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Evaluation of certain veterinary drug residues in food

Food and Agriculture Organization of the United Nations

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WHO Technical Report Series
988

Evaluation of certain veterinary drug residues in food

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

World Health Organization

visited on 6/29/2018

WHO Library Cataloguing-in-Publication Data:

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)

1.Food contamination. 2.Drug residues - analysis. 3.Drug residues - toxicity. 4.Veterinary drugs - toxicity. 5.Veterinary drugs - pharmacology. 6.Risk assessment. 7.Maximum allowable concentrations - standards. 8.No-observed-adverse-effect level. I.World Health Organization. II.Food and Agriculture Organization of the United Nations. III.Joint FAO/WHO Expert Committee on Food Additives. IV.Series.

ISBN 978 92 4 120988 5 (NLM classification: WA 712)

ISSN 0512-3054

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Typeset in India

Printed in Malta

visited on 6/29/2018

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Seventy-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Geneva, 5–14 November 2013

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List of abbreviations

ADI	acceptable daily intake
ARfD	acute reference dose
BMD	benchmark dose
BMDL	lower 95% confidence limit on the benchmark dose
bST	bovine somatotropin
bw	body weight
CAS	Chemical Abstracts Service
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EDI	estimated daily intake
EHC	Environmental Health Criteria monograph
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GEADE	global estimate of acute dietary exposure
GECDE	global estimate of chronic dietary exposure
GL36	Guideline 36 (VICH)
GLP	good laboratory practice
GVP	good veterinary practice
HPLC	high-performance liquid chromatography
IGF-I	insulin-like growth factor-I
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MIC_{50}	minimum concentration required to inhibit the growth of 50% of organisms

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MIC_{calc}	minimum inhibitory concentration derived from the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which the drug is active
MOE	margin of exposure
MRL	maximum residue limit
MR:TR	marker residue to total residue ratio
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
POD	point of departure
QuEChERS	Quick Easy Cheap Effective Rugged Safe
rbSTs	recombinant bovine somatotropins
TMDI	theoretical maximum daily intake
TRS	Technical Report Series
TTC	threshold of toxicological concern
USA	United States of America
USEPA	United States Environmental Protection Agency
UTL	upper tolerance limit
UV	ultraviolet
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization

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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. 69, 2014.

Residue monographs are issued separately by FAO under the title:

Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.

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1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 5 to 14 November 2013. 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 noted that the first JECFA meeting dedicated to veterinary drugs was the twelfth meeting, held in 1968, which pre-dated the formation of the Codex Committee on Veterinary Drug Residues in Foods (CCRVDF). The scope of the work has expanded since, and in 1989, at the thirty-fourth meeting, exposure assessment was introduced based on a standard food basket. In addition to the usual tasks of the Committee, there are a number of important general considerations on the agenda for this meeting that are related to the methods and principles based on which the Committee conducts its work, including further improvements on methods to estimate dietary exposure. Further developments in these areas are discussed with CCRVDF, illustrating the close interaction between JECFA as the risk assessment body and CCRVDF as the risk management body.

Dr Miyagishima reminded the Committee that participants have been invited to this meeting as independent experts and not as representatives of their organizations. He also reminded the Committee of the confidential nature of the meeting, which allows experts to freely express their opinions. Finally, Dr Miyagishima expressed his sincere gratitude to participants for providing their time and most importantly their expertise to this important work, which contributes to the core business of both WHO and FAO, providing science-based international norms and standards.

Nineteen 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* and *208*) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984 *(1)*.

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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 3 and Annex 2); and

— To respond to specific concerns raised by CCRVDF (see section 3 and Annex 2).

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the seventy-eighth meeting had completed declaration of interest forms. The following declared interests were discussed. Dr Susan Barlow had consulted for the producer of derquantel. This was recognized as a conflict of interest, and Dr Barlow was not present during the discussion of derquantel. Professor Alan Boobis has in the past undertaken consultancies related to specific non-pharmaceuticals or on generic issues related to human medicine. As these consultancies were not related to topics on the agenda, this was not considered to be a conflict.

1.2 Modification of the agenda

The agenda was modified to exclude apramycin (residues only) and sisapronil (phenylpyrazole), as no data were submitted by the sponsors, and to include ivermectin (recommendation of MRLs in muscle), as requested by the Twenty-first Session of CCRVDF.

[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 seventy-seven previous meetings of JECFA (Annex 1).

