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Safety and efficacy of a feed additive consisting of halofuginone hydrobromide (STENOROL®) for chickens for fattening and turkeys for fattening/reared for breeding (Huvepharma N.V.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a new scientific opinion on the coccidiostat halofuginone hydrobromide (STENOROL®) when used as a feed additive for chickens for fattening and turkeys for fattening/reared for breeding. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that the safety for turkeys for fattening established in its previous opinion can be extended to turkeys for breeding up to 12 weeks of age. Based on the new data provided on the safety for consumer, environment and efficacy, the Panel updates its previous conclusions as follows: halofuginone hydrobromide is not genotoxic. Applying an uncertainty factor of 100 to the lowest no observed adverse effect level (NOAEL) of 0.03 mg/kg body weight (bw) per day, an acceptable daily intake (ADI) of 0.3 μg halofuginone/kg bw is established. The chronic exposure of consumers to residues of halofuginone would amount to 6–19% of the ADI after 3 days of withdrawal. Therefore, the Panel considers that the additive is safe for the consumer of tissues obtained from chickens for fattening and turkeys for fattening fed the additive at a maximum level of 3 mg/kg complete feed at a 3-day withdrawal time. For control purposes, the Panel recommends the setting of the following maximum residue limits (MRLs): liver, 50 μg/kg; kidney, 40 μg/kg; muscle, 3 μg/kg; skin/fat, 10 μg/kg wet tissue. Based on an updated environmental risk assessment, no concern for groundwater is expected. Halofuginone is unlikely to bioaccumulate and the risk of secondary poisoning is not likely to occur. No safety concerns are expected for terrestrial and aquatic environments. The additive has the potential to control coccidiosis in chickens for fattening and turkeys for fattening/reared for breeding up to 12 weeks of age at a minimum level of 2 mg/kg complete feed.

Keywords: coccidiostats, halofuginone hydrobromide, chickens for fattening, turkeys for fattening, turkeys reared for breeding, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2021-00321

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defined the term of the authorisation by the Commission.

The applicant Huvepharma NV is seeking an authorization for halofuginone hydrobromide (Stenorol) when used as feed additive for chickens for fattening and turkeys, category: coccidiostats and histomonostats. (Table 1).

Table 1: Description of the additive

Category of additive	Coccidiostats and histomonostats
Functional group of additive	N/A
Description	halofuginone hydrobromide (Stenorol)
Target animal category	chickens for fattening and turkeys
Applicant	Huvepharma NV
Type of request	New opinion

On 30 September 2020, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority, in its opinion on the safety and efficacy of the product, could not conclude on the safety and efficacy of that additive.

After the discussion with the Member States at a meeting of the Standing Committee on Plants, Animals, Food and Feed (Animal Nutrition section), it was suggested to check for the possibility to demonstrate the safety and efficacy of the additive. As requested, a road map for the submission of the studies has been submitted by the applicant and agreed by the Member States.

The Commission gave the possibility to the applicant to submit complementary information and data in order to complete the assessment and to allow a revision of the Authority's opinion. The new data have been received on 18 March 2021 and were already transmitted to the Authority by the applicant. Following the road map, the data refer in particular to:

1. Outstanding issues on consumer safety:
   - Comet Assay.
2. Outstanding issues on environmental safety.
   - Earthworm study.
   - Green algae study.
   - Daphnia study.
   - Fish study.
3. Outstanding issues on efficacy.
   - Chickens for fattening AST.
   - Chickens for fattening floorpen study.
   - Turkeys for fattening AST.
   - Turkeys for fattening floorpen.

Future data will be submitted to complete the assessment.

However, in view to clarify some of the elements of concerns for a product already on the market, the Authority is kindly requested to provide an opinion of this first set of data.

In view of the above, the Commission asks the Authority to deliver a new opinion on halofuginone hydrobromide (Stenorol) as feed additive for chickens for fattening and turkeys based on the additional data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[1] to a previous application on the same product.[2] The dossier was received on 18 March 2021 and the general information and supporting documentation available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00321.

In accordance with Article 38 of the Regulation (EC) No 178/2002[3] 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,[4] a non-confidential version of the supplementary information has been published on Open.EFSA.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA to deliver the present output.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of halofuginone hydrobromide (STENOROL®) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

Halofuginone hydrobromide (halofuginone HBr) from STENOROL® is currently authorised for use in chickens for fattening and turkeys covering turkeys for fattening and reared for breeding up to a maximum age 12 weeks at the level of 2–3 mg/kg complete feedingstuffs with a withdrawal time of 5 days.[6]

In the current opinion, the safety of the additive for the consumer, including a new proposal for shorter withdrawal period, the safety for the environment, and the efficacy is assessed based on a second set of supplementary information submitted by the applicant.

The FEEDAP Panel adopted its first opinion on the re-evaluation of this additive in 2020 (EFSA FEEDAP Panel, 2020). In 2022, a second opinion was adopted on the supplementary data set covering the safety for target species (EFSA FEEDAP Panel, 2022) in which the Panel concluded on the safety for turkeys for fattening up to a maximum of 12 weeks of age; the safety for turkeys reared for breeding was not considered. In the current opinion, the safety of the additive for turkeys reared for breeding is also addressed.

3.1. Safety

The FEEDAP Panel noted that the information submitted to characterise the additive in 2020 (EFSA FEEDAP Panel, 2020), did not include suitable data to exclude the presence of small/nanoparticles as foreseen in the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA SC, 2021). Therefore, the applicant was requested to provide information in the context of the present assessment, choosing any of the appraisal routes indicated by the aforementioned guidance document.

[1] Dossier reference: EFSA-Q-2021-00321.
[2] Dossier reference: FAD-2010-0293.
[3] 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.
[4] Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[5] 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.
[6] Commission Directive 91/248/EEC of 12 April 1991 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs.

The applicant submitted particle size analysis data using a combination of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) technique.[7] The shortcomings identified in the submitted data performed with the additive STENOROL® did not allow the FEEDAP Panel to conclude on the absence of (a fraction of) small particles including nanoparticles in the formulation, which is constituted by 0.6% halofuginone HBr, 1% povidone (polyvinylpyrrolidone), 2% castor oil (macrogol glycerol ricinoleate) and 96.4% corn cobs.

In the absence of adequate data on the decision criteria for particle size, the FEEDAP Panel considered the following elements. The active substance is slightly soluble[8] in water (2.5 g/L)[9] and it is micronised (EFSA FEEDAP Panel, 2020). Furthermore, it is noted that the manufacturing of the additive includes a spray-drying step, in which the spraying liquid containing the micronised active substance, isopropyl alcohol, purified water, sulfuric acid, povidone (polyvinylpyrrolidone) and castor oil is sprayed onto corn cobs. No data was available if the active substance was completely dissolved in the spraying liquid. However, it is also noted that this process does not involve any chemical modification or binding/encapsulation.

The Panel further noted that the active ingredient and the formulated additive, subject of this assessment, and the test items used in the ADME, residue and toxicological studies, including tolerance studies, were produced by the same manufacturing process (EFSA FEEDAP Panel, 2020, 2022).

Considering the above and in line with the appraisal route described in Section 4 of the Guidance on technical requirements (EFSA SC, 2021), the Panel concludes that the safety of halofuginone HBr from STENOROL® can be adequately covered by the conventional risk assessment and any risks from particles that are potentially below 500 nm in size have already been covered by the existing data.

3.1.1. Safety for the target species

In its previous opinion, the FEEDAP Panel evaluated two tolerance studies with turkeys for fattening and concluded that 3 mg halofuginone HBr/kg complete feed is safe for turkeys for fattening up to 12 weeks of age without a margin of safety (EFSA FEEDAP Panel, 2022). The FEEDAP Panel extends the conclusions reached in the previous opinion to turkeys reared for breeding up to 12 weeks of age.

3.1.2. Safety for the consumer

3.1.2.1. Absorption, distribution, metabolism and excretion and residues

In 2020, the FEEDAP Panel re-evaluated the ADMER of halofuginone HBr and concluded the following: 'Halofuginone was absorbed but excreted unchanged at a large extent in chicken and turkey excreta. A major metabolite common to chicken and turkey excreta was also present in rat faeces. Biliary excretion was substantial. Comparative *in vitro* metabolic fate in chicken, turkey and rat, indicated quantitatively similar biotransformation pathways, but also a non-metabolic breakdown of the molecule. A major metabolite arising from the reduction of halofuginone was found to be common to the chicken and turkey, and likely the rat; this metabolite was shown to be present *in vivo* in chicken and turkey liver. A second metabolite (a glutathione-conjugate) was identified in rat liver incubations and shown to be present in chicken and turkey. Despite technical difficulties inherent to the chemical structure of halofuginone, the efforts made allow the FEEDAP Panel to conclude that the metabolic fate of this compound is very likely similar in the chicken, turkey and rat. In general, the residues of halofuginone in tissues and organs of chickens and turkeys are of the same magnitude and the ratios halofuginone vs. total residues were similar. Halofuginone is the marker residue and liver is the target tissue.'

