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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of *Macleaya cordata* (Willd.) R. Br. extract and leaves (Sangrovit® Extra) for suckling and weaned piglets and other growing Suidae (Phytobiotics Futterzusatzstoffe GmbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of *Macleaya cordata* (Willd.) R. Br. extract and leaves (Sangrovit® Extra) as a zootechnical feed additive for suckling and weaned piglets and other growing Suidae. The additive is standardised to contain a concentration of the sum of the four alkaloids sanguinarine, chelerythrine, protopine and allocryptopine of 1.25%, with 0.5% sanguinarine. Owing to the presence of the DNA intercalators sanguinarine and chelerythrine, a concern for genotoxicity was identified. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) had no safety concerns for the target species when the additive is used at the recommended level of 0.750 mg sanguinarine/kg complete feed for suckling and weaned piglets and other growing Suidae. Since in all consumer categories the exposure to sanguinarine and chelerythrine via the use of Sangrovit® Extra exceeds the threshold of toxicological concern of 0.0025 µg/kg bw per day for DNA reactive mutagens and/or carcinogens, the FEEDAP Panel could not conclude on the safety for the consumers. The additive was shown to be irritant to the eyes but not irritant to skin or a skin sensitiser. The FEEDAP Panel could not exclude the potential of the additive to be a respiratory sensitiser. When handling the additive, exposure of unprotected users to sanguinarine and chelerythrine may occur. Therefore, to reduce the risk, the exposure of users should be reduced. The use of Sangrovit® Extra as a feed additive under the proposed conditions of use was considered safe for the environment. The additive Sangrovit® Extra had the potential to be efficacious in improving performance of weaned piglets at 0.600 mg sanguinarine/kg complete feed. This conclusion was extended to suckling piglets and extrapolated to other growing Suidae.

KEYWORDS

otherzootechnical additives, efficacy, *Macleaya cordata* extract, safety, Sangrovit® Extra, sanguinarine, zootechnical additives

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1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Phytobiotics Futterzusatzstoffe GmbH[2] for the authorisation of the additive consisting of *Macleaya cordata* extract (Sangrovit® Extra), when used as a feed additive for suckling and weaned piglets and other growing *Suidae* (category: zootechnical additive; functional group: other zootechnical additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 20 March 2023.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of *Macleaya cordata* extract (Sangrovit® Extra), when used under the proposed conditions of use (see **Section 3.1.1**).

1.2 | Additional information

The additive under assessment, Sangrovit® Extra, consists of *Macleaya cordata* extract and leaves. The FEEDAP Panel issued an opinion on the safety and efficacy of Sangrovit® Extra for all poultry species (excluding laying and breeding birds) (EFSA FEEDAP Panel, 2023a).

It has not been previously authorised as a feed additive in the European Union.

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of *Macleaya cordata* extract and leaves (Sangrovit® Extra) as a feed additive. The dossier was received on 08/06/2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00357.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 20 March 2023 to 20 June 2023 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[5] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 2 February to 23 February 2024 for which no comments were received.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the sanguinarine (SG) in Sangrovit® Extra and in premixtures and feedingstuffs.[6]

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2]Phytobiotics Futterzusatzstoffe GmbH, Wallufer Str. 10a, D-65343, Eltville, Germany.

[3]Dossier reference: FEED-2021-2410.

[4]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[5]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[6]Evaluation report received on 17/7/2023 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Macleaya cordata* extract and leaves (Sangrovit® Extra) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023b), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019a) Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019b) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021).[8]

3 | ASSESSMENT

The additive under assessment (Sangrovit® Extra) consists of a *M. cordata* (Willd.) R. Br. extract and *M. cordata* processed leaves. It is intended for use as a zootechnical additive (functional group: other zootechnical additives) in feed for suckling and weaned piglets and other growing Suidae.

3.1 | Characterisation

The additive and the active substances were fully characterised in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2023a). The applicant stated that no changes in the manufacturing or composition of the additive have been introduced since the previous assessment.

The additive Sangrovit® Extra consists of *M. cordata* extract ([redacted]) and *M. cordata* processed leaves ([redacted]).

The additive is formulated with whole wheat flour ([redacted]), lignosulfonate ([redacted]) and sorbic acid ([redacted]) in order to achieve a target concentration of the sum of four *M. cordata* alkaloids of 1.25%, with 0.5% SG (selected as the marker substance).

The additive is specified to contain a minimum of 4000 mg SG/kg additive and a maximum 7000 mg SG/kg additive. Analysis of 10 batches of the additive (manufactured in 2019–2023) showed compliance with these specifications [redacted].[9]

All data pertaining to composition, purity, physicochemical properties and stability described thereof are considered valid also for this application (EFSA FEEDAP Panel, 2023a).

3.1.1 | Conditions of use

The additive is intended for use in feed for suckling and weaned piglets and other growing Suidae up to the age of 120 days or a body weight of 35 kg to provide a minimum SG content in complete feed of 0.600 mg/kg feed and a maximum SG content of 0.750 mg/kg feed. These levels are achieved with varying amount of the additive ranging between 90 and 150 mg Sangrovit® Extra/kg complete feed, depending on the SG concentration in the additive between 4000 and 7000 mg SG/kg Sangrovit® Extra.

3.2 | Safety

The applicant submitted the same data set that was already evaluated by the FEEDAP Panel in its previous assessment (EFSA FEEDAP Panel, 2023a). This included: (i) a structured literature search on the absorption, distribution, metabolism and excretion (ADME) of the alkaloids present in *M. cordata*, and the safety of the additive; (ii) toxicological studies, including genotoxicity, sub-chronic toxicity studies and studies aimed at demonstrating the safety of the additive for the user (skin and eye irritancy and skin sensitisation).

The previous conclusions on the ADME and toxicology of the *M. cordata* alkaloids, SG, chelerythrine (CH), protopine (PRO) and allocryptopine (ALL), are briefly summarised below.

[7]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[8]https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf.

[9]Annex II_1_3_2, Annex_ II_1_3_3.

In rat and in food-producing animals, SG and CH are poorly absorbed as such after oral administration. The two compounds are extensively metabolised in the gut mainly by reductive reactions. The resulting dihydrometabolites are absorbed, metabolised in the liver to conjugated derivatives and excreted via bile to the intestine and subsequently in faeces. Very low levels of the compounds or their metabolites were found in plasma, urine and in tissues. The biotransformation of SG and CH was similar in rat liver fractions and in human hepatocytes. PRO and ALL are absorbed in the rat to a greater extent than SG and CH, distributed in various tissues, extensively metabolised after absorption, and excreted in urine and faeces.

