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Fumagillin dicyclohexylamine

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Fumagillin dicyclohexylamine[1]

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Identity

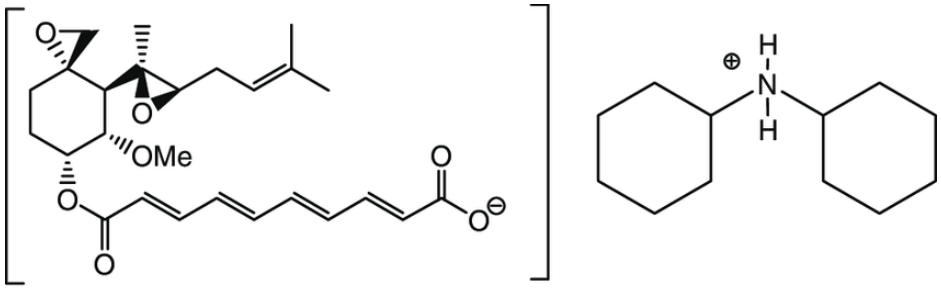
International Non-proprietary Names (INN): Fumagillin dicyclohexylamine

Synonyms: Fumagillin: Amebacilin, Flisint, Fugillin, BS-16576, Fumagillinum
Dicyclohexylamine: *N*-cyclohexylcyclohexanamine, cyclohexanamine, *N*-cyclohexyl-DCHA, DCH

IUPAC name: Fumagillin: (2E,4E,6E,8E)-10-{[(3R,4S,5S,6R)-5-methoxy-4-[(2R)-2-methyl-3-(3-methylbut-2-enyl)oxiran-2-yl]-1-oxaspiro[2.5]octan-6-yl]oxy}-10-oxodeca-2,4,6,8-tetraenoic acid
Dicyclohexylamine: *N*-cyclohexylcyclohexanamine

Chemical abstract service No.: Fumagillin: 23110-15-8
DCH: 101-83-7

Structural formula: Fumagillin (a) as the dicyclohexylamine salt (b)



a **b**

[1] Although the request of the CCRVDF was to evaluate fumagillin, the Committee interpreted the request as an evaluation of fumagillin DCH, as this is the form in which the compound is used as a veterinary drug.

Molecular formula: Fumagillin: $C_{26}H_{34}O_7$
DCH: $C_{12}H_{23}N$

Molecular weight: Fumagillin: 458.54 g/mol
DCH: 181.32 g/mol

Other information on identity and properties

Appearance: Fumagillin: light yellow needles, yellowish white powder
DCH: colourless or light-yellow liquid

Melting point: Fumagillin: 189–194 °C
DCH: -0.1 °C

Aggregate state at 20 °C: Fumagillin: liquid
DCH: solid

Solubility: Fumagillin: in water: 3.3 mg/L, in ethanol or DMSO: soluble;
DCH: sparingly soluble in water: 0.08 g/100 mL (at 25 ºC), soluble in ethanol, ether and benzene

UV_{max}: Fumagillin: 330–335 nm;
DCH: not applicable

Log K_{ow}: Fumagillin: 4.79;
DCH (neutral form): 4.37

Background

Fumagillin is a mycotoxin originally produced by the fungus *Aspergillus fumigatus* and currently used in a synthesized form. Fumagillin is registered as a veterinary drug in several Member States. It is used as an antimicrobial compound for the treatment of microsporidian infections in various fish species and in honeybees. Fumagillin has been used in human medicine for certain infectious diseases (Maillard *et al.*, 2021; Molina *et al.*, 2002; Guruceaga *et al.*, 2019), and to treat various cancers, by inhibiting the formation of new blood vessels around growing tumours (angiogenesis), thereby limiting their blood supply (Ingber *et al.*, 1990).

Fumagillin is poorly soluble in water and undergoes rapid ultraviolet and thermal degradation. Therefore, to increase its stability and water solubility, commercial formulations used in veterinary medicine contain fumagillin as the dicyclohexylamine (DCH) salt in a 1:1 stoichiometric ratio.

The mode of action of fumagillin is based on inhibition of type-2 methionine aminopeptidase (MetAP-2) activity via formation of a covalent bond with the histidine moiety (His231) of the enzyme. MetAP-2 is a cytosolic enzyme which removes the initial methionine from the amino terminus of newly synthesized proteins, for subsequent post-translational modifications, which affects the function of many proteins (Arico-Muendel *et al.*, 2009; Guruceaga *et al.*, 2019).

Fumagillin DCH has not previously been evaluated by the Committee.

The Committee evaluated fumagillin DCH at the present meeting at the request of the twenty-sixth session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), with a view to recommending maximum residue limits (MRLs) for fish and for honey. As fumagillin is only used in veterinary medicine as the DCH salt, the majority of data evaluated was on fumagillin DCH. However, because the fumagillin DCH salt dissociates into the two moieties and consumers would be exposed to the residues of both, the Committee evaluated fumagillin and DCH.

The sponsor provided unpublished proprietary studies as well as data from studies in the published literature to support the assessment.

In addition, the Committee conducted a search of peer-reviewed scientific literature in the following publicly accessible databases: Agricola, Web of Science, PubMed, Springer Protocols, Food Science and Technology Abstracts, PhishPharm, CABI VetMed Resource and ZB Med Search Portal. Keywords relevant to the use, metabolism and pharmacokinetics of fumagillin DCH and residue monitoring in fish species and honeybees were used (Table 1). The literature search resulted in 21 articles that were considered relevant for the evaluation.

Table 1. Inclusion and exclusion criteria for the literature search

Inclusion criteria	Exclusion criteria
Any article on fumagillin residues or on DCH residues in any fish species or honey	Any article on the efficacy of fumagillin
Any article on withdrawal periods of fumagillin in any fish species or honey	Any article on environmental contamination with fumagillin or DCH
Any article on analytical methods for determining fumagillin or DCH residues	Any article on resistance in target organisms
Any article on degradation products of fumagillin or DCH	Articles in languages other than English
Any article on kinetics and metabolism of fumagillin or DCH	
Any article on MRLs of fumagillin	
Any publication year	

Residues in food and their evaluation

Conditions of use

Fumagillin DCH is approved for the treatment of microsporidia infections in various fish species, including carp (*Sphaerospora renicola*, *Myxobolus cyprinid*), eels (*Pleistophora giardi*, *Myxobolus giardia*), and trout (*Sphaerospora sp.*, *Myxobolus cerebralis*) (Kano and Fukui, 1982; Molnar, Baska and Szekely, 1987; Rigos *et al.*, 2000). In apiculture, fumagillin is approved for treatment of infections caused by *Nosema apis* and *Nosema ceranae* (van den Heever *et al.*, 2016; Huang *et al.*, 2013). In several European countries (the United Kingdom of Great Britian and Northern Ireland, Spain, Belgium, Greece, Hungary, and Romania) exceptional temporary authorization has previously been given to use fumagillin under veterinary supervision to treat nosemosis in positively diagnosed apiaries (Higes *et al.*, 2011).

Dosage

The dosage used in fish is 15–50 mg of fumagillin base per kg bw in medicated feed for 30 consecutive days, or 60 mg fumagillin base per litre of water in an immersion bath for five consecutive days. According to good veterinary practice (GVP), the withdrawal period for use in fish is 28 days for both treatment regimens (water temperature not specified).

The inclusion rate for use in bees is 20–25 mg fumagillin base per litre of sugar, administered once weekly for 6–8 weeks. Bees should be treated in the autumn after honey supers have been removed, or in the spring, with treatment completed 4 weeks before the start of honey flow.

Fumagillin DCH is not currently registered for use as a pesticide.

Pharmacokinetics and metabolism

Pharmacokinetics and metabolism in laboratory animals

No data on the pharmacokinetics and metabolism of fumagillin in laboratory animals were available.

To provide further context on the pharmacokinetics and metabolism of the DCH component of fumagillin DCH, the Committee evaluated one published study on metabolism and excretion of DCH (administered as DCH alone) in laboratory animals (Suenaga, Wada and Ichibagase, 1983).

In rabbits and rats, absorption rate constants from the small intestines were determined as 0.44 and 0.33 per hour, respectively, after gavage treatment with DCH at doses of 50 mg/rabbit and 5 mg/rat for 23–43 days, indicating that intestinal absorption of DCH is rapid in both species. Urinary and faecal excretion of unchanged DCH was low in both species (0.08 and 0.30 percent of the administered dose in rabbits and 5.88 and 0.25 percent in rats, respectively) over 2 days after administration of DCH, suggesting that the substance is quickly metabolized. In liver supernatant from rabbits and rats *in vitro*, DCH was rapidly metabolized in rabbits but not in rats under aerobic conditions and was metabolized only slightly under anaerobic conditions in both species. The metabolites were not identified.

Pharmacokinetics and metabolism in food-producing animals

One published paper on the kinetics of fumagillin in fish (rainbow trout, *Oncorhynchus mykiss*) was available, and one study of metabolism in rainbow trout was provided by the sponsor.

Fumagillin

In a study reported in the literature (Laurén *et al.*, 1989), the plasma kinetics of fumagillin was evaluated in rainbow trout (both sexes; bw 100–300 g). The fish were kept at a water temperature of 15 °C, and fumagillin DCH was administered directly into the dorsal aorta at four doses (60, 30, 6 and 3 mg/kg bw). For the two high dosage groups (30 and 60 mg/kg bw), no pharmacokinetics results were reported due to mortality of the test animals (death within 360 minutes). Histological examination revealed extensive toxic alteration in liver and posterior kidneys. At the two lower dosage groups, plasma clearance of fumagillin at both dosage regimens fit a two-compartment model with a rapid alpha phase (estimated half-life, about 20 min) and a prolonged beta phase (5.4 days). As the slopes of the alpha and beta phases were similar at the two doses, the data were combined for calculation of kinetics parameters. The calculated volume of distribution was high (231 ± 64 mL/kg) (Table 2). DCH concentrations were not measured.

Table 2. Plasma pharmacokinetic parameters of fumagillin in rainbow trout after intra-aortal administration

Dose	6 mg/kg bw (*n* = 2)	3 mg/kg bw (*n* = 2)
Weight[a] (g)	313.6 ± 19.6	
C (µg/mL)	53.1, 32.7	21.1, 9.5
A (µg/mL)	46.9, 26.2	19.3, 7.3
α[a] (min^{-1})	0.0172 ± 0.0043	
T½ α (min)	20.7 ± 4.6	
B (µg/mL)	6.2, 6.5	2.2, 1.8
β[a] (min^{-1})	0.00004 ± 0.00002	
T½ β[a] (min)	7734 ± 2960	
Vdα[a] (mL/kg)	231 ± 64	

Notes: [a]Values from pooled data, *n*=4; C: concentration; A, α, B and β: macro-constants of the compartment model; T½ α: half-life of the alpha phase; T½ β: half-life of the beta phase; Vdα: volume of distribution of the alpha phase

Source: adapted from Laurén, D. J., Wishkovsky, A., Groff, J. M., Hedrick, R. P. & Hinton, D. E. 1989. Toxicity and pharmacokinetics of the antibiotic fumagillin in yearling rainbow trout (*Salmo gairdneri*). *Toxicology and Applied Pharmacology*, 98(3): 444–453.

A study of fumagillin metabolism in rainbow trout (one-year old; *n* = 50; 100 to 275 g bw), which was reported to be compliant with good laboratory practice (GLP), was provided by the sponsor (Kim, 2023). The test substance was a mixture of tritium-radiolabelled fumagillin and unlabelled fumagillin DCH. The fish received a dose of 50 mg/kg bw fumagillin by gavage. The water temperature was kept at 15 °C.

The sponsor reported that fumagillin was randomly labelled with tritium; as such, the precise positions of the tritium labels in the fumagillin molecule were unknown. The extent of exchange of the tritium radiolabel with water was assessed 6 hours after dosing. Most samples exceeded the acceptance criterion of ≤5 percent recommended by the Veterinary International Committee on Harmonization (VICH, 2011) (range, from -22.2 to +15.6 percent), suggesting that the tritium label was unstable. Almost 50 percent of the radioactive residues could not be extracted, although various solvents were tested, including toluene, acetonitrile acidified to pH 3 and acetonitrile adjusted to pH 10. No explanation was provided for the limited extractability of the radioactive residue.

The concentration of fumagillin in fillet ranged from 0.2 to 2.3 mg equiv/kg (0.2–2.1 percent of the total administered dose), whereas the concentrations of unextractable residues ranged from 0.4 to 1.4 mg equiv/kg (0.3–1.7 percent of the total administered dose) throughout the study. Only the parent compound, fumagillin, was identified in fillet. It was therefore proposed that fumagillin was not metabolized.

As no radiolabelled DCH was used in this study, no data were available on the metabolism and depletion of DCH in fish.

Like other veterinary drugs used in apiculture, fumagillin DCH does not appear to be metabolized in honeybees, and most fumagillin DCH is likely to end up in beeswax and honey.

Degradation products

The fumagillin portion of fumagillin DCH is subject to degradation under conditions relevant for the treatment of fish and honeybees (Figure 1). It can be degraded by exposure to light, producing biologically active degradation products with activity similar to that of fumagillin (Kochansky and Nasr, 2004), or hydrolysed under basic conditions to produce fumagillol, which has about 10 percent of the biological activity of fumagillin (Gochnauer and Furgala, 1962). Thermal degradation of fumagillin leads to formation of dihydroxyfumagillin, a biologically inactive compound (Kochansky and Nasr, 2004).

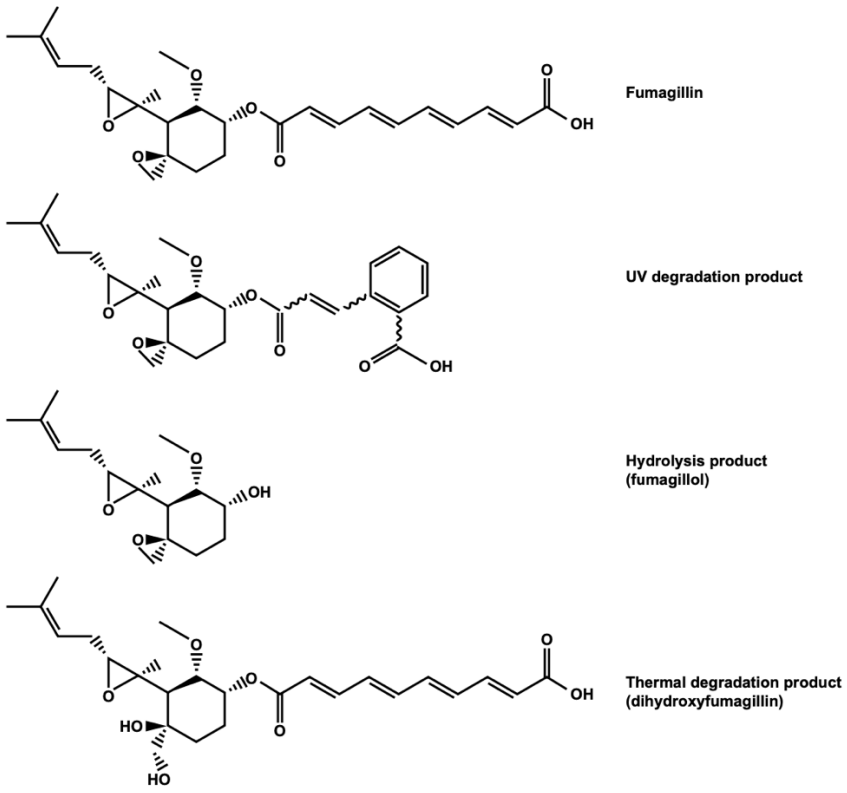
Based on structural considerations, the Committee concluded that fumagillin degradation products are unlikely to be of greater toxicological concern than the parent compound (see Fumagillin DCH toxicological monograph).