3.1.2.2. Toxicological studies, including genotoxicity

In 2020, the toxicological profile of halofuginone was re-evaluated by the FEEDAP Panel in its opinion on the safety and efficacy of STENOROL® for chickens for fattening and turkeys (EFSA FEEDAP Panel, 2020). The toxicological data set available allowed to identify the lowest no observed adverse effect level (NOAEL) of 0.03 mg halofuginone HBr/kg bw per day, based on reproductive effects and maternal toxicity seen in a rabbit teratology study. However, a final conclusion on the toxicity of halofuginone was not possible because the Panel identified a gap in the genotoxicity data set:

[7] Technical dossier/Additional data January 2023/Annex RTQ 08
[8] The value is below the threshold set in Section 2.3.1. of the Guidance on technical requirements (EFSA SC, 2021).
[9] FAD-2010-0293/Technical dossier/Section II

'Halofuginone HBr did not induce chromosome damage *in vivo* as observed by the micronucleus test in two studies showing negative results in the presence of target tissue exposure. The test item induced significant increase of gene mutations in bacteria, while no gene mutations were observed in mammalian cells *in vitro*; the *in vivo* UDS study was considered not sufficiently informative. Since an appropriate *in vivo* follow-up to exclude the mutagenic effect of halofuginone HBr was not available, the FEEDAP Panel cannot conclude on the genotoxicity of halofuginone HBr and considers that further testing is needed.'

For the current assessment, a new alkaline Comet assay[10] was performed in Han Wistar male rats to evaluate the potential halofuginone HBr (purity 98.5%) to induce DNA strand breaks *in vivo*. The study was conducted in accordance with OECD TG 489 (2016) and following good laboratory practice. Based on the results of the range-finder experiment, rats were treated orally at doses of 5, 10 and 20 mg/kg bw halofuginone HBr (equivalent to 25% of the maximum tolerated dose (MTD), 50% of the MTD and the MTD, respectively). The test item was administered twice at 0 and 22.5 h and rats were sacrificed 1.5 h after last administration. No clinical signs of toxicity were observed, while body weight reduction up to 2.4% was recorded in animals treated with the highest dose. Systemic exposure was confirmed by clinical chemistry and histopathological examinations. In detail, a dose-related increase of the incidence and severity of pale liver was observed together with atrophy of duodenum.

No increase in the percentage of hedgehog cells was reported in liver and duodenum, showing that treatment with halofuginone HBr did not cause excessive DNA damage that could interfere with comet analysis. Tail intensity values in the treated groups were comparable to the values observed in the concurrent vehicle controls and were within the range of historical vehicle control data. On this basis, the Panel concludes that halofuginone HBr did not induce DNA strand breaks *in vivo* under the experimental conditions applied in this study.

Conclusions on the toxicology

Halofuginone HBr is not genotoxic. The toxicological data set available allowed to identify the lowest NOAEL of 0.03 mg halofuginone HBr/kg bw per day, based on reproductive effects and maternal toxicity seen in the rabbit teratology study. This NOAEL can be considered as an appropriate basis for the health-based guidance value (acceptable daily intake (ADI)) of 0.3 μg halofuginone HBr/kg bw applying an uncertainty factor of 100. The FEEDAP Panel noted that this ADI corresponds to 0.25 μg halofuginone/kg bw. Considering that halofuginone is the marker residue, the rounded value of 0.3 μg halofuginone/kg bw is retained for the current assessment. The FEEDAP Panel noted that ADI is in line with the one established by the Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicine Agency (EMA) (EMA-CVMP, 1998 and 2000).

3.1.2.3. Assessment of consumer safety

Consumer exposure

The chronic exposure of consumers to halofuginone in chicken tissues was calculated (Table 2) following the methodology described in the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017) (for further details, see Appendix A) using the residue data originating from residue studies (EFSA FEEDAP Panel, 2020; Section 3.2.1.1, Table 1) summarised in Table 3.

Table 2: Chronic dietary exposure of consumers to halofuginone total residues based on residue data in chicken tissues after 3- and 4-day withdrawal – Summary statistics across European dietary surveys

Population class	Number of surveys	3-day withdrawal		4-day withdrawal	
		Highest exposure estimate (μg/kg bw per day)	% ADI(1)	Highest exposure estimate (μg/kg bw per day)	% ADI(1)
Infants	6	0.0400	13	0.0302	10
Toddlers	10	0.0563	19	0.0435	15
Other children	18	0.0572	19	0.0440	15

[10] Technical dossier/Ref-1.

Population class	Number of surveys	3-day withdrawal		4-day withdrawal	
		Highest exposure estimate (μg/kg bw per day)	% ADI(1)	Highest exposure estimate (μg/kg bw per day)	% ADI(1)
Adolescents	17	0.0264	9	0.0199	7
Adults	17	0.0413	14	0.0336	11
Elderly	14	0.0160	5	0.0121	4
Very elderly	12	0.0178	6	0.0142	5

(1): ADI: 0.3 μg/kg bw per day.

Table 3: Total residue concentration(1) of halofuginone (mg/kg) in tissues of chickens administered 3 mg halofuginone HBr/kg feed for 14 days followed by a withdrawal period of 3 and 4 days

Withdrawal time	Liver	Kidney	Muscle(2)	Skin/fat(2)
3 days	0.124	0.052	0.005	0.012
4 days	0.102	0.019	0.004	0.007

(1): total residue concentration (mean + 2× standard deviation).
(2): The residue concentration in muscle and skin/fat will be applied to the intake of meat at the following proportions: 90% muscle and 10% skin/fat (EFSA FEEDAP Panel, 2017). This corresponds to 0.0057 and 0.0043 mg/kg at 3 days and 4 days withdrawal, respectively.

The results showed that the highest chronic exposure calculated with residue data after 3-day withdrawal was for the age classes 'toddlers' and 'other children', with 0.06 μg/kg bw per day. These were followed by the age classes 'infants' and 'adults' with 0.04 μg/kg bw per day. The same trend was seen with residue data obtained after 4-day withdrawal. The exposure expressed as % ADI was < 19% in all cases (for detailed results per age class, country and survey, see Appendix A, Tables A.1 and A.2).

The FEEDAP Panel noted that residues of halofuginone in tissues and organs of chickens and turkeys were of the same magnitude, and the ratios halofuginone vs. total residues were also similar. Therefore, the results of exposure calculations obtained with residue data in chicken tissues can be extrapolated to consumer exposure to halofuginone residues from turkeys (EFSA FEEDAP Panel, 2020; Section 3.2.1.1).

The FEEDAP Panel noted that halofuginone is authorised in the EU also as veterinary medicine for bovines with the indication for use in non-ruminating calves of 4–15 days of age which are unlikely to be sent for slaughter during or immediately after treatment (EMA-CVMP, 2000); therefore, this use of halofuginone is not considered to contribute to the exposure of consumers to halofuginone residues in food of animal origin.

MRLs and withdrawal period

The exposure at 3-day withdrawal, and consequently at 4-day withdrawal, is well below the ADI. Considering the concentration of halofuginone measured in residue studies at 3-day withdrawal (see Table 4), the following maximum residue limits (MRLs) could be used for control purposes: liver, 50 μg/kg; kidney, 40 μg/kg; muscle, 3 μg/kg; skin/fat, 10 μg/kg wet tissue.

The safety of the proposed MRLs has been evaluated following the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017). The total residue concentrations calculated using the proposed MRLs and the ratio marker residue concentration to total residue concentration (RMTR) are shown in Table 4.

Table 4: Halofuginone total residues (mg/kg) calculated from proposed MRL values at withdrawal period of 3 days

	Liver	Kidney	Muscle(3)	Skin/fat(3)
MRC(1)	0.045	0.035	< 0.001	0.004
Proposed MRL	**0.050**	**0.040**	**0.003**	**0.010**

	Liver	Kidney	Muscle(3)	Skin/fat(3)
RMTR(2)	0.32	0.50	0.33(4)	0.36
TR_{MRL}	0.156	0.08	0.009	0.028

MRC: maker residue concentration, MRL: maximum residue limit; TRC: total residue concentration; RMTR: ratio MRC to TRC, TR_{MRL}: total residue concentration calculated from proposed MRLs applying the RMTR.
(1): MRC + 2 × SD; EFSA FEEDAP Panel, 2020 Section 3.2.1.1, Table 3.
(2): EFSA FEEDAP Panel, 2020 Section 3.2.1.1, Table 3.
(3): The residue concentration in muscle and skin/fat will be applied to the intake of meat at the following proportions: 90% muscle and 10% skin/fat (EFSA FEEDAP Panel, 2017). This corresponds to 0.011 mg/kg.
(4): For muscle, the MRC was below the LOQ. Therefore, the ratio was calculated dividing the LOQ by the TRC.

The chronic exposure of consumers to halofuginone total residues calculated from the proposed MRLs was calculated (Table 5) using the same methodology as above.

