Evaluation of certain veterinary drug residues in food

Eighty-fifth report of the Joint
FAO/WHO Expert Committee on
Food Additives

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Food and Agriculture Organization of the United Nations

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List of abbreviations

95/95 UTL	95/95 upper tolerance limit; upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ARfD	acute reference dose
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the concentration–time curve
AUC_{0-LOQ}	area under the concentration–time curve from time 0 to time of limit of quantification
bw	body weight
CAC	Codex Alimentarius Commission [Codex]
CAS	Chemical Abstracts Service
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CIA	Critically Important Antimicrobials [for Human Medicine]
CIFOCOss	FAO/WHO Chronic Individual Food Consumption Database – Summary statistics
5-CL	5-chloro-8-hydroxyquinoline or 5-chloroquinolin-8-ol (CHQ)
7-CL	7-chloro-8-hydroxyquinoline
5-CLG	5-chloro-8-hydroxyquinoline glucuronide conjugate
5-CLS	5-chloro-8-hydroxyquinoline sulfate conjugate
C_{max}	maximum concentration
CNS	central nervous system
5,7-DCL	5,7-dichloroquinolin-8-ol (DCHQ)
5,7-DCLG	5,7-dichloroquinolin-8-ol glucuronide conjugate
5,7-DCLS	5,7-dichloroquinolin-8-ol sulfate conjugate
EDI	estimated daily intake
EFSA	European Food Safety Authority
EHC 240	Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food
eq	equivalents
F_0	parental generation
F_1	first filial generation
FAO	Food and Agriculture Organization of the United Nations
GC–ECD	gas chromatography–electron capture detector
GC–MS	gas chromatography–mass spectrometry
GEADE	global estimate of acute dietary exposure

GECDE	global estimate of chronic dietary exposure
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GL	guideline
GLP	good laboratory practice
GVP	good practice in the use of veterinary drugs
HBGV	health-based guidance value
HPLC	high-performance liquid chromatography
HPLC–FL	high-performance liquid chromatography with fluorescence detection
HPLC–UV	high-performance liquid chromatography with ultraviolet detection
IBT Labs	Industrial Bio-Test Laboratories
IEDI	international estimate of daily intake
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDPE	low-density polyethylene
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
mADI	microbiological acceptable daily intake
MIC	minimum inhibitory concentration
MIC_{50}	minimum concentration required to inhibit the growth of 50% of organisms
MNU	*N*-methyl-*N*-nitrosourea
MR	marker residue
MR:TRR	marker residue to total radioactive residue ratio
MRL	maximum residue limit
MRT	mean residence time
n/N	sample size
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
PK/PD	pharmacokinetic/pharmacodynamic [modelling]
POD	point of departure
ppm	parts per million
PVC	polyvinyl chloride
rbST	recombinant bovine somatotropin

SD	standard deviation
T_{max}	time to reach the maximum concentration (C_{max})
TRR	total radioactive residue
UHPLC–MS/MS	ultra-high performance liquid chromatography–tandem mass spectrometry
USA	United States of America
USEPA	United States Environmental Protection Agency
UTL	upper tolerance limit
ULOQ	upper limit of quantification
v/v	volume per volume
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
VICH GL	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products guideline
w/w	weight per weight
WHO	World Health Organization

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. 74, 2018.

Residue monographs are issued separately by FAO under the title:

Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 21, 2018.

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1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 17 to 26 October 2017. The meeting was opened by Dr Kazuaki Miyagishima, Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), on behalf of the directors-general of WHO and the Food and Agriculture Organization of the United Nations (FAO).

Dr Miyagishima stated that WHO's continued priority was to build JECFA's global profile so that the work of the Committee receives the worldwide attention it deserves, noting that such an increase in visibility may facilitate future resource mobilization. Dr Miyagishima reminded the participants of recent developments on antimicrobial resistance by WHO, FAO and the United Nations, such as the Global Action Plan on Antimicrobial Resistance, adopted in 2015. The work of the Committee on the residues of antibiotics in food would contribute to building of an international stewardship framework in its broadest context.

Twenty-one meetings of the Committee had been held to consider veterinary drug 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* and *226*) in response to the recommendations of a 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 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) (see section 2);
- to evaluate the safety of residues of certain veterinary drugs (see section 4 and Annex 2); and
- to respond to specific concerns raised by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) (see sections 3 and 4 and Annex 2).

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[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 84 previous meetings of JECFA (Annex 1).

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eighty-fifth meeting had completed declaration of interest forms. No conflicts of interest were identified. There were no responses to the public posting of Committee members' work-related biographies.

1.2 Modification of the agenda

The agenda (see Annex 3) was modified to exclude bismuth sub-nitrate and diflubenzuron as no data were submitted by the sponsors.

2. General considerations

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

Dr Kevin Greenlees, Chairperson of CCRVDF, informed the Committee about relevant decisions of the Codex Alimentarius Commission and the principal outcomes and discussions of the Twenty-third Session of CCRVDF *(2)*, which was held after the eighty-first meeting of the JECFA in 2015 (Annex 1, reference *226*).

The Twenty-third Session of CCRVDF finalized work on the MRLs for lasalocid sodium, ivermectin and teflubenzuron that were recommended by the eighty-first meeting of JECFA (Annex 1, reference *226*); these MRLs were subsequently adopted by the Codex Alimentarius Commission at its Fortieth Session *(3)*. The Twenty-third Session of CCRVDF agreed to hold the MRLs for zilpaterol hydrochloride for consideration at the next Session of CCRVDF in light of the JECFA evaluation of the additional data provided by the pharmaceutical sponsor. The Fortieth Session of the Codex Alimentarius Commission noted that no request had been received to change the status of the draft MRLs for recombinant bovine somatotropins (rbSTs), which had been retained to allow further time to facilitate consensus while recognizing the validity of JECFA's risk assessments as the sound scientific basis for its deliberations on rbSTs *(3)*. The draft MRLs for rbSTs remain at step 8 at the Commission.

The Twenty-third Session of CCRVDF saw continued progress on the work on risk management recommendations for gentian violet. This will be further considered by the next session of CCRVDF.

Dr Greenlees informed the Committee that, in response to a request in the report of the eighty-first meeting of JECFA, the Twenty-third Session of CCRVDF had agreed to initiate discussion, through an electronic working group, on the feasibility of establishing MRLs for groups of fish species for veterinary drugs. Similarly, CCRVDF established an electronic working group to provide a definition for "edible offal" and specify edible offal tissues of interest in international trade. The Twenty-third Session of CCRVDF agreed to add information on the registration of a compound as a pesticide and, where applicable, information on the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluation to the form requesting information on compounds for evaluation by JECFA. This form is attached to the Circular Letter requesting proposals for inclusion in the Priority List for veterinary drugs requiring evaluation or re-evaluation by JECFA. The Executive Committee of the Codex Alimentarius encouraged fostering the collaboration between CCRVDF and Codex Committee on Pesticide Residues

(CCPR) when considering compounds used as both veterinary drugs and pesticides.

CCRVDF agreed to request FAO and WHO scientific advice on the unintended presence of veterinary drugs residues in food commodities resulting from the carry-over of drug residues into feed, that is, whether this constitutes a human health risk and which recommendations could be established to address the trade issues while protecting human health. The potential for unintended carry-over of residues of lasalocid sodium in eggs was provided as a working example.

The Twenty-third Session of CCRVDF agreed on a Priority List of veterinary drugs for evaluation (or re-evaluation) by JECFA. CCRVDF discussed at some length the difficulty in identifying data to support the evaluation of veterinary drugs by JECFA. An observer group for the veterinary drug industry (HealthforAnimals) will provide a discussion paper analysing the decline in new compounds in the CCRVDF Priority List.

The Twenty-fourth Session of the CCRVDF is scheduled to be held in Chicago, Ilinois, USA from 23 to 27 April 2018.

2.2 Chronic dietary exposure assessment of compounds used as veterinary drugs and pesticides

Following recommendation of JECFA at its seventy-eighth meeting (Annex 1, reference *217*) and of JMPR at the 2015 meeting *(4)*, an expert working group on the methodology applied by JECFA and JMPR to estimate chronic dietary exposure was convened. The working group was formed to address the issue of how to estimate less-than-lifetime exposure and dietary exposure to residues of substances used as both veterinary drugs and pesticides. For the dual-use exposure assessment, the working group examined two models:

- the global estimate of chronic dietary exposure (GECDE), used by JECFA, for assessing the dietary exposure from veterinary drugs; and
- the international estimate of daily intake (IEDI), used by JMPR, for assessing the dietary exposure from pesticide residues.

The aim was to develop a practical and scientifically sound harmonized model for estimating total exposure to residues of dual-use chemicals.

The working group assessed eight compounds that are used both as pesticides and veterinary drugs and that have been previously evaluated by both JECFA and JMPR: abamectin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, emamectin benzoate, teflubenzuron and thiabendazole. The working group did not examine the toxicological profiles of the compounds to

align them with the exposure model for this exercise; it was assumed that less-than-lifetime exposure was a potential concern.

A comparison of dietary exposure methodologies was carried out to assess whether:

- dual uses for the eight compounds resulted in dietary exposure estimates within the relevant ADIs;
- the current JMPR and JECFA dietary exposure methodologies, when applied to dual-use compounds, gave comparable estimates; and
- the current JMPR and JECFA dietary exposure methodologies gave estimates that were sufficiently protective when compared with national estimates of dietary exposure.

The median residues estimated by JMPR and JECFA were used to generate three separate sets of dietary exposure estimates. These dietary exposure estimates were:

- IEDI (the JMPR model), based on the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) cluster diets;
- GECDE (the JECFA veterinary drugs model), extended to cover plant products, using the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss) dataset; and
- National chronic dietary exposure assessments, conducted using food consumption data and national methodologies from Australia, Brazil, the People's Republic of China, the Republic of Korea, the Netherlands, New Zealand and the USA, and from 11 European Union member states, performed by the European Food Safety Authority (EFSA).

The estimations were conducted using two different approaches related to the median residues in animal commodities: the highest median residues from JECFA and JMPR, and combined median residues, the sum of the JECFA and JMPR medians.

Exposure was estimated in children, adults and the general population as a function of the information available, for median- and high-percentile consumers, to cover less-than-lifetime exposure, where possible. The working group noted that it was not possible to estimate exposure in children or in high-percentile consumers using the JMPR approach.

When there are dual-use compounds, residues may be present in animal commodities resulting from the use of the compound as a pesticide and

veterinary drug. In this case, the working group assumed that residues will be present in 100% of all animal commodities for both uses. This is consistent with the approaches currently used for the separate assessments of veterinary drugs and pesticides.

The results indicate that there were no marked differences between dietary exposure estimates based on the highest median residue or based on the sums of the median residues for the compounds assessed.

In principle, the GECDE is a suitable model for the assessment of lifetime and less-than-lifetime dietary exposure. However, the methodology needs to be refined to reflect the improvement of data quality. Consequently, refined GECDE-based dietary exposure estimates are expected to decrease compared with current estimates.

The IEDI is suitable for estimating chronic (lifetime) exposure from widely and regularly consumed staple commodities. However, the IEDI is not a suitable model for assessing less-than-lifetime dietary exposure.

For the adult population, the IEDI adequately covers the high percentiles obtained by the national estimates for six out of the eight assessed compounds. The GECDE adequately covers the high percentiles obtained by the national estimates for all compounds. It should be noted that in comparison to the IEDI, the GECDE is more conservative for all compounds (up to four times).

The IEDI does not specifically address exposure in children. The current IEDI estimates are below the national estimates (high percentiles) for seven out of the eight assessed compounds by a factor of up to four. The GECDE adequately covers the national estimates (high percentiles) for all compounds with a conservativeness of up to four times for the high-percentile group.

The working group concluded that, to appropriately link the exposure assessment with the hazard assessment, sensitive populations and relevant exposure duration need to be clearly identified from the toxicological profile for each compound under consideration.

The working group made the following recommendations.

In regard to compounds with dual use:

1. JECFA and JMPR are encouraged to always consider dual-use exposure.
2. In the immediate future, residue concentrations obtained from veterinary use and pesticide use in the same animal commodity should be added together to provide the residue data input for the dietary exposure assessment.
3. JECFA and JMPR are encouraged to harmonize their residue definitions to facilitate exposure assessment of dual-use compounds

(and subsequently facilitate harmonization of enforcement strategies).

4. The GECDE model should be refined to more accurately encompass national dietary exposure estimates.

In regard to less-than-lifetime exposure:

1. In order to appropriately link the exposure assessment with the hazard assessment, JECFA and JMPR should clearly identify sensitive populations and relevant exposure duration from the toxicological profile for each compound under consideration.

2. JECFA should implement this guidance in future evaluations of food chemicals where appropriate and, after some experience, revise it as appropriate.

3. JMPR should consider the use of individual food consumption data when it is indicated by the toxicological end-points.

In regard to the dietary exposure assessment methodology:

1. The GECDE, subject to further refinement, should be used to assess less-than-lifetime exposure.

2. Exposure to compounds under consideration should be assessed using each individual food consumption survey available in CIFOCOss.

3. The highest reliable percentile rather than the 97.5th percentile should be used for all cases.

In regard to food consumption data collection:

1. FAO and WHO should continue to update the CIFOCOss database to provide a more complete coverage of a broader range of countries and population groups.

2. Wherever possible, FAO and WHO should collect data based on the EFSA *Food classification and description system for exposure assessment, revision 2* (FoodEx2 classification) (*5*). The FoodEx2 classification is more detailed than the Codex classifications, and the mapping with the latter has been done.

3. A conversion table should be developed to approximately translate the foods of animal and plant origin for which food consumption statistics have been collected in CIFOCOss into Raw Agricultural Commodities.

The Committee agreed with the conclusions and recommendations of the working group and piloted the combined exposure approach for the two compounds with dual use that are on the present Committee meeting agenda (i.e. lufenuron and flumethrin). The details and the results of the combined exposure

assessment of each compound will be provided as an annex to the relevant JECFA monograph (to be published in 2018).

On the basis of the recommendations of the working group, the Committee further considered that the nature of the toxicological effect and the duration of exposure until the onset of effect be addressed as follows:

- Where the ADI is based on a developmental effect, pregnant women will be at potential risk and the critical exposure period may be only a few days or weeks. In such cases, it will be necessary to consider exposure in pregnant high-percentile consumers or an appropriate surrogate population.

- Where the point of departure (POD; e.g. the no-observed-adverse-effect level [NOAEL]) on which the ADI is based is not a developmental effect but is ≤3 times lower than the developmental POD, pregnant women will be at potential risk and the critical exposure period may be only a few days or weeks. In such cases, it will be necessary to consider exposure in pregnant high-percentile consumers or an appropriate surrogate population.

- Where the ADI is based on offspring toxicity, but the POD on which it is based is ≤3 times lower than the POD for long-term toxicity (e.g. 2-year rat study), infants and young children will be at potential risk. In such cases, it will be necessary to consider exposure in infants and young children who are typical (average) consumers.

- Where the POD on which the ADI is based in ≤3 times lower than the POD for offspring toxicity, infants and young children will be at potential risk. In such cases, it will be necessary to consider exposure in infants and young children who are typical (average) consumers.

- Where the ADI is based on offspring toxicity, and the POD on which it is based is >3 times lower than the POD for long-term toxicity (e.g. 2-year rat study), there will be particular concern about the potential risk to infants and young children. In such cases, it will be necessary to consider exposure in infants and young children who are high-percentile consumers.

- Where the ADI is based on effects observed in long-term studies (e.g. 2-year study of toxicity in rats) and the POD in a study (or studies) of shorter duration (e.g. 90-day rat or 90-day dog study of toxicity) is ≤3 times higher than the critical POD (the POD on which the ADI is based), there will be potential concern for less-than-lifetime exposure in the general population. In such cases, it will be necessary to consider exposure in high-percentile adult or general population consumers.

- Where the POD on which an acute reference dose (ARfD) is based is the same as the POD on which the ADI is based, if short-term exposures (children and general population) are not of concern, there will be no concern for less-than-lifetime exposure.
- In all other situations, there will be no specific concerns for less-than-lifetime exposure. In such cases, it will be sufficient to consider exposure in average adult or general population consumers.

2.3 Assessment of the relative bioavailability and/or pharmacological activity of incurred drug residues in animal tissues

Background and introduction

Recent JECFA assessments and publications have considered the potentially limited oral bioavailability and/or pharmacological activity of incurred drug residues[2] (*6*; Annex 1, references *217* and *223*).

At the request of the Twenty-first Session of CCRVDF *(7)*, the limited oral bioavailability of zilpaterol was considered by JECFA as part of its overall evaluation and exposure assessment at the seventy-eighth meeting (Annex 1, reference *217*). Although it was considered that MRLs could not be established at that time due to specific residue depletion data gaps, the Committee did establish an ADI of 0–0.04 μg/kg bw (0–0.000 04 mg/kg body weight) for zilpaterol. At the eighty-first meeting, the Committee considered the need to establish an ARfD and concluded that this should be based on the same end-point as the ADI with the same numerical value (Annex 1, references *226* and 228). Following the Committee's assessment at the seventy-eighth meeting, new data were submitted to JECFA to reassess the bioavailability of incurred zilpaterol residues. If the bioavailability of incurred residues was decreased in relation to oral administration by other routes (e.g. ampoule-containing water administration in humans in fasting condition, the route used in the toxicological study upon which the ADI/ARfD was derived), the human exposure assessments could be further refined.

JECFA assesses the bioavailability of non-extractable (i.e. bound) residues based on studies using the Gallo-Torres approach *(9)*. However, the bioavailability of total (including free or extractable) incurred residues is not routinely considered by JECFA in exposure assessments.

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[2] The International Programme on Chemical Safety defines "incurred residue" is as a "residue present in food or feed as a result of treatment with pesticides or veterinary drugs, for example, in the field (as opposed to residue resulting from spiking samples in the laboratory)" (*8*).

The Committee continues to assume, in the absence of evidence to the contrary, that all non-bound incurred residues are equally bioavailable as with other oral dosing regimens, as this provides the most conservative default position. However, the Committee may consider a lower bioavailability of incurred residues in the risk assessment, depending on the strength of evidence available. There is no current guidance on the most appropriate experimental design for studies on the bioavailability of incurred residues. In the following text, the Committee provides further considerations on what data may be useful for such an assessment. This guidance is restricted to a consideration of the toxicological implications of systemically available drug residues.

JECFA considerations on the design of incurred residue bioavailability and pharmacological activity studies

1. Selection of appropriate test animal models

There is no validated model established to assess the oral bioavailability of incurred residues, including the most appropriate test animal species (i.e. animals in which the bioavailability of incurred residues will be assessed). The species in which the residues are incurred (i.e. target animal) should be the food animal species for which the veterinary drug is approved (e.g. cattle, swine, poultry, fish). Ideally, a test species with bioavailability comparable to that in humans should be chosen. If it were possible to demonstrate comparable bioavailability of the compound in the test species and in humans (such as by oral tablet, capsule or solution), then this would provide confidence in the extrapolation of the results with incurred residues in the test species to humans.

The test animal species in which the bioavailability assessment will be conducted should have a gastrointestinal anatomy and physiology (especially proximal gastrointestinal tract) similar to that of humans. This would include comparable gastrointestinal pH and transit time. The pig is generally considered a suitable animal model to assess bioavailability in humans. However, the Committee noted that other animal models may also be suitable for generating relevant data. For example, although there may be greater difference in proximal gastrointestinal anatomy and transit time between dogs and humans than between pigs and humans (as noted in the eighty-first Committee's zilpaterol assessment), there remains substantial similarity in gastrointestinal anatomy and physiology between dogs and humans. Incurred residue bioavailability data generated from a dog test system may therefore be considered valid for JECFA's purposes, provided the sponsor includes appropriate justification. One potential reason for using the dog (as opposed to the pig) could be a greater willingness of dogs to ingest the amount of tissue necessary to achieve the desired dose from incurred residues.

2. Dosing strategies for achieving quantifiable tissue and plasma concentrations

For some veterinary drugs, it may be difficult to achieve high concentrations of incurred residues in the tissues of the target species (e.g. cattle). In such cases, in order for the test animal (e.g. pig or dog) to ingest a dose sufficient to achieve quantifiable plasma concentrations, it may be necessary to feed appreciable quantities of tissue containing incurred residues.

The Committee appreciates that the compound under evaluation may need to be administered to the target species at doses significantly higher than the label dose and the animals killed immediately after the final dose. Killing the target species immediately after the final dose may result in elevated concentrations of drug in plasma, whereas the actual plasma concentrations are likely negligible if the label withdrawal period is followed. This may distort the bioavailability assessment, as it is presumed that residues in plasma may have a higher or lower bioavailability than incurred residues in tissue.

Ingestion of a large quantity of tissue at one time by the test species can alter, for example, gastrointestinal motility compared with fasting animals receiving the drug via other oral regimens (e.g. gavage or capsule). Differences in gastrointestinal motility have the potential to alter the timing of residue absorption and thus the maximum concentration (C_{max}).

Deviations in drug dosing and withdrawal periods in the target species, and excess tissue ingestion in the test animal, may result in less realistic exposure from incurred residues and a subsequent over- or underestimation of the bioavailability. However, such estimates of bioavailability would provide a useful starting point for subsequent refinement of JECFA's exposure assessment.

3. Pharmacological activity of incurred residues (relay pharmacology)

Studies to assess the pharmacological potency of incurred residues (sometimes referred to as "relay pharmacology" studies) assess differences in physiological or pharmacological end-points in the test animal after administration of the drug via incurred residues compared with other oral administration methods (e.g. gavage, capsule or dietary admixture). Studies to determine the relative bioavailability of incurred residues ("bioavailability" studies) measure the plasma concentrations after ingestion of the drug via incurred residues and other oral administration methods, and derive the relevant pharmacokinetic parameters (C_{max} and area under the concentration–time curve [AUC]) from such data. In the former, all the pharmacologically active substances present contribute to the response measured; in the latter, only the parent compound is typically assessed.

Bioavailability and relay pharmacology are obviously related. In fact, a single study could assess both the relative bioavailability (pharmacokinetics) of incurred residues compared with other oral administration methods and the pharmacological activity (pharmacodynamics) observed after the various oral

doses are administered. Such a combination study may not be feasible in all cases due to technical challenges (e.g. collecting blood samples without biasing clinical end-points determined at the same time). However, the ability to integrate the pharmacokinetic and pharmacodynamic data (PK/PD modelling) would enable a clear relationship between the drug residues in plasma and their actual effect.

For example, although the pharmacokinetic parameter AUC is traditionally used for assessing bioavailability (drug exposure), the Committee considers that for some compounds having short reversible drug–receptor interactions, the magnitude of relevant effect may correlate more closely with the parameter C_{max} than with AUC.

4. Other issues regarding the assessment of relative bioavailability and relay pharmacology

As with any clinical study, the necessary sample size for a relative bioavailability or relay pharmacology study will depend on the magnitude of the expected differences between groups, as well as the degree of variance. For relative bioavailability or relay pharmacology studies, a crossover design with appropriate wash-out period (similar to bioequivalence studies) may be used to increase the study power and minimize the required sample size. Sponsors are encouraged to refer to Guideline 52 of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL52) *(10)* for further details regarding appropriate sample sizes and timing of plasma collection.

Relative oral bioavailability studies may not be feasible for incurred drug residues comprising multiple components (e.g. parent compound + metabolites). In order to determine the relative bioavailability of each incurred residue component, the concentrations of each component must be quantified in both the incurred tissue residues and the test animal plasma.

The doses used in a relay pharmacology study should be consistent with those known to cause a predictable pharmacological response in the test animal species. The primary outcomes measured should be a result of discrete pharmacological activity. Such outcomes should also be quantifiable, simple to measure and not persist for prolonged durations. Examples of appropriate outcome measure include changes in heart rate, blood pressure, respiration or motor activity.

If different oral bioavailability and/or pharmacological activities for incurred residues are claimed, supporting data can be provided for all the animal-derived tissues that significantly impact the human exposure assessment. For tissues for which data on bioavailability / relay pharmacology of incurred residues are not available, the Committee will assume the same bioavailability / pharmacological activity as by direct oral exposure.

2.4 Acute reference dose (ARfD) for residues of veterinary drugs

Following a recommendation of JECFA at the seventy-fifth meeting (Annex 1, reference *208*), WHO established a working group to elaborate principles to establish ARfDs for residues of veterinary drugs. Following public consultation, *Guidance document for the establishment of Acute Reference Dose (ARfD) for veterinary drug residues in food (11)* was published in May 2017 and adopted by WHO at the present meeting. The guidance was first applied in evaluations at the present meeting. The Committee considered whether it was necessary and how to establish an oral acute toxicological and microbiological reference dose for residues of all veterinary drugs evaluated at the meeting. The Committee established ARfDs for amoxicillin, ampicillin, ethion, flumethrin and halquinol.

2.5 Methodological approaches and types of data for assessment of antimicrobial residues in food

WHO list of Critically Important Antimicrobials for Human Medicine

The Committee noted ongoing activities of WHO on antimicrobial resistance and the upcoming publication of guidelines on the implications of the *WHO list of Critically Important Antimicrobials for Human Medicine* (WHO CIA list) for minimizing the emergence and spread of antimicrobial resistance in the food chain *(12)*. The Committee refers to the WHO CIA list in the "Explanation" section of the evaluation reports on the compounds the Committee evaluates at its meetings.

JECFA assesses veterinary drugs with microbiological activity for the potential risk of ingested residues to alter human intestinal microbiota and enhance the emergence of and selection for antimicrobial-resistant bacteria in the gastrointestinal tract. The recommended microbiological acceptable daily intakes (mADIs) set by JECFA ensure that drug residues in animal-derived food are at sufficiently low levels to minimize the potential selection of antibiotic-resistant bacteria in humans. JECFA will review the WHO guidelines on antimicrobial resistance and consider how it might modify its procedures to ensure that the issue of antimicrobial resistance is addressed to the extent possible within the remit of the Committee.

Microbiological ARfD

The Committee adopted the recently published *Guidance document for the establishment of Acute Reference Dose (ARfD) for veterinary drug residues in food (11)* at the present meeting. The document provides guidance on when and how to establish both a toxicological and a microbiological ARfD. Noted in the guidance is the distinct difference in the exposure of microorganisms in

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the gastrointestinal tract following acute intake of microbiologically active drug residues compared with that following chronic daily ingestion. This is addressed by using a dilution factor of 3 in the formula for calculating the microbiological ARfD. The remainder of the formula is the same as that used for calculating the mADI.

The formula includes a value for colon volume, which to date has been assumed to be 220 mL (based on mass of colon content of 220 g per day). This value was based on necropsy data of 17 accident victims *(13)*. In developing the guidance document on establishing ARfDs, the WHO expert working group reviewed more recent studies that used current imaging technology. These studies showed that the hydrated colon of healthy individuals is larger than the 220 g estimate.

Pritchard et al. *(14)* found, using three-dimensional abdominal magnetic resonance imaging techniques, that the 220 g estimate represents approximately the lower 95th percentile of colon volumes among 75 fasting human volunteers. The mean value of 561 mL for the colon volume, based on the combined volumes of the ascending colon, transverse colon and descending colon, provides a more robust estimate. The WHO expert working group noted that this estimate is still low: the measures did not take into account the volume of the lower sigmoid colon because the observations were from fasting individuals.

Based on this information, the expert working group concluded that the more appropriate value for the colon volume is 500 mL. This value has therefore been adopted for use in the formulae for calculating the mADI and microbiological ARfD for the evaluation of the effects of antimicrobial residues in food on the intestinal microbiota. The Committee at the present meeting used the colon volume of 500 mL in the microbiological evaluations of amoxicillin, ampicillin and halquinol.

Approaches for assessment of microbiological activity of veterinary drug residues in food

The Committee reviewed the methodological approaches and types of data it receives for assessments of veterinary drug residues in food with regard to their impact on human intestinal microbiota (disruption of the colonization barrier; emergence and selection for antimicrobial-resistant bacteria) with the goal of improving their safety evaluation. In determining mADIs, and now also microbiological ARfDs, JECFA typically:

- evaluates minimum inhibitory concentration (MIC) data and other in vitro datasets submitted by the sponsor; and

- reviews the published scientific literature on the susceptibility of selected human intestinal bacteria against antimicrobial agents for the end-point of disruption of the colonization barrier.

The MIC data on the susceptibility to antimicrobial agents of the intestinal microbiota can be very difficult to evaluate because the various laboratories use different procedures and MIC test methods, some of which are not performed according to internationally recognized standards, such as those of the Clinical and Laboratory Standards Institute *(15, 16, 17)*. In addition, in many cases the number of isolates tested ($n < 10$) is low, with a lack of MIC distribution information for the isolates. In some cases, the minimum concentrations required to inhibit the growth of 50% of organisms (MIC_{50}) are based on human faecal isolates from clinical infections, not healthy subjects.

The Committee recommends that MIC data used to derive mADIs and/or microbiological ARfDs come from studies that use standard internationally recognized methods with at least 10 strains of the relevant genera of intestinal bacteria sourced from faecal samples of healthy donors, as in Step 1 of VICH GL36(R) *(18)*. The selection of intestinal microbiota used in MIC tests should take into consideration recent scientific knowledge from molecular and metagenomic studies on intestinal microbial community composition.

In addition, data from in vitro studies (continuous culture flow chemostats) and in vivo models (human volunteers, animal models and human microbiota–associated animals) are evaluated by the Committee for both microbiological end-points. However, data from these studies can be problematic in determining an mADI and/or microbiological ARfD. This is due to the small sample size in the animal studies; insufficient data and low power of studies in human volunteers (because of small numbers of subjects); concentrations of antimicrobial agent generally not being adequate to determine a chronic or acute dose with no effect; and the lack of validation of the in vitro and in vivo test models. In addition, for the antimicrobial resistance end-point, many studies that the Committee evaluates determine the susceptibility and the emergence of resistance only of *Escherichia coli* and not of the other predominant microorganisms that inhabit the gastrointestinal tract.

Therefore, the Committee recommends that in vitro or in vivo studies be conducted using a range of concentrations of the antimicrobial agent, from residue levels to therapeutic levels. Such studies should address the predominant bacterial strains that inhabit the gastrointestinal tract when determining if levels of antimicrobial residues in animal-derived food after consumer ingestion can increase the population of antimicrobial-resistant intestinal bacteria in the gastrointestinal tract.

3. Comments on residues of specific veterinary drugs

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

3.1 Amoxicillin

Explanation

Amoxicillin (Chemical Abstracts Service [CAS] No. 26787-78-0) is a moderate-spectrum, semi-synthetic β-lactam aminobenzylpenicillin antimicrobial agent used for the treatment or control of bacterial infections in animals and humans. It exerts bactericidal effects by inhibiting the transpeptidase that catalyses the cross-linking of bacterial cell wall peptidoglycan. It is available as the sodium salt and the trihydrate salt.

Amoxicillin is categorized as a highly important antimicrobial agent in the WHO CIA list *(12)*.

Amoxicillin has been previously evaluated by the Committee at its seventy-fifth meeting (Annex 1, reference *208*). An mADI of 0–0.007 mg/kg body weight (bw) was established. The toxicological data available at that time did not allow for the establishment of a toxicological ADI. However, the Committee considered that the mADI would be protective for allergenicity, which was considered to be the most sensitive toxicological end-point. The Committee recommended MRLs for amoxicillin in cattle, sheep and pig tissue of 50 μg/kg and in cattle and sheep milk of 4 μg/L, the same as those established for benzylpenicillin and procaine penicillin at the thirty-sixth and fiftieth JECFA meetings (Annex 1, references *91* and *134*).

The Committee did not calculate an estimated daily intake (EDI) for amoxicillin at the previous meeting owing to the small number of quantifiable residue data points. Using the model diet of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 L milk with the MRLs recommended above, the theoretical maximum daily intake is 31 μg/person per day, which represents 74% of the upper bound of the ADI.

The Committee evaluated amoxicillin at the present meeting at the request of the Twenty-third Meeting of the CCRVDF *(2)*. The CCRVDF requested that JECFA recommend MRLs in finfish muscle plus skin in natural proportion.

A limited set of data was submitted to the present Committee. The data package did not include information upon which to base an evaluation for a toxicological ADI or for biochemical or microbiological effects.

The Committee conducted a literature search in PubMed (2011–2017). The following search string was used: "Amoxicillin/adverse effects" [Mesh] OR "Amoxicillin/contraindications" [Mesh] OR "Amoxicillin/poisoning" [Mesh] OR "Amoxicillin/toxicity" [Mesh]) OR ((Amoxycillin [TIAB] OR Hydroxyampicillin

[TIAB] OR Amoxicilline [TIAB] OR "BRL-2333" [TIAB] OR "BRL 2333" [TIAB] OR BRL2333 [TIAB] OR Clamoxyl [TIAB] OR Penamox [TIAB] OR Trimox [TIAB] OR Wymox [TIAB] OR Actimoxi [TIAB] OR Amoxic [TIAB]) AND ("adverse effect" [TIAB] OR "adverse effects" [TIAB] contraindication* [TIAB] OR poison* [TIAB] OR toxicit* [TIAB] OR toxic* [TIAB] OR "toxicity" [Subheading] OR "Toxicity Tests" [Mesh] OR "Poisons" [Mesh] OR "Poisons" [Pharmacological Action] OR "Toxicology" [Mesh] OR "Poisoning" [Mesh] OR "poisoning" [Subheading] OR "Poisons" [Mesh] OR "Poisons" [Pharmacological Action] OR "Toxicology" [Mesh] OR "Poisoning" [Mesh] OR "poisoning" [Subheading] OR "contraindications" [Subheading])).

The search retrieved 327 articles, of which 22 dealt with data relevant to the present toxicological and biochemical evaluation of amoxicillin. However, none of these articles provided any new information that changed the Committee's previous evaluation.

The submitted data package included information on three amoxicillin formulations, including a list of trade names and approved uses in fish; monitoring data on occurrence of amoxicillin in aquaculture products; and studies on pharmacology and residue depletion of amoxicillin in fish. English translations of the original studies were provided. In addition, raw data were provided by the sponsor on request for some of the studies. Information on established withdrawal periods for authorized products was missing. Proposals were made by the authors of the studies regarding possible withdrawal periods.

In addition, as part of the residue assessment, the Committee conducted a comprehensive review of peer-reviewed scientific literature from the following publicly accessible databases: Agricola, Embase, Web of Science, PubMed, Springer Protocols, Food Science and Technology Abstracts, Phish-Pharm, CABI VetMed Resource. The following searches were conducted in each of these databases:

- Amoxicillin AND fish AND residue
- Amoxicillin AND fish AND kinetics
- Amoxicillin AND fish AND withdrawal
- Amoxicillin AND fish AND metabolism
- Amoxicillin AND fish AND analytical method
- Amoxicillin AND (salmon OR trout) AND (residue OR kinetics OR withdrawal OR metabolism OR analytical method)

The criteria applied to filter the articles for the assessment at the present meeting are shown in 2.2Chronic dietary exposure assessment of compounds used as veterinary drugs and pesticides.

Table 1

Inclusion and exclusion criteria for the comprehensive literature search on amoxicillin

Inclusion criteria	Exclusion criteria
Any article focusing on:	Any article focusing on:
• amoxicillin concentrations in plasma of fish	• bacterial resistance to amoxicillin
• amoxicillin concentrations in edible tissues of fish	• amoxicillin use in food animal species other than fish
• residue determination in fish plasma / tissue	• kinetics/residues of antimicrobials other than amoxicillin (that
• bioavailability of amoxicillin residues in fish	do not include amoxicillin for comparison)
	• environmental issues
No restrictions concerning year of publication	Any article dealing with ornamental fish

The literature search identified 37 potentially relevant articles.