Although genotoxicity studies performed with *M. cordata* extract (in vitro) and with the formulated additive Sangrovit® Extra (in vitro and in vivo) gave negative results, based on the available information on individual components of the extract, the FEEDAP Panel concluded that SG 'has the potential to (i) intercalate between DNA base pairs; (ii) induce DNA strand breaks; (iii) and also induce DNA strand breaks associated with oxidative damage and the formation of reactive oxygen species (ROS). Therefore, SG has the potential to produce ROS in proximity of the double helix of DNA and to induce oxidative DNA damage. Based on the structural similarity with SG, the same conclusions apply to CH'. For the other two alkaloids, the FEEDAP Panel concluded that 'a direct interaction of PRO with DNA is not supported by the experimental data available. Based on the structural similarity between PRO and ALL, this conclusion can be extended to ALL'. Overall, a concern for genotoxicity was identified owing to the presence of the DNA intercalators SG and CH.

From a 90-day study with Sangrovit® Extra in Wistar rats,[10] the FEEDAP Panel identified a no observed adverse effect level (NOAEL) for SG of 95 mg/kg feed (corresponding to 7.7 mg/kg body weight (bw) per day) (EFSA FEEDAP Panel, 2023a).

In the next sections, only the data in pigs are described. These include ADME studies retrieved from the literature, a residue study and a tolerance study in weaned piglets conducted with the additive under assessment (Sangrovit® Extra).

3.2.1 | Absorption, distribution, metabolism and excretion in pigs

In the publication from Wu et al. (2020), which describes an in vitro metabolic study and an in vivo pharmacokinetics study of SG in pigs, incubation of SG with microsomes prepared from intestinal mucosa (added with nicotinamide adenine dinucleotide phosphate, NADPH), cytosol (added with nicotinamide adenine dinucleotide, NADH) and gut flora of pigs showed its reductive biotransformation to dihydrosanguinarine (DHSG). When pigs were administered a single oral dose of SG at 0.1 mg/kg bw, plasma SG and DHSG reached C_{max} (3.41 and 2.41 μg/L, respectively) at 2.75 h. For SG and DHSG, the area under the curve (AUC) was 15.6 and 9.1 μg h/L, and the half-life was 2.33 h and 2.20 h, respectively. A repeated dose administration was also carried out by orally giving to six pigs SG at a dose of 0.1 mg/kg bw three times a day, each dose interval of 8 h, for three consecutive days. Blood samples were collected at several time points up to 24 h post the last dose. AUC for SG and DHSG were 31 and 13 μg h/L, respectively, higher than after a single dose. Also, plasma C_{max} was higher after repeated doses, 5.9 and 2.9 μg/L for SG and DHSG, respectively, although attained at a similar T_{max} as for single dose (2.6 h). $T_{1/2}$ of SG and DHSG was 3.2 h and 2.4 h, respectively.

In the study from Kosina et al. (2004), three groups of pigs (three males and three females per group) were fed a standard diet (control group) or a diet added with an extract of *M. cordata* at 2 or 100 mg extract/kg feed,[11] (SG:CH of 3:1) for 90 days. The lower concentration tested contained 1.28 mg SG/kg feed and 0.44 mg CH/kg feed and the higher contained 64 mg SG/kg feed and 22 mg CH/kg feed. Blood was collected at day zero, and throughout the experiment at days 30, 60, and 90, and faeces at day 90. On day 91, the animals were killed and liver, muscle, gingiva, tongue, stomach and intestines collected for analysis of SG and CH by high-performance liquid chromatography (HPLC) with diode array and/or fluorimetric detectors (LOD: 0.001 μg/mL). SG was present in plasma in a concentration-dependent manner (4 and 108 μg/L for the low and high diet concentration); CH was not detected in plasma of the low concentration group, being present at 24 μg/L in the high concentration group. Plasma levels were constant throughout the experiment. The highest levels of SG in organs/tissues for both diet concentrations were reported in the gingiva (79 and 514 μg/kg), followed by the intestine (15 and 124 μg/kg), liver (13 and 113 μg/kg), tongue (10 and 32 μg/kg) and stomach (7 and 52 μg/kg). In the low concentration diet group, CH was only detected in the gingiva and liver (36 and 5 μg/kg, respectively) and in the gingiva, intestine and liver (50, 49 and 40 μg/kg, respectively) in the high concentration group. Both SG and CH were not detected in the muscle. The method of analysis did not include the search for metabolites. The very high contents of both compounds in faeces (1180 μg SG/kg and 842 μg CH/kg at the lowest diet concentration; 16,110 μg SG/kg and 8412 μg CH/kg at the highest diet concentration) indicate that this is the principal route of excretion.

The metabolism of SG was studied in microsomes and cytosol of pig liver in the presence of NADPH (Zhang et al., 2013). After incubation, the supernatant was analysed by liquid chromatography ion trap/time of flight mass spectrometry (LC-IT/TOFMS). In microsomes, seven metabolites were identified, while DHSG was the only metabolite identified in cytosol. The reduction of the iminium bond and O-demethylenation of SG were the main metabolic elucidated pathways. The amount of DHSG formed in liver cytosol was higher than that in liver microsomes in the presence of NADPH. This indicates that cytosolic enzymes are mainly responsible for the reduction of SA in the pig liver. The hydroxylated metabolites if formed in vivo are expected to be conjugated and excreted.

[10]Annex_III_2_2_3, Annex_III_2_2_3.xlsx.

[11]The daily dose of alkaloids for the 2 ppm and 100 ppm groups, respectively, was within 0.14 and 7 mg/kg bw at the beginning and 0.06 and 3 mg/kg bw at the end of the experiment, i.e. averaged doses over 90 days were 0.1 and 5 mg/kg bw, respectively.

3.2.1.1 | *Conclusions*

In pigs fed a diet with *M. cordata* extract for 90 days, the gingiva showed the highest contents of SG followed by the intestine, liver, tongue and stomach. CH was only detected in the gingiva, intestine and liver. Both SG and CH were not detected in the muscle. Based on the high contents of both compounds in faeces together with their absence in the muscle, the FEEDAP Panel concludes that faecal excretion is the principal route. The results from the studies in pigs are consistent with the results in laboratory animals described in a previous opinion (EFSA FEEDAP Panel, 2023a).