Table 5: Chronic dietary exposure of consumers to halofuginone total residues calculated from MRLs proposed for control purposes – Summary statistics across European dietary surveys

Population class	Number of surveys	Highest exposure estimate (μg/kg bw per day)	% ADI(1)
Infants	6	0.0756	25
Toddlers	10	0.1002	33
Other children	18	0.0936	31
Adolescents	17	0.0510	17
Adults	17	0.0556	19
Elderly	14	0.0269	9
Very elderly	12	0.0281	9

(1): ADI: 0.3 μg/kg bw per day.

The exposure expressed as % ADI was the highest for children (25–33%) and were between 9% and 19% for the other age classes (for detailed results per age class, country and survey, see Appendix A, Table A.3) Based on these results, the proposed MRLs are retained and recommended for control purposes in chicken and turkey tissues if a withdrawal period of 3 days is applied.

3.1.2.4. Conclusions on safety for the consumer

The chronic exposure to residues resulting from the use of the feed additive halofuginone HBr (STENOROL®) in chickens would amount up to 6–19% of the ADI after 3-day withdrawal. Based on this, the FEEDAP Panel considers that the additive is safe for the consumer of tissues obtained from chickens for fattening fed the additive under the proposed conditions of use including 3 days of withdrawal time. These conclusions are extrapolated to food products obtained from turkeys for fattening. For control purposes, the Panel recommends the setting of the following MRLs: liver, 50 μg/kg; kidney, 40 μg/kg; muscle, 3 μg/kg; skin/fat, 10 μg/kg wet tissue.

3.1.3. Safety for the environment

In 2020, the FEEDAP Panel adopted an opinion on the safety and efficacy of STENOROL® (halofuginone HBr) as a feed additive for chickens for fattening and turkeys (EFSA FEEDAP Panel, 2020). In this opinion, it was concluded that: 'Due to limitations in some of the ecotoxicological studies, no conclusions can be drawn on the safety of the additive for the environment.' For the present assessment, the applicant addressed the data gap highlighted in the previous opinion and submitted an updated environmental risk assessment, including new studies, performed according to the FEEDAP guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019). The environmental risk assessment of STENOROL® as a feed additive for chickens for fattening and turkeys is updated as follows.

In line with the approach followed in the opinion adopted in 2020, the environmental risk assessment is performed considering that halofuginone is the substance expected to be excreted and consequently reach the environment (EFSA FEEDAP Panel, 2020).

3.1.3.1. Phase I

Physico-chemical properties

The physico-chemical properties of halofuginone are summarised in Table 6.

Table 6: Physico-chemical properties of halofuginone(1)

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow} 25°C)	1.06 (pH 5)	–
	1.27 (pH 7)	
	2.58 (pH 9)	
Water solubility (20°C)	3.58	g/L
	6.63 (pH 5)	
	1.83 (pH 7)	
	1.52×10^{-2} (pH 9)	
Dissociation constant (25°C) (pKa)	8.07	–
Vapour pressure (VP)	8.1×10^{-7}	Pa

(1): EFSA FEEDAP Panel, 2020.

Fate and behaviour

The applicant submitted the same studies on adsorption/desorption in soil and degradation in soil that were previously evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2020).

In particular, in a study on adsorption/desorption, conducted in accordance with OECD guideline 106 on five soils, a lowest K_{FOC} value of 3.727 L/kg was identified as the most appropriate to calculate PEC in Phase I; in a study on the degradation of halofuginone in soil, conducted in accordance with OECD guideline 307, the arithmetic mean DT_{50} of 58 days was considered the reference value for the calculation of exposure. This value, normalised to 12°C using the Arrhenius equation,[11] corresponds to a DT_{50} of 123 days.

For the current assessment it is confirmed that the following values can be used for the assessment of the exposure in the different environmental compartments: K_{oc} of 3,727 L/kg and DT_{50} of 123 days at 12°C.

Predicted environmental concentrations (PECs)

The calculated PEC initial values for chickens for fattening and turkeys are given in Table 7. The highest dose recommended (3 mg halofuginone HBr/kg complete feed, equivalent to 2.51 mg halofuginone/kg complete feed) was considered for calculation of the initial PECs. Results show that turkeys represent the worst-case exposure, which covers also chickens for fattening.

Table 7: Initial predicted environmental concentration (PECs) of halofuginone in soil, groundwater, surface water and sediment

Property	Value	
Dose (mg/kg feed)	2.51	
Molecular weight (g/mol)	414.68	
Vapour pressure (Pa)	8.1×10^{-7}	
Solubility (mg/L) at pH 7	1,830	
K_{oc} (L/kg)	3,727	
DT_{50} in soil at 12°C (days)	123	
Output	**Chickens**	**Turkeys**
PEC_{soil} (µg/kg)	38	40
$PEC_{groundwater}$ (µg/L)	0.13	0.13

[11] The temperature correction was performed according to the scientific opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil (EFSA, 2007).

In Phase I, PEC trigger values are exceeded both for soil and for groundwater; a risk assessment for environment, according to Phase II, is therefore required.

3.1.3.2. Phase II

Exposure assessment

PECs calculation refined in Phase II

Considering the DT_{50} value of 123 days, a recalculation of the different PECs was performed to take into account possible accumulation. The $PEC_{plateau}$ for the different compartments are reported in Table 8. Since the calculated PECs are higher for turkey for fattening, the evaluation for environment will be referred to this species, which covers also chickens for fattening.

Table 8: Predicted environmental concentration at plateau of halofuginone in soil, groundwater, surface water and sediment

Output	Value
$PEC_{soil\ plateau}$ (µg/kg)	46
$PEC_{groundwater\ plateau}$ (µg/L)	0.15
$PEC_{surface\ water\ plateau}$ (µg/L)	0.051
$PEC_{sediment\ plateau}$ (µg/kg)	19

PEC_{soil} refined for metabolism

Halofuginone is excreted unchanged at a large extent in chicken and turkey excreta (Section 3.1.2.1); therefore, no refinement based on metabolism is possible.

$PEC_{groundwater}$ refinement

To refine $PEC_{groundwater}$, the simplified approach described in the Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) was used, which lays out requirements for the K_{OM} (= K_{oc}/1.7) as a function of the FOCUS leaching concentration. Considering the DT_{50} at 20°C of 58 days and the K_{oc} of 3,727 L/kg, halofuginone is considered not to pose a risk to groundwater.

$PEC_{surface\ water}$ and $PEC_{sediment}$ refinement

Concentrations in surface waters and sediment for halofuginone were assessed using the FOCUS Step 3 surface water assessment approach. The FOCUS surface water models PRZM, MACRO and TOXSWA were used.[12] The modelling was carried out in accordance with the EFSA guidance (EFSA FEEDAP Panel, 2019), considering a single application before emergence to winter cereals within Europe. The application rates in kg/ha were calculated from the initial PEC_{soil} considering a soil bulk density of 1500 kg/m^3 and a mixing depth of 5 cm: 0.028 kg/ha for chickens for fattening and 0.030 kg/ha for turkeys for fattening. In the following table, the input data used for modelling are reported (Table 9).

Table 9: Halofuginone property data for exposure modelling

Parameter	Unit	Value
Molecular weight	g/mol	414.68
Solubility in water (20°C)	mg/L	1,830
Saturated vapour pressure	Pa	8.1×10^{-7}
Mixing depth	m	0.05
DT_{50} water(1)	days	1,000
DT_{50} sediment(1)	days	1,000
DT_{50} soil(2)	days	42.7
K_{OC}	mL/g	3,726.5

[12] Technical dossier/Additional data January 2023/Annex RTQ 01.

Parameter	Unit	Value
K_{OM}	mL/g	2,161.5
1/n	–	0.92

(1): value not available; worst-case assumption.
(2): DT_{50} recalculated for a temperature of 20°C and soil moisture of pF2.

The four FOCUS scenarios relevant for the use of the additive in avian species were used (EFSA FEEDAP Panel, 2019). The test substance was incorporated into the soil and assumed to be uniformly mixed into the top 5-cm soil layer. Uptake by plant roots was set to zero.

The maximum predicted concentrations (global maximum) of halofuginone in surface water are 0.027 μg/L for applications to chickens for fattening and 0.029 μg/L for turkeys for fattening.

The maximum predicted concentration of halofuginone in sediment at any time was 3.7 μg/kg dry weight sediment for application to chickens for fattening and 3.9 μg/kg dry weight sediment for turkeys for fattening.

Conclusions on PEC used for risk assessment

The following exposure values are used for risk assessment: PEC_{soil} of 46 μg/kg, $PEC_{surface\ water}$ of 0.029 μg/L and $PEC_{sediment}$ of 3.9 μg/kg.

Ecotoxicity studies

Toxicity to soil organisms

Effects on plants

A study following OECD guideline 208 (OECD, 2006a) was performed to investigate the effect of halofuginone on six terrestrial plants (monocotyledon species *Allium cepa* and *Hordeum vulgare*; and dicotyledon species *Raphanus sativus*, *Solanum lycopersicum*, *Cucumis sativa* and *Helianthus annuus*). The study was already evaluated in 2020 and the same conclusions are reiterated in the present assessment: 'Overall, *S. lycopersicum* was the most sensitive species for the endpoint fresh weight biomass with median effective concentration (EC_{50}) value of 12.6 mg/kg.'