Conditions of use

Amoxicillin trihydrate is approved in a Member State for administration by the oral route at doses in the range of 20–40 mg/kg bw, once or twice a day, for a period of 4–7 days in yellowtail (*Seila quinqueradiata*) against furunculosis (*Photobacterium damselae* subsp. *piscicida*). The usual dose is 40 mg/kg bw. Based on the dose regimen approved for yellowtail, amoxicillin trihydrate is also used in flounder, rockfish and red sea bream.

Amoxicillin sodium is approved for use by the intramuscular route at a single dose of 12.5 or 40 mg/kg bw in olive flounder against streptococcosis (*Streptococcus iniae*).

Amoxicillin trihydrate is used in combination with florfenicol and is administered via intramuscular injection at a single dose of 10 mg/kg bw in olive flounder against streptococcosis and edwardsiellosis *(Edwardsiella tarda)*.

Literature available in the public domain describes use of amoxicillin preparations against furunculosis in Atlantic salmon and pseudotuberculosis in yellowtail.

The sponsor's submission did not provide any recommended withdrawal period but identified a national MRL of 50 μg/kg in fish.

An MRL of 50 μg/kg in edible tissues for the use of amoxicillin at an oral dose of 40 mg/kg bw in salmon, eel, perciform and other fish has been established in one Member State. In other Member States, the MRL in edible tissues of all animal species is 50 μg/kg.

Toxicological and microbiological evaluation

Observations in humans

JECFA previously concluded that the most critical effect of amoxicillin is

hypersensitivity (Annex 1, reference *208*). Hypersensitivity, even if rare, is the most relevant toxicological effect of amoxicillin and other β-lactam antibiotics in humans. Amoxicillin and other β-lactams are known to react chemically with proteins to form haptens. The limited information available suggests that amoxicillin is less chemically reactive than benzylpenicillin. The maximum tolerated concentrations of benzylpenicilloyl hapten in skin-prick tests and intradermal testing was 0.05 μmol/L compared with 51 700 μmol/L for amoxicillin, indicating that amoxicillin has significant lower reactivity than benzylpenicillin *(20)*.

At the thirty-sixth meeting, the Committee based its toxicological guidance value of 30 μg/person for benzylpenicillin on only four case studies of allergy to oral exposure to residues of penicillins. Considering that amoxicillin has a much lower allergenicity than benzylpenicillin, the Committee concluded that the mADI would be sufficient to be protective for potential allergenicity from residues of amoxicillin in food.

Microbiological data

The Committee revised the mADI from the value of 0–0.0007 mg/kg bw established at its seventy-fifth meeting to 0–0.0015 mg/kg bw based on the newly adopted colon volume of 500 mL currently used in the formula to derive the microbiological ADI.

The ARfD, also based on microbiological effects on the intestinal microbiota, was determined to be 0.0045 mg/kg bw, rounded to 0.005 mg/kg bw.

Evaluation

Considering that amoxicillin has a lower allergenicity than benzylpenicillin, the Committee established the mADI of 0–0.0015 mg/kg bw, rounded to 0–0.002 mg/kg bw, based on the effects of amoxicillin on the intestinal microbiota and using the newly adopted colon content volume of 500 mL. Because the majority of amoxicillin residue levels detected in target tissue were below the lowest MIC_{50} values of any of the representative human intestinal microbiota tested, it is unlikely that the development of resistance to amoxicillin residues would occur. The Committee also considered this ADI to be protective for potential allergenicity from residues of amoxicillin.

The Committee established an ARfD of 0.0045 mg/kg bw, rounded to 0.005 mg/kg bw, also based on microbiological effects on the intestinal microbiota. The Committee considered that this ARfD would be protective for potential allergenicity from residues of amoxicillin.

An addendum to the toxicological monograph was prepared.

Residue evaluation

Several studies with summary data were provided for the current evaluation; however, only a limited amount of raw data were available. A comprehensive literature search of public databases was conducted to complement the limited information submitted. Authors of recently published papers were contacted to provide raw data; however, only the raw data corresponding to one paper were sent.

Studies on pharmacokinetics and residue studies on amoxicillin sodium and trihydrate in the relevant species of fish were reviewed. The analytical methods used for the residue depletion studies were also assessed.

Data on pharmacokinetics and metabolism

The pharmacokinetics of amoxicillin in preruminating and ruminating cattle, lactating dairy cows, pigs and sheep, including lactating sheep, were evaluated at the seventy-fifth meeting. Metabolism studies were limited; but three metabolites, (5R,6R)-amoxicilloic acid and its epimer (5S,6R)-amoxicilloic acid and amoxicillin piperazine-2′,5′-dione, were found in pigs and in humans.

No absorption, distribution, metabolism and excretion (ADME) studies in fish or other species, using radiolabelled amoxicillin, were provided. No metabolism studies in fish were available.

Eleven studies investigating the pharmacokinetics of amoxicillin in fish were reviewed; these studies were not compliant with good laboratory practices. The studies included six different types of fish: eel, flatfish, sea bream, pompano, salmon and olive flounder. Three of these studies were conducted with amoxicillin sodium after intramuscular administration in olive flounder; the others were conducted with amoxicillin trihydrate.

The pharmacokinetics of amoxicillin trihydrate in olive flounder (*P. olivaceus*) were studied in fish (500 ± 20 g) maintained in seawater aquaria (20 ± 1.5 °C). Two groups were treated by gavage with medicated feed at a single dose of 40 or 80 mg/kg bw. Two other groups were treated intramuscularly at a dose of 30 or 60 mg/kg bw, and another group received an intravenous dose of 6 mg/kg bw. Blood was sampled at regular intervals. Amoxicillin plasma concentrations were quantified by high-performance liquid chromatography with ultraviolet detection (HPLC–UV) (limit of quantification [LOQ] of 0.1 μg/mL). The terminal half-lives were estimated to be 97, 27, 39, 259 and 285 hours after the intravenous (6 mg/kg bw), intramuscular (30 or 60 mg/kg bw) and oral (40 or 80 mg/kg bw) administrations, respectively. The fractions of amoxicillin absorbed after oral administration were 9% and 3.6% for 40 and 80 mg/kg bw, respectively. Bioavailability of amoxicillin in olive flounder treated intramuscularly were 86%

and 53% for 30 and 60 mg/kg bw, respectively.

The pharmacokinetics of amoxicillin after intramuscular administration in olive flounder (*P. olivaceus*) were studied in fish (mean body weight 140 ± 22 g) maintained in fresh water (23 ± 1 °C). Amoxicillin sodium was administered intramuscularly at two doses, 12.5 or 125 mg/kg bw. Amoxicillin concentration in plasma was quantified by HPLC–UV (LOQ of 0.04 µg/mL). Amoxicillin was rapidly absorbed and showed a dose proportionality for the AUC. Mean residence times (MRTs) were 18.8 and 14.4 hours, respectively.

The pharmacokinetics of amoxicillin after intramuscular administration in olive flounder were described in another published paper. Fish (mean body weight 821 ± 125 g) were maintained in sea water (22 ± 1 °C). Amoxicillin sodium was administered intramuscularly at two doses, 40 or 80 mg/kg bw. Amoxicillin concentration in plasma was quantified by HPLC–UV (LOQ of 0.54 µg/mL). Amoxicillin was rapidly absorbed. A dose proportionality for the AUC was observed. MRTs were 13.7 and 15.9 hours, respectively.

The pharmacokinetics of amoxicillin in eel, *Anguilla japonica*, was investigated after either single oral administration of amoxicillin trihydrate medicated feed (40 or 80 mg/kg bw) or a single intravenous injection (1 mg/kg bw). Fish (mean body weight 220 ± 10 g) were maintained at 28 ± 1.5 °C in a flow-through water tank. Amoxicillin concentration in plasma was quantified by HPLC–UV (LOQ of 0.2 µg/mL). The terminal half-life of intravenously administered amoxicillin was very long (2989 hours), and the MRT was 2527 hours. Amoxicillin was rapidly absorbed after oral administration, showing a dose proportionality for the AUC that was not observed for the C_{max}, and long terminal half-lives of 868 and 3139 hours for 40 and 80 mg/kg, respectively. After oral doses of 40 and 80 mg/kg bw, bioavailabilities were estimated at 1.6% and 1.1%, respectively.

The pharmacokinetics of amoxicillin in flatfish (species not specified) after intramuscular administration of 10, 20 or 40 mg/kg bw were described in a technical report. Plasma concentration was quantified by HPLC–UV (LOQ not reported). Proportionality of the AUC according to dose was demonstrated, and the MRTs were 12.0, 12.4 and 16.4 hours, respectively.

Amoxicillin depletion curves in serum and tissue in pompano (*Trachinotus blochii*) were compared after single or repeated oral doses (for 5 days) of amoxicillin trihydrate (40 mg/kg bw). Fish (mean body weight 160.4 ± 27.7 g) were maintained in pools with aerated sea water at 25–27 °C, 8–10 parts per million (ppm) oxygen and 3.3% salinity. Serum, muscle, liver and kidney concentrations of amoxicillin were determined by HPLC–UV (LOQ of 100 µg/kg). Peak concentrations of amoxicillin in serum, liver and kidney (7360 µg/L, 6170 µg/kg and 4270 µg/kg, respectively) were reached 0.5 hours after a single dose administration, whereas the peak concentration in muscle (1960 µg/kg)

was observed 2 hours post dose. The concentrations of amoxicillin in serum, liver, kidney and muscle declined with half-lives of 7.4, 18.3, 12 and 17.3 hours, respectively.

The pharmacokinetics of amoxicillin in sea bream after either a single intravenous dose (40 mg/kg bw) or an oral dose (80 mg/kg bw) via medicated feed were reported. Amoxicillin concentrations were determined by a bacteriological method and a radioimmunological test. A relative oral bioavailability of 0.33% was estimated.

Descriptive studies of amoxicillin serum concentration were reported for salmon after intramuscular administration and oral administration via feed or gavage. Concentrations were quantified by a bacteriological method. Concentrations were reported graphically in the papers. No pharmacokinetic parameters were reported.

Residue depletion data

No residue depletion studies of radiolabelled amoxicillin in cattle, pigs or sheep were submitted for evaluation by the Committee at its seventy-fifth meeting.
No residue depletion studies of radiolabelled amoxicillin in fish were provided to the present meeting.

Residue depletion studies with non-radiolabelled amoxicillin were performed in olive flounder, flatfish, grouper, bass, catfish, salmon, pompano, sea bream and rockfish after oral administration of amoxicillin trihydrate or intramuscular administration of amoxicillin sodium.

In all studies, amoxicillin residues in muscle were low and persisted for a few days after the final administration. In many studies, sampling frequency was inadequate to permit a detailed analysis of residue depletion in muscle. The data obtained from published papers were reported mainly as means and standard deviations or graphically. Unfortunately, requests for additional data for independent analysis resulted in only one set of additional individual animal data being sent to the Committee.

Olive flounder were treated for 7 consecutive days with amoxicillin trihydrate in medicated feed at a daily dose of 40 mg/kg bw in a first trial and at a daily dose of 80 mg/kg bw in a second trial. Water temperatures ranged from 13.4 to 15.6 °C in the first trial and from 16.8 to 21.4 °C in the second. Residues were determined by HPLC–UV (LOQ of 7 µg/kg) for 10 fish at each time point. At day 1 after the final administration, the mean concentrations were 189 ± 160 µg/kg and 89 ± 34 µg/kg in the first and second trials, respectively. The concentrations decreased more rapidly in the second trial than in the first, probably because of the higher water temperature. Amoxicillin concentrations were below the LOQ in all fish (except one) after 20 days in the first trial and after 13 days in all fish in

the second trial. Raw data were provided to the Committee by the authors.

Depletion studies in olive flounder were performed after intramuscular administration of a single dose of amoxicillin sodium at 40 or 400 mg/kg bw. Water temperature was 22 ± 1 °C. Amoxicillin residues were determined in muscle by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS; LOQ of 7.1 μg/kg). Amoxicillin concentrations in the muscle of olive flounder reached 11.3 ± 6.4 μg/kg at 7 days after the single intramuscular dose of 40 mg/kg bw. Mean amoxicillin concentrations reached 14.2 ± 3.5 μg/kg at 12 days after the single intramuscular dose of 400 mg/kg bw. Raw data from these studies were not provided.

A depletion curve was established for olive flounder after intramuscular administration of amoxicillin at 37.5 mg/kg bw per day for 7 days. Water temperature was 23 ± 1 °C. Concentrations were determined in fillet (muscle plus skin in natural proportion) by HPLC–UV (LOQ of 40 μg/kg). Mean tissue concentrations 1, 2 and 7 days after the final drug administration were 17 100, 4700 and 300 μg/kg. At 14 days, most concentrations were below the LOQ.

Amoxicillin depletion curves for muscle, liver and kidney were reported in olive flounder (*P. olivaceus*), rockfish (*S. schlegeli*) and red sea bream (*Pagrus major*) with mean weights of 360 ± 40 g, 470 ± 55 g and 450 ± 45 g, respectively. Water temperature was maintained at 25 °C. Amoxicillin trihydrate was orally administered via medicated feed at 400 mg/kg bw per day for 7 days. Amoxicillin concentration in muscle was determined by high-performance liquid chromatography with fluorescence detection (HPLC–FL; limit of detection [LOD] of 10 μg/kg) in five fish at each time point. Mean amoxicillin concentrations at day 1 post dose were 137 ± 39, 131 ± 43 and 172 ± 53 μg/kg in olive flounder, rockfish and red sea bream, respectively. Mean amoxicillin concentrations at day 3 post dose were 12 ± 8, 10 ± 9 and 17 ± 6 μg/kg, respectively, and were undetected at day 4 post dose.

Amoxicillin depletion curves for muscle were reported in olive flounder (*P. olivaceus*), rockfish (*S. schlegeli*) and red sea bream (*P. major*) with mean body weights of 350 ± 30 g, 450 ± 40 g and 420 ± 35 g, respectively. Water temperature was maintained at 25 °C. Amoxicillin trihydrate was administered orally at 400 mg/kg bw per day for 7 days. Amoxicillin concentration in muscle was determined using a solid-phase fluorescence immunoassay–based test (designed for analysis of milk; LOD less than 10 μg/kg) in five fish at each time point. Mean amoxicillin concentrations at day 1 post dose were 84.3 ± 2.1, 84.1 ± 2.0 and 96.2 ± 2.3 μg/kg in olive flounder, rockfish and red sea bream, respectively. Mean amoxicillin concentrations at day 3 post dose were 12.6 ± 0.5, 10.0 ± 0.4 and 13.6 ± 0.5 μg/kg, respectively, and were undetected at day 4 post dose.

Amoxicillin concentrations in flatfish (species not specified) after intramuscular administration of amoxicillin sodium at doses of 40 mg/kg bw or

400 mg/kg bw were determined. Water temperature was 22 ± 3 °C. Amoxicillin concentrations in muscle were determined using an LC–MS/MS method (LOQ not reported; LOD of 0.005 μg/kg). Two days after the administration of the 40 mg/kg bw dose, the mean amoxicillin concentration in muscle was 23.3 ± 14.1 μg/kg. Only one of the 10 fish sampled at 5 days had a detectable amoxicillin residue (0.21 μg/kg). Five days after administration of the 400 mg/kg dose, mean amoxicillin concentration in muscle of three of the 10 fish sampled exceeded 50 μg/kg; at 7 days, none of the samples exceeded that residue concentration.

The mean concentrations of amoxicillin in muscle of pompano (*T. blochii*) were graphically reported after oral treatment with a single dose of amoxicillin trihydrate at 40 mg/kg bw or repeated doses of 40 mg/kg bw per day for 5 days. Amoxicillin concentrations in muscle, liver and kidney were determined by HPLC–UV (LOD of 40 μg/kg). Amoxicillin residue concentrations were undetectable in muscle at day 1 post dose and in liver and kidney at day 3 post dose.

Amoxicillin depletion curves in muscle were reported graphically in largemouth black bass (*Micropterus salmoides*) after oral administration of amoxicillin trihydrate at 20 or 40 mg/kg bw per day for 5 days. Fish (230 ± 10 g) were maintained in fresh water (18 ± 0.8 °C). Amoxicillin concentrations in muscle were determined by HPLC–UV (LOD of 40 μg/kg; LOQ of 100 μg/kg) in six fish at each time point. Amoxicillin concentrations were below the LOQ at day 3 post dose.

Amoxicillin depletion curves for muscle were reported in grouper (*Epinephelus malabaricus*) after oral administration of amoxicillin trihydrate at 20 or 40 mg/kg bw per day for 5 days. Fish (285 ± 15 g) were maintained in salt water at 18 ± 0.8 °C. Amoxicillin concentrations in muscle were determined by HPLC–UV (LOD of 40 μg/kg; LOQ of 100 μg/kg) in five fish at each time point. Amoxicillin concentrations were reported graphically. Mean concentrations were 220 μg/kg at 24 hours after treatment with 20 mg/kg bw per day and 150 μg/kg at 48 hours after treatment with 40 mg/kg bw per day. The mean concentrations were below the LOQ at 48 hours post the final 20 mg/kg bw dose and undetectable at 72 hours post the final 40 mg/kg bw dose.

The depletion curve of amoxicillin in catfish fillets was determined after a single oral gavage dose of 110 mg/kg bw of amoxicillin trihydrate. Water temperature was 27.5 ± 1.4 °C. Amoxicillin concentrations were determined by a validated LC–MS/MS method (LOQ of 1.2 μg/kg). At 72 hours after treatment, amoxicillin concentrations were below the LOQ.

Concentrations of amoxicillin in Atlantic salmon muscle were determined 1 hour and 12 days after the last of four daily oral doses of 80 mg/kg bw amoxicillin trihydrate. Water temperature was 18 °C. Residues were quantified by a radioimmunological test (LOD of 5 μg/kg). No residues were detected 12

days after the final administration.

Residue depletion of amoxicillin was determined in sea bream (*Sparus aurata* L.) after oral administration of amoxicillin at 80 mg/kg bw per day for 10 consecutive days via medicated feed. Fish (body weight range 50–90 g) were maintained in salt water at 22–26 °C. A radioimmunological test (LOD of 5 μg/kg) was used to quantify amoxicillin in muscle, liver and skin. A poor distribution of amoxicillin, with a sharp decrease in residue concentrations after the final treatment, was observed.

Similarly, amoxicillin was undetectable in yellowtail muscle samples the first day after the final oral administration of a daily dose of 80 mg/kg bw per day for 10 days.

No information on the position of sampling sites in relation to injection sites was provided in the residue depletion studies. However, considering fish anatomy, the high solubility of amoxicillin sodium and the rapid absorption rate, the Committee considered it likely that concentrations at injection sites were similar to those in other muscle tissues.

Analytical methods

Qualitative and quantitative single or multi-residue methods are available to determine residues of amoxicillin, the main microbiologically active residue identified in muscle and fillet (muscle plus skin in natural proportion). Validated analytical methods, HPLC/UV, HPLC–FL and LC–MS/MS were reviewed.

Multi-residue LC–MS/MS methods for the screening, quantification and confirmation of amoxicillin residue in muscle of different animal species including fish were reported. These methods are appropriate for the monitoring of amoxicillin residues in fish muscle and fillet.

Maximum residue limits

In order to address the ambiguity of the terms "flatfish" and "finfish", the Committee decided that, for the purpose of this report, the term "finfish" would include all fish species. In recommending MRLs for amoxicillin in finfish, the Committee considered the following factors:

- An ADI of 0–0.002 mg/kg bw was established by the Committee based on a microbiological end-point.
- An ARfD of 0.005 mg/kg bw was established based on a microbiological end-point.
- The less-than-lifetime exposure scenarios will be covered by the acute exposure assessment.
- Amoxicillin is authorized for use in at least two fish species in one Member State. The withdrawal periods of approved use are not

known to the Committee.

- Amoxicillin is also used in other fish species in other Member States.
- Data on metabolism of amoxicillin in fish were not provided.
- Amoxicillin is not widely distributed in fish tissues, is not lipophilic and does not accumulate in fat.
- Pharmacokinetics and residue depletion curves were similar for different freshwater and saltwater representative fish species belonging to different orders (Salmoniformes, Perciformes, Siluriformes, Anguilliformes and Pleuronectiformes).
- Amoxicillin is the only microbiologically active residue and is suitable as a marker residue.
- Suitable validated routine analytical methods were available for monitoring purposes and can be applied to different fish species.
- The Codex MRL of 50 µg/kg established for cattle, sheep and pig muscle is suitable for the control of amoxicillin residue in muscle and fillet of finfish species.

The Committee recommended MRLs of 50 µg/kg for amoxicillin in finfish muscle and muscle plus skin in natural proportion.

Dietary exposure

Dietary exposure to amoxicillin is considered to occur only through its use as a veterinary drug. There is no registered use for amoxicillin as a pesticide. At its seventy-fifth meeting, the Committee considered the use of amoxicillin in cattle, sheep and pigs. The Committee did not calculate an EDI for amoxicillin at that time, owing to the small number of quantifiable residue data points. In the current dietary exposure assessment, fish was the sole contributor to dietary exposure, as other sources were considered to be negligible contributors based on currently available information.

Based on the hazard profile of the compound, acute dietary exposure is required for the general population and children. The less-than-lifetime exposure scenarios will be covered by the acute exposure assessment.

Dietary exposure was estimated based on the potential occurrence of amoxicillin residues in a single food (finfish muscle plus skin in natural proportion) after 7 days of treatment and 15 days withdrawal time (217 degree-days). Consumption inputs were based on the highest reported values available within the fish category (i.e. marine fish consumptions for means, freshwater fish consumption for the 97.5th percentile).

Based on a median residue concentration of 10 µg/kg at a withdrawal time of 15 days, the GECDE for the general population is 0.14 µg/kg bw per day,

which represents 7% of the upper bound of the mADI of 0–0.002 mg/kg bw.

In addition to the accepted GECDE methodology, further calculations 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 mean and ranges across surveys were reported. The mean of 25 estimates for adults or the general population was 0.024 µg/kg bw per day (1% of the upper bound of the ADI), with a range of 0.0006–0.14 (0.03–7% of the upper bound of the ADI).

Based on the upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentration (95/95 upper tolerance limit, or UTL) of 50 µg/kg at a withdrawal period of 15 days, the global estimate of acute dietary exposure (GEADE) for the general population is 1.4 µg/kg bw, which represents 28% of the microbiological ARfD of 0.005 mg/kg bw. The GEADE for children is 1.6 µg/kg bw, which represents 31% of the microbiological ARfD.

An addendum to the residue monograph was prepared.

Summary and conclusions
ADI
0–0.002 mg/kg bw, based on microbiological effects and using the newly adopted colon volume of 500 mL

ARfD
0.005 mg/kg, based on microbiological effects and using the newly adopted colon volume of 500 mL

Residue definition
Amoxicillin is the only microbiologically active residue and is suitable as a marker residue.

MRLs
50 µg/kg for finfish muscle and muscle plus skin in natural proportion.

Estimated chronic dietary exposure
The GECDE for the general population is 0.14 µg/kg bw per day, which represents 7% of the upper bound of the mADI of 0.002 mg/kg bw.

Estimated acute dietary exposure
The GEADE for the general population is 1.4 µg/kg bw, which represents 28% of

the microbiological ARfD of 0.005 mg/kg bw.

The GEADE for children is 1.6 µg/kg bw, which represents 31% of the microbiological ARfD.

3.2 Ampicillin

Explanation

Ampicillin (CAS No. 69-53-4) or α-aminobenzylpenicillin, is a semi-synthetic β-lactam antimicrobial agent with an extended antibacterial spectrum, used to treat a number of bacterial infections in humans and animals caused by Gram-positive and some Gram-negative bacteria. Ampicillin has a bactericidal effect and acts as an irreversible inhibitor of transpeptidase, an enzyme important for building the bacterial cell wall. Ampicillin may have a synergistic action with aminoglycosides and with β-lactamase inhibitors.

Ampicillin is used in several animal species, for example, cattle, sheep, pigs, fish, dogs and cats for treatment of diseases caused by different species of bacteria, e.g. *Streptococcus* spp., *Bordetella bronchiseptica, Pasteurella multocida, Trueperella pyogenes* (syn. *Arcanobacterium pyogenes, Corynebacterium pyogenes* and *Actinomyces pyogenes*), *Mannheimia haemolytica*, *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus* and *Staphylococcus* spp. It is ineffective against β-lactamase-producing organisms.

In aquaculture, ampicillin is used for the prevention and treatment of pseudotuberculosis, vibriosis, streptococcosis and edwardsiellosis. Ampicillin trihydrate is given as an oral powder in fish at a dose of 5–20 mg/kg bw once or twice a day for 5 days. Ampicillin sodium is administered via intramuscular injection at a single dose of 20 mg/kg bw.

In humans, ampicillin sodium or ampicillin trihydrate is mostly administered parenterally or, less often, orally at doses of 1000–2000 mg or approximately 17–33 mg/kg bw per day in adults (assumed weight 60 kg) to treat a number of diseases such as respiratory tract infections, urinary tract infections, meningitis, salmonellosis and endocarditis. Ampicillin has lower bioavailability than amoxicillin in humans.

Ampicillin is categorized as a highly important antimicrobial agent in the WHO CIA list *(12)*.

Ampicillin has not previously been evaluated by the Committee. The Committee evaluated ampicillin at the present meeting at the request of the Twenty-third Session of CCRVDF, with a view to establishing a suitable health-based guidance value (HBGV) and recommending MRLs in finfish muscle plus skin in natural proportion *(2)*. Other β-lactam antimicrobial agents, such as amoxicillin, have previously been evaluated by JECFA.

Data on ampicillin in flat fish were submitted to JECFA. The dataset did

not include biochemical, toxicological or microbiological data on ampicillin. As a result, the evaluation was based on information from relevant studies identified by a literature search, most of which did not have good laboratory practice (GLP) certification.

For the biochemical, toxicological and microbiological part of the evaluation, the Committee conducted a comprehensive review of peer-reviewed scientific literature retrieved from the following publicly accessible databases: Google Scholar, PubMed, Web of Science, BioOne and ScienceDirect. The search strategy for literature relevant for the biochemical and toxicological evaluation used the keywords "Ampicillin" together with "pharmacokinetics", "kinetics", "metabolism", "excretion", "bioavailability", "toxicity", "mutagenicity", "carcinogenicity", "reproduction", "fetus", "teratogenicity", "rat", "mice", "dog", "animals", "humans", "adverse effects", "hypersensitivity", "nephrotoxicity", "kidney", "liver", "allergy", "fetus", "gastrointestinal" and the Boolean operators ("AND", "OR" and "NOT"). Some search engines did not give any relevant information. Most articles were found in PubMed. For the search ("Ampicillin/toxicity"[Mesh]) OR ("Ampicillin/pharmacokinetics"[Mesh]) NOT (Amoxicillin OR "Amoxicillin-Potassium Clavulanate combination" OR Azlocillin OR Mezlocillin OR Piperacillin OR Pivampicillin OR Talampicillin) AND (rat OR rats OR mice OR animal or animals) NOT (human OR humans OR patient or resistance), 133 articles were retrieved. For the search "Ampicillin" and "adverse effects" and "gastrointestinal", 144 articles were retrieved, but this was reduced to 51 when "adverse effects" were added. For "Ampicillin" and "allergy" and "food", 19 articles were retrieved; for "ampicillin" and "fetus/drug effects", 20 articles were retrieved; for "ampicillin hypersensitivity" and "food", 17 articles were retrieved. Many articles were to do not with ampicillin but with new compounds that were derivates of ampicillin, and many articles were case–control studies that were not relevant for this evaluation.

The literature search identified 38 articles relevant for the biochemical and toxicological evaluation.

The search strategy for literature relevant for the microbiological evaluation used the keywords "ampicillin", "microbiome", "intestinal microbiota", "gut microbiota", "gut microbiome", "gastrointestinal microbiota", "gastrointestinal microbiome", "antimicrobial resistance", "susceptibility testing", "ampicillin metabolism", "excretion" and "bioavailability" as well as the genus/species and MIC values of specific intestinal bacteria with the Boolean operators (AND, OR and NOT).

The literature search identified 36 articles relevant for the microbiological evaluation of ampicillin.

For the residue evaluation, the sponsor submitted a dataset that included information on the two ampicillin formulations; a list of trade names and

Table 2

Inclusion and exclusion criteria for the comprehensive literature search on ampicillin

Inclusion criteria	Exclusion criteria
Any article focusing on: • ampicillin concentrations in plasma of fish • ampicillin concentrations in edible tissues of fish • residue determination in fish plasma / tissue • bioavailability of ampicillin residues in fish No restrictions concerning year of publication	Any article focusing on: • bacteria resistance to ampicillin • ampicillin use in food animal species other than fish • kinetics/residues of antimicrobials other than ampicillin (that do not include ampicillin for comparison) • environmental issues Any article dealing with ornamental fish

approved uses in fish; monitoring data on occurrence of ampicillin in aquaculture products; and studies on the pharmacology and residue depletion of ampicillin in flatfish, with English translations of the original studies. In addition, the sponsor provided raw data for one residue study and one pharmacokinetic study on request. Information on established withdrawal periods for authorized products was missing; the authors of the studies proposed possible withdrawal periods.

In addition, as part of the residue assessment the Committee conducted a comprehensive review of peer-reviewed scientific literature retrieved from the following publicly accessible databases: Agricola, Embase, Web of Science, PubMed, Springer Protocols, Food Science and Technology Abstracts, Phish-Pharm, CABI VetMed Resource. The following searches were conducted in each of these databases:

- Ampicillin AND fish AND residue
- Ampicillin AND fish AND kinetics
- Ampicillin AND fish AND withdrawal
- Ampicillin AND fish AND metabolism
- Ampicillin AND fish AND analytical method
- Ampicillin AND (salmon OR trout) AND (residue OR kinetics OR withdrawal OR metabolism OR analytical method)

The literature search retrieved 36 potentially relevant articles. A thorough review of the articles based on the criteria shown in Table 2 led to the exclusion of 20 articles. Sixteen articles were considered relevant and were used in the monograph.

None of the studies used in the evaluation indicated that they were GLP compliant. For one residue study retrieved via the literature search, only the abstract and tables were available in English; however, as only three other residue studies were available, the Committee decided to also include these data from the

abstract and tables in their evaluation.

Some Member States have established MRLs for ampicillin of 50 µg/kg for edible tissues of all food-producing species. One Member State has established MRLs for edible tissues of Salmoniformes (50 µg/kg), Anguilliformes (50 µg/kg), Perciformes (60 µg/kg) and other fish (50 µg/kg), whereas another has established an MRL of 50 µg/kg for fish tissues.

Toxicological and microbiological evaluation

Biochemical data

After oral administration, ampicillin was rapidly absorbed in mice, rats, dogs and humans, with a half-life in plasma of approximately 1 hour. The time to reach the maximum concentration (T_{max}) was between 1 and 2.4 hours, with C_{max} between 2.2 and 6.8 µg/mL at doses between 250 and 1000 mg per person. Bioavailability of ampicillin in rats was 23% and in humans was 46%. Plasma protein binding of ampicillin was only 17% in humans and cattle and 8% in horses. After oral treatment in humans and rats, ampicillin is metabolized to ampicillin piperazine-2′,5′-dione, (5R,6R)-ampicilloic acid and its epimer (5S,6R)-ampicilloic acid.

There was a large variation in excretion of ampicillin in urine in humans; following oral treatment, excretion ranged between 21.8% and 42.8% in non-fasting subjects. In the one study conducted with ^{35}S-labelled ampicillin, radioactivity in faeces was 39 ± 19% at 7 days after a single dose of 500 mg per person. Different foods ingested with ampicillin appear to affect excretion of ampicillin in urine.

Toxicological data

Acute oral toxicity was tested in rats and mice. The oral median lethal dose (LD_{50}) was more than 5000 mg/kg bw in adult mice and rats. The oral LD_{50} was 3300 mg/kg bw for 1-day-old rats and 4500 mg/kg bw for 83-day-old rats.

In rabbits treated with ampicillin at doses of 5, 15 and 50 mg/kg bw for 3 days, there was more than 50% lethality at the lowest dose. In rabbits, many antibiotics cause gastrointestinal problems, such as enteritis and disruption of the intestinal microbiota, that can cause death.

Mice were orally administered ampicillin trihydrate in corn oil at doses of 0, 200, 400, 800, 1600 or 2400 mg/kg bw per day for 14 days. The NOAEL was 1600 mg/kg bw based on weight loss in males and diarrhoea at 2400 mg/kg bw per day.

Mice were administered ampicillin trihydrate in corn oil, by gavage, for 5 days a week for 13 weeks, at doses of 0, 250, 500, 1000, 2000 or 3000 mg/kg bw per day (representing 0, 179, 357, 714, 1430 and 2140 mg/kg bw per day for 7 days a week). No adverse effects were reported. The NOAEL was 2143 mg/kg bw, the

highest dose tested.

Rats were administered ampicillin trihydrate in corn oil, by gavage, at doses of 0, 180, 370, 750, 1500 or 2 times 1500 mg/kg bw per day for 5 days a week (representing 0, 129, 264, 536, 1070 or 2140 mg/kg bw per day for 7 days a week) for 13 weeks. The NOAEL was 1070 mg/kg bw per day based on diarrhoea in the highest dose group.

Transgenic mice (Tg-rasH2) were orally treated, by gavage, with ampicillin trihydrate at 0, 350, 1000 or 3000 mg/kg bw per day for 26 weeks, while non-Tg mice were similarly treated with 3000 mg/kg bw per day. Tg-rasH2 mice treated with *N*-methyl-*N*-nitrosourea (MNU) as a single intraperitoneal dose of 75 mg/kg bw acted as positive control. Ampicillin trihydrate treatment did not affect mortality in either the Tg-rasH2 or non-Tg mice. Body weight was reduced in ampicillin trihydrate–treated male and female Tg-rasH2 mice from week 4 to 26 for the two lowest dose groups and week 5 to 26 for the highest dose group. Dilation of caecum was observed in ampicillin trihydrate–treated non-Tg mice and dose dependently in Tg-rasH2 mice. Several different tumours were found in the positive control group, demonstrating the carcinogenicity of MNU in Tg-rasH2 mice. Ampicillin trihydrate was not carcinogenic in Tg-rasH2 mice and non-Tg mice treated for 26 weeks. However, as decreased body weights and dilation of caecum were found in all treated groups, no NOAEL could be identified.

Mice were administered ampicillin trihydrate in corn oil, via gavage, over 103 weeks at doses of 0, 1500 or 3000 mg/kg bw per day, 5 days a week (representing 0, 1070 and 2140 mg/kg bw per day for 7 days a week). Increased salivation and decreased activity were considered treatment related. Hyperplasia (granulocytic) of the bone marrow was observed in male mice (incidences of 16%, 28% and 28%, respectively), and hyperplasia in the mandibular lymph node in female mice (incidences of 3%, 19% and 24%, respectively). There was no treatment-related increase in neoplasms. In view of the hyperplasia in the treated groups, a NOAEL could not be identified.

