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1041

Evaluation of certain veterinary drug residues in food

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

Food and Agriculture Organization of the United Nations

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World Health Organization

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(WHO Technical Report Series, No. 1041)

ISBN (WHO) 978-92-4-005758-6 (electronic version)

ISBN (WHO) 978-92-4-005759-3 (print version)

ISBN (FAO) 978-92-5-136900-5

ISSN 0512-3054

Suggested citation. *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. World Health Organization and Food and Agriculture Organization of the United Nations, 2022. Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data.
CIP data are available at http://apps.who.int/iris.

Dr Carl Cerniglia (1948–2021)

Food and Drug Administration of the United States of America

It was with great sadness that the Committee noted the passing of Dr Carl Cerniglia.

Carl was a long-standing Member of the Committee, and he played a key role in establishing the procedures for safety assessment that are used by the Committee and around the world. Carl's contribution to food safety risk assessment, and in particular to the work of JECFA, is without equal. Under his technical leadership JECFA pioneered the evaluation of microbiological effects of veterinary drug residues, which laid the foundation for this type of assessment at national and international level. Carl's contribution to the work of JECFA over the years is unique and was the foundation of solid, objective and consistent assessments.

His always-positive attitude and smile helped the Committee navigate through many difficult agendas. His warm personality, bright mind and great sense of humour will always be remembered.

Carl will be deeply missed by his peers and friends in the scientific community.

In recognition of his services, the Committee dedicates this report to the memory of Dr Carl Cerniglia.

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Virtual meeting, 16–27 May 2022

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List of abbreviations

AD	administered dose
ADI	acceptable daily intake
ALP	alkaline phosphatase
ALT	alanine transaminase
ARfD	acute reference dose
AUC	area under the concentration–time curve
BMD	benchmark dose
BMDL	lower 95% confidence limit on the benchmark dose
$BMDL_{05}$	BMDL for a 5% response over the controls
bw	body weight
CalEPA	California Environmental Protection Agency
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CI	confidence interval
CIFOCOss	FAO/WHO Chronic Individual Food Consumption Database – Summary statistics
C_{max}	maximum concentration
CNS	central nervous system
CVM	U.S. FDA Centre for Veterinary Medicine
DNC	4,4'-dinitrocarbanilide
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
equiv.	equivalent
EU	European Union
F0	parental generation
F1	first filial generation
FAO	Food and Agriculture Organization of the United Nations
FOB	functional observational battery
GABACls	γ-aminobutyric acid chloride channels
GD	gestation day
GEADE	global estimate of acute dietary exposure
GECDE	global estimate of chronic dietary exposure
GL36	Guideline 36 (VICH)
GLP	good laboratory practice
Glu-Cl	glutamate-gated chloride channel
GVP	good practice in the use of veterinary drugs
H_2B_{1a}	22,23-dihydroavermectin B_{1a}; ivermectin B_{1a}
H_2B_{1b}	22,23-dihydroavermectin B_{1b}; ivermectin B_{1b}
HBGV	health-based guidance value
HDP	2-hydroxy-4,6-dimethylpyrimidine

HPLC	high-performance liquid chromatography
HPLC-FLD	HPLC with fluorescence detection
HPLC-UV	HPLC with ultraviolet detection
ISO	International Organization for Standardization
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	liquid chromatography with mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
M4NPC	methyl-(4-nitrophenyl)carbamate
mADI	microbiological ADI
mARfD	microbiological ARfD
MOA	mode of action
MR	marker residue
MRL	maximum residue limit
MR:TRR	marker residue to total recovered radioactivity ratio
MRM	multiple reaction monitoring
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PNA	*p*-nitroaniline
PND	postnatal day
RIDA	reverse isotope dilution assay
RIVM	The Netherlands National Institute for Public Health
SD	standard deviation
tADI	toxicological acceptable daily intake
tARfD	toxicological acute reference dose
TRR	total recovered radioactivity
TSH	thyroid-stimulating hormone
TTC	threshold of toxicological concern
U.S EPA	United States Environmental Protection Agency
UTL	upper tolerance limit
UHPLC-MS/MS	ultra-high performance liquid chromatography-mass spectroscopy
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
v/v	volume per volume
WHO	World Health Organization
w/v	weight per volume

Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

- *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 85.

Residue monographs are issued separately by FAO under the title:

- *Residue evaluation of certain veterinary drugs*. FAO JECFA Monograph No. 28.

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1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a virtual meeting (see below in 1.1 Procedural matters) from 16 to 27 May 2022.

Twenty-three meetings of the Committee had been held to consider vetinerary residues in food (Annex 1, references *80, 85, 91, 97, 104, 110, 113, 119, 125, 128, 134, 140, 146, 157, 163, 169, 181, 193, 208, 217, 226, 236* and *243*) in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (*1*). The present meeting[1] was convened to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were to:

- elaborate further on principles for evaluating the safety of residues of veterinary drugs in food, for establishing acceptable daily intakes (ADIs) and acute reference doses (ARfDs) and for recommending maximum residue limits (MRLs) for such residues when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (GVP);
- evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2).

1.1 Procedural matters

Owing to the travel restrictions and lockdowns due to the COVID-19 pandemic in many countries, it was not possible to convene a physical meeting and it was instead decided to hold it online by videoconferencing. In view of the time differences in the countries of residence of the invited experts, the only possible time for a videoconference was restricted to a 3.5-hour time slot (12:00–15:30 CET) each day. This truncated daily meeting time limited the number of compounds that could reasonably be assessed by the Committee at the present meeting.

All participating experts reaffirmed that online meetings did not permit the necessary in-depth, robust scientific discussions that have been a characteristic of past JECFA physical meetings and therefore were not a suitable substitute. In particular, the experts felt that the online format did not foster the atmosphere of inclusiveness, engagement and openness that has marked all JECFA physical meetings. The experts considered that the success of the ninety-fourth meeting

1 As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been ninety-three previous meetings of JECFA (see Annex 1).

was mainly due to the cohesion between them, which stemmed from the trust built on the relationships they had formed during previous face-to-face meetings. The experts also commented on the significant difficulty of holding any informal meetings outside the scheduled meeting times because of the widely differing time zones. They noted that such informal interactions during the physical meetings were instrumental in solving problems and discussing issues in depth, bilaterally or in small groups, and added that such informal settings often gave rise to equitable solutions to difficult problems. These informal interactions are especially helpful when introducing new experts unfamiliar with JECFA processes; the absence of face-to-face discussions makes inclusion of new experts more challenging.

The experts emphasized that an invitation to a physical JECFA meeting at the FAO or WHO headquarters gives rise to a more significant recognition by the expert's employer of the weight, reach, responsibility and workload required for full participation in a JECFA meeting. The same degree of acknowledgement was not granted by employers for this online meeting, as the experts remained available locally. This lack of recognition of the workload and significance of participation in a JECFA meeting led to an increase in other demands on the experts, resulting in greater distractions and more frequent scheduling conflicts. The experts concluded that, cumulatively, such factors would be counterproductive for participation in future JECFA meetings if FAO and WHO maintained the online-only format.

In recognition of the difficulties and the tremendous efforts made, the Joint FAO/WHO Secretariat expressed its deep gratitude to all the experts for their commitment and flexibility, not least as the scheduled meeting times were exceedingly inconvenient for many.

1.2 Declaration of interests

The Secretariat informed the Committee that all experts participating in the ninety-fourth meeting had completed declaration of interest forms. Professor Alan Boobis, Professor Angelo Moretto and Dr Silvia Piñeiro declared interests. In each case these declared interests were carefully evaluated and were found to not constitute a conflict of interest.

2. General considerations

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

The Secretariat informed participants of the matters discussed at the 25th meeting of the CCRVDF which was held virtually in July 2021.

2.2 Comments on the parallel review process

As previously noted by the Committee at the eighty-eighth meeting, JECFA remains supportive of the parallel review process. Based on the experience gained through the evaluations of selamectin at the eighty-eighth and ninety-fourth (present) meetings, the Committee concluded that the process and requirements for this parallel review approach should be essentially the same as those for a compound that has already received registration in a Member State. This includes providing all necessary information required to establish a health-based guidance value (HBGV) and recommend maximum residue limits (MRLs) in the tissue(s) of interest, as is the mandate of JECFA. The Committee reiterates that specific MRLs cannot be recommended without established good veterinary practice (GVP) for a product in at least one Member State. As no GVP has been established yet for selamectin, a range of preliminary proposed MRL values, which may be useful in informing risk management, were derived for selamectin based on the currently available data.

2.3 Estimation of dietary exposure to veterinary drug residues as performed by JECFA

The current JECFA approach to acute and chronic dietary exposure is to derive estimates for two population groups; the general population and children. In some respects there is a degree of double-counting in this approach, as children are part of the general population.

In calculating the global estimate of chronic dietary exposure (GECDE) the maximum mean consumption and maximum highest reliable percentile consumption values, across surveys, are used to estimate dietary exposure. Food consumption data are derived from the FAO/WHO chronic individual food consumption database – summary statistics (CIFOCOss). Prior to the eighty-eighth meeting of JECFA, CIFOCOss changed to using the FoodEx 2 food description system and at the time of the eighty-eighth meeting of the Committee,

food consumption data were available expressed only on a "gram per day" basis. On this basis the highest food consumption levels for most foods will be by the adult population.

Since the eighty-eighth meeting of the Committee further work on CIFOCOss has resulted in food consumption data now being available on a "gram per day" or a "gram per kilogram body weight per day" basis. The latter presentation of the data has advantages, as no assumption need be made concerning the body weights of different populations. However, for food consumption expressed on this basis, in most cases the highest food consumption values will be for children (infants and toddlers). This has the potential to result in the GECDE estimates for children and the general population being identical, or very similar.

Food consumption data in CIFOCOss are available for a range of subpopulations. These subpopulations are assigned to one of four age classes: all (general population), adults and the elderly, children and adolescents, and infants and toddlers.

Use of the GECDE has been adopted for evaluations conducted by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as a measure of high consumer dietary exposure. JMPR routinely estimates mean and GECDE dietary exposure estimates for the classes: all (general population), all adults, adult females, children and adolescents, and infants and toddlers.

While further discussions are required to fully harmonize dietary exposure estimation methods between JECFA veterinary drugs and JMPR, it is proposed that a partial alignment of the subpopulations should be performed as an interim measure.

Recommendation

With the availability of food consumption information expressed on a unit body weight basis, it is recommended that these data be used preferentially to minimize the assumptions made in deriving the GECDE. It is further recommended that the population groups for which GECDE estimates are derived be amended to align with the age classes currently used in CIFOCOss:

- infants and toddlers (0–35 months),
- children and adolescents (3–14 years),
- and adults and the elderly (15 years and above).

It is further recommended that JMPR and JECFA continue to take opportunities to harmonize procedures for dietary exposure assessment.

2.4 A risk-based decision tree approach for the safety evaluation of residues of veterinary drugs

The Joint FAO/WHO Expert Committee on Food Additives is sometimes asked for advice on veterinary drugs for which the establishment of HBGVs and recommendation of MRLs is not appropriate, for example when the drugs are genotoxic carcinogens. In other situations there may not be a full data package, such as in the case of "old" drugs for which there is still a use, drugs with no commercial sponsor, drugs no longer in use but which cause contamination of food due to environmental persistence, or the misuse or abuse of drugs. In the early 2000s, a number of activities were undertaken to discuss possible approaches to these situations, including a Joint FAO/WHO technical workshop on residues of veterinary drugs without ADI/MRL, convened in Bangkok in 2004, and an FAO/RIVM/WHO workshop, "Updating the principles and methods of risk assessment: MRLs for pesticides and veterinary drugs", held in Bilthoven, The Netherlands in 2005. Subsequently this led to the publication of EHC 240, "Principles and methods for the risk assessment of chemicals in food", in 2009. The CCRVDF considered a report of a working group on residues of veterinary drugs without ADI/MRL at their sixteenth session, in Cancun, Mexico, in 2005.

This issue was raised at the sixty-sixth JECFA (February 2006), together with a number of related activities. The Committee concluded that there was need for an overarching approach, and recommended that the JECFA Secretariat convene a working group to develop a decision tree for the evaluation of veterinary drugs. This led to the development of a "Decision tree approach for the safety evaluation of residues of veterinary drugs", which was discussed at the seventieth meeting of JECFA (October 2008). The approach was endorsed by the Committee and a number of revisions suggested. The paper was revised accordingly and submitted as a "Risk-based decision tree approach for the safety evaluation of veterinary drugs" to CCRVDF for its eighteenth session (May 2009), as a work-in-progress. CCRVDF agreed with the proposed general principles and supported further work on the approach.

The scheme was discussed at the seventy-fifth meeting of JECFA (November 2011) and a number of follow-up actions were recommended. However, these were not taken up immediately due to resource limitations. The seventy-eighth JECFA (November 2013) reiterated the recommendations, which included the establishment of an e-working group to develop guidance for establishing ARfDs for residues of veterinary drugs. This was done, and guidance has been developed and adopted by JECFA (2017), including approaches for the establishment of a microbiological ARfD (mARfD).

A number of other recommendations to further develop the decision tree were made by the seventy-eighth JECFA (2013), which included undertaking

work on "preliminary risk assessment", and on the feasibility of using a threshold of toxicological concern (TTC) approach for residues of veterinary drugs. These were not followed up. A number of sections in the draft document noted that further extensive work was required. This included characterization of dietary exposure and management of risk. Since then, much work has been undertaken on dietary exposure assessment, but consideration has yet to be given to how this might be integrated into the decision tree. Guidance on some parts of the scheme was developed but has yet to be adopted by JECFA, such as on the identification of strengths and weaknesses in the risk assessment (uncertainties and sensitivity analysis).

The present Committee discussed the decision tree and concluded that there is a continuing need for such an approach. It was agreed that the approach should be finalized and published as guidance for JECFA. There was a need to develop some aspects further. There may be a need to include some additional aspects and there may be others that can be omitted. The Committee noted that the approach was essentially generic and would be applicable to additional committees that provide advice to the Codex Alimentarius on food safety, such as JMPR.

The Committee therefore recommended that the Joint Secretariat, together with other secretariats as appropriate, convene an electronic working group comprising experts from the three committees under JECFA, JMPR, and in dietary exposure assessment, to further develop the decision tree approach, with a view to its finalization in 2023 or 2024.

2.5 General considerations for microbiological effects

The impact of drug residues on the human intestinal microbiome is evaluated through a decision tree approach adopted by the sixty-sixth meeting of the Committee, which complies with VICH GL36(R) *(2)*. This entails answering three questions to determine the need for establishing a microbiological acceptable daily intake (mADI). Determine first if the drug residue, and/or its metabolites, are microbiologically active against representatives of the human intestinal microbiota. Second, determine if the drug residues enter the human colon, and third, whether the residues entering the human colon remain microbiologically active. If the answer to any of these questions is "no", then there is no need to calculate a mADI and the assessment does not need to be completed. However, if a mADI needs to be calculated, two end-points of concern for human health are considered for the assessment: disruption of the colonization barrier of the human intestinal microbiome, and increases in populations of resistant bacteria in the human intestinal microbiome. More recently, this was extended to consider

the possibility of acute effects and the need for a mARfD.

This guidance delineates a step-by-step approach and provides an explanation of test systems that sponsors can use to address the impact of animal drug residues on the human intestinal microbiome, as another toxicological target of concern.

When JECFA assesses the potential effects of residues of a veterinary drug on humans, the different toxicological targets of concern need to be addressed (reproductive, mutagenesis, carcinogenesis, and chronic toxicity, for example), either by information available in the public domain or by conducting a corresponding study. Because traditional toxicological studies have been done routinely for many years, it is readily understood that all these end-points need to be addressed. However, in the case of the effects of drug residues on the human intestinal microbiome, such a requirement is not so evident, since it is only in the last few years that an understanding the importance of the human intestinal microbiome to human health has developed and appreciation of this begun to spread. The human intestinal microbiome is now considered an additional target organ, in which changes in the composition and function of these intestinal microbes (microbiota dysbiosis) has been associated with diseases ranging from localized gastroenterologic disorders to neurologic, respiratory, metabolic, hepatic and cardiovascular illnesses *(3)*.

Thus, as one more toxicological target of concern, sponsors of drugs submitted for evaluation will need to address the effects of residues on the human intestinal microbiome, for both end-points of concern; the disruption of the colonization barrier and an increase in bacterial resistance. A drug, or its metabolite, might not be an antimicrobial but could still produce disruption and/or increase the population of resistant bacteria, to the extent that a mADI and/or mARfD need be calculated.

Therefore, sponsors need to fully address both of these concerns for potential impact of drug residues on the human intestinal microbiome, either using information available in the public domain or by running a corresponding study.

Furthermore, while current assessments consider only bacteria in the evaluation, it is now well established that the intestinal microbiome also includes bacteriophages and other viruses, archaea, fungi and protozoa, which play an important role in human health. JECFA will therefore consider how the impact of residues on some or all of the other components of the human intestinal microbiome might be addressed. It is recommended that the Secretariat convene a microbiome expert working group to explore developments in this evolving area.

3. Comments on residues of specific veterinary drugs

The Committee evaluated or re-evaluated four veterinary drugs. Information on the safety evaluations is summarized in Annex 2.

3.1 Imidacloprid

Explanation

Imidacloprid is the ISO-approved common name for (*E*)-1-(6-chloro-3-pyridinylmethyl)-*N*-nitroimidazolidin-2-ylideneamine (IUPAC), for which the Chemical Abstracts Service No. is 138261-41-3.

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

Imidacloprid acts on the central nervous system by causing prolonged activation and desensitization of the postsynaptic nicotinic cholinergic receptor, interfering with the transmission of nerve impulses, which results in paralysis and consequent death *(4)*. The selective toxicity of imidacloprid to target species is attributed to differences in the binding affinity or potency at the nicotinic acetylcholine receptor compared to mammals.

Imidacloprid has not previously been evaluated by the Committee, although it was evaluated by the JMPR as a pesticide in 2001 *(5)*. 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 *(6)*, CCRVDF requested an evaluation of imidacloprid for use in all fin fish, and for the Committee to recommend MRLs for muscle and fillet (muscle and skin in natural proportions). A toxicological re-evaluation was undertaken to establish health-based guidance values due to the time that had elapsed since its last review.

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

Toxicological and microbiological evaluation

The Committee reviewed a data package submitted by the sponsor. Additionally, the following databases of published literature were searched: Aquaculture Compendium (69 articles retrieved), Agris (24), CAB abstracts (1122), CAS (816), Embase (626), FSTA (136), PubMed (362), Scopus (489) and WOS (328). Of these, 32 papers were considered relevant to this assessment. The following sources of information were also scrutinized and information considered relevant included in this assessment: evaluations by JMPR from 2002 *(5)*, U.S. EPA prepared in 2003 *(7),* CalEPA prepared in 2006 *(8),* EFSA prepared in 2008 *(9)* and 2013 *(10)*, and the 2021 report from EMA *(11)* Many of the studies were not according to good laboratory practice (GLP) and where this was the case it is indicated.