Effect on earthworms

A study following OECD guideline 207 (OECD, 1984) was performed to investigate the effect of halofuginone (as halofuginone HBr) on *Eisenia fetida*.[13] Earthworms were placed in an artificial soil at a nominal concentration ranging 54.19, 97.55, 175.58, 316.05, 568.89 and 1,024 mg halofuginone HBr/kg dry weight soil (equivalent to 45.3, 81.6, 146.9, 264.5, 476.1 and 859.9 mg halofuginone/kg dry weight soil) and mortality assessed after 7 and 14 days. The study was considered valid, mortality in the controls was less than 10% at the end of the test (actual 0%) and the expected mortality was observed in the toxic reference. The 14-day LC_{50} was determined as 460.0 mg halofuginone HBr/kg dry weight soil (equivalent to 384.9 mg halofuginone/kg dry weight soil).

Effects on soil microorganisms

A study following OECD guideline 216 was performed to investigate the effect of halofuginone on soil microorganisms.[14] A sandy loam soil was treated with halofuginone at a rate of 45.55 and 455.5 μg/kg soil dry weight, equivalent to $PEC_{soil\ A\ plateau}$ and $PEC_{soil\ A\ plateau} \times 10$ (without considering refinement based on metabolism in the animal), respectively. Control and treated soils were incubated for 100 days and subsamples were taken on 0, 7, 14, 28 and 100 days after treatment and analysed for the nitrate concentration. The study met the validity criteria; variation in nitrate concentration of control replicates was less than 15% for all time points. Nitrate formation rate deviations from the controls were less than 25% for the $PEC_{soil\ A\ plateau} \times 10$ calculated using the incremental method (28–100 days) as well as overall (0–100 days) after treatment.

[13] Technical dossier/Ref-3.
[14] Technical dossier/Additional data January 2023/Annex RTQ 02.

Toxicity to aquatic organisms.

Effect on algae

The toxicity of halofuginone to a green alga *Pseudokirchneriella subcapitata* (with a recent taxonomic name of *Raphidocelis subcapitata* (Korshikov)) has been investigated in a 72-h static test.[15] The study was performed in 2020 and conducted in accordance with the OECD Guideline 201 (2006 and 2011) and GLP. Algal cells were exposed to 0.95, 3.05, 9.77, 31.25 and 100 μg halofuginone HBr/L (equivalent to 0.83, 2.72, 7.35, 24.51 and 78.84 μg halofuginone/L). The study met the validity criteria. To assess the stability of the test item, the concentration of halofuginone in the test media was determined at the start and end of the exposure period. During the test, halofuginone was not stable in the treated solutions with losses in concentrations ranging from 59 to 70% of the nominal concentrations. Therefore, the evaluation of biological endpoints was performed using geometric mean measured concentrations. The 72-h E_rC_{50} and E_rC_{10} values were 10 and 4.4 μg halofuginone/L, respectively. The E_rC_{10} of 4.4 μg halofuginone/L was used for the assessment.

Effect on crustaceans

The study was performed in 2021 and is conducted in accordance with the OECD Guideline 202 (2014a) and GLP. *Daphnia magna* specimens were exposed to 0.94, 1.7, 3.1, 5.6 and 10 μg halofuginone HBr/L (equivalent to 0.84, 1.42, 2.59, 4.69 and 8.37 μg halofuginone/L). The study was valid. To assess the stability of the test item, the concentration of halofuginone in the test media was determined at the start and end of the exposure period. Halofuginone was not stable over the 48-h exposure period with concentrations at the end of exposure between 68% and 76% of the nominal values. Therefore, the evaluation of biological endpoints was performed using geometric mean measured concentrations. The 48-h EC_{50} for immobilisation was 7.15 μg halofuginone HBr/L (equivalent to 5.98 μg halofuginone/L).

The effect of halofuginone on the reproductive capability of *Daphnia magna* has been investigated in 21-d semi-static test.[16] The study was performed in 2021 and conducted in accordance with the OECD Guideline 211 (2012) and GLP. *Daphnia magna* specimens were exposed to 0.05, 0.15 0.49 1.56, 5 μg halofuginone HBr/L (equivalent to 0.047, 0.127, 0.438, 1.361, 4.218 μg halofuginone/L). There were 10 replicates per treatment, each containing a single *Daphnia magna* neonate. The study was valid. Three media renewals were performed each week. To assess the stability of the test item, the concentration of halofuginone in the test medium was determined at the start and end of the renewal period. Halofuginone was not stable over the exposure period, the measured concentrations were not within 20% of the nominal concentrations in all cases. Therefore, the evaluation of biological endpoints was performed using geometric mean measured concentrations. There was a single adult mortality in the control, no other mortality was observed during the test. Therefore, the NOEC was determined to be 4.22 μg/L, the highest geometric mean measured concentration tested. This 21-day NOEC was used in the assessment.

Effect on fish

The toxicity of halofuginone to fish has been investigated in a 96-h static test.[17] The study was performed in 2021 and conducted in accordance with the OECD Guideline 203 (2019) and GLP. To limit *in vivo* vertebrate testing, the threshold approach was implemented in this study (OECD, 2010). The threshold concentration was derived from algal and acute invertebrate toxicity data. Initially the fish species *Danio rerio* were exposed to a nominal concentration of 100 μg halofuginone HBr/L (equivalent to 83.7 μg halofuginone/L). The study met the validity criteria. To assess the stability of the test item, the concentration of halofuginone in the test media was determined at the start and end of the exposure period. During the test, halofuginone was not stable and the threshold concentration after 96 h was 71.7% of the nominal concentration. Therefore, the geometric mean of the measured concentration was used to determine the relevant ecotoxicological endpoint. No mortality was observed at the threshold concentration and therefore, according to the guidance, it was not necessary to proceed to a dose–response experiment and LC_{50} is considered greater than the threshold concentration. The LC_{50} of $>$ 66.9 μg halofuginone/L was used for the assessment.

[15] Technical dossier/Ref-4.
[16] Technical dossier/Additional data January 2023/Annex RTQ 03.
[17] Technical dossier/Ref-6.

Effects on sediment-dwelling organisms

A GLP compliant study following the OECD guideline 218 (OECD, 2004b) was performed in 2015 to investigate the influence of halofuginone on the survival and development of the sediment-dwelling larvae of the midge *Chironomus riparius*.[18] For this purpose, first-instar larvae of *C. riparius* were exposed for 28 days in a sediment water system to 0.08, 0.2, 0.5, 1.25, 3.13, 7.81, 19.53 mg halofuginone hydrobromide/kg dry weight sediment (equivalent to 0.07, 0.17, 0.42, 1.04, 2.61, 6.51 and 16.28 mg halofuginone/kg dry weight sediment). The validity criteria of the study were met. The total number of adults emerged (emergence rate) and the time to emergence (development rate) were recorded. The concentration of halofuginone in the test medium was determined at the beginning and end of the exposure period and was not stable during the course of the test. Therefore, all reported results refer to geometric mean of measured concentrations (for the highest and lowest treatments) or account for the mean recovery (all intermediate treatments). The most sensitive endpoint was 28-day NOEC for the emergence rate which was 341 µg halofuginone HBr/kg dry weight sediment (equivalent to 285.4 µg halofuginone/kg dry weight sediment). This NOEC value has been normalised to an organic carbon content of 5% to allow a proper comparison of the PNEC with the PEC value in the risk assessment. The organic carbon normalised NOEC for emergence was established as 492.1 µg halofuginone/kg dry weight sediment.

Conclusions on the ecotoxic effect on soil, water and sediment

For the terrestrial compartment, studies are available for plants, earthworms and microorganisms. The plant study indicated that *S. lycopersicum* is the most sensitive species showing an EC_{50} of 12.6 mg/kg for fresh weight biomass; the study on microorganisms showed that halofuginone has no long-term influence on the nitrogen transformation functionality of soil.

For the aquatic compartment, studies are available for algae, crustaceans and fish. The study on crustaceans indicated the lowest NOEC of 4.22 µg/kg.

For the sediment, study is available for the sediment-dwelling larvae of the midge *Chironomus riparius*. The NOEC for emergence was established as 492.1 µg halofuginone/kg dry weight sediment.

Risk characterisation (Table 10, 11, 12)

Table 10: Risk characterisation (PEC/PNEC ratio) for halofuginone for the terrestrial compartment

Taxa	PEC_{soil} (µg/kg)	EC_{50}/LC_{50} (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm	46	384.9(1)	1000	384.9	0.12
Plants		12.6(2)	100	126.3	0.36

AF: assessment factor.
(1): 14-day LC_{50}.
(2): EC_{50}.