Rats (F344/N) were treated, by gavage, with ampicillin trihydrate in corn oil for 103 weeks at doses of 0, 750 or 1500 mg/kg bw per day for 5 days a week (representing 0, 536 or 1070 mg/kg bw per day for 7 days a week). Clinical effects related to ampicillin trihydrate were diarrhoea, chromodacryorrhoea and excessive urination. Mononuclear cell leukaemia was observed in control and treated rats; however, this was not dose-dependent. Mononuclear cell leukaemia was subsequently found to be a common spontaneous occurrence in aged rats in this strain. Focal cellular change of the adrenal cortex was increased in the treated rats (males: 1/50, 5/50 and 7/49, respectively; females: 6/50, 0/50 and 12/50, respectively) but within historical control ranges (15/49). Non-malignant pheochromocytomas of the adrenal medulla were increased in male rats at the highest dose (13/50, 12/50 and 23/49, respectively; historical control data was

247/1092 [22.3%]). Hyperplasia in bone marrow was observed in male and female rats (12% [controls], 33% and 34% for males; 26% [controls], 45% and 50% for females, respectively). Hyperkeratosis was increased in males (6%, 14% and 20%, respectively) but not females; acanthosis in the forestomach was also increased in males (0, 4.5% and 11%, respectively) but not in females. In addition, inflammation in the prostate increased in high-dose male rats. No haematological, clinical chemistry and urine analyses findings were reported. A NOAEL could not be identified because effects were seen at all doses.

Ampicillin was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Committee concluded that ampicillin is unlikely to be genotoxic.

No reproduction toxicity studies in laboratory animals have been found. In a developmental toxicity study, rats were treated orally with 0 and approximately 250 mg/kg bw per day of ampicillin trihydrate during gestation days 1–5, 6–11 or 12–17. Fetal body weights were decreased as a result of treatment on gestation days 6–11 and 12–17 and fetal cranio-caudal sizes were reduced as a result of treatment on gestation days 12–17, measured at day 20 of embryonic development. No teratogenicity was observed. Effects were observed at the only dose tested.

No firm conclusion on reproductive or developmental toxicity could be reached from studies in experimental animals.

Observations in humans

A population-based case–control study investigated if ampicillin given during the second and third months of pregnancy was teratogenic in humans. Data were from the Hungarian Congenital Abnormality Register (HCCSCA), covering a study population from the second trimester to the postnatal first year, 1980–1996. The oral dose of ampicillin was 250–500 mg four times daily for a mean duration of 5 days (range: 1–21 days) once or several times during pregnancy. Of the 22 865 women with progeny with congenital abnormalities, only 1643 (7.2%) were treated with ampicillin. Cleft palate was the only abnormality with a significantly elevated odds ratio (3.0; 95% confidence interval: 1.2–7.6) in the case–control pair analysis. Cleft palate was found after ampicillin use in the second and third months of pregnancy, the sensitive period for skeletal development, and the use of ampicillin was higher during pregnancy. The study authors noted that the observation of cleft palate was not consistent with reports from other studies. The Committee considered that no firm conclusion could be drawn from the study.

No evidence of cardiovascular effects was found in several case–control studies when ampicillin was given during pregnancy. In another case–control study, ampicillin was not shown to increase neural tube defects when mothers were treated for illnesses during the periconceptional period.

The most common adverse effects in humans after therapeutic treatment

(recommended doses 17–33 mg/kg bw per day) are diarrhoea and exanthema. In children treated orally with ampicillin at daily doses of 50–200 mg/kg bw for at least 7 days, mild to moderate diarrhoea and mild vomiting were common. Rare cases of acute interstitial nephritis after administration of ampicillin have been reported.

No data on hypersensitivity reactions caused by ampicillin residues in food were retrieved in the literature search. It is clear that there is cross reactivity with other β-lactam antibiotics. That ampicillin appears to have a lower allergenic potential than benzylpenicillin was shown in a study identifying maximum concentrations accepted in skin-prick tests and intradermal testing of immediate allergic reactions. The concentrations for ampicillin (54 000 μmol/L) and benzylpenicilloyl (0.05 μmol/L), indicating that ampicillin has a significant lower reactivity than benzylpenicilloyl *(20)*.

Microbiological data

A decision-tree approach adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) that complies with VICH GL36(R) *(18)* was used by the Committee to determine the need for and to establish, if necessary, an mADI for ampicillin.

Ampicillin residues may be present at low levels in foods consumed by humans; therefore, ampicillin-related residues could enter the colon of a person ingesting edible tissues of treated food-producing animals. Ampicillin is rapidly absorbed after oral administration in humans and animals, and a considerable amount of the administered ampicillin was detected as unmetabolized parent compound in urine. Therefore, ampicillin residues entering the human colon will remain microbiologically active.

There is potential for adverse effects in the human intestinal microbiota with disruption of the colonization barrier since MIC values for the most relevant and predominant bacteria in the gastrointestinal tract indicate that they are susceptible to ampicillin. The Committee also found published reports on the increase in the population of ampicillin-resistant bacteria in the intestinal tract after exposure to residual to therapeutic concentrations that were used to determine no-observed-adverse-effect concentrations (NOAECs). Therefore, both microbiological end-points were evaluated to derive the mADI.

Studies of microbiological activity of ampicillin against bacterial strains representative of the human colonic microbiota were evaluated. Ampicillin was active against *E. coli* (MIC_{50} = 2 μg/mL), *Enterococcus* (MIC_{50} = 1 μg/mL), *Bifidobacterium* (MIC_{50} = 0.12 μg/mL), *Clostridium* (MIC_{50} = 0.5 μg/mL), *Bacteroides* (MIC_{50} = 16 μg/mL), *Lactobacillus* (MIC_{50} = 0.5 μg/mL), *Fusobacterium* (MIC_{50} = 2 μg/mL), *Eubacterium* (MIC_{50} = 0.12 μg/mL) and *Peptostreptococcus* (MIC_{50} = 0.5 μg/mL).

$$ADI = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{body weight}}$$

The formula for deriving the mADI from MIC data is as follows:

where:

- MIC_{calc} for colonization barrier disruption represents the lower 90% confidence limit for the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera.

  The mADI was derived from in vitro MIC data, in accordance with Appendix C of VICH GL36 *(18, 19)*. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a MIC_{50} of *E. coli*, *Enterococcus, Bifidobacterium, Clostridium*, *Bacteroides*, *Lactobacillus*, *Fusobacterium*, *Eubacterium* and *Peptostreptococcus* (for individual MIC_{50} values, see above), the MIC_{calc} was calculated to be 0.387 μg/mL.

- Mass of colon content: The 500 mL value is based on the colon volume measured in humans.

- Fraction of oral dose available to the microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine.

  Ampicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. The lowest ampicillin urinary recovery data from the human studies was 21%. Therefore, the fraction of oral dose available would be 1 – 0.21 = 0.79.

- The body weight of an adult human is assumed to be 60 kg.

The upper bound of the mADI for colonization barrier end-point is calculated

$$ADI = \frac{0.387 \text{ μg/mL} \times 500 \text{ mL}}{0.79 \times 60 \text{ kg}} = 4.08 \text{ μg/kg bw}$$

as follows:

Therefore, an mADI of 0–0.004 mg/kg bw was derived from in vitro MIC susceptibility testing data.

The mADI for increase in the population(s) of resistant bacteria was derived as follows.

Several studies on the effects of orally administered ampicillin on the intestinal microbiota of humans and experimental animals were available. Compared with control subjects, ampicillin administered at 1.5 mg doses to six healthy volunteers for 21 days showed no change in total *E. coli* isolates in faecal samples analysed by bacterial plate counts. The population of *E. coli* resistant to ampicillin showed individual variability with very little or no change. Daily administration at 15 mg of ampicillin showed a significant increase in the population of resistant *E. coli* isolates. Therefore, the no effect level in humans would be 1.5 mg/kg bw. Because the composition of the intestinal microbiota varies within and between individuals, the Committee applied a factor of 10 to account for this variability, and derived an mADI of 0–0.0025 mg/kg bw.

The overall mADI for ampicillin was determined to be 0–0.0025 mg/kg bw based on studies on the increase in population of resistant bacteria in humans because the mADI value for this microbiological end-point is lower than that for microbiological effects on the disruption of the colonization barrier.

$$\frac{0.387 \text{ ug/mL} \times 3 \times 500 \text{ mL}}{0.79 \times 60 \text{ kg}} = 12.24 \text{ μg/kg bw}$$

An ARfD was calculated as follows:

Therefore, an ARfD of 0.012 mg/kg bw was derived on the basis of the disruption of the colonization barrier based on in vitro MIC susceptibility testing data.

Evaluation

Due to the absence of suitable data on ampicillin, it was not possible to establish a toxicological ADI. JECFA concluded that the most critical effect of penicillins used therapeutically is hypersensitivity (Annex 1, reference *208*). The limited information available suggests that ampicillin is less chemically reactive than benzylpenicillin. The maximum tolerated concentrations of benzylpenicilloyl in skin-prick tests and intradermal testing was 0.05 μmol/L compared with 54 000 μmol/L for ampicillin, indicating that ampicillin has significantly lower allergenicity than benzylpenicillin *(20)*.

Hypersensitivity, even if rare, is the most relevant toxicological effect of ampicillin and other β-lactam antibiotics in humans. However, no data on hypersensitivity reactions caused by ampicillin residues in food were retrieved in the literature search. The ampicilloyl metabolite of ampicillin was found to be the

main antigenic determinant. Well-documented cases showed hypersensitivity reactions could be caused by ingestion of less 40 μg of benzylpenicillin. The Committee, at its fiftieth meeting, recommended that the daily intake of benzylpenicillin from food be kept at less than 30 μg (Annex 1, references *134*).

The microbiological end-points of disruption of colonization barrier and resistance were compared to derive the mADI.

For the effects on the colonization barrier, an mADI of 0–0.004 mg/kg bw was derived from in vitro MIC susceptibility testing data.

Based on the increase in the population(s) of ampicillin-resistant bacteria in the intestinal tract after exposure to a range of dose levels in humans, a NOAEL of 1.5 mg per person (equivalent to 0.025 mg/kg bw) was identified. The Committee established an mADI of 0–0.0025 mg/kg bw for ampicillin, rounded to 0–0.003 mg/kg bw, on the basis of this NOAEL of 0.025 mg/kg bw and using a safety factor of 10.

The Committee established an overall mADI of 0–0.003 mg/kg bw based on the increase in population of ampicillin-resistant bacteria in humans, and using a safety factor of 10, as this microbiological end-point is lower than the microbiological ADI for effects on colonization barrier disruption.

In view of the large difference in sensitivity between benzyl penicillin and ampicillin, the Committee considered this ADI to be protective for potential allergenicity from residues of ampicillin.

The Committee established an ARfD of 0.012 mg/kg bw. The Committee considered that this ARfD would be protective for potential allergenicity from residues of ampicillin.

Residue evaluation

The present evaluation was performed based on studies provided by the sponsor and published papers retrieved via the literature search.

The Committee reviewed studies on the pharmacokinetics and metabolism of ampicillin and residue studies on ampicillin in the relevant species of finfish.

Data on pharmacokinetics and metabolism

In a study in olive flounder (*P. olivaceus*, mean body weight 300 g), ampicillin was administered via three routes: oral, intramuscular and water-bath treatment. There were nine treatment groups of 25 fish each. Fish were managed in a flow-through water tank at a temperature of 20 ± 1 °C.

Three groups of fish were treated at 10, 20 or 40 mg/kg bw via medicated feed (single treatment), with one pellet inserted into the mouth of each unanaesthetized fish. Fish in three other groups received a single injection of 5, 10 or 20 mg/kg bw into the muscle near the dorsal fin. For the water-bath

treatment, fish were placed for 1 hour into three different water tanks prepared with ampicillin doses of 10, 20 or 40 mg/kg bw. Blood samples were taken at 13 time points between 1 and 360 hours after dosing. Concentrations of residues in plasma were determined by HPLC–UV (LOD of 50 μg/kg; no LOQ was given).

The orally treated groups all reached C_{max} at 10 hours after dosing: 3.62 ± 0.97 μg/mL at 10 mg/kg bw, 5.20 ± 0.70 μg/mL at 20 mg/kg bw and 11.18 ± 0.87 μg/mL at 40 mg/kg bw. Ampicillin was eliminated more rapidly at lower doses and could not be detected in the blood at 144, 360 and 360 hours, respectively. The intramuscularly treated groups reached C_{max} at 5 hours after administration: 6.92 ± 1.29 μg/mL at 5 mg/kg bw, 9.89 ± 2.22 μg/mL at 10 mg/kg bw and 19.85 ± 2.97 μg/mL at 20 mg/kg bw. Ampicillin could not be detected in the blood at 216, 264 and 264 hours, respectively. For the water-bath treatment, all groups reached C_{max} at 3 hours after administration: 4.39 ± 1.10 μg/mL at 10 mg/kg bw, 9.57 ± 1.51 μg/mL at 20 mg/kg bw and 11.61 ± 1.95 μg/mL at 40 mg/kg bw. Ampicillin could not be detected in the blood at 264, 264 and 360 hours, respectively.

In a study in flatfish (species not specified; mean body weight 150 g) conducted at a water temperature of 22 ± 3 °C, ampicillin concentrations in blood after a single intramuscular injection were measured by HPLC–UV (no information on LOQ available). Elimination half-lives were 33.4, 67.8, 44.0 and 41.5 hours after treatment at doses of 5, 10, 20 and 40 mg/kg bw (5 samples per dosage group), respectively. T_{max} increased with dose from 0.25 to 1.00 hours.

No additional data on ADME of ampicillin in fish were available.

Residue depletion data

No residue depletion studies using radiolabelled ampicillin in fish were available for evaluation.

The residue depletion studies using unlabelled ampicillin were not reported to be GLP compliant. Three studies provided by the sponsor and one additional study identified via the literature search were included in the assessment. In addition, the sponsor provided monitoring data for several fish species.

Two of the studies investigated residues after oral administration of ampicillin.

The depletion of ampicillin from muscle tissue of olive flounder (*P. olivaceus*) was studied in fish treated with medicated feed at a dose of 20 mg/kg bw per day for 7 consecutive days at a water temperature range of 13.4–15.6 °C or 40 mg/kg bw per day for 7 consecutive days at a water temperature range of 16.8–21.4 °C. An HPLC–UV method was used to analyse the samples (LOQ of 5 μg/kg), and validation data were available. At the Committee's request, the sponsor provided data that were used to calculate summary statistics. Samples

were taken on days 1, 3, 7, 9 and 13 post treatment. At the lower dose of 20 mg/kg bw per day, ampicillin residue concentrations were highest at day 1 after final treatment, at a mean of 41 µg/kg in muscle. On day 3, the mean concentration was 23 µg/kg, and by day 13, this was below the LOQ. At the higher dose of 40 mg/kg bw, the residual amount of ampicillin was 30 µg/kg at day 1 of withdrawal. At day 3, the mean concentration was reduced to 19 µg/kg, and at day 9, this was below the LOQ.

In another study in olive flounder (*P. olivaceus*), rockfish (*S. schlegeli*) and red sea bream (*P. major*), residue depletion was investigated after 5 days of treatment with medicated feed at a dose of 100 mg/kg bw per day. Only the abstract and the tables were available in English. For analysis, an HPLC method was used (detection method unknown; no LOQ, but recovery values of 94–98% and 83–88% for the concentrations of 50 µg/kg and 100 µg/kg, respectively, were reported). Ampicillin concentrations at day 1 after treatment were 143, 138 and 187 µg/kg in muscle of olive flounder, rockfish and red sea bream, respectively; after a withdrawal period of 3 days, concentrations in muscle were 16, 12 and 21 µg/kg, respectively. Ampicillin was not detectable in any muscle sample 4 days after withdrawal of the medicated feed.

Two of the studies investigated residues after intramuscular administration of ampicillin.

Flatfish (species not specified, mean body weight 150 ± 20 g) were treated with ampicillin at 40 mg/kg bw or 200 mg/kg bw via single intramuscular injection. Fish were kept at a water temperature of approximately 22 °C. Samples from each dosage group (10 fish) were taken at days 2, 5, 7, 14, 21 and 30 after administration, and residue concentrations measured using LC–MS/MS analysis (no validation data were available). Residue concentrations were 35.5, 10.1 and 1.1 µg/kg at the lower dose and 422.7, 44.6 and 12.7 µg/kg at the higher dose at days 2, 5 and 7 after treatment, respectively.

As part of the same study, samples of internal organs and muscle from fish used in a clinical trial were assessed after single intramuscular treatment of 20 mg/kg bw. The fish were maintained at a water temperature range of 21–22 °C. Mean residue concentrations of 6.0, 2.5 and less than 0.001 µg/kg were reported in muscle at days 5, 7 and 14, respectively. Internal organs (combined liver, kidney and spleen) were sampled at day 5 only; the mean residue concentration was 200 µg/kg.

In the second study, residue concentrations after single intramuscular injection of ampicillin at doses of 20, 40 and 80 mg/kg bw in flatfish (species not specified, body weight 350–550 g) were investigated at a water temperature range of 22 ± 3 °C. The flatfish were harvested at days 5, 7 and 10. An LC–MS/MS method was used for analysis, and validation data were provided (LOQ of 10 µg/

kg). Ampicillin was not detected in any of the samples.

No information on the position of sampling sites in relation to injection sites was provided in the residue depletion studies. However, taking into account fish anatomy and the high solubility and rapid absorption rate of ampicillin sodium, the Committee considered it likely that concentrations of ampicillin in injection sites are similar to those in other muscle tissues apart from the injection site.

Data from a monitoring study on the occurrence of ampicillin in the aquaculture products of a Member State were available. A total of 958 saltwater and freshwater fish and crustaceans (halibut, flatfish, sea bass, convict grouper, sea bream, Korean bullhead, catfish, loach, croaker, lobster, rockfish, crucian carp, shrimp, trout, grey mullet, salmon, carp, eel, gizzard shad, leather carp) harvested at aquaculture farms were analysed. Ampicillin residues were not detected in any of the samples. However, no information on ampicillin treatments was given and, as such, the relevance of the results cannot be assessed.

Dietary exposure

Dietary exposure to ampicillin residues from food is considered to occur only from its use as a veterinary drug because there are no Codex MRLs for ampicillin. Based on the hazard profile of the compound, acute dietary exposure is required for the general population and children. Chronic exposure estimates for children and less-than-lifetime dietary exposure are not required. Dietary exposure was estimated based on the potential occurrence of ampicillin residues in a single food (finfish muscle plus skin in natural proportion) after 5 days of treatment (73 degree-days). Consumption inputs were based on the highest reported values available within the fish category (i.e. marine fish consumptions for means, freshwater fish consumption for the 97.5th percentile).

The GECDE for the general population is 0.29 μg/kg bw per day, which represents 10% of the upper bound of the ADI of 0.003 mg/kg bw set by JECFA at the present meeting.

In addition to the accepted GECDE methodology, further calculations were carried out. Instead of using the highest mean and the highest 97.5th percentile consumption across surveys, these calculations used the mean and the highest reliable percentile for each individual national survey from available datasets (CIFOCOss). The mean and ranges across surveys were reported. The mean of 25 estimates for adults or the general population was 0.05 μg/kg bw per day (2% of the upper bound of the ADI), with a range of 0.001–0.29 (0.03–10% of the upper bound of the ADI).

The GEADE for the general population is 1.9 μg/kg bw per day, which represents 16% of the ARfD of 0.012 mg/kg bw. The GEADE for children is 1.7

µg/kg bw per day, which represents 14% of the ARfD of 0.012 mg/kg bw. For both population groups, finfish muscle plus skin in natural proportion was the only contributor to acute dietary exposure.

Analytical methods
Qualitative and quantitative single and multi-residue methods are available to determine residues of ampicillin, the microbiologically active residue identified in fish muscle and in muscle plus skin in natural proportion. Validated analytical methods, HPLC–UV and LC–MS/MS were reviewed. LC–MS/MS methods are appropriate for screening, quantification and monitoring of ampicillin residues in fish tissues.

Maximum residue limits

In order to address the ambiguity of the terms "flat fish" and "finfish", the Committee decided that, for the purpose of this report, the term "finfish" would include all fish species. In recommending MRLs for ampicillin in finfish, the Committee considered the following factors:

- An ADI of 0–0.003 mg/kg bw was established based on a microbiological end-point.
- An ARfD of 0.012 mg/kg bw was established based on a microbiological end-point.
- The Committee concluded that there are no specific concerns for less-than-lifetime exposure.
- Ampicillin is authorized for use in at least two fish species in one Member State. The withdrawal periods of approved uses are unknown. Only withdrawal periods as proposed by the authors of the residue studies are available.
- Ampicillin is not lipophilic and is not widely distributed in fish tissues. Tissue residues deplete rapidly.
- Parent ampicillin is of relevance for the microbiological potential of the compound.
- No metabolism data in fish were available.
- Ampicillin is the relevant marker residue.
- Some residue data in flat fish muscle were available. However, they did not meet the data and validation requirements for residue studies.
- No residue data in fish skin were available.
- Suitable validated routine analytical methods are available for monitoring purposes and can be applied for tissues of different fish

species.

- The available data on ampicillin are not sufficient to establish an MRL for finfish. However, as the modes of action, the physicochemical properties and the toxicological and pharmacokinetic profiles of amoxicillin and ampicillin are very similar, the Committee recommended establishing the same MRLs for finfish for both substances.

- Based on the limited data available, median and 95/95 UTLs were calculated. These were suitable for use as inputs to estimate chronic and acute exposure to ampicillin from finfish.

The Committee recommended an MRL of 50 μg/kg for ampicillin in finfish muscle and in muscle plus skin in natural proportion.

A residue monograph was prepared.

Summary and conclusions

Uncertainty factor

10 (to account for the variability in the composition of the intestinal microbiota within and between individuals)

ADI

0–0.003 mg/kg bw based on the increase in population of ampicillin-resistant bacteria in humans

ARfD

0.012 mg/kg bw based on the microbiological end-point

Residue definition

Ampicillin is the relevant marker residue.

MRLs

The Committee recommended an MRL of 50 μg/kg for ampicillin in finfish muscle and in muscle plus skin in natural proportion.

Estimated dietary exposure

The GECDE for the general population is 0.29 μg/kg bw per day, which represents 10% of the upper bound of the ADI of 0.003 mg/kg bw set by the Committee at the present meeting.

The GEADE for the general population is 1.9 μg/kg bw per day, which

represents 16% of the ARfD of 0.012 mg/kg bw. The GEADE for children is 1.7 μg/kg bw per day, which represents 14% of the ARfD. For both population groups, finfish muscle plus skin in natural proportion was the only contributor to acute dietary exposure.

3.3 Ethion

Explanation

Ethion (International Union of Pure and Applied Chemistry [IUPAC] name: *O,O,O′,O′*-tetraethyl *S, S′*-methylene-bis (phosphorodithioate), CAS No. 563-12-2) is an organophosphate pesticide for use mainly in agriculture and as a veterinary drug.

Ethion was previously evaluated by JMPR in 1968, 1972, 1986 and 1990. Ethion is on the agenda for review by JECFA, using published data, at the request of the Twenty-third Session of the CCRVDF *(2)*. The request was specifically in relation to setting MRLs in edible tissues of cattle.

When used as a veterinary drug, ethion is indicated for the prevention of vector-borne diseases carried by the cattle tick, *Rhipicephalus microplus* (syn: *Boophilus microplus*), as well as being used as an acaricide in sheep, goats, swine and horses. It is also used to control lice and flies.

For cattle, ethion has been formulated into immersion bath treatments, pour-on treatments, sprays and ear tags, often in combination with cypermethrin (a pyrethroid insecticide).

Immersion bath treatments are marketed in the form of a concentrate solution containing, for example, 40% ethion and 10% cypermethrin, which is then diluted with water to a suitable concentration (e.g. 400 ppm ethion). The animals are then treated as a herd, by being corralled and driven through the bath one by one.

Pour-on products are marketed as solutions containing, for example, ethion at 150 g/L and cypermethrin at 50 g/L. Recommended doses are 5 mL for animals weighing 100–200 kg, 10 mL for animals weighing 200–400 kg and 20 mL for animals weighing more than 400 kg (range: 3.75–7.5 mg/kg bw for all weights of animals).

Ear tags can contain 36–40 g ethion per ear tag. These are left on the animals for a specific time (e.g. 120 days). Some products recommend using one ear tag per animal; others recommend using two.

Withdrawal periods for the approved immersion bath formulations vary from 15–45 days for meat, depending on the specific formulation and jurisdiction. The ear-tag product reviewed has approved withdrawal periods of 0 days for meat and 2 days for milk.

Comprehensive literature search

As part of the toxicological evaluation, the following bibliographical databases were searched using the term "ethion" and its synonyms[2] and CAS number: Agricola (1976–August 2017), CAB Abstracts (1958–August 2017), Cochrane Library (2000–August 2017), Embase (1963–August 2017), FSTA (1969–August 2017), International Pharmaceutical Abstracts (1970–August 2017), Global Health (1966–August 2017) and PubMed (1966–August 2017). In total, 2569 articles were recovered, of which 738 were duplicates and were removed. The 1831 remaining titles and abstracts were scanned for information on pharmacodynamics, pharmacokinetics, short- and long-term toxicity, genotoxicity, reproductive and developmental toxicity, endocrine disruption or carcinogenicity of ethion in humans, laboratory animals (mice, rats, rabbits), chickens, dogs, goats, sheep, pigs and cattle.

As part of the evaluation of ethion residues, the Committee performed a comprehensive literature search in April 2017 using the PubMed, B-on, Springer Nature, Science Direct and Web of Science online databases to identify any other relevant information.

The criteria applied to filter the articles for the assessment by the Committee at the present meeting are shown in Table 3.

Although no time limits were placed on the search results, studies published after 1994 were evaluated more thoroughly as these were not evaluated by the previous JMPR review of ethion.

The literature search retrieved no data useful in filling the identified gaps (see "Essential data needed to complete the assessment").

The majority of papers determined to be relevant for establishing the MRL of ethion in cattle concentrated on specific analytical methodologies, usually for use in national or regional surveillance and control of residues of pesticides in foods (including cattle tissues and milk), and these were usually multi-residue methods. All the methods described analysed for parent ethion only.

There was some potentially useful information on the stability of ethion

Table 3

Inclusion and exclusion criteria for the comprehensive literature search

Inclusion criteria	Exclusion criteria
Any article on:	Any article focusing on:
• ethion concentrations in plasma of cattle or other ruminants	• ethion efficacy against target parasites
• ethion concentrations in edible tissues of cattle or other ruminants	• parasite resistance to ethion
	• ethion use in food animal species other than ruminants
• ethion residue determination methods for cattle plasma/tissue	• kinetics/residues of organophosphates other than ethion (and not including ethion for comparison)
• ethion metabolism / metabolites in cattle	
• bioavailability of ethion residues in animals	• pharmacokinetics or pharmacodynamics of ethion in parasite species
Articles in all languages	

in various matrices during storage of samples before analysis.

There were no papers evaluating the pharmacokinetics or residues depletion of ethion or ethion metabolites in cattle.

Toxicological and microbiological evaluation

The present report was based on the summary JMPR evaluations from 1968, 1972, 1986 and 1990 (FAO/WHO, 1969, 1972, 1987, 1991); a United States Environmental Protection Agency (USEPA) report prepared in 1999; and a 2000 report and 2011 addendum from the Agency for Toxic Substances and Disease Registry (ATSDR). The Committee considered evaluations of studies on short- and long-term toxicity, reproductive and developmental toxicity, genotoxicity and carcinogenicity and delayed neurotoxicity and on observations in humans.

Biochemical data

Ethion is a small (molecular weight 384 g/mol), lipid-soluble molecule that can be absorbed by passive diffusion through the lungs, gastrointestinal tract and skin. A study in rats found oral absorption of ethion to be rapid. Gastrointestinal absorption of ethion appears to be 80% in the rat, based on residue studies performed with [^{14}C]ethion. In this study, 75–85% of the total label was excreted in urine and 4–8% in faeces, irrespective of whether labelled ethion was administered as the final dose after 14 days of dosing with unlabelled ethion.

Although general characteristics of organophosphate metabolism are known, most ethion metabolites have not been identified. Ethion is converted via oxidative desulfuration by hepatic cytochrome P450 enzymes to its active oxygen analogue, ethion monoxon. Ethion monoxon is a highly reactive and potent inhibitor of cholinesterases; it reacts with and inhibits neural acetylcholinesterase (AChE). It is not known if ethion monoxon can be further desulfurated to ethion dioxon. Ethion and ethion monoxon are further metabolized by blood and liver esterases. Cleavage of the monoxon at the P–S bond results in diethyl phosphate

and a transient intermediate (*O*,*O*-diethyl-*S*-mercaptomethyldithiophosphate). In humans, cleavage can occur at both the P–S bond and the S–C bond, based on the detection of diethyl phosphate (P–S cleavage of the monoxon), diethyl thiophosphate (P–S cleavage of ethion or S–C cleavage of the monoxon) and diethyl dithiophosphate (S–C cleavage of ethion) in the urine of pest control workers who use ethion; however, relative amounts of the identified substances were not reported. Evidence in mice of cleavage at the S–C bond and subsequent methylation of sulfur has also been reported.

Elimination of ethion is mainly via water-soluble metabolites in the urine. Analysis of urine of rats dosed with [^{14}C]ethion, using HPLC, found four to six unidentified metabolites.

Toxicological data

Most of the critical toxicological studies of ethion do not comply with GLP as the data were generated before the implementation of GLP standards. Overall, however, the Committee considered that the database was adequate to assess the risks of ethion.

Industrial Bio-Test Laboratories (IBT Labs) generated a substantial amount of data from toxicological studies of ethion. Because IBT Labs were subsequently found to have engaged in scientific misconduct, a joint programme called the IBT Review Program, led by the USEPA and Health and Welfare Canada (now Health Canada), re-examined the validity of the toxicological effects of many pesticides, including ethion, in 1983. Only those IBT studies of ethion that were validated by the IBT Review Program were included in the Committee's deliberations.

Overall, the Committee considered that the database was adequate to assess the risks of ethion.

Ethion had some acute toxicity when given to rats by subcutaneous, intraperitoneal and dermal routes; however, it was of high acute toxicity when given to mice and rats by the oral route. The oral LD$_{50}$ for ethion in female rats was cited by JMPR (FAO/WHO, 1969) as 27 mg/kg bw and ranged from 65 to 97 mg/kg bw in males. No evaluation of the AChE activity was performed in any of the single-dose studies.

Ethion was not irritating to the skin of rabbits. Ethion did not induce skin sensitization in a study in guinea-pigs.

The most sensitive effect observed in all species given repeated doses of ethion was inhibition of cholinesterase activity.

In a 13-week study, rats were fed diets containing ethion at concentrations of 0, 3, 10, 30 or 100 mg/kg feed (equal to 0, 0.15, 0.5, 1.5 and 5.0 mg/kg bw per day for males and females, respectively). Significant inhibition of erythrocyte

cholinesterase activities was observed at doses above 0.15 mg/kg/day. All animals exhibited normal physical appearance, behaviour, growth and feed consumption during the studies. No histopathological changes were noted at any level.

In a 13-week study, male and female rats received diets containing ethion at concentrations of 0, 300, 600, 1000 or 1500 mg/kg feed (equal to 0, 15, 30, 50 and 75 mg/kg bw per day, respectively). Complete inhibition of erythrocyte cholinesterase activity occurred in all test groups except in males at 15 mg/kg bw, where inhibition was marked but not complete. No treatment-attributable gross or histopathological changes were observed in any of the surviving rats.

Based on the decrease in erythrocyte AChE activity in both rat studies, the NOAEL was 0.15 mg/kg bw per day.

In a 13-week study in dogs, ethion was given in the diet at doses of 0, 0.5, 2.5, 25 or 300 mg/kg feed (equal to 0, 0.01, 0.06, 0.71 and 6.9 mg/kg bw per day for males and 0, 0.012, 0.07, 0.71 and 8.25 mg/kg bw per day for females, respectively). Reductions in brain and erythrocyte AChE activity at weeks 5, 9 and 13 were seen at 25 mg/kg feed. There were no adverse ophthalmological, haematological or histopathological changes. The NOAEL was 0.06 mg/kg bw per day based on inhibition of the brain AChE activity at 0.71 mg/kg bw per day.

In a chronic toxicity and carcinogenicity study, male and female mice received ethion 0, 0.75, 1.5 or 8 mg/kg feed (equal to 0, 0.11, 0.22 and 1.17 mg/kg bw per day for males and 0, 0.12, 0.24 and 1.28 mg/kg bw per day for females, respectively) for 105 weeks. No effects on body weight, food consumption, incidence of mortality, haematology, blood biochemistry or erythrocyte or brain cholinesterase were attributable to the administration of ethion. Incidences of tumours were not affected by treatment. Based on the absence of changes in erythrocyte AChE activity in all dose groups, the NOAEL was 1.17 mg/kg bw per day, the highest dose tested.

In a 24-month chronic toxicity and carcinogenicity study, male and female rats were given diets containing 0, 2, 4 or 40 mg/kg feed (equal to 0, 0.09, 0.18 and 1.8 mg/kg bw per day for males and 0, 0.11, 0.22 and 2.2 mg/kg bw per day for females, respectively). There was a treatment-related decrease of serum cholinesterase values in both male and female rats at the highest dose, indicating sufficient exposure and bioactivation of ethion; however, there were no significant differences between treated and control groups in erythrocyte and brain AChE activity. Haematological, blood biochemistry and ophthalmological examinations found no treatment-attributable effects. Incidences of tumours were not affected by treatment. Based on the absence of changes in erythrocyte AChE activity in all dose groups, the NOAEL was 1.8 mg/kg bw per day, the highest dose tested.

The genotoxic potential of ethion was investigated in an adequate range of in vitro and in vivo assays.

The Committee concluded that ethion was unlikely to be genotoxic.

The absence of any toxic effect elicited by the doses used in both mouse and rat chronic toxicity studies (maximum doses tested were 1.17 and 1.8 mg/kg bw per day, respectively) suggested to the Committee that an insufficient dose range had been used to adequately assess the carcinogenicity of ethion. However, the Committee also considered the margins of exposure for median and 95th percentile consumers to ethion of 33 400 and 4270, respectively. In view of the lack of genotoxicity and the large margin of exposure, the Committee concluded that ethion is unlikely to pose a carcinogenic risk to humans from residues in the diet.

In a three-generation reproduction study, rats received diets containing ethion at 0, 2, 4 or 25 mg/kg feed (equal to 0, 0.1, 0.19 and 1.2 mg/kg bw per day). No effect was observed on erythrocyte AChE in any group. Gross pathological examination of F_0, F_1 and F_2 parental animals and their progeny showed no alterations attributable to the administration of ethion at any dose level. Based on the absence of changes in erythrocyte AChE activity, the NOAEL was 1.2 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, rats were dosed orally by gavage with ethion at doses of 0, 0.2, 0.6 or 2.5 mg/kg bw per day on gestation days 6 through 15 (21). Treatment-related increased incidences of delayed ossification of pubes were observed in fetuses in the 0.6 and 2.5 mg/kg bw per day groups. There were no significant differences between treated and control groups in maternal body-weight gain or food consumption. The NOAEL for developmental and reproductive toxicity was 2.5 mg/kg bw per day, the highest dose tested. Based on skeletal variations, the NOAEL for embryo/fetal toxicity was 0.2 mg/kg bw.

