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1055

Evaluation of certain veterinary drug residues in food

Ninety-eighth report of the Joint FAO/WHO Expert Committee on Food Additives

Food and Agriculture Organization of the United Nations

World Health Organization

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Contents

List of abbreviations

ADI	acceptable daily intake
ARfD	acute reference dose
$BMDL_{05}$	benchmark dose lower bound for a 5% response
bw	body weight
CAS	Chemical Abstracts Services
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CHD-FA	carbohydrate-derived fulvic acid
CIFOCOss	Chronic Individual Food Consumption database summary statistics
DCH	dicyclohexylamine
ECHA	European Chemicals Agency
equiv.	equivalent
GD	gestation day
GEADE	global estimate of acute dietary exposure
GECDE	global estimate of chronic dietary exposure
GVP	good veterinary practice
HBGV	health-based guidance value
HPLC	high-performance liquid chromatography
HRP	highest reliable percentile
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LoP	level of protection
LOQ	limit of quantification
LPS	large portion size
mADI	microbiological acceptable daily intake
mARfD	microbiological acute reference dose
MetAP-2	methionine aminopeptidase 2
MIC	minimum inhibitory concentration
MOE	margin of exposure
MOS	margin of safety
MR	marker residue
MRL	maximum residue limit
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
POD	point of departure
SD	standard deviation
tADI	toxicological acceptable daily intake
tARfD	toxicological acute reference dose
TOS	total organic solids
TR	total residue
TRR	total radioactive residue
TRS	Technical Report Series
TTC	threshold of toxicological concern
USA	United States of America
UTL	upper tolerance limit
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

1. Introduction

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) met on 20–29 February 2024. The meeting was opened on behalf of the Director-General of the Food and Agriculture Organization of the United Nations (FAO) by Dr Divine Nije, Deputy Director, Agrifood Systems and Food Safety Division, and, on behalf of the Director-General of the World Health Organization (WHO), by Mr Soren Madsen, Department of Nutrition and Food Safety (WHO).

Mr Nije noted growing recognition of the importance of sustainable agrifood systems in the face of complex challenges such as climate change and rapid urbanization. Establishing safe levels of exposure to chemicals found in food to ensure safe food for everyone was an important priority of the United Nations Agenda 2030 for Sustainable Development. The work of JECFA ensured vigilance of scientific and technological advancements, thereby providing risk assessments and recommendations that were both reliable and relevant. He reminded participants that they were attending the meeting in their individual capacities as international experts, serving and advising the two sponsoring specialized United Nations agencies, FAO and WHO. He also pointed out the confidential nature of the meeting, which was open only to those expressly invited or authorized to attend.

Mr Madsen welcomed all meeting participants, noting that the agenda was limited to only a few veterinary drugs, which reflected the brevity of the priority list of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), which contained only a few drugs for which sufficient data were available. He recalled that, at the previous meeting of JECFA (veterinary drugs), the Committee had attempted to finalize a decision tree for evaluating veterinary drugs for which the dossier submitted by the sponsor was incomplete or outdated. Those discussions would be continued at the current meeting and might be helpful in making recommendations for the veterinary drugs to be considered.

1.1 Declarations of interests

As per usual JECFA practice, the biographies of all the invited experts were published online on the FAO and WHO websites for public notice and to allow for comments. No comments on the notice were received. All the invited experts, in addition to completing the forms relating to the JECFA code of conduct and on confidentiality, were also asked to declare any potential interests relevant to the subjects of the meeting. The Secretariat reviewed the declarations of interests submitted by the experts and concluded that, while some of the experts had

declared their employment, their capacity as a technical adviser and recipients of funding for research as interests, and some had disclosed that they provided expert opinions related to veterinary drugs residues, the Secretariat concluded that their declarations should not be perceived as possible conflicts of interests, as those activities reflect the precise expertise and the nature of their work experience that the Secretariat expects from its invited experts.

1.2 Adoption of the agenda

The draft agenda (see Annex 5) was amended to exclude ethoxyquin, as no data had been submitted in response to the call for data.

2. General considerations

2.1 Matters of interest arising from previous sessions of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)

The Chairperson of CCRVDF, Ms Brandi Robinson, reported the results and activities of the CCRVDF at its twenty-sixth session (CCRVDF26), held in February 2023. She reported that the maximum residue limits (MRLs) recommended by JECFA at its ninety-fourth meeting for nicarbazin in chicken tissues and for amoxicillin in sheep, goat and swine tissues had been advanced by CCRVDF and adopted by the Codex Alimentarius Commission at its the forty-sixth session (CAC46). She said that CCRVDF had extrapolated Codex MRLs for 10 compounds to apply to "all other ruminants" and had extrapolated MRLs for two compounds to fin fish with the extrapolation approach described in Risk analysis principles applied by CCRVDF (http://www.fao.org/fao-who-codexalimentarius/publications/en/). The extrapolated MRLs were advanced by CCRVDF26 and adopted by CAC46. The CCRVDF Chair also reported updates to the priority list and to the activities of the Joint CCRVDF/CCPR Electronic Working Group, which is harmonizing standards for compounds used as both pesticides and veterinary drugs and harmonizing food descriptors for both committees (CCRVDF and CCPR).

The CCRVDF Chair noted that CCRVDF continues to experience challenges in ensuring that compounds have robust dossiers for evaluation by JECFA. While many members are interested in having MRLs, many of the compounds in use for which they require MRLs are old, and the available information may not meet current standards. The CCRVDF Chair said that she would continue to encourage nomination of compounds for which there are robust dossiers and noted that Member States are working independently and cooperatively to ensure new data that could be evaluated by JECFA were available. She further noted that CCRVDF continues to seek innovative approaches to providing standards, such as a new extrapolation approach. The CCRVDF was discussing revision of the approach to allow extrapolation to additional species and tissues and preparation of a pilot review to shorten the time between national authorization and Codex MRLs. The CCRVDF Chair expressed her gratitude to JECFA for its advice and evaluations, which she said are critical to the work of CCRVDF.

2.2 Guidance for the Safety Evaluation of Residues of Veterinary Drugs with incomplete data packages

JECFA is sometimes asked to assess the risk of veterinary drug residues of compounds for which the data package is not comprehensive or is out of date. In such cases, generating a risk assessment that is of maximum utility for CCRVDF and other risk managers may require use of alternative approaches to those usually used by JECFA in assessing risk. JECFA first proposed guidance to address such situations at its sixty-sixth meeting, in 2006, and considered a first draft at its seventieth meeting, in 2008. Since then, the draft has been substantially revised and updated, including at the ninety-fourth meeting of JECFA, in 2022. At the current meeting, the Committee discussed the updated guidance (Annex 1) and added relevant considerations for toxicological, microbiological and residue evaluation and for evaluating dietary exposure. The Committee adopted the guidance and welcomes comments from CCRVDF .

2.3 JECFA Toolbox for Veterinary Drug Residues Risk Assessment

The process used by JECFA for assessing risks resulting from veterinary drug residues in food is based on sound scientific principles and procedures. In order for stakeholders and new JECFA experts to understand this process, the FAO Agrifood Systems and Food Safety Division is developing a Toolbox for Veterinary Drug Residues Risk Assessment. The aim is to strengthen understanding of JECFA procedures by stakeholders interested in veterinary drug residues in food, such as regulatory agencies responsible for veterinary drug approval or food safety standards, the pharmaceutical industry, producers in animal agriculture and veterinary associations. It is also designed for use by potential JECFA experts to broaden the pool of experts available for the JECFA roster and to ensure greater geographical representation, particularly from regions with previously low representation in FAO and WHO expert bodies. The Toolbox is intended to increase understanding of the principles, modalities and technical requirements of the Committee in assessing the risks of veterinary drug residues in food and in recommending MRLs. The Toolbox is also designed to expand understanding of the role of FAO experts on the Committee and their interaction with WHO experts in conducting full risk assessments during JECFA meetings and of critical information requirements. Additional sources of guidance listed in the Toolbox provide more detailed information about the specific steps in the risk assessment process.

3. Comments on residues of specific veterinary drugs

The Committee evaluated the safety of residues of two veterinary drugs for the first time and completed the evaluation of one other for which studies on the effects on bacteria in the human microbiome had been submitted. Information on the safety evaluations and specifications is summarized in Annex 2.

3.1 Clopidol

Explanation

Clopidol (International Union of Pure and Applied Chemists [IUPAC] name: 3,5-dichloro-2,6-dimethyl-*1H*-pyridin-4-one; Chemical Abstracts Services (CAS) No. 2971-90-6) is a pyridone-derivative that is structurally related to quinolones. Clopidol is a coccidiostat and is approved for use in broiler chickens and replacement layers (pullets) to prevent coccidiosis caused by certain *Eimeria* species. It exerts its antiprotozoal effect by inhibiting mitochondrial respiration for energy production in the early life-cycle stages of the parasite (sporozoites and trophozoites) *(1)*. The available products are not recommended for feeding to laying hens or to pullets after 16 weeks of age. The approved rate of inclusion of clopidol in chicken feed is 80–250 mg/kg feed, fed continuously as the sole ration. The withdrawal period for approved clopidol products is 0–7 days. Clopidol is not currently registered for use as a pesticide.

Clopidol has not previously been evaluated by the Committee. The Committee evaluated clopidol at the request of the CCRVDF at its twenty-sixth Session in order to establish relevant health-based guidance values and to recommend MRLs for residues in chicken liver, kidney, muscle and skin/fat.

Toxicological and microbiological evaluation

The Committee reviewed data provided by the sponsor. Additionally, the AGRIS, CAB Abstracts, CAS, Embase, PubMed, Scopus and WOS databases of published literature were searched with the terms "clopidol" and "tox": 17 articles of potential relevance were identified. Summary information on toxicology was also available in reviews by the American Conference of Governmental Industrial Hygienists (ACGIH) *(2)* and the Health Council of the Netherlands, Committee on Updating of Occupational Exposure Limits *(3)*.

For evaluation of the impact of residues of clopidol on the human intestinal microbiome, a search of literature in the public domain was conducted in a library catalogue that contains 228 databases, including PubMed and Scopus. Information relevant to the microbiological assessment was sought by using

combinations of the terms "clopidol", "microbiome", "microbiota", "bacteria", "gut", "gastrointestinal", "intestinal" and "antimicrobial resistance". The search did not identify any reference relevant to an impact of clopidol residues on the human intestinal microbiome.

Biochemical data

The pharmacokinetics of clopidol was studied in rats and rabbits, and metabolism was studied in rabbits. No in-vitro study of the metabolism of clopidol was found in the open literature or provided by the sponsor.

In a published study, after [^{36}Cl]-clopidol was administered orally to rats, most of the radioactivity was found in plasma, kidneys, blood, liver, lungs and heart. By 24 h after dosing, 60–65% of the radioactivity was detected in urine and 35–40% in faeces. The biological half-life of the radioactive material in rat tissues was estimated to be about 10 h, with no marked accumulation in any tissues *(4)*.

In another published study, rabbits received oral doses of [^{14}C]-clopidol daily for up to 5 days. The results indicated rapid absorption and excretion of [^{14}C]-clopidol, with over 90% of the dose excreted via urine within 24 h after the final dose. No accumulation of radioactivity was seen in the tissues, and no radioactivity was detected in expired air after repeated daily doses. Analysis of urine revealed three major radioactive components. The first was identified as unchanged clopidol and the second as 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol, constituting approximately 47% and 32%, respectively, of the total urinary radioactivity. The third component, which accounted for an average of 20% of urinary radioactivity, was considered most likely to be the *O*-glucuronide conjugate of the hydroxylated metabolite *(5)*.

Toxicological data

In rats, the oral median lethal dose (LD_{50}) of clopidol was > 2000 mg/kg bw.[1]

In an unpublished study that complied with good laboratory practice (GLP), rats were given clopidol by gavage for 13 weeks at a dose of 0, 20, 60 or 180 mg/kg bw per day. The no-observed-adverse-effect level (NOAEL) was 60 mg/kg bw per day, as decreases in body weight gain were seen at 180 mg/kg bw per day.[2]

Two unpublished non-GLP-compliant studies of the long-term toxicity of clopidol were described in minimal detail *(2,6)*. Clopidol was administered in the feed to rats for 24 months at a concentration of 0, 30, 100 or 300 mg/kg feed

1 Bae JS. A single-dose oral toxicity study of clopidol in Sprague-Dawley rats. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

2 Bae JS. A 13-week repeated-dose oral toxicity DRF study of clopidol in Sprague-Dawley rats. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023

(equivalent to 0, 1.5, 5.0 and 15 mg/kg bw per day). No alterations were found in haematology, clinical chemistry, histopathology or tumour incidence. Clopidol was administered in the diet of dogs at a concentration of 0, 20, 60 or 200 mg/kg feed (equivalent to 0, 0.5, 1.5 and 5 mg/kg bw per day) for 24 months. No alterations were found in haematology, clinical chemistry or histopathology.

Three GLP-compliant studies of genotoxicity with clopidol were submitted by the sponsor. Negative results were found for clopidol in an Ames test,[3] an in-vitro chromosome aberration test[4] and a micronucleus study in rats in vivo.[5]

The Committee concluded that clopidol is unlikely to be genotoxic.

In repeat-dose studies of up to 90 days' duration in rats, clopidol caused no preneoplastic changes relevant to humans, and it was not genotoxic in a range of well-conducted studies. The limited information available indicated that it was not carcinogenic after chronic administration to rats. The Committee concluded that clopidol is unlikely to pose a carcinogenic risk at the levels present in the diet from its use as a veterinary drug.

No reliable studies on the impact of clopidol on reproductive performance were provided by the sponsor or found in the published literature. A brief summary of one study, without details, was available from ACGIH *(2)*. Rats were given clopidol in their diet at 0, 30, 100 or 300 mg/kg feed (equivalent to 0, 1.5, 5 and 15 mg/kg bw per day) for three generations, each generation producing two litters. Neither reproduction nor fertility was impaired in any generation, and the animals showed normal growth and survival.

In an unpublished GLP-compliant study of developmental toxicity,[6] rats were given clopidol by gavage at 0, 40, 100 or 250 mg/kg bw per day on days 6–20 of gestation. The lowest-observed-adverse-effect level (LOAEL) for both maternal and developmental toxicity was 40 mg/kg bw per day, the lowest dose tested, which caused a decrease in maternal food consumption and body weight gain and decreased fetal body weight.

The Committee concluded that clopidol is not teratogenic in rats.

In view of the absence of an effect on reproduction (in a study reported in limited detail), the absence of genotoxicity in well-conducted studies and the absence of any other indication of an effect on reproductive organs in repeat-dose

3 Pak BS. Bacterial reverse mutation assay with clopidol. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

4 Pak BS. In vitro chromosome aberration test in Chinese hamster lung cells with clopidol. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

5 Kim MS. Evaluation of clopidol in male Sprague-Dawley rats bone marrow micronucleus assay (oral gavage study). Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

6 Bae JS. A 13-week repeated-dose oral toxicity study of clopidol followed by a 4-week recovery study in Sprague-Dawley rats. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

studies, the Committee concluded that residues of clopidol in the diet from its use as a veterinary drug are unlikely to be a risk to reproduction or to offspring.

Microbiological data

The impact of clopidol residues on the human intestinal microbiome was evaluated in a decision-tree approach, which complies with VICH guideline GL36 (R2) of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) *(7)*, adopted by the Committee at its sixty-sixth meeting *(8)*. The approach entails answering three questions to determine whether a microbiological acceptable daily intake (mADI) is required. Determine, first, whether the drug residues and/or its metabolites are microbiologically active against representative human intestinal microbiota; secondly, whether the drug residues enter the human colon; and, thirdly, whether residues that enter the human colon remain microbiologically active. If the answer to any of these questions is "No", there is no need to calculate an mADI. If an mADI is required, two end-points of concern for human health are considered: disruption of the colonization barrier of the human intestinal microbiome and increases in the populations of resistant bacteria in the human intestinal microbiome.

In a study of minimum inhibitory concentration (MIC), submitted by the sponsor and reported to be GLP-compliant,[7] a group of bacterial strains representative of the human intestinal microbiome were tested with clopidol to determine whether its residues directly impact the microbiome. Clopidol had no measurable activity against the tested isolates (MIC > 500 µg/mL). The answer to the first question of the assessment – whether clopidol residues are microbiologically active against representative human intestinal microbiota – is "No". Therefore, neither an mADI nor, by extension, a microbiological acute reference dose (mARfD), is necessary.

Evaluation

The Committee established an acceptable daily intake (ADI) for clopidol of 0–0.04 mg/kg bw based on a LOAEL of 40 mg/kg bw per day for decreased maternal body weight gain and fetal body weight in a developmental toxicity study in rats. An uncertainty factor of 1000 was applied, which comprises 100 for interspecies and intraspecies differences and additional factors of 2 to account for the use of a marginal LOAEL and 5 for database uncertainty.

The Committee concluded that, in view of the low acute oral toxicity of clopidol and the absence of developmental toxicity or any other toxicological

7 Jeong S, Jong-hwan K, Jeong-Ran M, Chang-hun L, Min-cheol S. A study on residue depletion of clopidol in edible tissues of chickens (Report No. RED22018). Asan: Hoseo Biomedical Science Research Center, Hoseo University; 2023.

effect likely to be elicited by a single dose, it was unnecessary to establish an acute reference dose (ARfD) for clopidol.

A toxicological monograph was prepared.

Residue evaluation

A published paper and a sponsor-provided study of metabolism in chickens, reported to be GLP-compliant, were evaluated. No studies were available to assess the pharmacokinetics in chickens or to compare the metabolism of clopidol in laboratory animals or chickens. A residue depletion study in broiler chickens, reported to be GLP-compliant, and a validated analytical method for clopidol in chicken tissues were assessed.

Literature search

As part of its assessment of clopidol, the Committee performed a literature search in PubMed, Google Scholar and Science Direct, to identify any further relevant information. The inclusion and exclusion criteria used in the search are shown in Table 1.

Table 1
Inclusion and exclusion criteria for the literature search

Inclusion criteria:	Exclusion criteria:
Any article that reported clopidol concentrations in tissues of poultry species	Any article on the efficacy of clopidol against target parasites
Any article on methods for determining clopidol residues in poultry plasma or tissue	Any article on environmental contamination with clopidol
Any article on the metabolism or metabolites of clopidol in poultry species	Any article on species other than poultry
Any publication year	Articles in languages other than English
	Any article on resistance in target organisms
	Articles that addressed only residues of clopidol in eggs
	Articles that reported only levels of clopidol in feed

The Committee also sought data on unavoidable and unintentional carry-over of clopidol into feed; however, no relevant data were found.