Table 11: Risk characterisation (PEC/PNEC ratio) for halofuginone for the freshwater compartment

Taxa	$PEC_{surface\ water}$ (µg/L)	E_rC_{10}, NOEC or LC_{50} (µg/L)	AF	PNEC (µg/L)	PEC/PNEC
Algae	0.029	4.4(1)	50	0.084	0.35
Crustaceans		4.22(2)			
Fish		> 66.9(3)			

AF: assessment factor.
(1): E_rC_{10}.
(2): NOEC.
(3): LC_{50}.

Table 12: Risk characterisation (PEC/PNEC ratio) for halofuginone for the sediment compartment

Taxa	PEC_{sed} (µg/kg)	NOEC (µg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Chironomus riparius	3.9	492.1	100	4.92	0.79

AF: assessment factor.

[18] Technical dossier/Additional data January 2023/Annex RTQ 04.

Bioaccumulation and risk for secondary poisoning

No data on bioaccumulation of halofuginone were submitted. The values of the octanol/water partition coefficient (K_{ow}) indicate that bioaccumulation of the substance is unlikely. Therefore, the risk of secondary poisoning for worm/fish eating birds and mammals is not likely to occur.

3.1.3.3. Conclusions on safety for the environment

The fate and behaviour in the environment were evaluated for halofuginone, which is the substance expected to reach the environment. Predicted environmental concentrations have been calculated for halofuginone in the different environmental compartments. No concern for groundwater is expected. Halofuginone is unlikely to bioaccumulate, and the risk of secondary poisoning is not likely to occur. No safety concerns are expected for terrestrial and aquatic environments.

These conclusions apply to the use of the additive in turkeys for fattening and can be extended to chickens for fattening.

3.2. Efficacy

3.2.1. Efficacy for chickens for fattening

In a previous opinion (EFSA FEEDAP Panel, 2020), the Panel could not conclude on the efficacy of the additive for chickens for fattening due to the insufficient number of studies with positive results. In that opinion, two floor pen trials and two anticoccidial sensitivity tests (ASTs) supported the coccidiostatic efficacy of the additive in chickens for fattening.

For the current assessment, the applicant submitted one floor pen study and one AST conducted with STENOROL® in chickens for fattening at the minimum proposed dose of 2 mg halofuginone HBr/kg complete feed.

3.2.1.1. Floor pen study in chickens for fattening

In a floor pen study performed in November 2020,[19] a total of 840 1-day-old male chickens (ROSS 308) were penned and distributed (10 replicates per treatment, 28 birds per replicate) into the experimental groups: an uninfected untreated control group (UUC), an infected untreated control group (IUC) and an infected STENOROL®-treated group (IT). The different groups were fed the same basal feed based on maize, wheat and soybean meal,[20] either not supplemented (in the two untreated groups (UUC and IUC)) or supplemented with 2 mg halofuginone HBr/kg feed in the treated group (IT), confirmed by analysis. The experimental diets were pelleted and offered for ad libitum access as well as water. In the infected groups, all birds were orally inoculated on day 14 via gavage with recent field isolates (collected in France in July 2020) of pathogenic *Eimeria* species.[21] Prior to the floor pen study, the virulence of the inoculum was tested in a dose titration study.[22] Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured weekly post-inoculation (PI), and feed to gain ratio calculated, per pen basis. Samples of excreta were analysed for oocyst excretion at 20, 21, 28 and 35 days of age. On the same days, intestinal lesions were scored on four birds per pen, following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The data were subjected to analysis of variance (ANOVA) using a general linear mixed model. The pen was the experimental unit for all endpoints, except for mortality and intestinal lesion score (ILS), which were assessed considering the bird as experimental unit. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests. If the treatment effect was significant, pairwise comparisons were made.

The total mortality after challenge (from 14 to 35 days of age) was significantly lower in the IT group (2.2%) compared to the IUC group (5.8%). Coccidiosis-related mortality, attributable to *E. tenella*, was observed in three birds of the IUC group and two birds of the IT group.

[19] Technical dossier/Ref-8.

[20] Birds were administered a commercial starter feed from day 1 till day 13 of age (calculated values: 21.5% crude protein (CP), 0.55% methionine (met), 12.2 MJ apparent metabolisable energy (AME)/kg) and a commercial grower feed from day 14 till day 35 of age (calculated values: 20.1% CP, 0.51% met, 12.7 MJ AME/kg).

[21] Number and type of oocysts administered per bird: 16,500 *E. acervulina*, 27,000 *E. tenella*, 5,500 *E. maxima* and 1,000 *E. mitis*.

[22] The dose selected resulted in lesion scores up to 2.7 (*E. maxima*) and 2.5 (*E. tenella*) and a weight gain reduction of 23% and 57% at days 6 and 7 PI, respectively; no mortality was observed.

Significant reductions in intestinal lesion scores (ILS) in the IT group compared to IUC were observed for *E. acervulina* on study days 20 (0.8 vs. 1.55) and 21 (0.98 vs. 1.98); for *E. maxima* on study day 20 (0.98 vs. 1.98); and for *E. tenella* on day 28 (0.65 vs. 1.38).

A significant reduction in the oocyst count per gram of excreta (OPG) was observed for *E. maxima* on study day 20 (0 vs. 9,914) and on study day 21 (207 vs. 34,370) in IT birds compared to IUC birds.

Regarding the zootechnical parameters calculated for the whole experimental period, feed intake was not influenced by the treatment, while final body weight, daily weight gain and feed to gain ratio were significantly improved in IT compared to IUC (2,193 g vs. 2,051 g; 55 g vs. 50 g and 1.48 vs. 1.56).

3.2.1.2. Anticoccidial sensitivity test in chickens for fattening

In an AST performed in August 2020,[23] a total of 150 1-day-old male birds (ROSS 308) were housed in the same building in battery cages, fed a commercial starter diet for 12 days and then switched to a commercial grower diet until the end of the study (21 days of age). On day 14, they were randomly allocated to the following experimental groups: a UUC, an IUC and an infected STENOROL®-treated group (IT) (10 replicates per treatment, 5 birds per replicate). At 14 days of age, all birds of the groups IUC and IT were orally inoculated via gavage with recent field isolates (collected in Norway in April 2019) of pathogenic *Eimeria* species.[24] The inoculum used in floor pen study was tested for its virulence in a dose titration study.[25] The feed of the IT group was supplemented with 2 mg halofuginone HBr per kg feed, confirmed by analysis. The experimental diets were pelleted and offered for ad libitum access as well as water. Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured, and feed to gain ratio calculated, per pen basis. Samples of excreta were analysed for oocyst excretion at 19, 20 and 21 days of age. Intestinal lesions were scored on two or three birds per pen on study days 19 and 21, following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The data were subjected to analysis of variance (ANOVA) using a general linear mixed model. The pen was the experimental unit for all endpoints except ILS, which was assessed considering the bird as experimental unit. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests. If the treatment effect was significant, pairwise comparisons were made.

No birds died after challenge. ILS for *E. acervulina* were significantly reduced in the IT group compared to IUC on both observation days (2.16 vs. 2.89 and 1.20 vs. 2.36). The low dose of *E. tenella* provided by the inoculum was not pathogenic (no intestinal lesions by *E. tenella*). Oocyst excretion was significantly reduced by the treatment on all days of observation; specifically, oocyst excretion for *E. acervulina* was lower on day 19 (25 vs. 5,185), day 20 (67,717 vs. 710,492) and day 21 (107,009 vs. 466,274) and for *E. mitis* oocyst excretion was lower on day 21 (147 vs. 10,248).

Considering the zootechnical parameters calculated from day 14 to day 21, feed intake was not influenced by the treatment, while daily weight gain and feed to gain ratio were significantly improved in IT compared to IUC (61 g vs. 53 g and 1.24 vs. 1.56).

Conclusions in chickens for fattening

In the floor pen study submitted, the efficacy of 2 mg halofuginone HBr/kg complete feed in chickens for fattening was demonstrated by a significant reduction in intestinal lesion score and oocyst excretion in the STENOROL® treated group (IT) compared to the untreated IUC group. Concerning the secondary parameters, the observed significantly higher final body weight, average daily weight gain and lower feed to gain ratio were related to the reduction in lesion scores. In the AST submitted, the efficacy of 2 mg halofuginone HBr/kg complete feed in chickens for fattening was demonstrated by a statistically significant reduction in intestinal lesion score and oocyst excretion compared to the IUC group. Improved weight gain and feed to gain ratio support this conclusion.

3.2.2. Efficacy for turkeys for fattening/reared for breeding

In its former opinion (EFSA FEEDAP Panel, 2020), the FEEDAP Panel could not conclude on the efficacy of the additive for turkeys due to the insufficient number of studies with positive results; two floor pen trials and three ASTs were indicative for the coccidiostatic efficacy of the additive in turkeys.

[23] Technical dossier/Ref-7

[24] Number and type of oocyst administered per bird: 87,360 *E. acervulina*, 2,520 *E. tenella*, 3,360 *E. mitis*.

[25] The dose selected resulted in lesion scores 2.9 and 1.9 (*E. acervulina*) and a weight gain reduction of 11% and 14% at day 6 and 7 PI, respectively; no mortality was observed.