The Committee concluded that ethion was unlikely to be teratogenic in rats.

In a study to determine the potential of ethion to cause delayed neurotoxicity, four groups of 10 chickens received a single gavage dose of 2792 mg/kg bw of ethion in corn oil after protection from acute cholinergic effects with 10 mg/kg bw atropine given intramuscularly, and were observed for 21 days. Preliminary experiments determined this dose to be equal to the LD_{50} for ethion in chickens with atropine prophylaxis. A positive control group received 500 mg/kg bw of the known delayed neurotoxic agent tri-*o*-cresyl phosphate in corn oil, and the negative control group received the vehicle only. Acute cholinergic signs were observed in the treated groups; 14 of the 40 dosed chickens died. However, after recovery from the acute effects, no clinical or histopathological signs of delayed neurotoxicity were observed in the treated groups. Evidence of delayed neurotoxicity was observed in the tri-*o*-cresyl phosphate–treated chickens.

The Committee concluded that ethion does not induce delayed neurotoxicity.

Observations in humans

A study in humans reviewed by JMPR, USEPA and ATSDR evaluated the effects of ethion on blood cholinesterase activities. Six male volunteers were given ethion in corn oil solutions in three divided doses (at 9:00, 12:00 and 17:00) of 0.05, 0.075, 0.1 or 0.15 mg/kg bw per day via gelatin capsule for 3 weeks at each dose level, except for the highest dose (0.15 mg/kg bw), which was given for 3 days only. Plasma and erythrocyte cholinesterase activities were determined five times during the 2 weeks of the pre-test period and 3 weeks of the test period. No adverse clinical signs (blood pressure, pulse rate, pupil size, light reflex, eye accommodation, chest sounds, muscle tone, knee jerk, tongue tremor and finger tremor) were observed. Although decreases in plasma cholinesterase were observed at 0.1 and 0.15 mg/kg bw dose levels, no statistically significant depression in erythrocyte AChE was observed at any time during the study *(22)*. Based on the absence of absence of effects on erythrocyte AChE activity at any dose tested, the NOAEL was 0.15 mg/kg bw, the highest dose tested.

Microbiological data

Considering the chemical structure and the mode of action of ethion, the Committee did not anticipate any adverse effects of ethion residues on human gastrointestinal microbiota.

Evaluation

In order to establish an ADI, the Committee considered four studies: (1) the human study, from which a NOAEL of 0.15 mg/kg bw per day was derived based on erythrocyte AChE inhibition; (2) the dog study, from which a NOAEL of 0.06 mg/kg bw was derived based on inhibition of brain AChE; (3) the 13-week rat toxicity study, from which a NOAEL of 0.15 mg/kg bw per day was derived based on inhibition of brain and erythrocyte AChE; and (4) the rat developmental toxicity study, from which a NOAEL of 0.2 mg/kg bw per day was derived based on embryotoxic effects.

The Committee compared the NOAELs for erythrocyte cholinesterase inhibition by ethion in rats (0.15 mg/kg bw per day), dogs (0.06 mg/kg bw per day) and humans (0.15 mg/kg bw per day). The Committee considered the dog study inappropriate to evaluate the effects of ethion on cholinesterase inhibition because dogs show considerably greater sensitivity to ethion than humans. Although the Committee considered the repeated-dose human study of AChE inhibition more appropriate than the corresponding 13-week rat study for evaluating this end-point, the Committee did not consider the data from the human study sufficient to assess the non-cholinergic effects of ethion. Hence,

an ADI of 0–0.002 mg/kg bw was established based on the rat developmental toxicity study, using the NOAEL of 0.2 mg/kg bw per day and a safety factor of 100.

The Committee established an ARfD of 0.02 mg/kg bw, based on the NOAEL of 0.15 mg/kg bw for erythrocyte AChE inhibition in the repeated-dose human study and using a 10-fold intraspecies safety factor. The Committee acknowledged that the ARfD was likely conservative, since an acute oral lowest-observed-adverse-effect level (LOAEL) for ethion has not been identified in humans.

As the ADI was based on developmental effect and is appreciably lower than the ARfD, there is a potential concern for exposure in pregnant women. Therefore, exposure in high-percentile pregnant consumers or a suitable surrogate population should be addressed. This exposure scenario will also be protective of children given the nature of the end-point on which the ADI is based.

Residue evaluation

Data on pharmacokinetics and metabolism

Ethion is a small, lipid-soluble compound that can be absorbed by passive diffusion through the lungs, gastrointestinal tract and skin. Absorption is relatively fast via the oral route compared with the dermal route, which may be due to the deposition of ethion in the epidermis and subcutaneous fat layer. Ethion is desulfurated by cytochrome P450 enzymes in the liver to its recognized active form, ethion monoxon, which causes acute toxicity due to its potent inhibition of neural AChE. Ethion and its monoxon metabolite are further metabolized by the action of esterases in the blood and liver, producing diethyl phosphate, diethyl thiophosphate, diethyl dithiophosphate and other metabolites that have not been characterized.

Elimination of ethion is mainly through excretion of water-soluble metabolites in the urine. Conjugation may occur; this is inferred from experiments where ^{14}C-labelled ethion was administered orally to rats and the radioactivity in urine analysed. Urine samples were extracted with ethyl acetate and the aqueous and organic phases were analysed by HPLC. More than 99% of the urine radioactivity was in the aqueous phase. Another sample was acidified to hydrolyse any conjugates and then extracted with ethyl acetate. Acidification converted about 30% of the radioactivity in the aqueous phase to an organosoluble form, which may indicate that some of the products of ethion metabolism are present in urine as conjugates. Four to six radiolabelled metabolites were detected by HPLC; none migrated with standards for ethion, ethion monoxon or ethion dioxon. None of the metabolites were specifically identified.

No pharmacokinetic data were available for the target species, cattle.

Toxicokinetic parameters and cumulative excretion were studied in goats

after intravenous, oral and dermal administration of unlabelled and ^{14}C-labelled ethion. Pharmacokinetic investigation after intravenous injection of 2 mg/kg bw demonstrated a half-life of 2 hours, a total body clearance of 3.2 L/kg per hour and a volume of distribution of 9.4 L/kg. Plasma concentrations of the ^{14}C label (ethion + metabolites) were much higher and more persistent than those of parent ethion. Cumulative excretion of [^{14}C]ethion was 78% of the dose with 66% in urine, 8% in faeces and 4% in milk over 14 days. Oral administration of 10 mg/kg bw resulted in low plasma concentrations of parent ethion, an absorption half-life of 10 hours and a bioavailability of less than 5%. Cumulative excretion over 14 days was 80% of the dose with 64% in urine, 14% in faeces and 1.7% in milk. Dermal application of 100 mg/kg bw demonstrated an absorption half-life of 85 hours and a bioavailability of 20%. Only 0.05% of the dose was excreted unchanged in milk.

It was concluded that (1) orally administered ethion is extensively metabolized in the gastrointestinal tract; (2) dermal application results in extended systemic absorption due to deposition in the dermal fat layer; and (3) systemically absorbed parent ethion is rapidly metabolized. The metabolites were not identified in this study.

Residue depletion data

No radiolabelled residue depletion studies in cattle or any other species were available for review.

There were no data available for pour-on or spray-on products.

Four studies investigating the depletion of ethion residues in cattle tissues after dermal administration (via immersion bath or ear tag) were provided by the requesting Member States; these were reviewed by the Committee. None of these studies investigated the depletion of any of the ethion metabolites. All the studies used parent ethion as the marker residue (see Table 4).

The Committee did not have access to the raw data for any of these studies, relying instead on either English translations of the study reports, which were originally in either Spanish or Portuguese, or on evaluation reports from the Member States in which the products are authorized.

One study investigated residues during and after treatment with an ear-tag product (40 g ethion per tag). Thirty cattle, divided into six groups of five animals each, were treated on day 0, with one ear tag applied to each animal and left in place for 120 days. The groups were slaughtered on days 7, 42, 65 and 79 (with the tags in place) and 120 (1 day after removal of the tags) and 142 (23 days after removal of the tags on day 119). Samples of fat, muscle, kidney and liver were taken for analysis by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS). Residues were highest in fat samples, although it was not recorded from which part of the animals' anatomy the fat samples were taken (i.e. whether they were subcutaneous or perirenal).

Table 4

Summary of available ethion residue depletion studies

No. of cattle tested	Ethion dose	Tissues collected	Sampling times	Assay	LOD & LOQ (µg/kg)	Issues
26	400 ppm bath (single administration)	Liver, kidney, lumbar muscle and perirenal fat	15–92 days post dose	LC–MS/MS	1–2; 5	ULOQ only 100 µg/kg. Early fat samples were above this range. Cannot use to model residue depletion in fat.
16	400 ppm bath (2×, 9 days apart)	Muscle, kidney, liver and subcutaneous and perirenal fat	5–20 days post final dose	GC–FPD	6.5; 19.5	1. LOD/LOQ are different in validation report. 2. Sampling duration too short to get true residue depletion curve in fat.
34	400 ppm bath (3×, 21 days apart)	Muscle (2×), kidney, liver, fat (2×)	13–105 days post final dose	GC–ECD, GC–MS and LC–MS/MS	1; 5–10 (1st lab) 0.5; 1 (2nd lab)	1. Products used are unknown. 2. Samples analysed at two labs with very different results. 3. Analytical method validation data for LC–MS/MS were not provided.
30	40 g ethion/100g ear tag	Muscle, kidney, liver, fat, milk	7–142 days after ear tag was applied (last 2 samples were 1 and 23 days after removal)	UHPLC–MS/MS	0.74–1; 5	1. Study not GLP compliant (claims GCP).

GC–ECD: gas chromatography–electron capture detector; GC–FPD: gas chromatography–flame photometric detector; GC–MS: gas chromatography–mass spectrometry; GCP: good clinical practice; GLP: good laboratory practice; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; ppm: parts per million; UHPLC–MS/MS: ultra-high-performance liquid chromatography–tandem mass spectrometry; ULOQ: upper limit of quantification

Table 5

Summary of available ethion residue depletion studies

Time point (days post application)		Mean residues in fat (µg/kg)[a,b]				
Tag still attached to ear at slaughter	7	49.45	137.92	29.67	83.73	90.89
	42	74.78	62.23	59.98	63.77	69.78
	65	29.59	47.72	65.97	76.86	52.27
	79	54.22	69.36	53.95	16.12	51.29
Tag removed from ear at slaughter	120	54.33	31.70	20.16	35.85	6.32
	142	12.28	5.77	6.20	15.24	6.12

LOD: limit of detection; LOQ: limit of quantification; UHPLC–MS/MS: ultra-high-performance liquid chromatography–tandem mass spectrometry

[a] Mean of $n = 5$ animals per time point.

[b] All samples were analysed in duplicate by UHPLC–MS/MS (LOD of 0.82; LOQ of 5.0). The values shown are means of two analyses of the same sample.

Table 5 shows the individual values.

There were no quantifiable residues in liver at any time point; no quantifiable residues in kidney at any time point except at day 79 (1/5 animals;

8.2 μg/kg); and no quantifiable residues in muscle except at day 79 (1/5 animals; 6.0 μg/kg) and day 120 (2/5 animals; 5.5 and 14.5 μg/kg) (LOQ of 5 μg/kg for all tissues). All samples were analysed in duplicate.

Three residue depletion studies that used immersion bath treatments were provided. These treatments consisted of near total immersion of the cattle in the insecticide solution. All the products used were of the same quantitative composition with respect to the active substances (40% ethion and 10% cypermethrin concentrates, mixed in water to give a 400 ppm concentration of ethion at point of administration).

Each of these studies used a different treatment protocol. In none of the studies was it possible to determine the exact dose received by each animal.

In the first study, the animals were treated once. Groups of four animals were slaughtered on days 15, 29, 43, 57, 69 and 92 post treatment. There were limited useful data derived from this study, as detectable residues in fat were mostly outside the validated range of the LC–MS/MS analytical method used (5–100 μg/kg in fat), but the results added to the evidence that ethion residues were highest in fat samples, with very few detectable ethion residues in any of the other edible tissues sampled (liver, kidney, muscle). Perirenal fat was sampled in this study.

In the second study, cattle were treated twice, 9 days apart. Groups of four animals (one male and three females) were slaughtered on days 5, 10, 15 and 20 post treatment. Samples of muscle, kidney, liver, subcutaneous and perirenal fat were taken from each animal. Muscle samples were taken from the loin (both left and right); samples of subcutaneous fat were taken from along the back line. Residues were undetectable (LOD of 6.5 μg/kg) in all samples except one kidney sample at day 10 and all fat samples at all time points. The withdrawal periods in the study were only up to 20 days, which is less than the approved withdrawal periods for immersion baths, but the depletion profile was comparable to the other data available.

In the third study, cattle were treated three times, 21 days apart. Groups of four animals were slaughtered on days 13, 34, 70, 90, 105 and 117 post final treatment. Samples of muscle (two types, loin and thigh); fat (initially only perirenal fat, but subcutaneous fat was also sampled from day 70 onwards); kidney; and liver were analysed using gas chromatography–electron capture detector (GC–ECD), gas chromatography–mass spectrometry (GC–MS) and LC–MS/MS. The LOD was 1 μg/kg for all tissues, and the LOQs were 5 μg/kg for fat (LC–MS/MS) and muscle, and 10 μg/kg for fat (GC–MS), liver and kidney. Samples were analysed in triplicate.

In addition, the samples of muscle and fat from slaughter day 34 onwards were re-analysed, by LC–MS/MS in a second laboratory. The LOQ was 1 μg/kg and the LOD was 0.5 μg/kg for both tissues. No validation data were available for

Table 6

Ethion residues in the same fat samples as determined by two different laboratories using different validated analytical methods

| Slaughter time point (days) | Animal ID | Sex | Residues in fat (µg/kg) | | | |
| | | | Lab 1[a] | | Lab 2[b] | |
			Perirenal	Subcutaneous	Perirenal	Subcutaneous
13	11589241	M	1 431	NS	NS	NS
	2218952	F	810	NS	NS	NS
	2463698	F	1 600	NS	NS	NS
	8005332	M	728	NS	NS	NS
34	22119487	F	184	70	320	NS
	22323061	F	797	460	246	NS
	6138971	M	337	217	658	NS
	17799836	M	563	392	722	NS
70	7884223	F	27	32	83	321
	7884228	F	77	623	180	532
	20763241	M	272	220	225	355
	17799904	M	29	20	NS	NS
90	17799819	M	13	20	18	37
	11589261	M	<LOQ	54	9.3	48
	25949491	F	<LOQ	72	5.0	133
	24881893	F	<LOQ	<LOQ	3.4	26
105	22118959	F	<LOQ	<LOQ	2.3	27
	26506995	F	<LOQ	<LOQ	4.2	53
	5417775	M	<LOQ	<LOQ	29	NS
	2489237	M	12	41	42	263

F: female; GC–ECD: gas chromatography–electron capture detector; GC–MS: gas chromatography–mass spectrometry; LC–MS/MS: liquid chromatography–tandem mass spectrometric detection system; LOD: limit of detection; LOQ: limit of quantification; M: male; NS: no sample analysed
[a] Samples analysed in triplicate by GC–ECD, GC–MS and LC–MS/MS. The LOD was 1 µg/kg (for all tissues), and the LOQs for fat were 5 µg/kg (LC–MS/MS) and 10 µg/kg (GC–MS).
[b] Samples re-analysed by LC–MS/MS. The LOD was 0.5 µg/kg and the LOQ was 1 µg/kg. No validation data were available for the analytical method used.
Results from all samples from time point 117 days were <LOD in all tissues.

the analytical method used in the second laboratory.

The results of analyses of the same samples were inconsistent in the two laboratories, bringing the reliability of these results into question (see Table 6). It is clear, however, that residues of parent ethion are detectable in fat at higher levels and for much longer after treatment than in any other edible tissue sampled. This is consistent with the half-life of the compound.

Analytical methods

The Committee noted that there are analytical methods available for the determination of parent ethion in cattle tissues. However, as the marker residue has not yet been determined, the Committee cannot comment on the suitability

of the analytical methods for use in residues control.

Maximum residue limits

In recommending MRLs for ethion in edible tissues of cattle, the Committee considered the following factors:

- An ADI of 0–0.002 mg/kg bw was established based on the NOAEL of 0.2 mg/kg bw per day in a developmental toxicity study in rats, and using a safety factor of 100.

- An ARfD of 0.02 mg/kg bw was established based on the NOAEL of 0.15 mg/kg bw for erythrocyte AChE inhibition in a repeated-dose study in male volunteers, and using a 10-fold intraspecies safety factor.

- As the ADI was based on developmental effects and is appreciably lower than the ARfD, there is a potential concern for exposure in pregnant women. Therefore, exposure in high-percentile pregnant consumers or a suitable surrogate population should be addressed. This exposure scenario will also be protective of children given the nature of the end-point on which the ADI is based.

- The residues of concern include all residues derived from ethion due to the lack of:
  - data relating to the toxicological end-point used to set the ADI to any specific residue or combination of residues, and
  - characterization of metabolites, either in the laboratory species used in the toxicological studies, or in the edible tissues of the target species, cattle.

- No data regarding total ethion residues in cattle were provided.

- A suitable marker residue could not be determined.

The Committee was unable to recommend MRLs for ethion at this time. A residue monograph was prepared.

Essential data needed to complete the assessment:

Pharmacokinetics and metabolism and residues depletion in cattle:

In order to enable a determination of a suitable marker residue(s), a metabolism study using radiolabelled ethion in cattle is required. The data should be sufficient to determine the ratios of the parent compound and metabolites (i.e. potential marker residues) to the total residues over the residue depletion period in edible tissues (e.g. liver, kidney, muscle and fat), and to identify the metabolites produced. This would also provide information on the relative distribution of the target compounds (parent ethion and/or active metabolites) in the various edible

tissues of cattle.

Cattle metabolites should be compared with the metabolites found in laboratory species to ensure that all residues of toxicological concern produced in cattle have been covered by the available toxicology studies.

Analytical methods:

Analytical method(s) that can measure suitable marker residues in all edible tissues (e.g. fat, kidney, liver, muscle) should be developed and validated in accordance with established guidance (CAC/GL 71-2009) *(23)*.

Summary and conclusions

Species / study (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 and carcinogenicity study (diet)	Males: 0, 0.11, 0.22, 1.17 Females: 0, 0.12, 0.24, 1.28	Erythrocyte AChE inhibition	1.17 [a]	_
Rat				
Thirteen-week studies	Study 1: 0, 0.15, 0.5, 1.5, 5.0 Study 2: 0, 15, 30, 50, 75	Erythrocyte AChE inhibition	0.15	0.5
Two-year and carcinogenicity study (diet)	Males: 0, 0.09, 0.18 1.8 Females: 0, 0.11, 0.22, 2.2	Erythrocyte AChE inhibition	1.8 [a]	–
Three-generation reproductive toxicity study (diet)	0, 0.1, 0.19, 1.2	Erythrocyte AChE inhibition	1.2 [a]	–
Developmental toxicity study (gavage)	0, 0.2, 0.6, 2.5	**Delays in ossification of the ischium and pubes of the pelvis, and hyoid and manubrium**	0.2 [*]	0.6
Dog				
Thirteen-week study (diet)	Males: 0, 0.01, 0.06, 0.71, 6.9 Females: 0, 0.01, 0.07, 0.71, 8.3	Brain AChE inhibition	0.06	0.71
Human				
Three-week study of effects of ethion on erythrocyte AChE activity (oral)[b]	0, 0.05, 0.075, 0.1, 0.15	**Erythrocyte AChE inhibition**	**0.15 [**,a]**	–

[*] **Pivotal study value for the derivation of the ADI (*21*)**
[**] **Pivotal study value for the derivation of the ARfD (*22*)**
[a] Highest dose tested.
[b] Group dosed with 0.15 mg/kg bw received ethion for 3 days only.

Studies relevant to risk assessment

Uncertainty factor

ADI: 100 (10 for interspecies variability and 10 for intraspecies variability)
ARfD: 10 (10 for intraspecies variability)

ADI

0–0.002 mg/kg bw based on rat developmental toxicity study

ARfD (based on toxicological effects)

0.02 mg/kg bw based on erythrocyte AChE inhibition in the repeated-dose human study

Residue definition

A suitable marker residue could not be determined and a marker residue to total radioactive residue ratio (MR:TRR) could not be established.

The Committee considered that the residues of concern include the total residues of ethion (i.e. the parent molecule and all metabolites) because the toxicological end-point on which the ADI was set was based on developmental effects, which could not be definitively related to the inhibition of acetylcholine esterase and could not therefore be linked to the known action of ethion monoxon. The metabolites have not been characterized in cattle.

MRLs

As there are several gaps in the available data, and the missing data are essential for setting MRLs, no MRLs for ethion in cattle tissues can be recommended at this time.

Estimated dietary exposure

No median or 95/95 UTL could be determined; therefore, no dietary exposure assessment could be conducted.

3.4   Flumethrin

Explanation

Flumethrin, the common name for (*RS*)-cyano-4-fluoro-3-phenoxybenzyl 3-(β,4-dichlorostyryl)-2,2-dimethylcyclopropanecarboxylate (IUPAC name), CAS No. 69770-45-2, is an α-3-phenoxyphenyl pyrethroid (type II) insecticide. Flumethrin has four centres of optical or steric isomerism. Current flumethrin-containing products consist of more than 90% trans-*Z*-1 and trans-*Z*-2 isomers, with minor contributions from cis-Z (<2%) and trans-E (<1%) isomers.

Flumethrin is used in the control of ectoparasites on food-producing and companion animals as pour-on treatments, sprays or dips. It is also used as impregnated plastic strips for treatment of honey bees.

Flumethrin has not been previously evaluated by the Committee, but has been previously evaluated by JMPR, in 1996, when an ADI of 0–0.004 mg/kg bw

was established on the basis of the NOAEL of 0.36 mg/kg bw per day in a two-generation study of reproductive toxicity in rats.

The Committee evaluated flumethrin at the present meeting at the request of the Twenty-third Meeting of the CCRVDF, with a view to establishing relevant HBGVs and recommending MRLs for honey *(2)*.

Flumethrin is registered in several countries for the diagnosis and control in honey bee colonies of varroatosis (also known as varroosis), a disease caused by the parasitic mite *Varroa destructor*. Usually a plastic (low-density polyethylene; LDPE) strip is impregnated with 3.6 mg flumethrin (0.5 mg/cm^3). This product is inserted between the combs in the brood chamber of the beehive. When used as recommended, no withdrawal period is required, although this is based on various restrictions.

This product is authorized for use in several Member States. The restrictions of use are as follows:

- The product should be used after the honey is harvested, usually in late summer. It should not be used during the period of honey flow. For use as a diagnostic tool, or in cases of severe infestation, the product can be used at any time of the year (all regions).
- In cases of treatment for high infestation during honey flow periods, the comb honey should not be sold (one Member State only; this is not stated directly in other regions, but it is implied).
- The usual dose recommended in all regions where the between-comb product is authorized is four strips per chamber in developed colonies and two strips per chamber in young colonies. The strips are suspended in the spaces between the combs in the central brood-rearing area. The strips should remain in the colony for 24 hours (diagnosis) or for 4–8 weeks (treatment), although the most common recommendation is for 6 weeks.

Another type of beehive strip, based on polyvinyl chloride (PVC) rather than LDPE, has also been authorized in some Member States. Instead of being inserted between the honey combs, this type of beehive strip is used as a gate at the entrance of the beehive.

The recommended dose for the beehive gate strips (275 mg flumethrin per strip) is two strips per beehive. The gate strips are fitted to the entrance of the hive so that the bees are forced to use the holes in the strips (15 per strip) to enter or leave the hive. The recommended duration of application is between 9 weeks and 4 months, just after honey flow and extraction.

Toxicological and microbiological evaluation

The Committee considered new studies on pharmacokinetics, acute toxicity, short- and long-term toxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity and neurotoxicity that had not been considered by JMPR in its evaluation of flumethrin in 1996. Pivotal studies were, in general, conducted according to GLP. Those that were not conducted under GLP-compliant conditions are identified in this report. A comprehensive literature search conducted in PubMed and ToxNet identified 12 additional papers, but these did not provide any relevant data additional to those submitted to the Committee.

Biochemical data

The pharmacokinetics of flumethrin were studied in rats, rabbits, dogs, cattle and sheep. A number of investigations were performed as part of toxicological studies.

Flumethrin was rapidly but incompletely absorbed after oral administration in all species investigated. After a single oral dose of [F-phenyl-U-^{14}C]flumethrin, approximately 30% of the administered radioactivity was absorbed in rats. Conversely, when rats were dosed with [Cl-phenyl-U-^{14}C]flumethrin, approximately 75% of the administered radioactivity was absorbed, highlighting the importance of the position of the radiolabel. Plasma levels of radioactivity reached a maximum by 8 hours, but the elimination half-life ranged from 130 to 160 hours. Renal clearance was negligible (<1.2 mL/kg bw per hour). The concentration of radiolabel in organs was 3- to 30-fold lower than in plasma. Radiolabel accumulated in the plasma after multiple dosing, with 9–20% of the administered radioactivity still present in the body 7 days after administration. The highest intensity of radioactivity was in the liver and the lowest in the central nervous system (CNS).

Toxicokinetic data from a two-generation reproductive toxicity study in F_0 rats at days 38/39 in the premating period showed that, at steady state, C_{max} and AUC of flumethrin were dose-dependent but not dose-proportional above 1 mg/kg bw per day; the T_{max} was 4 hours. In rabbits, the T_{max} after a single oral dose of 10 mg/kg bw was 5.4 hours, but the elimination half-life was 43.3 hours.

Topical administration of flumethrin in dogs, a lactating cow and sheep indicated very limited dermal absorption in these species, with plasma levels (of parent or radiolabel) generally less than the LOQ. In the lactating cow, the highest levels of detectable radioactivity were recovered from the application site and underlying skin, followed by the urine, bile, kidney and liver. Radioactivity was also found in milk.

The primary metabolic pathway involves hydrolysis of the central ester bond to form flumethrin acid and 3-phenoxy-4-fluorobenzyl alcohol. Flumethrin

acid accounted for 20–30% of the radiolabel recovered from the faeces of rats administered [Cl-phenyl-U-^{14}C]flumethrin. When [F-phenyl-U-^{14}C]flumethrin was administered, 3-(4-hydroxy-phenoxy)-4-fluorobenzoic acid and 3-phenoxy-4-fluorobenzoic acid were also identified in urine, accounting for the majority of the radioactivity present. Glycine conjugates of the primary metabolites (each <10% of urinary radiolabel) were also identified. In a dairy cow, flumethrin and flumethrin acid were found in all tissues; in milk, only flumethrin and an unknown metabolite were found. The glucuronidated form of the acid was also detected in the liver and kidney.

Toxicological data

Critical studies relevant to the risk assessment of flumethrin were identified. Most of the toxicity studies were conducted under GLP-compliant conditions, and the purity of the test substances used, where specified, was greater than 90%.

The oral acute toxicity of flumethrin in rats was dependent on the vehicle used. When flumethrin was given in peanut oil, the oral LD_{50} was 662 mg/kg bw; when given in miglyol, the oral LD_{50} was 2248 mg/kg bw; when given in acetone:peanut oil (1:10), the oral LD_{50} was 138 mg/kg bw; and when given in corn oil (50% volume per volume [v/v]), the oral LD_{50} was 175 mg/kg bw.

The dermal LD_{50} for flumethrin in corn oil was 1436 mg/kg bw in rats.

The inhalation median lethal concentration (LC_{50}) for flumethrin was 0.572 mg/L in rats.

Flumethrin was not irritating to the skin or the eyes of rabbits. Flumethrin was not sensitizing to the skin in guinea-pigs in Magnusson and Kligman maximization tests.

The primary target for toxicity was the CNS. Toxicity manifested as reduced motor activity, respiratory disorders, altered gait and salivation in multiple species. The onset of toxicity was rapid (1–15 minutes after administration), and the effects were comparatively long-lasting. In addition, the CNS-mediated paraesthetic effects common to other alpha-cyano pyrethroids were seen in mice, rats, rabbits and dogs and included intensive grooming and scratching behaviours leading to skin lesions.

In two 3-month dose-ranging studies, mice were fed diets containing flumethrin at concentrations of 0, 5, 10, 60, 120, 240 or 480 mg/kg feed (equal to 0, 0.9, 1.9, 9.9, 20.8, 42.2 and approximately 80 mg/kg bw per day for males and 0, 1.4, 2.5, 13.2, 26.5, 56 and approximately 100 mg/kg bw per day for females, respectively). The mortality was increased at doses of 60 mg/kg feed and greater, with all high-dose animals dying within the first week of treatment. The severity of clinical signs increased with dose, with mild skin changes (e.g. hair loss) at 5 and 10 mg/kg feed and piloerection at 10 mg/kg feed. Key histopathological findings were related to skin ulcers that occurred above 10 mg/kg feed. Mild skin

changes were the only pathological findings noted in the 5 mg/kg feed group and were not considered to be adverse.

The overall NOAEL in these two dose range–finding studies in mice was 5 mg/kg feed (equal to 0.9 mg/kg bw per day) based on clinical signs observed at 10 mg/kg feed.

In a 13-week non-GLP-compliant study, rats were fed diets containing flumethrin at concentrations of 0, 10, 50 or 150/250 mg/kg feed (150 mg/kg feed from the third week onwards) for 13 weeks (equal to 0, 0.70, 3.54 and 11.10 mg/kg bw per day for males and 0, 0.84, 4.21 and 13.20 mg/kg bw per day for females, respectively; highest dose given for 150 mg/kg feed). By 2 weeks, 10% of animals at 50 mg/kg feed and 50% of animals at 250 mg/kg feed showed inflammatory ulcerative skin changes. Unscheduled deaths occurred only in high-dose animals. The NOAEL was 10 mg/kg feed (equal to 0.7 mg/kg bw per day) based on inflammatory ulcerative changes in the skin at 50 mg/kg feed (equal to 3.54 mg/kg bw per day).

In a 15-week study, rats were fed diets containing flumethrin at concentrations of 0, 10, 40 or 160 mg/kg feed in the diet (equal to 0, 0.7, 2.9 and 11.9 mg/kg bw per day for males and 0, 0.8, 3.4 and 13.0 mg/kg bw per day for females, respectively). There were no unscheduled deaths. Two animals at 40 mg/kg feed had skin lesions, but these were less intense than the high-dose animals' skin lesions and generally reversible. The NOAEL was 10 mg/kg feed (equal to 0.7 mg/kg bw per day) based on isolated and reversible cases of scratching behaviour and skin lesions at 40 mg/kg feed (equal to 2.9 mg/kg bw per day).

In two non-GLP-compliant studies, dogs were fed diets containing flumethrin at concentrations of 0, 25, 50, 100 or 200 mg/kg feed (equal to 0, 0.88, 2.1, 4.7 and 9.6 mg/kg bw per day for males and 0, 0.94, 2.3, 5.0 and 9.0 mg/kg bw per day for females, respectively) for 13 weeks. There were no treatment-related deaths. Animals receiving 50 mg/kg feed and higher concentrations showed partial or complete hair loss and, in some instances, weeping ulcerative scabbed patches on the neck, back, tail, ears and limbs that had partially healed by the end of the study. The overall NOAEL for the two 13-week dog studies was 25 mg/kg feed (equal to 0.88 mg/kg bw per day) based on skin changes at 50 mg/kg feed (equal to 2.1 mg/kg bw per day).

In an 18-month study, mice were fed diets containing flumethrin at concentrations of 0, 1, 3, 15 or 30 mg/kg feed (equal to 0, 0.12, 0.39, 1.97 and 4.56 mg/kg bw per day for males and 0, 0.15, 0.52, 2.34 and 4.95 mg/kg bw per day for females, respectively). Clinical signs of toxicity at 1 and 3 mg/kg feed were limited to slight and transient red spots on the tails of both sexes. At 15 mg/kg feed, animals were observed with auricle loss, wounds on hairless areas and high stepping gait. Non-neoplastic changes (enlarged mandibular lymph nodes, plasmacytosis, increased lymphoid hyperplasia, enlarged or swollen spleens,

slight increase in the incidence of diffuse glandular hyperplasia in stomach, increased incidence of chronic nephropathy, decreased hepatocellular glycogen content and increased hepatocellular atrophy) were considered secondary to the skin changes and poor condition of the higher-dose groups. There were no dose-related or sex-related changes in the incidence of benign or malignant tumours or the total number of tumours. The NOAEL was 3 mg/kg feed (equal to 0.39 mg/kg bw per day) based on clinical signs and non-neoplastic histopathological findings at 15 mg/kg feed (equal to 1.97 mg/kg bw per day).

In a 2-year combined chronic toxicity and carcinogenicity study, rats were fed diets containing flumethrin at concentrations of 0, 0.7, 2 or 4 (reduced from 6 at week 18) mg/kg bw per day. Severe skin changes (wounds) resulted in a number of animals at 6 mg/kg bw per day being euthanized in week 17 of treatment. The highest dose was subsequently reduced to 4 mg/kg bw per day from week 18 in all surviving high-dose animals. Incidental skin changes were limited to only two animals at 0.7 mg/kg bw per day. With increasing dose, clinical signs of toxicity included hair loss and skin changes at 2 mg/kg bw per day and piloerection and more extensive hair loss and skin changes at 4 mg/kg bw per day. Increased vacuolation in the adrenal cortex was noted in males at 2 and 4 mg/kg bw per day. There were no significant organ weight changes. Histopathological changes noted at necropsy were related to the poor condition of the animals. There were no treatment-related increases in benign or malignant tumours in either sex. The NOAEL was 0.7 mg/kg bw per day, based primarily on clinical findings at 2 mg/kg bw per day.

The Committee concluded that flumethrin is not carcinogenic in mice or rats.

The genotoxicity of flumethrin was evaluated in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found, other than in one mouse micronucleus study. The potential for genotoxicity reported in this study was inconsistent with other studies and is likely to be related to the low purity of flumethrin in the study (~60% versus >90% in most other studies).

The Committee concluded that flumethrin is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that flumethrin is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study, rats received diets containing flumethrin at concentrations of 0, 1, 5 or 50 mg/kg feed (equal to 0, 0.07, 0.37 and 3.78 mg/kg bw per day in F_0 males and 0, 0.08, 0.40 and 4.05 mg/kg bw per day in F_0 females, respectively). No treatment-related clinical signs were noted at either 1 or 5 mg/kg feed in any generation. At 50 mg/kg feed, skin lesions developed in F_0 male and female animals and in F_1 females. Body weights of F_0 males and F_1 males and females were reduced in all phases. Survival in all F_1

and F_2 animals at 50 mg/kg feed was lower during the first 4 days after birth, and body weight gain was reduced. A higher incidence of pups with hypothermia and with cramped or hunched posture, stiff limbs in caudal posture and/or pectus carinatum (pigeon chest) was also seen at 50 mg/kg feed; vocalization was also more frequent. These observations were probably secondary to the toxic effects on the parents. No malformations were found. The NOAEL for parental toxicity was 5 mg/kg feed (equal to 0.37 mg/kg bw per day) based on skin lesions at 50 mg/kg feed. The NOAEL for offspring toxicity was 5 mg/kg feed (equal to 0.37 mg/kg bw per day) based on reduced survival and body-weight gain in pups at 50 mg/kg feed. The NOAEL for reproductive toxicity was 50 mg/kg feed (equal to 3.78 mg/kg bw per day), the highest dose tested *(24)*.