Biochemical data

The pharmacokinetics of imidacloprid have been well characterized in rats. Imidacloprid was rapidly and almost completely absorbed (92–99%) from the gastrointestinal tract of rats and was rapidly eliminated from the organism in the excreta (90% of administered dose [AD] within 24 hours), with no biologically significant differences shown between gender, different dose levels or routes of administration. Elimination was mainly renal (70–80% of AD), with 17–25% of AD in the faeces. The majority of faecal radioactivity originated from the bile. The radiolabel was rapidly distributed from the intravascular space into almost all organs and tissues, with the liver, kidney, lung, skin, and plasma being the major sites of distribution. Imidacloprid penetrated the blood–brain barrier to only a very limited extent. The maximum plasma concentration of radiolabel was reached between 1.1 and 2.5 hours after dosing *(12, 13*, *14*, *15, 16)*.

Metabolism of imidacloprid in mice and rats was rapid, the amount of unchanged parent compound representing 10–16% of AD. The parent imidacloprid initially underwent P450-dependent oxidation. The metabolites identified were 4-hydroxy-imidacloprid, 5-hydroxy-imidacloprid, 6-hydroxy-imidacloprid (6-hydroxynicotinic acid), 6-chloronicotinic acid (6-CNA), 2-imidazolidone, also olefin, guanidine and urea derivatives *(16, 17, 18, 19, 20)*. Based on a clinical study, in a single male volunteer, and two in vitro studies, the two metabolic pathways identified in the rat were confirmed in humans. One route is via imidazolidine hydroxylation and desaturation to give 5-hydroxy-imidacloprid and the olefins, respectively The other route is via nitroimine reduction and cleavage to yield the nitrosoimine, guanidine, and urea derivative products *(21*, *22, 23)*.

Toxicological data

From the JMPR 2001 toxicological monograph *(5)*, the oral median lethal dose (LD_{50}) of imidacloprid in mice was 130–170 mg/kg bw, and in rats 380–650 mg/kg bw. Behavioural and respiratory signs, disturbances in motility, narrowed palpebral fissures, transient trembling and spasms were seen in rats treated orally at doses of 71 mg/kg bw and above, and in mice at doses of 200 mg/kg bw and above. The clinical signs were reversed within six days.

In an acute oral toxicity study in the rat (GLP-compliant), submitted by the sponsor *(24)*, no clinical signs occurred at 130 mg/kg bw and the oral acute LD_{50} was estimated to be 1300 mg/kg bw (494–1740 mg/kg bw).

In relation to the short-term toxicity studies, the Committee reviewed the JMPR monograph (5) and relevant studies published in the scientific literature. Reduction in body weight was the most common toxic effect observed in oral studies with mice, rats and dogs. The liver was the principal target organ as demonstrated by hepatic necrosis or hypertrophy in rats and dogs, elevated activities of serum enzymes and alteration in clinical chemistry parameters.

In one study (not GLP-compliant), mice were fed diets containing imidacloprid for 107 days, at doses of 0, 0.17, 86 or 430 mg/kg bw per day. A no-observed-adverse-effect level (NOAEL) of 17 mg/kg bw per day was identified, based on an increase in alkaline phosphatase (ALP) activity and a reduction in body weight (in males) at 86 mg/kg bw per day *(25)*, as reported by JMPR 2001 *(5)*.

In another study, rats were exposed to imidacloprid daily for up to 98 days at concentrations of 0, 120, 600 or 3000 mg/kg feed (equal to 0, 11, 57 and 410 mg/kg bw per day for males, 0, 14, 78 and 510 mg/kg bw per day for females). The NOAEL was 120 mg/kg feed (equal to 11 mg/kg bw per day) based on a reduction in body weight at 600 mg/kg feed (equal to 57 mg/kg bw per day) *(26)*.

In a further study, rats received diets containing imidacloprid at concentrations of 0, 150, 600 or 2400 mg/kg feed (equal to 0, 14, 61 and 300 mg/kg bw per day for males, 0, 20, 83 and 420 mg/kg bw per day for females) for up to 96 days. Satellite groups received the test substance at concentrations of 0 or 2400 mg/kg feed over the same period, followed by a four-week observation period following cessation of treatment. The NOAEL was 150 mg/kg feed (equal to 14 mg/kg bw per day) based on liver toxicity and reduced body weight at 600 mg/kg feed (euql to 61 mg/kg bw per day). These liver effects were reversible after four weeks *(27)*.

In a study retrieved from the literature, female rats were administered imidacloprid orally by gavage for 90 days at doses of 0, 5, 10 or 20 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day, based on morphological, biochemical, haematological, and neuropathological changes in the brain, liver and/or kidney at 20 mg/kg bw per day *(28)*.

In another published study carried out by the same authors, imidacloprid was administered orally by gavage for 90 days to female rats at doses of 0, 5, 10 or 20 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day, based on clinical signs and reduced weight gain at 20 mg/kg bw per day *(29)*.

In an additional study conducted by the same researchers, female rats received imidacloprid by gavage for 90 days at doses of 0, 5, 10 or 20 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day based on clinical signs, reduced weight gain and changes in ovarian morphology and hormones at 20 mg/kg bw per day *(30)*.

In a 13-week study in dogs, animals received diets containing imidacloprid at concentrations of 0, 200, 600, or 1800/1200 mg/kg feed in the diet (equal to 0, 7.5, 24 or 67.5/45 mg/kg bw per day). A NOAEL was identified at 200 mg/kg feed (equal to 7.5 mg/kg bw per day), based on tremors occurring in the first week in animals treated with 600 mg/kg feed (equal to 24 mg/kg bw per day) *(31)*.

In a 52-week study, dogs received diets containing imidacloprid at 0, 200, 500 mg/kg feed or 1250 rising to 2500 mg/kg feed on week 17 (equal to 0, 6.1, 15, and 41 rising to 72 mg/kg bw per day). The NOAEL was 500 mg/kg feed (equal to 15 mg/kg bw per day) based on liver changes at 1250 mg/kg feed (equal to 41 mg/kg bw per day) *(32)*.

For evaluation of long-term toxicity and carcinogenicity, the studies provided to JMPR 2001*(5)* and a more recent one-year study submitted by the sponsor were assessed.

Imidacloprid was administered in the feed to mice for 24 months at concentrations of 0, 100, 330 or 1000 mg/kg feed (equal to 0, 20, 66 and 208 mg/kg bw per day for males, 0, 30, 104 and 274 mg/kg bw for females). There was no evidence of a carcinogenic effect. The NOAEL was 330 mg/kg feed (equal to 66 mg/kg bw per day) based on reduction in body weight at 1000 mg/kg feed (equal to 208 mg/kg bw per day) *(33, 34)*.

In a one-year, repeat-dose, oral toxicity study rats received imidacloprid in the diet at concentrations of 0, 100, 300 or 1000 mg/kg feed (equal to 0, 5.6, 16,3 and 55.8 mg/kg bw per day for males, 0, 6.7, 19.5 and 63.7 mg/kg bw per day for females). The NOAEL was 200 mg/kg feed (equal to 5.6 mg/kg bw per day) based on decreased body weight gain at 300 mg/kg feed (equal to16.3 mg/kg bw per day) *(35)*.

In a two-year toxicity/carcinogenicity study, imidacloprid was administered to rats at dietary levels of 0, 100, 300 or 900 mg/kg feed (equal to 0, 5.7, 17 and 51 mg/kg bw per day for males, 0, 7.6, 26 and 73 mg/kg bw per day for females). In a supplemental study to examine the maximum tolerated dose, rats were given diets containing imidacloprid for 24 months at concentrations of 0 or 1800 mg/kg feed (equal to 0 and 103 mg/kg bw per day for males, 0 and 144 mg/kg bw per day for females). There was no evidence of a carcinogenic effect. The NOAEL was 100 mg/kg feed (equal to 5.7 mg/kg bw per day) based on

an increase in incidence and severity of mineralized particles in the thyroid gland at 300 mg/kg feed (equal to 17 mg/kg bw per day) *(36, 37)*.

The Committee concluded that imidacloprid was not carcinogenic in rats or mice.

The genotoxicity of imidacloprid was assessed by JMPR 2001 *(5)* which stated the following:

> *"Imidacloprid gave negative results in an adequate range of assays for genotoxicity in vitro and in vivo. Weak induction of sister chromatid exchange was found in one test with Chinese hamster ovary cells in vitro, but not in vivo. The Meeting concluded that imidacloprid is unlikely to be genotoxic or to pose a carcinogenic risk to humans."*

Subsequently, three genotoxicity studies of imidacloprid were submitted by the sponsor. In these an Ames test was negative, an in vitro micronucleus test was negative following three hours incubation, but positive after 24 hours incubation *(38, 39)* and imidacloprid was shown to be negative in an adequately conducted in vivo micronucleus study in rats *(40)*. A number of in vitro and in vivo genotoxicity studies were also found in the open literature, however most of them were performed using commercial formulations of imidacloprid or had experimental limitations resulting in uninformative data.

The Committee concluded that imidacloprid is unlikely to be genotoxic in vivo at doses expected from the diet.

The Committee concluded that imidacloprid is unlikely to pose a carcinogenic risk to humans from the diet, given that it is unlikely to be genotoxic in vivo and it is not carcinogenic in rats or mice.

The evaluation of reproductive and developmental toxicity was based on data provided by JMPR 2001 *(5)* and studies submitted by the sponsor.

In a two-generation study described in detail by CalEPA *(8)*, rats received diets containing imidacloprid at a concentration of 0, 100, 250 or 700 mg/kg feed (equal to 0, 6.6, 17 and 47 mg/kg bw per day). The NOAEL for parental effects was 100 mg/kg feed (equal to 6.6 mg/kg bw per day) based on decreased premating body weights at 250 mg/kg feed (equal to 17 mg/kg bw per day). The NOAEL for reproductive effects was 700 mg/kg feed (equal to 47 mg/kg bw per day), the highest dose tested. The NOAEL for offspring effects was 2500 mg/kg feed (equal to 17 mg/kg bw per day) based on a decrease in pup body weight at 700 mg/kg feed (equal to 47 mg/kg bw per day) *(41)*.

In an extended one-generation reproductive toxicity study, rats received diets containing imidacloprid at concentrations of 0, 100, 300 or 1000 mg/kg feed (equal to 0, 5.25, 15.35 and 48.4 mg/kg bw per day for males, 0, 10.4, 30.43 and 85.6 mg/kg bw per day for females). The NOAEL for parental toxicity was

100 mg/kg feed (equal to 5.25 mg/kg bw per day) based on reduced body weight and food consumption at 300 mg/kg feed (equal to 15.35 mg/kg bw per day). The NOAEL for offspring toxicity was 100 mg/kg feed (equal to 10.4 mg/kg bw per day) based on reduced pup body weights at 300 mg/kg feed (equal to 30.43 mg/kg bw per day). The NOAEL for reproductive toxicity was 1000 mg/kg feed (equal to 48.4 mg/kg bw per day), the highest dose tested *(42)*.

In a prenatal study of developmental toxicity, imidacloprid was administered daily by gavage to mated female rats from gestation day (GD) 6 to15, at doses of 0, 10, 30 or 100 mg/kg bw per day. The NOAEL for maternal effects was 10 mg/kg bw per day based on weight gain. The NOAEL for embryo/fetal toxicity was 30 mg/kg bw per day based on a delay in embryo development at 100 mg/kg bw per day *(43)*.

In a study of developmental toxicity, rats were administered imidacloprid by gavage at doses of 0, 5, 15 or 50 mg/kg bw per day, from GD 6 to 19. The NOAEL was 15 mg/kg bw per day for maternal effects based on weight loss and reduced food intake at 50 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 50 mg/kg bw per day, the highest dose tested *(44)*.

In a study of developmental toxicity, pregnant rabbits were administered imidacloprid by oral gavage at doses of 0, 8, 24 or 72 mg/kg bw per day, on GD 6–18. The NOAEL for maternal toxicity was 8 mg/kg bw per day based on reduced food consumption and body weight gain observed at 24 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 24 mg/kg bw per day based on increased postimplantation loss, reduced body weight and delayed ossification at 72 mg/kg bw per day *(45)*.

The Committee concluded that imidacloprid is not teratogenic.

A study of the immunotoxicity of imidacloprid in mice was retrieved from the scientific literature and evaluated. Organ weights and a number of haematological and immunological parameters were measured. The Committee identified some inconsistencies in the findings, concluding that the study was not suitable for the risk assessment of imidacloprid *(46)*.

The immunotoxic effects of imidacloprid were evaluated in F1 rats from the extended one-generation reproductive toxicity study described above *(42)*. The F1 rats received imidacloprid in their feed as detailed earlier for up to 61 days, providing doses of 0, 11.4, 36.9 or 120.4 mg/kg bw per day for males, 0, 11.9, 35.5 or 121.0 mg/kg bw per day for females. The NOAEL for immunotoxicity was 121 mg/kg bw per day, the highest dose tested *(42)*.

In a study of acute neurotoxicity, rats were administered imidacloprid by gavage at doses of 0, 42, 151 and 307 mg/kg bw. Both neurobehaviour and neuropathology were assessed. No NOAEL could be identified, as

neurobehavioural effects were observed at all doses, although this is equivocal as effects observed at the lowest dose were not statistically significant *(47)*. Using the data from this study, CalEPA calculated a $BMDL_{05}$ (lower confidence limit on the benchmark dose for a 5% response) of 9 mg/kg bw, based on decreases in the motor and locomotor activity *(8)*.

In a 13-week neurobehavioural study of imidacloprid in adult rats fed 0, 140, 960 or 3000 mg/kg feed (equal to 0, 9.3, 63 and 196 for males, 0, 10.5, 69 and 215 for females), functional observational battery (FOB) changes were observed in males in the highest dose group. The NOAEL for systemic toxicity was 140 mg/kg feed (equal to 9.3 mg/kg bw per day) based on decreases in body weight gain and food consumption at the lowest-observed-adverse-effect level (LOAEL) of 960 mg/kg feed (equal to 63 mg/kg bw per day) *(48)*.

In a one-year toxicity study described above *(35)*, imidacloprid was administered in the diet to rats at levels of 0, 100, 300 or 1000 mg/kg feed (equal to 0, 5.6, 16.3 and 55.8 mg/kg bw per day for males, 0, 6.7, 19.5 and 63.7 mg/kg bw per day for females). Observations were made in the open-field arena and FOB responses recorded at various times. The NOAEL for neurotoxicity was 1000 mg/kg feed (equal to 55.8 mg/kg bw per day), the highest dose tested *(35)*.

In a developmental neurotoxicity study, imidacloprid was administered to female rats in the diet at doses of 0, 8, 19 and 54.7 mg/kg bw per day, from GD 0 until postnatal day (PND) 21. An offspring NOAEL was identified at 19 mg/kg bw per day based on reduction in body weight and decreased motor activity in the group treated with 54.7 mg/kg bw per day *(49)*.

The developmental toxicity and developmental brain neuropathology of imidacloprid were also evaluated in F1 rats from the extended one-generation reproductive toxicity study described above *(42)*. Animals received the same dietary concentrations of imidacloprid as the parental animals, that is 0, 100, 300 or 1000 mg/kg feed (equal to 0, 11.4, 36.9, 120.4 mg/kg bw per day for males, 0, 11.9, 35.5 and 121.0 mg/kg bw per day for females). Two cohorts were examined, one for acoustic startle, motor activity and FOB assessments, the other for brain histomorphometric evaluation. The NOAEL was 1000 mg/kg feed (equal to 120.4 mg/kg bw per day), the highest dose tested *(42)*.

Three studies evaluating the effects of imidacloprid on the male reproductive system in rats were retrieved from the literature. The Committee considered that these studies did not provide sufficient details and displayed inconsistent results, hence they could not be used for risk assessment. It was further noted that no effects indicative of male reproductive toxicity were identified in multigeneration or repeat-dose toxicity studies.

Studies on the acute oral toxicity and genotoxicity of several of imacloprid's metabolites were assessed by JMPR in 2001 *(5)*. The metabolites examined were:

1-(6-chloro-3-pyridylmethyl)-2-imidazolidinone;
1-(6-chloro-3-pyridylmethyl)-*N*-nitro(4-imidazolin-2-ylidene)amine;
1-(6-chloro-3-pyridylmethyl)imidazolidi-2-ylideneamine;
1-(6-chloro-3-pyridylmethyl)-*N*-nitroso(imidazolidin-2-ylidene)amine).

These metabolites were found to be less acutely toxic than the parent compound, and showed no evidence of genotoxicity.

Microbiological data

The impact of imidacloprid residues on the human intestinal microbiome was evaluated through a decision-tree approach adopted by the sixty-sixth meeting of the Committee (Annex 2, ref. *181*), which complies with the VICH guideline GL36(R) *(2)*. This entails answering three questions to determine the need for establishing a mADI. Determine first if the drug residue, and/or its metabolites, are microbiologically active against representatives of the human intestinal microbiota. Second determine if the drug residues enter the human colon, and third, whether the residues entering the human colon remain microbiologically active. If the answer to any of these questions is "no", then there is no need to calculate a mADI and the assessment does not need to be completed. However, if a mADI needs to be calculated, two end-points of concern for human health are considered for the assessment: disruption of the colonization barrier of the human intestinal microbiome and increases in populations of resistant bacteria in the human intestinal microbiome.

A published, non-GLP study in mice by Yang et al. *(50)* examined imidacloprid's effects on the intestinal microbiome. Mice were fed imidacloprid in drinking water at 0, 3, 10 or 30 mg/L (equivalent to 0, 0.5, 1.67 and 5 mg/kg bw per day) for 70 days. Results indicated that relative abundances of some colonic caecal bacteria appeared to increase relative to others in the treated group. The authors concluded that following imidacloprid exposure studies showed that the intestinal barrier function was greatly impaired, and that the sequencing of colonic contents revealed the balance of the gut microbiota was disrupted. However, this publication does not address the direct impact of imidacloprid on the intestinal microbiome. Thus, there was insufficient information for the Committee to assess whether residues of imidacloprid have direct impact in the intestinal microbiome. As a result, it was not possible to determine if there is a need for the calculation of a mADI for imidacloprid. This conclusion also applies to the need to determine a mARfD. The Committee was aware of an ongoing GLP study to elucidate the direct effects of imidacloprid on some human intestinal microbiome representative bacteria that would assist in the evaluation.

Evaluation

In view of the absence of a study to assess the impact of imidacloprid on representative human intestinal microbiota it was not possible to determine a mARfD or a mADI, thus the Committee was unable to establish a ARfD or an ADI for imidacloprid.

The Committee established a toxicological acceptable daily intake (tADI) of 0–0.05 mg/kg bw on the basis of a NOAEL of 5.25 mg/kg bw per day for decreased body weight gain in the extended one-generation reproduction study, with the application of a safety factor of 100 to allow for interspecies and intraspecies differences.

The Committee established a toxicological acute reference dose (tARfD) of 0.09 mg/kg bw based on a $BMDL_{05}$ of 9 mg/kg bw reported by Cal EPA for acute neurobehavioural effects in rats, and a safety factor of 100 to allow 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

A toxicological monograph was prepared.

Residue evaluation

Comprehensive literature search

As part of its assessment of imidacloprid, the Committee performed a comprehensive literature search in March 2022, using the Pubmed, Google Scholar, Science Direct and Web of Science online databases to identify any further relevant information.

Table 1
Inclusion and exclusion criteria for the comprehensive literature search

Inclusion criteria:	Exclusion criteria:
Any article regarding imidacloprid concentrations in tissues of fin fish	Any article focussing on imidacloprid efficacy against target parasites
Any article regarding imidacloprid residue determination methods for fin fish plasma/tissue	Any article focussing on environmental contamination
Any article regarding imidacloprid metabolism/metabolites in fin fish	Any article focussing on species other than fin fish
Articles in all languages were included	Any article focussing on parasite resistance

No time limits were placed on the search results, however no material published before 2016 was found that met the inclusion criteria.