For the current assessment, the applicant submitted one floor pen study and one AST conducted with STENOROL® in turkeys at a dose of 2 mg halofuginone HBr/kg complete feed.

3.2.2.1. Floor pen study in turkeys

In a floor pen study performed in November 2020,[26] a total of 860 1-day-old female turkey poults (Aviagen BUT Premium) were penned and distributed into three experimental groups, an UUC, an IUC and an infected STENOROL®-treated group (IT) with 12 replicates per treatment and 24 birds per replicate.[27] The STENOROL®-treated group received 2 mg halofuginone HBr/kg complete feed. The intended dietary concentrations of the coccidiostat were analytically confirmed. The experimental diets were fed for 84 days following four feeding phases.[28] The basal diet for all trials conducted was composed mainly of wheat, barley and soya. Compound feed was provided in crumble form during the pre-starter period and in pellet form later for ad libitum access so as water. In the infected groups, all birds were infected on day 15 via feed contaminated with recent field isolates (collected in Spain, February 2019) of pathogenic *Eimeria* species.[29] Prior to the study, the inoculum used in the floor pen study was tested for its virulence in a dose-titration study.[30] Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured on days 15, 29, 56 and 84, and feed to gain ratio calculated accordingly, per pen basis. Samples of excreta were analysed for oocyst excretion on days 20, 21, 22, 29 and 83. Intestinal lesions were scored on two birds per pen on days 21, 22 and 29, following the method of Gadde et al. (2020), similar to that described by Johnson and Reid (1970) for chickens.

The data were subjected to analysis of variance (ANOVA). The pen was the experimental unit for all endpoints except ILS, which was assessed on an individual basis. All hypothesis tests were conducted at the 0.05 level of significance. If the treatment effect was significant, pairwise comparisons were made.

Mortality was very low during the whole study and only six birds (2 in each experimental group) died with none of these deaths attributable to coccidiosis. Morbidity[31] related to coccidiosis was checked globally for each pen during the 14 days after infection. At day 5 PI, four out of 12 IUC pens[32] presented symptoms compatible with coccidiosis, while only two out of 12 UUC or IT pens did present such symptoms. These symptoms disappeared the days thereafter.

Results of ILS following the inoculation of *Eimeria* species at days 6, 7 and 14 PI showed very mild lesions (all scores below 1) and significant differences could therefore hardly be expected. The relevant FEEDAP guidance (EFSA FEEDAP Panel, 2018) contemplates the faecal score as an alternative endpoint to ILS in order to assess efficacy in turkeys, but no data on faecal scoring were provided.

Overall oocyst excretion was generally very low, particularly considering the high *Eimeria* oocyst dosage which was used for feed contamination. The OPG in the IT group was very low and measurable only at days 6 and 14 PI resulting in 152 and 106; at the corresponding time points, the OPG in the IUC group (15,300 and 6,480, respectively) was significantly higher.

Regarding the zootechnical parameters calculated for the whole experimental period, no significant difference was observed between any of the groups. Birds in the IT group reached a final body weight of 7,570 g, the daily feed intake resulted 180 g and the daily weight gain 89 g; feed to gain ratio was calculated to be 2.02.

3.2.2.2. Anticoccidial sensitivity test in turkeys

In an AST performed in August 2020,[33] a total of 216 1-day-old male turkey birds (Hybrid converter) were housed in the same building in battery cages and fed a starter diet for 14 days and

[26] Technical dossier/Ref-10.
[27] Except one pen with 22 birds in IT, four pens with 23 birds (one in UUC, one in IUC and two in IT) and two pens with 25 birds in UUC.
[28] Birds received pre-starter diet from day 0 to 15 days (calculated CP 28.1%, met 0.8%, AME 11.6 MJ/kg), starter from 15 to 29 days (calculated CP 26.1%, met 0.7%, AME 11.7 MJ/kg), grower diet from day 29 to 56 (calculated CP 23.5%, met 0.6%, AME 11.9 MJ/kg) and finisher diet from day 56 to 84 (calculated CP 26.1%, met 0.5%, AME 12.1 MJ/kg).
[29] Number and type of oocyst administered per bird: *E. meleagrimitis*/*E. meleagridis KCH* 318,000, *E. dispersa* 12,000, *E. adenoeides*/*E. meleagridis KR*/*E. gallopavonis* 81,000.
[30] The dose selected resulted in lesion scores up to 2.8 at day 6 PI and a weight gain reduction of 18% at day 7 PI; no mortality was observed.
[31] Presence of one or more of the following symptoms: ruffled feathers, mucoid diarrhoea or bloody faeces, listlessness, huddling, drooping wings.
[32] At least one-third of birds in the pen presenting symptoms.
[33] Technical dossier/Ref-9.

then switched to a grower diet until the end of the study (23 days of age). On day 14, birds were randomly allocated to the following experimental groups: an UUC, an IUC and an infected STENOROL®-treated group (IT) (8 replicates per treatment and 9 birds per replicate). The IT group received feed containing 2 mg halofuginone HBr/kg feed (analytically confirmed). The experimental diets were pelleted and offered for ad libitum access so as water. In the infected groups, on day 16 of age, all birds were orally inoculated via a syringe with recent field isolates (collected in Poland, May 2019) of pathogenic *Eimeria* species.[34] Prior to the study, the inoculum was tested for its virulence.[35] Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured; feed to gain ratio was calculated. On day 7 PI (23 days of age), samples of excreta were analysed for oocyst excretion and intestinal lesions were scored (scores 0–4) on five birds per pen, following the method El El-Sherry et al. (2018) and Gadde et al. (2020).

The data were subjected to analysis of variance (ANOVA) using a general linear mixed model. The pen was the experimental unit for all endpoints except mortality and ILS, which were assessed on an individual basis. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests. If the treatment effect was significant, pairwise comparisons were made.

Coccidiosis-related mortality was significantly higher in the IUC group (23.6%) than in UUC (0%) or IT (4.2%) groups. Intestinal lesion scores related to *E. adenoeides*/*E. gallopavonis* were significantly reduced in the IT group when compared to IUC (0.48 vs. 1.98).

Inoculation increased OPG significantly in IUC relative to UUC (260,257 vs. 0). However, no significant differences were observed between IT and IUC groups (total OPG 567,997 vs. 260,257).

In the period from day 14 until 23, daily weight gain (36 g in UUC vs. 24 and 20 g in IT and IUC, respectively) and feed to gain ratio (1.47 in the UUC vs. 1.84 and 1.97 in the IT and IUC, respectively) were negatively affected by the inoculation. However, no significant differences for both parameters were observed between IT and IUC birds.

Conclusions in turkeys for fattening/reared for breeding

The floor pen study currently submitted provides only weak evidence for a clinically relevant infection of turkey-specific *Eimeria* species. This could be related to an insufficient pathogenicity of the inoculum, or the route of administration of the inoculum. The IT group showed significantly lower oocyst excretion in the first 2 weeks of the study, the only parameter indicating a potential coccidiostatic efficacy of the additive. In contrast, the newly submitted AST provided clear evidence on the coccidiostatic effect of halofuginone HBr in turkeys by reducing coccidiosis-related mortality.

3.2.3. Conclusions on efficacy

Considering together the results of the floor pen trials and ASTs described and assessed in the previous FEEDAP opinion (EFSA FEEDAP Panel, 2020) and in the current opinion, halofuginone HBr from STENOROL® has the potential to control coccidiosis in chickens for fattening and turkeys for fattening/reared for breeding up to 12 weeks of age at the minimum level of 2 mg/kg complete feed.

3.3. Post-market monitoring

Field monitoring of *Eimeria* spp. resistance in chickens for fattening and turkeys to halofuginone HBr should be undertaken, preferably during the latter part of the period of authorisation.

4. Conclusions

The FEEDAP Panel concludes that halofuginone HBr, at a maximum concentration of 3 mg/kg complete feed is safe for turkeys reared for breeding up to 12 weeks of age.

Halofuginone HBr is not genotoxic. The toxicological data set available allowed to identify the lowest NOAEL of 0.03 mg halofuginone HBr/kg bw per day, based on reproductive effects and maternal toxicity seen in the rabbit teratology study. This NOAEL can be considered as an appropriate basis for the health-based guidance value (acceptable daily intake (ADI)) of 0.3 μg halofuginone

[34] Number and type of oocysts administered per bird: *E. meleagrimitis*/*E. adenoeides* 199,000, *E. meleagridis*/*E. gallopavonis* 43,000.

[35] The dose selected resulted in intestinal lesion scores of 2.0 for the small intestine, of 1.5 for the caecum and a weight gain reduction of 80% at day 5 PI; no mortality was observed. At day 6 PI, the intestinal lesion scores were 4.0 and 80% of mortality was registered.

HBr/kg bw applying an uncertainty factor of 100. Considering that halofuginone is the marker residue, a rounded ADI of 0.3 μg halofuginone/kg bw is retained for the assessment.