In a two-generation reproductive toxicity study, rats were administered flumethrin in corn oil vehicle at doses of 0, 0.5, 1 or 3 mg/kg bw. Unscheduled deaths were unrelated to the test substance, and clinical signs were generally unremarkable and limited to a small number of high-dose animals with hair loss and alopecia in F_0 animals. At 3 mg/kg bw, F_0 and F_1 males and F_1 females had significantly lower body weights compared with controls, and F_0 and F_1 dams had reduced food intake. There were no other significant treatment-related effects in these animals. Significant clinical findings in pups were limited to the high-dose group, in both F_1 and F_2 pups, and included a reduction in pups and litters with no milk spots and thin, relatively small appearance. These findings were concordant with significantly reduced mean pup weight throughout the lactation period in F_1 and F_2 pups. Sexual maturation in F_1 pups was slightly delayed. The NOAEL for parental toxicity was 1 mg/kg bw per day based on reduced body weight at 3 mg/kg bw per day. The NOAEL for offspring toxicity was 1 mg/kg bw per day based on reduced pup weight, reduced pup viability and clinical findings at 3 mg/kg bw per day. The NOAEL for reproductive toxicity was 3 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, rats were administered flumethrin, via gavage, in distilled water containing 5% Emulphor EL-719 (ethoxylated castor oil) and 5% ethanol at 0, 0.5, 1.0 or 2.0 mg/kg bw per day from gestation day 6 to 15. There were no unscheduled deaths. At 1.0 mg/kg bw per day, some dams exhibited salivation from the first day of dosing. At 2.0 mg/kg bw per day, hypoactivity, ptosis, ataxia, lachrymation and urine-stained ventral surface were also evident. In addition, the high-dose dams had a reduced mean feed consumption, mean body weight and mean body-weight gain. Fetotoxicity at 2.0 mg/kg bw per day was noted as a slight but significant reduction in median placental weights (0.48 g versus 0.53 g in controls) and median combined fetal weights (3.4 g versus 3.8 g in controls) and an increased incidence of reduced ossification at several sites, notably the skull bones (67% versus 42% in controls) and cervical vertebral arches (16% versus 1% in controls). The NOAEL for maternal toxicity was 0.5 mg/kg bw

per day based on clinical signs at 1.0 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1.0 mg/kg bw per day based on reduced placental weight, reduced fetal weights and an increase in the incidence of skeletal variations at 2.0 mg/kg bw per day. There was no evidence of teratogenicity *(25)*.

In a developmental toxicity study, rats were administered flumethrin, via gavage, in corn oil at 0, 0.75, 2 or 5 mg/kg bw per day from gestation day 6 to 19. Salivation was evident in dams at 2 and 5 mg/kg bw per day. Body weight loss was noted in the high-dose dams at gestation day 6–7, with reduced body-weight gain during the rest of the treatment period. Placental weight was significantly lower in high-dose dams (0.47 g versus 0.61 g in controls). Weights of fetuses from high-dose dams were also significantly reduced compared with controls (2.79 versus 3.62 g) and were below the historical control range. There were no significant external or visceral changes in fetuses. Delays in skeletal ossification and increased variations, noted mainly at the high dose, were not considered toxicologically significant as they were concordant with delayed development from reduced fetal weights. The NOAEL for maternal toxicity was 0.75 mg/kg bw per day based on salivation at 2 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 2 mg/kg bw per day based on reduced fetal weights at 5 mg/kg bw per day. There was no evidence of teratogenicity.

In a developmental toxicity study, rabbits were administered, via gavage, flumethrin in ethoxylated castor oil/ethanol vehicle at 0, 0.5, 1.7 or 6.0 mg/kg bw per day from gestation day 7 to 19. There were no unscheduled deaths, although four high-dose does aborted between gestation day 18 and 23. There were no significant clinical signs in any of the treated does. Animals at 6 mg/kg bw per day had reduced food intake and reduced body weights during the treatment period. At the high dose, there was a slight trend for a reduction in fetal weights (33.9 g versus 35.9 g in controls) that was lower than the testing laboratory historical control range (34.7–39.8 g). There were no statistically or toxicologically significant changes in the incidence of skeletal, external and visceral changes or malformations/variations in the fetuses. The NOAEL for maternal toxicity was 1.7 mg/kg bw per day based on reduced food intake and body weight at 6.0 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1.7 mg/kg bw per day based on reduced fetal weights at 6.0 mg/kg bw per day. There was no evidence of teratogenicity.

In another developmental study, rabbits were administered, via gavage, flumethrin in corn oil at 0, 0.5, 1.5 or 4.5 mg/kg bw per day from gestation day 6 to 28. There were no unscheduled deaths in the does. Four high-dose does aborted during gestation days 25–29. Limb swelling was apparent at both 1.5 and 4.5 mg/kg per day. The incidence of fused sternebrae was statistically significantly increased in the high-dose group on a fetal basis (17.9% versus 3.8% in controls), but not on a litter basis (47.1% versus 25% in controls) although this was at the

upper end of the historical control range (47.1%) for this strain of rabbit. The NOAEL for maternal toxicity was 0.5 mg/kg bw per day based on clinical signs at 1.5 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1.5 mg/kg bw per day based on an increase in skeletal variations at 4.5 mg/kg bw per day. There was no evidence of teratogenicity.

The Committee concluded that flumethrin is not teratogenic in rats or rabbits.

Observations in humans

There was limited information available on flumethrin-induced toxicity in humans.

Microbiological data

Considering the chemical structure and mode of action of flumethrin, the Committee did not anticipate any adverse effects of flumethrin residues on human gastrointestinal microbiota.

Evaluation

The Committee established an ADI of 0–0.004 mg/kg bw on the basis of a NOAEL of 0.37 mg/kg bw per day for skin lesions in parental animals and reduced survival and body-weight gain in pups in a two-generation toxicity study in rats, with application of a safety factor of 100 to account for interspecies and intraspecies variability, and rounded to one significant figure.

The Committee established an ARfD of 0.005 mg/kg bw on the basis of a NOAEL of 0.5 mg/kg bw for salivation in dams in a developmental toxicity study in rats, with application of a safety factor of 100.

In view of the toxicological profile of flumethrin, specific exposure scenarios are required to address exposure in pregnant women, infants and young children and high-percentile adult consumers.

A toxicological monograph was prepared.

Residue evaluation

Data on pharmacokinetics and metabolism

Flumethrin is a pyrethroid acaricide composed of a mixture of two diastereomers (trans-Z1 and trans-Z2, at an approximate ratio of 60:40).

There is no known metabolism of flumethrin by bees; the majority of the residues end up in the beeswax and honey where no biotransformation can take place. Therefore, the only way of reducing the concentration of flumethrin in honey is via a degradation process. However, investigations into the stability of flumethrin residues in honey stored at room temperature have shown that

no significant decrease in concentration was observed over a storage period of 9 months. This demonstrates that flumethrin does not degrade in honey. It should be noted that the Committee has not evaluated any data on the disposition of flumethrin in other commodities derived from beehives, such as propolis, royal jelly, etc.

Residue depletion data

No radiolabelled residue depletion studies have been conducted. These data are not required for substances in honey as there is no known metabolism of xenobiotics in honeybees and, in this case, there is also no degradation in honey.

Between-comb strips (honey):

Six non-GLP-compliant residues depletion studies of flumethrin in honey were conducted in various locations in Germany and the United Kingdom in the 1980s and 1990s. All these studies used LDPE between-comb strips impregnated with 3.6 mg flumethrin per strip.

In one study, 24 beehives were treated with four strips each, corresponding to a total dose of 14.4 mg flumethrin, for 6 months over the winter period. No residues were detected (LOQ of 3 µg/kg; LOD of 1 µg/kg) in the one sample analysed. In addition, 12 beehives were treated for 6 weeks with four strips per hive, from the beginning of September to the middle of October, over 2 years. No residues were detected in the four samples analysed.

In the second study, an unreported number of beehives were treated with four strips per brood chamber for 6 weeks, after the honey harvest. One sample taken during the spring honey flow was analysed; no residues were detected (LOQ of 3 µg/kg; LOD of 1 µg/kg).

In the third study, six beehives were treated for 6 weeks, from September to October, with four strips per frame. Six samples were taken in June of the following year and combined in pairs to form three samples for analysis. No residues were detected above the LOQ of 2 µg/kg.

In the fourth study, six beehives were treated for 5 months from October to March with four strips per frame. Six samples of freshly capped honeycomb were taken in June of the following year, after the early nectar flow. No residues were detected above the LOQ of 2 µg/kg.

In the fifth study, seven beehives were treated from early March to mid April with four strips per frame. At the end of the treatment period, these combs were labelled and suspended in the honey chamber until the last brood had hatched and fresh honey had been inserted and capped. No residues were detected above the LOQ of 2 µg/kg.

In the final study of between-comb strips, four beehives were treated for 4 months from May to September, during the honey flow period. The entire

honey harvest of each treated hive was centrifuged separately in the last week of August. No residues were detected above the LOQ of 2 µg/kg.

These studies found that even when flumethrin is used in ways that are contrary to good beekeeping practice (in doses higher than recommended or over extended periods of time) or when it is used during honey flow, no flumethrin residues are detected in honey. It should be noted, however, that these studies were not conducted according to current GLP standards; did not have comprehensive study reports; used slightly different analytical methods (based on HPLC–UV); and had inconsistencies in reporting that limit the usefulness of the results.

Between-comb strips (beeswax):

Five non-GLP-compliant residues depletion studies of flumethrin in beeswax were conducted in various locations in Germany and Switzerland in the 1980s to 1990s. All studies used LDPE strips impregnated with 3.6 mg flumethrin per strip. The samples in all studies were analysed using HPLC–UV.

In the first study, 15 beehives were treated as per product instructions (4 strips per brood chamber for 6 weeks, after the honey harvest) in three Swiss cantons. Thirteen samples of beeswax were analysed for flumethrin, and residues were found in the range <26–176 µg/kg; mean residues concentration was approximately 50 µg/kg. The LOQ of the analytical method used was reported to be 26 µg/kg.

In the second study, an unreported number of beehives were treated with 10 times the recommended number of strips (40 strips per hive). The analysis of four samples was reported. The results were in the range of 70–146 µg/kg (mean = 106 µg/kg). The LOQ of the analytical method used was reported to be 26 µg/kg.

In the third study, two beehives were treated with four strips per chamber for 6 weeks, just before the start of honey flow. Two samples per frame were analysed. Residues in the range <15–40 µg/kg were reported. The LOQ of the analytical method used was reported to be 15 µg/kg.

In the fourth study, six beehives were treated with four strips per brood chamber for 6 weeks, from September to October, after the honey harvest. The following year, samples were taken in June, after the early honey flow. One honeycomb was taken from each hive and combined with one from another beehive to form three samples, which were then analysed for flumethrin. The results of the analyses showed residues in the range of <20–50 µg/kg. The LOQ of the analytical method used was reported to be 20 µg/kg.

In the final study, four beehives were treated with four strips per brood chamber for 6 months, during the honey flow period. The strips were inserted in May and removed in September. Two combs from each hive that had been in the brood chamber were moved to the honey chamber in mid August until the brood

hatched. These combs were sampled and analysed. The results were in the range of 30–130 μg/kg. The LOQ of the analytical method used was reported to be 20 μg/kg.

These studies demonstrate that flumethrin has more affinity for beeswax than the honey, which is to be expected considering the lipophilic nature of flumethrin (log P_{ow} = 6.2). Again, it should be noted that these studies were not conducted according to current GLP standards; did not have comprehensive study reports; used slightly different analytical methods (based on HPLC–UV); and had inconsistencies in reporting that limit the usefulness of the results.

Beehive gates (honey and wax):

The sponsor provided one GLP-compliant field study conducted in beehives, using beehive gate strips, in 2015. Honeycombs were obtained from different test sites after application of flumethrin beehive gates (275 mg per gate). Both honey and beeswax were sampled and analysed from 32 honeycomb samples from four apiaries in Germany, 14 samples from two apiaries in Hungary, 12 samples from two apiaries in Spain and 18 samples from three apiaries in the Netherlands.

The maximum recommended application time at the hive entrance of 4 months was covered by data from Germany (122 or 120 days) and the Netherlands (119 days). In the other regions, application time was 92 days (Spain) and 102 days (Hungary).

For residue analysis, honey from all hives of one apiary were pooled, as was the wax. Equal amounts of the previously purified honey samples were combined and homogenized by stirring at 40 °C. Wax samples were melted at about 63 °C and homogenized, and contamination (e.g. hive detritus) was skimmed or sieved off. The honey and wax samples were analysed for flumethrin using a validated LC–MS/MS method.

Residue concentrations in honey were below the LOQ of 3 μg/kg. Concentrations in beeswax were above the LOQ of 25 μg/kg in three out of 11 samples. The highest residue in wax was 119 μg/kg.

Analytical methods

Studies of flumethrin residues in honey and wax after use of the between-comb strips were conducted in the 1980s to 1990s. Several versions of the HPLC–UV analysis method used were provided. However, none of these studies were well-reported or validated according to current standards.

The sponsor also provided the details of an LC–MS/MS method used to analyse flumethrin residues in honey and wax after use of the beehive gates. This study was conducted in a GLP-accredited laboratory, and the method was validated according to international requirements. The LOQ was 3 μg/kg in honey and 25 μg/kg in wax.

Maximum residue limits

In recommending an MRL for flumethrin in honey, the Committee considered the following factors:

- An ADI of 0–0.004 mg/kg bw was established by the Committee.
- An ARfD of 0.005 mg/kg bw was established by the Committee.
- In view of the toxicological profile of flumethrin, specific exposure scenarios are required to address exposure in pregnant women, infants and young children and high-percentile adult consumers.
- Flumethrin is used both as a pesticide and a veterinary drug.
- Flumethrin is authorized for use in beehives in several countries. The maximum recommended dose is 275 mg × 2, administered via PVC beehive gates, or 3.6 mg × 4, administered via between-comb LDPE strips.
- The withdrawal period for all products is 0 days.
- Flumethrin is the marker residue in honey.
- An MR:TRR value of 1.0 was calculated for honey and beeswax.
- A validated analytical method for the determination of flumethrin in honey and beeswax is available and may be used for monitoring purposes.

Although no quantifiable residues were found in honey after treatment with the flumethrin products evaluated, the Committee, in response to the request of the Twenty-third Session of CCRVDF *(2)*, set an MRL for honey at twice the LOQ of the analytical method used in the residue studies. Because the most reliable method was the more recent LC–MS/MS method, which had an LOQ of 3 µg/kg, the Committee recommended an MRL of 6 µg/kg.

Estimated dietary exposure

Exposure to flumethrin residues may occur through its use as a pesticide as well as its use as a veterinary drug.

Dietary exposure from pesticide residues (IEDI)

When used as a pesticide, the exposure of flumethrin was found to be below the upper bound of the ADI of 0.004 mg/kg bw. The sources of exposure considered were cattle meat and milk.

Dietary exposure from veterinary drug residues (GECDE)

The toxicological profile of flumethrin requires exposure estimates for children at the highest reliable percentile of food consumption based on consumers only.

When flumethrin is used as a veterinary drug, chronic and acute dietary exposures in the general population and in children were estimated based on the potential occurrence of residues in honey and beeswax. It was assumed that in all cases honey is consumed with beeswax (as occurs in comb honey and raw honey) and that the honey to wax ratio is 9:1. This is a conservative assumption as most commercial honey is likely to contain much less beeswax. Other honeybee products, such as propolis and pollen, were not included in the exposure assessment.

The GECDE for the general population is 0.008 µg/kg bw per day, which represents 0.2% of the upper bound of the ADI of 0.004 mg/kg bw set by the Committee at the present meeting. The GECDE for children was 0.006 µg/kg bw per day, which represents 0.2% of the upper bound of the ADI.

In addition to the accepted GECDE methodology, further calculations 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 mean of all the GECDE calculations across surveys were reported. The mean of 21 estimates was 0.002 µg/kg bw per day (0.05% of the upper bound of the ADI), with a range of <0.0001–0.008 µg/kg bw per day (<0.003–0.2% of the upper bound of the ADI). For children, chronic dietary exposure could be estimated from 43 individual studies. The mean of these 43 studies was 0.001 µg/kg bw per day (0.03% of the upper bound of the ADI), with a range of <0.0001–0.006 µg/kg bw per day (<0.003–0.2% of the upper bound of the ADI).

Dietary exposure from veterinary drug residues (GEADE)

The GEADE for the general population is 0.1 µg/kg bw per day, based on consumption of wax contained in honey, which represents 2.2% of the ARfD of 0.005 mg/kg bw per day. The GEADE for children is also 0.1 µg/kg bw per day, based on consumption of wax contained in honey, which represents 2.2% of the ARfD.

Combined chronic dietary exposure from pesticide and veterinary drug residues (extended GECDE)

Modified methods based on the GECDE were used to estimate combined chronic dietary exposure. The usual GECDE approach was extended to include additional commodities that were assessed for the compound by JMPR ("extended GECDE"). It should be noted that this new exposure assessment methodology is still being piloted. It should also be noted that the median residues used as inputs were extracted from JMPR publications and have not been validated for this assessment.

Combined chronic dietary exposure from veterinary drug and pesticide residues was considered for the general population and for children, based on the potential occurrence of residues in honey, beeswax, cattle meat and cattle milk. Assumptions for honey and beeswax were the same as for the GECDE.

The extended GECDE for the general population is 0.18 μg/kg bw per day, which represents 5% of the upper bound of the ADI of 0.004 mg/kg bw set by the Committee at the present meeting. The extended GECDE for children is 1.0 μg/kg bw per day, which represents 25% of the upper bound of the ADI. For both populations, cattle milk was the major contributor to chronic dietary exposure.

Risk management considerations

Beeswax that originates from a variety of sources may be present in food; because of this, and the fact that flumethrin accumulates in the wax, risk management measures regarding the use of beeswax that may contain residues of flumethrin could be applied.

An example is where beekeepers reuse the wax combs season after season in order to maximize honey production. This is common practice, as it takes a lot of energy for the bees to make the wax combs. It might therefore be prudent to advise beekeepers to limit reuse of the combs if they are using products containing flumethrin in their hives. Another measure might be to recommend avoiding using the same active ingredient in subsequent years, but rotating the available products between years. This may also reduce the likelihood of resistance to flumethrin of the target parasites.

No data on residues of flumethrin have been evaluated with regard to other products derived from beehives (e.g. propolis, royal jelly, etc.). Therefore, no risk management proposals can be made by the Committee for these commodities.

Summary and conclusions

Uncertainty factor

100 (10 for interspecies variability and 10 for intraspecies variability)

ADI (based on toxicological effects)

0–0.004 mg/kg bw based on the two-generation rat toxicity study

ARfD (based on toxicological effects)

0.005 mg/kg bw based on the rat developmental toxicity study

Studies relevant to risk assessment

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				
Three-month toxicity study (dietary)	Male: 0, 0.9, 1.9, 9.9, 20.8, 42.2, 80[a] Female: 0, 1.4, 2.5, 13.2, 26.5, 56.0, 100[a]	Hair loss, piloerection	0.9	1.9
Eighteen-month carcinogenicity (dietary)	Male: 0, 0.12, 0.39, 1.97, 4.56 Female: 0, 0.15, 0.52, 2.34, 4.95	Clinical signs of skin wounds and high stepping gait	Toxicity: 0.39	1.97
		Plasmacytosis, increased lymphoid hyperplasia & enlarged spleens	Carcinogenicity: none	–
Rat				
Thirteen-week toxicity study (dietary)	Male: 0, 0.70, 3.54, 11.1 Female: 0, 0.84, 4.21, 13.2	Inflammatory ulcerative changes in skin	0.7	3.54
Fifteen-week toxicity study (dietary)	Male: 0, 0.7, 2.9, 11.9 Female: 0, 0.8, 3.4, 13.0	Scratching behaviour and skin lesions	0.7	2.9
Two-year study of toxicity and carcinogenicity (dietary)	0, 0.7, 2, 4(6)[b]	Clinical signs of skin changes and hair loss. Increased vacuolation in the adrenal cortex	Toxicity: 0.7	2
			Carcinogenicity: none	–
Two-generation reproductive toxicity study (dietary)	Males: 0, 0.07, 0.37, 3.78 Females: 0, 0.08, 0.40, 4.05	Reproductive toxicity: Nil	3.78[c]	–
		Parental toxicity: Skin lesions	**0.37***	3.78
		Offspring toxicity: Reduced pup survival and body-weight gain	**0.37***	3.78
Two-generation reproductive toxicity study (gavage)	0, 0.5, 1, 3	Reproductive toxicity: Nil	3[c]	–
		Parental toxicity: Reduced body weight	1	3
		Offspring toxicity: Reduced pup weight, reduced pup viability and clinical findings	1	3
Developmental toxicity study (gavage)	0, 0.5, 1.0, 2.0	**Maternal toxicity: Salivation**	**0.5****	1.0
		Developmental toxicity: reduced placental weight, reduced fetal weights, increased skeletal variations	1.0	2.0
	0, 0.75, 2, 5	Maternal toxicity: Salivation	0.75	2
		Developmental toxicity: Reduced fetal weights	2	5
Rabbit				
Developmental toxicity study (gavage)	Study 1: 0, 0.5, 1.7, 6.0	Maternal toxicity: Reduced body weight, reduced food intake	1.7	6.0
		Developmental toxicity: Reduced fetal weights	1.7	6.0

Table (continued)

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)
Developmental toxicity study (gavage)	Study 2: 0, 0.5, 1.5, 4.5	Maternal toxicity: Limb swelling	0.5	1.5
		Developmental toxicity: Increased skeletal variations	1.5	4.5
Dog				
Thirteen-week toxicity study (dietary)	Study 1: Male: 0, 2.1, 4.7, 9.6 Female: 0, 2.3, 5.0, 9.0	Thinned hair/hairless and weeping, ulcerative, scabbed patches on skin	–	2.1[d]
	Study 2: Male: 0, 0.88 Female: 0, 0.94	Nil	0.88[c, e]	–

[*] **Pivotal study value for the derivation of the ADI (*24*)**
[**] **Pivotal study value for the derivation of the ARfD (*25*)**
[a] Estimated equivalent dose.
[b] Dose reduced from 6 to 4 mg/kg bw per day from week 18.
[c] Highest dose tested.
[d] Lowest dose tested.
[e] Overall NOAEL.

Residue definition

The marker residue in honey is flumethrin (trans-Z1 and trans-Z2 diastereomers).

MRLs

The Committee recommended an MRL in honey of 6 µg/kg.

Estimated dietary exposure

Exposure to flumethrin residues may occur through its use as a pesticide as well as a veterinary drug. When used as a pesticide, the exposure of flumethrin was found to be below the upper bound of the ADI of 0.004 mg/kg bw.

When used as a veterinary drug, dietary exposure was estimated based on the potential occurrence of residues in honey and wax and the assumption that the honey to wax ratio was 9:1.

The GECDE for the general population is 0.008 µg/kg bw per day, which represents 0.2% of the upper bound of the ADI of 0.004 mg/kg bw. The GECDE for children is 0.006 µg/kg bw per day, which represents 0.2% of the upper bound of the ADI.

The GEADE for the general population is 0.1 µg/kg bw per day, based on consumption of wax contained in honey, which represents 2.2% of the ARfD of 0.005 mg/kg bw. The GEADE for children is also 0.1 µg/kg bw per day, based on consumption of wax contained in honey, which represents 2.2% of the ARfD.

Halquinol

Explanation

Halquinol (CAS No. 8067-69-4) is composed of a mixture of chlorinated products of quinolin-8-ol. Chlorinating quinolin-8-ol yields a mixture that contains 5,7-dichloroquinolin-8-ol (5,7-DCL or DCHQ; 57–74% weight per weight [w/w]), 5-chloroquinolin-8-ol (5-CL or CHQ; 23–40% w/w) and 7-chloroquinolin-8-ol (7-CL; 0–4% w/w). Halquinol is a quinoline with broad spectrum antimicrobial activity, but a different mode of action than quinolones.

The halquinol formulation approved for veterinary use in medicated feed consists of 60% halquinol with silicon dioxide and chalk as inert excipients. It is indicated for use in poultry and swine for the enhancement of feed efficiency, and in swine for the control, treatment and prevention of scours/diarrhoea caused or complicated by *E. coli* and *Salmonella* spp. The approved halquinol inclusion rates in swine feed range from 60 to 600 mg/kg feed for up to 15 consecutive days. Based on an estimated daily feed consumption in swine of approximately 4% body weight, the resulting daily halquinol dose is approximately 2.4–24 mg/kg bw. Withdrawal periods for approved halquinol products range from 0–7 days.

Halquinol has not previously been evaluated by the Committee. The Committee evaluated halquinol at the request of the CCRVDF at its Twenty-third Session, with a view to establishing relevant HBGVs and recommending MRLs in swine tissues *(2)*.

Toxicological and microbiological evaluation

The Committee considered data on pharmacokinetics, short-term and long-term toxicity, reproductive and developmental toxicity, genotoxicity and microbiological safety. No carcinogenicity studies were submitted. Most of the submitted studies complied with GLP. A literature search identified 20 papers with information additional to what was submitted.

Biochemical data

None of the submitted studies considered the pharmacokinetics of halquinol. However, plasma levels of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol were measured in the 4- and 13-week rat and mini-pig toxicity studies and the 13- and 39-week dog toxicity studies. Other kinetic information was obtained using read-across from structurally similar compounds such as chloro-7-iodoquinolin-8-ol and 5,7-dibromoquinolin-8-ol in rats, guinea-pigs and rabbits. These oral pharmacokinetic studies showed that absorption of halogenated quinolin-8-ol compounds from the gastrointestinal tract was in the range of 30–40% in laboratory animal species. Following absorption, these compounds undergo extensive first-pass phase II metabolism to sulfate and glucuronide conjugates.

In vitro incubation of ^{14}C-labelled 5-7-dichloro-8-quinolinol with isolated hepatocytes prepared from rats, dogs, mini-pigs, swine and humans showed that the two major metabolites were glucose and glucuronide conjugates. In hepatic microsomes, the major phase I metabolite common to all the microsome samples was hydroxy-5,7-dichloro-8-quinolinol.

The predominant conjugate (sulfate or glucuronide) found in urine or bile/faeces differed between species. In rats, about 26% of a 15 mg/kg bw oral dose of radiolabelled 5,7-DCL and 5-CL (ratio 79:21) was excreted in urine while most of the remainder (69%) was detected in faeces less than 48 hours after dosing. In contrast, 80.5% was excreted in the urine of a ruminant (calf) administered an oral dose of 3 mg/kg bw by stomach intubation. Only about 10.5% of the radioactive dose was found in faeces, and the overall excretion rate was slower, with the radiolabel found in urine over 5–6 days. There was no evidence of bioaccumulation of radiolabelled compounds in the liver, kidney, muscle or fat.

Toxicological data

The acute toxicity of halquinol has been investigated in mice and rats. The oral LD_{50} was 470–850 mg/kg bw in mice and 700 mg/kg bw in rats. In a more recent study, there were no deaths in rats after oral administration of a single dose at 500, 1000 or 2000 mg/kg bw and no clinical signs at 500 or 1000 mg/kg bw. At 2000 mg/kg bw, one out of the three males showed piloerection 2 hours after the single-dose treatment.

In a non-GLP-compliant 28-day oral toxicity study in rats, halquinol was administered by gavage at doses of 0, 150, 450 or 1000 mg/kg bw per day. The NOAEL was 150 mg/kg bw per day based on histopathological changes in the liver (focal necrosis, congestion, hydropic degeneration) and kidney (cystic dilation of collecting tubules, necrosis of tubular epithelial lining) associated with a significant increase in clinical parameters.

In a 4-week oral toxicity study in rats, halquinol was administered by gavage at doses of 0, 150, 450 or 750 mg/kg bw per day. The LOAEL was 150 mg/kg bw per day based on green faeces, changes in appearance and colour and traces of blood and crystals in urine, and histopathological changes in kidney and lymph nodes.

In a 13-week oral toxicity study in rats with a 4-week recovery period, halquinol was administered by gavage at 0, 50, 150 or 450 mg/kg bw per day. The NOAEL was 50 mg/kg bw per day based on increase in kidney weights (absolute and relative) associated with histopathological changes.

In two toxicity studies in dogs, halquinol was administered in gelatin capsules at doses of 0, 3, 10, 60 or 150 mg/kg bw per day for 13 weeks and 0, 30, 60 or 90 mg/kg bw per day for 39 weeks. An overall NOAEL of 30 mg/kg bw per

day was established based on lower terminal body-weights at 60 mg/kg bw per day in males.

In a 1-year chronic toxicity study in rats, halquinol was administered by oral gavage at 0, 15, 50 or 150 mg/kg bw per day. The NOAEL was 15 mg/kg bw per day based on histopathological changes in kidney associated with an increase in mean absolute and relative kidney weights in females at 50 mg/kg bw per day.

Halquinol and its conjugates were tested in a range of in vitro tests including bacterial reverse cell mutation tests, mammalian cell mutation tests using L5178Y TK$^{+/-}$ mouse lymphoma cells and chromosomal aberration tests in cultured lymphocytes. Halquinol tested positive in mouse lymphoma and chromosome aberration tests in vitro. Two adequately conducted in vivo studies examining the potential of halquinol to induce chromosome damage in rat bone marrow cells were negative. The Committee concluded that the positive clastogenicity observed in vitro was not confirmed in vivo. However, the positive result in the mammalian mutagenicity study in vitro was not addressed by the in vivo studies (micronucleus and chromosome aberration), both of which were performed using bone marrow, because they did not assess the induction of a mutation. Moreover, structure–activity relationship (SAR) analysis indicated the presence of structural alerts for both mutagenicity and clastogenicity for all three components of halquinol.

In the absence of a carcinogenicity study or an in vivo gene mutation study, the Committee was unable to reach a definitive conclusion with respect to the genotoxic potential of halquinol.

In a two-generation reproductive toxicity study in rats, halquinol was administered by oral gavage at doses of 0, 50, 150 or 450 mg/kg bw per day. The NOAEL for parental toxicity was 50 mg/kg bw per day based on increased kidney and spleen weights at 150 mg/kg bw per day in F_0 and F_1 parents. The NOAEL for reproductive toxicity was 450 mg/kg bw per day based on the absence of any treatment-related effects. The NOAEL for offspring toxicity was 150 mg/kg bw per day based on histopathological kidney changes in pups at 450 mg/kg bw per day.

In a developmental toxicity study in mice, halquinol was administered by oral gavage on gestation days 6–17 at doses of 0, 30, 100 or 300 mg/kg bw per day. The NOAEL for maternal toxicity was 30 mg/kg bw per day based on clinical signs (round back, piloerection, pallor of extremities, emaciated appearance and/or hyperactivity/hypoactivity) at 100 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 30 mg/kg bw per day based on an increase in the incidence of delayed ossification at 100 mg/kg bw per day. No evidence of teratogenicity was observed.

In a developmental toxicity study in rats, halquinol was administered by oral gavage on gestation days 6–20 at doses of 0, 100, 300 or 1000 mg/kg bw

per day. The NOAEL for maternal toxicity was 300 mg/kg bw per day based on clinical signs (e.g. ptyalism) observed at 1000 mg/kg bw per day. The NOAEL for embryo/fetal toxicity could not be established because of lower mean female fetal body weights at 100 mg/kg bw per day, the lowest dose tested. No evidence of teratogenicity was observed.

The Committee concluded that halquinol was not teratogenic in mice and rats.

Observations in humans

No reliable information on toxicological effects of halquinol in humans was available.

Microbiological data

A decision-tree approach adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) that complies with VICH GL36 (*18*, *19*) was used by the Committee to determine the need for and to establish, if necessary, an mADI for halquinol. Studies of microbiological activity of halquinol against bacterial strains representative of the human colonic microbiota were evaluated. Halquinol was active against *E. coli* (MIC_{50} = 16 μg/mL), *Bifidobacterium* (MIC_{50} = 256 μg/mL), *Clostridium* (MIC_{50} = 32 μg/mL), *Bacteroides* (MIC_{50} = 32 μg/mL), *Lactobacillus* (MIC_{50} = 64 μg/mL), *Fusobacterium* (MIC_{50} = 32 μg/mL), *Eubacterium* (MIC_{50} = 32 μg/mL) and *Peptostreptococcus* (MIC_{50} = 32 μg/mL).

Halquinol residues would be microbiologically active in the human gastrointestinal tract. However, halquinol is extensively biotransformed to conjugates with considerably reduced to no activity before reaching the colon in humans. The MIC_{50} for the sulfate and glucuronide conjugates of 5-chloroquinolin-8-ol and 5,7-dichloroquinol-8-ol were greater than 256 μg/mL. However, because intestinal bacteria have hydrolytic enzymes such as β-glucuronidase and arylsulfatase, there is potential for the halquinol metabolites in the gastrointestinal tract to be deconjugated back to the microbiologically active halquinol.

Halquinol does not appear to induce or select for the development of bacterial resistance. Results from a study in which halquinol was fed to pigs over a 6-week period showed no induction of resistance to *E. coli*. Despite its long use in human and veterinary medicine, resistance development to halquinol has not been reported. Because resistance development is unlikely, the only potential adverse effect on human intestinal microbiota is disruption of the colonization barrier. Consequently, an mADI for halquinol residues wase derived.

The formula for deriving the mADI is as follows:

$$ADI = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{body weight}}$$

where:

- MIC$_{calc}$: In accordance with Appendix C of VICH GL36 (*18, 19*), calculation of the estimated NOAEC (MIC$_{calc}$) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC$_{50}$ for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC$_{calc}$ for halquinol were chosen according to these guidelines, which state that data on an intrinsically resistant bacterial genus should not be included in the calculation. Based on the MIC$_{50}$ values (see above), the MIC$_{calc}$ was 24.0395 µg/mL.

- Mass of colon content: The 500 mL value is based on the colon volume measured in humans.

- Fraction of oral dose available to the microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine.

- Halquinol is rapidly absorbed and is excreted in urine or bile, primarily conjugated with sulfate or glucuronide. The lowest halquinol urinary recovery data from rat studies was 30%. Similar recoveries were observed in the urine of humans treated with structurally related analogues. Therefore, based on similar rates of excretion in rats and humans, the fraction of a halquinol oral dose available in the colon would be $1 - 0.30 = 0.70$.

- The body weight of an adult human is assumed to be 60 kg.

The upper bound of the mADI for halquinol is calculated as follows:

$$\text{Upper bound of ADI} \ = \ \frac{24 \text{ µg/mL} \times 500 \text{ mL}}{0.70 \times 60 \text{ kg}} = 0.285 \text{ mg/kg bw}$$

The microbiological ARfD for halquinol was determined using the following formula:

$$\text{Microbiological ARfD} = \frac{(\text{MIC}_{\text{calc}} \text{ or NOAEC}) \times \text{correction factors} \times \text{colon volume}}{\text{fraction of oral dose available to microorganisms} \times \text{body weight}}$$

$$= \frac{24\ \mu g/mL \times 3 \times 500\ mL}{0.70 \times 60\ kg} = 0.857\ mg/kg\ bw$$

Evaluation

The Committee concluded that a toxicological ADI cannot be established due to the lack of information required to assess the in vivo mutagenicity and carcinogenicity potential of halquinol.