The results of the literature search provided some useful data on the pharmacokinetics of imidacloprid in additional fish species, including metabolism and distribution in trout, and distribution in a cichlid species (*Australoheros facetus*). Additionally, some papers covering analytical methodologies were found. However, the latter were not used in the evaluation,

as they were not reported in sufficient detail.

The Committee reviewed studies on the pharmacokinetics and metabolism of imidacloprid in rats and fish, including Atlantic salmon and rainbow trout. A study of the pharmacokinetics of radiolabelled imidacloprid in Atlantic salmon and several nonradiolabelled imidacloprid residue depletion studies in Atlantic salmon and rainbow trout were reviewed. In vivo distribution studies in *A. facetus*, Atlantic salmon, and rainbow trout were also reviewed.

The analytical method used to analyse seawater and tissue samples was assessed.

Data on pharmacokinetics and metabolism

The metabolic pathways of imidacloprid are similar in rodents and humans. Two main metabolic routes were identified. The first is oxidative cleavage which leads to the formation of 6-chloronicotinic acid, and subsequent conjugation with glycine to produce a hippuric acid conjugate. The second major pathway involves hydroxylation of the imidazolidine ring at the 4 or 5 position to yield 4- or 5-hydroxy-imidacloprid. However, the only metabolite identified in salmon and trout was 5-hydroxy-imidacloprid, which did not reach 10% of the total recovered radioactivity (TRR) at any time point studied. Imidacloprid is mainly excreted via the faeces in fish, but is also seen in the urine.

Atlantic salmon

One GLP-compliant study *(51)* was conducted using [^{14}C]imidacloprid to investigate tissue distribution and metabolism over time in Atlantic salmon. Twenty-six fish, around one year old, were held in the exposure bath (temperature 7–8°C) for a 60-minute period and then removed and returned to a holding tank containing fresh seawater. The concentration of imidacloprid in the treatment solution was 20.3–20.6 mg/L. Samples of salmon fillet (muscle and skin in natural proportions), liver (excluding the gall bladder), spleen, gut, gills, kidney, and the residual carcass were collected from six fish each at five hours, 25 hours, five days and 26 days after the cessation of treatment. Representative combined samples from the fillets and from the livers were extracted with acetonitrile, and the extractable residues were analysed by high performance liquid chromatography/ultraviolet detection (HPLC-UV). The limit of detection (LOD) of the method was 10 µg/kg for all tissue matrices. The parent substance was the major residue detected in all samples analysed, and accounting for 69.4–95.2% of TRR in fillet and 77.7–95.2% of TRR in liver. An unknown metabolite was detected at low concentrations (8.2% or less of TRR) in all extractable residues. This metabolite was isolated and identified as hydroxylated imidacloprid by liquid chromatography/mass spectrometry (LC-MS) analysis of a day 5 fillet sample. The radiolabelled imidacloprid had distributed to all tissues analysed.

Rainbow trout

One published paper was reviewed *(52)*. Studies (non-GLP) were conducted to determine the distribution and elimination of imidacloprid in rainbow trout. Trout (0.7–1.0 kg) were injected with a low (47.6 µg/kg), medium (117.5 µg/kg) or high (232.7 µg/kg) dose of imidacloprid directly into the arterial bloodstream. The trout were then sampled to characterize the tissue distribution of imidacloprid. In vitro biotransformation of imidacloprid was evaluated using trout liver S9 fractions. Imidacloprid was distributed to all tissues sampled (brain, kidney, liver, muscle, bile, plasma and urine). There was no evidence for hepatic biotransformation of imidacloprid in isolated trout liver fractions.

Chamaeleon cichlid (A. facetus)

One published paper was reviewed *(53)*. *Australoheros facetus* were exposed to three different concentrations (100, 300 or 2500 mg/L) of imidacloprid in fresh water, kept at a constant temperature of 15°C, and then sampled to determine how the imidacloprid had distributed around the tissues. Samples of brain, muscle, gills, gut, liver, and blood were analysed. Imidacloprid residues were determined by liquid chromatography/mass spectrometry. Distribution to all tissues was demonstrated, as observed in the salmon and trout studies summarized above. Metabolic aspects were not investigated.

Based on the results of these studies the Committee identified imidacloprid as the sole marker residue in salmon and rainbow trout fillet and determined that a value of 0.7 was appropriate for the marker residue to total recovered radioactivity ratio (MR:TRR). This was based on two main factors. Firstly, that there were no sample points between day 5 and day 26 (approximately 37–195 degree-days) of the TRR study in salmon, although the withdrawal period is likely to fall between those two time points. Secondly that the TRR study was conducted at a relatively low temperature (7–8°C) whereas it has been seen in some of the residue depletion studies that water temperature can reach 15–17°C under field conditions. It is known that water temperature affects the metabolic rate in fish, so it is possible that this increased metabolic rate at higher temperatures may result in a lower MR:TRR. Thus, with a choice between MR:TRRs of 0.9 determined at five days post treatment, or 0.7 determined at 26 days post treatment, the Committee considered that the lower value would lead to a more conservative approach and was therefore chosen in this case.

Residue data

The Committee reviewed residue depletion studies for Atlantic salmon and rainbow trout. None of these studies used radiolabelled imidacloprid (other than the Hobbs study *(51)* reviewed above). There were no residue depletion studies available in any other fin fish species.

Atlantic salmon

Three GLP-compliant studies were reviewed. In all three studies, fish were treated at a nominal concentration of 20 mg/L imidacloprid in seawater treatment baths. The effects were evaluated of the temperature of the treatment bath water (and the water in which the fish were kept after treatment), and the duration of exposure to the treatment baths, in particular how these factors affected the pattern of residue depletion. Two of the studies were conducted under controlled conditions and the other under field conditions. The same analytical method used for determining imidacloprid residues in salmon fillet was employed in all studies reported, and used liquid chromatography with tandem mass spectrometry (LC-MS/MS) with a validated limit of quantitation (LOQ) of 4 µg/kg. A non-GLP pilot residue depletion study was available *(54)*, but it did not provide any useful data.

In the first GLP-compliant study *(55)* salmon were exposed to 20 mg/L imidacloprid in seawater for approximately one hour at either 7°C or 15°C. The average weight of the fish in the 7°C group was 577 g, and in the 15°C group was 383 g. At each of the five sampling points during the 28 days post exposure for 15°C (days 1, 7, 14, 21, and 28, representing the range 15–420 degree-days), and 60 days post exposure for 7°C (days 1, 7, 21, 35, and 60, representing the range 7–420 degree-days), 10 treated salmon were harvested. Samples of muscle, liver, skin and fillet (muscle and skin) were collected from each salmon. At the higher water temperature (15°C) the initial residue concentrations were much higher and residue depletion over time was faster than at 7°C. At the lower temperature the absorption of imidacloprid from the immersion treatment was lower and the depletion of residues was slower than for salmon treated at 15°C.

Table 2
Mean results (data from *(55)*)

Mean concentration of imidacloprid (µg/kg) in salmon fillet at 7°C					
Time point	Day 1	Day 7	Day 21	Day 35	Day 60
	123.5	61.2	11.75	5.29	< LOQ
Mean concentration of imidacloprid (µg/kg) in salmon fillet at 15°C					
Timepoint	Day 1	Day 7	Day 14	Day 21	Day 28
	302	50.0	6.29	< LOQ	< LOQ

< LOQ: Below the limit of quantification (4 µg/kg)

In the second GLP-compliant study *(56)*, groups of salmon (average weight 409 g) were exposed to 20.9–21.8 mg/L imidacloprid in seawater for 60, 196 or 360 minutes at 15.2–16.0°C. At each of the sampling points (days 1, 7, 14, 21, 28 and 33 post exposure), 12 salmon per treatment group were harvested and fillets sampled. The longer the salmon were exposed to the treatment baths, the higher the residues measured at each time point.

Table 3
Mean concentrations (± SD) of imidacloprid (µg/kg) in Atlantic salmon fillet (data from *(56)*)

Sampling time points		Exposure time in immersion bath		
Days post treatment	Degree days	60 minutes	196 minutes	360 minutes
1	15.6	359 ± 63	740 ± 83	1372 ± 114
7	108.8	71 ± 15	160 ± 24	296 ± 56
14	206.8	12.73 ± 3.81	27.45 ± 9.87	54.73 ± 15.90
21	313.2	< LOQ	6.29 ± 2.28[a]	9.57 ± 3.70
28	422.1	< LOQ	< LOQ	2.68 ± 1.64[a]
33	508.6	< LOQ	< LOQ	< LOQ

< LOQ: Less than the limit of quantification (4 µg/kg);
[a] Where one or more samples were below the LOQ, the value for calculation of the mean was taken as 2 µg/kg (½ LOQ).

In the third GLP-compliant study *(57)*, conducted under field conditions, groups of salmon (weight range 1–4 kg) were bath treated at a concentration of 20 mg/L for 60 minutes using well-boats. Salmon from a total of eight sites were treated. Sampling time points were as follows: immediately prior to treatment, immediately after treatment, after 24 hours, then 3–5 days, 8–10 days, 19–21 days after treatment, and finally at 350 degree-days post treatment. Fillets were removed for residue analysis from five fish per pen, from two pens per site, at each time point. Site temperatures were regularly measured to determine degree days. Temperatures ranged from 5–17°C. Some sites were at temperatures of 5–10°C, while others were in the range 10–17°C.

Table 4
Concentration of imidacloprid in salmon fillet (data from *(57)*)

	Mean concentration in salmon fillet (µg/kg)			
Sampling time	Day 1	Day 5	Day 10	Day 21
Mean (µg/kg)	200.96	128.02	59.32	16.81
± SD (µg/kg)	71.86	46.01	40.81	13.37

As temperatures fluctuated both within and between study sites. time points could not be directly converted to degree-days.

Rainbow trout

One GLP-compliant study was reviewed. No additional data were available.

In this study *(57)*, conducted under field conditions, groups of rainbow trout (weight range 1.5–2.5 kg) were bath treated at a concentration of 20 mg/L in seawater for 60 minutes using well-boats. Treatment was carried out at four sites. Sampling time points were as follows: immediately prior to treatment, immediately after treatment, after 24 hours, 3–5 days, 8–10 days, 19–21 days after treatment, and in some cases an additional sampling at 350 degree-days post

treatment. Fillets were removed for residue analysis from five fish per pen from two pens per site, at each time point. Site temperatures were regularly measured to determine degree-days. Temperatures ranged from 13.5°C to 16.5°C.

Table 5
Concentration of imidacloprid in trout fillet (data from *(57)*)

	Sampling time (degree-days)					
	Pre-treatment	**Day 0 (0)**	**Day 1 (16)**	**Day 5 (80)**	**Day 10 (150)**	**Day 21 (300)**
Concentration in fillet						
Mean (µg/kg)	< LOQ	217.92	228.99	49.32	9.41	< LOQ
± SD (µg/kg)	NA	86.97	48.18	17.36	4.78	NA

< LOQ: Less than the limit of quantitation (4 µg/kg); NA: Not applicable;

As temperatures fluctuated both within and between study sites, time points could not be directly converted to degree-days; therefore, reported degree-days are approximate.

The Committee considered the residue depletion study by Longshaw *(56)* to be the pivotal study, as this gave the worst-case results in terms of extent and persistence of imidacloprid residues in salmon fillet. It was noted that this was because the salmon had been exposed to the treatment solution for longer than the approved duration (approximately six-fold longer); however, it was also noted that this was a practical consideration since the salmon had to be treated in well-boats in order to prevent exposure of the environment to imidacloprid. As a result, in some cases it can take longer than the approved treatment time to remove the salmon from the treatment bath. It was also noted that this was the study used to set the withdrawal period for the one product approved in a Member State.

Analytical methods

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

Imidacloprid was determined by a validated LC-MS/MS method.

In summary, tissue samples were mechanically homogenized, spiked with internal standard (imidacloprid-d4; a deuterium-labelled analogue), and extracted with methanol, followed by a solid-phase extraction clean-up. Imidacloprid was quantified by LC-MS/MS using the selected ion monitoring mode. The quantitation and confirmatory transitions were m/z 256→209 and m/z 256→175, respectively. The confirmatory transition was not evaluated during method validation, however, it could be employed to confirm the presence of imidacloprid in monitoring samples. Concentrations were determined using

a solvent calibration curve and peak area ratios. The LOQ for imidacloprid was 4 µg/kg for both salmon and trout fillet.

Estimated dietary exposure

Dietary exposure to imidacloprid may occur through its use as a veterinary drug or its multiple registered uses as a pesticide.

Dietary exposure to imidacloprid (in some cases in combination with other neonicotinoid insecticides) has been estimated in several studies. In 2012 JMPR *(58)* estimated exposure to be 2–5% of a ADI of 0.06 mg/kg bw from residues potentially occurring in plant and animal commodities.

Recently EFSA *(59)* estimated chronic and acute dietary exposure for all uses of imidacloprid. Dietary exposures calculated were compared with the toxicological reference value derived by EFSA *(9)*. The highest chronic exposure estimate represented 7% of the ADI. Exceedance of the EFSA ARfD was identified for some commodities.

In 2021 Crépet et al. *(60)* estimated acute dietary exposure to several pesticide residues, including imidacloprid, using probabilistic methods. They estimated that imidacloprid exposure for adults ranged from 0.07–0.78 µg/kg bw, depending on consumption patterns in six different countries. For children the range was 0.26–1.9 µg/kg bw. They concluded that estimated acute dietary exposures were much lower than the ARfD of 400 µg/kg bw established by JMPR.

Chronic dietary exposure estimates

When used as a veterinary drug, chronic dietary exposure was estimated based on the potential occurrence of imidacloprid residues in Atlantic salmon muscle. The adjusted (MR:TRR = 0.7) mean residue level in Atlantic salmon (fillet) was 486 µg/kg. This value relates to a withdrawal period of 98 degree-days. No ADI was available.

Based on incurred residues in Atlantic salmon (fillet) and a withdrawal period of 98 degree-days, the global estimate of chronic dietary exposure (GECDE) for adults and the elderly is 1.0 µg/kg bw per day. For children and adolescents the GECDE is 2.7 µg/kg bw per day. For infants and toddlers, the GECDE is 0.9 µg/kg bw per day.

Based on incurred residues in all fin fish meat and a withdrawal period of 98 degree-days, the GECDE for adults and the elderly is 1.8 µg/kg bw per day. For children and adolescents the GECDE is 3.8 µg/kg bw per day. For infants and toddlers the GECDE is 1.2 µg/kg bw per day.

Acute dietary exposure estimates

Acute dietary exposure (global estimate of acute dietary exposure, GEADE) was estimated for consumption of Atlantic salmon using food consumption values from the FAO/WHO large portion (97.5th percentile, one day) database and 95/95 upper tolerance limit (UTL) concentrations for imidacloprid. Acute dietary exposure was also assessed on the basis of total fish consumption using the same residue data. Data was taken GEMS/Food large portion size database (97.5th percentile, one day; see JECFA, 2013, Annex "Pilot of new approaches to estimate dietary exposure to veterinary drug residues" in Annex 2, ref. 217).

Acute dietary exposures were assessed at 98 degree-days post dose. The adjusted (MR:TRR = 0.7) 95/95 UTL concentrations used were 859 µg/kg. No ARfD was available. Estimates were made for both children and adults.

The GEADE based on consumption of Atlantic salmon was 6.2 and 6.6 µg/kg bw for adults and children respectively. The GEADE based on consumption of all fin fish was 34.1 and 23.8 µg/kg bw for adults and children respectively.

Maximum residue limits

In recommending MRLs for imidacloprid in fin fish fillet, the Committee considered the following factors:

- An ADI for imidacloprid could not be established by the Committee.
- An ARfD for imidacloprid could not be established by the Committee.
- Imidacloprid is used as a pesticide and a veterinary drug.
- Imidacloprid is authorized for use in salmon and trout in one Member State. The maximum recommended treatment regimen is 20 mg/L, once, via immersion in a seawater treatment bath for 60 minutes. The approved withdrawal period is 98 degree-days for Atlantic salmon and rainbow trout.
- Under field conditions it may not be possible to remove all the fish from the treatment bath immediately after 60 minutes, so exposures of up to 360 minutes were considered.
- Imidacloprid is the marker residue in salmon and trout fillet.
- The ratio of marker residue to total residue concentrations was established at 0.7 .
- Residue data for salmon and trout were provided using a validated analytical method to quantify imidacloprid in fillet.
- A validated analytical method for determining imidacloprid in salmon and trout fillet is available and may be used for monitoring purposes.

As the Committee could not establish an ADI or an ARfD, MRLs could not be recommended.

When considering the possibility of recommending the extrapolation of MRLs, the Committee referred to the discussion at the CCRVDF on the "Proposed approach for the extrapolation of maximum residue limits of veterinary drugs to one or more species"; see *(6)*, p.11.

Were an MRL to be recommended in salmon and trout, the Committee could recommend extrapolation of the MRL to all salmonids, and potentially to all fin fish.

Although there are no pharmacokinetic or residue depletion data currently available for species other than salmon and trout, experience shows that fish do not metabolise pharmaceutical compounds to a great extent. As such, it is considered to be unlikely that the MR:TR in non-salmonid fin fish species would be much different from that seen in Atlantic salmon.

It should be noted, however, that water temperature has a significant effect on the extent of absorption, extent of metabolism and rate of elimination in fish. The data available do not address rate or extent of metabolism at higher water temperatures. Many other farmed fin fish species are kept at higher temperatures than salmonid species, and so might have different metabolic profiles, including an increased metabolism which would lead to a lower MR:TR being calculated, leading to an underestimate of human dietary exposure.

Nonetheless, the Committee considered that, as the worst-case scenario had been used for estimating the likely dietary exposure, there would be a margin of safety for any proposed MRL that could take into account slight differences in metabolism between salmonids and non-salmonids. A final recommendation will be made once an ADI and ARfD have been established.

Interested parties may wish to provide data on the MR:TR when fish are exposed to higher water temperatures (that is, greater than 10°C) to allow the Committee to make a more informed recommendation.

A residue monograph was prepared.

Summary and conclusions

Studies relevant to risk assessment – imidacloprid

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)
Mouse				
Two-year toxicity study (dietary)	Male: 0, 20, 66, 208 Female: 0, 30, 104, 274	Reduction in body weight	66	208
Rat				
Acute neurotoxicity study (gavage)	0, 42, 151, 307 mg/kg bw	Decreased locomotor activity	9** ($BMDL_{05}$)	42***
One-year toxicity study (dietary)	Male: 0, 5.6, 16.3, 55.8 Female: 0, 6.7, 19.5, 63.7	Reduction in body weight	5.6	16.3

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)
Two-year study (dietary)	Male: 0, 5.7, 17 and 51 Female: 0, 7.6, 26, 73	Increased incidence and severity of mineralized particles in the thyroid gland	5.7	17
Extended one-generation study (dietary)	Male F0:0: 5.25, 15.35, 48.4 Female F0: 0, 10.4, 30.43, 85.6	**Reproductive toxicity**	48.4****	-
		Parental toxicity: lower mean body, decreased food consumption	5.25*	15.35
		Offspring toxicity: decrease in pups delivered, increased stillborn, decrease in live pups, increase T4 concentration, reduced weights, lower spleen and thymus weights	10.4	30.43
Developmental toxicity study (gavage)	0, 5, 15, 50	**Maternal toxicity**: reduced body weight and decreased food consumption	15	50
		Embryo/fetal toxicity:	50****	-
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 postimplantation loss, reduced body weight, delayed ossification	24	72
Dog				
90-day (dietary)	0, 7.5, 24, 67.5/45	Tremors in first week	7.5	24

LOAEL: Lowest-observed-adverse-effect level; NOAEL: No-observed-adverse-effect level
* Pivotal study for the derivation of the toxicological ADI *(42)*
** Pivotal study for the derivation of the toxicological ARfD *(47)*
*** Lowest dose tested; **** Highest dose tested

ADI

An ADI could not be established.