The chronic exposure to residues resulting from the use of the feed additive halofuginone HBr (STENOROL®) in chickens would amount to up to 6–19% of the ADI after 3 days of withdrawal. Based on this, the FEEDAP Panel considers that the additive is safe for the consumer of tissues obtained from chickens for fattening fed the additive under the proposed conditions of use including 3 days of withdrawal time. These conclusions are extrapolated to food products obtained from turkeys for fattening. For control purposes, the Panel recommends the setting of the following MRLs: liver, 50 μg/kg; kidney, 40 μg/kg; muscle, 3 μg/kg; skin/fat, 10 μg/kg wet tissue.

The fate and behaviour in the environment were evaluated for halofuginone, which is the substance expected to reach the environment. Predicted environmental concentrations have been calculated for halofuginone in the different environmental compartments. No concern for groundwater is expected. Halofuginone is unlikely to bioaccumulate and the risk of secondary poisoning is not likely to occur. No safety concerns are expected for terrestrial and aquatic environments.

Considering together the results of the floor pen trials and ASTs described and assessed in the previous FEEDAP opinion (EFSA FEEDAP Panel, 2020) and in the current opinion, halofuginone HBr from STENOROL® has the potential to control coccidiosis in chickens for fattening and turkeys for fattening/reared for breeding up to 12 weeks of age at a minimum level of 2 mg/kg complete feed.

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Abbreviations

ADI	acceptable daily intake
AST	anticoccidial sensitivity test
BW	body weight
DT_{50}	time to degradation of 50% of original concentration of the compound in the tested soils
DT_{90}	time to degradation of 90% of original concentration of the compound in the tested soils
EC_{50}	the concentration of a test substance which results in 50% of the test organisms being adversely affected, i.e. both mortality and sublethal effects
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
IT	infected treated group
IUC	infected untreated group
K_{OC}	adsorption or desorption coefficient corrected for soil organic carbon content
LOD	limit of detection
LOQ	limit of quantification
Log K_{ow}	logarithm of octanol–water partition coefficient
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
OPG	oocyst counts per gram of excreta
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
UUC	uninfected untreated group

Appendix A – Detailed results on chronic exposure calculated with FACE-model[36]

Table A.1: Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to halofuginone total residues based on residue data in chicken tissues after 3-day withdrawal

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Infants	Bulgaria	523	0.0400273741	95th
Infants	Germany	142	0.0072870337	95th
Infants	Denmark	799	0.0084513609	95th
Infants	Finland	427	0.0126353782	95th
Infants	United Kingdom	1,251	0.0178446048	95th
Infants	Italy	9	0.0000000000	50th
Toddlers	Belgium	36	0.0186372725	90th
Toddlers	Bulgaria	428	0.0562795720	95th
Toddlers	Germany	348	0.0122867586	95th
Toddlers	Denmark	917	0.0093772134	95th
Toddlers	Spain	17	0.0182692308	75th
Toddlers	Finland	500	0.0201838764	95th
Toddlers	United Kingdom	1,314	0.0194645700	95th
Toddlers	United Kingdom	185	0.0204198487	95th
Toddlers	Italy	36	0.0166465909	90th
Toddlers	Netherlands	322	0.0204488432	95th
Other children	Austria	128	0.0161443832	95th
Other children	Belgium	625	0.0232246646	95th
Other children	Bulgaria	433	0.0571683856	95th
Other children	Czech Republic	389	0.0350395280	95th
Other children	Germany	293	0.0133319416	95th
Other children	Germany	835	0.0128289263	95th
Other children	Denmark	298	0.0104822433	95th
Other children	Spain	399	0.0237361479	95th
Other children	Spain	156	0.0332199789	95th
Other children	Finland	750	0.0242448300	95th
Other children	France	482	0.0218288063	95th
Other children	United Kingdom	651	0.0173508581	95th
Other children	Greece	838	0.0169807028	95th
Other children	Italy	193	0.0178925111	95th
Other children	Latvia	187	0.0192807529	95th
Other children	Netherlands	957	0.0149173528	95th
Other children	Netherlands	447	0.0187841147	95th
Other children	Sweden	1,473	0.0140738210	95th
Adolescents	Austria	237	0.0111553979	95th
Adolescents	Belgium	576	0.0102037649	95th
Adolescents	Cyprus	303	0.0107875294	95th
Adolescents	Czech Republic	298	0.0264165695	95th
Adolescents	Germany	393	0.0098866484	95th
Adolescents	Germany	1,011	0.0080042553	95th
Adolescents	Denmark	377	0.0081976825	95th
Adolescents	Spain	651	0.0137855599	95th
Adolescents	Spain	209	0.0185001594	95th

[36] Dietary data from the UK were included in FACE when the UK was a member of the European Union.

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Adolescents	Spain	86	0.0164785347	95th
Adolescents	Finland	306	0.0103410916	95th
Adolescents	France	973	0.0136197089	95th
Adolescents	United Kingdom	666	0.0127167339	95th
Adolescents	Italy	247	0.0082250248	95th
Adolescents	Latvia	453	0.0117500071	95th
Adolescents	Netherlands	1,142	0.0140816278	95th
Adolescents	Sweden	1,018	0.0105967551	95th
Adults	Austria	308	0.0127748460	95th
Adults	Belgium	1,292	0.0106338999	95th
Adults	Czech Republic	1,666	0.0132364392	95th
Adults	Germany	10,419	0.0081198522	95th
Adults	Denmark	1,739	0.0053439691	95th
Adults	Spain	981	0.0128675373	95th
Adults	Spain	410	0.0128426581	95th
Adults	Finland	1,295	0.0103668502	95th
Adults	France	2,276	0.0114387279	95th
Adults	United Kingdom	1,265	0.0094039277	95th
Adults	Hungary	1,074	0.0269030902	95th
Adults	Ireland	1,274	0.0126689531	95th
Adults	Italy	2,313	0.0070499771	95th
Adults	Latvia	1,271	0.0105733833	95th
Adults	Netherlands	2,055	0.0118788103	95th
Adults	Romania	1,254	0.0412748121	95th
Adults	Sweden	1,430	0.0108155776	95th
Elderly	Austria	67	0.0112547489	95th
Elderly	Belgium	511	0.0093000476	95th
Elderly	Germany	2,006	0.0064599326	95th
Elderly	Denmark	274	0.0043219712	95th
Elderly	Finland	413	0.0083537976	95th
Elderly	France	264	0.0120274983	95th
Elderly	United Kingdom	166	0.0080392830	95th
Elderly	Hungary	206	0.0138860662	95th
Elderly	Ireland	149	0.0104970518	95th
Elderly	Italy	289	0.0082073486	95th
Elderly	Netherlands	173	0.0088070316	95th
Elderly	Netherlands	289	0.0074839603	95th
Elderly	Romania	83	0.0160090758	95th
Elderly	Sweden	295	0.0100713353	95th
Very elderly	Austria	25	0.0026850484	75th
Very elderly	Belgium	704	0.0095311468	95th
Very elderly	Germany	490	0.0067653510	95th
Very elderly	Denmark	12	0.0022567422	75th
Very elderly	France	84	0.0075081238	95th
Very elderly	United Kingdom	139	0.0057979970	95th
Very elderly	Hungary	80	0.0104367204	95th
Very elderly	Ireland	77	0.0104812971	95th
Very elderly	Italy	228	0.0069457409	95th
Very elderly	Netherlands	450	0.0074191750	95th
Very elderly	Romania	45	0.0177754919	90th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Very elderly	Sweden	72	0.0076905671	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are, respectively, required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than five observations were not included in this table.

Table A.2: Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to halofuginone total residues based on residue data in chicken tissues after 4-day withdrawal