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2. General considerations

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

The Codex Secretariat informed the Committee about relevant decisions of the Codex Alimentarius Commission and the principal outcomes and discussions of the Twentieth and Twenty-first Sessions of CCRVDF, which had been held since the seventy-fifth meeting of the Committee in 2011.

The Twentieth Session of CCRVDF *(2)* finalized work on the majority of the MRLs recommended by the seventy-fifth meeting of the Committee (Annex 1, reference *208*), which were subsequently adopted by the Codex Alimentarius Commission at its Thirty-fifth Session *(3)*. The MRLs for derquantel and monepantel in sheep tissues will be considered by the Twenty-second Session of CCRVDF (April 2015) on the basis of the outcomes of the current meeting of the Committee. The Twentieth Session of CCRVDF agreed to discontinue work on the MRLs for apramycin in cattle and chicken kidney. The Twentieth Session of CCRVDF also revised the *Risk analysis principles applied by the CCRVDF* and the *Risk assessment policy for residues of veterinary drugs in foods* to address risk management and risk communication recommendations for veterinary drugs with no ADI and/or MRL, which were adopted by the Thirty-fifth Session of the Codex Alimentarius Commission and included in the Procedural Manual.

The Thirty-fifth Session of the Codex Alimentarius Commission adopted the MRLs for ractopamine (in cattle and pig tissues), which had been held since its Thirty-first Session (2008). With regard to the MRLs for recombinant bovine somatotropins (rbSTs), the Codex Alimentarius Commission agreed to continue holding the MRLs for rbSTs, which had been held since its Twenty-third Session (1999), and to request the Committee to update the evaluation of rbSTs on the basis of new data and information and to consider the need to revise or maintain the ADI and MRLs; it was agreed that aspects of human antimicrobial resistance could be considered in the evaluation, as appropriate.

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With regard to the Twenty-first Session of CCRVDF in August 2013 *(4)*, the Codex Secretariat informed the Committee that CCRVDF had finalized work on several texts, which will be considered for adoption by the Thirty-seventh Session of the Codex Alimentarius Commission in July 2014. In particular, CCRVDF finalized risk management recommendations for eight veterinary drugs for which no ADI and/or MRL had been recommended by the Committee due to specific human health concerns: chloramphenicol, malachite green, carbadox, furazolidone, stilbenes, nitrofural, chlorpromazine and olaquindox. CCRVDF also requested that the Committee update the toxicological evaluation and exposure assessment for the four nitroimidazoles so that CCRVDF could take a decision regarding the risk management recommendations for these substances at its next session (April 2015).

CCRVDF finalized work on the Guidelines on Performance Characteristics for Multi-residues Methods for Veterinary Drugs, for inclusion in the *Guidelines for the design and implementation of national regulatory food safety assurance programmes associated with the use of veterinary drugs in food producing animals* (CAC/GL 71-2009).

CCRVDF completed work on extrapolation of MRLs to additional species and on the use of the Concern Form, for inclusion in the *Risk analysis principles applied by the CCRVDF*. CCRVDF provisions on extrapolation are high-level principles, which will complement the Committee's work on extrapolation. The Twenty-first Session of CCRVDF asked the Committee to address an additional nine questions on extrapolation of MRLs to additional species and to provide guidance on the appropriate terminology to be used (i.e. extrapolation or extension). The provisions on the use of the Concern Form aim to improve transparency and facilitate resolutions of issues between CCRVDF and the Committee by requiring Codex members to clarify the nature of their scientific concern or their request for clarification to JECFA.

CCRVDF developed some provisions to address the matter of establishing MRLs for honey; CCRVDF is planning to complete work on this matter at its next session in light of the Committee's work.

The Twentieth Session of CCRVDF agreed on a priority list of veterinary drugs for evaluation (or re-evaluation) by JECFA, which was revised by the Twenty-first Session. CCRVDF included ethoxiquin (a feed additive) on the priority list, subject to the Codex Alimentarius Commission's confirmation of the appropriateness of CCRVDF dealing with feed additives.

CCRVDF continued its work on the development of a database on countries' needs for MRLs, which now includes requests for MRLs in various species and tissues related to 83 veterinary drugs. The purpose of the database is

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to identify countries' needs and help countries to identify and collect data necessary for JECFA evaluation. In order to facilitate the move of substances from the database to the priority list, CCRVDF agreed to an alternative approach in which FAO and WHO could assist in the prioritization of substances for evaluation of gaps and ways to fill the data gaps.