The literature search identified a study on the pharmacokinetics of clopidol in chickens, some data on depletion of clopidol residues in chickens (although at a lower dose than the maximum approved under good veterinary practice [GVP]) and several papers on methods for analysing samples of chicken meat and offal for residues of clopidol.

Data on pharmacokinetics and metabolism

In the published paper *(9)*, chickens were continuously fed feed containing 0.0125% [^{36}Cl]-clopidol (125 mg/kg feed) for 7 consecutive days. Chickens were euthanized, and blood, muscle and liver samples were collected. Total radioactivity (TR) was measured after combustion, by liquid scintillation counting, and various analytical techniques were used to identify metabolites. Most radioactivity was present as unchanged clopidol in all tissues.

In a metabolism study submitted by the sponsor,[8] reported to be GLP-compliant, broiler chickens were given a single oral dose of 25 mg [^{3}H]-clopidol/kg bw. The extent of exchange of the tritium radiolabel with water was assessed 6 h and 1 day after dosing. Numerous samples taken at both times exceeded the VICH-recommended acceptance criterion of < 5% (–40.2% to +28.8%), suggesting that the tritium label was unstable. Broilers were euthanized at 6 h and 1, 3, 5 and 10 days after dosing. Liver, skin/fat, muscle and kidney tissues were collected and tested by radio-high-performance liquid chromatography (HPLC). Over 90% of the total radioactive residue (TRR) was extractable; unchanged clopidol accounted for > 80% of the extractable TRR at all times. A hydroxylated metabolite was identified as 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol (the same seen in the study in rabbits), which accounted for < 10% of the TRR. The level of radioactivity was not measured in urine or faeces.

No study on comparative metabolism was available. Nevertheless, the Committee noted similarities in the metabolism reported in published papers on laboratory animals and in the study in chickens, in which unchanged clopidol was identified as the major residue in tissues. In the study in chickens, a metabolite, 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol, was identified; however, this metabolite constituted < 10% of the TRR and was detected only at 6 h. While the same metabolite was seen in rabbits, it was not reported in rats, the laboratory species used in the toxicology studies to determine the ADI.

Residue data

In the unpublished study with [^{3}H]-clopidol[8], broiler chickens were given a single dose of 25 mg/kg bw by gavage. Four broilers were euthanized at 6 h and 1, 3, 5 and 10 days after dosing. Liver, skin/fat, muscle, and kidney tissues were collected. Total radioactivity was determined, after combustion, by liquid scintillation counting, and radioactive residue concentrations were measured by radio-HPLC. At 6 h after dosing, the total concentrations were highest in kidney (29.2 mg equiv/kg), followed closely by liver (28.8 mg equiv/kg), muscle (14.3 mg equiv/kg) and skin/fat (7.3 mg equiv/kg). The concentrations decreased rapidly in all tissues, to concentrations below the limit of quantification

8 Kim JH. Metabolism and residue kinetics of [3H]clopidol in broiler. Gyeongnam: Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology; 2023.

(LOQ) (kidney LOQ = 0.00882 mg equiv/kg; liver LOQ = 0.00151 mg equiv/kg; muscle LOQ = 0.00042 mg equiv/kg; skin/fat LOQ = 0.00166 mg equiv/kg) in all tissues, except muscle (0.086 mg equiv/kg), by day 5 after dosing. The clopidol residue concentrations, comprising more than 80% of the TRR, depleted most slowly from liver, followed by kidney, muscle and skin/fat. Clopidol residue concentrations determined by radio-HPLC were below the LOQ for the marker residue (MR) (kidney LOQ = 0.342 mg equiv/kg; liver and fat LOQ = 0.183 mg equiv/kg; muscle LOQ = 0.114 mg equiv/kg) in all tissues, except liver (0.357 mg equiv/kg), by day 3 post-dosing. The findings confirm that unchanged clopidol is a suitable MR. MR:TRRs could be calculated only at 6 h and 1 day after dosing in all tissues, because of the rapid elimination of clopidol. The sponsor reported MR:TRRs of 1.0 in kidney, muscle and skin/fat and 0.91 in liver 1 day after dosing. Because of uncertainty about the stability of the tritium radiolabel, the Committee considered it appropriate to evaluate dietary exposure with a range of MR:TRR values.

In a study with non-radiolabelled clopidol provided by the sponsor,[9] broiler chickens received feed containing clopidol at 125 or 250 mg/kg feed for 14 consecutive days. Groups of six animals were euthanized 1, 3, 5, 7 and 10 days after the final dose. Liver, kidney, muscle and skin/fat were collected and analyzed for clopidol with a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. Similar depletion profiles (residue concentrations and rates of depletion) for clopidol were observed in the two treatment groups. Marker residue concentrations depleted most slowly from skin/fat in both treatment groups. By days 7 and 10 after the final dose, clopidol concentrations in chickens treated with either inclusion rate were below the LOQ (50 µg/kg) in all tissues (Table 2). No explanation was provided for the similar residue concentrations observed despite the difference in inclusion rates.

Analytical methods

The Committee assessed the validation data against the requirements for analytical methods published in Codex Guideline CAC/GL 71-2009 *(10)*.

A validated LC-MS/MS method suitable for determination of clopidol was used in the study of non-radiolabelled residue depletion. The LOQ was 50 µg/kg for all tissues. Sample preparation involves extraction of clopidol from edible tissues in water/acetonitrile (2:10 v/v). After agitation, the mixture is centrifuged, and the resulting supernatant is collected and extracted by adding 10 mL *n*-hexane. After agitation, centrifugation and removal of the *n*-hexane layer, the remaining solvent is evaporated, and the resulting residue is resuspended in

9 Jeong S, Jong-hwan K, Jeong-Ran M, Chang-hun-L, Min-cheol-S. 2023. A study on residue depletion of clopidol in edible tissues of chickens. Hoseo Biomedical Science Research Center, Hoseo University, Report Number RED22018.

50% aqueous acetonitrile. The supernatant is filtered and analysed by LC-MS/MS. For detection and quantification of clopidol, selected reaction monitoring mode was used. The electrospray ionization source was used in positive ion mode. The quantification and confirmatory transitions were *m/z* 192.0 → 101.0 and *m/z* 192.0 → 87.1, respectively. Concentrations were determined on a matrix-matched calibration curve. The linear range of the matrix-matched calibration curve was 50–10 000 µg/kg, with a linear correlation coefficient > 0.99. Accuracy and precision were evaluated at four fortification levels (1000, 2500, 5000 and 10 000 µg/kg) on 3 days. The accuracy was 84.24–87.05% for muscle, 86.42–90.77% for skin/fat, 84.04–89.24% for liver and 84.38–86.13% for kidney. The precision was 1.8–2.6% for muscle, 4.1–7.9% for skin/fat, 6.4–12.6% for liver and 3.1–3.8% for kidney.

Table 2
Mean ± standard deviation (SD) clopidol residue concentrations (µg/kg) in tissues of broilers given 125 or 250 mg clopidol/kg feed

Dose (mg/kg)	Slaughter time (days)	Concentration ± SD (µg/kg)			
		Kidney	Liver	Muscle	Skin/fat
125	1	3016 ± 442.1	3420 ± 732.0	1537 ± 224.0	619.5 ± 279.3
	3	121.2 ± 68.7	160.1 ± 115.3	128.3 a	87.8 ± 15.4
	5	111.2[a]	< LOQ	< LOQ	73.2 ± 31.7
	7	< LOQ	< LOQ	< LOQ	< LOQ
	10	< LOQ	< LOQ	< LOQ	< LOQ
250	1	3293 ± 726.9	3747 ± 1118.6	1509 ± 381.3	886.1 ± 219.3
	3	196.6 ± 148.7	245.6 ± 143.4	152.3 ± 106.3	131.3 ± 49.8
	5	< LOQ	< LOQ	< LOQ	69.3 ± 16.5
	7	< LOQ	< LOQ	< LOQ	61.7 ± 10.0
	10	< LOQ	< LOQ	< LOQ	< LOQ

Six animals per time used for statistical analysis; < LOQ, below the limit of quantification (50 µg/kg)

[a] Only one sample with residue concentrations > LOQ

The Committee considered the method suitable for monitoring residues of clopidol in chicken tissues.

Chronic dietary exposure estimates

Dietary exposure to clopidol was estimated from the potential occurrence of clopidol residues in chicken tissues. Residue concentrations were derived from measurements made at 24-hour withdrawal (day 1) with an inclusion rate of 250 mg/kg feed, from the study with unlabelled residue reported above. Concentrations of residue were provided in terms of clopidol, the MR.

The study provided data on residues in both chicken liver and kidney; however, the available information on consumption of chicken kidney is based on a single individual. As chicken liver is much more commonly consumed and

contained higher residue concentrations than chicken kidney, chicken kidney was excluded from the assessment of chronic dietary exposure.

Given the uncertainty of the MR:TR, the sensitivity of dietary exposure estimates to this parameter was assessed by basing the estimates on three MR:TR values (1.0, 0.9 and 0.5) for all the tissue types assessed. The clopidol residue values used to estimate dietary exposure were derived by regression analysis of depletion for skin with fat and from the median residue determined at 24 h for chicken muscle and liver.

From incurred clopidol residues at 24-hour withdrawal in chicken muscle, liver and skin with fat and an MR:TR of 1.0, the global estimate of chronic dietary exposure (GECDE) values for adults and the elderly, children and adolescents, and infants and toddlers were 16.5, 16.8 and 14.3 µg/kg bw per day, respectively, which represent 41%, 42% and 36% of the upper bound of the ADI of 40 µg/kg bw.

From incurred clopidol residues at 24-hour withdrawal in chicken muscle, liver and skin with fat and an MR:TR of 0.9, the GECDE values for adults and the elderly, children and adolescents and infants and toddlers were 18.3, 18.6 and 15.9 µg/kg bw per day, respectively, which represent 46%, 47% and 40% of the upper bound of the ADI of 40 µg/kg bw.

From incurred clopidol residues at 24-hour withdrawal in chicken muscle, liver and skin with fat and a conservatively assigned MR:TR ratio of 0.5, the GECDE values for adults and the elderly, children and adolescents and infants and toddlers were 32.9, 33.5 and 28.6 µg/kg bw per day, respectively, which represent 82%, 84% and 71% of the upper bound of the ADI of 40 µg/kg bw.

Country-specific estimates of chronic dietary exposure were also derived. 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 for each individual national survey from available datasets (Chronic Individual Food Consumption database summary statistics [CIFOCOss]). The highest GECDE for each age class for each country was determined.

For an inclusion rate of clopidol of 250 mg/kg feed and the most conservative MR:TR ratio of 0.5, the mean (range) of 35 country-specific estimates for clopidol dietary exposure for adults and the elderly at 24-hour withdrawal was 8.5 (1–27.9) µg/kg bw per day, or 21% (2–70%) of the upper bound of the ADI. The mean (range) of 26 country-specific estimates of clopidol dietary exposure for children and adolescents at 24-hour withdrawal was 13.8 (0.6–33) µg/kg bw per day, or 35% (1–83%) of the upper bound of the ADI. The mean (range) of 18 country-specific estimates of clopidol dietary exposure for infants and toddlers at 24-hour withdrawal was 16.0 (2.7–27.9) µg/kg bw per day or 40% (7–70%) of the upper bound of the ADI.

As an ARfD was unnecessary, acute dietary exposure (global estimate of

acute dietary exposure [GEADE]) was not assessed for clopidol.

Maximum residue limits

In recommending MRLs for clopidol in chicken liver, kidney, muscle and skin/fat, the Committee considered the following factors:

- The Committee established an ADI of 0–0.04 mg/kg bw for clopidol.
- The Committee concluded that establishment of an ARfD for clopidol was unnecessary.
- Clopidol is registered for use in several Member States. The withdrawal periods range from 0–7 days for use of clopidol at inclusion rates of 80–250 mg/kg feed in broilers and replacement layers (pullets) up to 16 weeks of age.
- Clopidol is not authorized for use in laying hens.
- Clopidol is rapidly absorbed and excreted after oral administration.
- Clopidol is a suitable MR in all edible tissues of chickens.
- In the radiolabel study, numerous samples at both times exceeded the VICH-recommended acceptance criterion for exchange of tritium with water, suggesting that the tritium label was unstable. Because of this uncertainty, the Committee considered it appropriate to use a conservative MR:TR value of 0.5 in assessing dietary exposure.
- The study of non-radiolabelled residue depletion at the highest inclusion rate (250 mg/kg feed) was sufficient to determine the mean MR and 95/95 upper tolerance limit (UTL) concentrations in chicken skin/fat at 1-day withdrawal.
- Quantifiable residues in chicken kidney, liver and muscle were found at only two sampling times. Therefore, regression analysis could not be used to determine UTLs in those tissues. A 95/95 UTL was calculated at a single time point for these tissues, with the results of the 250 mg/kg feed inclusion rate at 1-day withdrawal.
- A validated LC-MS/MS method was considered suitable for routine monitoring of clopidol as the MR in chicken liver, kidney, muscle and skin/fat.

The Committee recommended MRLs of 10 400 µg/kg (liver), 8800 µg/kg (kidney), 4100 µg/kg (muscle) and 2600 µg/kg (skin/fat) in chickens.

For calculation of the UTL at a single time, the Committee followed the approach described by the US National Institute of Standards and Technology *(11)*. The general formula for any UTL is:

$$\text{UTL} = \text{mean residue concentration} + (k \times \text{standard deviation}),$$

where k is a factor chosen to ensure the specified coverage and confidence (95/95).

A residue monograph was prepared.

Summary and conclusions

Studies relevant to risk assessment – clopidol

Species/study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rat				
Short-term (13-week) study (gavage)	0 20, 60 and 180	Reduction in body weight gain, and kidney damage in the males	60	180
Developmental toxicity study (gavage)	0, 40, 100 and 250	**Maternal toxicity**: reduced body weight gain and decreased food consumption	-	40[a,b]
		Developmental toxicity: reduced fetal weight and decreased number of ossification centres		

LOAEL: Lowest-observed-adverse-effect level; NOAEL: No-observed-adverse-effect level;

[a] Lowest dose tested;

[b] Pivotal study for derivation of the ADI: Bae JS. A 13-week repeated-dose oral toxicity study of clopidol followed by a 4-week recovery study in Sprague-Dawley rats. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023

Microbiological effects

Clopidol had no measurable activity against the tested isolates from the human intestinal microbiome (MIC > 500 µg/mL). The Committee concluded that no mADI or mARfD was required.

ADI

The Committee established an ADI for clopidol of 0–0.04 mg/kg bw, based on a LOAEL of 40 mg/kg bw per day for decreased maternal body weight gain and fetal body weight in a developmental toxicity study in rats. An uncertainty factor of 1000 was applied, which comprises 100 for interspecies and intraspecies differences and additional factors of 2 to account for the use of a marginal LOAEL and 5 for database uncertainty.

ARfD

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

Residue definition

The MR for clopidol in chicken liver, kidney, muscle and skin/fat is clopidol.

Estimated dietary exposure

For clopidol included at 250 mg/kg feed at 24-hour withdrawal and the most conservative MR:TR considered of 0.5, the GECDE values for adults and the elderly, children and adolescents and infants and toddlers were 32.9, 33.5 and 28.6 µg/kg bw per day, respectively (82%, 84% and 71%, respectively, of the upper bound of the ADI of 40 µg/kg bw).

MRLs

The Committee recommended MRLs of 10 400 µg/kg (liver), 8800 µg/kg (kidney), 4100 µg/kg (muscle) and 2600 µg/kg (skin/fat) in chickens.

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3.2 Ethoxyquin

Although ethoyxquin was placed on the agenda, the sponsor did not provide any data for evaluation by the Committee, and the compound was withdrawn from consideration.

3.3 Fumagillin dicyclohexylamine [10]

Explanation

Fumagillin (IUPAC name: (2*E*,4*E*,6*E*,8*E*)-10-{[(3*R*,4*S*,5*S*,6*R*)-5-methoxy-4-[(2*R*)-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; CAS No. 23110-15-8) is a mycotoxin used as an antimicrobial agent for the treatment of microsporidial infections in honeybees and in various fish species.

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

The mode of action of fumagillin is based on inhibition of type-2 methionine aminopeptidase (MetAP-2) activity by 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 affect the function of many proteins.

Fumagillin DCH is used as a veterinary drug in feed for fish and honeybees or by immersion bath treatment for fish. The dosage used in fish is 15–50 mg of fumagillin base per kg bw in 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. Bees should be treated in the autumn after honey supers (boxes placed on a beehive for bees to store honey in) have been removed or in spring at least 4 weeks before the start of the honey flow.

Fumagillin has been used in human medicine for the treatment of certain infectious diseases and some forms of cancer *(1–4)*.

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

10 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 and because the fumagillin DCH salt dissociates into the two moieties and consumers would be exposed to the residues of both.

Fumagillin DCH has not previously been evaluated by the Committee.

The Committee evaluated fumagillin DCH at the present meeting at the request of the CCRVDF at its Twenty-sixth Session with a view to establishing relevant health-based guidance values and recommending 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.

Toxicological and microbiological evaluation

The Committee reviewed data provided by the sponsor and conducted a search of the scientific literature in the following publicly accessible databases, using the search terms (fumagillin OR dicyclohexylamine) AND "toxicity": Web of Science, PubMed and Scopus. In Google Scholar, the Committee searched for papers containing the terms: "fumagillin", "dicyclohexylamine and toxicity", "metabolism", "metabolites", "in vivo", "rats", "mice" and "human". The Committee also searched references in publications of the European Chemicals Agency (ECHA) and the International Agency for Research on Cancer. In total, 33 papers on fumagillin and 13 on DCH relevant to the assessment were identified.

For evaluation of the impact of residues on the human intestinal microbiome, a search for literature in the public domain was conducted in a library catalogue that covers 228 databases, such as PubMed and Scopus. The search was conducted with combinations of the terms "fumagillin", "DCH", "dicyclohexylamine", "microbiome", "microbiota", "bacteria", "gut", "gastrointestinal", "intestinal" and "antimicrobial resistance". No literature relevant to the impact of fumagillin residues on the human intestinal microbiome was identified.

Biochemical data

Fumagillin

No data on the metabolism or kinetics of fumagillin in mammalian species were submitted by the sponsor or found in the publicly available literature.

Dicyclohexylamine

No data on the metabolism or kinetics of DCH in mammalian species were submitted by the sponsor.

In a published study, DCH was readily absorbed after oral administration and was excreted via the urine in rabbits *(5)*.