The Committee noted that no information on concentrations of fumagillin degradation products in fish tissues or honey was available.

Comparative metabolism

No data was provided to the Committee, or available from the published literature, to allow comparison of the metabolism between species.

Figure 1. Fumagillin and its degradation products identified in honey



Sources: van den Heever, J. P., Thompson, T. S., Curtis, J. M. & Pernal, S. F. 2015a. Determination of Dicyclohexylamine and Fumagillin in Honey by LC-MS/MS. *Food Analytical Methods*, 8(3): 767–777; Nozal, M. A. J., Bernal, J. L., Martín, M. A. T., Bernal, J., Alvaro, A., Martín, R. & Higes, M. 2008. Trace analysis of fumagillin in honey by liquid chromatography-diode array-electrospray ionization mass spectrometry. *Journal of Chromatography A*, 1190(1-2): S. 224–231.

Tissue residue depletion studies

One study on the residue depletion of radiolabelled fumagillin in rainbow trout and 12 studies of residue depletion with unlabelled fumagillin DCH in rainbow trout, carp and eels were provided by the sponsor. All the studies were reported to be GLP-compliant. Studies with each fish species given unlabelled fumagillin DCH were conducted at two water temperatures and two administration routes (oral in feed and via immersion bath).

One study of residue depletion with non-radiolabelled fumagillin DCH in honeybees was provided by the sponsor.

Radiolabelled residue depletion studies

Fish

In the radiolabelled residue depletion study (Kim, 2023), 50 one-year-old rainbow trout (bw100–275 g), were treated with a mixture of [^{3}H]-fumagillin and unlabelled fumagillin DCH at a total dose of about 50 mg/kg bw of fumagillin. To achieve the intended dose, a solution of 50 000 mg/L was prepared from 0.02 mg [^{3}H]-fumagillin (as fumagillin base), and 499.98 mg unlabelled fumagillin (present as fumagillin DCH) added to 10 mL of 0.5 percent CMC solution (carboxymethylcellulose sodium salt in sterilized water). The test was performed in a circular tank system at a water temperature of 15 ± 3 °C.

For dose administration, test animals were anesthetized by bathing the fish for 2-3 minutes in ethyl 3-aminobenzoate methanesulfonate at a concentration of approximately 70 mg/L. Fish were treated via gavage based on their individual body weights within 3 minutes of being anaesthetised.

The fumagillin was randomly labelled with tritium. The extent of exchange of the tritium radiolabel with water was assessed 6 hours after dosing. Numerous samples exceeded the VICH-recommended acceptance criterion (VICH, 2011) of <5 percent (range, from -22.2 to +15.6), indicating that the tritium label was unstable.

A dose of 50 mg/kg bw of fumagillin was administered via gavage. Fillet samples from ten fish per timepoint were collected at 6 and 12 hours, and 1, 2, and 7 days after administration. Individual body weights were recorded after removing surface water from the fish and before tissue collection. The tissues were homogenized using a sample mixer and dry ice, after which they were stored at approximately -20 °C until analysis.

Storage stability samples were prepared by spiking a known amount (10 percent of total administration dose) of [^{3}H]-fumagillin into untreated samples. These samples were stored under the same conditions as the samples from the dosing group.

Validated liquid scintillation counting (LSC) and radio-HPLC (high performance liquid chromatography) methods were used to determine the concentrations of radiolabelled fumagillin. The radiochemical purity of [^{3}H]-fumagillin, determined with a radio-HPLC method, was reported to be 100 percent.

To assess the extent of exchange with water, the radioactivity values of both wet and dry samples were measured using LSC. The dry samples were prepared as follows: muscle samples (0.5 g) were dried at 50 °C for approximately 12 hours, and 5 mL of soluene-350 solution (tissue solubilizer) was added. The samples were shaken at 250 rpm and 50 ± 1 °C for approximately 12 hours until they were

completely dissolved. Subsequently, an aliquot (1 mL) was mixed with a scintillation cocktail (12 mL), and the radioactivity was measured by LSC. The wet samples collected from the rainbow trout were solubilized without drying and measured using the same method as described above.

For sample extraction, 10 g of muscle sample was transferred into a 50 mL tube and extracted up to two times with 80 percent acetonitrile in water first, followed by 0.1 percent formic acid in acetonitrile. The extracts were combined, and the total volume was measured. An aliquot (30 mL) of the extract was evaporated to approximately 0.05 mL using nitrogen gas, and subsequently brought up to 0.3 mL with a mixture of 5 mM ammonium formate and 0.1 percent formic acid in methanol. Afterwards, the solution was centrifuged at 12 000 rpm (4 °C) for 3 minutes, and 0.1 mL of the supernatant was mixed with 4 mL of scintillation cocktail for radioactivity quantification using LSC. Following this, the solution was analysed by radio-HPLC for metabolite characterization.

The remaining tissue after extraction was air-dried for an unknown time and thoroughly mixed. The total weight was measured, and an aliquot (*ca.* 0.1 g) of the dried sample was combusted in a sample oxidizer followed by LSC analysis to determine the amount of radioactivity in the non-extractable residues. Monophase-S was used as ^{3}H liquid scintillator.

The efficiency (recovery) of the radioactivity by the sample oxidizer was determined by combustion of the control tissue sample, spiked with a known amount of radiolabelled fumagillin, to check combustion and trapping efficiencies, which was 93.7–98.8 percent. The recovery test was performed before analysing the samples.

The radiochemical purity of [^{3}H]-fumagillin was determined, using a radio-HPLC method, to be 100 percent, indicating the absence of any other radioactivity apart from [^{3}H]-fumagillin. The identification of [^{3}H]-fumagillin was initially confirmed through HPLC using authentic analytical standards (non-radiolabelled fumagillin) by comparing the retention times.

Both analytical methods were validated and values for the limit of quantification (LOQ) and the limit of detection (LOD) are provided in Table 3. Linearity was observed between 0.0001 and 0.01 mg/L with a correlation coefficient greater than 0.999.

Table 3. Calculation of LOD and LOQ for LSC-analysis and for radio-HPLC analysis

LSC analysis	**Background (dpm)[a]**	**LOD (dpm)**	**LOQ (dpm)**	**Actual**			
				LOD		**LOQ**	
				%TRA[b]	**mg/kg**	**%TRA**	**mg/kg**
Muscle extract	21.5	13.4	58.0	0.000011	0.00010	0.000047	0.00044
Muscle unextractable	79.5	24.8	91.4	0.000020	0.00019	0.000074	0.00069
Radio-HPLC analysis		**LOQ (dpm)[c]**		**Actual**			
				LOQ			
				%TRA		**mg/kg**	
Muscle extract		12 400.00		0.01		0.09	

Notes: total radioactivity administered (TRA) average 123 630 000 dpm; specific activity 2 538 GBq/mmol (3.321 × 108 dpm/ug); [a]Measured from control sample; [b]Total radioactivity administered; [c]The lowest dpm detected by radio-HPLC

Source: adapted from Kim, J.-H. 2023. *Final Report: Metabolism and Residue Kinetics of [^{3}H]-Fumagillin in Rainbow Trout*. Republic of Korea, KRICT.

The injection precision of the radio-HPLC was assessed by injecting a standard solution containing 10 ng/mL of [^{3}H]-fumagillin six times. The precision, expressed as the coefficient of variation, was determined to be 0.6 percent.

The accuracies, represented by the average recoveries across all fortified levels, ranged from 96.9 to 102.3 percent. Precision (CV) was below 2 percent, meeting the acceptance criteria of ≤10 percent. Unextractable residues ranged from 1.9 to 4.4 percent and total recoveries were from 100.8 to 106.7 percent.

To evaluate the stability of fumagillin in tissue samples under frozen conditions below -20 °C, a study on storage stability was conducted. The mean storage stability of the tissue samples treated with [^{3}H]-fumagillin was 95.5 ± 1.1 percent for 14 days, showing that [^{3}H]-fumagillin in tissue was stable for at least 14 days.

The homogeneity of the dose formulation in 0.5 percent CMC solution was evaluated, yielding a coefficient of variation of 3.9 percent across concentrations at the top, middle and bottom, satisfying the acceptance criteria (≤10 percent). In addition, the concentration met acceptance criteria (100 ± 15 percent of the nominal concentration).

To assess the extent of tritium exchange with water, the wet and dry samples of each tissue were analysed by comparing the radioactivity of both samples. Most of the samples showed negative values; however, some fillet tissues exhibited a maximum ratio of 15.6 percent at 6 hours after administration.

The amount of fumagillin recovered in the extract accounted for 1.1 percent of the total administered dose (TAD) at 6 hours after administration, equivalent to 1.2 mg/kg. Fumagillin levels increased to 2.1 percent of TAD at 12 hours, corresponding to 2.3 mg equiv/kg. However, the percentage levels on days 1, 2, and 7 continuously declined to 1 percent, 0.9 percent, and 0.2 percent of TAD, respectively, corresponding to 1, 0.9, and 0.2 mg/kg, respectively.

The unextractable residue of the total radioactive residue (TRR) recovered accounted for 1.2 percent of TAD at 6 hours after administration, corresponding to 1.3 mg/kg. However, by day 7, the TRR decreased to 0.3 percent of TAD, corresponding to 0.4 mg/kg.

After administering fumagillin to rainbow trout, the unchanged fumagillin detected by radio-HPLC in the muscle tissues was collected and subjected to analysis using liquid chromatography-mass spectrometry (LC-MS) for identification of fumagillin. The mass spectra indicated the presence of ions at *m/z* 459 as the protonated molecular ion ($M+H^+$) and *m/z* 481 as the sodium adduct molecular ion ($M+Na^+$). The identification and confirmation of the unchanged fumagillin were achieved by comparison with an authentic fumagillin standard.

The TAD values obtained through additional extraction with toluene, acidic, and basic solutions ranged from 0.006 to 0.008 percent, 0.13 percent, and from 0.07 to 0.09 percent, respectively, corresponding to 0.006–0.007 mg/kg, 0.12–0.14 mg/kg, and 0.07–0.1 mg/kg. These findings indicate that a large percentage of the TRR (from 38 to 63.2 percent) was present in the fraction of the unextractable residues (Table 4).

Table 4. Distribution of radioactivity in tissues following oral administration of [3H]-fumagillin to rainbow trout, percent of total radioactive residues and mean values ± SD from 10 fish per time point

Time (days)	Fraction	Mean ± SD	
		%TRR	mg equiv/kg
6 h	Extract	48.5 ± 6.0	1.2 ± 0.4
	Unextractable	51.6 ± 6.0	1.3 ± 0.5
	Total	100.0 ± 0.0	2.5 ± 0.9
12 h	Extract	62.0 ± 14.0	2.3 ± 1.4
	Unextractable	38.0 ± 14.0	1.2 ± 0.5
	Total	100.0 ± 0.0	3.5 ± 1.3
1	Extract	41.5 ± 5.1	1.0 ± 0.4
	Unextractable	58.5 ± 5.1	1.4 ± 0.5
	Total	100.0 ± 0.0	2.4 ± 0.9
2	Extract	36.9 ± 10.7	0.9 ± 0.5
	Unextractable	63.2 ± 10.7	1.7 ± 0.9
	Total	100.0 ± 0.0	2.6 ± 1.2
7	Extract	55.8 ± 30.9	0.2 ± 0.0
	Unextractable	63.2 ± 6.0	0.4 ± 0.1
	Total	100.0 ± 0.0	0.5 ± 0.2

Notes: %TRR: percent of the total radioactivity residue; SD: standard deviation; LOQ: <0.09 mg/kg

Source: adapted from Kim, J.-H. 2023. *Final Report: Metabolism and Residue Kinetics of [^{3}H]-Fumagillin in Rainbow Trout.* Republic of Korea, KRICT.

The concentration of fumagillin in rainbow trout fillet increased from 1.2 mg equiv/kg at 6 hours after dosing to 2.3 mg equiv/kg at 12 hours, and then decreased to 0.2 mg equiv/kg on day 7.

The radiolabelled residue depletion study indicates that the parent compound, fumagillin, is a suitable marker residue.

Residue depletion studies with non-radiolabelled drug

Fish

The sponsor submitted residue depletion studies reported as GLP-compliant with non-radiolabelled fumagillin in rainbow trout, carp and eels (National Institute of Food and Drug Safety Evaluation (NIFDS), 2015a, 2015b, 2015c). For each species, studies were conducted at two water temperatures and two administration routes (oral via feed and immersion bath). A nominal dose of 50 mg/kg bw for 30 consecutive days was used for oral administration; for the immersion bath, fish were exposed to fumagillin at a concentration of 60 mg/L for 5 consecutive days.

The product used in these studies was Fumagil-C, containing 50 g/kg of fumagillin (administered as fumagillin DCH), and 13.6 g/kg of ascorbic acid, in glucose. Medicated feed was prepared by mixing Fumagil-C with feed under light-protected conditions, and fish oil was uniformly sprayed onto the medicated feed to prevent release of the drug into the water. Fumagillin was quantified in the medicated feed and constituted 80–110 percent of the intended concentration. Fumagillin was not quantified in the water of the immersion bath.

The feeding rate was 0.5–1.0 percent of the fish body weight, considering the specific appetite levels of the fish species tested. The medicated feed was administered every morning at the same time and the process was considered complete if consumption occurred within 30 minutes of supply. Details such as the exact quantity of feed administered and consumed, and the weight of the fish in each tank, were not provided.

In the studies provided, the assessment was solely of depletion of fumagillin. Residues of DCH were not quantified in the sampled tissues.

Ten fish were euthanized at each of 1, 3, 7, 14 and 28 days after the last oral dose and 1, 3 and 7 days after exposure in an immersion bath, and samples of fillet (muscle with skin in natural proportions) were collected. Fumagillin was quantified in the samples with a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method separately validated for each species. The LOQ for fumagillin in fish fillet for all species was 5 µg/kg.

Rainbow trout

The sponsor conducted four studies using non-radiolabelled fumagillin (test product Fumagil-C) in rainbow trout. These included two studies involving the oral administration of fumagillin via medicated feed at two different water temperatures (15 ± 3 and 22 ± 3 ºC), as well as two studies exposing fish to fumagillin in an immersion bath at two different water temperatures (13 ± 3 and 25 ± 3 ºC) (NIFDS, 2015a).

Rainbow trout, weighing 720 ± 90 g, were housed in a concrete tank measuring 5 × 5 × 1.5 m for acclimation, administration, and sampling purposes. Prior to the start of the experiment, a 2 to 4-week acclimation period was observed under laboratory conditions with voluntary feeding.

At the end of treatment, fillet and mixed organ samples were collected at various timepoints, and the quantification of fumagillin was carried out using a validated LC-MS/MS method. The calibration graph for fumagillin exhibited linearity ($r > 0.99$) over the concentration range of 5–500 µg/kg, with an LOQ of 5 µg/kg.