For the effects on the colonization barrier, an mADI of 0–0.285 mg/kg bw, rounded to 0–0.3 mg/kg bw, was derived from in vitro MIC susceptibility testing data and using the newly adopted colon volume of 500 mL. A microbiological ARfD of 0.857 mg/kg bw, rounded to 0.9 mg/kg bw, was established based on the effects of halquinol on the intestinal microbiota and using the newly adopted colon volume of 500 mL.

It was not possible to establish an ADI for halquinol in the absence of a toxicological ADI.

A toxicological monograph was prepared.

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

- Information to enable the assessment of the in vivo mutagenicity and carcinogenicity potential of halquinol.

Residue evaluation

The Committee reviewed studies on the pharmacokinetics of halquinol in Sprague Dawley rats, beagle dogs and mini-pigs. The Committee also evaluated published papers regarding the metabolism of halquinol in rats, guinea-pigs and rabbits. Halquinol metabolism studies in pigs were evaluated as were the comparative metabolism (in vitro) studies of halquinol in hepatocytes and hepatic microsomes of rats, dogs, mini-pigs, pigs and humans. Finally, halquinol residue depletion studies in pigs were assessed. Note that all pharmacokinetic and residue studies used halquinol formulations consisting of no more than 1% 7-CL. As plasma and tissue concentrations of 7-CL were expected to be negligible relative to the other halquinol components and their metabolites, and 7-CL represents only

0–4% of the approved formulation, this component was not analysed in any pharmacokinetic or residue depletion study.

Data on pharmacokinetics and metabolism

The pharmacokinetic data in laboratory animals were derived from toxicology studies in which oral halquinol was administered at doses ranging from 3 to 450 mg/kg bw per day for up to 13 weeks. Plasma samples were obtained at various time points throughout each study, from the first treatment day until the final dose. Methods of oral halquinol administration included gavage and capsule dosing. Due to significant differences in halquinol dose ranges, methods of oral administration, plasma sampling schedules and assay characteristics between studies, direct comparisons of pharmacokinetic parameters between species are not always possible.

5-Chloroquinolin-8-ol (5-CL) was not detectable in plasma samples from any laboratory animals administered halquinol at doses of less than 150 mg/kg bw. As the 5-CL glucuronide (5-CLG) and/or sulfate (5-CLS) metabolites appeared rapidly in plasma after halquinol administration in all species studied, it is presumed that 5-CL is absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism.

5,7-Dichloroquinolin-8-ol (5,7-DCL) was detectable after oral halquinol administration in all species studied, including when administered at the approved doses used in pigs (2.4–24 mg/kg bw per day). Plasma concentrations peaked and declined rapidly after administration and were typically quantifiable for 4–12 hours post dose.

No conclusions regarding bioaccumulation of halquinol could be made after repeated dosing in any species, although plasma concentrations of parent halquinol (5-CL and 5,7-DCL) and their glucuronide/sulfate metabolites did not increase after prolonged daily dosing regimens. There appeared to be no significant sex effects on halquinol pharmacokinetics in any species. However, the sample sizes for all studies were too small to draw definite conclusions.

The linearity of halquinol pharmacokinetics cannot be conclusively determined based on dose ranges used in the dog and rat studies. A trend towards decreasing dose-normalized halquinol exposure was observed with increasing dose in both rats and dogs, but only when administered at greater than 50 mg/kg per day (which is beyond the maximum approved label dose in pigs). At the approved dose range in pigs (2.4–24 mg/kg per day), halquinol pharmacokinetics were linear in dogs.

The oral bioavailability of halquinol was not evaluated in any species. The oral halquinol administration methods (gavage, capsule) used in the laboratory animal toxicology studies differ from the approved halquinol method

of administration (mixed in feed). It is not known if concurrent feed intake alters halquinol kinetics.

The proportion of halquinol metabolized to glucuronide or sulfate conjugates differed depending on laboratory animal species, dose and component (5-CL or 5,7-DCL). In general, the 5-CL component was metabolized primarily to the glucuronide rather than to the sulfate metabolite in the rat and mini-pig, whereas 5-CLS concentrations exceeded 5-CLG in the dog. For 5,7-DCL, the glucuronide pathway predominated in the mini-pig, whereas 5,7-DCLS exceeded 5,7-DCLG in the dog and rat.

Pharmacokinetic data in pigs were derived from a non-GLP-compliant crossover study in four male and four female pigs receiving halquinol by two oral dosing regimens: a single oral dose of 12 mg/kg bw administered as a 100 mg/mL halquinol suspension in 0.5% methylcellulose, and a 10-day dosage regimen of 6 mg/kg bw administered twice daily (12 mg/kg bw per day) in the same halquinol suspension. A wash-out period of 14 days was used between the dose regimens. The dose used is midway between the approved lower and upper dose regimens for halquinol in pigs (2.4–24 mg/kg per day).

Blood was collected at various time points following halquinol administration, and the concentrations of parent halquinol (5-CL and 5,7-DCL) and the glucuronide and sulfate metabolites were determined in plasma using an LC–MS/MS method (LOQ of 9 and 45 ng/mL for 5-CL and 5,7-DCL, respectively, and of 225 ng/mL for all metabolites). The 5-CL component was not quantifiable in any plasma samples whereas 5,7-DCL reached quantifiable concentrations. The glucuronide conjugates (5-CLG and 5,7-DCLG) were the primary halquinol metabolites produced for both parent components, indicating that both 5-CL and 5,7-DCL components were absorbed and rapidly metabolized to the glucuronide forms. Sulfate metabolites (5-CLS and 5,7-DCLS) were quantifiable in only five out of 104 plasma samples collected after the single halquinol dose and were not quantifiable in any plasma samples collected during the multi-dose portion of the study.

Limited pharmacokinetic analyses could be performed for 5-CL, 5-CLS and 5,7-DCLS due to lack of quantifiable plasma results. The ranges of T_{max} and C_{max} for the other quantifiable components are shown in Table 7.

In a GLP-compliant study, cryopreserved hepatocytes and hepatic microsomes prepared from male and female Sprague Dawley rats, beagle dogs, Göttingen mini-pigs, Landrace pigs and humans were incubated with 5 and 20 µmol/L ^{14}C-labelled 5,7-DCL. The metabolism of the 5-CL component was not assessed. Extensive metabolism of 5,7-DCL occurred in all species and sexes, and the predominant metabolite in all species was 5,7-DCLG. No sulfate conjugates of 5,7-DCL were observed in this in vitro study. 5,7-DCLS was not observed in pharmacokinetic studies in swine, although it was the predominant 5,7-DCL metabolite identified in dog and rat pharmacokinetic studies.

Table 7

Pharmacokinetic parameter range for 5,7-DCL, 5-CLG and 5,7-DCLG after administration of oral halquinol at 12 mg/kg bw in pigs

Parameter	5,7-DCL	5-CLG	5,7-DCLG
C_{max} (ng/mL)	67–494	552–2 960	242–1 770
T_{max} (h)	1–6	0.5–1	0.5–6

5-CLG: 5-chloro-8-hydroxyquinoline glucuronide conjugate; C_{max}: maximum plasma concentration; 5,7-DCL: 5,7-dichloroquinolin-8-ol; 5,7-DCLG: 5,7-dichloroquino-lin-8-ol glucuronide conjugate; T_{max}: time to reach the maximum concentration

Residue data

In a GLP-compliant radiolabelled study (McLean 2016), swine were dosed twice daily with oral gelatin capsules containing [^{14}C]halquinol at 6 mg/kg bw per dose for 7 days. Groups of four pigs were slaughtered at 3, 6, 12 or 48 hours after the final dose. The radioactivity was rapidly excreted in urine and faeces. Within 48 hours of the final dose, mean recoveries of total radioactive residues (TRRs) were 36% and 41% in faeces and urine, respectively. In urine, glucuronide metabolites and unchanged parent (5-CL and 5,7-DCL) accounted for 83% and 13% of the extracted radioactive residues, respectively. In faeces, unchanged parent accounted for 100% of the extractable radioactivity (no radiolabelled metabolites were present in the extractable faecal portion).

The quantitative distribution of radioactive residues was assessed by HPLC in six liver samples, four kidney samples, one muscle sample and one skin-with-fat sample. Extractability in muscle and skin-with-fat were 81.6% and 85.6% of TRR, respectively. Extractability was variable in the liver and kidney (18.3–50.7% and 15.9–87.6%, respectively) and was not uniform over the range of time points assessed.

Mean TRR and proposed marker residue concentrations were highest in kidney, followed by liver, skin-with-fat and muscle. 5-CL was not detected in any tissue. 5,7-DCL and both glucuronide metabolites (5-CLG and 5,7-DCLG) were quantified in all tissues. In liver, only 5,7-DCL and 5-CLG were quantifiable.

In liver, the radiolabelled halquinol and its glucuronide metabolites accounted for only 1–5% of the total radioactivity present. Other metabolites were detected, but the sum of all extracted radiolabelled components in liver accounted for only 18–42% of the total tissue radioactivity. In kidney, halquinol and its glucuronide metabolites accounted for 25–52% of the total tissue radioactivity. In muscle and skin-with-fat, halquinol and its glucuronide metabolites accounted for 60% and 73% of total tissue radioactivity, respectively. However, only one sample was analysed for each as the total tissue radioactivity was extremely low in all other muscle and skin-with-fat samples.

The sponsor proposed a marker residue (the sum of 5-CL, 5,7-DCL, 5-CLG and 5,7-DCLG) and a target tissue (kidney). Based on the radiolabelled study results, MR:TRR values over time could not be determined with confidence for any tissue. In muscle and skin-with-fat, MR:TRR values were based on only a single tissue sample (3 hours after final dose). In kidney and liver, the MR:TRR appeared to decrease rapidly over a short time (3–12 hours after final dose). Furthermore, confidence in the marker residue (MR) portion of the MR:TRR ratio derived in kidney and liver tissues is low due to the very low amounts of proposed marker residue present and the limited extractability of radiolabelled components.

In a GLP-compliant study, swine received a medicated feed containing halquinol at 700 ppm (mg/kg feed) ad libitum for 10 consecutive days. Groups of four animals were slaughtered at 8, 24, 48 or 120 hours post final dose. Liver, kidney, loin muscle, skin-with-fat and small intestine were collected and analysed for the proposed marker residue (the sum of 5-CL and 5,7-DCL and their glucuronide metabolites) using a validated LC–MS/MS method. Marker residue concentrations depleted most slowly from kidney. By 120 hours withdrawal, residues were detected only in kidney (3 of 4 samples) and skin-with-fat (1 of 4 samples). See Table 8 for halquinol marker residue concentrations in tissues.

In another effort to characterize the changing MR:TRR over time, a combination of MR results from one study and TRR results from another was considered. However, this approach was ultimately considered inappropriate due to significant differences in study design (doses administered, withdrawal periods, etc.) and discordant MR results between studies.

Analytical methods

A validated LC–MS/MS method suitable for the determination of the sponsor-proposed marker residue was used in the non-radiolabelled halquinol residue depletion study. The LOQ was 10 µg/kg. The halquinol marker residue was proposed by the sponsor to be the sum of 5-CL, 5,7-DCL, 5-CLG (expressed as 5-CL equivalents) and 5,7-DCLG (expressed as 5,7-DCL equivalents).

Maximum residue limits

In recommending MRLs for halquinol in swine, the Committee considered the following factors:

- An mADI of 0–0.3 mg/kg bw and a microbiological ARfD of 0.9 mg/kg bw were established. A toxicological ADI could not be established due to the lack of information required to assess the in vivo mutagenicity and carcinogenicity potential of halquinol. It was

Table 8

Halquinol marker residue concentrations in tissues in pigs fed halquinol for 10 days

Withdrawal period (hours)	Residue concentrations (µg/kg)			
	Muscle	Liver	Kidney	Skin-with-fat
8	28.5	210	1 482	96
	33.4	299	918	118
	23.4	206	2 569	96
	26.5	286	1 780	90
24	<LOQ	66	230	48
	<LOQ	38	219	25
	<LOQ	45	1 195	31
	<LOQ	26	192	21
48	<LOQ	<LOQ	65	<LOQ
	<LOQ	<LOQ	42	<LOQ
	<LOQ	<LOQ	20	<LOQ
	<LOQ	<LOQ	52	19
120	<LOQ	<LOQ	21	<LOQ
	<LOQ	<LOQ	23	<LOQ
	<LOQ	<LOQ	108	16
	<LOQ	<LOQ	<LOQ	<LOQ

LOQ (limit of quantification): 10 µg/kg for all tissues

not possible to establish an ADI for halquinol in the absence of a toxicological ADI.

- Withdrawal periods range from 0 to 7 days for approved veterinary uses in swine.

- Halquinol is extensively metabolized in swine, primarily to glucuronide metabolites.

- The Committee cannot confirm the suitability of the sponsor-proposed marker residue at this time due to insufficient characterization of the total metabolite profile in edible tissues.

- In the absence of an acceptable marker residue, the Committee cannot comment on the suitability of any analytical method for halquinol residue monitoring purposes.

- The non-radiolabelled halquinol residue depletion data were sufficient to determine median sponsor-proposed marker residues and 95/95 UTLs in muscle, liver, kidney and skin-with-fat for time periods up to 120 hours post withdrawal. However, the total residue of concern cannot be estimated with confidence from these proposed marker residue concentrations due to insufficient MR:TRR data. The

residue of concern is likely to be total halquinol residues and not any specific residue components.

MRLs could not be recommended for halquinol due to the lack of an established HBGV, incomplete characterization of residues in tissues (particularly liver and kidney) and a lack of data necessary to establish reliable MR:TRR ratios over time for calculation of total residues in tissues.

A residue monograph was prepared.

Estimated dietary exposure

No median or 95/95 UTL could be determined due to incomplete characterization of residues in tissues (particularly liver and kidney) and a lack of data necessary to establish reliable MR:TRR ratios over time for calculation of total residues in tissues; therefore, no dietary exposure assessment could be conducted.

Further information required to complete the residue assessment:

1. The characterization of specific halquinol metabolites in the radiolabelled study in pigs was incomplete, particularly for liver and kidney samples. Characterization of the non-extractable radiolabelled residues in tissues, as well as the extractable (but not defined) residues, is required.

2. Regarding the derivation of MR:TRR ratios over time, the Committee considered the proposed regression approach combining data from the radiolabelled and non-radiolabelled studies to be inappropriate. This was due to a number of factors, including:

   - a greater than 3-fold difference in doses used between the studies (acknowledging that while the pharmacokinetics of halquinol may be linear over this dose range in other species, this has not been demonstrated conclusively in pigs);
   - the discordance of the MR:TRR values derived from the radiolabelled study alone, and the regression approach derived from the combination of radiolabelled/non-radiolabelled data; and
   - the generally low amount of radioactivity observed in swine tissues may cause unacceptable uncertainty in MR and TRR counts.

The Committee acknowledges the sponsor's proposal to use the lower bound (more conservative approach) of estimated MR:TRR ratios. However, the total residues are predicted to be the residue of concern. The Committee considers

it inappropriate to predict total residues based on potentially unsound MR:TRR estimates, especially in view of the lack of characterization of total metabolite profile in swine tissues noted in point 1, above.

An accurate MR:TRR over the appropriate time in edible pig tissues after halquinol administration should be determined.

The Committee noted that new studies may be necessary to address these concerns.

Summary and conclusions

Toxicological effects

A toxicological ADI could not be established.

Microbiological effects

An mADI of 0–0.3 mg/kg bw was established.

A microbiological ARfD of 0.9 mg/kg bw was established based on the effects of halquinol on the intestinal microbiota.

ADI

An ADI could not be established in the absence of a toxicological ADI.

Residue definition

The sponsor-proposed marker residue could not be confirmed by the Committee.

The residue of concern is likely to be the sum of total halquinol residues. However, this cannot be confirmed until further characterization of halquinol metabolites in tissues is performed.

MRLs

MRLs could not be recommended for halquinol due to the lack of an established HBGV, incomplete characterization of residues in tissues (particularly liver and kidney) and a lack of data necessary to establish reliable MR:TRR ratios over time for calculation of total residues in tissues.

Estimated dietary exposure

The marker residue could not be confirmed, no median or 95/95 UTL could be determined due to incomplete characterization of residues in tissues (particularly liver and kidney) and no MRLs were recommended. Therefore, no dietary exposure assessment could be conducted.

Studies relevant to risk assessment

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				
Developmental study (gavage)	0, 30, 100, 300	Maternal toxicity: Clinical signs	30	100
		Embryo and fetal toxicity: Delayed bone ossification	30	100
Rat				
Thirteen-week toxicity (gavage)	0, 50, 150, 450	Histopathological lesions in the kidneys	50	150
One-year and carcinogenicity study (diet)	0, 15, 50, 150	Histopathological lesions in the kidneys	15	50
Two-generation reproduction study (gavage)	0, 50, 150, 450	Parental toxicity: Increase in kidney and spleen weights	50	150
		Offspring toxicity: kidney lesions in pups	150	450
		Reproductive toxicity: None	450	–
Developmental study (gavage)	0, 100, 300, 1 000	Maternal toxicity: Clinical signs	300	1 000
		Embryo and fetal toxicity: Lower mean fetal body weights correlating with delayed bone ossification	–	100
Dog				
Thirteen-week toxicity study (gelatin capsule)	0, 3, 10, 60, 150	Body weight loss	30[a]	60
Thirty-nine-week toxicity study (gelatin capsule)	0, 30, 60, 90	Body weight loss	30[a]	60

[a] Overall NOAEL

3.6 Lufenuron

Explanation

Lufenuron is the International Organization for Standardization (ISO)–approved common name for (*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-3-(2,6-difluorobenzoyl)urea (IUPAC name), for which the CAS No. is 103055-07-8.

Lufenuron is an insecticide initially registered for use on a wide range of crops for the control of the larvae of many insect pests. Lufenuron inhibits chitin synthesis, probably through enzymatic interference, and prevents the larvae from moulting.

Lufenuron is used as a veterinary drug in the treatment of sea lice in salmon when they are smolts in fresh water. It is coated onto non-medicated fish

feed pellets to achieve an intended dose of 5 mg/kg bw per day for 7 consecutive days. The withdrawal period is 2050 degree-days.

Lufenuron has not been previously evaluated by the Committee. Lufenuron was reviewed by the present Committee at the request of the Twenty-third Session of CCRVDF *(2)*. The Committee was asked to establish an ADI and recommend MRLs for lufenuron in finfish (salmon/trout) muscle plus skin in natural proportion.

The Committee noted that lufenuron had been evaluated by JMPR in 2015. At that meeting, JMPR established an ADI of 0–0.02 mg/kg bw for lufenuron based on the NOAEL of 1.93 mg/kg bw per day for tonic–clonic seizures and findings in lungs, gastrointestinal tract, liver and urinary tract in a 2-year dietary study in rats *(4)*. JMPR also concluded that it was not necessary to establish an ARfD for lufenuron in view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

Because the review of lufenuron by JMPR was so recent (in 2015), a literature search on lufenuron was not undertaken. The JECFA evaluation is essentially identical to the JMPR evaluation with minor editorial changes to accommodate formatting differences. In addition, a few unpublished studies previously not available to JMPR have been included in this review. These unpublished studies relate to the acute LD_{50}, genotoxicity and short-term dietary exposure to lufenuron metabolites. The only additional unpublished study on the parent compound was a pharmacokinetic and mass balance study in dogs. All critical studies contained statements of compliance with GLP.

Toxicological and microbiological evaluation

Biochemical data

In rats, lufenuron is only partially absorbed following a single oral dose. The extent of absorption is dose related; approximately 20% of a single 100 mg/kg bw dose appears to be absorbed, compared with about 70% of a single 0.1 or 0.5 mg/kg bw dose. A large proportion of the absorbed dose partitions into fat, with very much lower uptake by other tissues, including the brain. All tissue concentrations of radioactivity increased to a maximum 1 day after the last of 14 repeated low doses. The results suggest that most tissue concentrations would plateau within 2–3 weeks of similar repeated dosing. The depot in fat is slowly released, with a terminal half-life of up to 5–13 days at 0.5 mg/kg bw and 10–37 days at 100 mg/kg bw, leading to an increase in concentrations of lufenuron in the brain over long periods (see "Toxicological data", below). Excretion of the absorbed dose is predominantly via faeces; only about 1% of the dose is excreted in urine, independent of the dose. Metabolism of lufenuron is minimal; only about 1% of an oral dose is metabolized by deacylation followed by cleavage of the ureido

group. There is no marked sex difference in absorption, tissue distribution, metabolism or excretion. The pattern of excretion and metabolism is not affected by repeated dosing.

In a study in dogs not previously evaluated by JMPR, no more than 70% of a 10 mg/kg bw oral dose appeared to be absorbed, with C_{max} in blood observed 4 hours after capsule administration. The half-life of lufenuron in blood was estimated to be about 20 days. Judged by the large apparent volume of distribution (19 L/kg), much of the absorbed lufenuron readily distributes into tissues. Up to 54% of the oral dose was excreted via the faeces (mainly during the first 24–72 hours), but only a minor portion (0.8–2.2%) was eliminated in the urine. No parent compound was detected in faeces within 24 hours of administration, but increasing quantities of an unidentified major metabolite and three minor components appeared over the second and third days. Approximately 16% of the administered radioactivity was detected in the skin, 11% in skeletal muscle, 7% in adipose tissue, 1% in liver and bile combined, and 0.3% in blood.

Toxicological data

Lufenuron has low acute toxicity when administered orally or dermally (LD_{50} > 2000 mg/kg bw) or via inhalation (LC_{50} > 2.35 mg/L, maximal attainable concentration) to rats. Lufenuron was very slightly irritating to the skin and eyes of rabbits and was sensitizing to the skin of guinea-pigs.

The most significant toxicological end-point for lufenuron was convulsions, observed after prolonged treatment at high-dose levels. Convulsions are observable in all species treated with lufenuron at daily doses of more than 20 mg/kg bw for extended periods (2–3 months in rodents, >3 months in dogs). As lufenuron is a very lipophilic compound (log octanol–water partition coefficient [K_{ow}] = 5.12), it has the potential to accumulate in fatty tissues. Toxicity studies in mice, rats and dogs showed that after prolonged exposure to high doses of 20 mg/kg bw per day or more, fat compartments may become saturated. If exposure is continued after saturation, concentrations in the brain increase, leading to tonic–clonic convulsions.

In a dose range–finding study, lufenuron was fed to mice at a concentration of 0, 1000, 3000 or 9000 mg/kg feed (equal to 0, 151, 449 and 1470 mg/kg bw per day for males and 0, 189, 517 and 1440 mg/kg bw per day for females, respectively) for up to 65 days. As a result of mortality and neurotoxic effects (tonic–clonic seizures) of the test substance at all dose levels, it was concluded that the maximum tolerated dose was exceeded even at 1000 mg/kg feed (equal to 151 mg/kg bw per day).

In a second dose range–finding study, intended for test substance residue and blood level determination, lufenuron was fed to female mice at a concentration of 0, 4/8, 20, 100 or 1000 mg/kg feed (equal to 0, 0.47/1.1, 2.94,

14.5 and 143 mg/kg bw per day, respectively; from day 57 onwards, the diet of the low-dose group inadvertently contained 8 mg/kg feed instead of 4 mg/kg feed) for up to 91 days (only 71 days for the high dose). The NOAEL was 100 mg/kg feed (equal to 14.5 mg/kg bw per day) based on mortality and neurotoxicity (tonic–clonic seizures) at 1000 mg/kg feed (equal to 143 mg/kg bw per day).

In a 28-day range-finding study, rats were administered lufenuron in the diet at a concentration of 0, 50, 400, 3000 or 20 000 mg/kg feed (equal to 0, 4.10, 30.8, 254 and 1690 mg/kg bw per day for males and 0, 4.07, 32.6, 254 and 1740 mg/kg bw per day for females, respectively). The NOAEL was 400 mg/kg feed (equal to 30.8 mg/kg bw per day) based on decreased thymus weight at 3000 mg/kg feed (equal to 254 mg/kg bw per day).

In a 90-day toxicity study, rats were fed diets containing lufenuron at 0, 25, 150, 1500 or 15 000 mg/kg feed (equal to 0, 1.6, 9.68, 101 and 998 mg/kg bw per day for males and 0, 1.7, 10.2, 103 and 1050 mg/kg bw per day for females, respectively). The NOAEL was 150 mg/kg feed (equal to 9.68 mg/kg bw per day) based on clinical signs (tonic–clonic seizures), decreased body-weight gain and feed consumption, slight changes in haematology and clinical chemistry parameters and increased adrenal weights at 1500 mg/kg feed (equal to 101 mg/kg bw per day).

In a 4-week range-finding study, dogs received lufenuron in their diet at a concentration of 200 or 50 000 mg/kg feed (equal to 8.43 and 2200 mg/kg bw per day for males and 10.1 and 2650 mg/kg bw per day for females, respectively). The NOAEL was 50 000 mg/kg feed (equal to 2200 mg/kg bw per day), the highest dose tested.

In a 90-day toxicity study, dogs received lufenuron in their diet at a concentration of 0, 200, 3000 or 50 000 mg/kg feed (equal to 0, 7.8, 122 and 2020 mg/kg bw per day for males and 0, 7.9, 123 and 1930 mg/kg bw per day for females, respectively). The NOAEL was 200 mg/kg feed (equal to 7.8 mg/kg bw per day) based on increases in blood cholesterol levels and absolute and relative liver weights, reductions in blood potassium and phosphorus levels, and an increase in serum alkaline phosphatase activity in some animals at 3000 mg/kg feed (equal to 122 mg/kg bw per day).

In a 1-year toxicity study, dogs received lufenuron in their diet at a concentration of 0, 100, 2000 or 50 000 mg/kg feed (equal to 0, 3.97, 65.4 and 1880 mg/kg bw per day for males and 0, 3.64, 78.3 and 1980 mg/kg bw per day for females, respectively). The main target organs were the brain, adrenals, liver, thyroid and lungs. The NOAEL was 100 mg/kg feed (equal to 3.64 mg/kg bw per day) based on mortality, neuromuscular signs including convulsions, reduced body-weight gain, changes in clinical pathology parameters and histopathologicalical lesions in adrenals, liver, thyroid and lungs observed at 2000 mg/kg feed (equal to 65.4 mg/kg bw per day).

In another 1-year toxicity study, dogs received lufenuron in their diet at a concentration of 0, 10, 50, 250 or 1000 mg/kg feed (equal to 0, 0.31, 1.42, 7.02 and 29.8 mg/kg bw per day for males and 0, 0.33, 1.55, 7.72 and 31.8 mg/kg bw per day for females, respectively). The NOAEL was 250 mg/kg feed (equal to 7.02 mg/kg bw per day) based on treatment-related mortality and clinical findings, including convulsions and effects on body weight and on the liver and adrenals, with associated histopathological and/or clinical chemistry changes, at 1000 mg/kg feed (equal to 29.8 mg/kg bw per day).

An overall NOAEL of 250 mg/kg feed (equal to 7.02 mg/kg bw per day) was identified on the basis of the two 1-year dog studies. The 90-day dog study should not be included in the overall NOAEL as the observed effects (blood parameters and liver weights) are far less severe than the effects in the 1-year dog studies (e.g. mortality) at similar dose levels. This can be explained by the accumulation in fat, which is not yet saturated in the 90-day study; this accumulation leads to higher concentrations of the parent compound in the brain in the longer-term studies. In an 18-month dietary toxicity and carcinogenicity study, mice received lufenuron at a concentration of 0, 2, 20, 200 or 400 mg/kg feed (equal to 0, 0.222, 2.25, 22.6 and 62.9 mg/kg bw per day for males and 0, 0.217, 2.12, 22.0 and 61.2 mg/kg bw per day for females, respectively). As a result of high mortality in the high-dose group, surviving animals in this dose group were terminated in weeks 9 and 10. The NOAEL was 20 mg/kg feed (equal to 2.12 mg/kg bw per day) based on increased mortality, clinical signs (tonic–clonic convulsive episodes), increased incidences of fatty liver (in females, accompanied by necrotic changes) and a higher incidence of inflammatory changes in the prostate at 200 mg/kg feed (equal to 22.0 mg/kg bw per day). No treatment-related tumours were observed.

In a 2-year dietary toxicity and carcinogenicity study, rats received lufenuron at a concentration of 0, 5, 50, 500 or 1500 mg/kg feed (equal to 0, 0.19, 1.93, 20.4 and 108 mg/kg bw per day for males and 0, 0.23, 2.34, 24.8 and 114 mg/kg bw per day for females, respectively). As a result of overt toxicity at 1500 mg/kg feed, all animals in this group were terminated in week 14. The NOAEL was 50 mg/kg feed (equal to 1.93 mg/kg bw per day) based on clinical signs (tonic–clonic convulsions), decreased body weight, and (histo)pathological effects on lungs, liver, non-glandular stomach, intestines and urinary tract at 500 mg/kg feed (equal to 20.4 mg/kg bw per day). No treatment-related tumours were observed *(26)*.

The Committee concluded that lufenuron is not carcinogenic in mice or rats.

Lufenuron was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Committee concluded that lufenuron is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that lufenuron is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study, rats received lufenuron in their diet at a concentration of 0, 5, 25, 100 or 250 mg/kg feed (equal to 0, 0.41, 2.1, 8.3 and 20.9 mg/kg bw per day for males and 0, 0.44, 2.2, 8.9 and 22.2 mg/kg bw per day for females, respectively, based on mean intakes for combined P and F_1 generations during the premating period). The NOAEL for parental and reproductive effects was 250 mg/kg feed (equal to 20.9 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 100 mg/kg feed (equal to 8.3 mg/kg bw per day) based on the slight delay in righting reflex in pups at 250 mg/kg feed (equal to 20.9 mg/kg bw per day).

In a study of developmental toxicity, rats were administered lufenuron via gavage at a dose of 0, 100, 500 or 1000 mg/kg bw per day. The NOAEL for maternal toxicity was 500 mg/kg bw per day based on a transient reduction in body-weight gain on gestation days 7–9 and feed consumption on gestation days 6–9 at 1000 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity, rabbits were dosed at 0, 100, 500 or 1000 mg/kg bw per day with lufenuron via gavage. The NOAEL for maternal toxicity and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

The Committee concluded that lufenuron is not teratogenic.

In a repeated-dose neurotoxicity study, male rats received lufenuron in their diet at 0, 5, 25, 100 or 500 mg/kg feed (equal to 0, 0.26, 1.22, 5.43 and 27.0 mg/kg bw per day, respectively) for 4 months. No systemic toxicity was observed. The NOAEL for neurotoxicity was 100 mg/kg feed (equal to 5.43 mg/kg bw per day) based on spontaneous tonic–clonic convulsions or fasciculations observed in weeks 13–18 and facilitated pentylenetetrazol-induced generalized convulsions at 500 mg/kg feed (equal to 27.0 mg/kg bw per day).

Convulsions were observed in all species after prolonged treatment with lufenuron, owing to saturation of the accumulation in fatty tissues, with subsequent increased lufenuron levels in the brain. The neurotoxic effects are not expected to occur after a single dose.

The Committee concluded that lufenuron is not acutely neurotoxic, but is neurotoxic after prolonged treatment.

A study was performed to determine the effects of 3-week lufenuron treatment on the estrous cycle in female rats and various plasma hormone levels (estradiol, progesterone, corticosterone, aldosterone, prolactin, luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone and testosterone) in male and female rats administered a dietary concentration of 0,

500 or 1500 mg/kg feed (equal to 0, 30.5 and 92.5 mg/kg bw per day for males and 0, 39.4 and 120.1 mg/kg bw per day for females, respectively). The results of this investigation, focused on the pituitary, adrenal and genital organs, suggest that there is no effect of lufenuron on the endocrine system in rats of either sex. This conclusion is supported by the reproductive toxicity study in rats, which showed no effect of lufenuron on any reproductive end-point.

No specific studies on immunotoxicity were submitted. The available repeated-dose studies do not indicate an immunotoxic potential for lufenuron following exposure by the oral route.

Observations in humans

In reports on manufacturing plant personnel, no adverse health effects were noted. Several incident reports indicated no significant toxicity in humans.

Microbiological data

Considering the chemical structure and mode of action of lufenuron, the Committee did not anticipate any adverse effects of lufenuron residues on human gastrointestinal microbiota.

Evaluation

An ADI of 0–0.02 mg/kg bw was established on the basis of the NOAEL of 1.93 mg/kg bw per day for tonic–clonic seizures and findings in lungs, gastrointestinal tract, liver and urinary tract in the 2-year dietary study in rats, using a safety factor of 100.

The Committee concluded that it was not necessary to establish an ARfD for lufenuron in view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

The Committee concluded that for lufenuron there are no specific concerns for less-than-lifetime exposure.

A toxicological monograph was prepared.

Residue evaluation

The Committee reviewed studies on pharmacokinetics and metabolism of lufenuron in laboratory and food-producing animals, including Atlantic salmon and rainbow trout. A radiolabelled lufenuron residue depletion study in Atlantic salmon and a number of non-radiolabelled lufenuron residue depletion studies in Atlantic salmon and rainbow trout were reviewed. In vivo metabolism studies in bluegill and fathead minnow were also reviewed. The analytical method submitted by the sponsor to support the residue monitoring has been assessed.

Data on pharmacokinetics and metabolism

The metabolic pathway of lufenuron is similar in rodents (rats), poultry (chicken) and ruminants (goat). Lufenuron is metabolized to a very limited extent by cleavage of the benzoyl ureido bridge to 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea and 2,6-difluorobenzoic acid, and by cleavage of the urea bridge to the 2,6-difluorobenzamide. In fish, lufenuron is not metabolized and the main route of elimination is via faeces.

Atlantic salmon

Six GLP-compliant studies were conducted with unlabelled lufenuron in different geographical locations: Canada (three studies), Norway (two studies) and Chile (one study). Atlantic salmon smolts (mixed sex; average weight in the range of 62–125 g at the beginning of the treatment), held in a freshwater hatchery, were treated with lufenuron via feed at a nominal dose of 5 mg/kg bw for 7 consecutive days. Fish were transferred to sea cages after the end of treatment and kept in sea water until the end of the study. Water temperatures varied according to the geographical site where the studies were conducted: Canada (0.7–16 ºC), Norway (3.7–15.6 ºC) and Chile (9–18 ºC). Blood samples were collected up to 12 months after the last dose, and lufenuron was quantified by a validated UHPLC–MS/MS method (LOQ in blood of 10 ng/mL and in fillet [muscle plus skin in natural proportion] of 50 µg/kg). Lufenuron was rapidly absorbed, widely distributed throughout the fish and slowly eliminated primarily via faeces. It was noted that residues were mainly concentrated in fat. Blood and fillet profiles showed parallel depletion in all studies. For the study conducted in Chile, half-life estimates of lufenuron for fillet and blood were 568.1 degree-days (43.4 days) and 635.1 degree-days (53.7 days), respectively, based on an analysis that excluded some residues (the early and later time points of the depletion curves).