In a published study *(6)* in rabbits and rats, absorption rate constants from the small intestine were determined to be $0.44\,h^{-1}$ and $0.33\,h^{-1}$, 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% of the administered dose in rabbits and 5.88% and 0.25% 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.

Toxicological data

Fumagillin

In a published paper *(7)*, the oral LD_{50} of fumagillin in mice was reported to be > 2000 mg/kg bw.

In an unpublished GLP-compliant study,[11] fumagillin DCH at a dose of 0, 2.4, 6 or 15 mg/kg bw per day (equivalent to fumagillin at 0, 1.73, 4.32 and 10.8 mg/kg bw per day) was administered by gavage to rats for 13 weeks. The NOAEL for fumagillin was 1.73 mg/kg bw per day, based on decreased body weight gain at 4.32 mg/kg bw per day.

No studies of chronic toxicity or carcinogenicity were submitted by the sponsor or were found in the publicly available literature.

Fumagillin DCH was not mutagenic in a GLP-compliant bacterial reverse mutation assay.[12] In another GLP-compliant assay of mammalian cell chromosome aberrations in vitro,[13] equivocal evidence was found for the clastogenicity of fumagillin DCH, but only at cytotoxic concentrations. In a further unpublished GLP-compliant assay of bone marrow micronucleus formation in rats with fumagillin DCH,[14] no evidence of genotoxicity was observed.

In published papers, some evidence was reported that fumagillin DCH induced chromosomal aberrations and micronuclei in mice *(8–10)* and chromosomal aberrations, micronuclei and sister-chromatid exchanges in vitro *(11)*. These studies were considered to be unreliable because of inadequate reporting and design.

11 Bae JS. A 13-week repeated dose oral toxicity study of fumagillin dicyclohexylamine salt in Sprague-Dawley rats (study number: 23-RR-0223). Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

12 Pak BS. Bacterial reverse mutation assay with Fumagillin dicyclohexylamine salt. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

13 Kim MS. In vitro chromosome aberration test in Chinese hamster lung cells with fumagillin dicyclohexylamine salt. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

14 Kim MS. Evaluation of fumagillin dicyclohexylamine salt in female Sprague-Dawley rats bone marrow micronucleus assay. Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

The Committee concluded that fumagillin is unlikely to be genotoxic.

Fumagillin produced no preneoplastic changes in repeat-dose studies of up to 90 days' duration in rats, and it was not genotoxic. The Committee therefore concluded that fumagillin is unlikely to pose a carcinogenic risk at the levels present in the diet from its use as a veterinary drug.

No studies of reproductive toxicity were submitted by the sponsor or were found in the publicly available literature.

In an unpublished GLP-compliant study,[15] fumagillin DCH was administered to pregnant rats during organogenesis (gestation days [GD] 5–20) at a dose of 0, 2.4, 6 or 15 mg/kg bw per day by gavage (equivalent to fumagillin at 0, 1.73, 4.32 and 10.8 mg/kg bw per day, respectively). The NOAEL for maternal toxicity was 4.32 mg/kg bw per day based on decreases in absolute and relative weights of the ovaries and increases in the absolute and relative weight of both kidneys and the liver, without histopathological changes, at 10.8 mg/kg bw per day. The NOAEL for embryo-fetal toxicity was 1.73 mg/kg bw per day based on decreases in fetal body weight and associated morphological changes at 4.32 mg/kg bw per day.

The Committee concluded that fumagillin is not teratogenic in rats.

In the absence of genotoxicity and the absence of any indication of a relevant effect on reproductive organs in repeat-dose studies, the Committee concluded that residues of fumagillin in the diet from its use as a veterinary drug are unlikely to cause effects on reproduction or on the offspring.

Dicyclohexylamine

The oral LD_{50} of DCH in rats was reported to be 200–373 mg/kg bw *(12)*.

In an unpublished GLP-compliant study,[16] DCH was administered by gavage to rats for 28 days at a dose of 0, 20, 70 or 200 mg/kg bw per day. Clinical signs and mortality were observed after 4 days of administration at a dose of 200 mg/kg bw per day. The NOAEL was 20 mg/kg bw per day based on clinical signs and a significant decrease in ovarian weight, without histopathological changes, at 70 mg/kg bw per day.

In a GLP-compliant study reported by ECHA *(13)*, rats were given DCH at 0, 10, 30 or 90 mg/kg bw per day by gavage for 90 days. The NOAEL was 10 mg/kg bw per day based on haematological and clinical chemistry changes at 30 mg/kg bw per day.

DCH was tested in several Ames bacterial mutagenicity assays, with

15 Bae JS. An embryo–fetal development study of fumagillin dicyclohexylamine salt by oral administration in Sprague-Dawley rats (Study No: 22-RP-0209). Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023.

16 Ohara N. 28 days repeated-dose oral toxicity of dicyclohexylamine in rats. Kanagawa: Hatano Research Institute, Food and Drug Safety Center; 1998.

negative results *(12,14–17)*.

Equivocal results were found in a study of chromosomal aberrations in Chinese hamster lung cells due to significant cytotoxicity *(16)*.

A GLP-compliant study of bone marrow micronucleus formation in mice gave negative results (Krsmanovic et al., 1970, cited in *13*).

The Committee concluded that DCH is unlikely to be genotoxic.

As DCH caused no preneoplastic changes in repeat-dose studies of up to 90 days' duration in rats and was not genotoxic, the Committee concluded that residues of DCH resulting from use of fumagillin DCH as a veterinary drug are unlikely to pose a carcinogenic risk to humans exposed through the diet.

Only a preliminary screening test of reproductive toxicity was available. Rats were given DCH at a dose of 0, 20, 40 or 80 mg/kg bw per day by gavage *(18)*. The NOAEL for parental toxicity was 40 mg/kg bw per day, based on female mortality, reduced body weight gain and food intake and increased testicular weight in the absence of any histopathological changes at 80 mg/kg bw per day. The NOAEL for reproductive toxicity was 40 mg/kg bw per day, based on increased numbers of stillborn pups and decreased numbers of liveborn pups at 80 mg/kg bw per day. The NOAEL for offspring toxicity was 40 mg/kg bw per day, based on reductions in pup weights at 80 mg/kg bw per day, a dose that was maternally toxic.

In a GLP-compliant study of developmental toxicity reported by ECHA *(19)*, rats were given DCH at a dose of 0, 40, 80 or160 mg/kg bw per day by gavage during GD 5–19. The LOAEL for dams was 40 mg/kg bw per day, the lowest dose tested, based on marginal clinical effects. The NOAEL for embryo-fetal toxicity was 160 mg/kg bw per day, the highest dose tested.

The Committee concluded that DCH is not teratogenic in rats.

Observations in humans

Fumagillin was reported to cause reversible haematological changes in patients treated at 60 mg per day *(1,2,4)*. No information was available on the effects of DCH in humans.

Microbiological data

The impact of fumagillin DCH residues on the human intestinal microbiome was evaluated in a decision-tree approach adopted by the Committee at its sixty-sixth meeting *(19)*, which complies with VICH guideline GL36 (R2) *(20)*. It entails answering three questions to determine whether establishment of a microbiological acceptable daily intake (mADI) is necessary. Determine, first, whether the drug residues and/or its metabolites are microbiologically active against representative human intestinal microbiota; secondly, whether the

drug residues enter the human colon; and, thirdly, whether residues that enter the human colon remain microbiologically active. If the answer to any of these questions is "No", there is no need to calculate an mADI, and the assessment need not be completed. If an mADI is to be calculated, two end-points of concern for human health are considered: disruption of the colonization barrier of the human intestinal microbiome and increases in populations of resistant bacteria in the human intestinal microbiome.

In two unpublished studies of the MIC, reported to be GLP-compliant,[17,18] a group of representative bacterial strains of the human intestinal microbiome were tested against fumagillin DCH to determine whether its residues directly impact the human intestinal microbiome. In both studies, fumagillin DCH exerted no measurable activity against the tested isolates (MIC > 500 µg/mL). The Committee concluded that fumagillin DCH did not exert measurable antibacterial activity against the representative bacterial strains of the human intestinal microbiome tested in these studies. Thus, neither a microbiological ADI nor, by extension, a mARfD is necessary.

Metabolites or degradation products of fumagillin

Degradation products of fumagillin were identified in honey *(21)*. Based on structural considerations (Fig. 1), the Committee concluded that these degradation products are unlikely to be of greater toxicological concern than fumagillin.

Evaluation of fumagillin

The Committee established an ADI for fumagillin of 0–0.003 mg/kg bw, which was based on a NOAEL of 1.73 mg/kg bw per day for decreases in body weight gain in a 13-week study in rats and for decreases in fetal body weight and associated morphological changes in a developmental toxicity study in rats at 4.32 mg/kg bw per day. A safety factor of 500 was used, which comprised 100 for interspecies and intraspecies differences and an additional factor of 5 for database uncertainty.

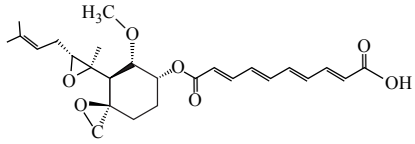
The Committee noted that the ADI provides a margin of exposure (MOE) of 333 for reversible clinical signs in humans treated with fumagillin for a number of infectious diseases.

17 Jeong SH. Final report: Risk assessment of fumagillin based on microbiological impact on human intestinal normal microflora. Asan: Hoseo Biomedical Science Research Center, Hoseo University; 2022.

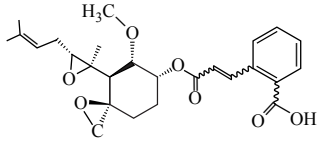
18 Jeong SH. Final Report (Revision): Microbiological impact of fumagillin on human normal gut microflora. Asan: Hoseo Biomedical Science Research Center, Hoseo University; 2024.

The Committee concluded that, in view of the low acute oral toxicity of fumagillin and the absence of developmental toxicity and of any other toxicological effects likely to be elicited by a single dose, it was unnecessary to establish an ARfD.

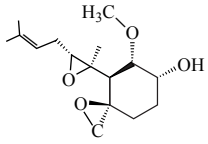
Figure 1.
Fumagillin and its degradation products identified in honey



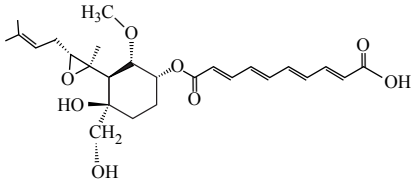
Fumagillin



Ultraviolet degradation product



Hydrolysis product (fumagillol)



Thermal degradation product
(dihydroxyfumagillin)

Evaluation of dicyclohexylamine

The Committee established an ADI for DCH of 0–0.02 mg/kg bw, based on a NOAEL of 10 mg/kg bw per day for haematological and clinical chemistry changes at 30 mg/kg bw per day in a 13-week toxicity study in rats. A safety factor of 500 was used, which comprised 100 for interspecies and intraspecies differences and an additional factor of 5 for database uncertainty.

The Committee established an ARfD for DCH of 0.7 mg/kg bw based on the NOAEL of 70 mg/kg bw per day for clinical signs and mortality after 4 days at 200 mg/kg bw per day in a 28-day toxicity study. A safety factor of 100 was applied to allow for interspecies and intraspecies differences.

A toxicological monograph was prepared.

Residue evaluation

The sponsor provided data on residues from proprietary studies. 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 (see 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	

Fumagillin

The Committee reviewed one published study on the pharmacokinetics and metabolism of fumagillin in rainbow trout. The Committee also reviewed a study on the pharmacokinetics of radiolabelled fumagillin in rainbow trout and several studies of non-radiolabelled fumagillin DCH residue depletion in rainbow trout, carp, eels and honey, which were submitted by the sponsor. Analytical methods submitted by the sponsor and available in the public literature to support residue monitoring were assessed.

Dicyclohexylamine

The Committee reviewed results on DCH residues in honey after feeding of sugar water containing fumagillin DCH to honeybees in one sponsor-provided study.[19]

No information on concentrations of DCH in fish was available.

Analytical methods available in the public literature to support DCH residue monitoring were also assessed.

19 Jeong SH. Final report: A study on fumagillin residue in honey samples and determining withdrawal period. Hoseo Biomedical Science Research Center, Hoseo University; 2023.

Data on pharmacokinetics and metabolism

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

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 rabbits and rats (see section Biochemical data on p 18 above).

Fumagillin

In a published study *(31)*, the plasma kinetics of fumagillin was evaluated in rainbow trout (both sexes; body weight: 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 two doses (3 or 6 mg/kg bw). Two additional dose groups were used (30 and 60 mg/kg bw), but no pharmacokinetics results were reported due to the deaths of the test animals. Plasma clearance of fumagillin at both dosage regimens could be fit by 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 to calculate kinetics parameters. The calculated volume of distribution was high (231 ± 64 mL/kg). DCH concentrations were not measured.

An unpublished study of fumagillin metabolism in rainbow trout, which was reported to be GLP-compliant, was provided by the sponsor[20]. The test substance was a mixture of tritium-radiolabelled fumagillin and unlabelled fumagillin DCH. The fish received a dose of 50 mg fumagillin per kg bw by gavage. The water temperature was kept at 15 °C. As the sponsor reported that fumagillin was randomly labelled with tritium, 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 h after dosing. Most samples exceeded the VICH-recommended acceptance criterion *(23)* of < 5% (range, –22.2 to +15.6), suggesting that the tritium label was unstable. Almost 50% 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 was 0.2–2.3 mg equiv/kg (0.2%–2.1% of the total administered dose), whereas the concentrations of unextractable residues were 0.4–1.4 mg equiv/kg (0.3%–1.7% of the total administered dose) throughout

20 Kim JH. Final report: metabolism and residue kinetics of [3H]-fumagillin in rainbow trout. Dejeon: Korea Institute of Toxicology; 2023.

the test. Only the parent compound, fumagillin, was identified in fillet extracts. It was concluded 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 some other veterinary drugs used in apiculture, fumagillin DCH does not appear to be metabolized in honeybees, and most fumagillin DCH is probably transferred intact to beeswax and honey.

Degradation products

Fumagillin

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 *(24)* or hydrolyzed under basic conditions to produce fumagillol, which has about 10% of the biological activity of fumagillin *(25)*. Thermal degradation of fumagillin leads to formation of dihydroxyfumagillin, a biologically inactive compound *(24)*.

The Committee concluded that fumagillin degradation products are unlikely to be of greater toxicological concern than the parent compound (see Metabolites or degradation products of fumagillin on p 22 above).

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

Dicyclohexylamine

No information was available on degradation of DCH.

Residue depletion data

One study of radiolabelled residue depletion 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 and immersion bath).

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

Radiolabelled residue depletion study

Fish

In the study of radiolabelled residue depletion (see footnote [14] on p 19), 50 one-year-old rainbow trout (body weight 100–275 g) were treated with a mixture

of [^{3}H]fumagillin and unlabelled fumagillin DCH at a total dose of about 50 mg fumagillin/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% CMC solution (sodium salt in sterile water). The fumagillin was randomly labelled with tritium. The extent of exchange of the tritium radiolabel with water was assessed 6 h after dosing. Numerous samples exceeded the VICH-recommended acceptance criterion of < 5% (range, –22.2 to +15.6), indicating that the tritium label was unstable. A dose of 50.0 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 h 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%.

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

This radiolabelled residue depletion study indicates that the parent compound, fumagillin, is a suitable MR.

Nonradiolabelled residue depletion studies

Fish

Residue depletion studies with unlabelled fumagillin in rainbow trout, carp and eels were assessed [21]. 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 administered orally, and, in 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). The 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 constituted 80–110% of the intended concentration in the feed.

Fumagillin was not quantified in the immersion bath. The studies provided assessed only depletion of fumagillin. Residues of DCH were also 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.

21 Final report. A study on residue depletion of fumagillin in rainbow trout; Final report. A study on residue depletion of fumagillin in carp; Final report. A study on residue depletion of fumagillin in eel. Chungcheongbuk-do: National Institute of Food and Drug Safety Evaluation; 2015.

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 an LC-MS/MS method separately validated for each species. The LOQ for fumagillin in fillet from all species was 5 µg/kg.

Rainbow trout (Oncorhynchus mykiss)

Four studies were provided[15]. In two studies, rainbow trout (average weight, 720 ± 90 g) received Fumagil-C in the feed for 30 consecutive days at two water temperatures (15 ± 3°C and 22 ± 3°C). In two additional studies, fish were exposed to Fumagil-C in an immersion bath at two water temperatures (13 ± 3°C and 25 ± 3°C).

After oral administration, the highest mean fumagillin concentration in fillet (1558 µg/kg; $n = 10$) was observed on day 1 after dosing at a water temperature of 22°C, 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).

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

Carp (Cyprinus carpio)

Four studies were provided[15]. In two studies, carp (mean ± SD weight, 750 ± 50 g) received Fumagil-C in the feed for 30 consecutive days at two water temperatures (13 ± 3°C and 25 ± 3°C). In two further studies, fish were exposed to Fumagil-C in immersion baths at water temperatures of 13 ± 3°C and 25 ± 3°C.

After oral administration, the highest mean concentration of fumagillin in fillet (2256 ± 1732 µg/kg, n=10) was found on day 1 after dosing at a water temperature of 25°C, 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).

In 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°C).

15 Bae JS. An embryo–fetal development study of fumagillin dicyclohexylamine salt by oral administration in Sprague-Dawley rats (Study No: 22-RP-0209). Gyeonggi-do: Nonclinical Research Institute, CorestemChemon Inc; 2023

Eels (Anguilla japonica)

Four studies were assessed (see footnote [15] on p 28). In two studies, eels (mean ± SD weight, 240 ± 55 g) received Fumagil-C in the feed for 30 consecutive days at two water temperatures (20 ± 3ºC and 28 ± 3 ºC). In two further studies, eels were exposed to Fumagil-C in immersion baths at the same two water temperatures.

After oral administration, the highest mean concentration ± SD of fumagillin in eel fillet (1714 ± 994 µg/kg; $n = 10$) was found on day 1 after dosing at a water temperature of 28ºC, with a maximum individual concentration of 3258 µg/kg. At 14 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 at 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 (28ºC).