ARfD

An ARfD could not be established.

Residue definition

The marker residue for imidacloprid in fillets of salmonids is the parent molecule, imidacloprid.

Dietary exposure

While estimates of dietary exposure were derived, there are no HBGVs with which to compare them.

MRLs

No MRLs could be established.

3.2 Ivermectin

Explanation

Ivermectin (Chemical Abstract Service No. 70288-86-7) is a macrocyclic lactone belonging to the avermectin family, widely used as a broad-spectrum antiparasitic drug against nematodes and arthropods in food-producing animals. It is also used in human medicine to treat various internal nematode infections, including onchocerciasis, strongyloidiasis, ascariasis, cutaneous larva migrans, filariasis, gnathostomiasis and trichuriasis, as well as for oral treatment of ectoparasitic infections such as pediculosis and scabies.

Ivermectin consists of two homologous compounds:
22,23-dihydroavermectin B_{1a} (H_2B_{1a}; not less than 80%), and
22,23-dihydroavermectin B_{1b} (H_2B_{1b}; not more than 20%).

Ivermectin is used in cattle, sheep, goats, pigs, horses, reindeer and American bison. It is available as injectable, topical (pour-on), premix and drench formulations.

Ivermectin was previously evaluated by the Committee at its thirty-sixth, fortieth, fifty-fourth, fifty-eighth, seventy-fifth, seventy-eighth, eighty-first, and eighty-eighth meetings (Annex 2, refs *91, 104, 116, 128, 208, 226, 243* respectively).

At the thirty-sixth meeting the Committee evaluated radiolabelled residue depletion studies in cattle, sheep and pigs and recommended maximum residue limits (MRLs) for these species.

At its fortieth meeting the Committee established an ADI of 0–1 µg/kg bw based on the developmental toxicity of ivermectin in CF-1 mice, and recommended MRLs of 40 µg/kg in fat and 100 µg/kg in liver for residues of ivermectin in cattle, with reference to the marker residue ivermectin H_2B_{1a}.

At its fifty-fourth meeting the Committee recommended a temporary MRL of 10 µg/kg for cattle milk, expressed as ivermectin H_2B_{1a}, which was confirmed at the fifty-eighth meeting of the Committee.

At its seventy-eighth meeting the Committee recommended an MRL of 4 µg/kg for cattle muscle, determined as ivermectin H_2B_{1a}, based on the depletion data contained in the residue monographs prepared by the thirty-sixth and fortieth meetings of the Committee, and based on a residue concentration of twice the limit of quantification of the analytical method employed.

At its eighty-first meeting the Committee was made aware that the MRL for ivermectin in bovine muscle recommended at the seventy-eighth meeting was in some cases ≥ 2.5 times lower than the MRLs established in some countries where ivermectin was being used. At that meeting, the Committee received new residue depletion data and recommended MRLs for cattle tissues: 400 µg/kg for fat, 100 µg/kg for kidney, 800 µg/kg for liver and 30 µg/kg for muscle. Also, at that meeting, an ADI of 0–10 µg/kg bw and an ARfD of 200 µg/kg bw were established,

as it had become apparent that the CF1 mouse was not an appropriate strain for evaluation of the toxicity of ivermectin.

At its eighty-eighth meeting, the Committee received a data set from one Member State, including two residue depletion studies in sheep. No residue depletion data were received for pigs or goats. At the eighty-eighth Meeting (Appendix 2, ref. *243*) the Committee recommended MRLs in sheep tissues: 15 µg/kg for kidney and 10 µg/kg for muscle. The Committee also confirmed the existing MRLs for fat at 20 µg/kg and for liver at 15 µg/kg. In addition, the Committee recommended maintaining the existing MRLs in pig fat (20 µg/kg) and pig liver (15 µg/kg) tissues, and extending the MRLs for sheep muscle to pig muscle (10 µg/kg) and sheep kidney to pig kidney (15 µg/kg). This extension was made considering the limited residue data for pigs and the similarity of the overall tissue distribution and residue depletion in both species. As no residue depletion data for ivermectin were received to calculate MRLs for goats, and based on the similarity of the residue distribution and depletion in different animal species, the Committee recommended extrapolation of the MRLs for sheep and pig tissues to goat tissues (10 µg/kg for muscle, 15 µg/kg for liver, 15 µg/kg for kidney and 20 µg/kg for fat).

Ivermectin was on the agenda at the present meeting at the request of the Codex Committee on Residues of Veterinary Drugs in Food for the re-evaluation of the MRLs for pig, sheep and goat tissues.

Residue evaluation

At the present meeting, the Committee considered data submitted by three sponsors that included information on residue depletion studies in pigs (one study employing radiolabelled ivermectin and two using non-radiolabelled ivermectin), sheep (one study employing radiolabelled ivermectin and two studies using non-radiolabelled ivermectin), and goats (one study using non-radiolabelled ivermectin). In addition, the Committee reviewed metabolism studies in pigs (one study) and sheep (one study). The metabolism study in pigs was considered at the eighty-eighth JECFA meeting but at that meeting only the summary had been available. The analytical methods submitted to support the ivermectin residue depletion study in sheep were also assessed.

Data on pharmacokinetics and metabolism

Metabolism studies in pigs and sheep using radiolabelled ivermectin were provided.

Pigs

In a metabolism and depletion study (*(61),* GLP compliance not stated) a single subcutaneous injection of tritium labelled ivermectin (tritium in C_{22}– C_{23} position)

was administered to 12 pigs (weights not provided) at a dose of 0.4 mg/kg bw. Three animals per group were slaughtered at 1, 7, 14, and 28 days post dose. Muscle, liver, kidney and fat tissues were collected and assayed for H_2B_{1a} and H_2B_{1b} using reverse isotope dilution assay (RIDA). Composite liver samples from pigs slaughtered seven and 14 days after dosing were examined for the presence of drug metabolites by use of solvent fractionation and a combination of reversed-phase and normal-phase high-performance liquid chromatography (HPLC). Radioactive residues in the liver samples were identified or classified based on chromatographic polarity. Two metabolites were identified by comparison with the metabolites generated by in vitro pig liver microsome incubations. The fat tissues of one pig slaughtered 14 days after dosing were analysed for the presence of drug metabolites in a similar way to the liver samples.

The radioactive residues were extractable in organic solvents in their entirety, which indicated that no covalently bound residues would need to be considered. The unaltered drug (H_2B_{1a} plus H_2B_{1b}) accounted for about 42% and 28% of TRR in liver at seven and 14 days post dose, respectively. In fat these values were 51% and 37% respectively, for the same days. Due to the low levels of radioactive residues in the tissues, isolation and purification of the metabolites for structural elucidation was not possible. In vitro incubations of ivermectin with pig liver microsomes were performed for metabolite identification. The metabolites were purified by reversed-phase and normal-phase HPLC and the products were analysed by ultraviolet spectrometry, nuclear magnetic resonance (NMR) spectrometry and fast atom bombardment mass spectrometry (FAB-MS). Among the metabolites isolated and purified from the in vitro study, two metabolites were characterized: 3″-*O*-desmethyl-H_2B_{1a} and 3″-*O*-desmethyl-H_2B_{1b}. These metabolites had been considered by the Committee at the eighty-eighth Meeting (Annex 2, ref. 243).

Sheep

In a metabolism study, GLP compliance not stated, 12 sheep received a single intraruminal dose of 0.3 mg/kg bw of [*22,23*-^{3}H]ivermectin *(62)*. Groups of three animals were slaughtered on each of days 1, 3, 5 and 7 post dose. Tissue samples were taken from the liver, kidney, fat and muscle to measure the radioactive residues. These edible tissues were assayed for both components of the unaltered drug (H_2B_{1a} and H_2B_{1b}) either by RIDA or by direct fluorescence assay. By a combination of solvent fractionations and reverse-phase HPLC, the radioactive residues in liver and fat were identified or classified based on chromatographic polarity. Unaltered H_2B_{1a} (marker) and H_2B_{1b} were the major components at all slaughter timepoints. The radioactive residues in the tissues were extractable in organic solvents (dichloromethane), indicating that there were no significant bound residues. The liver of one animal slaughtered on day 5 post dose was

assayed for the presence of metabolites. The analysis included solvent extraction and liquid chromatography. Radioactive residues were identified based on chromatographic polarity. Overall, 68% of the TRR in the liver was identified. The remaining 32% of the residue consisted of at least two products, both less polar than the parent compound, and another seven compounds more polar than ivermectin. At least four metabolites more polar than the parent compound were identified by co-chromatography with in vitro metabolites prepared from cattle liver microsome incubation with ivermectin. The fat samples of two animals slaughtered five days and seven days post dose contained the unaltered drug at 29.9% and 30.5% of TRR, respectively. The distribution of polar metabolites in sheep liver was similar to that observed in cattle and rat livers. The profile of the radioactive metabolites in sheep fat indicated that most of the metabolites were less polar than the parent drug. A decline in the proportion of the polar residues was also observed, accompanied by an increase in nonpolar metabolites with increasing time post dose.

The identified metabolites were consistent with the findings of Chiu & Lu *(63)* from the in vivo liver metabolism of tritium-labelled ivermectin in sheep, pigs, cattle and rats. The Committee had reviewed this study at its eighty-eighth meeting (Annex 2, ref. 243).

Goats

No metabolism studies were available to the Committee for ivermectin in goats.

Residue depletion data

Two residue depletion studies using radiolabelled ivermectin in pigs and sheep were provided by one sponsor for evaluation at the present meeting.

The Committee assessed five additional residue depletion studies with non-radiolabelled ivermectin; two studies in pigs, two studies in sheep and one study in goats.

Residue depletion studies with radiolabelled drug

Pigs

One study, GLP compliance not stated, using [*22,23*-^{3}H]ivermectin was considered. Twelve pigs (body weights not stated) were dosed with 0.4 mg/kg bw tritium-labelled ivermectin via a single subcutaneous injection *(61)*. The purity, specific activity, and solvent in which the drug was prepared were not reported. Three animals were slaughtered at 1, 7, 14 and 28 days post dose and muscle, liver, kidney and fat tissues collected. Concentrations of H_2B_{1a} and H_2B_{1b} were determined using RIDA. The LOD was 1.2 µg/kg for liver and fat, 0.8 µg/kg for kidney and 3 µg/kg for muscle. Fat tissues contained the highest mean H_2B_{1a}

concentrations (51.5 µg equiv./kg at day 1 and 80.5 µg equiv./kg at day 7 post dose), followed by liver (48 µg equiv./kg at day 1 and 39 µg equiv./kg at day 7 post dose). The marker residue concentrations in muscle were lower than 15 µg equiv./kg at all sampling times. No residues were detected in muscle after 14 days post dose. Concentrations of H_2B_{1a} were at least 2–3 times higher than the homologue H_2B_{1b} in all tissues analysed. The ratios of mean marker residue to total radioactive residues (MR:TRR) varied with time post dose, and are shown in Table 6. The Committee noted that the changes in MR:TRR with time were not monotonic, except in kidney.

Table 6
Mean marker residue (H_2B_{1a}) to TRR ratio at different times post dose in pigs

	MR:TRR ratio			
Time post dose (days)	**Muscle**	**Liver**	**Kidney**	**Fat**
1	0.35	0.24	0.29	0.13
7	0.44	0.35	0.45	0.53
14	Not detected	0.25	0.50	0.20

Sheep

In a radiolabelled study, GLP compliance not stated, four groups of three sheep were administered a single intraruminal dose of 0.3 mg/kg bw [*22,23*-^{3}H]ivermectin *(62)*. Groups of animals were slaughtered on days 1, 3, 5 and 7 post dose. Liver, kidney, fat and muscle were sampled and assayed for H_2B_{1a} and H_2B_{1b} either by RIDA or by direct fluorescence assay. The highest mean H_2B_{1a} concentrations were recorded in fat (218 µg eq/kg) at day 1 post dose, followed by liver (128 µg eq/kg), kidney (37 µg eq/kg) and muscle (37 µg eq/kg). The MR:TRR ratios declined from day 1 to day 5 post dose in all tissues (Table 7).

Table 7
Mean marker residue (H_2B_{1a}) to TRR ratio at different times post dose in sheep

	MR:TRR ratio			
Time post dose (days)	**Muscle**	**Liver**	**Kidney**	**Fat**
1	0.67	0.54	0.51	0.71
3	0.52	0.51	0.44	0.51
5	0.54	0.56	0.08	0.25

All the residues were extractable into organic solvents, indicating that none of the residues were covalently bound to tissues.

Residue depletion studies with non-radiolabelled drug

Five studies (two in pigs, two in sheep and one in goats) with non-radiolabelled ivermectin were provided by three sponsors and assessed by the current Committee.

Pigs

In the first study *(64)*, GLP compliance not stated, 35 pigs (22.8–32.3 kg, barrows, and gilts) were administered a single subcutaneous dose of ivermectin of 0.4 mg/kg bw. The drug formulation contained 40% v/v glycerol formal, and propylene glycol to make up 100%. The approved withdrawal period for this formulation is 14 days. The animals were slaughtered in groups of five at 1, 3, 5, 7, 10, 14 and 28 days post dose and the concentration of H_2B_{1a} was determined in the edible tissues and in an injection site sample using HPLC with a fluorescence detector (HPLC-FLD). The recovery was assessed by fortification of blank tissues at four concentration levels (10, 20, 50 and 100 µg/kg). The mean recoveries were: 88% for muscle, 82% for liver, 87% for kidney and 90% for fat. The LOD of the method was in the range of 1–2 µg/kg, and the LOQ was 10 µg/kg. The injection sites contained the highest ivermectin concentrations (mean ± standard deviation [SD]) at all time points (day 1 post dose, 139 333 ± 5857 µg/kg; day 14 post dose, 260 ± 254 µg/kg). At 14 days post dose the mean concentrations ± SD of the marker residue were: 4.1 ± 2.4 µg/kg in muscle, 15.4 ± 3.8 µg/kg in liver, 5.7 ± 2.2 µg/kg in kidney, 26.9 ± 9.0 µg/kg in fat, and 260 ± 254 µg/kg in the injection site sample. At day 28 post dose residues in all five pig tissues were lower than the LOD of the method.

In the second study *(*GLP-compliant), 22 pigs (11 barrows and 11 gilts; body weight range 92.4–122.2 kg) were administered a single subcutaneous injection of ivermectin at a dose of 0.3 mg/kg bw *(65)*. Twenty animals were separated into five groups. A group of four animals was slaughtered at each of 7, 14, 21, 28 and 35 days post dose and tissues (muscle, liver, kidney, fat and the injection site) collected. Ivermectin quantitation (H_2B_{1a}) was carried out using a validated HPLC-FLD method *(66)*. The LODs for the method were: 0.11 µg/kg for muscle and liver, 0.56 µg/kg for fat, and 0.10 µg/kg for kidney. The LOQ was 2 µg/kg for all tissues. Recoveries from fortified blank samples at different concentration levels ± SD were: 91% ± 8.1% for muscle, 86% ± 5.5% for liver, 91% ± 7.3% for kidney, and 96% ± 8.1% for fat. Individual dosing data were not provided, therefore the Committee could not establish the actual dose administered to each animal. Fat tissues contained the highest residue concentrations at all time points (at day 7 post dose, 112.8 ± 63.8 µg/kg and at day 28 post dose 6.7 ± 3.8 µg/kg). At 14 days post dose the mean concentrations ± SD of the marker residue were 7.7 ± 6.0 µg/kg in muscle, 18.8 ± 5.6 µg/kg in liver, 11.1 ± 3.0 µg/kg in kidney, 72.9 ± 20.6 µg/kg in fat, and 16.1 ± 7.9 µg/kg in the injection site sample. At 35 days post dose the

residue concentrations in muscle, liver and in the injection site sample were below the LOQ of the method, while the mean concentrations ± SD determined in kidney and fat were 1.08 ± 0.9 µg/kg and 3.8 ± 2.2 µg/kg, respectively.

Sheep

In the first study *(67)*, GLP compliance not stated, 24 wether and ewe lambs (39.6–58.1 kg) were administered a subcutaneous dose of ivermectin of 0.3 mg/kg bw once a week for three weeks. The drug formulation contained 40% v/v glycerol formal, and propylene glycol to make up 100%. The approved withdrawal period for this formulation is 22 days. The animals were slaughtered in groups of four or five at 3, 7, 10, 14 and 28 days post dose, and the concentration of H_2B_{1a} was determined in the edible tissues and at the injection site using HPLC-FLD. The recovery was assessed by fortification of blank tissues at four concentration levels (10, 20, 50 and 100 µg/kg). Mean recoveries were: 93% for muscle, 89% for liver, 87% for kidney and 95% for fat. The LOQ of the method was about 10 µg/kg. The injection site samples contained the highest H_2B_{1a} concentrations at all time points (at day 1 post dose 18 358 ± 18 613 µg/kg, and at day 28 post dose 235 ± 185 µg/kg). At 14 days post dose, the mean concentrations ± SD of the marker residue were: 32.0 ± 14.7 µg/kg in muscle, 61.6 ± 28.3 µg/kg in liver, 22.5 ± 6.2 µg/kg in kidney, 104.0 ± 54.6 µg/kg in fat, and 480.0 ± 294.9 µg/kg in the injection site sample. At 28 days post dose the mean residues of H_2B_{1a} in muscle, liver, kidney, and fat were below 13.9 µg/kg and in the injection site samples, below 235 µg/kg.

In the second study (GLP-compliant) 20 ewes (52–89 kg) were administered a single subcutaneous dose of ivermectin of 0.2 mg/kg bw *(68)*. The animals were slaughtered in groups of five at 28, 35, 42, and 49 days post dose, and the concentration of H_2B_{1a} was determined in muscle, liver, kidney, fat and an injection site sample, by a validated HPLC-FLD method. The calibration was in the range of 5–200 µg/kg, and the LOQ of the method was 5 µg/kg for all tissues. All 100 tissue samples analysed contained concentrations of H_2B_{1a} below the LOQ, with the exception of seven samples collected at 28 days post dose (5.2 µg/kg in kidney, 14.3 µg/kg in liver, 30.8 and 12.0 µg/kg both in fat, and 5.7 µg/kg in an injection site sample), and 49 days post dose (44.9 µg/kg and 6.4 µg/kg in injection site samples). The Committee could not establish depletion curves for the four tissues due to a lack of data above the LOQ.