Oral administration

Two separate studies were conducted at two water temperatures (15 and 22 ºC). Ten fish were euthanized at each of 1, 3, 7, 14 and 28 days after the last treatment and fillet and mixed organ samples (liver, kidney, spleen, stomach, and intestine) were collected. The concentrations of fumagillin determined in the fillet samples at 15 °C and 22 °C are shown in Table 5 and Table 6, respectively.

Table 5. Concentration of fumagillin in fillet from rainbow trout with time after oral daily dose (medicated feed) of fumagillin (50 mg/kg bw) for 30 days at 15 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard-deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	15	15	10	678.38	10	444.31	1 452.67	319.59
3	45	15	10	504.98	10	86.24	1 208.00	381.35
7	105	15	10	75.52	6	<LOQ	205.20	70.62
14	210	15	10	<LOQ	0	<LOQ	<LOQ	-
28	420	15	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ (5 µg/kg); DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015a. *Final Report (A): A Study on Residues Depletion of Fumagillin in Rainbow Trout.* Republic of Korea, NIFDS.

Table 6. Concentration of fumagillin in fillet from rainbow trout with time after oral daily dose (medicated feed) of fumagillin (50 mg/kg bw) for 30 days at 22 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	Temp (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard-deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	22	22	10	1 558.46	10	566.50	3 258.40	915.60
3	66	22	10	496.93	10	58.41	1 113.45	329.76
7	154	22	10	47.14	4	<LOQ	105.2	43.09
14	308	22	10	<LOQ	0	<LOQ	<LOQ	-
28	616	22	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ (5 µg/kg); DD: degree-days

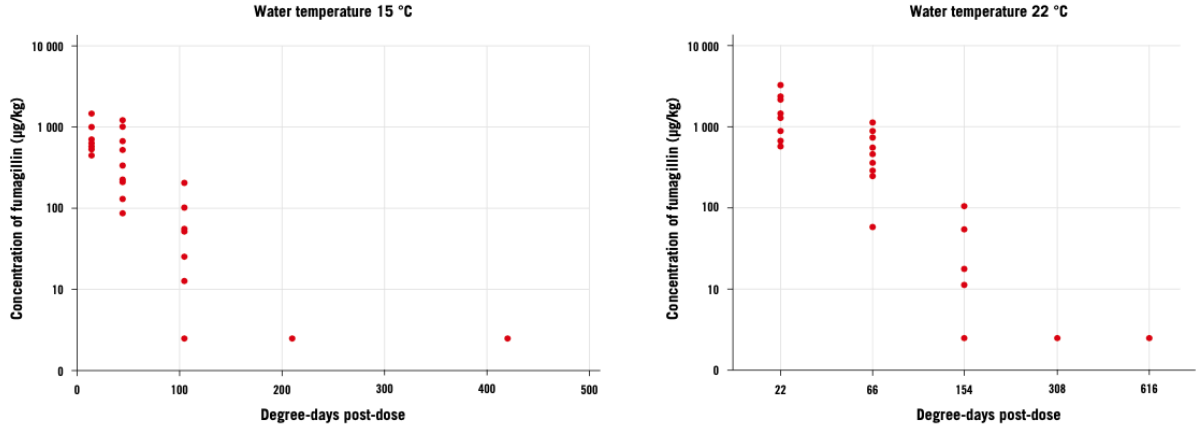
Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015a. *Final Report (A): A Study on Residues Depletion of Fumagillin in Rainbow Trout*. Republic of Korea, NIFDS.

In the mixed organs of fish maintained at 15 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 1 299.43 ± 694.65 µg/kg on day 1 and 897.36 ± 656.25 µg/kg on day 3. On day 7, fumagillin was detected in 7 out of 10 mixed organ samples with levels of 96.68 ± 104.89 µg/kg. On day 14, fumagillin was quantified in 4 out of 10 samples at a level of 7.72 ± 2.82 µg/kg. After that, no fumagillin was detected in any of the samples.

In the mixed organs of fish maintained at 22 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 2 787.44 ± 1 623.04 µg/kg on day 1 and 950.15 ± 660.31 µg/kg on day 3. On day 7, fumagillin was detected in 5 out of 10 samples with levels of 44.75 ± 45.85 µg/kg. On day 14, fumagillin was quantified in 4 out of 10 samples at a level of 11.48 ± 6.55 µg/kg. After that, no fumagillin was detected in any of the samples.

Figure 2 shows the concentration of fumagillin in rainbow trout fillet versus degree-days post-dose from the residue depletion studies conducted at two different water temperatures.

Figure 2. Residue depletion profile of fumagillin in rainbow trout fillet at two water temperatures after in-feed administration of fumagillin



Source: Authors' own elaboration, based on data submitted to the Committee.

Immersion bath

Rainbow trout were exposed to fumagillin in an immersion bath at a concentration of 60 mg/L for 5 days at two water temperatures, 13 ± 3 ºC and 25 ± 3 ºC.

Ten fish were euthanized at each of 1, 3 and 7 days after treatment and fillet samples were collected. The concentrations of fumagillin determined for the lower and higher temperatures are shown in Table 7 and Table 8, respectively.

Table 7. Concentration of fumagillin in fillet from rainbow trout with time after immersion bath of fumagillin (60 mg/L) for 5 days at 13 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard-deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	13	13	10	22.21	9	<LOQ	94.43	27.71
3	39	13	10	<LOQ	0	<LOQ	<LOQ	-
7	91	13	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ (5 µg/kg); DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015a. *Final Report (A): A Study on Residues Depletion of Fumagillin in Rainbow Trout.* Republic of Korea, NIFDS.

Table 8. Concentration of fumagillin in fillet from rainbow trout with time after immersion bath of fumagillin (60 mg/L) for 5 days at 25 ± 3 ºC

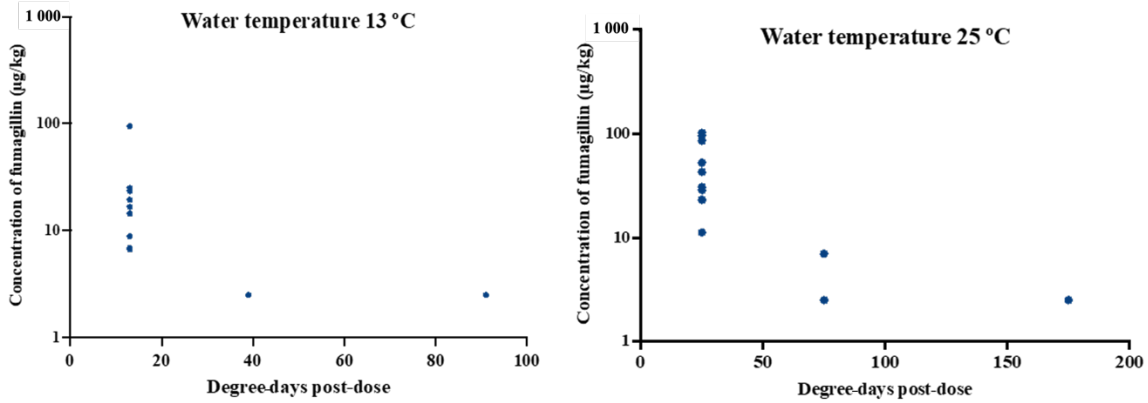
Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	25	25	10	50.11	10	11.25	103.43	33.70
3	75	25	10	7	1	<LOQ	7	-
7	175	25	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ (5 µg/kg); DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015a. *Final Report (A): A Study on Residues Depletion of Fumagillin in Rainbow Trout.* Republic of Korea, NIFDS.

Figure 3 shows the concentration of fumagillin in rainbow trout fillet versus degree-days post-dose from the depletion studies at two different water temperatures.

Figure 3. Residue depletion profile of fumagillin in rainbow trout fillet at two water temperatures after immersion bath treatment with fumagillin at a dose of 60 mg/L



Source: Authors' own elaboration, based on data submitted to the Committee

In the published literature, one residue depletion study of fumagillin, as DCH salt, in rainbow trout (>50 g) is described by Guyonnet *et al.* (1995). Two doses were evaluated; 3 mg/kg bw and 15 mg/kg bw. The fish were held at constant water temperatures (16–17ºC) and a water flow of 150 L/min. Fumagillin was administered as medicated feed (5 g of fumagillin as the DCH salt per 100 g of feed) twice per day for 10 consecutive days. Twelve fish per timepoint were euthanized at 1 hour, and at 4, 10 and 21 days after the end of the treatment. Muscle samples were collected and fumagillin determined by an HPLC-UV method. The LOQ and LOD of the method were 20 µg/kg and 7 µg/kg, respectively. The mean concentrations of fumagillin (± SD) determined at 1 hour post-dose was 30 ± 8 µg/kg and 46 ± 24 µg/kg for the doses of 3 and 15 mg/kg bw, respectively. In the muscle sampled on 4, 10 and 21 days post-dose, no fumagillin was detected.

Carp

Four studies were conducted using non-radiolabelled fumagillin (test product Fumagil-C) in Carp (*Cyprinus carpio).* These comprised two studies involving the oral administration of fumagillin via medicated feed at two water temperatures (13 ± 3 ºC and 25 ± 3 ºC), and two studies exposing fish to fumagillin via immersion bath at two water temperatures (13 ± 3 ºC and 25 ± 3 ºC) (NIFDS, 2015b).

The carp (750 ± 50 g bw) were housed in a concrete tank (5 × 5 × 1.5 m) for acclimation, administration, and sampling. Prior to the start of the experiment, they were acclimated under laboratory conditions for 2–4 weeks with voluntary feeding.

Fillet and mixed organs were sampled and fumagillin was quantified by a validated LC-MS/MS method. The calibration graph for fumagillin was linear (r > 0.99) in the concentration range of 5 to 500 µg/kg, with an LOQ of 5 µg/kg.

Oral administration

Carp were treated with fumagillin, once a day, via feed at a nominal dose of 50 mg/kg bw for 30 consecutive days. Two studies were carried out at different water temperatures (13 and 25 ºC).

Ten fish were euthanized at each of 1, 3, 7, 14 and 28 days after the treatment and fillet and mixed organ samples (liver, kidney, spleen, stomach, and intestine) were collected. Fumagillin was quantified by a validated LC-MS/MS method. The concentrations of fumagillin determined in the samples analysed in the two studies are shown in Table 9 and Table 10, respectively.

Table 9. Concentrations of fumagillin in carp fillet with time after oral daily dose (medicated feed) of fumagillin (50 mg/kg bw) for 30 days at 13 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard-deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	13	13	10	1 224.13	10	534.28	2 566.93	765.80
3	39	13	10	404.50	10	79.31	885.45	272.76
7	91	13	10	37.26	6	<LOQ	87.33	28.15
14	182	13	10	8.5	1	<LOQ	8.5	-
28	364	13	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015b. *Final Report (B): A Study on Residues Depletion of Fumagillin in Carp.* Republic of Korea, NIFDS.

Table 10. Concentrations of fumagillin in carp fillet with time after oral daily dose (medicated feed) of fumagillin (50 mg/kg bw) for 30 days at 25 ± 3 ºC

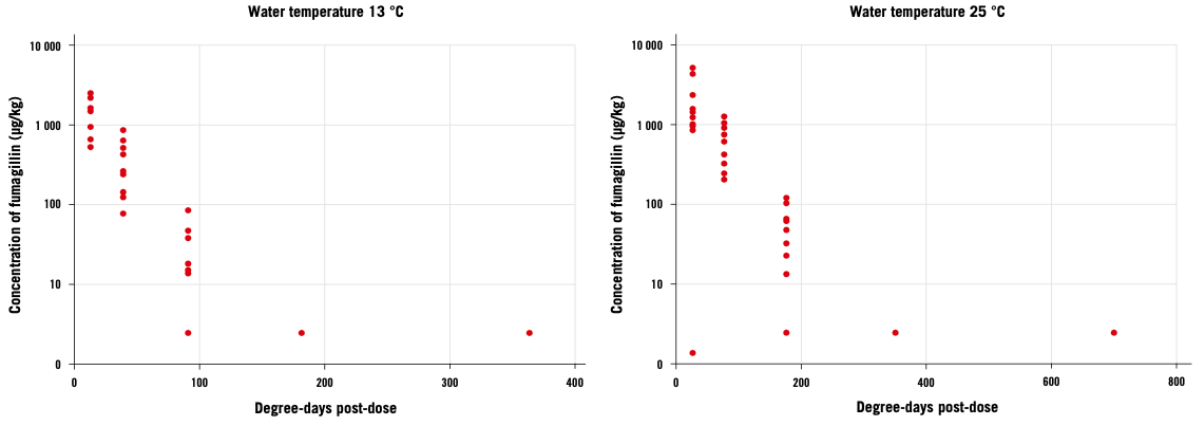
Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	n >LOQ	Minimum	Maximum	
1	25	25	10	2 256.13	10	867.45	5 234.27	1 732.10
3	75	25	10	658.01	10	205.35	1 282.00	356.94
7	175	25	10	59.02	8	<LOQ	122.34	38.45
14	350	25	10	<LOQ	0	<LOQ	<LOQ	-
28	700	25	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015b. *Final Report (B): A Study on Residues Depletion of Fumagillin in Carp*. Republic of Korea, NIFDS.

Figure 4 shows the concentrations of fumagillin in carp fillet versus days post-dose from the depletion studies at two different water temperatures.

Figure 4. Residue depletion profile of fumagillin in carp fillet at two water temperatures after oral administration of fumagillin



Source: Authors' own elaboration, based on data submitted to the Committee.

In the mixed organs of carp maintained at 13 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 1 894.68 ± 880.31 µg/kg on day 1 and 724.06 ± 488.24 µg/kg on day 3. On day 7, fumagillin was quantified in 7 out of 10 samples with levels of 76.65 ± 89.81 µg/kg. On day 14, fumagillin was quantified in 4 out of 10 samples at a level of 10.77 ± 7.84 µg/kg. After that, no fumagillin was detected in any of the samples.

In the mixed organs of carp maintained at 25 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 2 926.57 ± 1 785.48 µg/kg on day 1 and 1 171.26 ± 635.35 µg/kg on day 3. On day 7, fumagillin was quantified in 8 out of 10 samples with levels of 130.20 ± 82.68 µg/kg. On day 14, fumagillin was quantified in 2 out of 10 samples at a level of 6.78 ± 0.61 µg/kg. After that, no fumagillin was detected in any of the samples.

Immersion bath

Carp were exposed to fumagillin via an immersion bath at a concentration of 60 mg/L for 5 days at two water temperatures, 13 ± 3 ºC and 25 ± 3 ºC.

Ten fish were sampled at each of 1, 3 and 7 days after the treatment and fillets were collected. Fumagillin was quantified by a validated LC-MS/MS method. The concentrations of fumagillin determined in the fillet samples analysed in the two studies at 13 and 25 ºC are shown in Table 11 and Table 12, respectively.

Table 11. Concentrations of fumagillin in carp fillet with time after immersion bath of fumagillin (60 mg/L) for 5 days at 13 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	13	13	10	26.48	10	6.71	81.31	24.44
3	39	13	10	17.22	4	6.60	28.50	9.75
7	91	13	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015b. *Final Report (B): A Study on Residues Depletion of Fumagillin in Carp.* Republic of Korea, NIFDS.