Summary of fish studies

Depletion of fumagillin was observed after several withdrawal times after treatment by both routes and at both 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 [22]. Sugar water was provided from a honeybee feeder, and all was consumed within 1 day of supply. The applied dosages were reported

22 Jeong SH. Final report: A study on fumagillin residue in honey samples and determining withdrawal period. Hoseo Biomedical Science Research Center, Hoseo University; 2023.

to be lower (75.6–98.6%) than the intended doses, and the treatment duration (4–5 weeks) was shorter than that required by GVP (6–8 weeks). Treatment was administered in spring, 1 week before onset of honey flow, and honey samples were taken from 1 week after onset of honey flow (although GVP requires that the treatment be finished 4 weeks before the start of honey flow). Honey from each beehive was collected before treatment and at various times after the last treatment. In one apiary, four samples each were taken on days 16, 31, 36, 42 and 45; in the second apiary, only two samples per beehive were taken on days 24 and 29 after the last treatment; and, in the third apiary, two samples were taken on days 22 and 44 after the last treatment. No samples were taken from beeswax. An LC-MS/MS method was used for determination of fumagillin concentrations in honey (LOD 2.0 µg/kg; LOQ 5.0 µg/kg). Only fumagillin itself was measured (i.e. not 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 ± 10.0 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. In the third apiary, no fumagillin residues were detected on days 22 and 44 after treatment.

DCH was determined in one apiary only, with an 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 1698.1 ± 1160.5 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 < LOQ in all samples.

The Committee noted that DCH concentrations in honey were at least one 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 GL56 *(26)* 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 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 methods

Fumagillin

The Committee assessed the validation data for determination of fumagillin against the requirements for analytical methods published in Codex Guideline CAC/GL 71-2009.

Fumagillin was determined in trout, carp and eel fillet with an LC-MS/MS method [23]. The sample preparation involved adding acetonitrile with 0.1% formic acid to 5 g of homogenized fish fillet. After agitation, the mixture was centrifuged, and the resulting supernatant was collected and the solvent removed. Subsequently, water was added to the residue, and the solution was cleaned-up by solid phase extraction. The electrospray ionization source was operated in the positive mode. Quantification was performed by acquisition of ions in the selected reaction-monitoring mode, with the transition of m/z 459.3 → 131.0 for quantification of fumagillin. For identity confirmation, two additional transitions were monitored: m/z 459.3 → 103.1 and m/z 459.3 → 177.1. The linear range of the matrix-matched calibration curve was 5–500 µg/kg, with a linear correlation coefficient > 0.99. Precision and accuracy were evaluated at 5 and 10 µg/kg. The accuracy was 74.1–88.3%, and the precision was 9.4–19.2%. The estimated LOQ was 5 µg/kg.

Fumagillin in honey was determined with LC-MS/MS. A matrix-matched calibration curve spanning a concentration range of 5–250 µg/kg was used for quantification. No details of the analytical method were provided. The accuracy, evaluated on 3 days and at four fortification levels (5.0, 10.0, 100.0 and 500 µg/kg), was 85.8–115.5%, while the precision varied from 0.4% to 8.1%. For inter-day accuracy and precision, the average accuracy across the 3 days was 99.4–107.0%, with an average precision of 3.6–13.8%.

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 *(27)* indicates that fumagillin is not stable when exposed to ultraviolet 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

23 Final report. A study on residue depletion of fumagillin in rainbow trout; Final report. A study on residue depletion of fumagillin in carp; Final report. A study on residue depletion of fumagillin in eel. Unpublished reports from the National Institute of Food and Drug Safety Evaluation, Chungcheongbuk-do, Republic of Korea.

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 *(27)*, for which the LOQ was 10 µg/kg.

In summary, honey samples were diluted in water and cleaned up by solid phase extraction. Chromatographic separation was performed on a C18 column. The electrospray ionization source was operated in the positive ion mode. Quantification was performed by acquisition of ions in the selected reaction-monitoring mode, with the transitions of *m/z* 182.0 → 83.0 for quantification and *m/z* 182.0 → 100.0 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 3 days. The inter-day accuracy ranged from 98.3% to 104.0% and precision ranged from 5.9% to 9.7%. The estimated LOQ was 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 as 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. Residue concentrations were reported in terms of fumagillin (the MR). 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 time, 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% of the TRR was recovered from rainbow trout tissue by extraction. Therefore, an MR:TR of 0.5 was used to estimate chronic dietary exposure.

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% over 28 days *(27)*. 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.

According to the assumptions described above, the global estimates of 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%, 3% and 4% of the upper bound of the acceptable daily intake (ADI) of 3 µg/kg bw.

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% (0.1–1.8%) 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% (0.2–3.0%) 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% (0.3–3.2%) of the upper bound of the ADI.

As no 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. 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 (0.018 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);
- 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 1030 µg/kg (rounded to 1000 µg/kg). The calculation was based on food consumption by infants and toddlers, the age group with the highest food consumption per kg bw.

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 (LPS) (97.5th percentile consumers only, food consumption from single-day food surveys). LPS were available for two population groups: children and the general population. For LPS 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 as follows:

$$GECDE = LPS \times C_{fish\ or\ honey}$$

where

- the LPS is:
  - 0.0313 kg/kg bw for fish consumption by children,
  - 0.0278 kg/kg bw for fish consumption by the general population,
  - 0.0055 kg/kg bw for honey consumption by children

or the general population;

and

- C_{fish} or C_{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_{fish} or C_{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 within a 24 h 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 and 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°C after a single oral dose. No studies of radiolabelled DCH were available.
- Fumagillin was identified as the MR 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 MR in honey and is considered more suitable for monitoring residues than fumagillin, which is unstable in this matrix. An MR:TR of 1.0 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. DCH concentrations were available for a subset of samples.
- Suitable LC-MS/MS analytical methods are available for the determination of the MRs (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 MR, 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 < 1000 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 MR, DCH.

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

Fish

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

Nevertheless, the Committee noted that no quantifiable fumagillin residue (LOQ, 5 µg/kg) was 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°C, 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 in time (4 weeks) and not in 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°C).

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 MR 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 < 1000 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 revise 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 for 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 MR 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 MR (10 µg/kg).

A residue monograph was prepared.

Summary and conclusions

Studies relevant to risk assessment – fumagillin

Species/study type (route of administration)	Doses (mg/kg bwper day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rat				
13-week (gavage) study of toxicity (GLP)	Fumagillin: 0, 1.73, 4.32 and 10.8 (Fumagillin DCH: 0, 2.4, 6 and 15)	Decrease in body weight gain	1.73 [a]	4.32
Developmental toxicity (gavage) study (GLP)	Fumagillin: 0, 1.73, 4.32 and 10.8 on GD 5–20 (Fumagillin DCH: 0, 2.4, 6 and 15)	Maternal toxicity: significant decreases in absolute and relative weights of the ovaries and increases in absolute and relative weight of both kidneys and of liver	4.32	10.8
		Embryo-fetal toxicity: decreases in fetal body weight and associated morphological changes	1.73 [a]	4.32

LOAEL: Lowest-observed-adverse-effect level; NOAEL: No-observed-adverse-effect level

* Pivotal studies: Bae JS. A 13-week repeated dose oral toxicity study of fumagillin dicyclohexylamine salt in Sprague-Dawley rats (study number: 23-RR-0223); Bae JS. An embryo-fetal development study of fumagillin dicyclohexylamine salt by oral administration in Sprague-Dawley Rats (study number: 22-RP-0209). Nonclinical Research Institute, CorestemChemon Inc.; 2023.

Studies relevant to risk assessment – dicyclohexylamine

Species/study type (route of administration)	Doses (mg/kg bwper day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rat				

Species/study type (route of administration)	Doses (mg/kg bwper day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
28-day (gavage) study of toxicity	0, 20, 70 and 200	Clinical signs (salivation and convulsions) and decreased ovarian weight	20[a]	70
13-week (gavage) study of toxicity (GLP)	0, 10, 30 and 90	Haematological and clinical chemistry changes	10[b]	30
Preliminary reproductive toxicity (gavage) study (GLP)	0, 20, 40 and 80	Parental toxicity: female mortality, reduced body weight gain and food intake and increased testicular weight	40	80
		Reproductive toxicity: increased number of stillborn pups and decreased number of liveborn pups	40	80
		Offspring toxicity: Reduced pup weights	40	80
Developmental toxicity (gavage) test (GLP)	0, 40, 80 and 160 on GD 5–ca GD 19	Maternal toxicity: marginal clinical effects	-	40[c]
		Embryo-fetal toxicity: none	160[d]	-

LOAEL: Lowest-observed-adverse-effect level; NOAEL: No-observed-adverse-effect level;
[a] The NOAEL for acute effects (clinical signs and mortality after 4 days) was 70 mg/kg bw per day (basis of ARfD);
[b] Pivotal study value: *(13);* [c] Lowest dose tested*;* [d] Highest dose tested

Microbiological effects

Fumagillin had no measurable activity against the tested isolates (MIC > 500 µg/mL). The Committee concluded that neither a mADI nor a mARfD was required.

ADI for fumagillin

The Committee established an ADI for fumagillin of 0–0.003 mg/kg bw based on a NOAEL of 1.73 mg/kg bw per day for decreased body weight gain in a 13-week study in rats and for decreased fetal body weight and associated morphological changes in a developmental toxicity study in rats at 4.32 mg/kg bw per day. A safety factor of 500 was used, which comprised 100 for interspecies and intraspecies differences and an additional factor of 5 for database uncertainty.

ADI for dicyclohexylamine

The Committee established an ADI for DCH of 0–0.02 mg/kg bw based on a NOAEL of 10 mg/kg bw per day for haematological and clinical chemistry changes at 30 mg/kg bw per day in a 13-week toxicity study in rats. A safety factor of 500 was used, which comprised 100 for interspecies and intraspecies differences and an additional factor of 5 for database uncertainty.

ARfD for fumagillin

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

ARfD for dicyclohexylamine

The Committee established an ARfD for DCH of 0.7 mg/kg bw based on the NOAEL of 70 mg/kg bw per day for clinical signs and mortality after 4 days at 200 mg/kg bw per day in a 28-day toxicity study in rats. A safety factor of 100 was used to allow for interspecies and intraspecies differences.

Residue definition

The marker residue for fumagillin DCH in fish fillet is fumagillin.
The marker residue for fumagillin DCH in honey is DCH.

Estimated dietary exposure

Based on potential fumagillin residues in fish fillet and honey, the GECDE values 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%, 3% and 4% of the upper bound of the acceptable daily intake (ADI) of 3 µg/kg bw.

There was insufficient information to estimate dietary exposure (chronic or acute) to DCH.

MRLs

The Committee recommended an MRL in fish fillet of 10 µg/kg for the MR 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 < 1000 µg/kg, 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 for the MR DCH

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3.4 Imidacloprid (microbiological assessment)

Explanation

Imidacloprid is the International Standards Organization (ISO)-approved common name for (*E*)-1-(6-chloro-3-pyridinylmethyl)-*N*-nitroimidazolidin-2-ylideneamine (IUPAC), for which the CAS number is 138261-41-3.

Imidacloprid is a neonicotinoid parasiticide in the chloronicotinyl nitroguanidine chemical family. It is used to control sea lice on farmed fish and to control sucking insects, chewing insects (including termites), soil insects and fleas on pets. Imidacloprid may be applied to structures, crops and soil and can be used as seed treatment.

One product, formulated as 100% imidacloprid, is approved for treatment of sea lice (*Lepeophtheirus salmonis*) in Norway. It is supplied as a powder for dissolution for bath treatment of pre-adult and adult sea lice infestation of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). The product is authorized for use only in closed containment vessels (well boats) because of environmental concerns. The dose regimen is 20 mg/L in seawater for 60 min. The authorized withdrawal period is 98 degree-days.

Imidacloprid was previously evaluated in 2002 by the JMPR as a pesticide *(1)*. At that meeting, an ADI of 0–0.06 mg/kg bw and an ARfD of 0.4 mg/kg bw were established.

At its twenty-fifth meeting, the CCRVDF requested JECFA to evaluate imidacloprid for use in all fin fish and to recommend MRLs for muscle and fillet (muscle with skin in natural proportions).

At its ninety-fourth meeting *(2)*, JECFA derived a toxicological ADI (tADI) of 0–0.05 mg/kg bw and a toxicological ARfD (tARfD) of 0.09 mg/kg bw. In the absence of complete information to assess the direct impact of imidacloprid on representative human intestinal microbiota, neither an mARfD nor a microbiological ADI (mADI) could be derived. Therefore, the Committee was unable to establish an ARfD or an ADI for imidacloprid, and MRLs could not be recommended.

At the present meeting, imidacloprid was placed on the agenda so that the Committee could complete its assessment, including evaluation of microbiological data that were submitted by the sponsor.

Toxicologicaland microbiological evaluations

Toxicological data

No new toxicological information was available for consideration at the current meeting.

At its ninety-fourth meeting, the Committee derived a tADI of 0–0.05 mg/kg bw, which was based on a NOAEL of 5.25 mg/kg bw per day for

decreased body weight gain in an extended one-generation reproductive toxicity study[24] and application of a safety factor of 100 to allow for interspecies and intraspecies differences.

At its ninety-fourth meeting, the Committee derived a tARfD of 0.09 mg/kg bw, which was based on a $BMDL_{05}$ of 9 mg/kg bw reported by the California (USA) Environmental Protection Agency for acute neurobehavioural effects in rats *(3)* and application of a safety factor of 100 for interspecies and intraspecies differences. This value was supported by a NOAEL of 7.5 mg/kg bw per day for tremors occurring during the first week of treatment in a 90-day toxicity study in dogs, although it is not known whether tremors occurred after the first dose.

Microbiological data

The impact of imidacloprid residues on the human intestinal microbiome was evaluated in a decision-tree approach adopted by the Committee at its sixty-sixth meeting *(4)*, which complies with VICH guideline GL36 R2 *(5)*. The approach entails answering three questions to determine whether an mADI should be established. Determine, first, whether the drug residue and/or its metabolites are microbiologically active against representative human intestinal microbiota; secondly, whether the drug residues enter the human colon; and thirdly, whether the residues that enter the human colon remain microbiologically active. If the answer to any of these questions is "No", there is no need to calculate an mADI. If an mADI is required, two end-points of concern for human health are considered in the assessment: disruption of the colonization barrier of the human intestinal microbiome and increases in populations of resistant bacteria in the human intestinal microbiome.

In two unpublished studies of the MIC[25, 26], reported to be GLP-compliant, that were submitted to the current meeting, imidacloprid had no or minimal activity against a representative group of bacterial strains on the human intestinal microbiome.

A search was performed of literature in the public domain in a library catalogue that contains 228 databases, including PubMed and Elsevier

24 Holalagoudar S. PAQ008: Oral (dietary) extended one-generation reproductive study in the rat (OECD443). Study No. 8382174, by Covance Laboratories Ltd, Harrogate, United Kingdom; 2019. Submitted to FAO/WHO by Benchmark Animal Health Ltd, Edinburgh, Scotland, United Kingdom.

25 Pridmore A. Study report: Determination of minimal inhibitory concentrations (MICs) for imidacloprid and reference antimicrobial agent ampicillin against 90 bacterial strains representing the normal human intestinal microbiota. Don Whitley Scientific Ltd, Bingley, United Kingdom ; 2022.

26 Pridmore A. Study report: Determination of minimal inhibitory concentrations (MICs) for imidacloprid and reference antimicrobial agent ampicillin against *Escherichia coli* and *Enterococcus* strains isolated from the normal human intestinal microbiota. Don Whitley Scientific Ltd, Bingley, United Kingdom ; 2024.

ScienceDirect Journals Complete. The strategy for searching for information relevant to a microbiological assessment involved use of combinations of the terms "imidacloprid", "microbiome", "microbiota", "bacteria", "gut", "gastrointestinal", "intestinal" and "antimicrobial resistance". The search did not identify any reference relevant to the impact of imidacloprid residues on the human intestinal microbiome.

As imidacloprid exerted very low or no measurable antibacterial activity against the representative bacterial strains of the human intestinal microbiome tested, the Committee concluded neither an mADI nor an mARfD was required.

Evaluation

The Committee established an ADI of 0–0.05 mg/kg bw, based on a NOAEL of 5.25 mg/kg bw per day for decreased body weight gain in the extended one-generation reproductive toxicity study, with application of a safety factor of 100 to allow for interspecies and intraspecies differences.

The Committee established an ARfD of 0.09 mg/kg bw, based on a $BMDL_{05}$ of 9 mg/kg bw for acute neurobehavioural effects in rats and a safety factor of 100 to allow for interspecies and intraspecies differences.

An addendum to the toxicological monograph was prepared.

Residue evaluation

No new data on residues were available for consideration at the current meeting.

Estimated dietary exposure

At its ninety-fourth meeting, JECFA estimated the GECDE for imidacloprid residues based on their potential occurrence in Atlantic salmon muscle and all fin fish meat. No health-based guidance values were established at that meeting.

At its ninety-fourth meeting, the Committee noted that dietary exposure to imidacloprid may also occur due to its many registered uses as a pesticide. A review of previous assessments of pesticide residues showed that exposure from this source is low (< 5% of the upper limit of the ADI) and would probably not contribute substantially to overall dietary exposure.

Estimated dietary exposure

The current meeting established an ADI of 0–0.05 mg/kg bw (0–50 µg/kg bw).

The GECDE for adults and the elderly (1.0 µg/kg bw per day), based on incurred residues in Atlantic salmon (fillet) and a withdrawal period of 98 degree-days, is 2% of the upper limit of the ADI. The GECDE for children and adolescents (2.7 µg/kg bw per day) is 5% of the upper limit of the ADI, and that for infants and toddlers (0.9 µg/kg bw per day) is 2% of the upper limit of the ADI.

The GECDE for adults and the elderly (1.8 µg/kg bw per day), based on total consumption of fin fish meat, is 4% of the upper limit of the ADI; that for children and adolescents (3.8 µg/kg bw per day) is 8% of the upper limit of the ADI, and that for infants and toddlers is 2% of the upper limit of the ADI.

Acute dietary exposure estimates

At the current meeting, the Committee established an ARfD of 0.09 mg/kg bw (90 µg/kg bw).

The GEADE for adults and children (6.2 and 6.6 µg/kg bw, respectively), based on consumption of Atlantic salmon, was 7% of the ARfD, and those based on consumption of fin fish (34.1 and 23.8 µg/kg bw) were 38% and 26% of the ARfD for adults and children, respectively.

Maximum residue limits

In recommending MRLs for imidacloprid in fin fish fillet (muscle with skin in natural proportions) and muscle, the Committee considered the following.