Dr Steve Vaughn, Chairperson of CCRVDF, expressed his appreciation for the work of JECFA in support of the work of CCRVDF. He asked for continuing flexibility for improving coordination between JECFA and CCRVDF, in particular in light of the challenges posed by limited resources and the different schedules of the CCRVDF and JECFA meetings. He thanked JECFA for the flexibility exhibited to date in providing advice to Codex through electronic working groups and other means.

Dr Vaughn highlighted the importance of extrapolation and noted that JECFA's work in this area would also assist Codex in responding to developing countries' needs for more MRLs, which often are related to the use of veterinary drugs in "minor" species. The importance of the JECFA work on honey for CCRVDF was also emphasized, as well as its complementarity with the ongoing work in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH).

With regard to antimicrobial resistance, Dr Vaughn noted the growing concern of consumers on this issue; he recalled the work on "Risk analysis of foodborne antimicrobial resistance", recently completed by the Codex Task Force on Antimicrobial Resistance, and the Codex *Code of practice to contain and minimise antimicrobial resistance* (CAC/RCP 61-2005), developed some years ago by CCRVDF. While there is no need to change JECFA's consideration of antimicrobial resistance, he asked the Committee to continue to pay attention to the developments and discussions in this area.

Dr Vaughn noted that the next CCRVDF session in April 2015 will consider the outcome of this meeting and that the recommendations for zilpaterol hydrochloride and rbSTs will be particularly challenging to the CCRVDF discussion. He asked the Committee to have clear and defensible conclusions, to the extent possible, to guide the risk management decisions in CCRVDF.

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

Following consideration of the outcome of the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADIs/MRLs, held in Bangkok in 2004, and related information, the Committee at its sixty-sixth meeting (Annex 1, reference *181*) recommended that the JECFA

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Secretariat convene a working group to develop a general decision-tree for the evaluation of veterinary drugs, which would identify different options for hazard identification, hazard characterization and exposure assessment.

Following the sixty-sixth meeting, a working group was convened, and a draft of "A hypothesis-driven decision tree approach for the safety evaluation of residues of veterinary drugs" was presented at the seventieth meeting of JECFA (Annex 1, reference *193*). Following discussion, the draft was revised, and the Committee recommended that the concept paper be submitted to CCRVDF for consideration.

The paper was submitted as "A risk-based decision tree approach for the safety evaluation of residues of veterinary drugs" to the Eighteenth Session of CCRVDF *(5)* and presented by the JECFA Joint Secretary, who pointed out that the document had to be considered as "work-in-progress". CCRVDF agreed with the proposed general principles and supported further work on this matter, noting that sufficient time and opportunity for input and comments by members were necessary.

At its seventy-fifth meeting (Annex 1, reference *208*), JECFA considered further the proposed decision-tree approach for the safety evaluation of residues of veterinary drugs. It was noted that the expert meeting on dietary exposure assessment methodologies, held on 7–11 November 2011 in Rome, was in part a follow-up activity to the decision-tree approach. The Committee further recommended that the JECFA Secretariat establish an electronic working group to elaborate principles to establish acute reference doses (ARfDs) for residues of veterinary drugs, taking the guidance developed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) into account *(6)*, as well as ongoing efforts by VICH.

The present Committee noted that it had not been possible to follow up on this recommendation of the seventy-fifth meeting due to resource limitations. The Committee further discussed the decision-tree and agreed to the following:

- Preliminary risk assessment, as envisaged in the decision-tree, would be most readily accomplished by Member States, when considering suggesting compounds for evaluation by JECFA. The Committee recommended that an electronic working group should be established to develop guidance on what would comprise a preliminary risk assessment, taking into account the risk analysis principles applied by CCRVDF.
- There are a number of issues that would need to be addressed in applying the threshold of toxicological concern (TTC) approach to residues of veterinary drugs. In particular, some pharmacologically active compounds are very potent, and it is possible that the current

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TTC values, based primarily on toxicological end-points, would not be applicable. The Committee recommended that an electronic working group should be established to perform a feasibility exercise on the application of the TTC approach to residues of veterinary drugs and, if appropriate, to make specific recommendations for developing such an application.