Goats

In a residue depletion study *(69)*, 12 bucks and 12 does (18–36 kg) were administered a single subcutaneous dose of ivermectin of 0.2 mg/kg bw. The animals were slaughtered in groups of four at 1, 7, 21, 28, 35 and 42 days post

dose and the concentration of H_2B_{1a} was determined in liver and fat tissues by HPLC-FLD. Data on ivermectin residues in the kidney and muscle were not reported. Quantitation was performed using a solvent calibration curve, and not all the method validation parameters were reported. An LOD for the marker residue of 4.2 µg/kg was reported. The mean recoveries were: 90% for muscle, 78% for liver, 76% for kidney and 85% for fat. The documentation presented was incomplete. The samples had been stored longer than the amount of time for which stability was demonstrated.

Analytical methods

The Committee noted that the methods used in the residue depletion studies assessed by the present meeting were outdated and did not fulfil all the requirements for analytical methods as published in the Codex Guideline CAC/GL 71-2009. The methods were validated in the 1980s and 1990s using complex multistep sample preparation procedures, and utilized HPLC-FLD. The calibration graphs were obtained in solvent instead of using the matrix-matched calibration curves more commonly employed nowadays. As a result, all residues reported in edible tissues needed to be corrected for recoveries.

Suitable validated analytical methods for the determination of H_2B_{1a} in tissues are available for monitoring purposes and were assessed by the Committee at the eighty-eighth meeting (Annex 2, ref. 243).

Monitoring data

The Committee received a report of findings for residues of ivermectin in pigs, goat and lambs from one competent national authority for residues control. Samples of liver and muscle were collected between 2015 and 2021 and analysed using LC-MS/MS or HPLC-FLD, for which the LOQ was 2 µg/kg. Of the samples tested, only 15 out of 4634 (0.32%) of pig tissues and two out of 1471 samples of lamb tissues (0.14%) were positive for H_2B_{1a} residues. For goat tissues, no residues of H_2B_{1a} were detected in the 123 samples analysed. The highest concentration (7.4 µg/kg) was determined in a sample of pig liver.

Estimated dietary exposure

Chronic dietary exposure assessment

Dietary exposure to ivermectin may occur only through its use as a veterinary drug. There is no registered use for ivermectin as a pesticide.

When used as a veterinary drug, dietary exposure was estimated based on the potential occurrence of ivermectin residues in cattle, sheep, pig and goat tissues.

Median residue levels in cattle tissues (muscle, liver, kidney and fat) were taken from the evaluation carried out at the eighty-first meeting of the Committee. These values relate to a withdrawal period of 14 days (Annex 2, ref. *226*).

For pigs and sheep, several residue depletion studies were available. However, a range of different dosing regimens were used and for the current assessment a conservative approach was taken, with the study that reported the highest tissue residue levels being used to determine chronic dietary exposure.

For sheep, residue levels were derived from the 1984 study of Wood et al. *(67)*. Data were taken from 22 days post dose (0.3 mg/kg bw administered subcutaneously, once a week for three weeks). In this study, tissues were analysed at 14 and 28 days post final dose, but not at 22 days. Residue concentrations for dietary exposure assessment were determined by linear regression. The study of Chiu & Jacob *(62)* determined MR:TRR ratios at 1, 3, and 5 days post dose (0.3 mg/kg bw subcutaneously). Ratios at day 5 were applied to the day 22 residue concentrations. The ratios were: 0.54 for muscle, 0.56 for liver, 0.08 for kidney and 0.25 for fat.

For pigs, residue data were available from the 1981 study by Wood et al. *(64)*. Data were taken from 14 days post dose (0.4 mg/kg bw, subcutaneously), the shortest withdrawal period reported for ivermectin use in pigs. The 1982 study by Chiu & Lu *(61)* determined MR:TRR ratios at 1, 7, and 14 days post dose (0.4 mg/kg bw, subcutaneously). Ratios for day 14 were applied to the day 14 median residue concentrations, except for muscle, for which only day 1 and day 7 ratios were available, so the day 7 ratio was used. The ratios were: 0.44 for muscle, 0.25 for liver, 0.50 for kidney and 0.20 for fat.

No suitable residue data were available for ivermectin in goat tissues and the values derived for sheep were used as surrogates.

The Committee had previously evaluated milk residue data and recommended an MRL of 10 µg/kg for milk in cattle, expressed as H_2B_{1a}. However, there are currently no approvals for the application of ivermectin formulations to lactating dairy cattle and dietary exposure to ivermectin residues in milk was not considered in the current evaluation. There are no MRLs for ivermectin residues in milk from other species.

Based on incurred residues in cattle, sheep, pig and goat tissues (muscle, liver, kidney and fat) and a withdrawal period of 14 days for cattle and pigs and 22 days for sheep and goats, the global estimate of chronic dietary exposure (GECDE) for adults and the elderly is 0.72 µg/kg bw per day, which represents 7.2% of the upper bound of the ADI of 10 µg/kg bw. For children and adolescents, the GECDE is 0.93 µg/kg bw per day, which represents 9.3% of the upper bound of the ADI. For infants and toddlers, the GECDE is 0.48 µg/kg bw per day, which represents 4.8% of the upper bound of the ADI.

As part of the GECDE methodology, further estimates of chronic dietary exposure were carried out. Instead of using the highest mean and the highest 97.5th percentile consumption across surveys, the calculations were carried out using the mean and the highest reliable percentile for each individual national survey from available datasets (CIFOCOss). The highest GECDE for each age class for each country was determined.

The mean and range of 35 country-specific estimates for adults and the elderly at 14 days (cattle and pigs) and 22 days (sheep and goats) after final treatment was 0.095 µg/kg bw per day, range 0.002–0.61 µg/kg bw per day, or 1.0% (range 0.02–6.1%) of the upper bound of the ADI. The mean and range of 25 country-specific estimates for children and adolescents was 0.12, range 0.005–0.82 µg/kg bw per day, or 1.2% (range 0.05–8.2)% of the upper bound of the ADI. The mean and range of 18 country-specific estimates for infants and toddlers was 0.11 µg/kg bw per day, range 0.018–0.35 µg/kg bw per day or 1.1% (range 0.2–3.5%) of the upper bound of the ADI.

Acute dietary exposure assessment

Acute dietary exposure (as GEADE) was assessed for consumption of cattle, sheep or pig muscle using food consumption values from the FAO/WHO large portion (97.5th percentile, one-day) database and 95/95 UTL concentrations for ivermectin at the injection site. No injection site residue data were available for goats. Acute dietary exposures for cattle and pigs were assessed at 14 days post dose and sheep at 22 days post dose. The 95/95 UTL concentrations used were 5447 µg/kg for cattle (from eighty-first JECFA), 4466 µg/kg for sheep (from *(67)*) and 1860 µg/kg for pigs (from *(64)*). No MR:TRR ratios were available for the injection site, and it was assumed that little metabolism would occur at this site (MR:TRR = 1). Estimates were considered for both children and the general population and these were compared to the ARfD of 200 µg/kg bw. However, for all species the highest large-portion values were for children and, consequently, the large-portion sizes for children and the general population, were identical. The GEADE was 69 µg/kg bw (35% of the ARfD) from consumption of cattle muscle for the general population and children. The GEADE was 73 µg/kg bw (37% of the ARfD) from consumption of sheep muscle for the general population and children. The GEADE was 30 µg/kg bw (15% of the ARfD) from consumption of pig muscle for the general population and children.

Maximum residue limits

In recommending MRLs for ivermectin in pigs, sheep and goats the Committee considered the following factors:

- The ADI previously established by the Committee was 0–10 µg/kg bw.
- The ARfD previously established by the Committee was 200 µg/kg bw.
- Ivermectin B_{1a} (synonym for *22,23*-dihydroavermectin B_{1a} or H_2B_{1a}) is the marker residue in pigs, sheep and goats.
- Ivermectin is authorized for use in sheep, goats and pigs in many Member States.
- Data on the metabolism of ivermectin in pigs and sheep were provided by one sponsor (two studies). No metabolism data were received for goats.
- Tissue distribution of ivermectin residues was similar in pigs and sheep, with the highest residue levels in fat and liver tissues comparable to those described in cattle.
- The ratios of marker residue to total residue in pigs of 0.20 in fat, 0.50 in kidney, 0.25 in liver and 0.44 in muscle were used (day 14 post dose for all tissues except muscle, day 7 post dose for muscle).
- The ratios of marker residue to total residue in sheep of 0.25 in fat, 0.08 in kidney, 0.56 in liver and 0.54 in muscle were used (all day 5 post dose).
- One complete study was available for deriving upper tolerance limits (UTLs) in pig tissues. The animals were dosed once (0.4 mg/kg bw) with a 1% ivermectin formulation; the indicated withdrawal period for this formulation is 14 days.
- One complete study was available for deriving UTLs in sheep tissues. The animals were dosed (0.3 mg/kg bw) three times at weekly intervals with a 1% ivermectin formulation; the indicated withdrawal period for this formulation is 22 days. The Committee noted that the dose administered in this study was not the indicated dosing regimen for this product, which is a single injection.
- The analytical methods used for the residue depletion studies in pigs, sheep and goats were adequate for the time that they were used, however, they are not fully validated based on current requirements. Validated analytical methods for the determination of ivermectin in all edible tissues of all the species considered are available and are suitable for monitoring purposes.

MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of total residue concentrations

(95/95 UTL) in pig and sheep tissues derived from the data provided.

The Committee recommended the following MRLs in pig tissues: 15 µg/kg for muscle, 30 µg/kg for liver, 20 µg/kg for kidney and 50 µg/kg for fat, all based on the UTLs at 14 days.

The Committee recommended the following MRLs in sheep tissues: 30 µg/kg for muscle, 60 µg/kg for liver, 20 µg/kg for kidney and 100 µg/kg for fat, based on the UTLs at 22 days. MRLs based on UTLs for shorter withdrawal periods were not recommended because estimates of acute exposure based on injection site residues resulted in an exceedance of the ARfD (130%).

The residue depletion study of ivermectin in goats was incomplete. Data were provided for liver and fat only, and it was not possible to derive UTLs. Based on the similarities between small ruminant species, the Committee recommended extension of the MRLs for sheep to goat tissues.

An addendum to the residue monograph was prepared.

Summary and conclusions

ADI

The ADI previously established by the Committee was 0–10 µg/kg bw.

ARfD

The ARfD previously established by the Committee was 200 µg/kg bw.

Residue definition

The marker residue in sheep, pigs and goats is ivermectin B_{1a} (H_2B_{1a}, or 22,23-dihydroavermectin B_{1a}).

Dietary exposure

The global estimate of chronic dietary exposure (GECDE) was 0.72, 0.93 and 0.48 µg/kg bw per day (7.2%, 9.3% and 4.8% of the upper bound of the ADI of 10 µg/kg bw) for adults and the elderly, children and adolescents, and toddlers and infants, respectively.

The global estimate of acute dietary exposure (GEADE) was 69, 73 and 30 µg/kg bw (35%, 37% and 15% of the ARfD) from consumption of cattle, sheep and pig muscle respectively, for both the general population and children.

MRLs

Pigs

15 µg/kg for muscle, 30 µg/kg for liver, 20 µg/kg for kidney and 50 µg/kg for fat.

Sheep and goats

30 µg/kg for muscle, 60 µg/kg for liver, 20 µg/kg for kidney and 100 µg/kg for fat.

3.3 Nicarbazin

Explanation

Nicarbazin 1,3-*bis*(4-nitrophenyl)urea;4,6-dimethyl-*1H*-pyrimidin-2-one (IUPAC); Chemical Abstract Service No. 330-95-0 is a carbanilide used for the prevention of faecal and intestinal coccidiosis in chickens, as well as in some other poultry species. Nicarbazin is used as a feed additive or as a veterinary drug for oral use in feed.

Nicarbazin is an equimolar complex of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). It is practically insoluble, but will dissociate completely in aqueous conditions, such as the digestive tract. DNC is the active anticoccidial component while HDP has no anticoccidial activity. The absorption of DNC is greatly enhanced when the two components are complexed together. The mode of action (MOA) of DNC is unclear but may involve the inhibition of mitochondrial electron transport.

Nicarbazin was evaluated for toxicology and residues by the Committee at its fiftieth meeting (Appendix 2, ref. 134). An ADI of 0–400 µg/kg bw nicarbazin (24 mg/person for a 60 kg person) was established. MRLs for chicken muscle, liver, kidney and skin/fat (in natural proportions) were recommended at 200 µg/kg nicarbazin, using DNC as the marker residue.

The Committee evaluated nicarbazin at the present meeting at the request of the twenty-fifth session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) with a view to recommending maximum residue limits (MRLs) for edible chicken tissues. A toxicological re-evaluation was also undertaken to establish health-based guidance values due to the time that had elapsed since its last review. The sponsor provided unpublished proprietary studies as well as data from studies in the published literature to support the assessment.

The inclusion rate for nicarbazin provided by the sponsor was 125 mg/kg nicarbazin per day in complete feeding stuffs to be used in chickens for fattening. For this dose, withdrawal periods range from 1–10 days for edible tissues. The Committee noted that a higher inclusion rate of 200 mg/kg feed is approved in at least one Member State, with a withdrawal period of five days, but no residue data were provided for this dosing regimen. When used in combination with either narasin or monesin the nicarbazin inclusion rate is lower at 50 mg/kg feed and withdrawal periods range from 0–8 days. Products are not intended for use in animals producing eggs for human consumption.

Nicarbazin is not currently used as a plant protection product or as a human medicine.

Toxicological and microbiological evaluation

The Committee conducted a comprehensive search of the scientific literature from the following publicly accessible databases: Web of Science (371 papers identified), PubMed (252), Google Scholar (3720), CAB Abstracts (1292). Of these, 36 were considered relevant for this assessment. Further, information in reports from the European Food Safety Authority (EFSA) in 2003*(70)*, 2010 *(71)*, 2017*(72)*, 2018*(73)* and 2021*(74)*, and the United States Food and Drugs Administration Centre for Veterinary Medicine (CVM) in 2018 *(75)* were considered in making this assessment. Many of the studies were not performed according to GLP and where this was the case it is indicated.

Biochemical data

Rats received single oral doses of 1, 5, or 10 mg/kg bw nicarbazin. Low concentrations of DNC were detected at six hours, but not at 18 hours. By contrast HDP was found at considerably higher concentrations, which increased between six hours and 18 hours. Qualitatively similar findings were obtained in rats given oral doses for eight days. In urine collected at five hours, concentrations of HDP were an order of magnitude higher than those of DNC *(76)*.

Male rats received single oral doses by gavage of nicarbazin at 0, 50, 150 or 450 mg/kg bw, DNC at 0, 150, 450 or 900 mg/kg bw, or a DNC/HDP mixture at 0/0, 35/15, 106/44, or 319/131 mg/kg bw. Maximum concentration (C_{max}) in plasma for DNC occurred at around two hours in all treated groups. The half-life of DNC was around 12 hours (6–16 h) and was independent of dose and the form in which it was administrated. The bioavailability of DNC when given alone was less than 2.5% relative to that when given in the form of nicarbazin. When given as a mixture with HDP, the bioavailability of DNC was less than 3.3% relative to that when given in the form of nicarbazin *(77)*.

In a study summarized by EFSA in 2003, [^{14}C]DNC nicarbazin was administered to rats in their diet at about 100 mg/kg feed (1 mg/rat per day) for five days. DNC and metabolite M1 (DNC with one nitro group reduced and acetylated) were the major constituents found in the faeces. M1 and M3 (DNC with both nitro groups reduced and acetylated) were the major constituents found in the urine *(70)*.

Studies on the physicochemical properties of DNC alone and in complex with HDP *(78)* indicated that the presence of an intermolecular bonded moiety such as HDP, as in nicarbazin, was necessary for the effective absorption of DNC.

Toxicological data

The acute oral toxicity of nicarbazin in rodents was low, the LD_{50} values being greater than 25 000 mg/kg bw in mice and greater than 10 000 mg/kg bw in rats.

The individual components of nicarbazin also displayed low acute toxicity; the oral LD_{50} in mice being 4000 mg/kg bw for HDP and greater than 18 000 for DNC.

Only summaries of the short-term studies on nicarbazin were available, and these reports were inadequate for detailed evaluation as they contained minimal detail of the protocols used, limited data on toxicological findings, and were often in the form of progress reports. There was evidence of kidney damage associated with crystalline deposits in the collecting tubules in rats at oral doses of 500 mg/kg bw per day and above. In dogs, bile duct proliferation was the principal finding following an oral dose of 1600 mg/kg bw per day *(76)*.

More recently, 13-week studies of toxicity in rats have been conducted to more closely examine the toxicity of nicarbazin and DNC. These studies were conducted in accordance with current Organisation for Economic Co-operation and Development (OECD) guidelines and complied with GLP. Nicarbazin was administered orally to rats for 13 weeks via the diet, to provide dosages of approximately 200, 600 or 1000 mg/kg bw per day *(77)*. There were significant changes in clinical chemistry and urinalysis parameters indicative of renal toxicity at 200 mg/kg bw per day or greater. A NOAEL could not be identified. Another 13-week toxicity study was conducted in order to identify a NOAEL, and this examined the toxicity of DNC alone. The top dose level was chosen to be equivalent to the dose of DNC that would result from 1000 mg/kg bw per day of nicarbazin. DNC was administered to rats by oral gavage at 106, 284 or 709 mg/kg bw per day for 91 days *(79)*. There were no effects on any of the parameters evaluated including histopathological changes in the kidneys. The NOAEL for DNC was 709 mg/kg bw per day (the molar equivalent of 1000 mg/kg bw per day of nicarbazin), the highest dose tested.

In an OECD guideline study reported in summary by EFSA in 2017 *(72)*, rats were fed for 13 weeks a diet containing nicarbazin to provide a dose of 100 mg/kg bw per day, or an equimolar mixture of DNC/HDP to provide a dose of 71/29 mg/kg bw per day. A range of histopathological effects were seen in the kidneys of nicarbazin-treated animals, with associated changes in clinical chemistry and urinalysis. By contrast, for a mixture of DNC plus HDP the only dose tested, 71/29 mg/kg bw per day, was the NOAEL for the mixture.

In an OECD guideline study reported in summary by EFSA in 2021, rats received a diet for 13 weeks containing nicarbazin [sic], at dose levels providing 0 + 0, 50 + 17, 150 + 50 or 300 + 100 mg/kg bw per day (DNC + HDP). The NOAEL was 300 + 100 mg/kg bw per day DNC + HDP, the highest dose tested *(74)*.

In an OECD guideline study reported in summary by EFSA in 2021, dogs received nicarbazin [sic] by gavage for 90 days, at dose levels providing 0 + 0, 60 + 20, 180 + 60 or 600 + 200 mg/kg bw per day (DNC + HDP). The NOAEL was 600 + 200 mg/kg bw per day DNC + HDP, the highest dose tested *(74)*.

In an OECD guideline study rats were fed for 52 weeks a control diet or

a DNC + HDP mixture at 20 + 8, 50 + 20.5 and 154 + 63 mg/kg bw per day. The NOAEL was 20 + 8 mg/kg bw per day on the basis of the occurrence of crystals in the urine and associated histopathological changes in the kidney, including tubular basophilia, interstitial chronic inflammation, mononuclear inflammatory infiltrate, tubular dilation, cysts, intraductal inflammatory cells, hyaluronic acid casts, and papillary oedema, at 50 + 20.5 mg/kg bw per day. As summarized in the EFSA report, the effects were described as "rather slight". The full study report was not available, so it was not possible to assess the data in detail *(72)*.