- The ADI for imidacloprid is 0–0.05 mg/kg bw.
- The ARfD for imidacloprid is 0.09 mg/kg bw.
- Imidacloprid is used as a pesticide and as a veterinary drug.
- Imidacloprid is authorized for use in Atlantic salmon and rainbow trout. The maximum recommended dose is 20 mg/L once, by immersion in a seawater treatment bath for 60 min. The withdrawal period is 98 degree-days for both species.
- Under field conditions, it may not be possible to remove all the fish from the treatment bath immediately after 60 min; therefore, extended exposure up to 360 min was considered.
- Imidacloprid is the marker residue in Atlantic salmon and rainbow trout muscle and fillet.
- The ratio of the concentration of marker residue to that of total residue was calculated to be 0.7 .
- Data on residues in Atlantic salmon and rainbow trout were provided that were derived with a validated analytical method for quantifying imidacloprid in muscle and fillet.
- A validated analytical method for determining imidacloprid in Atlantic salmon and rainbow trout muscle and fillet is available and may be used for monitoring purposes.

- The MRL recommended for salmon and trout muscle and/or fillet is based on the 95/95 UTL at 98 degree-days from a study of unlabelled residue depletion in Atlantic salmon.
- The Committee recommended an MRL for Atlantic salmon and rainbow trout fillet (muscle with skin in natural proportions) and/or muscle of 600 µg/kg.

The Committee recommended that the MRL be extrapolated to all fin fish.

An addendum to the residue monograph was prepared.

Summary and conclusions

Studies relevant to risk assessment – imidacloprid

Species/study type (route of administration)	Doses	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Mouse				
Two-year toxicity study (diet)	Males: 0, 20, 66 and 208 Females: 0, 30, 104 and 274	Reduction in body weight	66	208
Rat				
Acute neurotoxicity study (gavage)	0, 42, 151, 307	Decreased locomotor activity in females	9 [a] ($BMDL_{05}$)	- [b]
One-year toxicity study (diet)	Males: 0, 5.6, 16.3 and 55.8 Females: 0, 6.7, 19.5 and 63.7	Reduction in body weight	5.6	16.3
Two-year study (diet)	Males: 0, 5.7, 17 and 51 Females: 0, 7.6, 26 and 73	Increased incidence and severity of mineralized particles in the thyroid gland	5.7	17
Extended one-generation study (diet)	Males F0: 0, 5.25, 15.35 and 48.4 Females F0: 0, 10.4, 30.43 and 85.6	**Reproductive toxicity:**	48.4 [c]	-
		Parental toxicity: lower mean body weight, decreased food consumption	5.25 [d]	15.35
		Offspring toxicity: decreased number of pups delivered, increased number of stillborn pups, decreased number of live pups, increased T4 concentration, reduced pup weights, reduced spleen and thymus weights.	10.4	30.43
Developmental toxicity study (gavage)	0, 5, 15 and 50	**Maternal toxicity:** reduced body weight gain and decreased food consumption	15	50
		Embryo-fetal toxicity:	50 [c]	-

(continued on next page)

Species/study type (route of administration)	Doses	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rabbit				
Developmental toxicity study (gavage)	0, 8, 24, 72	**Maternal toxicity:** reduced body weight gain and decreased food consumption	8	24
		Embryo–fetal toxicity: increased post implantation loss, reduced body weight, delayed ossification	24	72
Dog				
90-day (diet)	0, 7.5, 24 or 67.5/45	Tremors in first week	7.5	24

[a] Pivotal study for derivation of the tARfD (3);

[b] The report of the ninety-fourth meeting of JECFA inadvertently included a value for a lowest-observed-adverse-effect level (LOAEL). This was redundant, as the $BMDL_{05}$ was used as the point of departure for this study. The LOAEL has therefore been deleted from the report of the present meeting;

[c] The highest dose tested;

[d] Pivotal study for derivation of the toxicological ADI;

LOAEL: Lowest-observed-adverse-effect level; NOAEL: No-observed-adverse-effect level

Microbiological data

Imidacloprid exerted very low or no measurable antibacterial activity against the representative bacterial strains of the human intestinal microbiome tested.

The Committee concluded that no mADI or mARfD was required.

ADI

The Committee established an ADI of 0–0.05 mg/kg bw, based on a NOAEL of 5.25 mg/kg bw per day for decreased body weight gain in an extended one-generation reproductive toxicity study in rats, with application of a safety factor of 100 to allow for interspecies and intraspecies differences.

ARfD

The Committee established an ARfD of 0.09 mg/kg bw based on a $BMDL_{05}$ of 9 mg/kg bw for acute neurobehavioural effects in rats and a safety factor of 100 to allow for interspecies and intraspecies differences.

Residue definition

The marker residue for imidacloprid in fin fish is the parent molecule, imidacloprid.

Estimated dietary exposure

For Atlantic salmon only, the GECDE was 1.0, 2.7 and 0.9 µg/kg bw per day (2%, 5% and 2% of the upper bound of the ADI of 50 µg/kg bw) for adults and the elderly, children and adolescents, and toddlers and infants, respectively.

For all fin fish, the GECDE was 1.8, 3.8 and 1.2 µg/kg bw per day (4%, 8% and 2% of the upper bound of the ADI of 50 µg/kg bw) for adults and the elderly, children and adolescents, and toddlers and infants, respectively.

The GEADE, based on consumption of Atlantic salmon, was 7% of the ARfD for adults and children (6.2 and 6.6 µg/kg bw, respectively); the GEADE for all fin fish was 38% and 26% of the ARfD (34.1 and 23.8 µg/kg bw) for adults and children, respectively.

MRLs

The Committee recommended an MRL for Atlantic salmon and rainbow trout fillet (muscle with skin in natural proportions) and/or muscle of 600 µg/kg. It further recommended that the MRL be extrapolated to all fin fish.

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3. Risk characterization document for imidacloprid. Sacramento (CA): California Environmental Protection Agency; 2006. (http://www.cdpr.ca.gov/docs/risk/rcd/imidacloprid.pdf).

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5. VICH GL36(R). Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. Guidance for industry. Brussels: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products; 2019 (https://www.vichsec.org/en/guidelines/pharmaceuticals/pharma-safety/antimicrobial-safety).

4. Future work and recommendations

Recommendations relating to specific veterinary drugs, including ADIs and proposed MRLs, are given in section 3.

This section presents recommendations related to future work by the JECFA Secretariat.

4.1 Guidance for the safety evaluation of residues of veterinary drugs with incomplete data packages

The Committee adopted the guidance and welcomes comments from the CCRVDF.

4.2 JECFA Toolbox for Veterinary Drug Residues Risk Assessment

The toolbox is expected to be available by the end of 2024 and will be publicly available on the FAO website.

Acknowledgements

FAO and WHO acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the ninety-eighth meeting of JECFA.

Annex 1

Guidance for the safety evaluation of residues of veterinary drugs with incomplete data packages

Annex 1 Contents

Preface

JECFA expects sponsors to provide comprehensive, high-quality dossiers to enable informed recommendations for risk management from the risk assessment.

Under some circumstances, incomplete dossiers introduce additional uncertainty that can be accounted for by establishing more conservative HBGVs, with additional safety factors, or by using limits of quantification or similar values instead of the actual amounts of residues to estimate dietary exposure. The outcome is recommendation of more conservative MRLs than would be the case.

when there is less uncertainty.

In some cases, when the uncertainties due to missing information are too great to be bridged by assumptions, it will not be possible to complete a full assessment or to recommend MRLs.

It should be noted that the absence of a "traditional" study on a given effect does not necessarily mean that a conclusion cannot be reached from other relevant information, such as on carcinogenic potential.

JECFA is committed to using the highest-quality science in its risk assessments to provide advice to risk managers. This commitment will be maintained in assessments based on incomplete information and high levels of uncertainty. In such cases, JECFA will clearly communicate the outcomes, limitations, uncertainties and assumptions that frame the risk assessment.

A1.1 Purpose

JECFA can fully assess the risk of a veterinary drug residue only with comprehensive information on the compound; however, JECFA is sometimes asked to assess the risk of veterinary drug residues with less than comprehensive information and/or where the study designs are outdated.

Current JECFA guidance is not clear about how such situations should be managed. The purpose of this annex is to outline processes, approaches, minimal data requirements and limitations of risk assessments undertaken in response to such requests.

A1.2 Introduction

JECFA provides scientific advice on veterinary drugs to the CCRVDF, FAO, WHO and their Member States. The terms of reference of JECFA (veterinary drugs) are to elaborate principles for evaluating the safety and for quantifying the risks of residues of veterinary drugs; establish ADI values for chronic exposure and other guidance values for acute exposure; recommend MRLs for target tissues; and determine appropriate criteria for and evaluate methods of analysis for detecting and/or quantifying residues in food.

JECFA in its call for data requests a comprehensive data package on compounds, such as would be sufficient to enable registration by national and regional authorities. JECFA assesses the toxicological risk (including the risk of effects on the human intestinal microbiome) from exposure to residues of the veterinary drug and establishes, as appropriate, an ADI and an ARfD based on toxicological or intestinal microbiome effects, data permitting.

It is important to note that, when this document refers to microbiological risk, effects or data, it is referring (unless otherwise specified) to the impact of

residues on the human intestinal microbiome, as described in VICH GL36 (R2) *(1)*. A microbiological risk assessment is not conducted of the potential development of resistance to a given antimicrobial drug to select for resistant bacteria of human health concern (antimicrobial resistance, AMR) under the intended use in food producing animals, as described in VICH GL 27 *(2)* and FDA GFI 152 *(3)*.

JECFA also assesses residue depletion from tissues and analytical methods and estimates dietary exposure in order to derive a MRL. MRLs are recommended based on use according to GVP and are not intended to cover misuse or abuse *(4)*.

While the approach has served well to protect public health from adverse health effects of residues of veterinary drugs, it is inadequate to accommodate information gaps (for example, lack of a study on reproductive toxicity or incomplete data on metabolism); it is also predicated on the existence of a threshold for the adverse response. Unless an ADI or ARfD and MRL can be recommended, the approach provides no mechanism for the provision of meaningful advice to risk managers.

JECFA is sometimes asked to assess veterinary drug residues for which there is no comprehensive data package. As discussed below, situations in which this may occur include "old" drugs, drugs with no commercial sponsor, drugs no longer in use that contaminate food because of environmental persistence, unavoidable and unintentional carryover of drugs into feed or misuse or abuse. Current JECFA guidance should be adapted to address such issues.

Despite data gaps, however, requests may be received to assess the risks of residues, albeit with suboptimal data. This is the fundamental premise for consideration of alternative approaches to drug residue risk assessment, which should remain suitably robust despite potential data deficiencies. In some instances, such deficiencies are significant enough to preclude a risk assessment or recommendation of MRLs until suitable data become available.

Failure to complete a risk assessment, however, also has consequences. Delays in a JECFA risk assessment due to inadequate information and subsequent adoption of MRLs by Codex can impede international trade. Furthermore, jurisdictions with limited capacity and resources for conducting independent risk assessments of veterinary drug residues rely strongly on JECFA's risk assessments and Codex MRLs. Therefore, delays in a JECFA assessment may delay approval of such drugs in those jurisdictions, possibly depriving food-producing animals in those regions of useful medications.

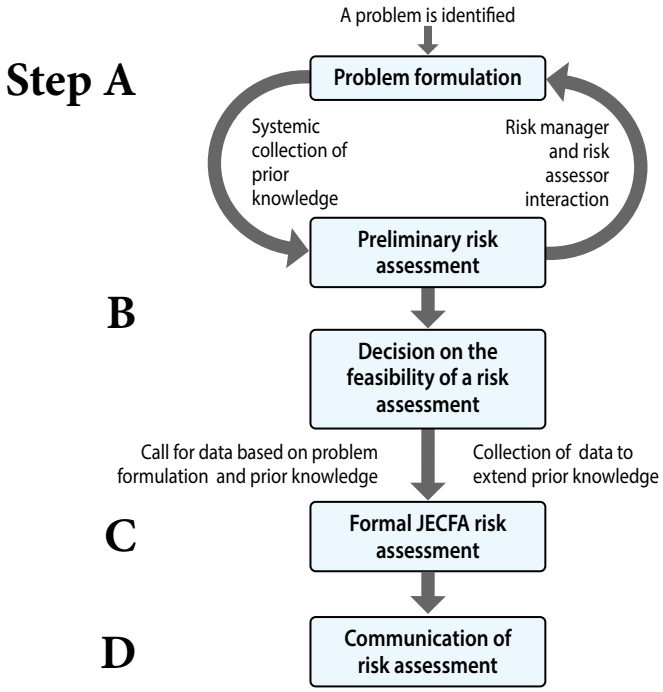
Hence, a more flexible approach is required to enable JECFA to respond effectively to requests related to such substances from CCRVDF, Member States, FAO and WHO. This document describes an approach, based on the principles of risk analysis, to address such situations.

Risk analysis framework for risk assessment of veterinary drugs

A key component of the proposed approach is problem formulation, which requires close dialogue between risk assessors and risk managers. The approach recognizes the importance of a preliminary risk assessment in identifying available data and data gaps and helps to refine the risk questions that drive the analysis. It is intended to provide greater consistency and transparency to the evaluation of residues of veterinary drugs, while offering greater flexibility and adaptability to changes in technology, pharmacology and safety concerns.

The basic approach is derived from the publication by Renwick et al. in 2003 (5). This publication presents a decision tree approach for evaluating the safety of chemicals in food. While it is not specifically designed for residues of veterinary drugs, the publication proposes a hypothesis-driven risk analysis approach that calls for interaction between the developer of the safety data (here, the pharmaceutical sponsor), the risk manager (CCRVDF) and the risk assessor (JECFA). The framework for such an approach is summarized in Fig. A1.1.

Figure A1.1.
Risk analysis framework for risk assessment of veterinary drugs



An important consideration is that, while a decision-tree approach based on risk analysis may be anticipated to provide greater flexibility in the evaluation of certain categories of veterinary drugs, the requirement for sufficient data of adequate quality cannot be over-emphasized. It might sometimes be possible to accommodate some deficiencies in the available information by using additional safety factors. When this is not possible, the risk analysis approach may help to identify critical information requirements early in, or during, the evaluation and lead to generation of critical information that will permit successful evaluation of the veterinary drug.

A1.3 Risk analysis of veterinary drugs

The most common reason for referring a compound for evaluation to JECFA is to obtain recommendations for MRLs for consideration by CCRVDF for veterinary drugs in commercial use in one or more Member State. This requires consideration of the level of residues in food from a veterinary drug that would not be a human health concern. This necessitates establishment of a HBGV, usually the ADI and/or ARfD, based on toxicological, microbiological and other relevant data (e.g. on kinetics), to be compared with dietary exposure estimates. It also requires derivation and recommendation of MRLs for veterinary drugs in foods based on evaluation of residue depletion and other relevant studies. Recommended MRLs must be compatible with the ADI, defined as a daily intake with no appreciable health risk, when the drug is used according to GVP.

Veterinary drugs considered by JECFA are usually products with a main commercial producer ("sponsor"), that is, a veterinary pharmaceutical company, which would be expected to generate appropriate data to allow establishment of HBGVs and for recommending MRLs. The sponsor is, however, not always a large pharmaceutical company; it may be a small commercial organization, an academic institution or a national government that produces one or a small number of products, more for their essential use in certain parts of the world than for international distribution. Alternatively, there may be local production by small companies of generic pharmaceuticals that are no longer under patent. Sometimes, older drugs have changed producers, for example through takeovers or mergers. Consequently, the data generated for their original registration, to protocols and standards that are most likely now outdated, may be only partially available and of limited utility for a contemporary evaluation. It is very unlikely that a data package that meets current standards would be available for these substances. Nevertheless, JECFA may be asked to establish HBGVs and to recommend MRLs for such drugs.

In formulating its advice to risk managers, JECFA describes the strengths and weaknesses of the evaluation and provides an estimate of the uncertainty in

its conclusions.

JECFA might have to indicate the potential consequences to human health if specific risk management options are not feasible. This last aspect would require prior discussion with risk managers, as indicated above.

A1.3.1 Problem formulation (step A)

Problem formulation is intended to identify and characterize the problem to be addressed and to determine risk management goals. Problem formulation is undertaken by a risk manager in discussion with a risk assessor. Hence, in formulating the problem to be addressed, it is of paramount importance that a dialogue be maintained between JECFA (through the Secretariat) and the risk managers who are requesting advice, usually CCRVDF. Among the issues that will probably have to be resolved are:

- whether the compound is supported by a commercial sponsor;
- whether the compound is registered or likely to be registered in a country or region;
- whether the compound is a product of commercial value;
- whether there is sufficient information to enable a meaningful evaluation;
- the nature of specific concern (duration of exposure, population exposed, source of residue in food);
- whether risk management options are available should a dietary exposure estimate not be acceptable;
- the form of advice that would be most helpful to the risk manager; and
- if such advice cannot be provided (for example because of data limitations), the alternative advice that might be of value.

Request for an ADI or ARfD and MRL of a veterinary drug

When a compound is supported by a commercial sponsor, it is anticipated that registration by national and regional authorities would be sought. This would involve substantial data requirements, such as those normally requested by JECFA. In such cases, JECFA would anticipate that a full data package, comprising all relevant information, would be submitted for consideration, and that the studies would meet current standards. Details can be found in Environmental Health Criteria 240 and its updates *(4,6)* and Guidance for JECFA Monographers *(7)*.

When the sponsor is not a major pharmaceutical company, registration is likely to be limited, and the availability of data would vary widely. While JECFA expects a full data package, it is recognized that there might be certain limitations. Information in the open literature can be particularly important

for such compounds, as can information generated for other uses (e.g. human medicine, industrial chemical). JECFA would consider such submissions case by case.

Similarly, for older drugs, the information available is unlikely to comprise a full data package to current standards. In these cases, the information required would be such that it would be possible to address the key questions in the decision-tree approach discussed below. Hence, it should be possible to match information on toxicological end-points with duration and extent of exposure and exposed populations (e.g. infants). There should be sufficient information on the compound's fate in the target species to estimate dietary exposure. It will also be necessary to weigh studies conducted to GLP, in which there is full disclosure of the raw data and records, to permit detailed evaluation of the study, with studies in the open literature. The latter are often not conducted according to any agreed standards, the raw data will not be available, and they may have been conducted before establishment of GLP.

Request for advice on protecting human health from contaminants related to veterinary uses

While most of the questions referred to JECFA relate to the veterinary use of drugs, concerns about the effect on human health of pharmaceutical residues in food may arise in other situations, such as contamination of livestock with drugs that are no longer legally used, due either to environmental persistence or to illegal use. Examples in which JECFA has undertaken such assessments include chloramphenicol *(8)* and malachite green *(9)*. While this is clearly an area that overlaps with that of JECFA (additives and contaminants), it is likely that, on occasion, such issues will be referred to JECFA (residues of veterinary drugs), because of its expertise in veterinary medicine.