3.2.2 | Residue study in pigs

The applicant submitted an efficacy study in weaned piglets, from which some information on residues of the four alkaloids SG, CH, PRO and ALL can be obtained (see Section 3.3.1).

Tissue samples were taken from eight weaned piglets per treatment (four males/four females).[12] The tissues collected at the end of the study (after 70 days) were muscle and skin+fat in natural proportion and organs (liver and kidneys) from the not supplemented (control) group and from the group fed with the maximum recommended level (0.750 mg SG/kg feed). All alkaloids were quantified by ultraperformance liquid chromatography tandem mass spectrometry (UPLC–MS/MS).

In samples from the control group, [redacted]

The results of the analyses of the samples of tissues and organs of the animals fed with the maximum recommended level are summarised in Table 1.[13]

TABLE 1 Residue data of sanguinarine, chelerythrine, protopine and allocryptopine in tissues (skin + fat, kidney, liver and muscle) and excreta samples collected at the end of the efficacy study (after 70 days) from the animals administered with the maximum recommended level (0.750 mg SG/kg feed). The results are presented as the mean (SD) for $n \geq 6$ or mean (range) when $n < 6$.

Sample	Sanguinarine µg/kg	Chelerythrine µg/kg	Protopine µg/kg	Allocryptopine µg/kg
Skin+fat	1.38 (0.70) [redacted]	[redacted]	< 0.5 ($n = 3$)	n.d.
Kidney	0.63 [redacted]	[redacted]	n.d.	n.d.
Liver	1.13 (0.62) [redacted]	[redacted]	0.59 (< 0.5–1.10) ($n = 4$)	< 0.5 ($n = 1$)
Muscle	0.7 [redacted]	[redacted]	< 0.5 ($n = 6$)	< 0.5 ($n = 8$)

Note: Sanguinarine (SG), chelerythrine (CH), protopine (PRO) and allocryptopine (ALL) [redacted] LOQ: 0.5 µg/kg for SG, CH, PRO and ALL in all tissues; LOD: 0.1 µg µg/kg for SG, CH, PRO and ALL in all tissues, [redacted]; for ALL: 0.1 µg/kg in muscle, 0.19 µg/kg in skin/fat, 0.13 µg/kg in liver and 0.16 µg/kg in kidney.

Abbreviation: n.d., not detected.

[redacted][14]

PRO was below the LOQ in all samples, except in four liver samples and it was detectable in few skin+fat ($n = 3$) ad muscle samples ($n = 6$). ALL was < LOQ in all samples and was detectable in one liver sample and in all muscle samples ($n = 8$).[15]

3.2.3 | Safety for the target species

According to the General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021), genotoxicity and carcinogenicity endpoints are not considered relevant for short-living animals. Short-living animals are defined as those animals raised for fattening whose lifespan under farming conditions makes it very unlikely to develop cancer as a result of the exposure to genotoxic and/or carcinogenic substances in the diet. Therefore, for these species, the safety evaluation of additives containing substances which are genotoxic and carcinogenic can be based on the outcome of the tolerance trials in the target species

[12] Annex_IV_3_3.

[13] Annex_III_2_1_1_Conf, Annex_III_2_1_2_Conf, Annex_II_6_13_Conf.

[14] Annex_III_2_1_2_Conf.

[15] Annex_II_6_13_Conf.

(EFSA FEEDAP Panel, 2021). In the context of this assessment, the definition of short-living animals includes growing pigs and other growing Suidae but does not include pigs and Suidae reared for reproduction which are considered to be long-living/reproductive animals and for which genotoxicity and carcinogenicity endpoints are considered relevant.

3.2.3.1 | *Tolerance trial in weaned piglets*

To support the safety for the target species, the applicant submitted a tolerance-efficacy study with the additive under assessment.[16]

A total of 800 hybrid[17] weaned piglets (26 days old, initial body weight ≈ 6.1 kg; 50%♀: ♂) coming from four consecutive batches were categorised by body weight, distributed in 80 single-sex pens of 10 animals each, and randomly allocated to five groups (16 replicates per group, eight of each sex).

Two basal diets (pre-starter, from day 1 to 14; starter from day 15 to 42) based on maize, soybean meal and wheat were either not supplemented (control) or supplemented with the additive to provide 0.45 (0.6× maximum proposed use level), 0.60 (0.8×), 0.75 (1×) and 15 (20×) mg SG/kg complete feed (equivalent to 90, 120, 150 or 3000 mg Sangrovit® Extra/kg complete feed).[18] The diets were offered ad libitum as pellets for 42 days.

Mortality and health status were checked every day and the most likely reason of death/culling provided. The animals were individually weighed at the start of the trial. Thereafter, the body weight and the feed intake were recorded on days 14 and 42. The average daily feed intake, average daily weight gain and feed-to-gain ratio were calculated and corrected for mortality for the overall period. Blood samples were obtained from two randomly selected piglets per pen on day 42 for haematology and biochemistry analysis.[19]

The zootechnical data were analysed with a general linear model with the weaning batch and the diet as fixed effects and the initial body weight as a covariate, including the pen as the experimental unit. For the blood haematology and biochemistry, a generalised linear model was used, with the diet as a fixed effect and the animal as the experimental unit. When differences were observed, group means were compared with Tukey test. The significance level was set at 0.05. The piglets' zootechnical data were subjected to a non-inferiority test. For that purpose, the lower limits of the 95% confidence interval of the 1× and 20× diets mean minus the control were compared with the established margins for each parameter (body weight = −1.73 kg; average daily weight gain = −30.46 g; average daily feed intake = −40.83 g; feed-to-gain ratio = 0.055). The significance level was set at 0.05.

The overall mortality and culling rate were 5.4% on average (4.5%–6.3%) and no statistical difference was observed between groups. The inclusion of the additive up to 20× the maximum proposed use level showed no significant differences in any of the zootechnical performance and blood haematology parameters recorded in comparison with the control. Regarding the blood biochemistry data, the 0.6×, 0.8× and 1× groups showed lower serum cholesterol content compared to the control; the creatine kinase serum concentration was significantly decreased in the 1× group; and the serum C-reactive protein and potassium concentration and the lactate dehydrogenase (LDH) activity were higher in the 0.8× group compared to the control. The differences observed in the biochemistry parameters were small and not dose related. Therefore, those were not considered relevant for the safety assessment.

