BMABMDR] version 0.1.18 for the underlying calculations.

C. Methods and choices made

The BMD is identified as the specific dose that corresponds to the desired BMR level. To assess the uncertainty associated with the BMD, a 90% credible interval is estimated, with the lower bound denoted as BMDL and the upper bound as BMDU. The BMDL and BMDU values help to quantify the range within which the true BMD value is expected to lie. The BMR for the study by Agata et al. (1995) was set at 10% as recommended for quantal animal data (EFSA Scientific Committee, 2022). Estimation method used to provide average BMD and credible interval is based on Bridge sampling, the recommended method.

D. Results

In order to assess if the models used were fitting sufficiently well the data at hand, the best fitting model was compared to the saturated model and the resulting Bayes factor in favor of saturated model is 2.51, indicating that at least one of the models in the suite of models fit well the data given that this value is smaller than 10.

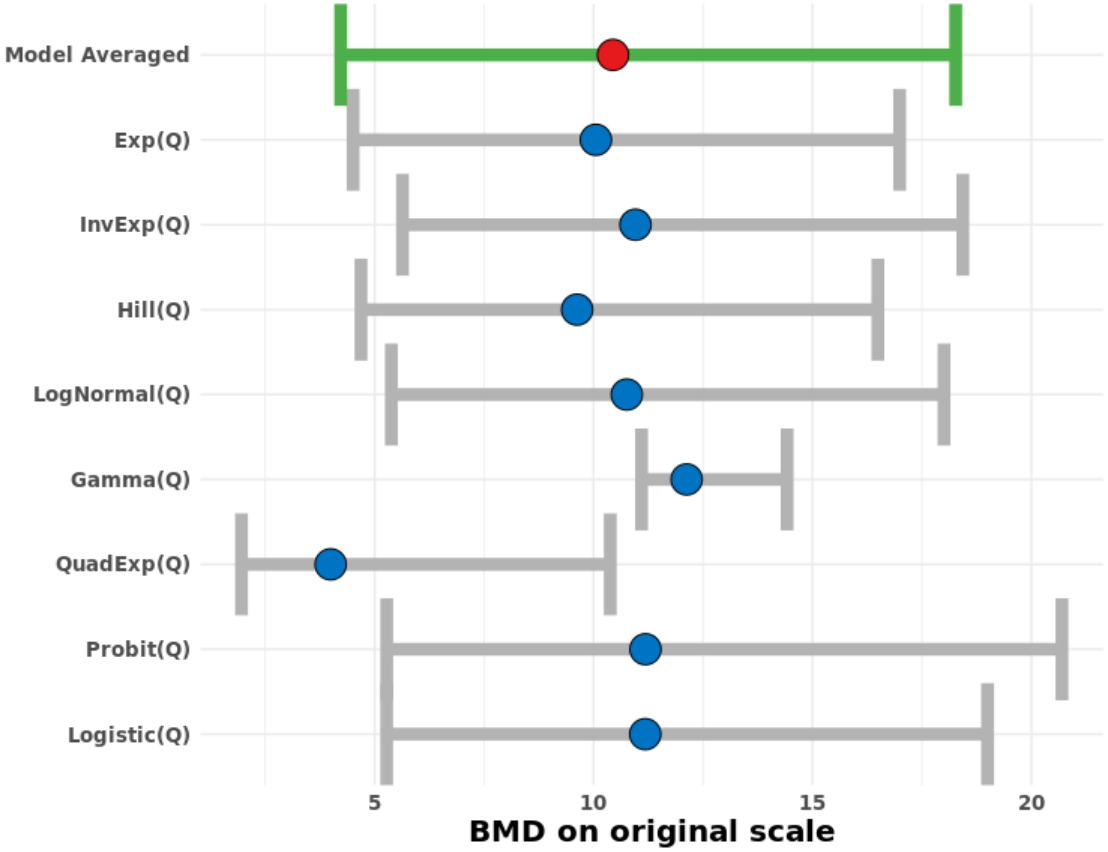
Model Averaged BMD in µg cereulide/kg bw per day:

Model	Type	BMDL	BMD	BMDU
Model Averaged	BS	4.221	10.374	17.979

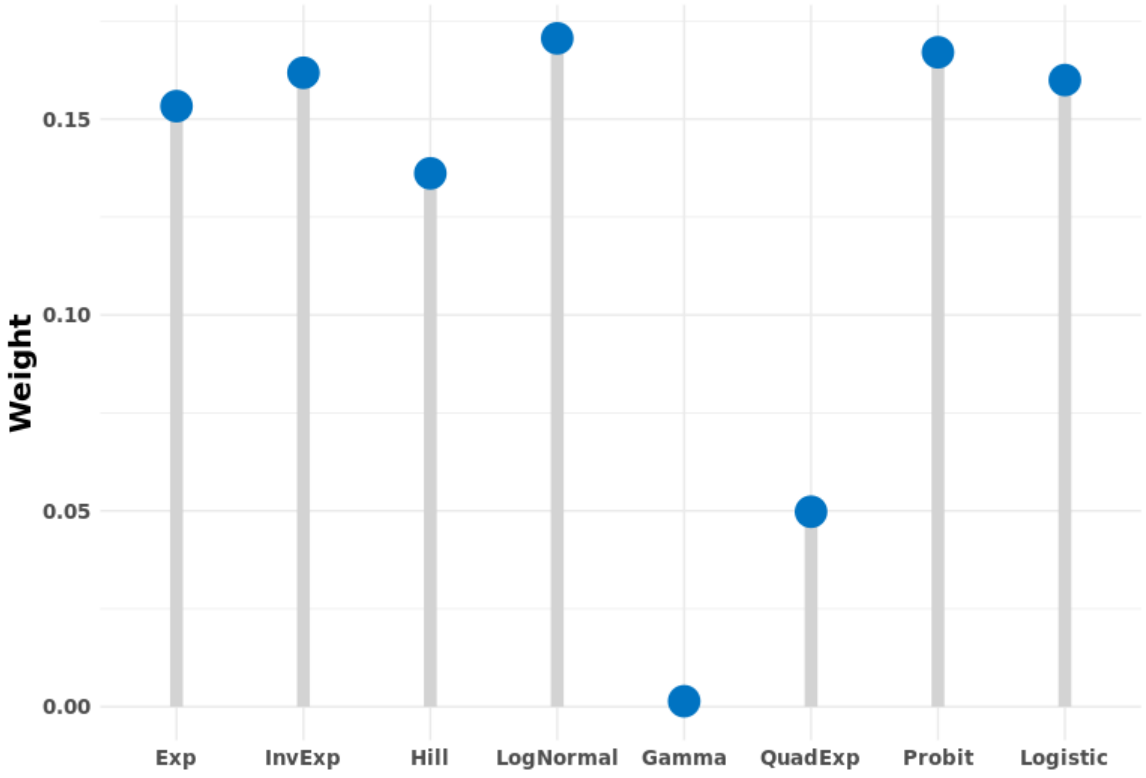
The table below presents the estimated BMDs, credible intervals, weights in the final average model as well as the indicator for convergence for each of the 8 models used.

Model	BMDL	BMD	BMDU	Model Weights	Converged
E4_Q	4.508	10.052	16.991	0.153	1
IE4_Q	5.636	10.957	18.434	0.162	1
H4_Q	4.687	9.624	16.493	0.136	1
LN4_Q	5.382	10.759	18.004	0.171	1
G4_Q	11.096	12.126	14.421	0.001	0
QE4_Q	1.959	3.995	10.380	0.050	1
P4_Q	5.276	11.185	20.699	0.167	1
L4_Q	5.274	11.180	18.999	0.160	1

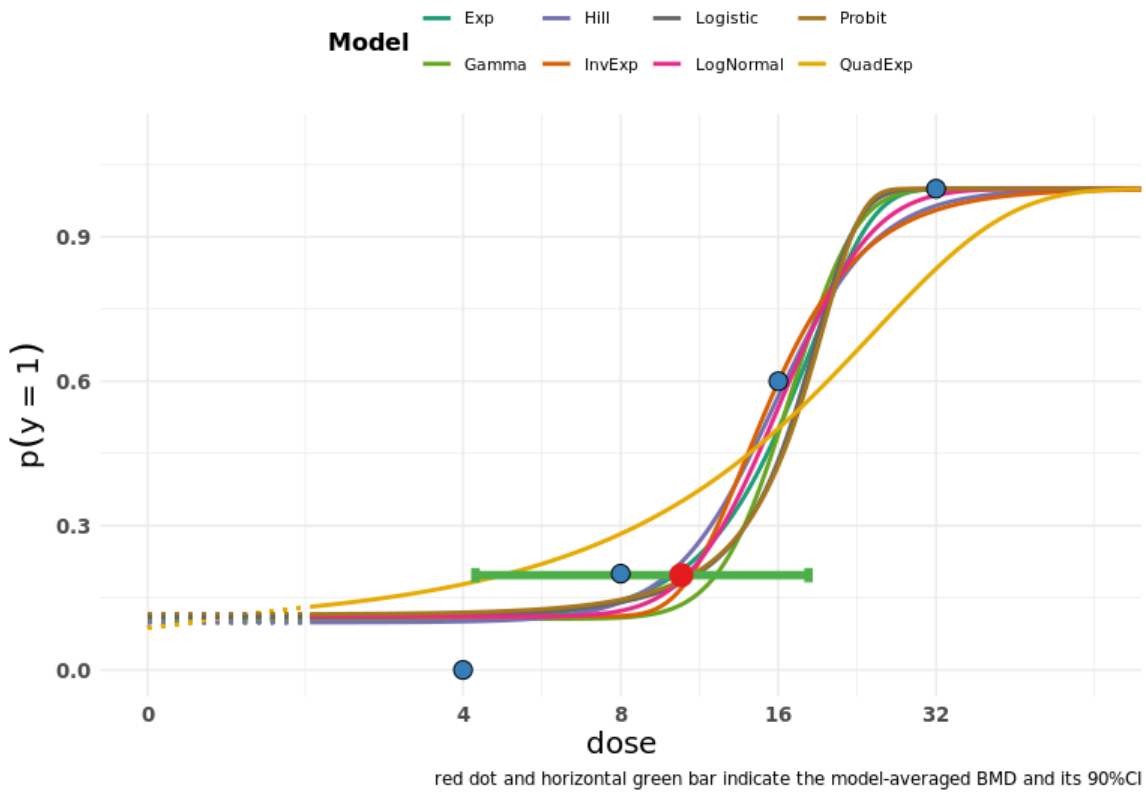
Plots of Fitted Models credible intervals and the averaged model in green