As for older drugs, as described above, it is highly unlikely that a full data package will be available. As there may be no sponsor for the compound for any use, JECFA will consider whatever data are available (e.g. in the open literature or generated for other uses, such as for an industrial chemical or consumer product), following a request to do so. As above, information would be required such that it is possible to match the toxicological data relevant to the duration and extent of exposure and the exposed populations. JECFA would consider the data case by case and would provide an assessment of the uncertainty in any conclusion regarding the acceptability or otherwise of residue levels to which consumers are exposed. When it is not possible to establish a HBGV such as an ADI or ARfD due to data limitations, it may still be possible for JECFA to derive a MOE or some other risk estimate (see below).

Another situation in which JECFA may be asked for advice is that of abuse, misuse or unauthorized use of a compound, such as GVP not being followed or

not available or when a drug is used illegally. The route of administration, species, dosage regimen or some other aspect of treatment may not be as approved, but risk managers may request advice on potential consequences to human health.

Systematic collection of prior knowledge (step A)

Systematic collection of prior knowledge is intended to identify all information that may contribute to the risk assessment. For the assessment of risk in the consumption of residues of veterinary drugs, areas in which information is required are described in the Guidance for JECFA Monographers *(7,10)* and are as follows:

- general characteristics – details of chemical and physical characteristics of the drug, including impurities, purity and quality of final product; substances should be registered as veterinary drugs in at least one country, and details should be provided (but see above);
- use patterns – good veterinary or animal husbandry practices, including purpose of use, doses, methods of administration, target species and withdrawal times;
- pharmacological characteristics;
- analytical criteria;
- metabolism and pharmacokinetics;
- toxicology data;
- microbiological data; and
- residue depletion studies in field conditions.

One of the changes proposed in the current approach is to more explicitly problem formulation for the safety assessment of residues of a particular veterinary drug in food. Problem formulation, as discussed earlier, is the process in which questions about safety and paths to address those questions are identified. An important first step is to identify information that is already available for a preliminary assessment of the veterinary drug, before an in-depth review by JECFA experts. The preliminary assessment is a means to identify the strengths and gaps of the existing knowledge base and allows further refinement of the problem formulation.

The nature of the prior knowledge that would be useful in problem formulation and in an initial risk assessment before a formal JECFA evaluation is not markedly different from that of the information necessary for the JECFA evaluation. It is anticipated, however, that systematic collection of information and initial risk assessment will identify areas in which additional information is required and help to shape the final problem formulation.

Prior knowledge on the substance would be useful in the following areas.

Identity and use of the substance.

This information includes the name, chemical structure and what is known of the substance's physicochemical properties. Additionally, information may be available on the use or uses of the substance, including as a human or veterinary drug, a pesticide, a human or animal biological, a human or animal food additive or other uses. Taken together, this information provides understanding of the substance that is being proposed for evaluation.

Human exposure to the substance

This includes the dose and duration of administration if the substance is used as a human or veterinary drug but also includes information on the concentrations of the substance in food or water. In addition, information can be provided about the prevalence of human exposure and the human populations or subpopulations that are exposed.

Biological effects

This information is used to characterize the biological hazard presented by the substance. It includes data on absorption, distribution, metabolism and excretion and on pharmacokinetics and pharmacodynamics in mammalian species. Information on biological, toxicological and microbiological effects (see above) could include data from in-vitro studies and studies conducted in humans and other species. Epidemiological information can be useful for determining the effects of the substance on human populations.

Evaluations by regulatory authorities

While JECFA is committed to an independent scientific evaluation of the safety of a substance in food, it is often useful to consider evaluations by regional and national regulatory authorities, the nature of the information available for those evaluations and the conclusions that have been reached from the information. Such evaluations may indicate additional sources of data, specific toxicological or other concerns and unique approaches to the evaluation and thus help to frame the context of the JECFA evaluation.

A1.3.2 Preliminary risk assessment (step B)

Close interaction between risk management and risk assessment during the initial problem formulation and subsequent systematic collection of prior knowledge allow development of a preliminary risk assessment that will best address risk management needs. This may, however, be constrained by the time for the JECFA process. It is possible that the outcome of a JECFA evaluation will essentially be a preliminary risk assessment, in which the further information required has been identified.

A preliminary risk assessment should be based on the question(s) identified during the initial problem formulation by risk management. The risk assessment should then identify and characterize the hazards and exposures and, if possible, provide a preliminary characterization of the risk. It is anticipated that a preliminary risk assessment will be particularly useful in identifying the additional information required and other issues that may impact a formal JECFA risk assessment. Continued close interaction between risk management and risk assessment is critical to provide focus and direction for the preliminary risk assessment. The preliminary risk assessment, in turn, may result in refinement of the problem formulation. The interaction should continue until there is good understanding of the available information and the impact of the information on problem formulation. Once agreement is reached between risk management and risk assessment, the results should be used to inform a decision on whether a formal JECFA risk assessment is necessary and/or feasible and the form it should take.

A1.3.3 Decisions on the feasibility of risk assessment (step B)

Problem formulation, as modified by a preliminary risk assessment, will determine the feasibility of a formal JECFA risk assessment or, otherwise, the form that the risk assessment should take. It is important that problem formulation result in clear questions to be addressed by JECFA, so that expectations are met. In view of the policy considerations discussed above, JECFA may be requested, for example, to provide:

- a formal risk assessment to establish a HBGV, such as an ADI or ARfD, and to recommend MRLs, the traditional role of JECFA in support of CCRVDF;
- a formal risk assessment, recognizing that there are insufficient data to establish an ADI, ARfD and/or MRLs but intended to identify what data are currently available and the key information required (see section 4.1). While this has sometimes been the outcome of previous JECFA assessments, it was almost always incidental to an unsuccessful attempt to establish an ADI and/or recommend an MRL due to data gaps, for example imidacloprid (28). It should be recognized that it may be the stated objective of the risk assessment from the outset, depending on problem formulation, that is, a preliminary risk assessment;
- a formal risk assessment to provide estimates of risk other than an ADI and/or ARfD, such as a tolerable daily intake for an out-of-use veterinary drug present as a contaminant, to assist risk management decisions;

- an assessment of the risk from use of an illegal drug, perhaps expressed as a MOE;
- an assessment of the risk of more than one risk management option; and
- a scientific assessment of other issues related to the safety of veterinary drug residues for human consumption.

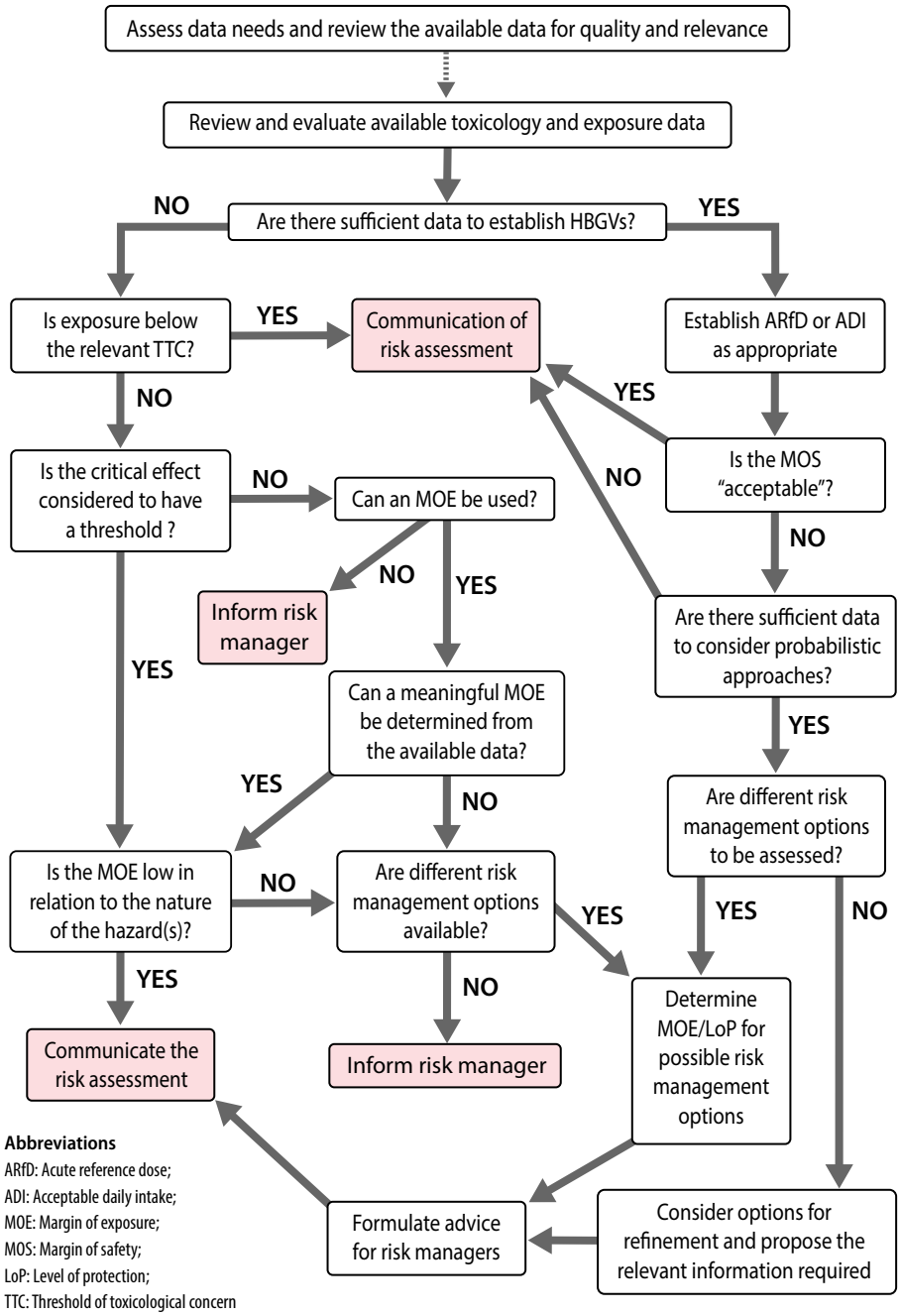
One result of the preliminary risk assessment is that it can help to identify the (types of) information that could focus the data call by JECFA and subsequent literature search.

It is also possible that the preliminary risk assessment leads to a conclusion that a more formal risk assessment is unlikely to satisfactorily address the questions posed by the problem formulation. In these circumstances, there may be a mutual decision between risk management and risk assessment that the desired risk assessment is not possible currently, ideally with identification of the information requirements that would enable a risk assessment to proceed. Possible risk management options would then have to be considered, usually by CCRVDF, for example withdrawal of existing MRLs.

A1.3.4 Risk assessment (step C)

Should the preliminary risk assessment result in the conclusion that a detailed risk assessment is required, a formal risk assessment is conducted in accordance with the general principles of the risk assessment paradigm, as described in EHC 240 and its updates *(4,6)* and in the Guidance for JECFA Monographers *(7)*. The decision tree shown in Fig. A1.2 and described below should be used for this purpose.

Figure A1.2.
Decision-tree approach in the risk assessment of residues of veterinary drugs; step C
(The top box is not part of this step, but is covered in steps A and B, described above)



A1.3.5 Communication of the risk assessment (step D)

The scope and purpose of a particular commissioned risk assessment should be clearly stated and be in accordance with the risk assessment policy of the relevant risk management body, usually the CCRVDF.

The following scheme could be considered, in that order, to describe the outcome of the risk assessment to the risk managers who commissioned it, depending on the data availability and the choices made during the risk assessment.

- Provide HBGV(s) if supported by the risk assessment.
- If risk assessment does not support the establishment of HBGV(s):
  - describe and recommend the additional information required, and
  - provide other estimate of risk.
- Offer additional advice as warranted to the risk managers, for example risk resulting from different risk management options.

There are several sources of uncertainty in risk assessment of residues of veterinary drugs. The degree of uncertainty in the available scientific information will depend on the type of assessment, being greater for non-standard assessments such as those described above. The results of risk assessments by JECFA should be explained clearly and transparently, including uncertainties and constraints and how they were managed in the risk assessment. Guidance on uncertainty analysis can be found in chapter 7 of EHC 240 *(4)*

A1.4 Formal JECFA risk assessment – decision-tree approach (step C)

The nature of the risk assessment is determined by the problem formulation, informed by a preliminary risk assessment (if this was possible) and the availability of critical data.

As noted in the Introduction, the basic approach to the evaluation of the safety of veterinary drugs by JECFA is described in EHC 240 and its updates *(4,6)* and in the Guidance for JECFA Monographers *(7)*.

The expanded JECFA risk assessment approach proposed here is presented diagrammatically in Fig. A1.2. The flow chart does not provide details of the risk assessment, either for hazard or exposure characterization, but rather illustrates the major steps in the assessment and some key decision points.

A1.4.1 Collection of data

In addition to the collection of prior knowledge for the preliminary risk assessment, the JECFA Secretariat issues a formal call for data. While the collection of prior knowledge is based on publicly available information, the call

for data is for both proprietary and public data, particularly those data that have been used to support the approval or registration of the veterinary drug with a national or regional authority.

Requests for evaluations of certain veterinary drugs and other relevant substances for which a hazard has been identified and consideration of issues of a more general nature by the Committee may come from several sources:

- CCRVDF refers substances to JECFA according to the priorities it establishes with the criteria that it has developed, which are in accordance with accepted procedures of the Codex Alimentarius Commission, in particular relating to the risk analysis policy adopted for CCRVDF.
- FAO and WHO Member States may directly request the inclusion of veterinary drugs on the agenda of JECFA.
- For veterinary drugs not previously evaluated by JECFA, an industry sponsor may forward a request for evaluation through the government of a Member State to CCRVDF, with a commitment to provide the relevant data.
- Requests for re-evaluation of a veterinary drug that was previously reviewed by JECFA may be forwarded directly to the JECFA Secretariat.
- The JECFA Secretariat may place a veterinary drug on the agenda for re-evaluation, even when no outside request has been received, for example because of a potential public health concern.

Before inclusion of a substance on an agenda, the JECFA Secretariat will have received a firm indication from CCRVDF that one or more entities will submit data for the evaluation, or that the data are available from other sources, such as a government organization or the published literature. For substances that are being re-evaluated, the Secretariat assumes that the sponsor of the original evaluation will provide the necessary data, unless informed otherwise.

Literature searches

JECFA uses all available data to perform assessments. Accordingly, sponsors should perform a literature search on their compound, as should JECFA. It is extremely important that literature searches be performed, especially on substances that have been used for a long time in human and/or animal medicine. This is also the case for substances other than approved veterinary drugs for which requests for a risk assessment by JECFA are made. Risk assessments by other authorities should also be reviewed, to identify any studies that were not submitted to JECFA. JECFA will contact the sponsor and request submission of studies that they know have been reported elsewhere but were not included in

the data package, if they are likely to be relevant to the evaluation. If the sponsor does not respond to this request, JECFA should note the potential impact of the missing data on the evaluation. If full reports of studies are not submitted, JECFA will request them, including the raw data, from the sponsor.

A1.4.2 Identification and characterization of hazards

JECFA normally requires sponsors to provide information from a comprehensive series of toxicological tests, most of which are described by guidelines of the Organisation for Economic Co-operation and Development, to detect general or specific toxic effects. The effects investigated should also include pharmacological and microbiological effects (see above) that might help characterize the hazards of residues of the substance, unless a scientific case can be made that this is not necessary. When a species-specific metabolite is found only in a food-producing animal, studies of the parent compound in experimental animals will provide no information on its toxicological effects. Such metabolites should be dealt with case by case, which may include some limited toxicological studies of the metabolite itself. Such testing results in identification of the range of effects of the compound and serves as the basis for hazard characterization. Further details of toxicity testing of veterinary drugs can be found in EHC 240 and its updates *(4,6)* and the Guidance for JECFA Monographers *(7)*.

JECFA is open to submission of relevant information generated by scientifically reliable, new approach methodologies (such as computational [in silico] and in-vitro approaches) in support or in place of data from studies in laboratory animals.

A number of compounds used as veterinary drugs will have been developed for use in humans, as clinical medicines, or have undergone exploratory studies for such potential use. Clinical and pre-clinical data on the safety of the substance obtained in such studies should also be submitted to JECFA in response to the call for data.

The objective of hazard characterization is to determine the relations between the extent of exposure to a chemical agent and the severity and/or frequency of associated adverse health effects. Except in exceptional circumstances (and in agreement with risk managers), JECFA establishes HBGVs and recommends MRLs only for compounds for which there is considered to be a threshold in the dose–response relationships for all of their adverse effects. For each adverse effect, a reference point , usually the NOAEL, or the lower 95% confidence limit for the BMDL is identified. The lowest relevant reference point, known as the point of departure (POD), is used as the basis for establishing a HBGV. Guidance on dose–response assessment and the establishment of HBGVs can be found in EHC 240, update to chapter 5 *(6)*. While relevant information

on the potential hazard to human health of residues of veterinary drugs usually comes from studies in laboratory animals, relevant human data may be available, particularly for substances that have or are being developed for use in human medicine. While such information is particularly valuable, there are a number of unique issues in its assessment (see EHC 240 and updates for details; *4,6*).

JECFA determines whether to establish microbiological HBGVs for residues of veterinary drugs with a decision-tree approach *(11)* that complies with VICH GL36 (R2) *(1)*. The approach initially determines whether microbiologically active veterinary drug residues enter the human colon in a stepwise approach. If the answer is "No" at any step, no microbiological HBGV is necessary. If, however, residues could be present, two end-points of public health concern are considered: disruption of the colonization barrier and an increase in the population(s) of resistant bacteria. For acute effects, unless there is evidence to the contrary, only the first end-point is considered. It is possible to provide scientific justifications to eliminate testing (i.e. the need for a microbiological HBGV) for either one or both end-points. The MIC_{calc} or the no-observed-adverse-effect concentration (NOAEC) is used as the POD.

A1.4.3 Hazard characterization from an incomplete data package

As discussed in 3.3, there may be situations in which JECFA is asked to assess the risk, and to recommend MRLs, for veterinary drugs for which the information provided is not complete (i.e. that currently required for submission for national authorization or registration) and for which there is little or no prospect of obtaining further information. JECFA first considered approaches to the hazard characterization of such compounds in 1993 and again in 1995, under "Evaluation of veterinary drugs with a long history of use" *(12,13)*.

For such compounds, it should be possible, at a minimum, to assess their pharmacological effects, general toxicity, reproductive toxicity, embryo/fetal toxicity, genotoxicity, carcinogenicity, microbiological effects, other effects identified as being of importance, metabolism, tissue residues and analysis. It might be possible to address these concerns by a combination of animal studies and alternative sources of information. When information is not available on a specific compound, an approach analogous to read-across [27] should be considered.