Based on the confidence intervals obtained in the non-inferiority test for body weight (−0.66 to 1.01), average daily weight gain (−18.84 to 24.52), average daily feed intake (−16.77 to 36.23) and feed-to-gain ratio (−0.03 to 0.06), the 20× group was not inferior to the control group.

To complement the safety for the target species, the applicant provided six published studies involving the dietary supplementation of weaned piglets either with *M. cordata* extracts or SG. All the studies showed limitations that prevent using them as evidence for the safety of growing pigs. Four of them[20] included no overdose levels (maximum level applied of 0.75 mg SG/kg complete feed), recorded a limited number of endpoints relevant for the safety (no data on blood haematology/biochemistry or gross pathology) and two of them had a short duration (21 days). One of the studies[21] was designed as a challenge trial in which all the animals from all groups were inoculated with *Salmonella* Typhimurium. The other study[22] included one group supplemented with a high level of SG (up to 53 times the content supplied in the maximum proposed use level); however, the Panel notes that the test item used might not be representative of Sangrovit® Extra, the duration of the study was limited to 14 days, and no blood data or gross pathology evaluation were monitored. In any case, no adverse effect was observed in any of the parameters evaluated in growing pigs in any of the trials reported.

[16] Annex_III_1_1.

[17] ((Piétrain × Duroc) × (Landrace × Large White)).

[18] Analysed Sanguinarine content in Pre-starter/Starter supplemented with Sangrovit® Extra (SE) (mg sanguinarine/kg feed): 90 mg SE/kg: 0.44/0.38; 120 mg SE/kg: 0.52/0.79; 150 mg SE/kg:0.63/0.80; 3000 mg SE/kg: 15.90/15.90.

[19] Analyses: total red blood cell counts (RBC), packed cell volume, haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total and differential leucocyte counts (WBCB), platelet counts, prothrombin time and fibrinogen, sodium, potassium, chloride, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, urea/uric acid, cholesterol, creatinine, bilirubin, acute phase proteins (haptoglobin and C-reactive protein), amylase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, gamma glutamyltransferase, alkaline phosphatase and creatine kinase.

[20] Chen_et_al_2018, Chen_et_al_2019, Goodarzi_et_al_2018, Kantas_et_al_2015.

[21] Liu_et_al_2018.

[22] Robbins_et_al_2013.

3.2.3.2 | *Conclusions on safety for the target species*

Based on the results of the tolerance trial in weaned piglets, the Panel concludes that Sangrovit Extra® is safe for weaned piglets at 0.750 mg SG/kg complete feed (achieved with 150 mg Sangrovit® Extra/kg complete feed) with a wide margin of safety. This conclusion is extended to suckling piglets for the period in which solid feed is given and other growing Suidae up to an age of 120 days or a body weight of 35 kg.

The Panel notes that these conclusions do not cover pigs and minor porcine species reared for reproduction, which are considered long-living/reproductive animals.

3.2.4 | Safety for the consumer

Assessment of consumer exposure

The FEEDAP Panel performed an exposure assessment following the methodology described in the Guidance on consumer safety (EFSA FEEDAP Panel, 2017a) (Appendix A).

The residue values for SG and CH from the residue study in weaned piglets (see Section 3.2.2) were used as input data for the exposure calculation and are reported in Table 2. For SG, quantified in [redacted], the arithmetic mean plus two standard deviations was calculated. For SG in [redacted] the highest value was considered. CH was quantified [redacted] (the highest analysed value was used). [redacted] where CH was detected (> LOD) but not quantified (< LOQ), a value corresponding to half the LOQ was used.

TABLE 2 Input values used to calculate consumer exposure to sanguinarine and chelerythrine residues (mg/kg) in mammal tissues (pigs).

	Input values sanguinarine + chelerythrine mg/kg wet tissue
Mammal fat tissue	0.00368
Mammal liver	0.00261
Mammal meat[a]	0.00150
Mammal offal and slaughtering products (other than liver)	0.00165

[a]The residue concentrations in muscle (0.00095 mg/kg) and skin/fat (0.00368 mg/kg) have been used to calculate the intake of meat at the following proportions: 80% muscle and 20% skin/fat (EFSA FEEDAP Panel, 2017b).

The values of chronic exposure of consumers to SG + CH for the different population categories are reported in Table 3.

In the absence of a reference point for SG and CH, the estimated exposure can be related to the value that, according to threshold of toxicological concern (TTC) approach, is considered of low probability of adverse health effects (EFSA Scientific Committee, 2019b). For substances that have the potential to be DNA reactive mutagens and/or carcinogens, the TTC value corresponds to 0.0025 μg/kg bw per day (equal to 2.5 ng/kg bw per day). Table 3 reports the estimated exposure related to the TTC of 2.5 ng/kg bw per day.

TABLE 3 Chronic exposure of consumers to sanguinarine and chelerythrine residues based on residue data in pig tissues.

Population class	Highest exposure estimate[a] ng/kg bw/day	% TTC value[b]
Infants	10.53	421
Toddlers	13.18	527
Other children	14.64	586
Adolescents	11.95	478
Adults	9.18	367
Elderly	6.38	255
Very elderly	5.96	239

Abbreviations: bw, body weight; TTC, threshold of toxicological concern.

[a]Highest reliable percentile: 95th percentile.

[b]TTC value for substances that have the potential to be DNA reactive mutagens and/or carcinogens: 0.0025 μg/kg bw per day (equal to 2.5 ng/kg bw per day).

When considering the residue data in pig tissues, the estimated 95th percentile ranged between 2.31 and 14.64 ng/kg bw per day. The highest exposure at the 95th percentile occurs in the category 'other children'.

For all consumer categories, exposure in the individual countries was constantly above the TTC value of 2.5 ng/kg bw per day. Above this threshold, a non-TTC approach (e.g. substance-specific risk assessment) is required in order to reach a conclusion on potential adverse health effects.

Based on the available data, the FEEDAP Panel cannot conclude on the safety of the additive for the consumers.

The Panel notes that there was an application for the authorisation of this additive in growing poultry. When considering the combined exposure of consumers to SG and CH residues from pig and poultry tissues,[23] the estimated exposure[24] was comparable to the exposure from pig tissues only. The FEEDAP Panel notes that consumer exposure to SG + CH is essentially due to the consumption of pig tissues, in particular to the consumption of fat.