- The Committee confirmed the importance of developing guidance for the acute risk assessment of residues of veterinary drugs. It was recommended that an electronic working group should be established to develop guidance for establishing ARfDs for residues of veterinary drugs, addressing situations in which it would be necessary to establish an ARfD and how this would be done. Consideration should also be given to compounds for which the ADI is based on an acute effect (e.g. pharmacological effects, antimicrobial effects). The working groups should include an expert from JMPR who is experienced in the establishment of ARfDs.

The Committee identified volunteers for the above electronic working groups and agreed to prepare suitable reports for consideration at the next JECFA meeting on veterinary drug residues in food.

2.3 Dietary exposure to veterinary drug residues

Explanation

Dietary exposure assessment plays an essential part in quantifying risk and is central to the work of JECFA. There has been an ongoing need to improve the approaches used to estimate dietary exposure to veterinary drug residues in foods. The seventieth meeting of the Committee (Annex 1, reference *193*) identified further work on new approaches in this area, considering methods for chronic and acute dietary exposure assessment. In response, CCRVDF requested FAO and WHO to convene an expert meeting on dietary exposure assessment methodologies for residues of veterinary drugs.

The expert meeting, held in November 2011, proposed new methods for acute and chronic dietary exposure assessment for veterinary drug residues, taking the key findings, concerns and recommendations of stakeholders into consideration. Subsequently, it was recommended that the new approaches should be piloted at the seventy-eighth meeting of JECFA.

The purpose of the pilot study was to explore the new calculations for dietary exposure assessment, compare them with estimates calculated using the model diet approach, identify the practical impact of using the new methods and make recommendations for dietary exposure assessment at future meetings. As summarized below, dietary exposures were calculated

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for four veterinary drug residues using the model diet approach as well as the new methods for chronic and acute dietary exposure estimation.

Dietary exposure assessment approaches

The current model diet used for veterinary drug residues is intended to cover chronic high consumers of animal products. The model assumes that the food consumption applies to an adult with a body weight of 60 kg and is intended to also cover the consumption of all processed foods with these foods as ingredients. All muscle tissues are equivalent, so meat and fish consumed are considered as equivalent in the calculations.

For estimating chronic dietary exposures to veterinary drug residues, JECFA uses the median of the residue depletion to derive the estimated daily intake (EDI). The contribution to the EDI from consumption of individual tissues is calculated by multiplying the amount of tissue in the model diet by the median concentration of marker residue corresponding to the MRL. The EDI itself is the sum of the individual intakes resulting from all tissues. Where a median residue cannot be derived, the MRL may be substituted for the median residue to calculate the theoretical maximum daily intake (TMDI).

The two new methods for estimating dietary exposure are the global estimate of acute dietary exposure (GEADE) and the global estimate of chronic dietary exposure (GECDE). Both methods differ from the EDI by having the capacity to estimate specific dietary exposure for additional population groups (children aged 12 months and older and infants younger than 12 months) and by using more realistic global consumption amounts as inputs into the calculations. Consumption data used are based on surveys and can be expressed per person, to be compared with the current approach, or per kilogram body weight, based on values reported in food consumption surveys. Instead of the set amounts of food in the model diet, more detailed food consumption data are used where available. For example, muscle tissue is differentiated by species, and finfish are considered separately from molluscs and crustaceans.

It should be noted that consumption amounts for infants are not reported for some categories (e.g. mammalian fat, poultry fat and skin) and therefore are not included in estimates. Other categories were not reported separately as consumed according to the surveys used to derive consumption amounts. In such cases, the broader categories have been used, with the highest residue concentration used as the input. For example, “mammalian kidney” consumption is not reported for infants; therefore, the residue found in kidney would be assigned to “All mammalian offal”, which is the best available match for kidney consumption in this population.

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The current approach to estimating dietary exposure does not adequately estimate acute dietary exposure, which should be based on the highest probable exposure from a single commodity on a single day. The GEADE is an explicit estimate of acute dietary exposure, combining consumption at the 97.5th percentile with the 95th percentile residue concentration. Unlike the EDI, estimates can be derived specifically for children as well as for the general population, following the principle that dietary exposure assessments should cover the whole population, including children.

The GECDE uses median residues combined with two different types of consumption data to estimate chronic dietary exposure. Firstly, the highest exposure at the 97.5th percentile of consumption is selected from all the foods relevant to exposure. This value is derived from chronic consumers of the food; that is, the percentile consumption is calculated from consumers of the food only and is different from the 97.5th percentile of consumption used in acute exposure, which reflects a single eating occasion (acute). Secondly, the mean dietary exposures from all the other relevant foods are then added to estimate total exposure. The mean dietary exposure is derived from the total population; in other words, non-consumers of the food are included in the mean calculation. In addition to the general population and children, dietary exposure of infants can also be estimated.