The long-term effects of veterinary drugs are usually assessed on the basis of the results of chronic studies in rats (2 years), sometimes also in mice, and shorter-term studies in rodents and dogs, typically 90 days and 1 year (in dogs). Most authorities have now concluded, however, that 1-year studies in dogs provide little additional information over that obtained in a 90-day study and

27 Read-across involves interpolation of the effects of a compound from those of one or more close structural analogues.

have therefore dropped the requirement for the longer study. While, occasionally, effects are observed in rats after exposure for 1 or 2 years that are not observed after 90 days, the differences are usually quantitative rather than qualitative. It is now considered possible to assess the potential chronic toxicity of a veterinary drug from the results of a 90-day study in rats, with consideration of potential precursor effects (e.g. changes in clinical chemistry, organ weights), supported by read-across from analogous compounds, when possible. In such situations, an additional safety factor may be warranted (see below), but this will depend on the nature of the effect and its dose- and time-responses.

Assessment of the genotoxic potential of a veterinary drug should encompass the key end-points of gene mutation, aneugenicity and clastogenicity. Understanding of the biological and mechanistic basis of these effects is now such that computational approaches can be used with reasonable confidence to assess the genotoxic (mutagenic) potential of compounds, at least for the end-point of most concern – gene mutation. Other end-points can be addressed by mechanistic considerations and MOEs. This is described in the updated chapter 4.5 of EHC 240 on "Genotoxicity" *(6)*. Hence, it may be possible to assess genotoxic potential with a combination of in-silico and read-across approaches.

Assessment of carcinogenicity has traditionally relied on lifetime (2 years) testing in two species, typically rats and mice. The usefulness of such studies in assessing human-relevant carcinogenic potential has, however, been increasingly questioned, and several alternative approaches, obviating the need for rodent bioassays, have been proposed. These rely on current understanding of chemical carcinogenesis, whereby tumours can arise due to chemical exposure by clonal expansion of a population of cells with either a pre-existing mutation or a new one that they have acquired *(14,15)*. The mechanisms by which this can occur are direct genotoxicity, with de-novo mutation, or an increase in the probability of mutation events by mitogenic stimulation by growth factors or hormones, an increase in cell proliferation secondary to cytotoxicity (regenerative repair) or immunosuppression resulting in a decrease in the rate of cell death. Hence, it may be possible to assess carcinogenic potential from a combination of read-across, genotoxicity evaluation (including in-silico methods), the results of an adequately conducted study of sub-chronic toxicity (e.g. 90-day study in rats) and pharmacological studies (e.g. for endocrine effects). Such an assessment would always be conducted case by case and would rely to an appreciable extent on expert judgement.

For older drugs, studies of reproductive or developmental toxicity conducted according to current standards are often lacking. These effects must be assessed case by case, and, again, with appreciable judgement. If a developmental toxicity study is available that was not conducted to current standards, the questions to be asked include: Is there evidence for embryo-fetal-specific effects?

Is the compound genotoxic (e.g. aneugenic)? Is it possible to determine a likely mechanism for any effects observed, for example from primary pharmacology[28] or read-across? What is the MOE for any effect? In the case of reproductive toxicity, in addition to results from specific studies, is there evidence for effects on organs of reproduction in repeat-dose toxicity studies? Is there evidence for potential effects from primary pharmacology or read-across? Are there any endocrine effects? Are effects observed in any developmental toxicity studies?

For toxicological studies that are informative but do not meet contemporary standards, it might be possible to compensate for inadequacies by increasing the safety factor used in establishing the HBGV. Importantly, however, it is emphasized that a lack of any information about a potential toxicological hazard cannot be compensated for in this way.

When JECFA relies on "non-standard" approaches to hazard characterization of a veterinary drug (such as those described above), the Committee report should include sufficient detail to explain the lines of evidence and the scientific basis for the conclusions reached. As described below, the report should include explicit consideration of the sources of uncertainty in the assessment. The report should identify the type of information and data that could be helpful for refining its assessment. In cases in which the deficiencies were such that it was not possible to establish an ADI (or ARfD, if necessary), the information required for this purpose should be indicated and should be specified in terms of toxicological domains (e.g. carcinogenic potential, potential to impair development) rather than indicating specific studies (e.g. 2-year bioassay in rats, developmental toxicity study in rabbits).

Individual sponsors may be reluctant to generate data on veterinary drugs for which they do not have exclusive commercial rights. Nevertheless, data adequate to enable establishment of HBGVs and to recommend appropriate MRLs is essential. Hence, when the available data are deficient, establishment of consortia to provide the necessary resources to generate the required data is encouraged. Groups that might be considered include drug sponsors, government agencies and manufacturers' groups or associations. In addition, when information is known to exist, for example from exploratory clinical studies or from a previous major producer, best endeavour should be used to acquire the information, including purchasing it, if necessary, for submission to JECFA.

A1.4.4 Health-based guidance values

A tADI is determined by the application of a suitable safety factor (more appropriately known as an uncertainty factor) to the lowest, toxicologically

28 Primary pharmacology is the study of the biological effect produced by interaction with the therapeutic target for the drug.

relevant NOAEL or BMDL (POD). The mADI is determined from the MIC_{calc} or NOAEC or NOAEL, with application of safety factors, as appropriate. The ADI for a compound is established as the lower of the tADI and the mADI and is the quantity of residues that can be ingested daily over a lifetime by a consumer with no appreciable health risk.

The ADI is not a suitable HBGV for assessing the risks of acute (≤ 24 h) exposure to residues of veterinary drugs, as safety assessed with the ADI is based on the assumption of regular exposure at the upper bound of the ADI every day over a lifetime. A suitable HBGV, reflecting the potential acute effects of a compound, is the ARfD. Just as there could be a toxicological or microbiological basis for the ADI, this is also the case for the ARfD. Hence, the ARfD for a compound is established as the lower of the tARfD and the mARfD. Details of establishing ARfDs for veterinary drugs are provided by WHO *(16)*.

The default value of the safety factor used to establish a tADI (or tARfD) from a NOAEL or BMDL from a study in laboratory species is 100. This incorporates allowance for uncertainty and variation in extrapolating from an experimental species to sensitive humans. The default factors for these are 10 for interspecies differences and 10 for interindividual variation within the human population, the product of which gives a combined factor of 100 (10 × 10).

On occasion, additional factors may be used, for example to extrapolate from a LOAEL to a NOAEL, from subchronic to chronic exposure, for the severity of the endpoint and for incompleteness of the database. This will be particularly important in nonstandard assessments, in which there may be incomplete but sufficient data to complete the assessment.

If specific quantitative information is available on interindividual variation or interspecies differences in toxicokinetics or toxicodynamics, it might be possible to use this to determine chemical-specific adjustment factors, which can be used to replace some of the default factor of 100 *(4,17,18)*.

Establishment of HBGVs can be considered as the final stage of hazard characterization.

A1.4.5 Threshold of toxicological concern (TTC)

The goal of most risk assessments is to establish a safe intake level for a chemical. The TTC concept refers to establishment of a level of exposure for any chemical, below which there would be no appreciable risk to human health *(19,20)*. A major advantage of the TTC concept is that it provides a method for ensuring public health protection in the absence of substance-specific data on toxicity.

The TTC concept has been refined over the past three decades. A full description is available *(21)*. A decision tree is used to determine the appropriate TTC value for a given chemical from its structure. Some structural classes are excluded from the TTC approach, including those that contain particularly potent

genotoxins (aflatoxin-like, *N*-nitroso and azoxy compounds), bioaccumulative compounds (dioxin-like) and nonessential metals. Compounds are then categorized as potential DNA-reactive genotoxins according to structural alerts (in silico), organophosphates or carbamates, or Cramer class III, II or I based on the scheme of Cramer et al. *(22)*.

JECFA (food additives) developed a decision tree for assessing flavours, which is based on the TTC concept, with the TTC values for the three Cramer structural classes *(23)*, which was updated at its eighty-second meeting *(24)* to reflect the conclusions of a European Safety Authority/WHO workshop in 2014 *(21)*. JECFA (food additives) excludes potentially genotoxic compounds, based on structural alerts, from further consideration. The remaining compounds are allocated a TTC of 90, 540 or 1800 µg/day, depending on whether they are in Cramer class III, II or I, respectively.

JMPR has adopted the TTC approach as one means for assessing metabolites of pesticides present as residues in food. In this case, the full scheme is used, so that, after exclusion of compounds to which the scheme is not applicable, TTC values for potentially DNA-reactive genotoxins, potential inhibitors of acetyl cholinesterase and Cramer class III, II and I compounds are allocated as appropriate. Again, this is based only on structural considerations.

A few issues should be considered before using the TTC approach for assessing residues of veterinary drugs. First, there are considerations in relation to exposure. As, in the TTC approach, human exposure threshold values are compared with dietary exposure data, sound estimates of human dietary exposure are required, and consideration must be given to acute versus chronic exposure. Recent developments in the approach used by JECFA for dietary exposure assessment should provide such estimates. Secondly, the chemical nature of the compounds considered by JECFA should be considered and whether they fit into the structural classes described above. The Cramer classification is based on the concept that specific structural features dictate toxicological potency. Assessments might be required to investigate the suitability of these structural classes for veterinary drugs by analysing the toxicity data for such compounds. Of note and of relevance for residues of veterinary drugs in food, the decision tree is based on compounds for which exposure was only by the oral route. Some veterinary drugs are administered parenterally.

A1.5 Characterization of exposures

A1.5.1 Estimation of dietary exposure

JECFA uses the GEADE and the GECDE, details of which can be found in EHC 240, updated chapter 6: Dietary exposure assessment for chemicals in food *(6)*.

A1.5.2 Dual-use compounds

Compounds used as both a pesticide and a veterinary drug are known as "dual-use" compounds. Dietary exposure to these compounds is frequently assessed separately for each use, although, in practice, consumers may be exposed to residues from both uses. If individual dietary exposure assessments are available for each use, risk managers may develop management options without considering combined dietary exposure to such chemicals.

Estimates of dietary exposure by JECFA for dual-use compounds are based on dietary exposure from both uses. In many cases, the data available on residues and exposure from pesticide use are limited for estimating exposure to veterinary drug residues (especially if the compound has been used as a pesticide for a long time). When there are such uncertainties and data gaps, the dietary exposure assessment must be based on conservative assumptions. In these situations, it is in the best interest of sponsors to provide as much information as possible, so that the assessor can obtain a complete picture of usage and residues.

For compounds to which there is already high potential dietary exposure from use as pesticides, the high baseline may affect recommendation of veterinary drug MRLs. The risk assessors will provide risk managers with enough information to enable establishment of MRLs for substances used for both applications that are adequately protective of public health. It is in the best interest of sponsors to provide information about current usage and residues from pesticide use when this information may not be readily available or is outdated.

A1.5.3 Dietary exposure assessment in the absence of a HBGV

In the absence of a HBGV, dietary exposure can still be estimated for a preliminary risk assessment and may be used to characterize risk with approaches such as TTC (e.g. evaluation of the 4-chloroaniline metabolite of diflubenzuron *(25)*) and MOE.

A1.5.4 Dietary exposure assessment with limited or no data on residues

When there are limited or no data on residues, an exposure assessment can still provide some insight into the potential health impacts of residues.

JECFA may explore a range of scenarios based on assumptions to account for uncertainties due to data gaps. For example, if there is a high level of uncertainty regarding the MR:TR, dietary exposure can be calculated with more conservative ratios (e.g. clopidol evaluation, this report). Other assumptions and scenarios may be used to provide data for exposure estimates. Dietary exposure assessments that are based on highly conservative assumptions and yet do not indicate any public health concern can be the basis of recommendations to risk managers. If, however, the conservative assumptions lead to exceedance of the

HBGV, further refinement may be required.

Another possibility is use of LODs or LOQs as a proxy for residue data. This approach can be particularly useful when the acceptable withdrawal period is extended to a point at which it is highly unlikely that any residues remain (e.g. evaluation of fumagillin DCH, this report).

A further option is a back-calculation approach. In this situation, if the amount consumed of the food of interest is known, it can be combined with the HBGV to estimate the residue concentration equivalent to a target exposure, for example, < 100% of the HBGV (e.g. DCH in the fumagillin DCH evaluation, this report). Another option is to assume a residue concentration and calculate the amount of food that would have to be consumed to reach the HBGV. Comparison with the typical consumption of the food then indicates whether there are realistic scenarios that could pose a public health concern.

A1.5.5 Sources of food consumption data

The food consumption of a population can be estimated in surveys, ranging from the most granular individual level (individual dietary surveys) to approximations at national level from food supply data, providing annual estimates of the national availability of food commodities. The quantities of the food supply provide an average estimate of food available per capita for consumption over a specified reference period (typically 5 years) but cannot provide information for subpopulations by age and/or gender. Per-capita data are used to estimate population average consumption over a lifetime. In contrast, individual dietary surveys provide information on inter-individual variation in food consumption in well-defined groups of individuals. Dietary surveys are preferably conducted over an entire year to cover seasonal variations, while consumption data for individuals in such a survey are collected over short periods, generally 2–7 consecutive or nonconsecutive days.

The food consumption dataset that has been used by JECFA since 2011 is the FAO/WHO CIFOCOss *(26)*. To be included in CIFOCOss, data must have been collected on at least two non-consecutive days per subject to account for intra-individual variation in consumption. Data collected on only a single day per subject are considered inappropriate for chronic dietary exposure assessment, and their use is limited to estimation of acute dietary exposure.

It should be noted that consumption data may be limited for some foods, such as foods from minor species. In these cases, the dietary exposure expert will select the consumption value of a similar food or aggregate of foods (e.g. consumption of all mammalian meat).

Sometimes, no consumption data are available. For example, residues may accumulate in beeswax (e.g. evaluation of flumethrin *(27)*). While data on

wax consumption are not usually reported in food consumption surveys, since it is not consumed on its own and/or people may not realise that they are consuming it, it is present in raw honey and honeycomb and is widely used as a food additive. Dietary exposure experts use a variety of sources to create realistic exposure scenarios case by case. Often, sponsors will have detailed knowledge about the end-use of their products that can be helpful in making realistic assumptions and informing a better assessment of dietary exposure.

A1.5.6 Considerations in use of the TTC approach for dietary exposure

Use of the TTC approach (see above) depends on reliable estimates of dietary exposure. These may be based on a worst-case scenario or a plausible high level of consumption. Use of scenarios (upper, mid and lower bounds) can be a useful approach for further understanding of exposure scenarios.

A1.5.7 Other dietary exposure scenarios

While assessment of chronic exposure to residues of most veterinary drugs assessed by JECFA is based on median residue concentrations as an input, this is not appropriate or possible in some situations. Similarly, for acute exposure, the upper one-sided 95% confidence limit over the 95th percentile residue concentration may not be the most suitable in some cases.

A special case is the infrequent (but plausible) consumption of injection-site muscle residues, in which acute exposure is of interest (e.g. evaluation of ivermectin *(28)*). JECFA will develop a plausible model of the presence of residues at the injection site to provide a concentration (or concentrations) that can be used as an input into a modified GEADE, that in an acute exposure scenario.

Other exceptional cases include compounds not approved as veterinary drugs but evaluated as contaminants (e.g. malachite green) for which no MRL will be recommended, substances with no major sponsor and substances for which the data package is insufficient to support recommendation of MRLs.

A1.5.8 Characterization of risk

Margin of safety (MOS)

For compounds for which HBGVs (ADI and/or ARfD) can be established, risk is usually characterized by comparing the HBGV with an appropriate estimate of exposure. This has been termed the "margin of safety" (MOS).

Thus: MOS = HBGV (ADI or ARfD)/estimate of exposure.

An MOS < 1 would raise concern about the adequacy of consumer protection. The MOS should be calculated for all subpopulations of potential concern.

In the MOS approach, uncertainty and variation are implicit in the value established for the HBGV. The primary utility of the MOS is in benchmarking actual exposure estimates with respect to the HBGV. JECFA may be requested to provide advice on the consequences of scenarios other than GVP, such as environmental exposure to a veterinary drug as a contaminant or due to specific misuse or abuse. If a HBGV existed or could be established, the MOS would be of value in formulating such advice.

Interpretation of the MOS requires consideration of the toxicological/microbiological basis of the HBGV, how steep the dose–response curve is, the anticipated duration of exceedance of the HBGV, the basis of the safety factors used to establish the HBGV, and the margin by which the MOS is < 1.

Margin of exposure (MOE)

Reporting a HBGV to a risk manager is a strong statement of confidence in the database. It implies that the database is sufficiently robust and comprehensive that all relevant end-points are covered by the HBGV reported, if necessary with application of additional safety factors. There may be situations in which the data have key deficiencies or uncertainties such that a HBGV cannot be established with confidence. Nevertheless, the risk manager may still require advice. In such instances, on a case-by-case basis and after discussion with the risk manager as appropriate, JECFA may report a MOE.

An alternative situation is a compound that is genotoxic and carcinogenic. While such substances would not usually be acceptable for use as veterinary drugs if they give rise to detectable residues in human food, in some situations JECFA is requested to provide advice after exposure to such compounds in the diet. Examples are illegal use, former use leading to residues as contaminants, or impurities or metabolites in drugs that are not themselves genotoxic or carcinogenic. JECFA (additives and contaminants) at its sixty-fourth meeting *(29)* made recommendations on use of the MOE approach for this purpose and applied it to several such compounds in order to provide advice.

The MOE is derived by using the ratio of the POD (e.g. NOAEL, $BMDL_{10}$) to an estimate of the exposure of a high consumer, for both acute and chronic exposure scenarios. For compounds that are genotoxic and carcinogenic, no threshold in their dose–response relation can be assumed, and use of an NOAEL is considered inappropriate (although this would be possible for calculating an MOE). For such compounds, a BMDL is determined, or, if this is not possible, the T_{25} (the chronic daily dose in mg/kg bodyweight that will give 25% of animals tumours at a specific tissue site, after correction for spontaneous incidence, over the standard life span of that species) *(30)* may provide an alternative POD. The suitability of the T_{25} for a given data set should, however, be considered case by

case, and the BMDL, if the data allow, is preferred.