The residue values for PRO and ALL from the residue study in weaned piglets (see Section 3.2.2)[25] were used as input data for the exposure calculation and are reported in Table 4. PRO was quantified only in four liver samples (the highest value was considered). In the other samples, where PRO was detected (> LOD) but not quantified (< LOQ), a value corresponding to half the LOQ was used. PRO and ALL were not detected in the kidney; ALL was not detected in fat.

TABLE 4 Input values used to calculate consumer exposure to protopine and allocryptopine residues (mg/kg) in mammal tissues (pigs).

	Input values protopine + allocryptopine mg/kg wet tissue
Pigs	
Mammal fat tissue	0.00025
Mammal liver	0.00135
Mammal meat[a]	0.00045
Mammal offal and slaughtering products (other than liver)	n.d.

Abbreviation: n.d., not detected.

[a]The residue concentrations in muscle (0.00050 mg/kg) and skin/fat (0.00025 mg/kg) have been used to calculate the intake of meat at the following proportions: 80% muscle and 20% skin/fat (EFSA FEEDAP Panel, 2017b).

Chronic exposure of consumers to PRO and ALL for the different population categories are reported in Table 5.

In the absence of a reference point for PRO and ALL, the estimated exposure can be related to the value that, according to TTC approach, is considered of low probability of adverse health effects (EFSA Scientific Committee, 2019b). For substances belonging to Cramer class III, the TTC value corresponds to 1.5 µg/kg bw per day (equal to 1500 ng/kg bw per day). For all consumer categories, exposure in the individual countries was consistently below 4 ng/kg bw per day, corresponding to < 0.26% of the value of TTC. Below this threshold, the probability of adverse effects is low.

TABLE 5 Chronic exposure of consumers to protopine and allocryptopine residues based on residue data in pig tissues.

Population class	Highest exposure estimate[a] ng/kg bw per day	% TTC value[b]
Infants	2.79	0.19
Toddlers	3.64	0.24
Other children	3.96	0.26
Adolescents	3.11	0.21
Adults	2.30	0.15
Elderly	1.63	0.11
Very elderly	1.59	0.11

Abbreviations: bw, body weight; TTC, threshold of toxicological concern.

[a]Highest reliable percentile: 95th percentile.

[b]TTC value for CC III: 1.5 µg/kg bw per day (equal to 1500 ng/kg bw per day).

When considering the residue data in pig tissues, the 95th percentile ranged between 0.007 and 3.96 ng/kg bw per day. The highest exposure at the 95th percentile occurs in the category 'other children'.

[23]SG + CH in poultry tissues: Fat 0.00029 mg/kg; kidney: 0.00245 mg/kg; liver: 0.00187 mg/kg, meat: 0.00038 mg/kg.

[24]When considering the combined exposure to poultry and pigs the estimated 95th percentile tanged between 7.48 and 14.97 ng/kg bw per day.

[25]Annex_II_6_13_Conf.

When considering the combined exposure of consumers to PRO and ALL residues from pig and poultry tissues,[26] the estimated exposure[27] was more than doubled compared to the exposure from pig tissues only and was comparable to the exposure calculated for poultry tissues only (EFSA FEEDAP Panel, 2023a). The FEEDAP Panel notes that consumer exposure to PRO + ALL is essentially due to the consumption of poultry liver. However, since consumer exposure is < 1% of the TTC value for Cramer Class III compounds, the probability of adverse effects is low.

Conclusions on safety for the consumer

In all consumer categories, the exposure to SG and CH exceeds the threshold of toxicological concern of 0.0025 μg/kg bw per day (equal to 2.5 ng/kg bw per day) for DNA reactive mutagens and/or carcinogens. Therefore, the FEEDAP Panel cannot conclude on the safety of Sangrovit® Extra for the consumers.

3.2.5 | Safety for the user

The safety for the user was previously evaluated (EFSA FEEDAP Panel, 2023a, 2023b). The FEEDAP Panel is not aware of any additional data or information that would lead to a change of the conclusions previously reached. The additive is considered irritant to the eyes but not to the skin. The additive is not a skin sensitiser; however, the FEEDAP Panel cannot exclude the potential of the additive to be a respiratory sensitiser. When handling the additive, exposure of unprotected users to SG and CH may occur. Therefore, to reduce the risk, the exposure of users should be minimised.

3.2.6 | Safety for the environment

M. cordata is a natural occurring plant primarily distributed in temperate areas in North America and eastern Asia. It was introduced in Europe in the late part of the 18th century, where it is widely distributed as an ornamental garden plant.

SG, the main component of *M. cordata* extract, is widely distributed in nature and can be found in plants of the families Papaveraceae, Fumariaceae and Rutaceae.

The use of Sangrovit® Extra in animal nutrition at the proposed conditions of use is not expected to increase the concentration of SG and other alkaloids in the environment, and therefore is considered of no concern for the environment.

3.3 | Efficacy

3.3.1 | Efficacy in weaned piglets

The applicant submitted five trials aiming at supporting the efficacy of Sangrovit® Extra to improve the performance of weaned piglets.

One of the trials[28] showed a non-optimal animal health status (reflected by the outspread of colibacillosis in some pens of the control group and a mortality rate up to 9.5% in the group supplemented with 90 mg Sangrovit® Extra/kg complete feed). Owing to this limitation, the study was not further considered for the assessment of the efficacy.

The other four trials shared a similar design (see Table 6). In all trials, the animals were fed two basal diets (starter, days 1–14; grower days 15–42), either not supplemented (control) or supplemented with Sangrovit® Extra from 60 to 150 mg/kg complete feed. The level of Sangrovit® Extra in the experimental feeds was checked based on the analysis of SG and CH as primary and secondary markers, respectively. In all trials, the experimental feeds were offered ad libitum for 42 days. The first trial included an external marker in the grower diet to measure the apparent ileal digestibility of phosphorus and total amino acids.

[26]PRO+ALL in poultry tissues: fat 0.00037 mg/kg; kidney: 0.00284 mg/kg; liver: 0.02473 mg/kg, meat: 0.00068 mg/kg.

[27]When considering the combined exposure to poultry and pigs, the estimated 95th percentile for PRO + ALL ranged between 0.007 and 10.25 ng/kg bw per day.

[28]Annex_IV_3_3.

TABLE 6 Trial design and use level of the efficacy trials performed in weaned piglets.