In a GLP study based on OECD TG 452, rats were fed a diet containing 3:1 mixtures of DNC + HDP at 0 + 0, 52.5 + 17.5, 150 + 50 or 300 + 100 mg/kg bw per day for up to 52 weeks. The NOAEL was 52.5 + 17.5 mg/kg bw per day DNC + HDP based on chronic inflammation in the kidneys which correlated macroscopically with rough surface and/or tan discolouration in animals exposed to 150 + 50 mg/kg bw per day or more of DNC + HDP *(74)*.

Rats were fed diets containing a mixture of DNC and HDP (purity unspecified) for two years at concentrations calculated to give doses of 0, 50, 150 or 300 mg/kg bw per day of DNC and 0, 17, 50 or 100 mg/kg bw per day of HDP. The administration of the DNC and HDP mixture did not affect the incidence of tumours and there were no signs of any dose-related gross or histopathologic changes, so the highest dose tested was identified as the NOAEL, which was 300 mg/kg bw per day for DNC, and 100 mg/kg bw per day for HDP *(80)*.

The Committee concluded that DNC was not carcinogenic in rats.

Dogs were fed diets containing a mixture of DNC and HDP (purity unspecified) in a ratio of 3:1 for six days per week for two years. The actual intakes were 0, 60, 180 or 600 mg/kg bw per day of DNC, and 0, 20, 60 or 200 mg/kg bw per day of HDP. Changes in alanine transaminase (ALT) were seen in some dogs at the highest dose and one dog in this group showed slight bile duct proliferation. Although the relationship between the hepatic findings and treatment was unclear, JECFA 1998 concluded that the conservative NOEL in this study was 240 mg/kg bw day (DNC + HDP) *(81)*. Correcting for duration of exposure on six days per week, the NOAEL was 154 mg/kg bw per day for DNC and 51 mg/kg bw per day for HDP, equivalent to 200 mg/kg bw per day day of nicarbazin.

The genotoxic potential of nicarbazin was investigated in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was observed, including from in vitro mammalian gene mutation assays, other than a weak positive response, a two-fold increase in revertant colonies at 1000 µg/plate, in *Salmonella* Typhimurium TA98 with and without metabolic activation.

Nicarbazin can contain *p*-nitroaniline (PNA) and methyl-(4-nitrophenyl) carbamate (M4NPC) as impurities *(72, 73)* In addition, when

chicken meat is subjected to heat treatment, PNA can be expected as a product of DNC breakdown *(82)*. Negative or weakly positive results from the Ames test have been reported for PNA in strain TA98 *(83)*. In 2019 JECFA reviewed 4-chloroaniline (PCA), a close structural analogue of PNA, and concluded that PCA does not exhibit DNA-reactive genotoxicity in vivo. In 2017 EFSA *(72)* concluded that no safety concern would arise from the impurity PNA if the maximum content in nicarbazin of 0.1% were respected. The impurity M4NPC was also considered safe for the consumer provided a maximum concentration of 0.4% in nicarbazin is not exceeded.

The Committee concluded that nicarbazin was unlikely to be genotoxic in vivo.

As nicarbazin (and DNC) are unlikely to be genotoxic in vivo, any carcinogenic effect would be secondary to prolonged preneoplastic damage, the only indication of which in repeat-dose studies was renal toxicity due to crystal formation. As this is not observed in long-term studies with DNC, which is the residue of concern, and the mixture of DNC and HDP was not carcinogenic in rats, the Committee concluded that nicarbazin was unlikely to pose a carcinogenic risk to humans from its use as a veterinary drug.

In a three-generation study of reproductive toxicity, rats were fed diets containing a mixture of DNC + HDP, at doses of 0 + 0, 50 + 17, 150 + 50 or 300 + 100 mg/kg bw per day. The NOAELs for parental, reproductive and offspring toxicity of the DNC + HDP mixture were 300 + 100 mg/kg bw per day, the highest dose tested.

In a two-generation reproductive toxicity study, based on OECD guidelines, mixtures of DNC and HDP were administered to rats in their feed to provide DNC + HDP doses of 0 + 0, 100 + 33, 300 + 100 or 580 + 193 mg/kg bw per day. Detailed data were not submitted by the sponsor. A NOAEL could not be identified as renal crystal deposits and associated histopathological effects in the kidney were observed at 100 + 33 mg/kg bw per day and greater. The NOAEL for reproductive and offspring toxicity was 580 mg/kg bw per day for DNC and 193 mg/kg bw per day for HDP, the highest dose tested.

In a two-generation reproductive toxicity study based upon OECD TG 416, rats were administered a 3:1 mixture of DNC and HDP in the diet at 0 + 0, 52.5 + 17.5, 150 + 50 or 300 + 100 mg/kg bw per day. The NOAELs for parental, reproductive and offspring toxicity were all 300 + 100 mg/kg bw per day DNC + HDP, the highest dose tested *(74)*.

In a developmental toxicity study, pregnant rats were given nicarbazin by gavage at doses of 0, 70, 200 or 600 mg/kg bw per day on GD 7–17. No teratogenic effects were observed. The NOAEL for maternal toxicity was 200 mg/kg bw per day on the basis of reduced food intake and body weight gain, and increased mortality

at 600 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 200 mg/kg bw per day based on reduced fetal body weight and delayed ossification, suggesting retarded fetal development possibly secondary to maternal toxicity. This study was considered by the forty-eighth meeting of JECFA in 1998 in establishing the ADI (Appendix 2, ref. 128).

In a GLP developmental toxicity study based upon OECD TG 414, time-mated rats were administered either vehicle or a 3:1 mixture of DNC and HDP orally by gavage from GD 6–20. Dosage levels were 0 + 0, 52.5 + 17.5, 150 + 50 or 450 + 150 mg/kg bw per day DNC + HDP. The NOAELs for maternal and embryo/fetal toxicity were both 450 + 150 mg/kg bw per day DNC + HDP, the highest dose tested *(74)*.

In an GLP-compliant, OECD guideline study of developmental toxicity in rabbits, nicarbazin was administered by gavage at dose levels of 0, 30, 60 or 120 mg/kg bw per day from GD 6–28 *(84)*. The NOAEL for maternal toxicity was 60 mg/kg bw per day based on the occurrence of prominent liver lobulation in two of 24 animals at the LOAEL of 120 mg/kg bw per day. A similar finding had been noted in the preliminary study where two of six animals were similarly affected at both 200 and 400 mg/kg bw per day, and therefore an association with treatment could not be discounted. The NOAEL for embryo/fetal toxicity was 120 mg/kg bw per day, the highest dose tested. In an OECD TG 414 study of development toxicity, pregnant rabbits were treated daily by oral gavage with nicarbazin at doses of 0, 60, 120 and 240 mg/kg bw per day, from GD 6 to 28. The NOAEL for maternal toxicity was 240 mg/kg bw per day, the highest dose tested. The NOAEL for embryo/fetal toxicity was 120 mg/kg bw per day, based on changes in skeletal ossification indicative of developmental retardation at 240 mg/kg bw per day *(72)*.

In an oral developmental toxicity study based upon OECD TG 414, time-mated rabbits received a mixture of DNC and HDP by gavage from GD 7–28, at doses of 0 + 0, 22.5 + 7.5, 45 + 15 or 90 + 30 mg/kg bw per day DNC + HDP The NOAELs for maternal and embryo/fetal toxicity were both 90 + 30 mg/kg bw per day DNC + HDP, the highest doses tested *(74)*.

Microbiological data

The impact of nicarbazin residues on the human intestinal microbiome was evaluated through a decision-tree approach adopted by the sixty-sixth meeting of the Committee, which complies with VICH GL36(R) *(2)*. This entails answering three questions to determine the need for establishing a mADI. Determine first if the drug residue, and/or its metabolites, are microbiologically active against representatives of the human intestinal microbiota. Second, determine if the drug residues enter the human colon, and third, whether the residues entering the human colon remain microbiologically active. If the answer to any of these

questions is "no", then there is no need to calculate a mADI and the assessment does not need to be completed. However, if a mADI needs to be calculated, two end-points of concern for human health are considered for the assessment: disruption of the colonization barrier of the human intestinal microbiome and increases in populations of resistant bacteria in the human intestinal microbiome.

A non-GLP study *(85)* determined nicarbazin minimum inhibitory concentrations (MICs) against two strains of each of the following species: *Staphylococcus aureus, Salmonella* Typhimurium, and *Clostridium perfringens*. Similarly three strains were examined of the following species: *Klebsiella pneumoniae, Escherichia coli.* MIC results for all 12 isolates tested were greater than 128 µg/mL.

A publication from 2010 *(86)* described nicarbazin MICs against 51 strains of *Clostridium perfringens* isolated from broilers. Results showed that nicarbazin did not inhibit in vitro growth of the tested strains up to a concentration of 128 µg/mL.

A GLP study *(87)* determined MICs for nicarbazin, DNC and HDP against a panel of 45 bacterial strains, five from each of the following groups, some of which are representative of the human intestinal microbiome: *Salmonella, Staphylococcus, Enterococcus, Escherichia coli, Proteus, Lactobacillus, Campylobacter, Clostridium* and *Bacteroides*. Nicarbazin and DNC had no antimicrobial activity against any bacterial strains tested (MICs greater than 256 µg/mL). HDP had no measurable activity against 44 of the 45 bacterial strains tested, with MICs greater than 256 µg/mL, while one strain of *Campylobacter jejuni* had an MIC of 256 µg/mL.

Three microbiological studies therefore showed that nicarbazin exhibits no, or almost no, antimicrobial activity, with high MIC values (equal to or greater than 128 µg/mL) against all 108 isolates tested; which included anaerobic and aerobic microorganisms.

Further, a search on literature available in the public domain did not produce any result indicating antimicrobial activity of nicarbazin against bacteria representative of the human intestinal microbiome. It was concluded that the answer to the first step of the assessment, which asks whether the drug residues, and/or their metabolites, are microbiologically active against representatives of the human intestinal microbiota, was negative, therefore there was no need to determine a mADI. The same conclusion applies to determination of a mARfD, for which there is also no need.

Evaluation

The Committee established a ADI for nicarbazin (as DNC) of 0–0.9 mg/kg bw on the basis of a NOAEL of 60 mg/kg bw per day (equivalent to 42.5 mg/kg bw per day of DNC) due to prominent liver lobulation, observed in a study of developmental toxicity in the rabbit, applying a safety factor of 50. DNC is the toxic component of nicarbazin, and its absorption alone or in a mixture with HDP is substantially less (< 5%) than when released from ingested nicarbazin. As DNC is the residue of concern and there is no nicarbazin in products from treated animals, the Committee concluded that despite limitations in the database, a reduction in the default safety factor of 100 used to account for interspecies and intraspecies variability, would be justified. The Committee was unable to quantify just how much of a reduction would be appropriate, but concluded that 50 could certainly be supported, and would still result in a conservative evaluation.

The Committee concluded that it was not necessary to establish an ARfD for nicarbazin (or DNC) in view of their low acute oral toxicity, the absence of developmental toxicity or of any other toxicological effects that would be likely to be elicited by a single dose.

A toxicological monograph was prepared.

Residue evaluation

The Committee reviewed radiolabelled and non-radiolabelled studies on the pharmacokinetics, metabolism and residue depletion of nicarbazin in chickens. The analytical method used to analyse tissue samples was assessed.

Data on pharmacokinetics and metabolism

The sponsor proposed a possible metabolic pathway in rats and chickens based on radiolabelled studies. In the intestinal tract nicarbazin is entirely dissociated into its two components, DNC and HDP. Nicarbazin parent does not appear as a residue in tissues.

HDP is excreted much faster than DNC and primarily as parent HDP. The DNC component is metabolised and three resulting metabolites have been identified.

- Metabolite M1 was identified as monoacetylamino-DNC, corresponding to the reduction and acetylation of one nitro group.
- Metabolite M2 was identified as *N*,*N*′-1,4-phenylene-*bis*(acetamide) resulting from the cleavage, reduction and acetylation of the molecule.
- Metabolite M3 was identified as diacetylamino-DNC, resulting from the reduction and acetylation of two nitro groups.

Residue depletion data

Three radiolabelled residue depletion studies were available for evaluation. In one study nicarbazin radiolabelled either on the HDP or the DNC moiety was used. The second and the third studies used either [^{14}C]DNC or [^{14}C]HDP.

In one study *(88),* GLP-compliance not stated, chickens were administered nicarbazin with a radiolabel on either the HDP or DNC moiety of the molecule for 2–7 consecutive days at 125 mg/kg feed. No radiolabelled residues were found five days post withdrawal of nicarbazin labelled on the HDP moiety. In the chickens fed nicarbazin labelled on the DNC moiety, only liver retained detectable radioactivity up to day 8 post withdrawal. Other tissues were clear of radioactivity by day 5 post withdrawal.

The second study (GLP-compliant) was conducted to provide total residue depletion data in tissue samples following multiple oral administrations of nicarbazin containing [^{14}C]DNC to 18 broiler chickens *(89)* . Animals were dosed for seven days with nicarbazin at a target inclusion rate of 125 mg/kg feed. Doses were prepared in gelatine capsules and administered twice daily. The major route of elimination for radioactivity was via excreta. The major component in all pooled excreta samples was parent DNC which represented approximately 90% of the extracted radioactivity. The highest mean total radioactive residues at all time points were observed in the liver, followed by kidney, skin with fat, and muscle. The mean total radioactive residues of DNC in the liver at 24 hours post dose (one day withdrawal) was 27.8 mg equiv./kg (range 25.9–30.3 mg equiv./kg). Levels of total radioactive residues decreased to 0.05 mg equiv./kg (range 0.04–0.08 mg equiv./kg) at 240 hours post dose (nine days withdrawal). Analysis by radio-HPLC and LC-MS/MS confirmed that the major component in all pooled tissue samples at 24 hours after the last morning dose (zero day withdrawal) was 4,4-dinitrocarbanilide, that is parent DNC. The highest concentration of DNC residues for each tissue was observed in tissues collected at 12 hours withdrawal. The highest mean concentration was measured in liver, followed by kidney, skin with fat, and muscle.

The third study (GLP-compliant) evaluated residue depletion after oral administration of nicarbazin containing [^{14}C]HDP, at a target inclusion rate of 125 mg/kg feed *(90)*. Doses were prepared in gelatine capsules and administered twice daily for seven days. Elimination of total radioactivity was rapid, with a mean of 96.7% (range 92.9–99.1%) of the total dose recovered within 16 hours of the final dose. Concentrations of total radioactivity from HDP in plasma were low at each time point, ranging from 0.036–0.093 mg equiv./kg. Tissue residue concentrations at one day withdrawal were highest in the kidney (0.13 mg/kg), followed by skin with fat (0.11 mg/kg), liver (0.095 mg/kg), and muscle (0.084 mg/kg). Total radioactivity decreased with each time point, with

radioactivity almost undetectable at nine days withdrawal. Additional HPLC analysis indicated that the principal component of the total radioactivity was HDP at 24 hours post final dose. Overall, HDP residues were extremely low in plasma and all tissues, and parent HDP was the principal component of all residues examined.

From the radiolabelled residue depletion studies, it can be concluded that HDP residues deplete quickly, while DNC residues reach greater tissue concentrations and deplete more slowly. Radioactive HDP represents less than 1% of the total radioactive nicarbazin residues at 24 hours withdrawal. Other metabolites have been identified but are present at less than 10% of the total residues. DNC is the most appropriate marker residue. Liver is the target tissue based on the distribution and decline of the [^{14}C]DNC administered to chickens. For DNC at 24 hours withdrawal, marker residue to total recovered radioactivity (MR:TRR) ratios of 0.43, 0.36, 0.24, and 0.47 were calculated for liver, kidney, muscle and skin with fat respectively.

Two residue depletion studies using unlabelled nicarbazin were provided. In one study, nicarbazin was administered at a target inclusion rate of 125 mg/kg feed and in the other study, nicarbazin was administered in combination with narasin, each at a target inclusion rate of 50 mg/kg feed.

In the GLP-compliant study *(91)* using nicarbazin only, chickens received feed containing nicarbazin at 125 mg/kg feed for 28 days, then six animals were slaughtered on each of days 1, 5, 6, 9, 11 and 14. DNC was detected in all tissues at day 1 following withdrawal of the test diet. Limits of quantitation for the LC-MS/MS method used were 50, 100, 25, 25 and 50 µg/kg in liver, kidney, muscle, skin with fat and fat, respectively. Residues detected ranged from: 7564–12 595 µg/kg in liver, 1194–4110 µg/kg in kidney, 1342–2688 µg/kg in muscle, 1678–2798 µg/kg in skin with fat, and 1811–2866 µg/kg in fat. At day 5 post withdrawal, residues for kidney had declined below the LOQ in all birds tested. The ranges at day 5 were 411–544 µg/kg in liver, 33.4–56.6 µg/kg in muscle, 90.3–176 µg/kg in skin with fat, and 62.9–93.5 µg/kg in fat. At days 7, 9 and 11 post withdrawal, residues in liver, kidney, muscle and fat had declined to below the LOQ in all birds. At these sampling times residue levels in skin with fat ranged from below the LOQ to 41.7 µg/kg. At day 14 post withdrawal, all residues were below the LOQ in all tissues and for all birds.

In another GLP-compliant residue depletion study *(92)*, nicarbazin was administered in combination with narasin (50 mg/kg feed each) to broiler chickens. Three females and three males were necropsied at day 0 (immediately after feed was withdrawn) and at days 3, 5, and 7 post withdrawal, and tissues including the kidneys, liver, muscle, skin with fat, and the abdominal fat pad were removed from each bird. DNC concentrations in samples were analysed using a validated LC-MS/MS method (LOQ: 50 µg/kg for liver, 100 µg/kg

for kidney and 25 µg/kg for muscle and fat). Individual residues of DNC in tissues were below 750 µg/kg at five days following the last dose. The relative concentrations of DNC in the tissues at five days withdrawal were: liver > skin with fat > muscle > kidney. The concentrations at seven days after the last dose were less than 87.8 µg/kg for liver, less than 25.9 µg/kg for skin with fat, less than the LOQ (25 µg/kg) for muscle and less than the LOQ (100 µg/kg) for kidney.

Using either treatment regimen, residue concentrations were consistently highest in liver tissues. The Committee considered both residue depletion studies as suitable to derive 95/95 upper tolerance limits for the two dosage regimens used in veterinary practice.

Analytical methods

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

An LC-MS/MS method has been developed and validated for the nicarbazin marker residue (DNC) depletion studies in chickens. The LOQ of the method is 17 µg/kg for liver tissues and 13, 18, and 18 µg/kg for kidney, muscle, and skin with fat, respectively. The stability of samples was adequately demonstrated for normal conditions of laboratory handling.

Estimated dietary exposure

Dietary exposure to nicarbazin may occur only through its use as a veterinary drug. There is no registered use for nicarbazin as a pesticide.

When used as a veterinary drug, dietary exposure was estimated based on the potential occurrence of DNC residues in chicken tissues. Residue concentrations were taken from measurements made at 24 hours withdrawal (day 1) for an inclusion rate of 125 mg/kg feed *(93)*, or at day 0 for nicarbazin at an inclusion rate of at 50 mg/kg feed *(92)*. These studies reported residue concentrations in terms of DNC (the marker residue).

The above studies provide residue data for both chicken liver and kidney. However, the available food consumption data are for chicken offal, without further distinction. Residue data from the tissue with the higher residue concentrations (chicken liver) were used for the dietary exposure assessment.