One GLP-compliant study evaluated the metabolism of lufenuron in Atlantic salmon. In this study, post-smolt salmon (107–185 g), held at water temperature of 4–14 °C, received a dose of 5.34 mg/kg bw of [^{14}C]lufenuron for 7 consecutive days via medicated feed. Ten fish were sampled at 1, 30, 90 and 178 days post dosing. Samples of fillet, liver, hindgut and residual carcasses were collected, and the TRR determined by liquid scintillation counting and [^{14}C]lufenuron quantified by HPLC with radiometric detector. The TRR was almost completely extracted from fillet and faeces (>91.2%). The TRR declined progressively from day 1 to 178 post dose in all tissues. Lufenuron was the only residue detected in all samples analysed, accounting for 79.2–95.3% of the TRR in the salmon fillet. The mean concentration of TRR in fillet (24.8 ± 7.7 mg equivalents/kg [eq/kg]) was determined 1 day post dose. The mean concentrations on days 30, 90 and 178 were 14.3 ± 8.2, 10.5 ± 5.1 and 2.7 ± 1.4 mg eq/kg, respectively. In addition, the concentration of TRR was also determined

in blood, bladder, brain, brown fat, muscle, gill-arch, gill-rakes, gill-whole, heart muscle, intestine wall, kidney, liver, skeleton, spinal cord, spleen, stomach wall, swim bladder wall and white fat (mean of three fish) at 35, 90 and 178 days post dose. Lufenuron was excreted unchanged in the faeces, and no metabolism was detected in Atlantic salmon over the 6-month period. Quantitative whole body autoradiography analysis of longitudinal fish sections indicated that lufenuron is located primarily in the fat and viscera. The TRR data indicate a slow depletion of radioactivity in all tissues. The highest concentration of TRR in white fat was determined with a mean concentration of 67.6 ± 35.9 mg eq/kg at 35 days post dose. The high extractability of radioactivity, which was entirely associated with lufenuron, indicated that there were no other residues.

Rainbow trout

In a GLP-compliant study conducted in Chile, two groups of rainbow trout (*Oncorhynchus mykiss*) smolts (average weight 212 g at the beginning of the treatment) were treated with lufenuron-medicated feed at nominal doses of 5 mg/kg bw or 10 mg/kg bw for 7 consecutive days. Fish were transferred to sea cages after the end of treatment and kept in sea water until the end of the study. The seawater temperature ranged from 9.2 to 17.1 ºC. Blood samples drawn from the caudal vein were collected at 1, 6, 21, 49, 78, 106, 134, 163, 190, 218, 245 and 275 days after the final dose. Six fish per time point were sampled. Based on data from days 78 to 275 days post final dose, the half-life of lufenuron in blood was calculated to be 591.1 degree-days or 54.1 days for the 5 mg/kg bw dose group and 584.1 degree-days or 53.4 days for the 10 mg/kg bw dose group.

Based on the results of these studies, the Committee identified lufenuron as the marker residue in salmon and rainbow trout fillet and determined that a value of 1.0 was appropriate for the MR:TRR.

Residue data

The Committee reviewed residue depletion studies for Atlantic salmon and rainbow trout. Although a radiolabelled study in salmon was provided, the results of this study were not used because the growth of fish was impaired, which is characteristic of fish held in confined tanks.

Atlantic salmon

Six GLP-compliant residue depletion studies were submitted. In all the studies, Atlantic salmon were treated at a nominal dose of 5 mg/kg bw per day for 7 days via medicated feed and then transferred to sea cages and kept in sea water until the end of the studies. The fish were harvested at various time points after treatment. Lufenuron was quantified in fillet using a validated UHPLC–MS/MS method. Recoveries were above 90%, and the LOQ of the method was 50 μg/kg.

In a study carried out in Chile (*CHI-12-002*), Atlantic salmon smolts (average weight 125 g) received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose of 33.9 mg/kg bw) via medicated feed. The seawater temperature ranged from 9 to 18 ºC. Fillet samples were collected at 15 time points. The mean concentrations of lufenuron in fillet are shown in Table 9.

Two studies were carried out in Norway. In the first (*NAH-14-14, Voldnes Sea*), Atlantic salmon smolts (average weight 102 g) received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose of 36.2 mg/kg bw) via medicated feed. The seawater temperature ranged from 5.8 to 15.6 ºC. Fillet samples were collected on days 104, 167, 222, 246, 279 and 347 post treatment. The maximum individual lufenuron concentration was determined at the first time point (4300 µg/kg). In the second study (*NAH-14-114, Langholmen Sea)*, the seawater temperature ranged from 3.7 to 13.3 ºC and the smolts (mean body weight 98 g) received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose of 35.0 mg/kg bw) via medicated feed. Fillet samples were collected on days 62, 127, 184 and 246 post treatment. The highest individual lufenuron concentration was determined on day 62 (15 900 µg/kg). The mean concentrations of lufenuron in fillet in both studies are shown in Table 10.

Three other studies were carried out in Canada. In the first (*GSO-13-005*), Atlantic salmon smolts (average weight 115 g) received a nominal dose of lufenuron of 5 mg/kg bw per day for 7 days (actual total dose of 33.9 mg/kg bw) via medicated feed. The seawater temperature ranged from 1.7 to 13.7 ºC. Fillet samples from six fish were collected at each time point (1, 8, 37, 96, 192, 283 and 369 days post dose) except at the last time point (369 days) when only two fish were sampled. In the second study carried out in Canada (*NAH-13-071*), Atlantic salmon smolts (average weight 88 g) received a dose of 5 mg/kg bw per day for 7 days (actual total dose of 34.2 mg/kg bw) via medicated feed. The seawater temperature ranged from 0.7 to 13.2 ºC. Fillet samples from 12 fish were collected at four time points post final dose. The mean concentrations of lufenuron in fillet in both studies are shown in Table 11.

In the third study carried out in Canada (*NAH-15-014*), Atlantic salmon smolts (average weight 62 g) received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose of 33.8 mg/kg bw) via medicated feed. The seawater temperature ranged from 4 to 16 ºC. The fish growth in the hatchery had been delayed due to the unusually low temperature of the fresh water. Fillet samples were collected after 2, 4, 6 and 8 months post treatment. The concentrations of lufenuron were 3659 ± 2295, 492 ± 342, 96 ± 78 and 110 ± 56 µg/kg, respectively. Unlike the previous study results, it was not possible to establish the depletion curve due to the difficulty in assigning correct degree-days because the sampling times were reported in months.

Table 9

Mean concentration of lufenuron in Atlantic salmon fillet (study CHI-12-002)[a]

Time post dose (days)	21	49	76	104	132	160	189	217	246	274	315	343	371	401	430
Concentration (µg/kg)	8 349	4 908	2 908	734	813	322	167	111	109	123	83	94	54	72	59
SD	4 178	2 651	1 700	320	473	179	90	47	38	81	25	38	4	13	–

bw: body weight; SD: standard deviation

[a] Smolts (average body weight at start of study 125 g) kept in sea water at 9–18 ºC received a daily dose of 5 mg/kg bw in feed for 7 days (actual total dose 33.9 mg/kg bw).

Table 10

Mean concentration of lufenuron in Atlantic salmon fillet (study NAH-14-114)

Time post dose (days)	Voldnes Sea[a]					Langholmen Sea[b]			
	104	167	222	279	347	62	127	184	246
Concentration (µg/kg)	2 498	1 255	862	446	158	10 981	2 010	438	178
SD	843	526	547	154	56	3 858	358	88	149

bw: body weight; SD: standard deviation

[a] Smolts (average weight at start of study 102 g) kept in in sea water at 5.8–15.6 ºC received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose 36.2 mg/kg bw).

[b] Smolts (mean body weight at start of study 98 g) kept in sea water at 3.7–13.3 ºC received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose 35.0 mg/kg bw).

Rainbow trout

One GLP-compliant study (*CHI-12-003*) was provided. Rainbow trout (*O. mykiss*) smolts (average weight 212 g) held in freshwater hatchery tanks received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose 35.0 mg/kg bw). At 8 days post treatment, the fish were transferred to sea cages and kept in sea water until the end of the study. The seawater temperature ranged from 9.2 to 17.1 ºC. Whole fillet samples were collected at days 21, 49, 78, 106, 134, 163, 190 and 245 post treatment. Four to six fish were sampled at each time point, and lufenuron quantified by a validated UHPLC–MS/MS method. The highest individual lufenuron concentration was determined on day 49 (12 800 µg/kg) (Table 12).

Analytical methods

The Committee assessed the validation data against the requirements for analytical methods as published in the Codex Guideline CAC/GL 71-2009 *(23)*.

Lufenuron was determined by a validated UHPLC–MS/MS method using a matrix-matched calibration curve. In summary, the fish fillet is mechanically

Table 11

Mean concentration of lufenuron in Atlantic salmon fillet (study GSO-13-005 and NAH-13-071)

Time post dose (days)	Study GSO-13-005[a]							Study NAH-13-071[b]			
	1	8	37	96	192	283	369	127	187	247	307
Concentration (µg/kg)	12 315	14 223	10 428	4 088	2 693	564	77	3 023	1 964	776	324
SD	3 342	4 766	3 594	1 261	1 187	378	19	858	958	464	283

bw: body weight; SD: standard deviation

[a] Six fish (average weight at start of the study 115 g) kept in in sea water at 1.7–13.7 ºC received a nominal dose of 5 mg/kg bw per day for 7 days (actual total dose of 33.9 mg/kg bw) sampled per time point. On day 369, only two fish were sampled.

[b] Twelve fish (average weight at start of the study 88 g) received a dose of 5 mg/kg bw per day for 7 days (actual total dose 34.2 mg/kg bw) sampled per time point.

Table 12

Mean concentration of lufenuron in rainbow trout fillet

Time post dose (days)	21	49	78	106	134	163	190	245
Concentration (µg/kg)[a]	7 142	5 892	1 451	1 359	728	711	586	372
SD (µg/kg)	2 148	3 635	381	633	485	396	164	181

bw: body weight; SD: standard deviation.

[a] Four to six trout (average weight at start of the study 212 g) at a nominal dose of lufenuron of 5 mg/kg bw per day for 7 days (actual total dose of 35.0 mg/kg bw) sampled per time point.

homogenized and fluazuron (*N*-((4-chloro-3-((3-chloro-5-(trifluoromethyl) pyridin-2-yl)oxy)phenyl)carbamoyl)-2,6-difluorobenz-amide) is added as the internal standard. The analytes are extracted from the sample with acetonitrile. After centrifugation, the supernatant is diluted with water and loaded onto a C18 solid-phase extraction cartridge. The sorbent is washed with acetonitrile:water at 30:70 v/v and dried under vacuum, and the retained analytes are eluted with acetonitrile:water 70:30 v/v. The eluate is filtered and analysed by UHPLC–MS/MS using the electrospray ionization source in the negative mode. The chromatographic separation is carried out on a reversed phase CSH (charged surface hybrid) C18 column and a mobile phase of formic acid and acetonitrile under gradient elution. Quantitation is performed using acquisition of ions in the selected reaction-monitoring mode, using the transitions of m/z 508.9→326.0 for lufenuron and m/z 504.1→305.1 for fluazuron. For identification and identity confirmation two additional transitions for lufenuron were monitored: m/z 508.9→174.0 and m/z 508.9→201.9. A plot of the area–response ratio of lufenuron to fluazuron versus concentration of lufenuron was fitted to a quadratic

regression model with 1/× weighting. Linearity was in the range of 2.5–200 ng/mL (equivalent to 50–4000 µg/kg).

The method was validated for the determination of lufenuron in salmon and rainbow trout fillet. The linear range was from 50 to 4000 µg/kg, with a linear correlation coefficient greater than 0.99. The precision and accuracy were evaluated at three concentration levels: 50, 600 and 3500 µg/kg. The within-run precision ranged from 3.0% to 14.1% and the between-run precision is acceptable with a coefficient of variance of less than 8.9%. The accuracy ranged from 98.9% to 106.7%. The matrix effect of lufenuron and the internal standard was less than +19% and the recovery in the range of 90–93%.

The estimated LOQ was 18 and 21 µg/kg for salmon and trout fillet, respectively. However, for this method the lowest calibration point was 50 µg/kg and this was set as the LOQ. The selectivity of the method was demonstrated and also incurred samples were used in the validation procedure.

The method is fully validated and suitable for the depletion studies summarized above. However, the Committee noted that fluazuron, an approved veterinary drug, could affect the analytical result and therefore may not be an appropriate internal standard for the method used for monitoring purposes.

Maximum residue limits

In recommending MRLs for lufenuron in salmon and trout fillet, the Committee considered the following factors:

- An ADI of 0–0.02 mg/kg bw was established.
- An ARfD was considered unnecessary.
- The Committee considered that there are no specific concerns for less-than-lifetime exposure.
- Lufenuron is used as both a pesticide and a veterinary drug.
- Lufenuron is authorized for use in salmon in one Member State. The maximum recommended dose is 5 mg/kg bw per day for 7 consecutive days, administered through medicated feed. The withdrawal period is 2050 degree-days.
- Lufenuron is the marker residue in fillet.
- An MR:TRR value of 1.0 was calculated.
- Residue data for salmon and trout were provided using a validated analytical method to quantify lufenuron in fillet.
- A validated analytical method for the determination of lufenuron in salmon and trout fillet is available and may be used for monitoring purposes, with the reservation noted (see "Analytical methods").

An MRL was calculated based on the 95/95 UTL in salmon fillet derived from the data provided, in accordance with GVP and a withdrawal period of 2050 degree-days.

The Committee recommended an MRL of 1350 µg/kg for lufenuron in salmon fillet.

Using the same approach, the Committee recommended an identical MRL for trout should lufenuron be approved for use in trout in Member States. This MRL is compatible with a withdrawal period of 2643 degree-days based on the residue data provided.

The Committee could not extrapolate the MRL to other fish species or to finfish in general considering that:

- lufenuron is a lipophilic drug and its concentration is higher in fatty tissues, the fat content in fish depends on the species and growing conditions;
- the decrease in concentration of lufenuron in studies in Atlantic salmon and in rainbow trout is dependent on the time after the final drug administration as well as the increase in body weight, both of which are dependent on the water temperature; and
- no depletion data were provided for species other than salmonids.

A residue monograph was prepared.

Estimated dietary exposure

Exposure to lufenuron residues may occur through its use as a pesticide as well as its use as a veterinary drug.

Dietary exposure from pesticide residues (IEDI)

When used as a pesticide, the exposure of lufenuron was 0–4% of the upper bound of the ADI.

Dietary exposure from veterinary drug residues (GECDE)

When used as a veterinary drug, chronic dietary exposure to lufenuron in the general population was estimated. Based on the toxicological profile of the compound, dietary exposure estimates for children, less-than-lifetime dietary exposure or acute dietary exposure were not required.

Dietary exposure was estimated based on the potential occurrence of residues in salmon and trout muscle plus skin in natural proportion. Other finfish were not included in the exposure estimate.

The GECDE for the general population is 1.1 µg/kg bw per day, which represents 5.5% of the upper bound of the ADI of 0.02 mg/kg bw per day. Salmon

was the major contributor to chronic dietary exposure. It should be noted that no reliable high-percentile consumption value was available for trout.

In addition to the accepted GECDE methodology, further calculations 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 mean and ranges across surveys were reported. The mean of 25 estimates for adults or the general population was 0.35 μg/kg bw per day (2% of the upper bound of the ADI), with a range of 0.0001–1.1 μg/kg bw per day (<0.01–5.5% of the upper bound of the ADI).

Combined chronic dietary exposure from pesticide and veterinary drug residues (extended GECDE)

Modified methods based on the GECDE were used to estimate combined chronic dietary exposure. The usual GECDE approach was extended to include additional commodities that were assessed for the compound by JMPR ("extended GECDE"). It should be noted that this new exposure assessment methodology is still being piloted. It should also be noted that the median residues used as inputs were extracted from JMPR publications and have not been validated for this assessment.

Combined chronic dietary exposure from veterinary drug and pesticide residues was considered for the general population based on the potential occurrence of residues in salmon, trout, mammalian meat, mammalian fats, edible mammalian offal, milk, poultry meat, poultry fats, edible poultry offal, eggs and plant products (cucumber, melons, except watermelon, sweet pepper, potato, tomato, tomato juice and tomato products).

The extended GECDE for the general population is 1.8 μg/kg bw per day, which represents 9% of the upper bound of the ADI of 0.02 mg/kg bw per day. Milk is the major contributors to combined lufenuron chronic dietary exposure.

Summary and conclusions
Uncertainty factor
100 (10 for interspecies variability and 10 for intraspecies variability)

Toxicological effects
A toxicological ADI of 0–0.02 mg/kg bw could be derived.

Microbiological effects
A microbiological ADI was not derived.

Studies relevant to risk assessment

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				
Eighteen-month toxicity and carcinogenicity study (diet)	Males: 0, 0.222, 2.25, 22.6, 62.9 Females: 0, 0.217, 2.12, 22.0, 61.2	Toxicity: Mortality, tonic–clonic seizures, increased incidence of fatty liver	2.12	22.0
		Carcinogenicity: None	61.2[a]	–
Rat				
Ninety-day toxicity study (diet)	Males: 0, 1.6, 9.68, 101, 998 Females: 0, 0, 1.7, 10.2, 103, 1 050	Tonic–clonic seizures, increased adrenal weights	9.68	101
Two-year toxicity and carcinogenicity study (diet)	Males: 0, 0.19, 1.93, 20.4, 108 Females: 0, 0.23, 2.34, 24.8, 114	**Tonic–clonic seizures, histopathological effects on lungs, liver, non-glandular stomach, intestines and urinary tract**	1.93*	20.4
Two-generation reproductive toxicity study (diet)	Males: 0, 0.41, 2.1, 8.3, 20.9 Females: 0, 0.44, 2.2, 8.9 and 22.2	Reproductive toxicity: None	20.9[a]	–
		Parental toxicity: None	20.9[a]	–
		Offspring toxicity: Slight delay in righting reflex	8.3	20.9
Developmental toxicity study (gavage)	0, 100, 500, 1 000	Maternal: Transient reduction in body-weight gain and feed consumption	500	1 000
		Embryo/fetal toxicity: None	1 000[a]	–
Repeated-dose neurotoxicity study (diet)	Males: 0, 0.26, 1.22, 5.43, 27.0	Tonic–clonic seizures	5.43	27.0
Rabbit				
Developmental toxicity study (gavage)	0, 100, 500, 1 000	Maternal toxicity: None	1 000[a]	–
		Embryo/fetal toxicity: None	1 000[a]	–
Dog				
One-year toxicity studies (diet)[b]	Study 1: Males: 0, 3.97, 65.4, 1 880 Females: 0, 3.64, 78.3, 1 980 Study 2: Males: 0, 0.31, 1.42, 7.02, 29.8 Females: 0, 0.33, 1.55, 7.72, 31.8	Toxicity: mortality, convulsions, reduced body-weight gains and histopathological lesions in adrenals, liver, thyroid and lungs and/or clinical chemistry changes	7.02[c]	29.8[d]

* **Pivotal study value for the derivation of the ADI (*26*)**
[a] Highest dose tested.
[b] Two or more studies combined.
[c] Overall NOAEL.
[d] Overall LOAEL.
[e] Lowest dose tested

ADI (based on toxicological effects)

0–0.02 mg/kg bw

Residue definition

The marker residue in salmon and trout fillet is lufenuron.

MRLs

The Committee recommended an MRL for lufenuron in salmon fillet (muscle plus skin in natural proportion) of 1350 μg/kg. The same MRL could be recommended for trout should lufenuron be approved for use in trout in Member States.

Estimated dietary exposure

Based on the toxicological profile of the compound, dietary exposure estimates for children, less-than-lifetime dietary exposure or acute dietary exposure were not required.

Estimated dietary exposure

Dietary exposure was estimated based on the potential occurrence of residues in salmon and trout muscle plus skin in natural proportion. Other finfish were not included in the exposure estimate.

The GECDE for the general population is 1.1 μg/kg bw per day, which represents 5.5% of the upper bound of the ADI of 0.02 mg/kg bw per day. Salmon was the major contributor to chronic dietary exposure. It should be noted that no reliable high-percentile consumption value was available for trout.

3.7 Monepantel

Explanation

Monepantel (CAS No. 887148-69-8) is an anthelminthic of the amino-acetonitrile derivative class. In the form of an oral drench, it is used for the treatment of nematodes in sheep. Monepantel is the *S*-enantiomer of an optically active molecule. It exerts its nematocidal action through activation of a nematode-specific subfamily of nicotinic acetylcholine receptors. Monepantel was previously reviewed by the Committee at its seventy-fifth meeting, and an ADI of 0–20 μg/kg bw per day (0–0.02 mg/kg bw per day) was established, corresponding to an upper bound of acceptable intake of 1200 μg/day for a 60 kg person (Annex 1, reference *208*).

The ADI was established on the basis of a NOAEL of 1.8 mg/kg bw per day for an increased incidence of fatty change in the liver in female mice at 5.5

mg/kg bw per day in a 78-week oral dosing study, with an application of a safety factor of 100 to account for interspecies and intraspecies variability and rounding to one significant figure.

At the seventy-fifth meeting, the Committee recommended MRLs, determined as monepantel sulfone, in sheep tissues of 300 μg/kg in muscle, 700 μg/kg in kidney and 3000 μg/kg in liver and 5500 μg/kg in fat. The Twentieth Session of the CCRVDF requested JECFA to consider if higher MRLs (700 μg/kg in muscle, 5000 μg/kg in liver, 2000 μg/kg in kidney and 7000 μg/kg in fat) were compatible with the ADI and consistent with the JECFA process of MRL derivation *(27)*. Monepantel was subsequently reviewed by the Committee at its seventy-eighth meeting, and the ADI of 0–20 μg/kg bw (0–0.02 mg/kg bw per day) was confirmed (Annex 1, reference *217*). The Committee recommended revised MRLs of 500 μg/kg in muscle, 1700 μg/kg in kidney, 7000 μg/kg in liver and 13 000 μg/kg in fat. These were adopted at the Thirty-eighth session of Codex Alimentarius Commission in 2015 *(28)*.

The Committee evaluated monepantel at the present meeting at the request of the Twenty-third Session of CCRVDF, with a view to establishing MRLs in cattle tissues (not including milk) *(2)*. As JECFA had implemented the recently published *Guidance document for the establishment of Acute Reference Dose (ARfD) for veterinary drug residues in food (11)*, the Committee evaluated the acute effects of monepantel to consider the need to establish an ARfD.

Toxicological and microbiological evaluation

The Committee considered the 2012 JECFA evaluations (Annex 1, reference *208*) of studies on the short-term and long-term, reproductive and developmental toxicity, genotoxicity and carcinogenicity of monepantel. The Committee also considered information found as a result of literature searches on monepantel. All critical studies contained statements of compliance with GLP.

Biochemical aspects

Absorption and elimination of a single oral dose of monepantel at 2.5 mg/kg bw were rapid in rats. Monepantel showed significant first-pass metabolism with approximately 30% absorption and 9.4% bioavailability. T_{max} in blood was between 2 and 8 hours after oral administration in rats.

Toxicological data

Single-dose toxicity studies were conducted in rats and sheep. The oral LD_{50} was greater than 2000 mg/kg bw in rats.

A single oral or intraduodenal treatment with monepantel at 2000 mg/kg bw in rats did not induce any significant changes in intestinal motility, stomach

weight, general behaviour, cardiovascular parameters and respiratory rates and volumes of respiration.

A single oral dose of 37.5 mg/kg bw, or repeated oral doses of 0, 3.75, 11.25 or 18.75 mg/kg bw administered to sheep every 3 weeks a total of eight times, had no effect on clinical signs, body weight or haematological, blood biochemistry and histological findings at 6, 14 and 21 days after exposure.

In a single escalating-dose study, sheep were treated orally with doses of monepantel at 0, 50, 75 or 100 and 125 mg/kg bw, administered at 14-day intervals. There were no treatment-related effects on body weight, feed consumption, body temperature, heart rate, behaviour, respiratory rate or sounds, general health and behaviour, skin and mucous membranes, digestion and posture, clinical chemistry and haematology over the 21-day observation period.

In a 13-week study in mice fed monepantel at dietary concentrations of 0, 30, 120, 600 or 6000 mg/kg feed (equal to 0, 4.53, 18.0, 97.5 and 959 mg/kg bw per day for males and 0, 5.27, 22.0, 115 and 1210 mg/kg bw per day for females, respectively), no clinical signs were observed.

In a 4-week study in rats fed monepantel at dietary concentrations of 0, 1000, 4000 or 12 000 mg/kg feed (equal to 0, 86, 346 and 1040 mg/kg bw per day for males and 0, 90, 362 and 1020 mg/kg bw per day in females, respectively), no clinical effects were seen during the first week of treatment.

In a 13-week study in rats fed monepantel at dietary concentrations of 0, 50, 200, 1000 or 12 000 mg/kg feed (equal to 0, 3.61, 14.6, 73.6 and 900 mg/kg bw per day for males and 0, 3.98, 15.2, 81.5 and 947 mg/kg bw per day for females, respectively), no clinical effects were seen.

In a 4-week dose range–finding study in dogs fed monepantel at dietary concentrations of 0, 5000, 15 000 or 40 000 mg/kg feed (equal to 0, 161, 566 and 1220 mg/kg bw per day in males and 0, 184, 561 and 1470 mg/kg bw per day in females, respectively), no clinical signs were observed.

In a 13-week study in dogs fed monepantel at dietary concentrations of 0, 300, 3000 or 30 000 mg/kg feed (equal to 0, 9.90, 107 and 963 mg/kg bw per day for males and 0, 10.7, 96.8 and 1180 mg/kg bw per day for females, respectively), no clinical signs were seen during daily observations.

Although systemic toxicity was observed in some of the studies described above, the Committee concluded that none of the effects seen are likely to occur following acute exposures.

Monepantel was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Committee concluded that monepantel is unlikely to be genotoxic.

In a two-generation reproductive toxicity study in rats fed monepantel at dietary concentrations of 0, 200, 1500 or 12 000 mg/kg feed (equal to, respectively, 0, 13.3, 99.8 and 798 mg/kg bw per day for F_0 males; 0, 15.8, 119 and 950 mg/

kg bw per day for F_0 females; 0, 16.8, 125 and 1010 mg/kg bw per day for F_1 males; and 0, 18.6, 141 and 1110 mg/kg bw per day for F_1 females), no evidence of clinical signs and reproductive toxicity was found.

In developmental toxicity studies in rats and rabbits with oral doses, by gavage, of up to 1000 mg/kg bw per day from gestation day 6 to 20 (in rats) and from gestation day 6 to 27 (in rabbits), no adverse effects were seen in maternal or fetal animals. The NOAEL for maternal toxicity and for embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested, in both rats and rabbits.

The Committee concluded that monepantel is not teratogenic in rats or rabbits.

Monepantel does not have any microbiological effects. Considering the chemical structure and the mode of action of monepantel, the Committee did not anticipate any adverse effects of monepantel residues on the human gastrointestinal microbiota.

Evaluation

Overall, no toxicological effects were seen after a single oral dose of monepantel up to 2000 mg/kg bw in rats or 125 mg/kg bw in sheep. There were no acute effects below the cut-off level of 500 mg/kg bw. In addition, monepantel is not administered by injection in target animals, and therefore exposure to high residue levels at injection sites will not occur.

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

Residue evaluation

The Committee reviewed studies on pharmacokinetics and metabolism of monepantel in laboratory and food-producing animals, including studies originally provided to the seventy-fifth meeting of JECFA. In addition, radiolabelled and non-radiolabelled monepantel residue depletion studies in cattle were reviewed. The analytical method submitted to support the residue monitoring has been assessed.

Data on pharmacokinetics and metabolism

The pharmacokinetics of monepantel in laboratory animals were evaluated by the Committee at the seventy-fifth meeting.

A GLP-compliant oral ADME study was conducted in cattle using [^{14}C]monepantel (3.75 mg/kg bw). Systemic absorption was relatively high, with TRR reaching 216 μg eq/kg in blood and 268 μg eq/kg in plasma at 24 hours after dosing. Absolute bioavailability was not determined in the study. Initial elimination was rapid with a half-life of about 36 hours, but this slowed progressively at later times. Residues of approximately 5 μg eq/kg were still detectable in blood and plasma at the final sampling time point. Bile TRR was

highest on day 3 (~4500 µg eq/kg) but declined progressively to approximately 100 µg/kg on day 21. Approximately 21% and 36% of the dose was eliminated in the urine and faeces, respectively, over 3 days, with the remaining compound distributed in the tissues.

In a second GLP-compliant study, the pharmacokinetic profiles of monepantel and monepantel sulfone in blood were investigated in cattle dosed orally three times, 21 days apart, at 3.75 mg/kg bw monepantel, as part of the pivotal cold residue depletion study. Monepantel sulfone was the dominant residue in the blood. Concentrations peaked at 24 hours after each dosing and declined thereafter, with a terminal half-life of approximately 3 days. Monepantel concentrations peaked at approximately 24 hours after the first and second dosing, with the maximum concentration of monepantel occurring 12 hours after the third dosing. Monepantel residues were less than the LOQ (0.25 ng/mL) at day 13. No accumulation of monepantel and monepantel sulfone in blood was evident following repeated administration of the test item. Overall, the blood profile was similar to that observed in the cattle ADME study where only TRR was measured.

The pharmacokinetics of monepantel and monepantel sulfone in the blood and plasma of sheep were evaluated by the Committee at the seventy-fifth meeting.

The ADME of monepantel in the rat was also evaluated by the Committee at its seventy-fifth meeting. Metabolism of monepantel in the rat proceeds by rapid oxidation of sulfur to the sulfone with slower hydroxylation of the phenoxyl ring. Excretion of the sulfate or glucuronide conjugates of these metabolites is via the urine and faeces. (For a summary of the metabolic pathways in the rat, see Annex 1, reference *208*).

Samples of edible tissues, blood, bile and excreta from the GLP-compliant ADME study in cattle were investigated for extractability of residues and metabolite profiles. Radioactivity was extracted readily from fat, kidney and muscle with simple solvent extraction. Less than 5% of the TRR was unextractable. Liver residues proved more difficult to extract, especially at later sampling times. Unextracted residues increased from 37% at day 3 to 75% at day 14. Simple solvent extraction could remove about half of the TRR at day 3 but this dropped to approximately 13% on day 21. Extracted day 21 TRR was too low to proceed with metabolite profiling. The higher bound residues in cattle liver are consistent with the shorter terminal half-life of monepantel sulfone in the blood of cattle (~3 days following three oral doses of monepantel) compared with the terminal half-life of monepantel sulfone in the blood of sheep (nearly 6 days after a single intravenous dose of monepantel or more than 4 days after a single intravenous dose of monepantel sulfone).

The major component present in all tissues co-eluted with monepantel sulfone. Monepantel was a minor constituent, present only at the early time points in fat, liver and kidney and was not detected in the muscle. Minor unknown components of high polarity were detected in the liver, kidney and muscle. In liver, these metabolites (designated L1 and L2) were determined to be from cleavage of previously identified metabolites. Kidney profiles were similar to those in liver, including the presence of small amounts of the two cleavage metabolites, L1 and L2. A third polar metabolite was observed only at day 3, and it was less than 5% of the TRR. A component detected only in fat was determined to be the cyclized product of a previously identified metabolite. The small amount of TRR in muscle limited profiling to the day 3 samples. Only a trace of L1 was detected. The major metabolite in blood was monepantel sulfone. There was a small amount of monepantel and several minor metabolites. The metabolic profile in bile was complex. In addition to monepantel, there was a trace of monepantel sulfone. Bile contained a number of polar compounds, including a peak identified as L1. In urine, there was one polar metabolite and four minor polar peaks. No monepantel, monepantel sulfone or hydroxylated monepantel sulfone was detected. Faecal residues were extracted easily with simple solvent extraction (>80%).

The metabolism of monepantel in tissues of sheep was evaluated by the Committee at the seventy-fifth meeting.

A non-GLP in vitro comparative metabolism study was conducted to investigate the metabolism of monepantel in rat, cattle and sheep hepatocytes. ^{14}C-labelled monepantel was used in the study. The activity of rat and bovine hepatocytes was consistent with historical controls. Sheep hepatocytes were about 30% less active than hepatocytes from cattle and rats, but there were no historical data for comparison. Following quenching, incubations were profiled by HPLC with radiodetection. Metabolites in these extracts were identified by LC–MS techniques, and the extent of covalent binding was determined by further extracting the protein pellet with various solvents.

Covalent binding was minimal but occurred in all three species. Binding was highest in bovine hepatocytes, intermediate in rat hepatocytes and lowest in sheep hepatocytes. Radioactivity recovered in the initial methanol extracts of the rat, bovine and sheep incubations at 1, 2 and 4 hours ranged from 84% to 93% of the applied radioactivity or 96% to 105% of the time 0 radioactivity. Results were comparable across the species.

Metabolism was greatest with rat hepatocytes. Less than 1% monepantel remained after 2 hours incubation. With bovine and sheep hepatocytes, 13% and 66% of monepantel remained after 4 hours. Incubations with rat, sheep and cattle hepatocytes resulted in qualitatively similar metabolic profiles. Three new metabolites were observed in the in vitro study, one in rat hepatocytes and two

in cattle hepatocytes. No new metabolites were observed in sheep hepatocytes. Reasonable metabolic pathways were used with the mass spectrometry data to propose the metabolic structures. The two new metabolites found in cattle hepatocytes were present at very low concentrations (≤5% extracted radioactivity). In addition, these new metabolites are likely of no toxicological significance because, based on the presumptive metabolite identification, they can be hydrolysed back to previously identified metabolites. Five chemical adducts were observed in 0-hour incubation samples from cattle, were observed for only the dominant peaks in the metabolic profile, and did not increase over time. The adducts were concluded to be chemical artefacts formed during the sample workup procedure.

Residue data

Selected samples of edible tissues from the GLP-compliant ADME study were investigated for TRR. After a single oral dose of monepantel at 3.75 mg/kg bw, the TRR was determined using combustion and liquid scintillation counting. The calculated TRR half-lives were 4–5 days for fat, kidney and muscle, but about 10 days for liver, consistent with presence of bound residues. The order of residue concentrations in tissues was fat > liver > kidney > muscle and was the same as seen previously for sheep. As the major residue is monepantel sulfone, it is the marker residue identified for monepantel in cattle tissues. It also is the marker residue previously identified for sheep tissues. The MR:TRR in cattle tissues was determined as part of the method validation study. The MR:TRR for muscle, 67%, could be determined only for the initial sampling time, at day 3. In fat, the MR:TRR values were 84%, 88%, 79% and 70% at days 3, 7, 14 and 21, respectively. In kidney, the MR:TRR values were 55% and 62% at days 3 and 7, respectively, the only sampling times when the MR:TRR could be determined. In liver, the MR:TRR was low; 30%, 20%, 13% and 6% at days 3, 7, 14 and 21, respectively.

No radiolabelled residue data on the use of monepantel in lactating dairy cattle were provided; radiolabelled data on the use of monepantel in sheep were evaluated by the Committee at its seventy-fifth meeting.