Trial	Total no animals (animals × replicate) replicates × treatment	Breed sex (duration, days)	Feed composition (form)	Groups				
				mg Sangrovit® extra/kg complete feed	mg sanguinarine/kg complete feed		mg chelerythrine/kg complete feed	
					Intended	Analysed*	Intended	Analysed*
1[29]	42 (2) 7	DanBred × Piétrain 1:1 ♂/♀ (42)	Maize, wheat and soybean meal (mashed)	0 60 120	0 0.29 0.58	< 0.05 0.39 0.81	0 0.22 0.45	< 0.05 0.16 0.25
2[30]	80 (5) 8	DanBred × Piétrain 1:1 ♂/♀ (42)	Maize, wheat and soybean meal (mashed)	0 120	0 0.67	< 0.05 0.95	0 0.53	< 0.05 0.29
3[31]	120 (15) 2	DanBred × Piétrain 1:1 ♂/♀ (42)	Wheat, soybean meal and maize (mashed)	0 90 120 150	0 0.49 0.65 0.81	< 0.05 0.48 0.57 0.74	0 0.24 0.32 0.40	< 0.05 0.19 0.22 0.25
4[32]	1440 (24) 12	DanBred 1:1 ♂/♀ (42)	Wheat, soya and barley (mashed and pelleted)	0 60 90 120 150	0 0.33 0.49 0.65 0.81	< 0.05 0.27 0.43 0.59 0.72	0 0.16 0.24 0.32 0.40	< 0.05 0.19 0.27 0.33 0.47

*Average values of starter and grower diets combined.

In all trials, the mortality and health status were checked every day. The animals were individually weighed at the start of the trial. Thereafter, the body weight and feed intake were recorded on a pen basis once a week and the average feed intake, average weight gain and feed-to-gain ratio were calculated and corrected for mortality for the overall period.

The experimental data were analysed with a generalised linear model with the pen as the experimental unit and the diet as the fixed effect. When differences were observed, group means were compared with Tukey test. The significance level was set at 0.05.

The results of the four trials are presented in Table 7. Mortality values were within commercial standards in all trials, with no difference between groups. In all trials, the piglets that received Sangrovit® Extra at the minimum use level (120 mg Sangrovit® Extra/kg complete feed) showed improved performance (higher body weight gain and better feed-to-gain ratio) in comparison with the control. In trial 3, the piglets supplemented with the proposed use level also showed a higher final body weight. In trial 1, the results of the digestibility trial showed that the apparent ileal digestibility of phosphorus and total amino acids was significantly higher in the group supplemented with 120 mg Sangrovit® Extra/kg complete feed. These results support the findings observed in the zootechnical data.

TABLE 7 Effects of Sangrovit® Extra on the zootechnical performance of weaned piglets.

Trial	Groups (mg Sangrovit® extra/kg complete feed)	Feed intake[1] (kg or kg/day)	Final body weight (kg)	Weight gain[2] (kg or g/day)	Feed-to-gain ratio	Mortality and culling (%)
1	0	25.9	24.1	17.7[a]	1.46[a]	0
	60	26.1	24.6	18.2[b]	1.43[b]	0
	120	26.1	25.1	18.7[b]	1.40[c]	7.1
2	0	0.69	26.6	480[a]	1.46[a]	0
	120	0.73	28.5	520[b]	1.40[b]	2.5
3	0	0.71	25.8[a]	442[a]	1.62[a]	0
	90	0.72	26.2[ab]	453[ab]	1.59[ab]	3.3
	120	0.74	26.9[b]	470[b]	1.56[b]	0
	150	0.75	27.2[b]	478[b]	1.57[b]	3.3

(Continues)

[29]Annex_IV_3_1.

[30]Annex_IV_3_2.

[31]Annex_IV_3_4.

[32]Annex_IV_3_5.

TABLE 7 (Continued)

Trial	Groups (mg Sangrovit® extra/kg complete feed)	Feed intake[1] (kg or kg/day)	Final body weight (kg)	Weight gain[2] (kg or g/day)	Feed-to-gain ratio	Mortality and culling (%)
4	0	0.72	25.7	464.9[a]	1.53[a]	3.5
	75	0.73	26.2	477.9[ab]	1.52[ab]	2.1
	90	0.73	26.4	483.7[ab]	1.50[ab]	2.1
	120	0.72	26.7	488.5[b]	1.48[b]	2.4
	150	0.73	26.7	491.4[b]	1.49[b]	2.1

[a,b]Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.

[1]Values for feed intake expressed as total feed intake in trial 1 and as average daily feed intake in trials 2–4.

[2]Values for weight again expressed as total weight gain in trial 1 and as average daily weight gain in trials 2–4.

3.3.2 | Conclusions on efficacy

The additive has the potential to be efficacious for weaned piglets at the minimum use level of 0.600 mg SG/kg complete feed (achieved with 120 mg Sangrovit® Extra/kg complete feed). This conclusion is extended to sucking piglets for the period in which solid feed is given and extrapolated to other growing Suidae up to an age of 120 days or a body weight of 35 kg.

3.4 | Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[33] and Good Manufacturing Practice.

4 | CONCLUSIONS

The following conclusions apply to Sangrovit® Extra, which consists of a *M. cordata* extract (0.5%–1.2%) [redacted] and of *M. cordata* processed leaves (30%–64%) and is formulated to contain a concentration of the sum of the four alkaloids (SG, CH, PRO and ALL) of 1.25%, with 0.5% SG (0.4%–0.7%).

Owing to the presence of the DNA intercalators SG and CH, a concern for genotoxicity is identified.

Based on the results of the tolerance trial in weaned piglets, the FEEDAP Panel has no safety concerns for the target species when the additive Sangrovit® Extra is used up to the maximum recommended level of 0.750 mg SG/kg complete feed for weaned piglets. This conclusion was extended to suckling piglets and extrapolated to other growing Suidae. The Panel notes that these conclusions do not cover pigs and minor porcine species reared for reproduction, which are considered long-living/reproductive animals.

Since in all consumer categories the exposure to SG and CH exceeds the threshold of toxicological concern of 0.0025 μg/kg bw per day for DNA reactive mutagens and/or carcinogens, the FEEDAP Panel cannot conclude on the safety of Sangrovit® Extra for the consumers.

The additive is irritant to the eyes but not irritant to skin or a skin sensitiser. The FEEDAP Panel cannot not exclude the potential of the additive to be a respiratory sensitiser. When handling the additive, exposure of unprotected users to SG and CH may occur. Therefore, to reduce the risk, the exposure of users should be reduced.