When the toxicological database is incomplete, the most sensitive end-point may not have been evaluated. When MRLs cannot be recommended, exposure should be estimated from the available data on residues with the same principles used for deriving an MRL, to the extent possible. The MOE is then reported as a numerical value, with a narrative emphasizing the applicability of the MOE, for example with respect to duration of exposure, type of end-point and nature of the diet, to reflect the limitations in the database used in its derivation. Interpretation of the MOE should also be discussed. In general, the acceptability of an MOE is based on considerations similar to those used in establishing a HBGV. When a MOE is based on data for experimental animals, as a default, a MOE of at least 100 would be considered acceptable for effects with a biological threshold. For effects for which it is considered that a threshold cannot be identified (e.g. genotoxicity), JECFA (additives and contaminants) has suggested that a MOE > 10 000 would be of low concern. In interpreting the MOE, consideration should be given to all relevant factors, including the conservatism of assumptions, the completeness of the database (whether all potentially relevant end-points have been assessed), whether the response might be considered to show a biological threshold and whether residues arise through permitted use, inadvertently or unavoidably. These factors should be clearly described in the report.

JECFA will report a MOE only when the deficiencies in the database are such that a HBGV cannot be established with confidence or when the nature of the end-point is such that establishment of a HBGV is inappropriate. Hence, a MOE provided by JECFA should not be considered a reliable estimate of the acceptability of exposure; however, values of the MOE well above the "target" value can provide some assurance to risk managers. The MOE is invaluable in evaluating the effectiveness of risk reduction strategies, for comparing strategies and for assessing the consequences of misuse or abuse of a veterinary drug.

Level of protection (LoP)

The LoP is the percentage of the population whose estimated exposure is below a HBGV. The LoP is valuable for exploring the consequences of different exposure assumptions or risk management options.

The Committee usually uses a deterministic approach in establishing HBGVs and point estimates of exposure for risk assessment of residues of veterinary drugs. Estimation of the LoP requires construction of distributions of hazard and/or estimated exposures, based on individual toxicity predictions and/or actual consumption data. Residues data could be realistic, worst case or actual, for example from routine monitoring studies, depending on the question

addressed. The choice of centile for the LoP is a risk management decision, and there has been no discussion to date about what a suitable value might be for exposure of global subpopulations. At national level, values >99% are often used, such as 99.9% or 99.99%. To use the LoP, JECFA would require adequate information on regional consumption patterns of food likely to contain residues of veterinary drugs.

Qualitative estimates

In providing guidance to risk managers on estimates of risk, it is recognized that there may be circumstances in which neither a HBGV nor some other quantitative estimate of risk can be determined. Nevertheless, it may be possible to inform risk management decisions with qualitative estimates of risk (e.g. unlikely, appreciable concern).

A1.6 Maximum residue limits

A1.6.1 Recommendation of MRLs

MRLs for veterinary drugs are the maximum concentrations of residues permitted in or on a food. To calculate and recommend MRLs, information must be available on the nature and disposition of residues.

MRLs can be derived only once the following processes have been completed: establishment of HBGVs, characterization of residues in tissues, determination of the MR, analytical method(s) available for monitoring, and determination of MR:TR.

To characterize the residues in food after administration of a veterinary drug, metabolism studies are conducted in the target (food) animal species, typically with radiolabelled drugs. The total residues in edible tissues are determined from total radioactivity, and individual residue components (parent + metabolites) are quantified, usually by LC-MS/MS or radiometric profiling techniques. The major residue components (≥10% of TRR) are identified (i.e. their structure determined). The individual metabolites may also be characterized according to toxicological or microbiological potency, if known (i.e. potency relative to that of the parent compound). The drug's metabolic profile is compared in laboratory and target food animal species to ensure that no additional metabolites of concern are present only in food animal tissues.

A MR is determined for regulatory monitoring purposes. The MR may be the parent compound, a metabolite, or some combination thereof.

The MR:TR is determined in each edible tissue. In some cases, certain components of the total residue do not pose a risk to human safety. This may be because some metabolites are biologically inactive or are not bioaccessible or bioavailable in the human gastrointestinal tract (e.g. bound tissue residues) *(31)*.

In such cases, a "residue of concern" excluding the inactive or bound components, is defined in lieu of "total residues" *(32)*. In many evaluations, a significant amount of data is required to adequately characterize the MR:TR, as ratios may differ between edible tissues and within the same tissue over time since final treatment.

As a radiolabel study may be performed with an alternative formulation, dose or animal class than those intended for the final drug product, a nonradiolabel MR depletion study is also performed to examine residues under field-use conditions and GVP. Regression analysis is used to estimate the rate of residue depletion in each tissue. The median MR concentrations are determined over time since last drug administration. The median total residue (or residue of concern) is then estimated over time, based on the median MR concentration divided by the appropriate MR:TR for that specific tissue and time point.

MRL derivation begins by estimating human dietary exposure to drug residues from animal-derived foodstuffs from estimates of drug residue concentrations and amounts of foodstuffs consumed. At various times after the last drug administration (consistent with the range of approved withdrawal periods of the drug product in various Member States), the median total residue is determined for each edible tissue. Combined dietary exposure to the drug residue (GEADE and GECDE) is estimated from estimates of acute and chronic human food consumption for each edible tissue *(31)*.

Estimated human dietary exposure to drug residues at such withdrawal times is compared with the relevant HBGVs (ADI and/or ARfD). If the dietary exposure estimates are lower than the relevant HBGVs (i.e. safe for human consumption), potential MRLs can be calculated for that tissue. The MRL is derived from the upper limit of the one-sided 95% confidence interval over the 95th percentile of MR concentrations (95/95 UTL). The proposed MRLs must be suitably health-protective and considerate of international trade implications. In uncommon cases, exposure estimates derived from the range of approved withdrawal times may be higher than the HBGVs. In this situation, JECFA will either note that GVP (withdrawal periods) should be updated or that MRLs cannot be recommended.

A1.6.2 Recommendation of MRLs when there is an incomplete data package

If JECFA cannot establish a suitable HBGV (i.e. no ADI/ARfD), an MRL cannot be derived. Even when suitable HBGVs have been established, deficiencies in residue data may obviate derivation of MRLs.

When there are deficiencies in residue data (such as lack of suitable radiolabelled studies for determining total residues, insufficient characterization of metabolites or suboptimal time points in residue depletion studies), alternative approaches may be considered. These may include extrapolation of

residue depletion regression lines, combination of residue depletion data from several studies, or weighted residue modelling approaches. When the TR data are limited or do not allow robust estimates of MR:TR, alternative approaches include use of conservative (low) MR:TR estimates for predicting total dietary exposure (see section 5). When data from a MR depletion study preclude use of traditional methods to derive MRL values (i.e. regression for generating UTLs), other approaches may be used. Specific examples include calculating a single-timepoint upper tolerance limit, recommending MRLs based on the LOQ of the analytical method, or extrapolation of potential residue concentrations from other species. Alternative approaches used to assess residue concentrations are shown in Table A1.1.

Table A1.1.
Selected examples of JECFA evaluations for which alternative residue assessment approaches were used

Alternative approach	Compound	Reference
Data from multiple residue depletion studies combined	Ethion	*(27)*
	Fumagillin DCH (fish)	This report
Conservative MR:TR estimates	Halquinol	*(25)*
	Clopidol	This report
Single-time point UTL	Nicarbazin	*(28)*
	Clopidol	This report
MRL based on LOQ	Fumagillin DCH	This report
	Colistin	*(11)*

A1.7 Identification of strengths and weaknesses in a risk assessment

Identification and evaluation of the assumptions and sources of uncertainty in a risk assessment are important to ensure transparency and promote consistency in risk assessment. The Codex Working Principles for Risk Analysis *(33)* state that:

> Constraints, uncertainties, and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner. Expression of uncertainty or variability in risk estimates may be qualitative or quantitative but should be quantified to the extent that is scientifically achievable.

It is clearly not feasible, or indeed necessary, to quantify all sources of variation and uncertainty in a risk assessment. When qualitative consideration of a source of uncertainty provides sufficient confidence for risk managers to reach a decision

(e.g. the assessment is clearly conservative), quantitative assessment would be unnecessary. A flexible, tiered approach to evaluation of uncertainty is therefore recommended:

- All identifiable sources of uncertainty should be reported.
- All of these should be evaluated qualitatively.
- When uncertainty remains about their qualitative impact on the assessment, the sources of uncertainty should be quantified to the extent necessary to provide sufficient reassurance to enable risk management decisions.

JECFA acknowledges that atypical assessments, such as for "old" drugs, are more uncertain than those made with up-to-date data packages. Hence, a clear description of the uncertainties in such assessments and their probable impact on the Committee's conclusions is particularly important. More information on the assessment of uncertainty is provided in EHC 240 *(4)*.

A1.8 Conclusion

JECFA will continue to refine the processes and approaches it uses to assess residues of veterinary drugs for which there are incomplete data packages.

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Annex 2

Recommendations on compounds on the agenda

Clopidol (coccidiostat)

Acceptable daily intake	The Committee established an ADI for clopidol of 0–0.04 mg/kg bw based on a lowest-observed-adverse-effect level (LOAEL) of 40 mg/kg bw per day for decreased maternal body weight gain and fetal body weight in a developmental toxicity study in rats. An uncertainty factor of 1000 was applied, which comprises 100 for interspecies and intraspecies differences and additional factors of 2 to account for the use of a marginal LOAEL and 5 for database uncertainty.
Acute reference dose	The Committee concluded that, in view of the low acute oral toxicity of clopidol and the absence of developmental toxicity or any other toxicological effects likely to be elicited by a single dose, it was unnecessary to establish an ARfD for clopidol.
Estimated chronic dietary exposure	For clopidol included at 250 mg/kg in feed at 24 hour withdrawal and the most conservative ratio of marker residues to total residues (MR:TR) considered of 0.5, the global estimates of chronic dietary exposure (GECDEs) are: for adults and the elderly 32.9 µg/kg bw per day. for children and adolescents 33.5 µg/kg bw per day. for infants and toddlers 28.6 µg/kg bw per day. (representing 82%, 84% and 71%, respectively, of the upper bound of the ADI of 40 µg/kg bw)
Residue definition	The marker residue for clopidol in chicken liver, kidney, muscle and skin/fat is clopidol.
Maximum residue limits	The Committee recommended MRLs of 10 400 µg/kg in liver, 8800 µg/kg in kidney, 4100 µg/kg in muscle and 2600 µg/kg in skin/fat of chickens.

Fumagillin dicyclohexylamine (mycotoxin)

In veterinary medicine, fumagillin is administered only as the dicyclohexylamine (DCH) salt. As the fumagillin DCH salt dissociates into the two moieties, consumers would be exposed to residues of both. The Committee evaluated both fumagillin and DCH.

Acceptable daily intake	The Committee established an ADI for fumagillin of 0–0.003 mg/kg bw based on a NOAEL of 1.73 mg/kg bw per day for decreased body weight gain in a 13-week study in rats and for post-implantation loss, decreased fetal body weight and associated morphological changes in a developmental toxicity study in rats at 4.32 mg/kg bw per day. A safety factor of 500 was used, which comprised 100 for interspecies and intraspecies differences and an additional factor of 5 for database uncertainty. The Committee established an ADI for DCH of 0–0.02 mg/kg bw based on a NOAEL of 10 mg/kg bw per day for haematological and clinical chemistry changes at 30 mg/kg bw per day in a 13-week toxicity study in rats. A safety factor of 500 was used, which comprised 100 for interspecies and intraspecies differences and an additional factor of 5 for database uncertainty.
Acute reference dose	The Committee concluded that it was unnecessary to establish an ARfD for fumagillin. It established an ARfD of 0.7 mg/kg bw for DCH based on the NOAEL of 70 mg/kg bw per day for clinical signs and mortality after 4 days at 200 mg/kg bw per day in a 28-day toxicity study in rats. A safety factor of 100 was used to allow for interspecies and intraspecies differences.
Residue definition	The marker residue for fumagillin DCH in fish fillet is fumagillin.
Estimated dietary exposure	Based on potential fumagillin residues in fish fillet and honey, the global estimates of chronic dietary exposure (GECDEs) are: for adults and the elderly 0.06 µg/kg bw per day.

	for children and adolescents 0.10 µg/kg bw per day. for infants and toddlers 0.11 µg/kg bw per day. (representing 2%, 3% and 4%, respectively, of the upper bound of the ADI of 3 µg/kg bw)
Maximum residue limits	The Committee recommended an MRL in fish fillet of 10 µg/kg for the MR 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 < 1000 µg/kg, 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 would need to be developed. The Committee recommended an MRL in honey of 20 µg/kg for the MR DCH.

Imidacloprid (neonicotinoid parasiticide)

Acceptable daily intake	The Committee established an ADI of 0–0.05 mg/kg bw, based on a NOAEL of 5.25 mg/kg bw per day for decreased body weight gain in an extended one-generation reproductive toxicity study in rats, with application of a safety factor of 100 to allow for interspecies and intraspecies differences.
Acute reference dose	The Committee established an ARfD of 0.09 mg/kg bw based on a $BMDL_{05}$ of 9 mg/kg bw for acute neurobehavioural effects in rats and a safety factor of 100 to allow for interspecies and intraspecies differences.
Residue definition	The marker residue for imidacloprid in fin fish is the parent molecule, imidacloprid.
Estimated dietary exposure	For Atlantic salmon only, the global estimates of chronic dietary exposure (GECDEs) are: for adults and the elderly 1.0 µg/kg bw per day. for children and adolescents 2.7 µg/kg bw per day. for infants and toddlers 0.9 µg/kg bw per day. (representing 2%, 5% and 2%, respectively, of the upper bound of the ADI of 50 µg/kg bw) For all fin fish, the global estimates of chronic dietary exposure (GECDEs) are: for adults and the elderly 1.8 µg/kg bw per day. for children and adolescents 3.8 µg/kg bw per day. for infants and toddlers 1.2 µg/kg bw per day. (representing 4%, 8% and 2%, respectively, of the upper bound of the ADI of 50 µg/kg bw) The GEADE, based on consumption of Atlantic salmon, was 7% of the ARfD for adults and children (6.2 and 6.6 µg/kg bw, respectively); the GEADE for all fin fish was 38% and 26% of the ARfD (34.1 and 23.8 µg/kg bw) for adults and children, respectively.

Maximum residue limits	The Committee recommended an MRL for Atlantic salmon and rainbow trout fillet (muscle with skin in natural proportions) and/or muscle of 600 µg/kg. It further recommended that the MRL be extrapolated to all fin fish.

Annex 3

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).

2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).

3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).

4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).

5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).

6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).

7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).

8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).

9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38 A, 1965; WHO/Food Add/24.65 (out of print).

10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40 A, B, C; WHO/Food Add/67.29.

13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.

15. Toxicological evaluation of some flavouring substances and non nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44 A, 1968; WHO/Food Add/68.33.

16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.

17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.

18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45 A, 1969; WHO/Food Add/69.34.

19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.

20. 20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46 A, 1970; WHO/Food Add/70.36.

21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.

22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48 A, 1971; WHO/Food Add/70.39.

24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.

25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.

26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some

antioxidants (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50 A, 1972; WHO Food Additives Series, No. 1, 1972.

28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.

30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51 A, 1972; WHO Food Additives Series, No. 4, 1972.

32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).

33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53 A, 1974; WHO Food Additives Series, No. 5, 1974.

34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.

35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54 A, 1975; WHO Food Additives Series, No. 6, 1975.

37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.

38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances. (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.

39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55 A, 1975; WHO Food Additives Series, No. 8, 1975.

40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series,

No. 599, 1976.

42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.
43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.
46. Specifications for identity and purity of some food additives, including antioxidant, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.
49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.
50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.
52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.
53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.
55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.
56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.
58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.
59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.
61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.

65. Guide to specifications—General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.

66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.

67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.

68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.

69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.

70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.

71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.

72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.

73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.

74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.

75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.

76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.

77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.

78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.

79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.

80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23.

Cambridge University Press, 1988.

82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.
83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.
87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.
90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.
91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.
93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.
96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. Toxicological evaluation of certain veterinary residues in food. WHO Food Additives Series, No. 29, 1991.
99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. Guide to specifications—General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Ref. 2, 1991.
101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.

103. Compendium of food additive specifications: addendum 1. FAO Food and Nutrition Paper, No. 52, 1992.

104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.

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Annex 4

List of participants

Ninety-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives
Rome, 20–29 February 2024

FAO members

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Dr Vittorio Fattori, Agrifood Systems and Food Safety Division, FAO, Rome, Italy (*FAO JECFA Secretary*)

Ms Elisabeth Heseltine, France (*WHO editor*)

Ms Ngai Yin Ho, Department of Nutrition and Food Safety, WHO (*WHO Consultant*)

Dr Markus Lipp, Agrifood Systems and Food Safety Division, FAO (*FAO Secretariat*)

Mr Soren Madsen, Department of Nutrition and Food Safety WHO (*WHO JECFA Secretary*)

Dr Keya Mukherjee, Agrifood Systems and Food Safety Division, FAO, Rome, Italy

Annex 5

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

NINETY-EIGHTH JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)

Rome, Italy, 20–29 February 2024

1. Opening
2. Declarations of Interests
3. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
4. Adoption of Agenda
5. Matters of interest arising from previous Sessions of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)
6. Critical issues and questions from working papers (first brief round of discussion on all subjects to inform the full Committee)
7. Evaluations
   Veterinary drug residues
   - Clopidol
   - Ethoxyquin
   - Fumagillin
   - Imidacloprid
8. General considerations
9. Other matters as may be brought forth by the Committee during discussions at the meeting.
10. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain veterinary drug residues in food
Ninety-fourth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No.1041, 2022.

Evaluation of certain veterinary drug residues in food
Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No.1023, 2019.

Evaluation of certain veterinary drug residues in food
Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 997, 2016.

Evaluation of certain veterinary drug residues in food
Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 988, 2014.

Evaluation of certain veterinary drug residues in food
Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 911, 2002.

Pesticide residues in food – 2001 Joint FAO/WHO meeting on pesticide residues, Evaluations 2001. Part II – Toxicological. World Health Organization, Geneva (2002).
WHO/PCS/02.1. ISBN 92 4 166517 3

Evaluation of certain veterinary drug residues in food
Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 888, 1999.

Evaluation of certain food additives and contaminant
Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 859, 1995.

Evaluation of certain veterinary drug residues in food
Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 832, 1993.

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Evaluation of certain veterinary drug residues in food

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of residues of certain veterinary drugs in food and to recommend maximum levels for such residues in food. The first part of the report considers general principles regarding the evaluation of residues of veterinary drugs within the terms of reference of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It covers topics such as the parallel review process; estimation of dietary exposure to veterinary drug residues; a risk-based decision tree approach for safety evaluation; assessment of the potential effects of residues on the human intestinal microbiome. Summaries follow the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: two antiparasitic agents (imidacloprid, ivermectin) and one coccidiostat (nicarbazin). Additionally, further evaluation of the parasiticide selamectin is included as part of a pilot in support of the proposed parallel review process. Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes and proposed maximum residue limits.

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