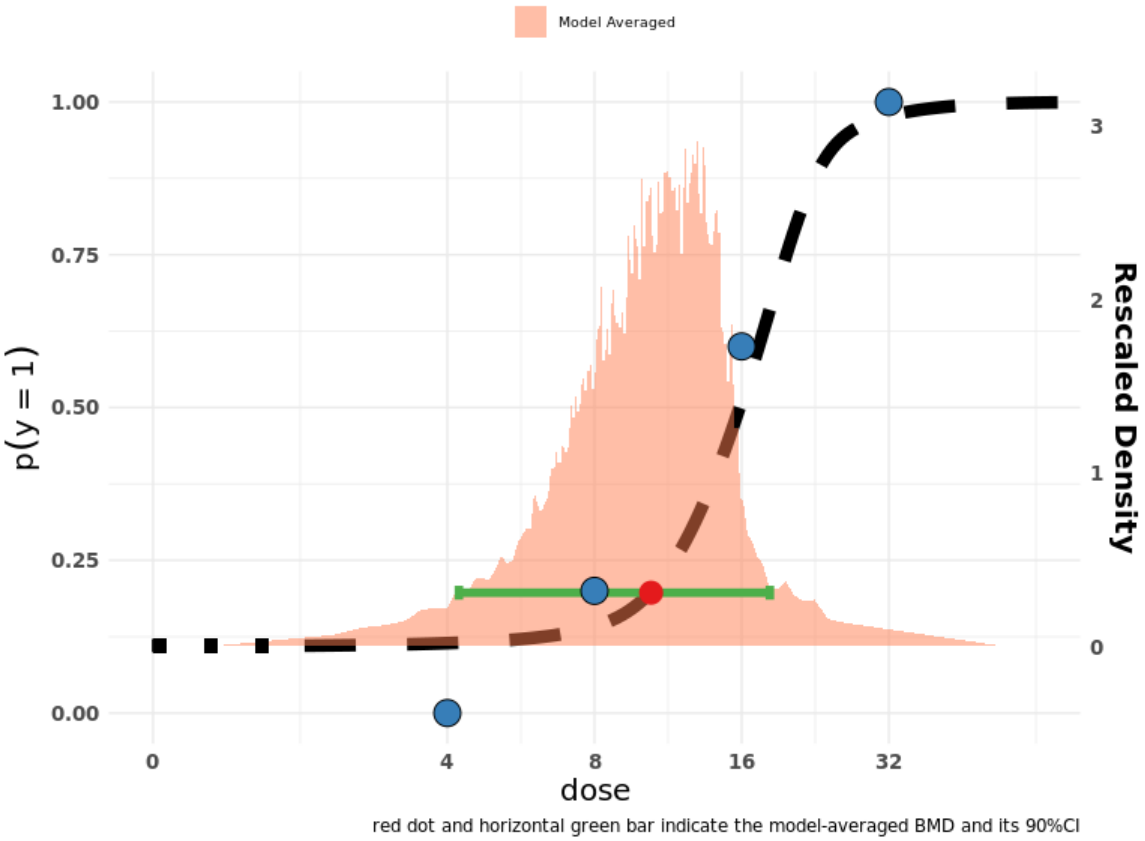
The following plot presents the contribution of each model to the average model.



The fitted models, observed frequencies of emesis and the credible interval is presented in the graph below.



The following plot presents the model average dose-response curve, together with the posterior distribution of the BMD and the observed frequencies of emesis in the data for each dose.



E. Conclusions

Considering the results of the BMD modelling and the statistical tests indicate a dose-response effect and that at least one of the models fits sufficiently well. The estimated $BMDL_{10}$ of 4.2 µg cereulide/kg bw and credible interval can therefore be used to derive an ARfD.