Table 12. Concentrations of fumagillin in carp fillet with time after immersion bath of fumagillin (60 mg/L) for 5 days at 25 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	25	25	10	34.39	7	<LOQ	67.21	22.70
3	75	25	10	11.2	1	11.20	11.20	-
7	175	25	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

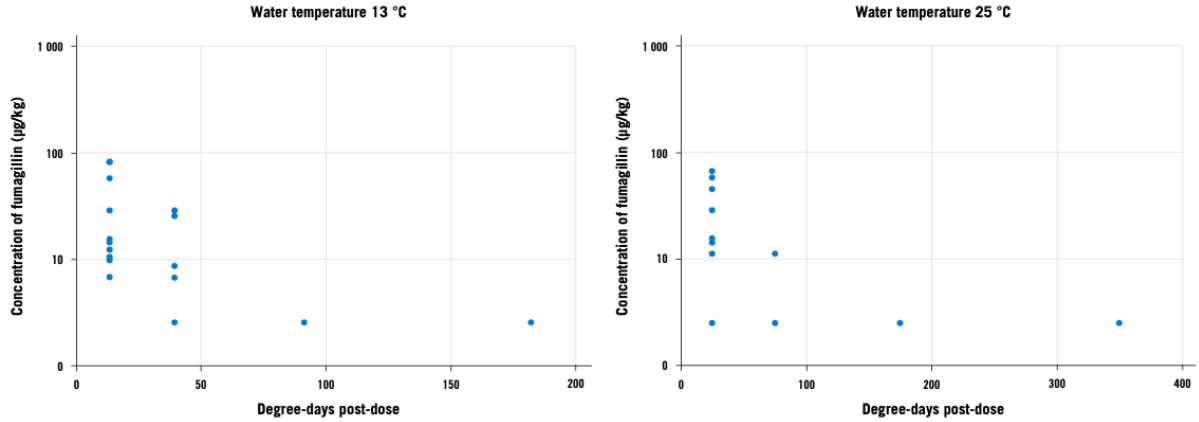
Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015b. *Final Report (B): A Study on Residues Depletion of Fumagillin in Carp.* Republic of Korea, NIFDS.

Figure 5 shows the concentrations of fumagillin in carp fillet versus degree-days post-dose from the depletion studies at two different water temperatures (treatment immersion bath).

In the mixed organs of carp maintained at 13 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 33.30 ± 30.72 µg/kg on day 1 and 14.24 ± 12.36 µg/kg on day 3. On day 7 and day 14, fumagillin was not detected in any of the samples analysed.

In the mixed organs of carp maintained at 25 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 40.30 ± 27.08 µg/kg on day 1 and 9.67 ± 3.11 µg/kg on day 3. On day 7 and day 14, fumagillin was not detected in any of the samples analysed.

Figure 5. Residue depletion profile of fumagillin in carp fillet at two water temperatures after immersion bath with fumagillin at a dose of 60 mg/L



Source: Authors' own elaboration, based on data submitted to the Committee.

Eels

Four studies were conducted using non-radiolabelled fumagillin (test product Fumagil-C) in eels (*Anguilla japonica).* These comprised two studies involving the oral administration of fumagillin in medicated feed at different water temperatures (20 ± 3 ºC and 28 ± 3 ºC), and two studies exposing fish to fumagillin in an immersion bath at different water temperatures (20 ± 3 ºC and 28 ± 3 ºC) (NIFDS, 2015c).

The eels (240 ± 55 g bw) were housed in a concrete tank (3 × 1 × 0.9 m) for acclimation, administration and sampling. Prior to the start of the experiment, they were acclimated under laboratory conditions for 2–4 weeks with voluntary feeding.

Fillet and mixed organs were sampled and fumagillin quantified by a validated LC-MS/MS method. The calibration graph for fumagillin was linear (r >0.99) in the concentration range of 5 to 500 µg/kg, with an LOQ of 5 µg/kg.

Oral administration

Eels were treated with fumagillin, once a day, via feed, at a nominal dose of 50 mg/kg bw for 30 consecutive days. Two studies were carried out at different water temperatures (20 ºC and 28 ºC).

Ten eels were euthanized at each of 1, 3, 7, 14 and 28 days after the treatment and fillet and mixed organ samples (liver, kidney, spleen, stomach, and intestine) were collected. Fumagillin was quantified by a validated LC-MS/MS method. The concentrations of fumagillin determined in the samples analysed in the two studies are shown in Table 13 and Table 14, respectively.

Table 13. Concentrations of fumagillin in eel fillet with time after oral daily dose (medicated feed) of fumagillin (50 mg/kg bw) for 30 days at 20 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	20	20	10	904.04	10	440.32	2 300.23	569.39
3	60	20	10	425.39	10	143.20	1 256.29	357.37
7	140	20	10	14.24	2	<LOQ	22.35	11.48
14	280	20	10	20.21	2	<LOQ	23.40	3.40
28	560	20	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015c. *Final Report (C): A Study on Residues Depletion of Fumagillin in Eel.* Republic of Korea, NIFDS.

Table 14. Concentration of fumagillin in eel fillet with time after oral daily dose (medicated feed) of fumagillin (50 mg/kg bw) for 30 days at 28 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	28	28	10	1 714.26	10	555.89	3 257.54	994.01
3	84	28	10	540.80	10	109.44	1 209.65	309.05
7	196	28	10	31.98	5	<LOQ	78.42	33.26
14	392	28	10	18.78	2	<LOQ	26.79	8.02
28	784	28	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

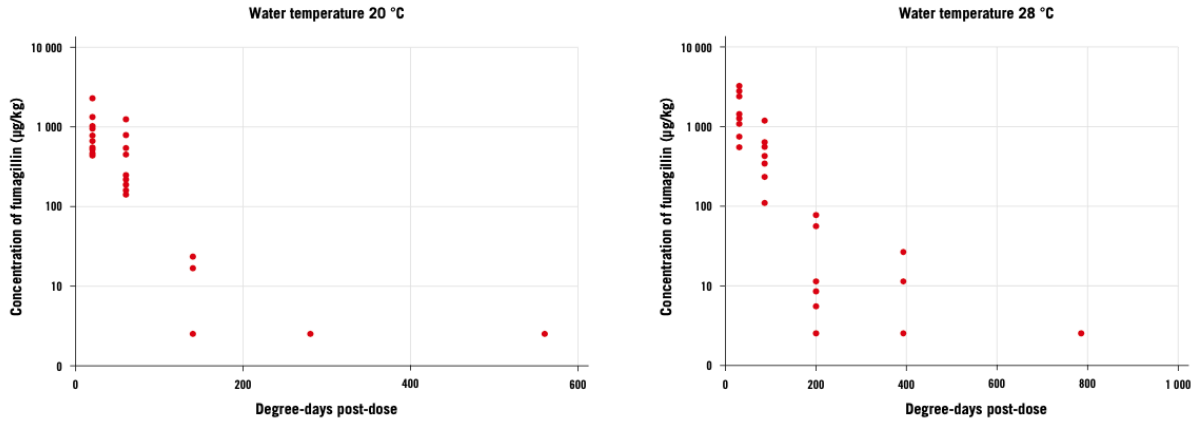
Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015c. *Final Report (C): A Study on Residues Depletion of Fumagillin in Eel.* Republic of Korea, NIFDS.

Figure 6 shows the concentrations of fumagillin in eel fillet versus days post-dose from the depletion studies at two different water temperatures.

In the mixed organs of eels maintained at 20 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) were 1 563.49.68 ± 1 232.08 µg/kg on day 1 and 658.44 ± 497.80 µg/kg on day 3. On day 7, fumagillin was detected in 4 out of 10 samples with levels of 96.55 ± 82.72 µg/kg. On day 14, fumagillin was quantified in 3 out of 10 samples at a level of 12.94 ± 7.50 µg/kg. No fumagillin was detected in any of the samples collected at day 28.

In the mixed organs of eels maintained at 28 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) of fumagillin were 4 303.64 ± 2 592.14 µg/kg on day 1 and 702.45 ± 577.11 µg/kg on day 3. On day 7, fumagillin was quantified in 3 out of 10 samples with levels of 36.05 ± 39.30 µg/kg. On day 14, fumagillin was quantified in 2 out of 10 samples at a level of 6.44 ± 0.76 µg/kg. No fumagillin was detected in any of the samples collected at day 28.

Figure 6. Residue depletion profile of fumagillin in eel fillet at two water temperatures after oral administration of fumagillin



Source: Authors' own elaboration, based on data submitted to the Committee.

Immersion bath

Eels were exposed to fumagillin in an immersion bath at a concentration of 60 mg/L for 5 days at two water temperatures: 20 ± 3 ºC and 28 ± 3 ºC.

Ten fish were euthanized at each of 1, 3 and 7 days after the treatment and fillets and mixed organ samples were collected. Fumagillin was quantified by a validated LC-MS/MS method. The concentrations of fumagillin determined in the samples analysed in the two studies are shown in Table 15 and Table 16, respectively.

Table 15. Concentration of fumagillin in eel fillet with time after immersion bath of fumagillin (60 mg/L) for 5 days at 20 ± 3 ºC

Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	20	20	10	41.35	9	<LOQ	85.34	22.57
3	60	20	10	20.38	6	<LOQ	54.2	17.63
7	140	20	10	<LOQ	0	<LOQ	<LOQ	-
14	280	20	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values >LOQ; DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015c. *Final Report (C): A Study on Residues Depletion of Fumagillin in Eel.* Republic of Korea, NIFDS.

Table 16. Concentrations of fumagillin in eel fillet with time after immersion bath of fumagillin (60 mg/L) for 5 days at 28 ± 3 ºC

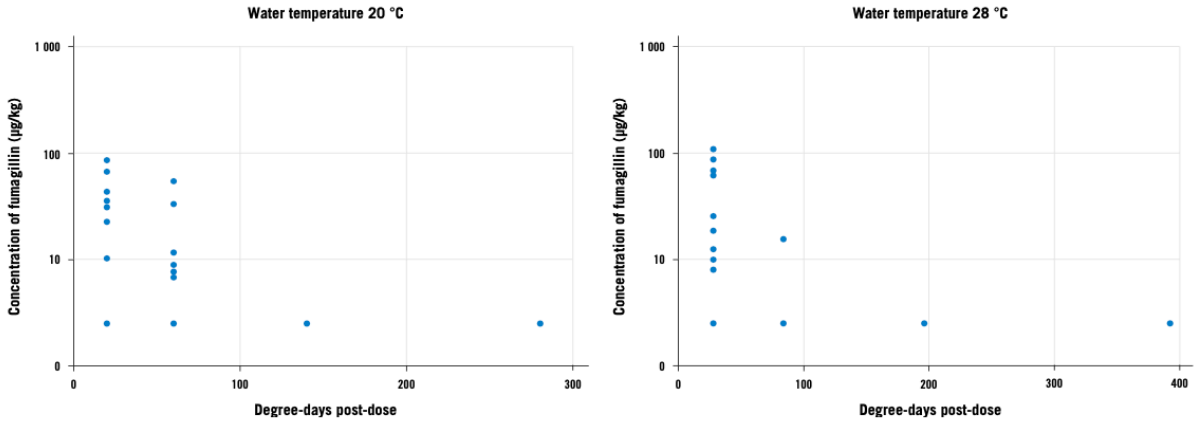
Time post-dose (day)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of fumagillin (µg/kg)				Standard deviation[a] (µg/kg)
				Mean[a]	*n* >LOQ	Minimum	Maximum	
1	28	28	10	44.11	9	<LOQ	106.87	37.34
3	84	28	10	15.43	1	<LOQ	15.43	-
7	196	28	10	<LOQ	0	<LOQ	<LOQ	-
14	392	28	10	<LOQ	0	<LOQ	<LOQ	-

Notes: Data not corrected for recovery. [a]Considering only the values > LOQ; DD: degree-days

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015c. *Final Report (C): A Study on Residues Depletion of Fumagillin in Eel.* Republic of Korea, NIFDS.

Figure 7 shows the concentrations of fumagillin in eel fillet versus days post-dose from the depletion studies at two different water temperatures (treatment via immersion bath).

Figure 7. Residue depletion profile of fumagillin in eel fillet at two water temperatures after immersion bath with fumagillin at a dose of 60 mg/L



Source: Authors' own elaboration, based on data submitted to the Committee.

In the mixed organs of eels maintained at 20 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) of fumagillin were 55.10 ± 27.78 µg/kg (*n*=9) on day 1, 32.60 ± 39.91 µg/kg (*n*=6) on day 3 and 5.98 ± 0.05 µg/kg (*n*=2) on day 7. On day 14, fumagillin was quantified in one out of 10 samples, at a concentration of 6.10 µg/kg.

In the mixed organs of eels maintained at 28 ºC, the mean (± SD) residue levels of fumagillin (considering only the values above the LOQ) of fumagillin were 4.52 ± 42.82 µg/kg (*n*=9) on day 1, 32.81 ± 23.68 µg/kg (*n*=3) on day 3 and 5.76 ± 0.63 µg/kg (*n*=3) on day 7. On day 14, fumagillin was not detected in any of the samples.

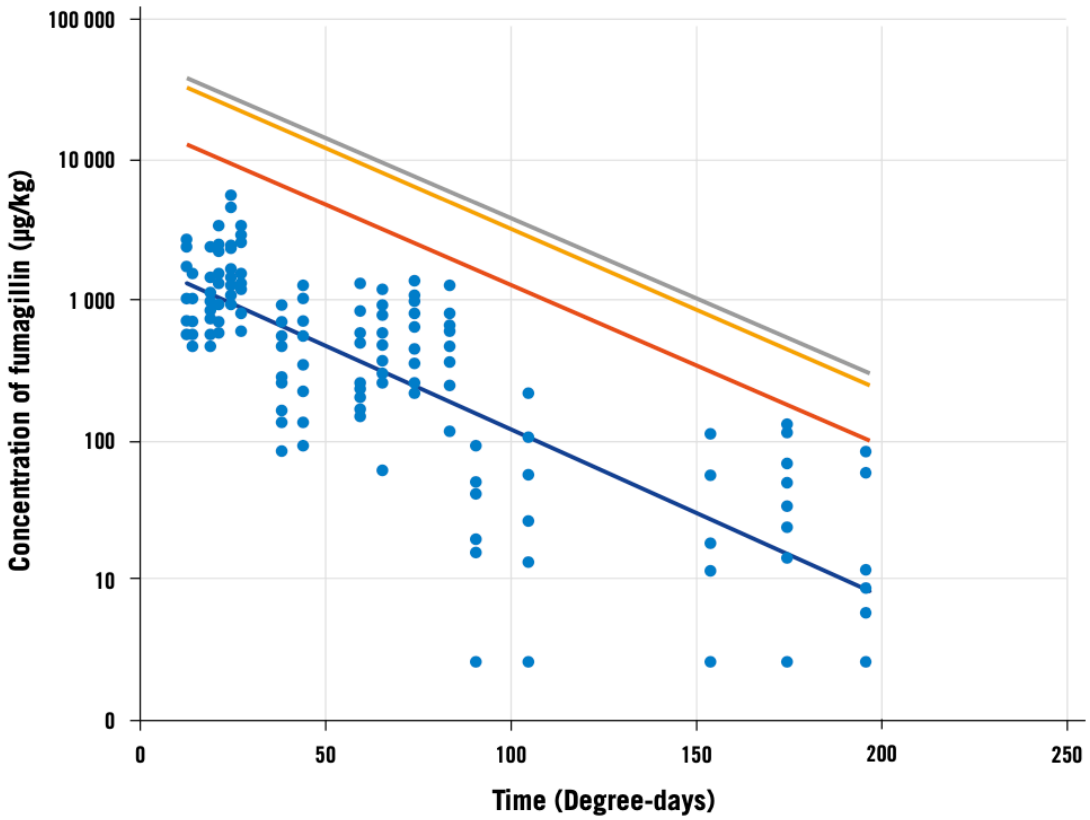
Conclusions

Levels of fumagillin were determined at several timepoints after both administration routes and water temperatures in all three fish species. Slightly higher concentrations of fumagillin residues in fillet were generally found in fish euthanized at the higher water temperature in all studies.