Based on incurred DNC residues at 24 hours withdrawal time in chicken muscle, offal, and skin with fat (125 mg/kg feed) the global estimates of chronic dietary exposure (GECDE) for adults and the elderly, children and adolescents, and for infants and toddlers were 120, 160 and 210 µg/kg bw per day, respectively, which represent 13%, 18% and 23% respectively of the upper bound of the acceptable daily intake (ADI) of 900 µg/kg bw.

Based on incurred DNC residues in chicken muscle, offal, and skin with fat at zero days withdrawal time (50 mg/kg feed) the GECDE for adults and the elderly, children and adolescents, and infants and toddlers were 95, 120 and 160 µg/kg bw per day, respectively, which represent 11%, 14% and 18% respectively of the upper bound of the ADI of 900 µg/kg bw.

As part of the GECDE methodology, further estimates of chronic dietary exposure were carried out. Instead of using the highest mean and the highest 97.5th percentile consumption across surveys, the calculations were carried out using the mean and the highest reliable percentile for each individual national survey from available datasets (CIFOCOss). The highest GECDE for each age class for each country was determined.

For the inclusion rate of nicarbazin at 125 mg/kg feed:

- the mean (range) of 35 country-specific estimates for DNC dietary exposure for adults and the elderly at 24 hours withdrawal was 32 (4–100) µg/kg bw per day, or 3.5% (0.4–11.1%) of the upper bound of the ADI;
- the mean (range) of 26 country-specific estimates of DNC dietary exposure for children and adolescents at 24 hours withdrawal was 53 (2–160) µg/kg bw per day, or 5.9% (0.2–17.9%) of the upper bound of the ADI;
- the mean (range) of 19 country-specific estimates of DNC dietary exposure for infants and toddlers at 24 hours withdrawal was 67 (10–210) µg/kg bw per day or 7.4% (1.1–23.4%) of the upper bound of the ADI.

For the inclusion rate of nicarbazin at 50 mg/kg feed:

- the mean (range) of 35 country-specific estimates of DNC dietary exposure for adults and the elderly at 0 days withdrawal was 25 (3–76) µg/kg bw per day or 2.7% (0.3–8.4%) of the upper bound of the ADI;
- the mean (range) of 26 country-specific estimates of DNC dietary exposure for children and adolescents at 0 days withdrawal was 41 (2–120) µg/kg bw per day or 4.5% (0.2–13.5%) of the upper bound of the ADI;
- the mean (range) of 19 country-specific estimates of DNC dietary exposure for infants and toddlers at 0 days withdrawal was 51 (7–160) µg/kg bw per day or 5.7% (0.8–17.8%) of the upper bound of the ADI.

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

Maximum residue limits

In recommending MRLs for nicarbazin in chickens, the Committee considered the following factors:

- An ADI, expressed as DNC, of 0–0.9 mg/kg bw was established by the Committee.
- Withdrawal periods range from 1–10 days for use of nicarbazin at an inclusion rate of 125 mg/kg feed in chickens for fattening. Withdrawal periods range from 0–8 days for use of nicarbazin at an inclusion rate of 50 mg/kg feed when applied in combination with narasin or monensin.
- Nicarbazin is not intended for use in laying hens.
- Nicarbazin is an equimolar complex of DNC and HDP that fully dissociates in aqueous conditions, including the gastrointestinal contents. While HDP residues quickly deplete, DNC residues reach a greater concentration in tissues and deplete more slowly. Neither component of nicarbazin is extensively metabolized in chickens; metabolites are present at less than 10% of the total residues.
- DNC is the marker residue (MR) and is considered to be suitable for residue monitoring purposes.
- The non-radiolabelled nicarbazin marker residue depletion data were sufficient to determine mean MR and 95/95 UTL concentrations in chicken muscle, liver, kidney, and skin with fat, at 24 hours withdrawal for use of nicarbazin only (125 mg/kg feed), and at 0 hours withdrawal for use in combination with narasin at the lower inclusion rate of 50 mg/kg feed.
- The residue of concern (DNC) can be estimated from the non-radiolabelled residue depletion data, along with MR:TRR data.
- A validated analytical method (LC-MS/MS) for the determination of nicarbazin marker residue (DNC) in chicken liver, kidney, muscle, and skin with fat is available and may be used for monitoring purposes.

Available residue depletion data are not suitable for linear regression analysis. Quantifiable residue values (below the LOQ) were measured in all edible tissues only at 24 hours and five days after withdrawal of treatment after use of nicarbazin at an inclusion rate of 125 mg/kg feed *(93)* and at zero days and three days after use of nicarbazin at an inclusion rate of 50 mg/kg feed *(92)*.

As an alternative, tolerance limits were calculated based on the one-sided tolerance interval calculation *(94)*. Upper tolerance limits of DNC residues at one day were calculated for the use of nicarbazin at an inclusion rate of 125 mg/kg feed, as well as at zero days for the use of nicarbazin in

combination with narasin at an inclusion rate of 50 mg/kg feed for each. Upper tolerance limits were highest in liver tissues and in the same order of magnitude for both patterns of use.

Maximum residue limits were calculated based on the upper limit of the one-sided 95% confidence interval over the 95th percentile of marker residue concentrations (95/95 UTL) in chicken liver, kidney, muscle and skin with fat.

The Committee recommended MRLs based on the marker residue DNC, as DNC is the residue of toxicological concern. The Committee recommended increasing the MRLs proposed in 1998 to 15 000, 8000, 4000, and 4000 µg/kg for DNC residues in chicken liver, kidney, muscle, and skin with fat, respectively. These MRLs are based on nicarbazin inclusion rates in feed of 125 and 50 mg/kg and withdrawal periods of 1 and 0 days respectively. As no residue data were available for other inclusion rates, the Committee could not assess whether these recommended MRLs are compatible with such inclusion rates and corresponding GVPs.

Table 8

Upper tolerance limit calculations for DNC residues in chicken tissues after administration of nicarbazin at 125 mg/kg feed at one day withdrawal (DNC concentrations from *(93)*) and after administration of 50 mg/kg feed with narasin at 50 mg/kg feed at 0 days withdrawal (DNC concentrations from *(92)*) and proposed MRLs for edible tissues

Dose	Withdrawal period		Liver	Kidney	Muscle	Skin with fat
Nicarbazin at 125 mg/kg feed	1 day	Mean DNC concentration (µg/kg) $n = 6$	9249	3007	2110	2327
		Standard deviation	1804	1094	506	473
		95/95 Upper Tolerance Limits (µg/kg)	**15 937**	7065	**3988**	**4081**
Nicarbazin at 50 mg/kg feed	0 days	Mean DNC concentration (µg/kg) $n = 6$	9193	4293	1610	2043
		Standard deviation	953	1036	149	480
		95/95 Upper Tolerance Limits (µg/kg)	12 727	**8133**	2163	3822
Proposed MRLs (based on DNC)			**15 000**	**8000**	**4000**	**4000**

An addendum to the residue monograph was prepared.

Summary and conclusions

Studies relevant to risk assessment – nicarbazin

Species/study type (route of administration)	Doses	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rat				
91 days study of toxicity (gavage)	DNC 106, 284 or 709 mg/kg/day	–	709 mg/kg bw per day for DNC [a]	–
Two-year study of toxicity/ carcinogenicity (dietary)	DNC/HDP, 50/17, 150/50 or 300/100 mg/kg bw/day	– No increase in tumour incidences	300/100 [a]	–
Three-generation study (dietary)	DNC/HDP, 50/17, 150/50 or 300/100 mg/kg bw/day	**Reproductive toxicity:**	300/100 [a]	–
		Parental toxicity:	300/100 [a]	–
		Offspring toxicity:	300/100 [a]	–
Developmental study (oral gavage)	Nicarbazin, 70, 200, or 600 mg/kg bw per day	**Maternal toxicity:** mortality, decreased food intake and body weight gain	200	600
		Embryo/fetal toxicity: reduced fetal body weight and delayed ossification,	200	600
Rabbit				
Developmental toxicity study (oral gavage)	Nicarbazin 30, 60 and 120 mg/kg bw per day	**Maternal toxicity:** Prominent liver lobulation	60*	120
		Developmental toxicity:	120 [a]	–
Dog				
Two-year toxicity study (dietary)	DNC/HDP, 60/20, 180/60 and 600/200 mg/kg bw per day, 6 days per week	Elevated serum ALT and single incidence of mild bile duct proliferation	DNC: 154 HDP: 51 (correcting for duration of exposure on six days per week)	DNC: 514 HDP: 171

* Pivotal study for the ADI *(84)*; GD: Gestation day; [a] The highest dose tested
LOAEL: Lowest-observed-adverse-effect level; NOAEL: No-observed-adverse-effect level

Toxicological effects

The lowest NOAEL was 60 mg/kg bw per day (equivalent to 42.5 mg/kg bw per day of DNC) due to an increased incidence of prominent liver lobulation at 120 mg/kg bw per day, observed in a study of developmental toxicity in the rabbit.

Toxicological ADI

The Committee concluded that the toxicological ADI was 0–0.9 mg/kg bw (DNC).

Microbiological effects

Nicarbazin and/or its metabolites show no antimicrobial activity towards representative bacteria of the human intestinal microbiota.

Microbiological ADI

The Committee concluded that there was no need to determine a mADI.

ADI

The ADI for nicarbazin was established at 0–0.9 mg/kg bw based on toxicological effects.

Uncertainty factor

When considering nicarbazin it is DNC that is the toxic component, and its absorption alone or in a mixture with HDP is substantially less (< 5%) than when released from ingested nicarbazin. As DNC is the residue of concern and there is no nicarbazin in products from treated animals, the Committee concluded that despite limitations in the database, a reduction in the default safety factor of 100 used to account for interspecies and intraspecies variability, would be justified. The Committee was unable to quantify just how much of a reduction would be appropriate, but concluded that 50 could certainly be supported, and would still result in a conservative evaluation.

ARfD

The Committee concluded that it was not necessary to establish an ARfD.

Residue definition

The marker residue in chickens is DNC.

Dietary exposure

For a nicarbazin inclusion rate of 125 mg/kg feed, the global estimate of chronic dietary exposure (GECDE) was 120, 160 and 210 µg/kg bw per day (13%, 18% and 23% of the upper bound of the ADI of 900 µg/kg bw) for adults and the elderly, children and adolescents, and toddlers and infants, respectively.

For a nicarbazin inclusion rate of 50 mg/kg feed, the global estimate of chronic dietary exposure (GECDE) was 95, 120 and 160 µg/kg bw per day (11%, 14% and 18% of the upper bound of the ADI of 900 µg/kg bw) for adults and the elderly, children and adolescents, and toddlers and infants respectively.

MRLs

Chicken: 4000 µg/kg for muscle, 15 000 µg/kg for liver, 8000 µg/kg for kidney, and 4000 µg/kg for skin with fat.

3.4 Selamectin

Explanation

Selamectin has the IUPAC name (2*aE*,4*E*,5′*S*,6*S*,6′*S*,7*S*,8*E*,11*R*,13*R*,15*S*,17*aR*,20*Z*,20*aR*,20*bS*)-6′-cyclohexyl-3′,4′,5′,6,6′,7,10,11,14,15,17*a*,20,20*a*,20*b*-tetradecahydro-20*b*-hydroxy-20-hydroxyimino-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl-2,6-dideoxy-3-*O*-methyl-*a*-*L*-arabino-hexopyranoside; Chemical Abstract Service No. 165108-07-6). It is a semisynthetic macrocyclic lactone compound of the avermectin class, a large family of broad-spectrum parasiticides. It is widely used as an endectocide against nematode and arthropod parasites in dogs and cats.

Selamectin, like other avermectins, acts mainly on a glutamate-gated chloride channel (GluCl) that is present in both neuronal and muscle membranes of invertebrates, but is not present in vertebrates. Normally avermectins are also agonists of γ-aminobutyric acid chloride channels (GABACls) in the central nervous system (CNS) of invertebrates and vertebrates; however, the binding affinity of selamectin in the mammalian brain is 100-fold lower than the affinity for binding sites in invertebrates *(95)*. In addition, selamectin has a much lower binding affinity to the GABACls in the vertebrate CNS than ivermectin *(96)*.

Selamectin is not currently approved for use in food-producing animals. However the Committee has evaluated selamectin as part of a pilot programme in which it conducts a parallel review of the information at the same time as the sponsor pursues approval in the proposed species with national authorities, as discussed at the twenty-fourth session of CCRVDF *(97)*. Selamectin is under development for the control of sea louse infestations in Atlantic salmon (*Salmo salar*). It is intended as a seven-day, in-feed ectoparasiticide additive for treatment and prevention of all parasitic stages of sea lice on Atlantic salmon in seawater, ranging from smolts to market-weight fish,. The product is to be administered in feed to fish for seven days at a proposed dose rate of 100 µg/kg biomass per day. A withdrawal period has not been established.

Selamectin was previously evaluated by the Committee at its eighty-eighth meeting. An ADI of 0–0.01 mg/kg bw and an ARfD of 0.4 mg/kg bw were established. However MRLs could not be recommended for selamectin due to incomplete characterization of residues in fillet, lack of data necessary to establish reliable ratios of marker residue to total recovered radioactivity (MR:TRR ratios) over time, and lack of an analytical method for monitoring (Annex 2, ref. 243).

In support of the continuing evaluation of selamectin, the sponsor submitted additional data on residues. The sponsor also submitted a more comprehensive report of the critical one-year study of toxicity in rats evaluated at the eighty-eighth meeting.

Toxicological and microbiological evaluation

The Committee reviewed the one-year toxicity study of selamectin in rats *(98)*, including the previously unavailable information. The Committee concluded that the NOAEL for the one-year study was 5 mg/kg bw per day, based on increased serum ALP, liver weight (absolute and relative to body and brain) and uterus/cervix weight (absolute and relative to body and brain) at 15 mg/kg bw per day.

In addition, a literature search was conducted but no new information relevant to the assessment was identifed.

Evaluation

The Committee withdrew the previous ADI and established an ADI of 0–0.05 mg/kg bw, based on a NOAEL of 5 mg/kg bw per day for increased liver and uterus/cervix weights at 15 mg/kg bw per day in a one-year study in rats, with application of a safety factor of 100 to account for interspecies and intraspecies variability. Although the NOAEL for effects seen in a 13-week dietary neurotoxicity/toxicity study in rats, assessed by the Committee at its last meeting (*99*; Annex 2, ref 243), was 1 mg/kg bw per day, the LOAEL, at 15 mg/kg bw per day, and the effects observed were the same as those on which the ADI is based. The Committee concluded that the ADI established at the present meeting would be sufficiently protective of these findings.

The Committee concluded that the ARfD of 0.4 mg/kg bw established at the eighty-eighth meeting was still appropriate.

Residue evaluation

The current Committee received additional information from the sponsor for evaluation. The dossier also contained information previously submitted and evaluated by the eighty-eighth Committee. For completeness of the evaluation, those studies are summarized again below. The newly submitted studies included a pharmacokinetic study in Atlantic salmon comparing two selamectin formulations, residue profiling of samples from the radiolabelled residue depletion study, a non-radiolabelled residue depletion study in Atlantic salmon, an in vitro study of the stability of [^{3}H]selamectin that was used in the radiolabelled study, and an analytical method. The studies previously submitted and evaluated by the eighty-eighth Committee were a radiolabelled depletion study in Atlantic salmon (TRR counts only), a study attempting to identify a major metabolite in semisolid effluent (faeces and uneaten feed), and an in vitro comparative metabolism study with liver microsomes.

Data on pharmacokinetics and metabolism

In one study, the pharmacokinetics of selamectin in salmon from two different formulations were compared *(100)*. Atlantic salmon (*Salmo salar*, mean mass 406 g at dosing) held in seawater with a salinity range of 31–33 g/L and temperature range of 9–16°C, were fed feed top-coated using cod liver oil with either one of two different formulations containing 12% selamectin, (T01, T02), at a rate of 100 µg/kg bw per day for seven days. Four fish per tank (14 tanks, seven tanks/treatment group) were harvested on days 0, 3, 6, 7, 14, 28, 42, 56, 70, 98 and 112, and blood collected for plasma analysis and analysed using LC-MS/MS. Tank level data was used to estimate pharmacokinetic parameters. The mean plasma C_{max} was 871 ng/mL (90% confidence interval: 799, 950) for formulation T01, and 843 ng/mL (90% CI: 774, 919) for formulation T02. Mean area under the plasma concentration–time curve values ($AUC_{0\text{-}t(last)}$), were 436 000 and 402 000 ng h/mL for T01 and T02, respectively. The mean plasma half-life values were also similar across groups at 501 hours (20.9 days) and 417 hours (17.4 days) for formulations T01 and T02, respectively.

In another study, Atlantic salmon maintained in tanks of seawater at 8°C were dosed with [^{3}H]selamectin in feed at a nominal rate of 100 µg/kg bw per day for seven consecutive days (*101*; Annex 2, ref 243*)*. Gut contents were pooled from six fish per tank at three hours and 12 hours, and at 1, 3, 7, 14, 30, 40, 60 and 90 days after the final dose. Semisolid effluent (faecal material and any uneaten feed) samples were collected from each tank on the first, third and fifth day of treatment, and at 1, 3, 7, 14, 30, 45, 60 and 90 days after the final dose. Mean TRR concentrations in gut contents peaked at one day withdrawal and then rapidly declined. Total recovered radioactivity in semisolid effluent increased during the treatment period of the medicated diet, peaked at 24 hours after the last treatment, reaching a mean of 4660 µg equiv./kg, then rapidly declined over the next two weeks, followed by a continual slow decline to reach a mean of 195 µg equiv./kg 90 days after the final dose. Parent selamectin was identified as the predominant residue in fillet, kidney, liver, gut contents, remaining carcass, and semisolid effluent samples *(102)*. A prominent metabolite (10% or more of TRR) of selamectin was found in gut contents, semisolid effluent and liver samples. A study (not GLP-compliant) was conducted to identify this metabolite in semisolid effluent. Analysis of samples by ultra-high performance liquid chromatography-mass spectroscopy (UHPLC-MS/MS) suggested that the metabolite is a mono-oxidation product of selamectin (*103*; Annex 2, ref 243). Another metabolite was detected in liver but was not identified, as liver is not considered an edible tissue in fish and it was 10% of the TRR or less at only one time point.

One non-GLP, in vitro study *(104)* examined the stability of the [^{3}H]selamectin that was used in the radiolabelled depletion and metabolism

study. The Committee concluded that this study combined with the results from the TRR and metabolism study were sufficient to demonstrate stability of the tritium label.

In a comparative study of in vitro metabolism, (*105*; Annex 2, ref 243), [^{3}H]selamectin at final nominal concentrations of 1 µM and 10 µM was incubated with liver microsomes from rats, rabbits, dogs, salmon and humans at about 37°C (25°C for salmon). Metabolite characterization and identification were accomplished by LC-MS with online radiodetection. Structures of metabolites were proposed by interpretation of their mass spectral fragmentation patterns and comparison with available reference standards. Unchanged selamectin accounted for more than 70% of the total radioactivity following incubation with liver microsomes from all species.

Five metabolites were identified that were common to all species studied. These metabolites were derived from pathways involved in mono-oxidation at different positions, *O*-demethylation, and/or epoxidation accompanying hydroxylation on the epoxide ring. Only two metabolites were seen in the fish liver microsome preparations, and these were conserved across species. All the metabolites seen with liver microsomes from humans (male and female) were detected with female rabbit liver microsomes.