The use of Sangrovit® Extra as a feed additive under the proposed conditions of use is considered safe for the environment.

The additive Sangrovit® Extra has the potential to be efficacious in improving performance of weaned piglets at 0.600 mg SG/kg complete feed. This conclusion is extended to sucking piglets for the period in which solid feed is given and other growing Suidae up to the age of 120 days or a body weight of 35 kg.

5 | RECOMMENDATIONS

The specification for SG in Sangrovit® Extra should not exceed 0.7%. The specifications for the sum of the four alkaloids should not exceed the highest analysed concentration (1.4%).

ABBREVIATIONS

ALL	allocryptopine
AUC	area under the curve
bw	body weight

[33]Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

CH	chelerythrine
DHSG	dihydrosanguinarine
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HGB	haemoglobin
HPLC	high-performance liquid chromatography
LC-IT/TOFMS	liquid chromatography ion trap/time of flight mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NOAEL	no observed adverse effect level
PRO	protopine
RBC	red blood cell count
SG	sanguinarine
TTC	threshold of toxicological concern
UPLC–MS/MS	ultraperformance liquid chromatography tandem mass spectrometry

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

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APPENDIX A

Detailed results of chronic exposure calculation

TABLE A.1 Chronic dietary exposure of consumers to residues of the sum of sanguinarine and chelerythrine per population class, country and survey (ng/kg body weight per day) based on residue data in pigs.

Population class	Survey's country	Number of subjects	Mean	Highest reliable percentile value	Highest reliable percentile description
Infants	Bulgaria	523	1.369	7.046	95th
Infants	Germany	142	1.281	6.186	95th
Infants	Denmark	799	3.350	10.526	95th
Infants	Finland	427	2.173	5.932	95th
Infants	Italy	9	1.679	0.022	50th
Infants	United Kingdom	1251	1.571	6.465	95th
Toddlers	Belgium	36	6.870	11.149	90th
Toddlers	Bulgaria	428	4.682	12.488	95th
Toddlers	Germany	348	4.163	10.845	95th
Toddlers	Denmark	917	6.656	13.181	95th
Toddlers	Spain	17	5.777	9.065	75th
Toddlers	Finland	500	5.275	11.624	95th
Toddlers	Italy	36	4.587	10.295	90th
Toddlers	Netherlands	322	4.750	10.846	95th
Toddlers	United Kingdom	1314	3.395	9.332	95th
Toddlers	United Kingdom	185	4.232	10.631	95th
Other children	Austria	128	4.627	10.739	95th
Other children	Belgium	625	5.429	11.839	95th
Other children	Bulgaria	433	5.759	13.124	95th
Other children	Germany	293	4.129	8.933	95th
Other children	Germany	835	4.121	9.410	95th
Other children	Denmark	298	5.766	10.105	95th
Other children	Spain	399	5.766	12.743	95th
Other children	Spain	156	5.903	14.642	95th
Other children	Finland	750	6.021	12.172	95th
Other children	France	482	5.230	10.409	95th
Other children	Greece	838	4.576	10.509	95th
Other children	Italy	193	5.408	11.950	95th
Other children	Latvia	187	4.373	11.279	95th
Other children	Netherlands	957	3.977	9.563	95th
Other children	Netherlands	447	3.945	10.003	95th
Other children	Sweden	1473	5.654	11.376	95th
Other children	Czechia	389	5.264	12.126	95th
Other children	United Kingdom	651	3.543	8.779	95th
Adolescents	Austria	237	3.134	6.491	95th
Adolescents	Belgium	576	2.459	6.112	95th
Adolescents	Cyprus	303	2.005	4.870	95th
Adolescents	Germany	393	3.286	7.468	95th
Adolescents	Germany	1011	2.524	6.863	95th
Adolescents	Denmark	377	3.295	7.101	95th
Adolescents	Spain	651	4.177	9.184	95th
Adolescents	Spain	209	4.390	10.739	95th
Adolescents	Spain	86	4.445	9.037	95th
Adolescents	Finland	306	2.729	5.788	95th

(Continues)

TABLE A.1 (Continued)

Population class	Survey's country	Number of subjects	Mean	Highest reliable percentile value	Highest reliable percentile description
Adolescents	France	973	3.182	6.274	95th
Adolescents	Italy	247	3.553	7.163	95th
Adolescents	Latvia	453	3.696	8.750	95th
Adolescents	Netherlands	1142	2.846	6.897	95th
Adolescents	Sweden	1018	3.813	7.249	95th
Adolescents	Czechia	298	5.060	11.953	95th
Adolescents	United Kingdom	666	2.291	5.210	95th
Adults	Austria	308	2.103	6.484	95th
Adults	Belgium	1292	2.328	5.787	95th
Adults	Germany	10,419	2.296	5.874	95th
Adults	Denmark	1739	2.482	4.929	95th
Adults	Spain	981	3.580	7.248	95th
Adults	Spain	410	2.740	6.538	95th
Adults	Finland	1295	2.263	5.765	95th
Adults	France	2276	2.527	4.917	95th
Adults	Hungary	1074	3.709	8.119	95th
Adults	Ireland	1274	2.427	5.589	95th
Adults	Italy	2313	2.332	4.937	95th
Adults	Latvia	1271	2.955	7.704	95th
Adults	Netherlands	2055	2.219	5.470	95th
Adults	Romania	1254	3.105	6.752	95th
Adults	Sweden	1430	2.895	6.408	95th
Adults	Czechia	1666	3.812	9.178	95th
Adults	United Kingdom	1265	1.749	4.255	95th
Elderly	Austria	67	1.950	4.817	95th
Elderly	Belgium	511	2.417	5.459	95th
Elderly	Germany	2006	2.072	4.920	95th
Elderly	Denmark	274	2.198	4.080	95th
Elderly	Finland	413	1.866	4.759	95th
Elderly	France	264	2.250	4.056	95th
Elderly	Hungary	206	2.820	6.382	95th
Elderly	Ireland	149	2.455	5.429	95th
Elderly	Italy	289	1.977	4.434	95th
Elderly	Netherlands	173	2.296	5.179	95th
Elderly	Netherlands	289	2.002	4.595	95th
Elderly	Romania	83	2.090	4.909	95th
Elderly	Sweden	295	2.330	5.237	95th
Elderly	United Kingdom	166	1.543	3.502	95th
Very elderly	Austria	25	1.839	2.311	75th
Very elderly	Belgium	704	2.265	5.214	95th
Very elderly	Germany	490	1.886	4.617	95th
Very elderly	Denmark	12	2.091	2.616	75th
Very elderly	France	84	2.153	4.247	95th
Very elderly	Hungary	80	3.186	5.964	95th
Very elderly	Ireland	77	2.516	5.536	95th
Very elderly	Italy	228	1.738	3.829	95th
Very elderly	Netherlands	450	2.045	4.617	95th
Very elderly	Romania	45	2.308	5.276	90th
Very elderly	Sweden	72	2.344	5.457	95th
Very elderly	United Kingdom	139	1.707	3.677	95th

TABLE A.2 Chronic dietary exposure of consumers to residues of the sum of protopine and allocryptopineper population class, country and survey (ng/kg body weight per day) based on residue data in pigs.