The Committee noted that the residue concentrations of fumagillin in fish exposed via immersion baths were almost 100 times lower than after oral administration. The Committee also noted that the higher water temperatures used in these studies may not be optimal for all species used.

When the Committee combined the data on residue depletion in fish after oral administration of fumagillin DCH (normalized to degree-days), the depletion profiles were similar for the three species (Figure 8). The Committee also noted that no quantifiable fumagillin residues were found at the approved GVP withdrawal period (28 days), including in the most conservative scenario (coldest water temperature 13 °C, corresponding to 364 degree-days).

Figure 8. Residue depletion profile combined of fumagillin in rainbow trout, carp and eel fillet data at different water temperatures following oral administration of fumagillin DCH at a dose of 50 mg/kg bw for 30 consecutive days. Regression line (blue), UTL 95/95 regression line (orange), UTL 95/99 regression line (yellow) and UTL 99/99 regression line (grey).



Sources: NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015a. *Final Report (A): A Study on Residues Depletion of Fumagillin in Rainbow Trout.* Republic of Korea, NIFDS; NIFDS. 2015b. *Final Report (B): A Study on Residues Depletion of Fumagillin in Carp.* Republic of Korea, NIFDS; NIFDS. 2015c. *Final Report (C): A Study on Residues Depletion of Fumagillin in Eel.* Republic of Korea, NIFDS. Authors' own elaboration.

Bees

In one study in honeybees, reported to be GLP-compliant, six beehives at each of three apiaries were treated with Fumidil-B (containing 20 g fumagillin per kg product as fumagillin DCH) at a dose of 25 g product dissolved in 20 L of sugar water (corresponding to 25 mg/L fumagillin solution), once a week for 4 or 5 consecutive weeks (Jeong, 2023). Sugar water was provided in a honeybee feeder (0.8 L sugar water containing 20 mg fumagillin per hive), and all was consumed within 1 day of supply.

The applied doses were reported to be lower (75.6–98.6 percent) than the intended doses (Table 18). The sponsor explained the lower concentrations were due to storage of the formulation in a transparent container, allowing UV degradation to occur. The treatment duration (4–5 weeks) was shorter than approved according to GVP (6–8 weeks).

Table 18. Concentration ranges in sugar water used for treatment and percent of the intended doses of fumagillin

Apiary	Fumagillin concentration	% of intended dose
A	23.68–23.88 mg/L	95.07%
B	16.52–21.09 mg/L	75.62%
C	24.49–24.84 mg/L	98.61%

Source: adapted from Jeong, S.-H. 2023. Final Report: A Study on Fumagillin Residues in Honey Samples and Determining Withdrawal Period (Study No. RED22023). Republic of Korea, HBSRC.

The treatment was administered in spring, 1 week before onset of honey flow (from 8 March to 9 April). Honey samples were taken starting 1 week after onset of honey flow (from 14 April to 16 May), although GVP requires that the treatment should be finished by 4 weeks before the start of honey flow. The exact location of sampling in the beehives (i.e., whether samples were taken from honey supers or elsewhere) was not reported.

Robinie, or 'Black Locust' (*Robinia pseudoacacia*) was identified as the main honey crop for all three apiaries. Information on climate conditions on treatment and sampling days for each apiary was provided, but no further data on agro-ecological conditions for the three apiaries were available.

Honey from each beehive was collected before treatment and at various times after the last treatment. In one apiary (Apiary A), four samples were taken on each of days 16, 31, 36, 42 and 45; in the second apiary (Apiary B), only two samples per beehive were taken, on days 24 and 29 after the last treatment; and, in the third apiary (Apiary C), two samples were taken, on days 22 and 44 after the last treatment. Honey samples from all apiaries were analysed for residues of fumagillin, and samples from Apiary A were also analysed for DCH concentrations. No samples of beeswax were taken. No details on sampling (e.g., amount of pooled honey, pH, and moisture content of all pooled honey samples) were provided.

An LC-MS/MS method was used for determination of fumagillin concentrations in honey (LOD 2 µg/kg, LOQ 5 µg/kg). Only fumagillin itself was measured (i.e without degradation products). The accuracy, evaluated on three separate days and at four fortification levels (5, 10, 100 and 500 ng/mL), exhibited a range from 85.8 percent to 115.5 percent, while precision varied from 0.4 to 8.1 percent. For inter-day accuracy and precision, the average accuracy across the three days was between 99.4 percent and 107 percent, with average precision ranging from 3.6 to 13.8 percent.

In all three apiaries, the residue concentrations of fumagillin in honey samples decreased with time after treatment (Table 19). In one apiary, fumagillin was detected at concentrations (mean ± SD) of

110.7 ± 97.3 µg/kg, 19.01 ± 10 µg/kg, 5.882 ± 1.2 µg/kg, <LOQ, and <LOQ on days 16, 31, 36, 42, and 46 after treatment, respectively. In the second apiary, fumagillin concentrations of 20.45 ± 17.67 µg/kg and 16.59 µg/kg (only one quantifiable sample) were measured on days 24 and 29 after treatment, respectively. In the third apiary, no fumagillin residues were detected in samples collected on days 22 and 44 after treatment.

Table 19. Fumagillin concentrations in honey from three apiaries with samples from six beehives per site, values <LOQ were excluded from calculations of mean values

Apiary	Treatment duration	Day after final treatment	Mean ± SD (µg/kg)
A	4 weeks	16	110.700 ± 97.300
		31	19.010 ± 10.000
		36	5.882 ± 1.200
		42	<LOQ
		45	<LOQ
B	5 weeks	24	20.450 ± 17.670
		29	16.590 ± 0.000
C	4 weeks	22	N/D
		44	N/D

Notes: SD: Standard deviation, LOQ: Limit of quantification (5 µg/kg), N/D: not detected

*Source***:** adapted from Jeong, S.-H. 2023. Final Report: A Study on Fumagillin Residues in Honey Samples and Determining Withdrawal Period (Study No. RED22023). Republic of Korea, HBSRC.

DCH was determined in one apiary only, with an LC-MS/MS method (LOD 12 µg/kg, LOQ 20 µg/kg). The intraday accuracy ranged between 95.4 percent and 106.8 percent and precision between 1.2 percent and 3.5 percent on day 1. On day 2, accuracy ranged between 110.2 percent and 126.9 percent and precision between 1.4 percent and 10.1 percent. On day 3, accuracy ranged between 101.9 percent and 115.0 percent and precision between 0.6 percent and 5.1 percent. In terms of inter-day accuracy and precision, the average accuracy over the 3 days was between 102.5 percent and 116.2 percent, and the average precision was between 5.9 percent and 9.7 percent.

Residue levels of DCH in honey samples decreased with time after treatment (Table 20). On days 16, 31, 36 and 42 after the last treatment, DCH was detected at mean concentrations ± SD of 1 698.1 ± 1 160.5 µg/kg, 524.9 ± 261.5 µg/kg, 296.9 ± 106.2 µg/kg and 32.5 ± 3.1 µg/kg, respectively. On day 45, the residue levels of DCH were below the LOQ in all samples.

Table 20. DCH concentrations in honey from Apiary A, values <LOQ were excluded from calculations of mean values

Day after treatment	Mean ± SD
16	1 698.1 ± 1 160.5
31	524.9 ± 261.5
36	296.9 ± 106.2
42	32.5 ± 3.1
45	< LOQ

Notes: SD: Standard deviation, LOQ: Limit of quantification (20 µg/kg)

Source: Jeong, S.-H. 2023. *Final Report: A Study on Fumagillin Residues in Honey Samples and Determining Withdrawal Period (Study No. RED22023).* Republic of Korea, HBSRC.

The Committee noted that DCH concentrations in honey were at least an order of magnitude higher than those of fumagillin on each day of sampling.

The concentrations of fumagillin and DCH residues differed among the beehives, with a wide range; standard deviations were close to the mean at higher concentrations and in the order of half the mean at lower concentrations. The Committee noted that several recommendations from VICH GL 56 (VICH, 2018) were not met. For example, treatment was not performed according to GVP, only three apiaries were included instead of four, and no information was available on agro-ecological conditions or beekeeping management practices. Validation of the analytical method provided by the sponsor was insufficiently described for fumagillin, and no description of the method and no validation data were available for DCH.

Methods of analysis for residues in tissues

It is noteworthy that the prevailing focus in the literature on analytical methods primarily revolves around the determination of fumagillin, with limited attention given to the quantification of DCH.

Fumagillin

Fumagillin, due to its physicochemical properties, has been quantified in fish tissues and honey using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Additionally, older methods employing high performance liquid chromatography (HPLC) with a photodiode array detector (DAD) or ultraviolet (UV) detector have been documented in the literature for the determination of fumagillin, capitalizing on its absorption in the UV region (Fekete *et al.*, 1995; Guyonnet, Richard and Hellings, 1995). Nevertheless, contemporary analytical preferences in food analysis favour mass spectrometry detection due to its selectivity and the attainability of lower limits of quantitation. In general, the electrospray ionization source is operated in the positive mode, resulting in protonated fumagillin as the precursor ion and different mass/charge (*m/z*) transitions have been monitored, including *m/z* 459.2→177.0, 459.1→233.3 and/or 459.2→131 (Dmitrovic and Durden, 2013; Thompson, van den Heever and Pernal, 2018; van den Heever *et al.*, 2015a; Lopez *et al.*, 2008). It is important to mention that most of the analytical methods reported in the literature only focus on the determination of fumagillin, without considering the quantitation of DCH.

The chromatographic separation of fumagillin is typically conducted in the reverse phase mode using non-polar stationary phases, such as C18 or C8 (Guruceaga *et al.*, 2019). For the mobile phase, acetonitrile has been the most used organic modifier. Nevertheless, in some methods, methanol or a mixture of acetonitrile and methanol has been used (Kanda *et al.*, 2011; van den Heever *et al.*, 2015a). As additives of the mobile phase, formic acid, acetic acid, ammonium formate/formic acid and ammonium formate have all been used (Lopez *et al.*, 2008; Nozal *et al.*, 2008; Higes *et al.*, 2011; Dmitrovic and Durden, 2013; van den Heever *et al.*, 2015a). Exploiting the acidic nature of fumagillin, Guyonnet *et al.* (1995) developed an ion-pairing method using a mobile phase with a pH of 7.8, where tetrabutylammonium acts as a cation to retain fumagillin onto a C8 column. Finally, an alternative method using a normal-phase liquid chromatographic method was proposed by Fekete *et al.* (1995) for the determination of fumagillin in fish tissues using a silica gel stationary phase and a hexane-dichloromethane-dioxan-2-propanol-acetic acid as mobile phase.

For the sample preparation procedure, fumagillin has been extracted with water or acetonitrile containing formic acid from the food matrix. The Quick, Easy, Cheap, Effective, Rugged and Safe

(QuEChERS) approach has also been used. Clean-up of the extracts has been carried out employing solid phase extraction (SPE) using polymeric sorbents and weak anion exchange cartridges (Guyonnet, Richard and Hellings, 1995; Dmitrovic and Durden, 2013; Kanda *et al.*, 2011; van den Heever *et al.*, 2015a; Nozal *et al.*, 2008).

Nozal *et al.* (2008) proposed a method for the determination of residues of fumagillin in honey using liquid chromatography coupled to a diode array detector and mass spectrometer (LC-DAD-MS). The sample preparation procedures involve solid-phase extraction on polymeric cartridges (Strata X-33 µm) to isolate fumagillin from diluted honey. Chromatographic separation of fumagillin was conducted in isocratic mode on a C18 column (150 × 3 mm, 5 µm). The mobile phase consists of a mixture of 20 mM ammonium formate in water and acetonitrile (61:39, v/v) at 35 °C, with a flow rate set at 1 mL/min. As internal standard, roxithromycin was used. Average analyte recoveries, influenced by botanical origin, range from 88 percent to 96 percent. The LOQ of the LC-DAD-MS method varied between 3 and 10 µg/kg. This method has been applied to determine fumagillin residues in honey samples collected from veterinary-treated beehives infected by *Nosema ceranae* and fed with Fumidil-B at different doses. The authors verified that fumagillin is very unstable under irradiation, and it rapidly degrades into four compounds with the same molecular mass, which must be diastereoisomers of neofumagillin, formed by the cyclization of the chain. These compounds are eluted at higher retention times than fumagillin. Degradation products at lower retention times than fumagillin were also verified in the chromatograms. One suggested compound is dihydroxyfumagillin, formed by hydrolysis of the unstable epoxide.

Screening methods

In the literature, an enzyme-linked immunosorbent assay (ELISA) method is reported as a screening method for fumagillin in honey, with detection levels of at least 20 µg/kg (Assil and Sporns, 1991).

Confirmatory methods

Numerous confirmatory techniques employing LC-MS/MS are documented in the literature for the determination of fumagillin alone or in the presence of other residues of veterinary drugs (multiclass methods) in food matrices.

Honey

Lopez *et al.* (2008) described a multiclass method including the determination of fumagillin in honey using LC-MS/MS. The separation was achieved using a Phenomenex Polar RP Synergy column (50 × 2 mm, 4 µm) with a guard column of the same stationary phase. The mobile phase was a mixture of water and acetonitrile with 0.1 percent formic acid added under gradient elution. The ionization of fumagillin was conducted by operating the electrospray ionization source in positive mode. For quantitation and identity confirmation, the following transitions were monitored: *m/z* 459.1→233.3 and *m/z* 459.1→215.3.

Kanda *et al.* (2011) reported a method of the determination of residues of fumagillin in honey using the QuEChERS approach followed by LC-MS/MS quantitation. The chromatographic separation was conducted in gradient mode on a C8 column (100 × 2 mm, 5 µm) maintained at 40 °C. The mobile phase comprised a mixture of a 2 mM ammonium formate added to 0.01 percent formic acid solution and methanol, with a flow rate set at 0.2 mL/min. Fumagillin extraction was carried out using acetonitrile containing 0.1 percent formic acid and adding the QuEChERS salts (sodium chloride, trisodium citrate dihydrate, and 4 g magnesium sulfate). Clean-up was carried out using solid-phase

extraction with an Oasis® mixed-mode weak anion-exchange cartridge. The electrospray ionization source was operated in the negative ion mode. For quantitation and identity confirmation, the following transitions were monitored: fumagillin *m/z* 456.9→131 (quantitation) and *m/z* 456.9→102.8 and 456.9→175 (identity confirmation). The limit of quantitation (LOQ) was established at 0.1 µg/kg. The combination of LC-MS/MS with the QuEChERS method demonstrated good potential for the accurate determination of fumagillin residues in honey.