Gender-dependent selamectin metabolism was not observed in human liver microsomes, but in rat liver microsomes selamectin metabolism differed between males and females. The Committee noted that the incubation temperature for salmon microsomes was not reflective of the normal water temperature range for salmon. However, the Committee also noted that the metabolite profiles found in vitro were consistent with metabolite patterns observed in vivo.

Residue data

One study using [^{3}H]selamectin was performed (*106*; Annex 2, ref 243). Atlantic salmon maintained in tanks of seawater at 8°C were dosed with [^{3}H]selamectin in feed at a nominal rate of 100 µg/kg bw per day for seven consecutive days. Samples were collected from six fish each at 3 and 12 hours, and at 1, 3, 7, 14, 30, 40, 60 and 90 days after the final dose. Liver, kidney and fillet were collected individually. Samples of carcass (defined as bones, head, any meat that did not come off in the filleting, all the viscera, scales and the fins that were removed during filleting) were pooled from each tank at each time point listed above and analysed for TRR. Mean TRRs were highest in liver (2948 µg equiv./kg), followed by kidney (1275 µg equiv./kg), carcass (649 µg equiv./kg) and then fillet (569 µg equiv./kg). Highest mean TRR concentrations in liver and kidney were at 12 hours withdrawal, and highest mean TRR concentrations in fillet and carcass occurred at seven days withdrawal. After reaching maximum, TRR concentrations in all tissues showed a gradual decline during 90 days after the final dose. Selamectin

was the primary component in all tissues across all time points. Fillet extracts were analysed by LC-MS/MS. Parent selamectin was considered to be an appropriate marker residue (MR). Mean MR:TRR values in fillet ranged from 0.8 to 1.

Based on some of the uncertainties in the data provided from this study, the Committee considered that the lowest MR:TRR value of 0.8 would lead to a more conservative approach and it was therefore chosen in this case.

In one study, postsmolt (four tanks, mean weight 331 g) and adult (two tanks, mean weight 3.07 kg) Atlantic salmon in seawater were treated with selamectin-medicated feed at a target dose of 100 µg/kg biomass per day *(107)*. Groups of 15 postsmolt fish and 12 adult fish per tank were harvested 2, 4, 10, 22, 58 and 88 days post treatment. Selamectin concentrations were determined in fillet using a validated LC-MS/MS method (LOQ, 2.8 µg/kg). Mean concentrations (± SD) of selamectin in fillet in the treated postsmolt fish were 400 ± 135 µg/kg at two days post last dose (22 degree-days), and this decreased to 160 ± 61 µg/kg at 88 days post last dose (542 degree-days). Mean concentrations (± SD) in the fillet of the adult fish were 333 ± 99 µg/kg at two days post last dose (22 degree-days), and this decreased to 130 ± 64 µg/kg at 88 days post last dose (544 degree-days). The Committee noted that four of the six tanks (two postsmolt, both adult tanks) received substantially less than the target dose (67–87% of target dose).

Analytical methods

An LC-MS/MS method has been developed and validated to determine the marker residue (parent selamectin) in Atlantic salmon fillet. Samples were extracted by acetonitrile/water (70:30 v/v) and partitioned twice with hexane. The analyte was reconstituted in acetonitrile/water (80:20 v/v) and injected into LC-MS/MS equipped with an C18 column maintained at 50°C. Elution was carried out using water/formic acid (100:0.3 v/v) as mobile phase A and acetonitrile/formic acid (100:0.3) as mobile phase B with a flow rate of 0.65 mL/minute. For detection and quantification of selamectin, multiple reaction monitoring (MRM) was used. The electrospray ionization was used in positive ion mode. Quantification of selamectin (m/z 770 → 608) was performed using selamectin $D_2{}^{15}N$ as internal standard (m/z 773 → 612). Standard curves were generated by linear regression using a $1/x^2$ weighting factor. The calculated LOD and LOQ for fillet were 0.943 µg/kg and 2.83 µg/kg respectively.

Estimated dietary exposure

Chronic dietary exposure assessment

Dietary exposure to selamectin may occur only through its use as a veterinary drug. There is no registered use for selamectin as a pesticide.

When used as a veterinary drug, dietary exposure was estimated based on the potential occurrence of selamectin residues in Atlantic salmon muscle. Adjusted (MR:TRR = 0.8) median residue levels in Atlantic salmon (muscle, adult fish) were 410 and 179 µg/kg, relating to withdrawal periods of 22 and 544 degree-days respectively.

Based on incurred residues in Atlantic salmon muscle, the global estimates of chronic dietary exposure (GECDEs) and the percentage they represent the upper bound of the ADI of 50 µg/kg bw are as shown below in Table 9.

Table 9
Estimates of chronic dietary exposure (GECDE)

	Adults and elderly		Children and adolescents		Infants and toddlers	
	GECDE µg/kg bw per day	% ADI	GECDE µg/kg bw per day	% ADI	GECDE µg/kg bw per day	% ADI
22 degree-days	0.8	2	2.2	4	0.8	2
544 degree-days	0.4	1	1.0	2	0.3	1

Acute dietary exposure assessment

Acute dietary exposure (global estimate of acute dietary exposure; GEADE) was assessed for consumption of Atlantic salmon muscle (adult fish) using food consumption values from the FAO/WHO large portion (97.5th percentile, one day) database and 95/95 and 99/95 UTL concentrations for selamectin residues.

Adjusted (MR:TRR = 0.8) UTL 95/95 residue levels in Atlantic salmon (muscle, adult fish) were 1068 µg/kg and 454 µg/kg, relating to withdrawal periods of 22 and 544 degree-days respectively. Adjusted (MR:TRR = 0.8) UTL 99/95 residue levels in the same tissue were 1653 µg/kg and 695 µg/kg, also relating to withdrawal periods of 22 and 544 degree-days respectively

Based on incurred residues in Atlantic salmon muscle, GEADEs and the percentage they represent of the ARfD of 400 µg/kg bw, are as shown in Table 10.

Table 10
Global estimates of acute dietary exposure for selamectin residues in Atlantic salmon

	General population				Children			
	GEADE µg/kg bw	% ARfD	GEADE µg/kg bw	% ARfD	GEADE µg/kg bw	% ARfD	GEADE µg/kg bw	% ARfD
UTL	95/95	95/95	99/95	99/95	95/95	95/95	95/95	99/95
22 degree-days	7.7	2	12.0	3	8.2	2	12.7	3
544 degree-days	3.3	1	5.0	1	3.5	1	5.3	1

Maximum residue limits

Selamectin is not yet approved for use in salmon in any Member State. Using the process followed by the Committee, specific MRLs cannot be recommended at this time due to a lack of established GVP in at least one Member State. In evaluating a range of MRLs for selamectin in salmon, the Committee considered the following factors:

- Selamectin is not approved for use in any Member State and was evaluated by the Committee under the pilot parallel review program as requested by CCRVDF.
- An ADI of 0–0.05 mg/kg bw was established by the Committee.
- An ARfD of 0.4 mg/kg bw was previously established by the Committee.
- Selamectin is predominantly unmetabolized.
- Selamectin is the marker residue.
- The ratio of the concentration of marker residue to the concentration of total residue (MR:TRR) is 0.8 for fillet in salmon.
- A non-radiolabelled depletion study in postsmolt and adult Atlantic salmon was available. The Committee noted several deficiencies in the study, including most fish not receiving the target dose.
- A validated analytical method for the determination of selamectin in fillet of salmon is available and may be used for monitoring purposes.

Based on the lack of a registration in a Member State and lack of GVP, and the study deficiencies noted above, specific MRLs could not be recommended. Based on the information the Committee received regarding preliminary proposed GVP, the following ranges could be considered. These were based on the 95/95 and 99/95 UTLs from the non-radiolabelled residue depletion study using the treated adult fish, as they represent those which will enter the market for human consumption. They correspond to withdrawal periods of 22 degree-days (two calendar days; the earliest withdrawal time point in the residue depletion study) and 544 degree-days (88 calendar days; the longest withdrawal time point). Proposed MRLs at 22 degree-days would be in the range of 900 µg/kg to 1300 µg/kg, and those at 544 degree-days would be in the range of 400 µg/kg to 600 µg/kg.

No further recommendations can be made without full registration in a Member State, including GVP.

An addendum to the residue monograph was prepared.

Summary and conclusions

Residue definition

The marker residue in Atlantic salmon fillet is selamectin.

Maximum residue limits (MRLs)

Specific MRLs were not recommended.

Estimated dietary exposure

Dietary exposure was assessed for some possible scenarios, but no GVP has been established.

ADI

The ADI for selamectin was established at 0–0.05 mg/kg bw based on a NOAEL of 5 mg/kg bw per day for increased liver and uterus/cervix weights at 15 mg/kg bw per day in a one-year study in rats, with application of a safety factor of 100.

ARfD

An ARfD for selamectin of 0.4 mg/kg bw was established at the eighty-eighth meeting of the Committee and was reaffirmed by the present Committee.

4. Future work and recommendations

Recommendations relating to specific veterinary drugs, including ADIs, ARfDs and proposed MRLs, are given in section 3 and Annex 2. This section includes recommendations relating to future work by the JECFA Secretariat.

Imidacloprid

Additional information that would assist in the further evaluation of the compound:

- Further information on disruption of the colonisation barrier and on the selection for, and emergence of, resistance in the microbiota in the gastrointestinal tract.

Selamectin

Further information required to complete the residue assessment:

- Full registration in a Member State, including GVP.

Estimation of dietary exposure to veterinary drug residues as performed by JECFA

With the availability of food consumption information expressed on a body weight basis, it is recommended that these data be used preferentially to minimize the assumptions made in deriving the GECDE.

It is also recommended that the population groups for which GECDE estimates are derived be amended to align with the age classes currently used in CIFOCOss: infants and toddlers (0–35 months), children and adolescents (3–14 years), and adults and the elderly (15 years and above).

It is further recommended that JMPR and JECFA continue to take opportunities to harmonize procedures for dietary exposure assessment.

A risk-based decision tree approach for the safety evaluation of residues of veterinary drugs

The Committee recommends that the Joint Secretariat, together with other secretariats as appropriate, convene an electronic working group comprising experts from the three committees under JECFA, JMPR, and in dietary exposure assessment, to further develop the decision tree approach, with a view to its finalization in 2023 or 2024.

General considerations for microbiological effects

The Committee recommends that the Secretariat convene a microbiome expert working group to explore developments in this evolving area.

Acknowledgements

The Committee wishes to thank Dr Russell Parry for his assistance in the preparation of the report.

FAO and WHO wish to acknowledge the significant contributions of the experts, especially under the challenging circumstances posed by numerous virtual meetings, and also to their institutions (where relevant), to the work of the 94th meeting of JECFA.

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Annex 1

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.

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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.

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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.

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

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81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.

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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.

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Annex 2

Recommendations on compounds on the agenda

Imidacloprid (parasiticide)

Acceptable daily intake	In view of the absence of a study to assess the impact of imidacloprid on representative human intestinal microbiota, it was not possible to determine a mADI, thus the Committee was unable to establish an ADI for imidacloprid. The Committee established a toxicological acceptable daily intake (tADI) of 0–0.05 mg/kg bw on the basis of a NOAEL of 5.25 mg/kg bw per day for decreased body weight gain in the extended one-generation reproduction study, with the application of a safety factor of 100 to allow for interspecies and intraspecies differences.
Acute reference dose	In view of the absence of a study to assess the impact of imidacloprid on representative human intestinal microbiota, it was not possible to determine a mARfD, thus the Committee was unable to establish an ARfD for imidacloprid. The Committee established a toxicological acute reference dose (tARfD) of 0.09 mg/kg bw based on a $BMDL_{05}$ of 9 mg/kg bw reported by Cal EPA for an acute neurotoxicity study in rats and a safety factor of 100 to allow for interspecies and intraspecies differences. This value was supported by a NOAEL of 7.5 mg/kg bw per day for tremors in a 90-day toxicity study in dogs occurring during the first week of treatment, although it is not known whether tremors occurred after the first dose.

Estimated chronic dietary exposure	While estimates of dietary exposure were derived, there are no HBGVs with which to compare them. Based on incurred residues in Atlantic salmon (fillet) and a withdrawal period of 98 degree-days: The GECDE for adults and the elderly is 1.0 µg/kg bw per day. The GECDE for children and adolescents is 2.7 µg/kg bw per day. The GECDE for infants and toddlers is 0.9 µg/kg bw per day. Based on incurred residues in fish meat and a withdrawal period of 98 degree-days: The GECDE for adults and the elderly is 1.8 µg/kg bw per day. The GECDE for children and adolescents is 3.8 µg/kg bw per day. The GECDE for infants and toddlers is 1.2 µg/kg bw per day.
Estimated acute dietary exposure	Acute dietary exposures were assessed at 98 degree-days post dose. The adjusted (MR:TRR = 0.7) 95/95 UTL concentrations used were 859 µg/kg. No ARfD was available. Based on consumption of Atlantic salmon: The GEADE for adults is 6.2 µg/kg bw per day. The GEADE for children is 6.6 µg/kg bw per day. Based on consumption of all fin fish: The GEADE for adults is 34.1 µg/kg bw per day. The GEADE for children is 23.8 µg/kg bw per day.
Residue definition	The marker residue for imidacloprid in fillets of salmonids is the parent molecule, imidacloprid.
Maximum residue limits	As the Committee could not establish an ADI or an ARfD, no MRLs could be recommended for imidacloprid.

Ivermectin (broad-spectrum antiparasitic agent)

Acceptable daily intake	The Committee established an ADI of 0–10 µg/kg bw at the eighty-first meeting.
Acute reference dose	The Committee established an ARfD of 200 µg/kg bw at the eighty-first meeting.
Residue definition	The marker residue in sheep, pigs and goats is ivermectin B_{1a} (H_2B_{1a}, or 22,23-dihydroavermectin B_{1a}).
Estimated chronic	The GECDE for adults and the elderly is 0.72 µg/kg bw per day, which represents 7.2% of the upper bound of the ADI of 10 µg/kg bw. The GECDE for children and adolescents is 0.93 µg/kg bw per day, which represents 9.3% of the upper bound of the ADI of 10 µg/kg bw. The GECDE for infants and toddlers is 0.48 µg/kg bw per day, which represents 4.8% of the upper bound of the ADI of 10 µg/kg bw.
Estimated acute dietary exposure	The GEADE for cattle muscle, applicable to children and the general population, is 69 µg/kg bw, which represents 35% of the ARfD of 200 µg/kg bw. The GEADE for sheep muscle, applicable to children and the general population, is 73 µg/kg bw, which represents 37% of the ARfD of 200 µg/kg bw. The GEADE for pig muscle, applicable to children and the general population, is 30 µg/kg bw, which represents 15% of the ARfD of 200 µg/kg bw.

Recommended maximum residue limits (MRLs) for ivermectin

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Pigs	15	30	20	50
Sheep and goats	30	60	20	100

Nicarbazin (coccidiostat)

Toxicological effects	The NOAEL was 60 mg/kg bw per day (equivalent to 42.5 mg/kg bw per day of DNC) due to an increased incidence of prominent liver lobulation, observed in a study of developmental toxicity in the rabbit.
Toxicological ADI	The tADI for nicarbazin was established at 0–0.9 mg/kg bw (DNC).
Microbiological effects	Nicarbazin and/or its metabolites show no antimicrobial activity towards representative bacteria of the human intestinal microbiota.
Microbiological ADI	The Committee concluded that it was not necessary to establish a mADI for nicarbazin.
Acceptable daily intake	The ADI for nicarbazin was established at 0–0.9mg/kg bw based on toxicological effects.
Uncertainty factor	When considering nicarbazin it is DNC that is the toxic component, and its absorption alone or in a mixture with HDP is substantially less (< 5%) than when released from ingested nicarbazin. As DNC is the residue of concern and there is no nicarbazin in products from treated animals, the Committee concluded that despite limitations in the database, a reduction in the default safety factor of 100 used to account for interspecies and intraspecies variability, would be justified. The Committee was unable to quantify just how much of a reduction would be appropriate, but concluded that 50 could certainly be supported, and would still result in a conservative evaluation.
Acute reference dose	The Committee concluded that it was not necessary to establish an ARfD for nicarbazin.
Residue definition	The marker residue in chickens is DNC.

Estimated dietary exposure

Based on incurred DNC residues in chicken muscle, offal, and skin with fat, at 24 hours withdrawal time and 125 mg/kg feed:

The GECDE for adults and the elderly is 120 µg/kg bw per day, which represents 13% of the upper bound of the ADI of 900 µg/kg bw.

The GECDE for children and adolescents is 160 µg/kg bw per day, which represents 18% of the upper bound of the ADI of 900 µg/kg bw.

The GECDE for infants and toddlers is 210 µg/kg bw per day, which represents 23% of the upper bound of the ADI of 900 µg/kg bw.

Based on incurred DNC residues in chicken muscle, offal, and skin with fat, at zero days withdrawal time and 50 mg/kg feed:

The GECDE for adults and the elderly is 95 µg/kg bw per day, which represents 11% of the upper bound of the ADI of 900 µg/kg bw.

The GECDE for children and adolescents is 120 µg/kg bw per day, which represents 14% of the upper bound of the ADI of 900 µg/kg bw.

The GECDE for infants and toddlers is 160 µg/kg bw per day, which represents 18% of the upper bound of the ADI of 900 µg/kg bw.

Recommended maximum residue limits (MRLs) for nicarbazin

Species	Liver (µg/kg)	Kidney (µg/kg)	Muscle (µg/kg)	Skin with fat (µg/kg)
Chicken	15 000	8000	4000	4000

Selamectin (broad-spectrum parasiticide)

Acceptable daily intake	The Committee withdrew the previous ADI and established an ADI of 0–0.05 mg/kg bw, based on a NOAEL of 5 mg/kg bw per day for increased liver and uterus/cervix weights at 15 mg/kg bw per day in a one-year rat study, with application of a safety factor of 100 to account for interspecies and intraspecies variability. Although the NOAEL for effects seen in a 13-week dietary neurotoxicity/toxicity study in rats, assessed by the Committee at its last meeting was 1 mg/kg bw per day, the LOAEL, at 15 mg/kg bw per day, and the effects observed were the same as those on which the ADI is based. The Committee concluded that the ADI established at the present meeting would be sufficiently protective of these findings.
Acute reference dose	The Committee concluded that the ARfD of 0.4 mg/kg bw established at the eighty-eighth meeting was still appropriate.
Residue definition	The marker residue in Atlantic salmon fillet is selamectin.
Estimated dietary exposure	Dietary exposure was assessed for some possible scenarios, but no GVP has been established.
Maximum residue limits	Specific MRLs could not be recommended at this time due to a lack of an established GVP.

Annex 3

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

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

Evaluation of certain veterinary drugs

Remote, 16–27 May 2022

Agenda

1. Opening

2. Declarations of Interests (information by the Secretariat on any declared interests and discussion).

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
   - Imidacloprid
   - Ivermectin
   - Nicarbazin
   - Selamectin

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
Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 799, 1990.

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.

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
Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 888, 1999.

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WHO/PCS/02.1. ISBN 92 4 166517 3

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Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 911, 2002.

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WHO Technical Report Series, No. 988, 2014.

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
Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No.1023, 2019.

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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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