Two GLP-compliant residue depletion studies were conducted in cattle using unlabelled monepantel. In the first study, cattle were treated orally three times at a nominal dose of monepantel at 3.75 mg/kg bw. Doses were administered 21 days apart. Groups of five animals were slaughtered 4, 7, 10 and 13 days after the final treatment. Samples of muscle, kidney, liver, renal fat and subcutaneous fat were collected and analysed for monepantel sulfone. Concentrations of monepantel sulfone residues were highest in the day 4 samples: 4110, 4680, 1090, 478 and 231 µg/kg for subcutaneous fat, renal fat, liver, kidney and muscle, respectively. At the final sampling time, maximum residues were 1720, 770, 146, 61.8 and 22.8 µg/kg for subcutaneous fat, renal fat, liver, kidney

and muscle, respectively. Although initial residue concentrations were the highest in renal fat, by the later sampling times residue concentrations were highest in subcutaneous fat. The half-lives for muscle, kidney, liver, renal and subcutaneous fat were estimated to be 2.8, 2.7, 2.7, 2.9 and 5.1 days, respectively. The second study employed the same dosing design but animals were not slaughtered until 21, 42, 56 and 85 days after the final treatment. The maximum monepantel sulfone residue concentrations were observed in the day 21 samples: 1010, 583, 143, 63.4 and 14.5 µg/kg for subcutaneous fat, renal fat, liver, kidney and muscle, respectively. At the final sampling time, all residues were below the method LOQ of 5 µg/kg). The incurred residues at the first sampling time in the second study (21 days post treatment) were generally consistent with the depletion trend seen for the incurred residues at the last sampling time in the first study (13 days post treatment).

No unlabelled residue depletion data on the use of monepantel in lactating dairy cattle were submitted; unlabelled depletion data for the use of monepantel in sheep were evaluated by the seventy-fifth meeting of the Committee (Annex 1, reference *208*).

Analytical methods

An analytical procedure, validated under GLP, with an LOQ of 5 µg/kg and demonstrated to be suitable for routine analysis of monepantel in bovine tissue matrices, was available. In addition, the procedure met the requirements of the validation criteria established by the CCRVDF, as contained in CAC/GL 71–2009 *(23)*.

In summary, a ground tissue sample is extracted with acetonitrile and centrifuged, and the supernatant diluted with acetonitrile/methanol/water solvent. Once diluted, the extract is injected into the LC–MS/MS, which is calibrated with injections of varying concentrations of pure standards. Detection is by tandem MS with electrospray ionization in the negative mode. Monepantel sulfone (the marker residue) is quantified with the transition ion (m/z 503.8→186) and its identity confirmed with the two qualifying transitions at m/z 504→166 and m/z 504→146 at the appropriate chromatographic retention time.

There was no interference with co-extractives from various sources of control tissue matrices. Experiments confirmed that the method was selective, would accurately detect negative control samples as negative and that the presence of other veterinary drugs used in food animal production would not interfere with the quantification of monepantel sulfone.

The method met the acceptance criteria for accuracy and precision.

Monepantel sulfone was demonstrated to be stable when stored under normal analytical conditions (i.e. in the autosampler chamber at room or cool room temperature; in matrix at 6 °C; and as an incurred residue after short-term

storage at room temperature, long-term storage at approximately 10–22 °C and over 3 freeze/thaw cycles).

Maximum residue limits

In recommending MRLs for monepantel in cattle, the Committee considered the following factors:

- A complete toxicological re-evaluation was not undertaken at the present meeting. An ADI of 0–0.02 mg/kg bw was previously established by the Committee.
- An ARfD was considered unnecessary.
- The metabolite monepantel sulfone is the marker residue in cattle tissues.
- Fat contains the highest concentration of monepantel sulfone at all sampling times, followed by liver, kidney and muscle. Liver and fat can serve as the target tissues.
- The MR:TRR values are 0.88 in fat, 0.20 in liver and 0.62 in kidney at 7 days post treatment. The MR:TRR in muscle, 0.67, is available only at the first sampling time, 3 days post treatment.
- A correction factor of 0.94 is applied to account for the mass difference between monepantel sulfone (the marker residue) and monepantel.
- A validated analytical method for the determination of monepantel sulfone in edible cattle tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.
- MRLs were calculated on the basis of 95/95 UTL at a withdrawal period of 5 days, which is consistent with GVP.

The Committee recommended MRLs determined as monepantel sulfone, expressed as monepantel, in cattle tissue of 300 μg/kg in muscle, 1000 μg/kg in kidney, 2000 μg/kg in liver and 7000 μg/kg in fat.

An addendum to the residue monograph was prepared.

Estimated dietary exposure

Exposure to monepantel residues is considered to occur only through its use as a veterinary drug because there are no Codex MRLs other than for its veterinary use.

When used as a veterinary drug, dietary exposure was estimated based on the potential occurrence of monepantel residues in the muscle, liver, kidney and fat of sheep and other ovines and cattle and other bovines.

The GECDE for the general population is 13.7 µg/kg bw per day, which represents 68% of the upper bound of the ADI of 0.02 mg/kg bw. The GECDE for children is 5.0 µg/kg bw per day, which represents 25% of the upper bound of the ADI of 0.02 mg/kg bw. The GECDE for infants is 4.4 µg/kg bw per day, which represents 22% of the upper bound of the ADI of 0.02 mg/kg bw.

Summary and conclusions

Residue definition

The marker residue in cattle tissue is monepantel sulfone, expressed as monepantel.

MRLs

The Committee recommended MRLs in cattle tissue of 300 µg/kg in muscle, 1000 µg/kg in kidney, 2000 µg/kg in liver and 7000 µg/kg in fat.

Estimated dietary exposure

The GECDE for the general population is 13.7 µg/kg bw per day, which represents 68% of the upper bound of the ADI of 0.02 mg/kg bw. The GECDE for children is 5.0 µg/kg bw per day, which represents 25% of the upper bound of the ADI of 0.02 mg/kg bw. The GECDE for infants is 4.4 µg/kg bw per day, which represents 22% of the upper bound of the ADI of 0.02 mg/kg bw.

3.8   Sisapronil

Sisapronil was evaluated by JECFA at the eighty-first meeting when it was not possible to establish an ADI because of potential concerns about effects observed in a 3-month repeated-dose oral toxicity study in dogs (Annex 1, reference *226*).

No data were submitted to the present meeting, but the sponsor requested further clarification on alternative ways to address the data gaps.

Additional information that would assist in the further evaluation of sisapronil

There are appreciable differences between rats and dogs in both the toxicokinetics and toxicological effects of sisapronil. Although the half-life of sisapronil was not determined with any accuracy in either species, it is clear that elimination in the dog is much slower than in the rat; while steady state was likely to have been achieved in the rat in the available 1-year repeated-dose oral chronic toxicity study, this was not the case in the dog in the available 3-month study. It would take appreciably longer than 3 months for steady state to be achieved in this species. Although, it has in general been accepted that the repeated-dose oral toxicity of chemicals such as pesticides can be characterized in dogs with only a 3-month study and that

there is no need for a 1-year study, this will not be the case for compounds such as sisapronil that take longer than 3 months to reach steady state.

The target organs in both rat and dog following repeated-dose oral administration of sisapronil were the liver and the thyroid. Although a mode of action has been established for the thyroid (and liver) effects in rat, this is not the case for the dog. The thyroid effects in the rat are due to induction of hepatic conjugation of thyroid hormones, leading to a reduction in circulating hormone levels, de-repression of thyroid-stimulating hormone synthesis and stimulation of the thyroid gland. In the dog, despite histopathological changes in the thyroid gland, there were no changes in the circulating levels either of thyroid hormones or of thyroid-stimulating hormone. No information was available on the effects of sisapronil on hepatic conjugation of thyroid hormones. Hence, the toxicological significance of the effects on the thyroid in dogs could not be dismissed. In the absence of a study in which steady state levels of sisapronil had been achieved, the long-term potency of sisapronil for these effects could not be characterized.

Information on the comparative pharmacokinetics in rats, dogs and humans is not available. In the absence of such information, the Committee made the health-protective assumption that the toxicokinetics of sisapronil in humans might resemble those in dogs. It was not possible for the Committee to interpret the toxicological significance of the findings in the dog in the absence of further information from suitable studies. Hence, the Committee concluded that the findings in dogs should form the basis for the critical NOAEL in the available database for sisapronil but that this hazard has not been adequately characterized.

Information that would assist in the further evaluation of sisapronil include:

- comparative toxicokinetic data in rat, dog and human;
- effects of sisapronil at steady state following repeated-dose oral administration in the dog; and
- determination of the relevance of the effects on the thyroid observed in the dog.

Although not all the toxicokinetic data would necessarily have to be generated in vivo, the approach used would have to be suitably validated (e.g. physiologically based toxicokinetic model verified in vivo in rat and dog).

3.9 Zilpaterol hydrochloride

Explanation

Zilpaterol hydrochloride has been assessed by the Committee at its seventy-eighth and eighty-first meetings (Annex 1, references *217* and *226*) at the request of the

Twenty-first and Twenty-second Sessions of the CCRVDF, respectively *(7, 29)*. The eighty-first meeting of the Committee established an ARfD of 0.04 µg/kg bw (0.000 04 mg/kg bw), the same value as the upper bound of the ADI previously established by the seventy-eighth Committee. MRLs of 3.3 µg/kg in kidney, 3.5 µg/kg in liver and 0.5 µg/kg in muscle were recommended, based on 95/95 UTLs derived at 77 hours post dose.

In their assessment, the Committee considered the limited bioavailability of the non-extractable (bound) portion of incurred residues, as per the Gallo-Torres model *(9)*. A bioavailability correction factor of 0.05 was used for all non-extractable residues in liver, kidney and muscle. The Committee did not accept the sponsor's assertion that the submitted data demonstrated lower bioavailability for all incurred zilpaterol residues in tissue compared with administration of zilpaterol by other oral routes. Therefore, in its assessment of zilpaterol exposure the Committee assumed that all extractable zilpaterol residues were fully bioavailable. It was noted that additional data would be required to conclusively demonstrate reduced oral bioavailability of all (both extractable and non-extractable) incurred zilpaterol residues.

As the sponsor planned to provide JECFA with additional data, the Twenty-third Session of CCRVDF agreed to hold the MRLs at Step 4 so that JECFA could evaluate the additional data and thus provide the best possible risk assessment of the compound *(2)*.

Residue evaluation

Bioavailability of incurred zilpaterol residues

A GLP-compliant study investigating the oral bioavailability of incurred zilpaterol residues from cattle tissues was performed in pigs. The objective was to compare the zilpaterol plasma concentration profile in pigs after oral administration via two different methods: an aqueous solution containing zilpaterol hydrochloride and consumption of edible cattle tissues containing incurred zilpaterol residues. Sixteen male Landrace × Piétrain pigs (67–75 kg) were allocated to one of four treatment groups (*n* = 4 per group):

- A: Gavaged via gastric tube with 53 mL of a 9.34 µg/mL aqueous solution of zilpaterol hydrochloride (0.5 mg zilpaterol hydrochloride per dose, equivalent to 0.44 mg zilpaterol free base);
- B: Offered 440 g of cattle liver containing 0.44 mg incurred zilpaterol (free base) residues;
- C: Offered 246–272 g of cattle kidney containing 0.44 mg incurred zilpaterol (free base) residues; or
- D: Offered 1222–1392 g cattle muscle containing 0.22 mg incurred zilpaterol (free base) residues.

Incurred zilpaterol tissue residues were obtained from an earlier study in cattle treated with zilpaterol hydrochloride at doses ranging from 1.5–2.0 mg/kg bw per day for 12–13 days (study S16308–00). The cattle were slaughtered around the time of anticipated maximum zilpaterol plasma concentration (14–17 hours after the final dose). The high-dose level (10 times the recommended dose) and the early slaughter time point were necessary to ensure sufficient residue concentrations in the cattle tissues the pigs were to consume to enable subsequent detection of zilpaterol in pig plasma. Zilpaterol residues in cattle tissues were quantified in a separate study (study S16314–00; Table 13).

All frozen cattle tissues were thawed at room temperature overnight before being fed to pigs. Cattle tissues were mixed with feed pellets to encourage complete consumption. Two pigs did not consume the entire allotment of cattle liver, and their resulting plasma zilpaterol concentrations were dose-corrected to reflect the decreased zilpaterol dose. Likewise, for the pigs consuming 0.22 mg zilpaterol via incurred residues in cattle muscle, plasma zilpaterol concentrations were doubled to reflect a consistent 0.44 mg dose for all groups.

Blood samples were collected by venepuncture at pretreatment and again at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 24 hours post treatment. Blood was centrifuged and plasma stored frozen until analysis of zilpaterol free base concentrations using a validated LC–MS/MS method with a lower LOQ of 0.05 ng/mL (ZIL-PV001-01).

The pharmacokinetic parameters C_{max}, T_{max} and area under the concentration–time curve from 0 to the limit of quantification (AUC_{0-LOQ}) were determined and analysis of variance (ANOVA) performed (α = 0.05, pairwise comparisons performed using Tukey's honest significant difference test). Results are shown in Fig. 1 and Table 14.

Group A (zilpaterol via aqueous solution) had the highest C_{max} (mean 2.00 ng/mL) observed at mean 0.9 hours after dosing. Zilpaterol was more rapidly absorbed after oral gavage of the aqueous formulation than after consumption of edible cattle tissue containing incurred zilpaterol residues. The T_{max} of the groups administered zilpaterol as incurred residues was delayed (mean 2–4.5 hours). The highest T_{max} was observed in group D, possibly due to the comparatively large quantity of muscle tissue consumed.

The mean AUC_{0-LOQ} values were not statistically significantly different between any treatment groups, indicating that although zilpaterol absorption was delayed after ingestion of incurred residues, it was nonetheless comparably absorbed.

Summary and conclusions

Pigs fed cattle tissues containing incurred zilpaterol residues had delayed absorption and generally lower peak concentrations of zilpaterol compared

Table 13
Incurred zilpaterol residue concentrations in various cattle tissues fed to pigs

Tissue	Cattle ID	Concentration of zilpaterol free base (mg/kg)	Tissue amount consumed by pigs (kg)	Amount of zilpaterol consumed by pigs (mg)
Liver	68422	1.00	0.44	0.44
Kidney	32392	1.62	0.272	0.44
	68422	1.79	0.246	0.44
Muscle	32390	0.158	1.392	0.22
	68404	0.180	1.222	0.22

Fig. 1
Mean zilpaterol plasma concentrations with time in pigs consuming 0.44 mg[a] zilpaterol via oral gavage or incurred residues in cattle tissues
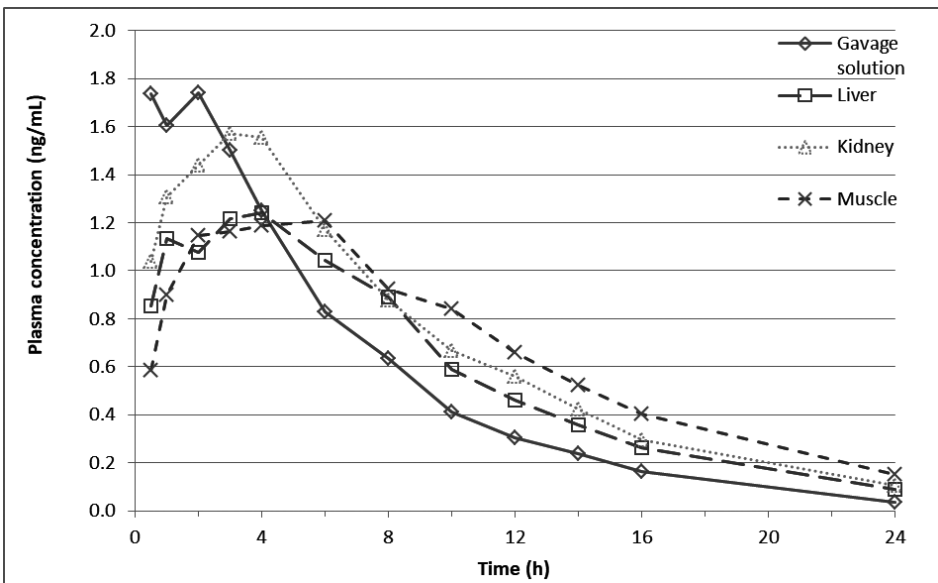
[a] All plasma concentrations dose-corrected to reflect a dose of 0.44 mg zilpaterol

with pigs administered zilpaterol via aqueous solution. However, the extent of absorption (AUC) was equivalent in all groups. Although the absolute bioavailability of orally administered zilpaterol was not assessed in this study, the relative oral bioavailability of incurred zilpaterol residues is not lower than zilpaterol administration via oral gavage.

Table 14

Pharmacokinetic parameters in pigs after administration of zilpaterol hydrochloride by various oral formulations

Zilpaterol dosage form (total dose administered)[a]	Mean measure ± SD		
	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0–LOQ}$ (ng·h/mL)
Gavage (0.44 mg)	2.00 ± 0.18[A]	0.88 ± 0.75[A]	12.7 ± 1.7[A]
Liver (0.44 mg)	1.28 ± 0.47[B]	3.25 ± 0.96[AB]	13.2 ± 5.1[A]
Kidney (0.44 mg)	1.68 ± 0.16[AB]	2.13 ± 1.18[AB]	16.2 ± 3.2[A]
Muscle (0.22 mg)	1.27 ± 0.03[B]	4.50 ± 1.91[B]	16.0 ± 2.1[A]

$AUC_{0–LOQ}$: area under the concentration–time curve from 0 to the limit of quantification; C_{max}: maximum concentration; SD: standard deviation; T_{max}: time to reach the maximum concentration

[a] Pharmacokinetic parameters based on dose-corrected plasma concentrations to reflect equivalent 0.44 mg dose.

[A–B] Within columns, mean values with different superscript letters differ significantly ($P < 0.05$).

During the previous zilpaterol exposure assessment, at its eighty-first meeting, the Committee corrected for limited oral bioavailability only the non-extractable (bound) zilpaterol residues in cattle tissues. The remaining (extractable) zilpaterol residues were considered to be fully bioavailable. The most recently submitted bioavailability data support the approach used in the previous assessment. Following evaluation of these data, the MRLs recommended by the Committee at its eighty-first meeting remain unchanged.

No additional monograph was prepared.

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.

Ethion

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

- A metabolism study using radiolabelled ethion in cattle should identify suitable marker residue(s) and determine their relative distribution in edible tissues.
- Metabolites in cattle should be compared with metabolites in laboratory species to ensure that all residues of toxicological concern produced in cattle are covered by the available toxicology studies.
- Analytical method(s) that can measure suitable marker residues in all edible tissues should be developed and validated in accordance with established guidance (CAC/GL71-2009).

Halquinol

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

- The in vivo mutagenicity and carcinogenicity potential of halquinol
- Characterization of the non-extractable residues as well as the extractable (but not defined) residues
- Accurate MR:TRR values over the appropriate times in edible tissues of pig after halquinol administration because the total residues are predicted to be the residue of concern

The Committee noted that new studies may be necessary to address these concerns.

Sisapronil

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

- Comparative toxicokinetic data in rat, dog and human
- Effects of sisapronil at steady state following repeated-dose oral administration in the dog
- Relevance of the effects on the thyroid observed in the dog

Although not all the toxicokinetic data would necessarily have to be generated in vivo, the approach used would have to be suitably validated (e.g. a physiologically based toxicokinetic model verified in vivo in rat and dog).

Zilpaterol hydrochloride

In its assessment of zilpaterol exposure, the Committee assumed that all extractable zilpaterol residues were fully bioavailable. Additional data would be required to conclusively demonstrate reduced oral bioavailability of all (both extractable and non-extractable) incurred zilpaterol residues.

Chronic dietary exposure assessment of compounds used as veterinary drugs and pesticides

The Committee agreed with the conclusions and recommendations of the expert working group on the methodology applied by JECFA and JMPR to estimate chronic dietary exposure (see "2.2 Chronic dietary exposure assessment of compounds used as veterinary drugs and pesticides"). On the basis of the recommendations of the working group, JECFA considered that the nature of the toxicological effect and the duration of exposure until the onset of effect be addressed as follows:

- Where the ADI is based on a developmental effect, pregnant women will be at potential risk and the critical exposure period may be only a few days or weeks. In such cases, it will be necessary to consider exposure in pregnant high-percentile consumers or an appropriate surrogate population.
- Where the POD (e.g. NOAEL) on which the ADI is based is not a developmental effect but is ≤3 times lower than the developmental POD, pregnant women will be at potential risk and the critical exposure period may be only a few days or weeks. In such cases, it will be necessary to consider exposure in pregnant high-percentile consumers or an appropriate surrogate population.
- Where the ADI is based on offspring toxicity, but the POD on which it is based is ≤3 times lower than the POD for long-term toxicity (e.g. 2-year rat study), infants and young children will be at potential risk. In such cases, it will be necessary to consider exposure in infants and young children who are typical (average) consumers.
- Where the POD on which the ADI is based in ≤3 times lower than the POD for offspring toxicity, infants and young children will be at potential risk. In such cases, it will be necessary to consider exposure in infants and young children who are typical (average) consumers.

- Where the ADI is based on offspring toxicity, and the POD on which it is based is >3 times lower than the POD for long-term toxicity (e.g. 2-year rat study), there will be particular concern about the potential risk to infants and young children. In such cases, it will be necessary to consider exposure in infants and young children who are high-percentile consumers.

- Where the ADI is based on effects observed in long-term studies (e.g. 2-year study of toxicity in rats) and the POD in a study (or studies) of shorter duration (e.g. 90-day rat or 90-day dog study of toxicity) is ≤3 times higher than the critical POD (the POD on which the ADI is based), there will be potential concern for less-than-lifetime exposure in the general population. In such cases, it will be necessary to consider exposure in high-percentile adult or general population consumers.

- Where the POD on which an ARfD is based is the same as the POD on which the ADI is based, if short-term exposures (children and general population) are not of concern, there will be no concern for less-than-lifetime exposure.

- In all other situations, there will be no specific concerns for less-than-lifetime exposure. In such cases, it will be sufficient to consider exposure in average adult or general population consumers.

JECFA considerations on the design of incurred residue bioavailability and pharmacological activity studies

1. Selection of appropriate test animal models

- The target animal species should be the food animal species for which the veterinary drug is approved (e.g. cattle, swine, poultry, fish).

- The test animal species chosen (i.e. in which the bioavailability assessment is conducted) should have bioavailability comparable to that in humans.

- The test animal species should have a gastrointestinal anatomy and physiology (especially proximal gastrointestinal tract) and gastrointestinal pH and transit time similar to that of humans. However, other animal models may also be suitable for generating relevant data.

2. Dosing strategies for achieving quantifiable tissue and plasma concentrations

Although deviations in drug dosing and withdrawal periods as well as excess tissue ingestion by the test animal (to achieve high concentrations of incurred residues in the target species) may result in less realistic exposure from incurred residues and over- or underestimation of bioavailability, such estimates of bioavailability would provide a useful starting point for subsequent refinement of JECFA's exposure assessment.

3. Pharmacological activity of incurred residues (relay pharmacology)

Bioavailability studies measure the plasma concentrations after ingestion of incurred residues and other oral administration methods, and derive the relevant pharmacokinetic parameters from such data. However, in many cases a single study could compare the relative bioavailability (pharmacokinetics) of incurred residues with other oral administration methods and the pharmacological activity (pharmacodynamics) after the various oral doses are administered. PK/PD modelling would enable a clear relationship between the drug residues in plasma and their actual effect.

4. Other issues regarding the assessment of relative bioavailability and relay pharmacology

- Sponsors are encouraged to refer to VICH GL52 (*10*) for appropriate sample sizes and timing of plasma collection for relative bioavailability or relay pharmacology studies.
- Relative oral bioavailability studies may not be feasible for incurred drug residues comprising multiple components (e.g. parent compound + metabolites). The concentrations of each component must be quantified in both the incurred tissue residues and the test animal plasma.
- The doses used in a relay pharmacology study should be consistent with those known to cause a predictable pharmacological response in the test animal species. The primary outcomes measured should be a result of discrete pharmacological activity and should be quantifiable, simple to measure and not prolonged.
- If oral bioavailability and/or pharmacological activities for incurred residues differ, supporting data can be provided for all the animal-derived tissues that significantly impact the human exposure assessment. For tissues for which data on bioavailability / relay pharmacology of incurred residues are not available, the Committee will assume the same bioavailability / pharmacological activity as by direct oral exposure.

Methodological approaches and types of data for assessment of antimicrobial residues in food

In determining mADIs and microbiological ARfDs, the Committee typically evaluates MIC data and other submitted in vitro datasets, and reviews the literature on the susceptibility of selected human intestinal bacteria (disruption of the colonization barrier; emergence and selection for antimicrobial-resistant bacteria) to the antimicrobial agent. The Committee recommends that:

- these MIC data come from studies that use standard internationally recognized methods with at least 10 strains of the relevant genera of bacteria sourced from faecal samples of healthy donors, as in Step 1 of VICH GL36(R) (*18*);

- results from recent molecular and metagenomic studies on the composition of intestinal microbial communities guide the selection of microbiota used in the MIC tests;

- in vitro or in vivo studies be conducted using a range of concentrations of the antimicrobial agent, from residue levels to therapeutic levels, and the predominant bacterial strains, and not just *E. coli*.

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Annex 1

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

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208. Evaluation of certain veterinary drug residues in food (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.

209. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 66, 2012.

210. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 12, 2012.

211. Evaluation of certain food additives (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.

212. Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

213. Compendium of food additive specifications. FAO JECFA Monographs 13, 2012.

214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.

215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.

216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.

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

218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.

219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.

220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.

221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.

222. Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.

223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.

224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.

225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.

226. 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.

227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.

228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.

229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.

230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.

231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.

232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.

233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No.1002, 2017.

234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No 1007, 2017.

Annex 2

Recommendations on the substances on the agenda

Amoxicillin (antimicrobial agent)

Acceptable daily intake	The Committee established an mADI of 0–0.002 mg/kg body weight (bw) based on the effects of amoxicillin on the intestinal microbiota.
Acute reference dose	The Committee established an ARfD of 0.005 mg/kg bw based on microbiological effects on the intestinal microbiota.
Estimated chronic dietary exposure	The GECDE for the general population is 0.14 µg/kg bw per day, which represents 7% of the upper bound of the mADI.
Estimated acute dietary exposure	The GEADE for the general population is 1.4 µg/kg bw, which represents 28% of the microbiological ARfD. The GEADE for children is 1.6 µg/kg bw, which represents 31% of the microbiological ARfD.
Residue definition	Amoxicillin

Recommended maximum residue limits (MRLs)

Species	Fillet[a] (µg/kg)	Muscle (µg/kg)
Finfish[b]	50	50

[a] Muscle plus skin in natural proportion.
[b] The term "finfish" includes all fish species.

Ampicillin (antimicrobial agent)

Acceptable daily intake	The Committee established an overall mADI of 0–0.003 mg/kg bw based on a NOAEL equivalent to 0.025 mg/kg bw per day for increase in population(s) of ampicillin-resistant bacteria in the gastrointestinal tract in humans, and using a safety factor of 10 (for the variability in the composition of the intestinal microbiota within and between individuals).
Acute reference dose	The Committee established an ARfD of 0.012 mg/kg bw based on the microbiological end-point.

Estimated chronic dietary exposure	The GECDE for the general population is 0.29 μg/kg bw per day, which represents 10% of the upper bound of the ADI.
Estimated acute dietary exposure	The GEADE for the general population is 1.9 μg/kg bw per day, which represents 16% of the ARfD. The GEADE for children is 1.7 μg/kg bw per day, which represents 14% of the ARfD.
Residue definition	Ampicillin
Maximum residue limits	The Committee recommended an MRL of 50 μg/kg for ampicillin in finfish muscle and in finfish muscle plus skin in natural proportion, the same as that recommended for amoxicillin, because the modes of action, the physicochemical properties and the toxicological and pharmacokinetic profiles of amoxicillin and ampicillin are very similar.

Ethion (acaricide)

Acceptable daily intake	The Committee established an ADI of 0–0.002 mg/kg bw based on the NOAEL of 0.2 mg/kg bw per day for embryotoxic effects in a rat developmental toxicity study, and using a safety factor of 100 (10 for interspecies variability and 10 for intraspecies variability).
Acute reference dose	The Committee established an ARfD of 0.02 mg/kg bw based on the NOAEL of 0.15 mg/kg bw for erythrocyte acetylcholinesterase inhibition in a repeated-dose human study, and using an intraspecies safety factor of 10.
Estimated dietary exposure	No dietary exposure assessment could be conducted.
Residue definition	None. A suitable marker residue could not be determined and a marker to total residue ratio could not be established.
Maximum residue limits	The Committee was unable to recommend MRLs for ethion.

Flumethrin (type II pyrethroid insecticide)

Acceptable daily intake	The Committee established an ADI of 0–0.004 mg/kg bw based on the NOAEL of 0.37 mg/kg bw per day for skin lesions in parental animals and reduced survival and body-weight gain in pups in a two-generation toxicity study in rats, and using a safety factor of 100 (10 for interspecies variability and 10 for intraspecies variability).
Acute reference dose	The Committee established an ARfD of 0.005 mg/kg bw based on the NOAEL of 0.5 mg/kg bw for salivation in dams in a developmental toxicity study in rats, and using a safety factor of 100 (10 for interspecies variability and 10 for intraspecies variability).
Estimated chronic dietary exposure	As flumethrin is also used as pesticide, the overall dietary exposure was estimated. The assumptions and detailed results will be presented in the JECFA 85 report. The results below are only for the use of flumethrin as a veterinary drug.
	The GECDE for the general population is 0.008 µg/kg bw per day, which represents 0.2% of the upper bound of the ADI.
	The GECDE for children is 0.006 µg/kg bw per day, which represents 0.2% of the upper bound of the ADI.
Estimated acute dietary exposure	The GEADE for the general population is 0.1 µg/kg bw per day, which represents 2.2% of the ARfD.
	The GEADE for children is 0.1 µg/kg bw per day, which represents 2.2% of the ARfD.
Residue definition	Flumethrin (trans-Z1 and trans Z2 diastereomers at a ratio of approximately 60:40).

Maximum residue limits	The Committee set an MRL for honey of 6 µg/kg, which is twice the limit of quantification (LOQ; 3 µg/kg) of the most reliable analytical method (liquid chromatography coupled with tandem mass spectrometry; LC–MS/MS) used in the residues studies.

Halquinol (broad spectrum antimicrobial)

Acceptable daily intake	In the absence of information required to assess the in vivo mutagenicity and carcinogenicity potential of halquinol, the Committee was unable to establish an ADI for halquinol. An mADI of 0–0.3 mg/kg bw was derived from in vitro MIC susceptibility testing data.
Acute reference dose	A microbiological ARfD of 0.9 mg/kg bw was established based on the effects of halquinol on the intestinal microbiota.
Estimated dietary exposure	No dietary exposure assessment could be conducted.
Residue definition	None due to incomplete characterization of residues in tissues.
Maximum residue limits	The Committee was unable to recommend MRLs for halquinol.

Lufenuron (insecticide)

Acceptable daily intake	The Committee established an ADI of 0–0.02 mg/kg bw based on the NOAEL of 1.93 mg/kg bw per day for tonic–clonic seizures and findings in lungs, gastrointestinal tract, liver and urinary tract in a 2-year dietary study in rats, and using a safety factor of 100 (10 for interspecies variability and 10 for intraspecies variability).

Acute reference dose	The Committee concluded that it was unnecessary to establish an ARfD for lufenuron in view of its low acute oral toxicity and the absence of developmental toxicity and other toxicological effects likely to be elicited by a single dose.
Estimated chronic dietary exposure	As lufenuron is also used as pesticide, the overall dietary exposure was estimated. The assumptions and detailed results will be presented in the JECFA 85 report. The results below are only for the use of lufenuron as a veterinary drug. The GECDE for the general population is 1.1 μg/kg bw per day, which represents 5.5% of the upper bound of the ADI.
Residue definition	Lufenuron

Recommended maximum residue limits (MRLs)

Species	Fillet[a] (µg/kg)
Salmon	1 350
Trout	1 350

[a] Muscle plus skin in natural proportion.

Monepantel (anthelminthic)

Acceptable daily intake	The ADI of 0–0.02 mg/kg bw per day established by the Committee at the seventy-fifth meeting (WHO TRS No. 969, 2012) remains unchanged.
Acute reference dose	The Committee concluded that it was unnecessary to establish an ARfD.
Estimated dietary exposure	The GECDE for the general population is 13.7 μg/kg bw per day, which represents 68% of the upper bound of the ADI. The GECDE for children is 5.0 μg/kg bw per day, which represents 25% of the upper bound of the ADI. The GECDE for infants is 4.4 μg/kg bw per day, which represents 22% of the upper bound of the ADI.
Residue definition	Monepantel sulfone

Recommended maximum residue limits (MRLs)[a]

Species	Fat (μg/kg)	Kidney (μg/kg)	Liver (μg/kg)	Muscle (μg/kg)
Cattle	7 000	1 000	2 000	300

[a] Determined as monepantel sulfone, expressed as monepantel.

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Sisapronil (ectoparasiticide)

No additional data were submitted. As a result, the ADI remains unestablished.

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Zilpaterol hydrochloride (β2-adrenoceptor agonist)

Following evaluation of the bioavailability data submitted, the MRLs recommended by the Committee at its eighty-first meeting (WHO TRS No. 997, 2016) remain unchanged.

Annex 3

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

85ᵗʰ JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
WHO Headquarters, Geneva 17-26 October 2017

Draft Agenda

1. Opening at the Chateau de Penthes on 17 October 2017 at 09:30

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. Other matters of interest arising from FAO and WHO

   a. Update on FAO/WHO CIFOCOss database

7. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full Committee)

8. Evaluations

   Veterinary drug residues
   - Amoxicillin
   - Ampicillin
   - Bismuth sub-nitrate
   - Diflubenzuron
   - Ethion
   - Flumethrin
   - Halquinol

- Lufenuron
- Monepantel
- Sisapronil
- Zilpaterol hydrochloride

9. General considerations

- Report from the JECFA/JMPR working group for dietary exposure assessment of compounds used as veterinary drug and pesticide.
- Bioavailability
- Upcoming guidance on hazard characterization (Benchmark dose and genotoxicity studies)

10. Other matters as may be brought forth by the Committee during discussions at the meeting

11. Adoption of the report

12. Closure on 26 October at 16:00

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of Certain Food Additives
Eighty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1007, 2017 (92 pages)

Evaluation of Certain Contaminants in Food
Eighty-third Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1002, 2017 (166 pages)

Evaluation of Certain Food Additives
Eighty-second Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1000, 2016 (162 pages)

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 (110 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 72, 2016 (162 pages)

Evaluation of Certain Food Additives and Contaminants
Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 995, 2016 (114 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 71, 2015 (132 pages)

Evaluation of Certain Food Additives
Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 990, 2015 (124 pages)

Safety Evaluation of Certain Food Additives
Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 70, 2015 (369 pages)

Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 988, 2014 (127 pages)

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), including chronic dietary exposure assessment of compounds used as veterinary drugs and pesticides, assessment of the relative bioavailability and/or pharmacological activity of incurred drug residues in animal tissues, acute reference doses (ARfDs) for residues of veterinary drugs and methodological approaches and types of data for assessment of veterinary drug residues in food.

Summaries follow of the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: three antimicrobial agents (amoxicillin, ampicillin and halquinol), an acaricide (ethion), an antiparasitic agent (flumethrin), an insecticide (lufenuron) and an anthelminthic (monepantel). In addition, the Committee considered issues raised by the Codex Committee on Residues of Veterinary Drugs in Foods concerning sisapronil, an ectoparasiticide, and zilpaterol hydrochloride, a β2-adrenoceptor agonist. Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes (ADIs), ARfDs and proposed maximum residue limits (MRLs).