Van den Heever *et al.* (2015a) detailed a method for the determination of fumagillin, DCH and fumagillin degradation products in honey. Quantitative analysis was conducted using LC-MS/MS with two internal standards, namely DCH-d_{10} and roxithromycin. For quantitation and identity confirmation, the following transitions were monitored: fumagillin *m/z* 459.2→177 and 459.2→102.8, DCH *m/z* 182.0→83 and 182→100, DCH-d10 *m/z* 192.2→83 and 192.2→100 and roxithromycin *m/z* 837.4→158. The chromatographic separation was achieved using an Xterra MS-C18 column (4.6 × 100 mm, 3.5 µm) maintained at 30 ºC, with a mobile phase consisting of a mixture of water and methanol, with ammonium formate/formic acid added to both. The sample preparation procedure involved weighing 5 g of honey and adding the internal standards and water (10 mL). After agitation using a vortex and mechanical shaker for 1 hour, the mixtures underwent centrifugation, and the supernatant was subjected to clean up using a reversed polymeric phase in SPE. Following washing and drying of the sorbent, the retained analytes were eluted with acetonitrile containing 5 percent formic acid. Quantitation relied on matrix matched calibration curves within the concentration range of 10–500 ng/g. The LOQ of the method was established at 10 ng/g.

Method provided by the sponsor for the determination of fumagillin in rainbow trout, carp, and eel tissues

The Committee assessed the validation data for determination of fumagillin against the requirements for analytical methods published in the Codex Guideline CAC/GL 71-2009 (FAO and WHO, 2014).

Fumagillin was determined in trout, carp and eel fillets with a LC-MS/MS method (NIFDS, 2015a, 2015b, 2015c). In summary, the sample preparation involves the addition of 20 mL of acetonitrile with 0.1 percent formic acid to 5 g of homogenized tissue. Following agitation, the mixture undergoes centrifugation at 4 ºC and 2 600 *g* for 15 min. The resulting supernatant is collected, and the solvent removed (leaving a residue of 0.5 mL) using a rotary evaporator at 40 ºC. Subsequently, 5 mL of water is added to the residue, and the solution undergoes clean-up using a Strata-X cartridge previously conditioned with methanol and water. The cartridge is washed with 5 mL water: methanol 60:40 v/v. Fumagillin is then eluted with 10 mL of acetonitrile containing 0.1 percent formic acid. The solvent is removed using a rotary evaporator at 40 ºC and the resulting residue is resuspended in 1 mL of methanol containing 0.1 percent formic acid and 10 mM ammonium formate. The mixture is agitated, followed by centrifugation at 4 ºC, 15 000 *g* for 10 minutes. The supernatant is filtered (0.2 µm) and subjected to analysis by LC-MS/MS.

The separation of fumagillin is performed on a Phenomenex Luna C18 column (2 x 100 mm, 3 µm), at 35 ºC, utilizing a mobile phase containing aqueous 0.1 percent v/v formic acid and 10 mM ammonium formate (solvent A), and methanol containing 0.1 percent v/v formic acid and 10 mM ammonium formate (solvent B). The flow rate is set at 0.25 mL/min, employing a gradient elution as follows: from 0 to 2 min, 95:5 v/v A:B; 5 min to 5:95 v/v A:B; 10 min 5:95 v/v A:B; 15 min 95:5 v/v A:B; 20 min 95:5 v/v A:B). The mass spectrometer conditions were: electrospray source operating in the positive mode and source temperature of 350 ºC. Quantitation is performed using acquisition of ions in the selected reaction-monitoring mode, using the transition of *m/z* 459.3→131 for fumagillin. For identity

confirmation, two additional transitions for fumagillin are monitored: *m/z* 459.3→103.1 and *m/z* 459.3→177.1. Linearity is observed in the range of 5 to 500 µg/kg. The validation parameters are shown in Table 21.

Table 21. Validation parameters of the LC-MS/MS method for the determination of fumagillin in fillet of trout, carp and eel

Parameter	Trout fillet	Carp fillet	Eels fillet
Precision (CV, *n*=5)	19.2% (5 µg /kg) 16.6% (10 µg /kg)	12.4% (5 µg /kg) 9.4% (10 µg /kg)	13.9% (5 µg /kg) 12.7% (10 µg /kg)
Accuracy (*n*=5)	80.6% (5 µg /kg) 88.3% (10 µg /kg)	79.2% (5 µg /kg) 81.5% (10 µg /kg)	74.1% (5 µg /kg) 79.4% (10 µg /kg)
LOQ	5 µg/kg	5 µg/kg	5 µg/kg
Analytical range (µg/kg)	5–500	5–500	5–500
Linearity (r)	>0.99	>0.99	>0.99
Specificity/selectivity	No interference observed	No interference observed	No interference observed

Source: adapted from NIFDS (Korean National Institute of Food Drug Safety Evaluation). 2015a. *Final Report (A): A Study on Residues Depletion of Fumagillin in Rainbow Trout.* Republic of Korea, NIFDS; NIFDS. 2015b. *Final Report (B): A Study on Residues Depletion of Fumagillin in Carp.* Republic of Korea, NIFDS; NIFDS. 2015c. *Final Report (C): A Study on Residues Depletion of Fumagillin in Eel.* Republic of Korea, NIFDS.

Method provided by the sponsor for the determination of fumagillin in honey

Fumagillin in honey underwent analysis utilizing LC-MS/MS, with quantitation performed using a matrix-matched calibration curve spanning a concentration range of 5 to 250 µg/kg. However, the absence of details regarding the analytical method impedes the verification of data reliability. The accuracy and precision of the method are outlined in Table 22.

Table 22. Validation parameters of the LC-MS/MS method for the determination of fumagillin in honey

Parameter	Honey
Precision (CV, *n*=5)	13.8% (5 µg /kg) 5.1% (10 µg /kg) 4.6% (100 µg/kg) 3.6% (500 µg/kg)
Accuracy (*n*=5)	101.9% (5 µg /kg) 107.0% (10 µg /kg) 100.8% (100 µg/kg) 99.4% (500 µg/kg)
LOQ	5 µg/kg
Analytical range (µg/kg)	5–250
Linearity (r)	>0.99
Specificity/selectivity	No interference observed

Source: adapted from Jeong, S.-H. 2023. *Final Report: A Study on Fumagillin Residues in Honey Samples and Determining Withdrawal Period (Study No. RED22023).* Republic of Korea, HBSRC.

The stability of fumagillin in honey and fish tissue samples was not adequately demonstrated for normal conditions of laboratory handling or for typical storage conditions.

Information in the literature (van den Heever *et al.*, 2015a) indicated that fumagillin is not stable when exposed to UV light or at common temperatures in hives (about +34 °C).

Dicyclohexylamine

No methods were submitted by the sponsor for the analysis of DCH in fish tissues or honey. An LC-MS/MS method for analysis of DCH in honey was described in the published literature (van den Heever *et al.*, 2015a), which had an LOQ of 10 µg/kg.

In summary, honey samples are diluted with water and cleaned up by solid phase extraction. Chromatographic separation is performed on a C18 column. The electrospray ionization source is operated in the positive ion mode. Quantification is performed by acquisition of ions in the selected reaction-monitoring mode, with the transitions of *m/z* 182→83 used for quantification and *m/z* 182→100 used for identity confirmation. The linear range of the matrix-matched calibration curve, with DCH-d10 as internal standard, was 10–500 µg/kg, with a linear correlation coefficient >0.99. Precision and accuracy were evaluated at three concentrations, 10, 100, and 500 µg/kg and on three days. The inter-day precision ranged from 5.9 percent to 9.7 percent and the accuracy from 98.3 percent to 104 percent. The estimated limit of quantitation (LOQ) was 10 µg/kg.

Overall comment on validation of the analytical methods

Fumagillin

The information on the performance of the analytical methods for fumagillin in fish and honey provided by the sponsor consisted only of a summary of validation data, making it difficult to confirm whether the methods adhered to full validation parameters in accordance with Codex Guideline CAC/GL 71-2009 or VICH guidelines. The Committee considered that, while the lack of full validation reports was a source of uncertainty, the methods were suitable for monitoring purposes.

Dicyclohexylamine

The Committee considered that the method described in the publicly available literature (van den Heever *et al.*, 2015a) is suitable for monitoring DCH residues in honey.

Stability of fumagillin

Fumagillin is composed of a decatetraenedioic acid linked to a cyclohexane through an ester bond. The cyclohexane is further characterized by the presence of a methoxy group, an epoxide, and an aliphatic chain derived from a terpene, featuring an additional epoxide. The primary functional groups contributing to the instability of the molecule are the epoxides. Numerous published papers in the literature explore the stability of fumagillin, with a particular emphasis on photodegradation and thermal degradation.

Fumagillin undergoes degradation in both acidic (1 mol/L HCl) and alkaline (1 mol/L NaOH) conditions. Furthermore, when exposed to standard fluorescent light, approximately 40 percent degradation occurs within a six-hour period (Guruceaga *et al.*, 2019).

As outlined by Higes *et al.* (2011), the efficacy of Fumidil-B is influenced by various factors, encompassing storage, treatment preparation, and the quantity consumed by bees. Notably, exposure to UV radiation, such as sunlight, markedly diminishes the initial concentration of fumagillin within a few hours, while temperature exerts an influential impact on its degradation. To evaluate the stability of fumagillin, the authors conducted assessments in 50 percent sugar syrup at three different

concentrations of Fumidil-B (1, 1.5, and 2.5 g in 250 mL of sugar syrup). Stability analyses were performed for each concentration in the absence and presence of UV radiation (6 W short/longwave UV lamp) at temperatures of 4 °C, 22 °C (room temperature), 30 °C (drying oven), and 40 °C (drying oven). Results indicated that fumagillin underwent rapid decomposition under light exposure, with this degradation accelerated by higher temperatures. Specifically, after 70 days, samples exhibited 30 percent decomposition at 4 °C, 60 percent decomposition at 22 °C, and 65 percent decomposition at 30 °C. At 40 °C, under light exposure, fumagillin became undetectable after 20 days. In the absence of light (utilizing amber vials), fumagillin demonstrated greater stability at lower temperatures, with only 12 percent decomposition observed at 4 °C after 70 days. However, exposure to UV irradiation, even with amber vials, resulted in complete decomposition of fumagillin after 40 days, with UV-decomposed fumagillin products detectable for up to 60 days.

According to the sponsor, fumagillin prepared in sugar water (25 mg/L) undergoes degradation within a day when exposed to light. However, it maintains stability for up to 7 days when stored under light protection.

The stability of fumagillin and DCH in honey under simulated hive and ambient storage conditions, both in the presence and in the absence of light was investigated by van de Heever *et al.* (2015a). Honey samples (Buram honey Company, Turkey) were weighed (5 g) into either amber coloured or clear 50 mL centrifuge tubes. The samples were fortified at 500 µg/kg with fumagillin (free acid form) and with DCH, followed by shaking for 30 min on a mechanical shaker to homogenize the samples as well as possible. Simulated shelf condition stability samples (n = 93) were weighed into clear 50 mL centrifuge tubes to enable exposure of the honey to ambient fluorescent light. Amber coloured centrifuge tubes were divided into two sets consisting of 93 tubes, with one set being stored in an incubator at 34 °C in darkness, to simulate hive conditions, and the other set kept at 21 °C in the dark, simulating bulk storage conditions in drums. Fumagillin and DCH were quantified by a validated LC-MS/MS method (LOQ of 10 µg/kg). The results are shown in Figure 9.

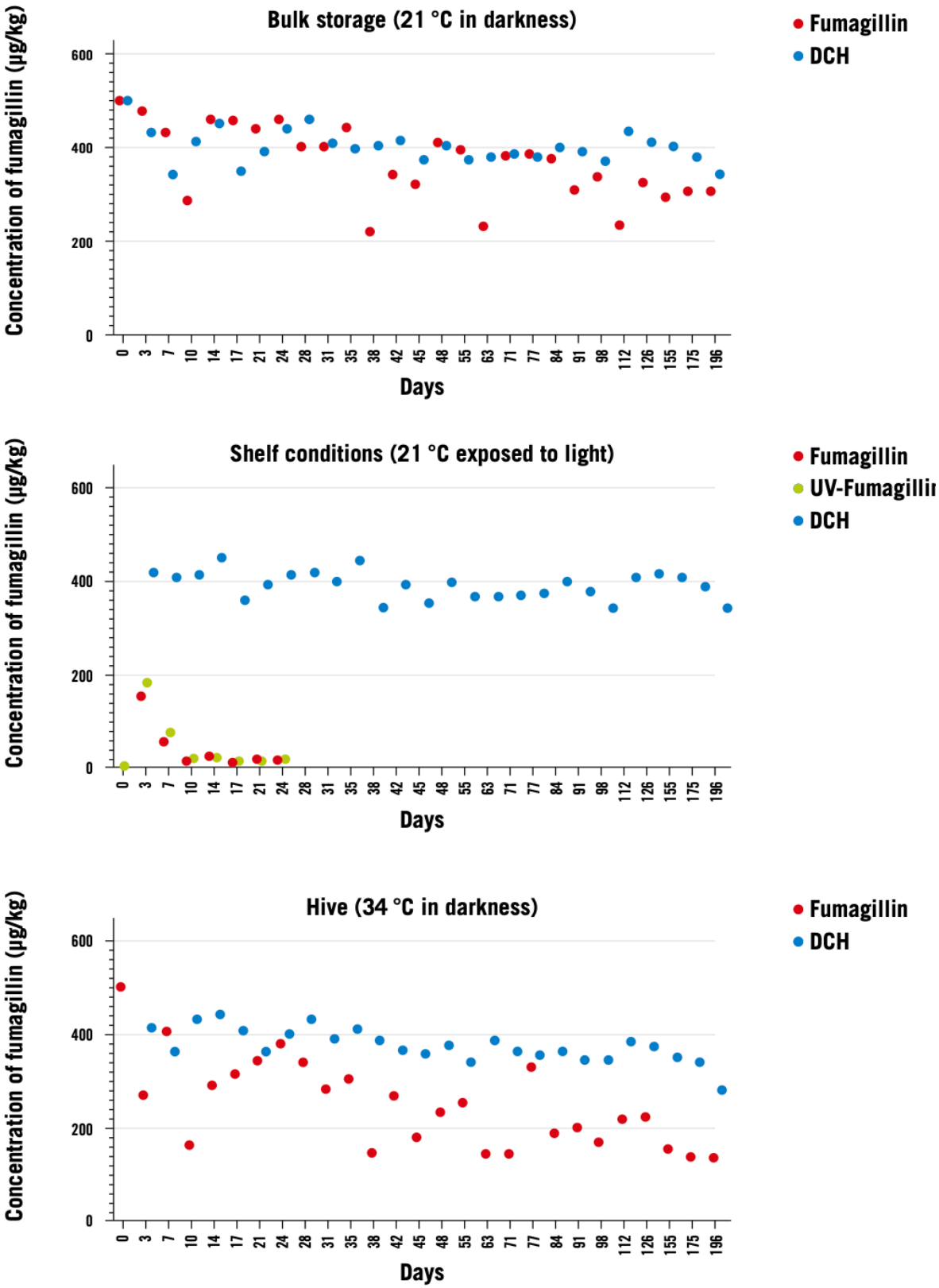
Fumagillin and its UV degradation products exhibit high instability when exposed to fluorescent light. This rapid degradation of both fumagillin and its UV decomposition products contrasts with the comparatively stable behavior observed for DCH under identical conditions. In the absence of UV-decomposed fumagillin standards, the calibration curve of fumagillin was utilized for the quantification of UV-decomposed fumagillin. Furthermore, DCH demonstrates significantly greater stability than fumagillin at hive and room temperatures, particularly in the absence of light. At room temperature upon exposure to light, fumagillin has an estimated half-life of 3 days, compared to that of 829 days for DCH. The authors indicate that it is unlikely that fumagillin or its UV degradation products will be detected in any appreciable amounts in honey destined for human consumption, when the commercial formulations are used according to GVP.