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Infants	Bulgaria	523	0.0301960892	95th
Infants	Germany	142	0.0054972359	95th
Infants	Denmark	799	0.0063755880	95th
Infants	Finland	427	0.0095319520	95th
Infants	United Kingdom	1,251	0.0134617194	95th
Infants	Italy	9	0.0000000000	50th
Toddlers	Belgium	36	0.0140596968	90th
Toddlers	Bulgaria	428	0.0435142505	95th
Toddlers	Germany	348	0.0092689582	95th
Toddlers	Denmark	917	0.0070740382	95th
Toddlers	Spain	17	0.0137820513	75th
Toddlers	Finland	500	0.0152264331	95th
Toddlers	United Kingdom	1,314	0.0146837984	95th
Toddlers	United Kingdom	185	0.0154044473	95th
Toddlers	Italy	36	0.0125579545	90th
Toddlers	Netherlands	322	0.0154263203	95th
Other children	Austria	128	0.0121790961	95th
Other children	Belgium	625	0.0175203610	95th
Other children	Bulgaria	433	0.0440037161	95th
Other children	Czech Republic	389	0.0262702801	95th
Other children	Germany	293	0.0100574296	95th
Other children	Germany	835	0.0096779619	95th
Other children	Denmark	298	0.0079076572	95th
Other children	Spain	399	0.0179062169	95th
Other children	Spain	156	0.0250606859	95th
Other children	Finland	750	0.0192200281	95th
Other children	France	482	0.0171520685	95th
Other children	United Kingdom	651	0.0130892438	95th
Other children	Greece	838	0.0128100038	95th
Other children	Italy	193	0.0134978593	95th
Other children	Latvia	187	0.0145451294	95th
Other children	Netherlands	957	0.0112534416	95th
Other children	Netherlands	447	0.0141704725	95th
Other children	Sweden	1,473	0.0106170930	95th
Adolescents	Austria	237	0.0084154756	95th
Adolescents	Belgium	576	0.0076975770	95th
Adolescents	Cyprus	303	0.0081379608	95th
Adolescents	Czech Republic	298	0.0199282893	95th
Adolescents	Germany	393	0.0074583488	95th
Adolescents	Germany	1,011	0.0060382979	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Adolescents	Denmark	377	0.0061842166	95th
Adolescents	Spain	651	0.0103996329	95th
Adolescents	Spain	209	0.0139562606	95th
Adolescents	Spain	86	0.0124311753	95th
Adolescents	Finland	306	0.0078011743	95th
Adolescents	France	973	0.0107414124	95th
Adolescents	United Kingdom	666	0.0095933256	95th
Adolescents	Italy	247	0.0062048433	95th
Adolescents	Latvia	453	0.0088640405	95th
Adolescents	Netherlands	1,142	0.0106229824	95th
Adolescents	Sweden	1,018	0.0079940433	95th
Adults	Austria	308	0.0096371645	95th
Adults	Belgium	1,292	0.0080220648	95th
Adults	Czech Republic	1,666	0.0099853840	95th
Adults	Germany	10,419	0.0061591251	95th
Adults	Denmark	1,739	0.0040314153	95th
Adults	Spain	981	0.0097070896	95th
Adults	Spain	410	0.0096883210	95th
Adults	Finland	1,295	0.0078681239	95th
Adults	France	2,276	0.0087653129	95th
Adults	United Kingdom	1,265	0.0070941911	95th
Adults	Hungary	1,074	0.0161310588	95th
Adults	Ireland	1,274	0.0095572804	95th
Adults	Italy	2,313	0.0053184038	95th
Adults	Latvia	1,271	0.0079764120	95th
Adults	Netherlands	2,055	0.0089612078	95th
Adults	Romania	1,254	0.0336356731	95th
Adults	Sweden	1,430	0.0081591199	95th
Elderly	Austria	67	0.0084904246	95th
Elderly	Belgium	511	0.0070158254	95th
Elderly	Germany	2,006	0.0047917251	95th
Elderly	Denmark	274	0.0032604344	95th
Elderly	Finland	413	0.0063019876	95th
Elderly	France	264	0.0091116334	95th
Elderly	United Kingdom	166	0.0060647222	95th
Elderly	Hungary	206	0.0104754534	95th
Elderly	Ireland	149	0.0079188286	95th
Elderly	Italy	289	0.0061915086	95th
Elderly	Netherlands	173	0.0066439010	95th
Elderly	Netherlands	289	0.0056457946	95th
Elderly	Romania	83	0.0120770221	95th
Elderly	Sweden	295	0.0075976740	95th
Very elderly	Austria	25	0.0020255629	75th
Very elderly	Belgium	704	0.0072303193	95th
Very elderly	Germany	490	0.0051036858	95th
Very elderly	Denmark	12	0.0017024546	75th
Very elderly	France	84	0.0056963000	95th
Very elderly	United Kingdom	139	0.0043739275	95th
Very elderly	Hungary	80	0.0078733154	95th
Very elderly	Ireland	77	0.0079069434	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Very elderly	Italy	228	0.0052397695	95th
Very elderly	Netherlands	450	0.0055969215	95th
Very elderly	Romania	45	0.0141544033	90th
Very elderly	Sweden	72	0.0058016559	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are, respectively, required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than five observations were not included in this table.

Table A.3: Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to halofuginone total residues calculated based on MRLs proposed for control purposes

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Infants	Bulgaria	523	0.0756008628	95th
Infants	Germany	142	0.0140626965	95th
Infants	Denmark	799	0.0163096438	95th
Infants	Finland	427	0.0243840632	95th
Infants	United Kingdom	1,251	0.0340644832	95th
Infants	Italy	9	0.0000000000	50th
Toddlers	Belgium	36	0.0359666662	90th
Toddlers	Bulgaria	428	0.0964821626	95th
Toddlers	Germany	348	0.0233931142	95th
Toddlers	Denmark	917	0.0180963768	95th
Toddlers	Spain	17	0.0352564103	75th
Toddlers	Finland	500	0.0389513405	95th
Toddlers	United Kingdom	1,314	0.0373503527	95th
Toddlers	United Kingdom	185	0.0379261442	95th
Toddlers	Italy	36	0.0321250000	90th
Toddlers	Netherlands	322	0.0394626799	95th
Other children	Austria	128	0.0311558273	95th
Other children	Belgium	625	0.0440085949	95th
Other children	Bulgaria	433	0.0897383675	95th
Other children	Czech Republic	389	0.0672030420	95th
Other children	Germany	293	0.0252736323	95th
Other children	Germany	835	0.0245193388	95th
Other children	Denmark	298	0.0202288905	95th
Other children	Spain	399	0.0458066013	95th
Other children	Spain	156	0.0641087313	95th
Other children	Finland	750	0.0383993590	95th
Other children	France	482	0.0350688463	95th
Other children	United Kingdom	651	0.0334841120	95th
Other children	Greece	838	0.0327697773	95th
Other children	Italy	193	0.0345294074	95th
Other children	Latvia	187	0.0372084706	95th
Other children	Netherlands	957	0.0287878738	95th
Other children	Netherlands	447	0.0362500459	95th
Other children	Sweden	1,473	0.0271600055	95th
Adolescents	Austria	237	0.0215279608	95th
Adolescents	Belgium	576	0.0196914761	95th
Adolescents	Cyprus	303	0.0208180392	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Adolescents	Czech Republic	298	0.0489124719	95th
Adolescents	Germany	393	0.0188278196	95th
Adolescents	Germany	1,011	0.0154468085	95th
Adolescents	Denmark	377	0.0158200890	95th
Adolescents	Spain	651	0.0266037122	95th
Adolescents	Spain	209	0.0357020621	95th
Adolescents	Spain	86	0.0291627043	95th
Adolescents	Finland	306	0.0199564925	95th
Adolescents	France	973	0.0229533852	95th
Adolescents	United Kingdom	666	0.0245410655	95th
Adolescents	Italy	247	0.0158728548	95th
Adolescents	Latvia	453	0.0226754524	95th
Adolescents	Netherlands	1,142	0.0271750712	95th
Adolescents	Sweden	1,018	0.0204498783	95th
Adults	Austria	308	0.0246532115	95th
Adults	Belgium	1,292	0.0200396429	95th
Adults	Czech Republic	1,666	0.0242764958	95th
Adults	Germany	10,419	0.0152912919	95th
Adults	Denmark	1,739	0.0103129228	95th
Adults	Spain	981	0.0248205128	95th
Adults	Spain	410	0.0247840770	95th
Adults	Finland	1,295	0.0198293012	95th
Adults	France	2,276	0.0202854159	95th
Adults	United Kingdom	1,265	0.0181479307	95th
Adults	Hungary	1,074	0.0371094846	95th
Adults	Ireland	1,274	0.0244488568	95th
Adults	Italy	2,313	0.0135907302	95th
Adults	Latvia	1,271	0.0204047748	95th
Adults	Netherlands	2,055	0.0229240199	95th
Adults	Romania	1,254	0.0514782367	95th
Adults	Sweden	1,430	0.0208721673	95th
Elderly	Austria	67	0.0217196909	95th
Elderly	Belgium	511	0.0176382882	95th
Elderly	Germany	2,006	0.0118953957	95th
Elderly	Denmark	274	0.0083406463	95th
Elderly	Finland	413	0.0157844664	95th
Elderly	France	264	0.0180621175	95th
Elderly	United Kingdom	166	0.0155144057	95th
Elderly	Hungary	206	0.0267976716	95th
Elderly	Ireland	149	0.0202574684	95th
Elderly	Italy	289	0.0158387429	95th
Elderly	Netherlands	173	0.0169960258	95th
Elderly	Netherlands	289	0.0144427304	95th
Elderly	Romania	83	0.0263892888	95th
Elderly	Sweden	295	0.0194359103	95th
Very elderly	Austria	25	0.0051816724	75th
Very elderly	Belgium	704	0.0183934413	95th
Very elderly	Germany	490	0.0130559405	95th
Very elderly	Denmark	12	0.0043551165	75th
Very elderly	France	84	0.0144623430	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Very elderly	United Kingdom	139	0.0111891169	95th
Very elderly	Hungary	80	0.0195831541	95th
Very elderly	Ireland	77	0.0202270645	95th
Very elderly	Italy	228	0.0134040615	95th
Very elderly	Netherlands	450	0.0143177062	95th
Very elderly	Romania	45	0.0281203008	90th
Very elderly	Sweden	72	0.0148414453	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are, respectively, required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than five observations were not included in this table.