Population class	Survey's country	Number of subjects	Mean	Highest reliable percentile value	Highest reliable percentile description
Infants	Bulgaria	523	0.412	2.206	95th
Infants	Germany	142	0.348	1.486	95th
Infants	Denmark	799	0.915	2.792	95th
Infants	Finland	427	0.652	1.780	95th
Infants	Italy	9	0.504	0.007	50th
Infants	United Kingdom	1251	0.453	1.809	95th
Toddlers	Belgium	36	1.990	3.283	90th
Toddlers	Bulgaria	428	1.370	3.638	95th
Toddlers	Germany	348	1.127	2.808	95th
Toddlers	Denmark	917	1.769	3.412	95th
Toddlers	Spain	17	1.749	2.421	75th
Toddlers	Finland	500	1.559	3.453	95th
Toddlers	Italy	36	1.315	3.089	90th
Toddlers	Netherlands	322	1.286	2.926	95th
Toddlers	United Kingdom	1314	0.939	2.597	95th
Toddlers	United Kingdom	185	1.163	2.839	95th
Other children	Austria	128	1.287	2.892	95th
Other children	Belgium	625	1.546	3.390	95th
Other children	Bulgaria	433	1.629	3.701	95th
Other children	Germany	293	1.129	2.373	95th
Other children	Germany	835	1.130	2.482	95th
Other children	Denmark	298	1.601	2.787	95th
Other children	Spain	399	1.634	3.675	95th
Other children	Spain	156	1.667	3.962	95th
Other children	Finland	750	1.690	3.437	95th
Other children	France	482	1.530	3.090	95th
Other children	Greece	838	1.324	3.091	95th
Other children	Italy	193	1.570	3.524	95th
Other children	Latvia	187	1.199	3.288	95th
Other children	Netherlands	957	1.080	2.572	95th
Other children	Netherlands	447	1.155	2.880	95th
Other children	Sweden	1473	1.526	3.002	95th
Other children	Czechia	389	1.427	3.325	95th
Other children	United Kingdom	651	0.977	2.416	95th
Adolescents	Austria	237	0.873	1.849	95th
Adolescents	Belgium	576	0.711	1.710	95th
Adolescents	Cyprus	303	0.603	1.477	95th
Adolescents	Germany	393	0.892	1.990	95th
Adolescents	Germany	1011	0.683	1.853	95th
Adolescents	Denmark	377	0.938	1.996	95th
Adolescents	Spain	651	1.209	2.655	95th
Adolescents	Spain	209	1.254	3.112	95th
Adolescents	Spain	86	1.266	2.513	95th
Adolescents	Finland	306	0.777	1.663	95th
Adolescents	France	973	0.915	1.819	95th
Adolescents	Italy	247	1.032	2.097	95th
Adolescents	Latvia	453	1.000	2.399	95th
Adolescents	Netherlands	1142	0.819	2.019	95th

(Continues)

TABLE A.2 (Continued)

Population class	Survey's country	Number of subjects	Mean	Highest reliable percentile value	Highest reliable percentile description
Adolescents	Sweden	1018	1.050	1.989	95th
Adolescents	Czechia	298	1.316	2.912	95th
Adolescents	United Kingdom	666	0.656	1.492	95th
Adults	Austria	308	0.604	1.747	95th
Adults	Belgium	1292	0.680	1.711	95th
Adults	Germany	10,419	0.625	1.589	95th
Adults	Denmark	1739	0.709	1.403	95th
Adults	Spain	981	1.024	2.104	95th
Adults	Spain	410	0.779	1.839	95th
Adults	Finland	1295	0.634	1.571	95th
Adults	France	2276	0.720	1.415	95th
Adults	Hungary	1074	0.968	2.110	95th
Adults	Ireland	1274	0.706	1.625	95th
Adults	Italy	2313	0.672	1.430	95th
Adults	Latvia	1271	0.820	2.121	95th
Adults	Netherlands	2055	0.642	1.593	95th
Adults	Romania	1254	0.836	1.810	95th
Adults	Sweden	1430	0.819	1.788	95th
Adults	Czechia	1666	0.953	2.296	95th
Adults	United Kingdom	1265	0.506	1.215	95th
Elderly	Austria	67	0.566	1.391	95th
Elderly	Belgium	511	0.699	1.604	95th
Elderly	Germany	2006	0.558	1.370	95th
Elderly	Denmark	274	0.633	1.206	95th
Elderly	Finland	413	0.519	1.322	95th
Elderly	France	264	0.635	1.126	95th
Elderly	Hungary	206	0.735	1.631	95th
Elderly	Ireland	149	0.704	1.611	95th
Elderly	Italy	289	0.577	1.313	95th
Elderly	Netherlands	173	0.667	1.498	95th
Elderly	Netherlands	289	0.582	1.338	95th
Elderly	Romania	83	0.578	1.415	95th
Elderly	Sweden	295	0.660	1.506	95th
Elderly	United Kingdom	166	0.450	1.095	95th
Very elderly	Austria	25	0.501	0.680	75th
Very elderly	Belgium	704	0.661	1.562	95th
Very elderly	Germany	490	0.494	1.173	95th
Very elderly	Denmark	12	0.590	0.733	75th
Very elderly	France	84	0.619	1.224	95th
Very elderly	Hungary	80	0.849	1.580	95th
Very elderly	Ireland	77	0.731	1.577	95th
Very elderly	Italy	228	0.507	1.136	95th
Very elderly	Netherlands	450	0.600	1.344	95th
Very elderly	Romania	45	0.632	1.366	90th
Very elderly	Sweden	72	0.657	1.592	95th
Very elderly	United Kingdom	139	0.497	1.090	95th