Conclusion

UV decomposition products of fumagillin retain their biological activity, whereas the thermally degraded fumagillin does not. The hydrolysed product (fumagillol) also retains some biological activity, albeit only about 10 percent of that of fumagillin.

Based on this, fumagillin is likely to represent the major residue. However, it may not represent the total activity, since degraded and hydrolysed products still have some biological activity. Exposure to light results in a decrease of fumagillin concentration, but some activity of the degraded fumagillin would remain. After exposure to light the M:T ratio would be <1.

Figure 9. Concentrations of fumagillin, UV-fumagillin and DCH under simulated storage conditions



Source: adapted from van den Heever, J. P., Thompson, T. S., Curtis, J. M. & Pernal, S. F. 2015a. Determination of Dicyclohexylamine and Fumagillin in Honey by LC-MS/MS. *Food Analytical Methods,* 8(3): 767–777.

Appraisal

Introduction

Fumagillin (IUPAC name: (2E,4E,6E,8E)-10-{[(3R,4S,5S,6R)-5-methoxy-4-[(2R)-2-methyl-3-(3-methylbut-2-enyl)oxiran-2-yl]-1-oxaspiro[2.5]octan-6-yl]oxy}-10-oxodeca-2,4,6,8-tetraenoic acid; Chemical Abstract Service No. 23110-15-8) is a mycotoxin used as an antimicrobial agent for the treatment of microsporidian infections in honeybees and in various fish species.

Fumagillin is poorly soluble in water and undergoes rapid ultraviolet and thermal degradation. Therefore, to increase its stability and water solubility, commercial formulations used in veterinary medicine contain fumagillin as the dicyclohexylamine (DCH, IUPAC name N-cyclohexylcyclohexanamine; Chemical Abstract Services No. 101-83-7) salt in a 1:1 stoichiometric ratio.

Fumagillin DCH is used as a veterinary drug in feed for fish and honeybees or via immersion bath treatment for fish. The mode of action of fumagillin is based on inhibition of type-2 methionine aminopeptidase (MetAP-2) activity via formation of a covalent bond with the histidine moiety of the enzyme. MetAP-2 is a cytosolic enzyme, which removes the initial methionine from the amino terminus of newly synthesized proteins for subsequent post-translational modifications, which affects the function of many proteins.

Fumagillin DCH has not previously been evaluated by the Committee. The Committee evaluated fumagillin DCH at the present meeting at the request of the twenty-sixth session of the CCRVDF with a view to recommending maximum residue limits (MRLs) for fish and for honey. In veterinary medicine, fumagillin is administered only as the DCH salt; however, because the fumagillin DCH salt dissociates into the two moieties and consumers would be exposed to the residues of both, the Committee evaluated both fumagillin and DCH.

The dosage used in fish is 15–50 mg of fumagillin base per kg bw in medicated feed for 30 consecutive days, or 60 mg fumagillin base per litre of water in an immersion bath for 5 consecutive days. The GVP withdrawal period for use in fish is 28 days for both treatment regimens (water temperature not specified). The inclusion rate for use in bees is 20–25 mg fumagillin base per litre of sugar syrup, administered once weekly for 6–8 weeks. Bees should be treated in the autumn after honey supers have been removed, or in spring, when the treatment should be completed at least 4 weeks before the start of the honey flow.

Fumagillin DCH is not currently registered for use as a pesticide.

Metabolism

A study of fumagillin metabolism in rainbow trout, which was reported to be GLP-compliant, was provided by the sponsor (Kim, 2023). The fish received a dose of 50 mg fumagillin per kg bw (mixture of tritium-radiolabelled fumagillin and unlabelled fumagillin DCH) by gavage. The water temperature was kept at 15 °C. The precise positions of the tritium labels in the fumagillin molecule were unknown. The extent of exchange of the tritium radiolabel with water was assessed 6 hours after dosing. Most samples exceeded the VICH-recommended acceptance criterion (VICH, 2011) of < 5 percent (range, -22.2 to +15.6), suggesting that the tritium label was unstable. Almost 50 percent of the radioactive residues could not be extracted, although various solvents were tested. No explanation was provided for the limited extractability of the radioactive residue. Only the parent compound, fumagillin, was

identified in fillet extracts. It was therefore proposed that fumagillin was not metabolized. As no radiolabelled DCH was used in this study, no data were available on the metabolism and depletion of DCH in fish.

Like other veterinary drugs used in apiculture, fumagillin DCH does not appear to be metabolized in honeybees, and most fumagillin DCH probably appears in beeswax and honey.

Degradation products

The fumagillin portion of fumagillin DCH is subject to degradation under conditions relevant for the treatment of fish and honeybees. It can be degraded by exposure to light, producing biologically active degradation products with activity similar to that of fumagillin (Kochansky and Nasr, 2004), or hydrolysed under basic conditions to produce fumagillol, which has about 10 percent of the biological activity of fumagillin (Gochnauer and Furgala, 1962). Thermal degradation of fumagillin leads to formation of dihydroxyfumagillin, a biologically inactive compound (Kochansky and Nasr, 2004).

The Committee concluded that fumagillin degradation products are unlikely to be of greater toxicological concern than the parent.

The Committee noted that no information on concentrations of fumagillin degradation products in fish tissues or honey was available.

No information on degradation of DCH was available.

Residue depletion

Radiolabelled residue depletion study

Fish

In the study of radiolabelled residue depletion (Kim, 2023), 50 one-year-old rainbow trout, bw 100–275 g) were treated with a mixture of [^{3}H]-fumagillin and unlabelled fumagillin DCH at a total dose of about 50 mg of fumagillin per kg bw. To achieve the intended dose, a solution of 50 000 mg/L was prepared from 0.02 mg [^{3}H]-fumagillin (as fumagillin base), and 499.98 mg unlabelled fumagillin (present as fumagillin DCH) were added to 10 mL of 0.5 percent CMC solution (carboxymethylcellulose sodium salt in sterilized water). The fumagillin was randomly labelled with tritium. The extent of exchange of the tritium radiolabel with water was assessed 6 hours after dosing. Numerous samples exceeded the VICH-recommended acceptance criterion of <5 percent (range, from -22.2 to +15.6), indicating that the tritium label was unstable. A dose of 50 mg/kg bw of fumagillin was administered via gavage. Fish were euthanized and fillet samples collected from each of 10 fish at 6 and 12 hours and 1, 2 and 7 days after administration. The tissues were homogenized and stored at about -20 °C until analysis.

Validated LSC and radio-HPLC methods were used to determine the concentrations of radiolabelled fumagillin. The radiochemical purity of [^{3}H]-fumagillin, determined with a radio-HPLC method, was reported to be 100 percent.

The concentration of fumagillin in fish fillet increased from 1.2 mg equiv/kg at 6 hours after dosing to 2.3 mg equiv/kg at 12 hours and decreased to 0.2 mg equiv/kg on day 7.

The radiolabelled residue depletion study indicates that the parent compound, fumagillin, is a suitable marker residue.

Non-radiolabelled residue depletion studies

Fish

Residue depletion studies with unlabelled fumagillin in rainbow trout, carp and eels were assessed (NIFDS, 2015a, 2015b, 2015c). For each species, studies were conducted at two water temperatures and two administration routes (oral and immersion bath). A nominal dose of 50 mg/kg bw for 30 consecutive days was used for oral administration, and for the immersion bath, fish were exposed to fumagillin at a concentration of 60 mg/L for 5 consecutive days. The product used in these studies was Fumagil-C, containing 50 g of fumagillin per kg (administered as fumagillin DCH). Medicated feed was prepared by mixing Fumagil-C with feed under light-protected conditions, and fish oil was uniformly sprayed onto the medicated feed to prevent release of the drug into the water. Fumagillin, quantified in the medicated feed, constituted 80–110 percent of the intended concentration.

Fumagillin was not quantified in the immersion bath. In the studies provided, the assessment was solely of depletion of fumagillin. Residues of DCH were not quantified in the sampled tissues. Details such as the exact quantity of feed administered and consumed and the weight of the fish in each tank, were not provided.

Ten fish were sampled at 1, 3, 7, 14 and 28 days after the last oral dose and 1, 3 and 7 days after exposure in an immersion bath, and fillet (muscle with skin in natural proportions) was collected. Fumagillin was quantified in the samples with a LC-MS/MS method separately validated for each species. The LOQ for fumagillin in fish fillet for all species was 5 µg/kg.

Rainbow trout *(Oncorhynchus mykiss)*: After oral administration, the highest mean ± SD fumagillin concentration in fillet (1 558 ± 916 µg/kg, *n*=10) was observed on day 1 after dosing at a water temperature of 22 ºC, and the peak individual concentration was 3258 µg/kg. By 14 days after treatment cessation at both temperatures, all fumagillin concentrations were below the LOQ of the method (5 µg/kg).

For the immersion bath treatment, fumagillin residues were quantifiable only on day 1 after dosing (mean concentration ± SD of 22 ± 28 µg/kg, *n*=9) at a water temperature of 13 ± 3 ºC. At 25 ± 3 ºC, the mean concentration ± SD of fumagillin determined on day 1 was 50 ± 34 µg/kg (*n*=10); on day 3, one of the 10 fish fillets sampled contained a concentration of 7 µg/kg.

Carp (*Cyprinus carpio)*: After oral administration, the highest mean concentration of fumagillin in fillet (2 256 ±1 732 µg/kg, *n*=10) was found on day 1 after dosing at a water temperature of 25 ºC, with an individual peak concentration of 5234 µg/kg. By 28 days after treatment cessation, all fumagillin concentrations in fillet were below the LOQ of the method (at both temperatures).

For the immersion bath treatment, quantifiable fumagillin residues in fillet persisted until day 3 after dosing at both water temperatures. All mean concentrations were <34 µg/kg; the maximum concentration was 81 µg/kg in a sample collected on day 1 (13 ºC).

Eels (*Anguilla japonica)*: After oral administration, the highest mean concentration ± SD of fumagillin in eel fillet (1 714 ± 994 µg/kg, *n*=10) was found on day 1 after dosing at a water temperature of 28 ºC, with a maximum individual concentration of 3 258 µg/kg. At 14 days and 28 days after treatment cessation, all fumagillin concentrations in fillet were below the LOQ (5 µg/kg) of the method for the lower and higher water temperatures, respectively.

In the immersion bath treatment, quantifiable fumagillin residues persisted until day 3 after dosing for both water temperatures. All the mean concentrations were <44 µg/kg; the maximum individual concentration was 107 µg/kg, in a sample collected on day 1 (28 ºC).

Summary of fish non-radiolabelled residue depletion studies

Depletion of fumagillin was observed after several withdrawal times after both treatment routes and water temperatures in all three fish species. Slightly higher residue concentrations of fumagillin in fillet were generally found in fish harvested at the higher water temperature in all studies.

The Committee noted that the residue concentrations of fumagillin in fish exposed in immersion baths were almost 100 times lower than after oral administration. The Committee also noted that the higher water temperatures used in these studies may not be optimal for all species used.

When the Committee combined the data on residue depletion in fish after oral administration of fumagillin DCH (normalized to degree-days), the depletion profiles were similar for the three species. The Committee also noted that no quantifiable fumagillin residues were found at the label withdrawal period (28 days), including in the most conservative scenario (coldest water temperature 13 °C, corresponding to 364 degree-days).

Honeybees

In one study in honeybees, reported to be GLP-compliant, six beehives each at three apiaries were treated with Fumidil-B (containing 20 g fumagillin per kg product as fumagillin DCH) at a dose of 25 g product dissolved in 20 L of sugar water (corresponding to 25 mg/L fumagillin solution), once a week for 4 or 5 consecutive weeks (Jeong, 2023). Sugar water was provided from a honeybee feeder, and all was consumed within 1 day of supply. The applied dosages were reported to be lower (75.6–98.6 percent) than the intended doses, and the treatment duration (4–5 weeks) was shorter than that according to GVP (6–8 weeks). Treatment was administered in spring, 1 week before onset of honey flow, and honey samples were taken starting 1 week after onset of honey flow (although GVP requires that the treatment should be finished 4 weeks before the start of honey flow). An LC-MS/MS method was used for determination of fumagillin concentrations in honey (LOD 2 µg/kg, LOQ 5 µg/kg). Only fumagillin itself was measured (i.e., without degradation products).

In all three apiaries, the residue concentrations of fumagillin in honey samples decreased with time after treatment. In one apiary, fumagillin was detected at concentrations (mean ± SD) of 110.7 ± 97.3 µg/kg, 19.01 ± 10 µg/kg, 5.882 ± 1.2 µg/kg, <LOQ on days 16, 31, 36, 42, and 46 after treatment, respectively. In the second apiary, fumagillin concentrations of 20.45 ± 17.67 µg/kg and 16.59 µg/kg (only one quantifiable sample) were measured on days 24 and 29 after treatment. In the third apiary, no fumagillin residues were detected on days 22 and 44 after treatment.

DCH was determined in one apiary only, with a LC-MS/MS method (LOD 12 µg/kg, LOQ 20 µg/kg). Residue levels of DCH in honey samples decreased with time after treatment. On days 16, 31, 36 and 42 after the last treatment, DCH was detected at a mean concentration ± SD of 1 698.1 ± 1 160.5 µg/kg, 524.9 ± 261.5 µg/kg, 296.9 ± 106.2 µg/kg and 32.5 ± 3.1 µg/kg, respectively. On day 45, the residue levels of DCH were below the LOQ in all samples.

The Committee noted that DCH concentrations in honey were at least an order of magnitude higher than those of fumagillin.

The concentrations of fumagillin and DCH residues differed among the beehives, with a wide range and standard deviations close to the mean at higher concentrations and in the order of half the mean at lower concentrations. The Committee noted that several recommendations from VICH GL 56 (VICH, 2018) were not met. For example, treatment was not performed according to GVP, there were too few study sites, no information was available on agro-ecological conditions or beekeeping management practices, validation of the analytical method provided by the sponsor was insufficiently described for fumagillin, and no description of the method and no validation data were available for DCH.

Analytical method

Fumagillin. The Committee assessed the validation data for determination of fumagillin against the requirements for analytical methods published in the Codex Guideline CAC/GL 71-2009 (FAO and WHO, 2014).

An LC-MS/MS method has been developed and validated for analysis of fumagillin in trout, carp and eels (NIFDS, 2015a, 2015b, 2015c) as well as in honey (Jeong, 2023). The estimated LOQ was 5 µg/kg for fish fillet and honey.

The stability of fumagillin in fish tissue and honey samples was not adequately demonstrated under normal conditions of laboratory handling or typical storage conditions.

Information in the literature (van den Heever *et al.*, 2015a) indicated that fumagillin is not stable when exposed to UV light or at common temperatures in hives (about +34°C).

The information on the performance of the analytical methods for fumagillin in fish and honey consisted only of a summary of validation data, making it difficult to confirm whether the methods adhered to full validation parameters in accordance with Codex Guideline CAC/GL 71-2009 or VICH guidelines. The Committee considered that, while the lack of full validation reports was a source of uncertainty, the methods were suitable for monitoring purposes.

Dicyclohexylamine. No methods were submitted for the analysis of DCH in fish tissues or honey. An LC-MS/MS method for analysis of DCH in honey was described in the publicly available literature (van den Heever *et al.*, 2015a), which had an LOQ of 10 µg/kg. The Committee considered that the method is suitable for monitoring DCH residues in honey.

Estimated dietary exposure

Chronic dietary exposure assessment

Fumagillin

Dietary exposure to fumagillin was estimated according to the potential occurrence of fumagillin residues in fish fillet and honey.

For fish, residue concentrations were taken from measurements in rainbow trout, carp and eels that received a nominal dose of fumagillin of 50 mg/kg bw per day. The studies reported residue concentrations in terms of fumagillin (the marker residue). Data for the three species were combined, and regression analysis was used to estimate residue concentrations at a withdrawal period of 364 degree-days (28 days at 13 °C). At this withdrawal period, the regression line concentration of fumagillin was < LOQ (5 µg/kg). For estimation of dietary exposure, a fumagillin residue concentration of LOQ/2 (2.5 µg/kg) was applied. While no metabolites of fumagillin in fish were identified, only

37–62 percent of the TRR was recovered from rainbow trout tissue by extraction. Therefore, a marker residue to total residue ratio (MR:TR) of 0.5 was used to estimate chronic dietary exposure.

Food consumption information in the FAO/WHO Chronic Individual Food Consumption – Summary Statistics database (CIFOCOss) were combined for all fish types to give total fish consumption.

In apiculture, fumagillin is not used during honey flow; therefore, residues of fumagillin should not be present in honey. As no residue data were available from studies conducted according to GVP, the actual fumagillin concentrations in honey are not known. Therefore, for estimation of dietary exposure, the concentration of fumagillin residue in honey was assumed to be at the LOQ (5 µg/kg).

Fumagillin is not metabolized in honey, although substantial degradation may occur. In honey in a hive, the concentration of fumagillin was reported to decrease by 32 percent over 28 days (van den Heever *et al.*, 2015b). Although the toxicological significance of the fumagillin degradation products has not been investigated (see toxicological and microbiological evaluation), the Committee concluded that they are unlikely to have greater toxicological activity than fumagillin. Therefore, a conservative MR:TR of 0.5 was used to estimate dietary exposure.

Food consumption information in CIFOCOss were combined for all honey types to give total honey consumption.

According to the assumptions described above, the global estimates of chronic dietary exposure (GECDE) for adults and the elderly, children and adolescents, and infants and toddlers were 0.06, 0.10 and 0.11 µg/kg bw per day, respectively, which represent 2 percent, 3 percent and 4 percent of the upper bound of the acceptable daily intake (ADI) of 3 µg/kg bw. Details of the GECDE estimates are included in Table 23.

Country-specific estimates of chronic dietary exposure were also determined. Instead of using the highest mean and the highest reliable percentile consumption from all surveys, the calculations were made with the mean and the highest reliable percentile in each national survey from available datasets (CIFOCOss). The highest GECDE for each age class for each country was determined.

In accordance with the assumptions described above, the mean (range) of 43 country-specific estimates for fumagillin dietary exposure for adults and the elderly was 0.015 (0.003–0.053) µg/kg bw per day or 0.5 percent (0.1–1.8 percent) of the upper bound of the ADI (3 µg/kg bw). The mean (range) of 32 country-specific estimates of fumagillin dietary exposure for children and adolescents was 0.027 (0.006–0.090) µg/kg bw per day, or 0.9 percent (0.2–3.0 percent) of the upper bound of the ADI. The mean (range) of 23 country-specific estimates of fumagillin dietary exposure for infants and toddlers was 0.037 (0.008–0.097) µg/kg bw per day or 1.3 percent (0.3–3.2 percent) of the upper bound of the ADI.

As no acute reference dose (ARfD) was necessary, acute dietary exposure, GEADE was not assessed for fumagillin.

Dicyclohexylamine

Information was available on residues of DCH in honey; however, the design of the study did not allow assessment of DCH residues when the veterinary drug is used in accordance with GVP. For estimation of dietary exposure, it was assumed that DCH should not be present in honey, and the LOQ (10 µg/kg) was used as an estimate of the median residue concentration after chronic dietary exposure. As DCH is

not metabolized in honey and DCH is reasonably stable in honey, an MR:TR of 1 was used to estimate chronic dietary exposure.

No information was available on the concentration of DCH residues in fish. In order to provide guidance on a potential target level for DCH in fish (C_{fish}), the maximum residue concentration consistent with the upper bound of the ADI (20 µg/kg bw) was back-calculated from the following equation:

$$GECDE = (HRP_{Fish} \times C_{Fish}) + (Mean_{Honey} \times C_{Honey})$$

where

- HRP_{Fish} is the highest reliable percentile consumption of fish (0.019 kg/kg bw);
- C_{Fish} is the maximum concentration of DCH in fish (in µg/kg);
- $mean_{Honey}$ is the population mean consumption of honey (0.0014 kg/kg bw); and
- C_{Honey} is the assigned concentration of DCH in honey (10 µg/kg).

Setting the GECDE to the upper bound of the ADI (20 µg/kg bw) results in an approximate value of C_{fish} of 1 050 µg/kg (rounded to 1 000 µg/kg). The calculation was based on food consumption by infants and toddlers, the age group with the highest food consumption per kg bw.

Table 23. Global estimate of chronic dietary exposure (GECDE) for fumagillin in fish and honey

Category	Type	Median concentration[1] (µg/kg)	Mean consumption, whole population[2] (g/kg bw per day)	HRP consumption, consumers only[3] (g/kg bw per day)	MR:TR ratio	Exposure µg/kg bw/day		GECDE[4]	
						Mean	HRP	µg/kg bw/day	%ADI
Adults and the elderly									
Fish and seafood	Fish	2.5	2.38	10.6	0.50	0.012	0.052	0.052	
Honey	Honey	5	0.28	0.95	0.50	0.003	0.0.009	0.003	
TOTAL								0.055	2
Children and adolescents									
Fish and seafood	Fish	2.5	3.14	18.0	0.50	0.016	0.090	0.090	
Honey	Honey	5	1.21	1.48	0.50	0.012	0.015	0.012	
TOTAL								0.102	3
Infants and toddlers									
Fish and seafood	Fish	2.5	2.69	19.3	0.50	0.013	0.097	0.097	
Honey	Honey	5	1.41	8.76	0.50	0.014	0.088	0.014	
TOTAL								0.111	4

Notes: MR: marker residue, TR: total residue, HRP: highest reliable percentile, GECDE: global estimates of chronic dietary exposure

[1]For fish, no residues greater than the LOQ were detected at the specified withdrawal time and a value of LOQ/2 was used as the median residue concentration. Residues of fumagillin should not be present in honey and the LOQ of the available analytical method was used as the median residue concentration, expressed as fumagillin; [2]Highest mean consumption figures based on whole population considered from the available dataset; [3]Highest reliable percentile food consumption figures based on consumers only considered from the available dataset; [4]GECDE is the sum of the highest exposure at the highest reliable percentile of consumption for a food and the mean dietary exposures of the other food

Source: Authors' own elaboration.

Acute dietary exposure assessment

The Committee concluded that it was unnecessary to establish an ARfD for fumagillin.

The Committee established an ARfD for DCH of 0.7 mg/kg bw. No information was available to derive appropriate residue concentrations of DCH in fish or honey for estimation of acute dietary exposure (GEADE). In the GEADE method, the information on food consumption is on large portion sizes (97.5th percentile consumers only food consumption from single-day food surveys). Large portion sizes are reported for two population groups; children and the general population. For large portion sizes of fish and honey for children and the general population, the maximum DCH residue concentrations that would not result in exceedance of the ARfD are 22 000 and 25 000 µg/kg in fish for children and the general population, respectively, and 130 000 µg/kg in honey for both children and the general population. The details of this approach are outlined below.

$$GEADE = LPS \times C_{Fish\ or\ Honey}$$

where:

- LPS is the large portion size (0.0313 kg/kg bw for fish consumption by children, 0.0278 kg/kg bw for fish consumption by the general population and 0.0055 kg/kg bw for honey consumption by children or the general population).
- $C_{\text{fish or honey}}$ is the maximum concentration of DCH in fish or honey (µg/kg), consistent with the ARfD.

The GEADE was set to the ARfD (700 µg/kg bw) and $C_{\text{fish or honey}}$ was calculated. In the GEADE method, each relevant food is considered individually, as it is assumed that an individual would not be a high consumer of more than one food in a 24-hour period.

Maximum residue limits

In recommending MRLs for fumagillin DCH in fish and honey, the Committee considered the following factors:

- The Committee established an ADI of 0–0.003 mg/kg bw for fumagillin. The Committee established an ADI of 0–0.02 mg/kg bw for DCH.
- The Committee concluded that it was unnecessary to establish an ARfD for fumagillin. The Committee established an ARfD of 0.7 mg/kg bw for DCH.
- Fumagillin DCH is approved in one Member State for use in fish. For application to fish, fumagillin DCH is administered in feed at a dose of 15–50 mg fumagillin base/kg bw for 30 consecutive days or in immersion baths containing fumagillin base at a concentration of 60 mg/L for 5 consecutive days. A withdrawal period of 28 days is applied for either use in fish (no water temperature specified).
- Fumagillin DCH is approved in several Member States for use in honeybees. For application to honeybees, fumagillin DCH is incorporated into a sugar solution. The inclusion rate is 20–25 mg of fumagillin base per litre, administered once weekly for 6–8 weeks. Honeybees should be treated in the autumn after honey supers have been removed or in spring, when treatment should be completed 4 weeks before start of honey flow.

- Data from a study with radiolabelled fumagillin were used to assess the depletion of fumagillin in rainbow trout at a water temperature of 15 ºC after a single oral dose. No studies of radiolabelled DCH were available.
- Fumagillin was identified as the marker residue in fish fillet and is considered suitable for monitoring residues. As no reliable MR:TR for fumagillin in fish fillet was identified, a conservative MR:TR value of 0.5 was applied in the dietary exposure assessment.
- DCH was identified as the marker residue in honey and is considered more suitable for monitoring residues than fumagillin, which is unstable in this matrix. An MR:TR of 1 was used for DCH in the dietary exposure assessment, as it is not metabolized in honey, and DCH is reasonably stable in this matrix.
- Data on residue depletion after administration of non-radiolabelled fumagillin DCH were available in rainbow trout, carp and eels. Only the concentrations of fumagillin were measured in tissues and not those of DCH.
- Data were available on the concentration in honey of non-radiolabelled fumagillin and for a subset of samples, DCH concentrations were available.
- Suitable LC-MS/MS analytical methods are available for the determination of the marker residues (fumagillin in fish and DCH in honey) and may be used for monitoring.
- No suitable analytical method is currently available for the determination of DCH in fish.

The Committee recommended an MRL in fish fillet of 10 µg/kg, which corresponds to twice the LOQ of the analytical method for the marker residue fumagillin. The Committee recommended that residues of DCH (including any potential metabolites) be monitored when fumagillin DCH preparations are used in fish to ensure that the concentration is < 1 000 µg/kg, which is a target level compatible with the upper bound of the ADI. The Committee noted that a suitable analytical method for the determination of DCH in fish fillet should be developed.

The Committee recommended an MRL in honey of 20 µg/kg, which corresponds to twice the LOQ of the analytical method for the marker residue DCH.

Data limitations and explanation of the approach taken by the Committee for recommending MRLs

Fish

Given the data challenges and sources of uncertainty in the fish residue depletion studies provided (no confirmation of the dose administered/consumed, weight of fish not stated, unconsumed feed not measured, treatment water not analysed, DCH residues not determined), the Committee acknowledged that the fumagillin residue concentrations reported in such studies may not accurately reflect fumagillin residue concentrations under GVP.

Nevertheless, the Committee noted that no quantifiable fumagillin residue concentrations (LOQ 5 µg/kg) were observed in fish fillet in any study at the sampling time corresponding to GVP (withdrawal period of 28 days, no temperature indicated). At the approved withdrawal period and the lower water temperature (13 ºC, resulting in 364 degree-days), the Committee recommended an MRL for fumagillin in fish fillet of 10 µg/kg (twice the LOQ of the analytical method). The Committee considered it unlikely that fish harvested according to GVP (28 days or 364 degree-days) would contain fumagillin residues in fillet that exceed this value.

The Committee noted that the withdrawal period according to GVP was reported as time (4 weeks) and not degree-days, and that other risk management options (such as longer withdrawal periods) could be considered if fumagillin DCH is to be used at water temperatures outside the range of those used in the studies reviewed (13–28 ºC).

No data were provided or were available in the scientific literature on residue concentrations of DCH in fish fillet after administration of fumagillin DCH. No studies were available of use of radiolabelled DCH in fish, and its metabolism remains unknown. Therefore, the Committee was unable to define a marker residue or to assess depletion of DCH residues in fish.

The MRLs recommended by the Committee for fumagillin residues in fish are protective of consumers and compatible with GVP. As fumagillin is currently used only in association with DCH in veterinary medicine, however, DCH residues may also be present when fumagillin residues are detected. The Committee used the limited information available to the current meeting to provide guidance on a potential target level for DCH in fish (see exposure section). The Committee recommended that residues of DCH (including any potential metabolites) be monitored when fumagillin DCH preparations are used in fish to ensure that the concentration is < 1 000 µg/kg, a target level compatible with the upper bound of the ADI. The Committee noted that a suitable analytical method for determination of DCH in fish fillet should be developed.

Should JECFA receive sufficient data on DCH residues resulting from fumagillin DCH use in fish, the Committee may refine its recommendations.

Honeybees

The Committee noted that the data on fumagillin DCH residues in honey were not generated according to GVP and are likely to be overestimates of the residue concentrations that might be present if GVP were followed. When fumagillin DCH is used according to GVP, residues of neither fumagillin nor DCH should be present in honey. No data on the concentrations of bioactive degradation products of fumagillin or DCH in honey were provided with the residue studies. In view of the reported instability of fumagillin residues and the relative persistence of DCH in honey, the Committee recommended that DCH be used as the marker residue for this matrix. The Committee recommended an MRL in honey for fumagillin DCH of 20 µg/kg, which corresponds to twice the LOQ of the analytical method for the marker residue (10 µg/kg).

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