Pilot study results[2]

Estimated dietary exposure to derquantel residues

There were insufficient data to establish median residues for derquantel. Therefore, a TMDI was calculated using the MRLs set for liver, kidney, muscle and fat. Based on the established model diet, the TMDI was estimated to be 6.8 µg/person per day. This represents approximately 38% of the upper bound of the ADI of 0–0.3 µg/kg body weight (bw) per day.

The GECDE was calculated from the consumption of sheep and other ovine muscle, mammalian liver, mammalian kidney and mammalian trimmed fat. Using the established MRLs as input, the GECDE for the general population was 7.1 µg/person per day (0.12 µg/kg bw per day), very similar to the TMDI. The estimated exposure of children was 0.19 µg/kg bw per day; it was estimated that infants were exposed at 0.17 µg/kg bw per day.

None of the GECDEs exceeded the upper bound of the ADI (general population 39%, children 64% and infants 55%). For all population groups, mammalian offal was the major contributor to estimated dietary exposure from derquantel residue.

[2] Percentages may not calculate exactly because of rounding.

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Estimated dietary exposure to emamectin benzoate residues

In this dietary exposure assessment, fish was the only contributor to dietary exposure. The EDI for emamectin benzoate was calculated on the basis of median residues found in fish muscle. The estimated dietary exposure was 11.2 µg/person per day, which represents approximately 37% of the upper bound of the ADI of 0–0.5 µg/kg bw per day (or 30 µg/person per day).

Using the median residue and fish consumption as inputs, the GECDE for the general population was 24.2 µg/person per day (0.40 µg/kg bw per day), which respresents approximately 81% of the upper bound of the ADI. The higher exposure estimate compared with the EDI was due to the higher consumption of fish used in the GECDE, 10 g/kg bw per day (655 g/person), compared with 300 g of muscle (fish) per person used in the model diet. The consumption data for fish are based on 1043 consumers from a Brazilian nutrition survey and are considered to be a robust estimate of high-level chronic fish consumption. This estimate is considered to be conservative for the 97.5th percentile of the global population.

In children, the GECDE was 0.56 µg/kg bw per day, which represented 111% of the upper bound of the ADI. This estimate above the ADI was due to a consumption amount of fish (226 g) that was very similar to the model diet being combined with the lower body weight of the population group, resulting in a comparatively high exposure on a body weight basis. Exposure of infants was estimated to be lower, at 0.24 µg/kg bw per day, because fish consumption in infants is 10% of that in the model diet.

The Committee derived an "ARfD"[3] of 10 µg/kg bw for emamectin residues. Therefore, an estimate of acute dietary exposure was carried out to characterize the risk of dietary exposure. The GEADE was calculated based on the 95th percentile residues, adjusted for the marker to total residue ratio. The GEADE for the general population was approximately 1.97 µg/kg bw per day. For children, the GEADE was approximately 1.77 µg/kg bw per day. For the general population, the GEADE represented approximately 20% of the "ARfD". For children, the acute dietary exposure estimate was 18% of the "ARfD".

Estimated dietary exposure to lasalocid residues

The EDI for lasalocid residues was calculated from the median residues found in poultry muscle, liver, kidney and fat. Based on the established model diet, the dietary exposure to lasalocid expressed as the EDI was 80 µg/person per day. Dietary exposure was estimated to be 17% of the upper bound of the ADI of 0–5 µg/kg bw per day.

[3] The Committee developed an "ARfD" for emamectin benzoate solely for the purpose of this pilot study.

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The GECDE for lasalocid was calculated from the consumption of poultry muscle, poultry fat and skin and total poultry offal. Exposure estimates for the general population were 111.3 µg/person per day, or 1.86 µg/kg bw per day.

The GECDEs for children and infants were 3.37 and 2.99 µg/kg bw per day, respectively. None of the dietary exposure estimates using the new methodology exceeded the upper bound of the ADI; the GECDEs were 37% (general population), 67% (children) and 60% (infants) of the upper bound of the ADI of 0–5 µg/kg bw per day. For all population groups, poultry offal was the major contributor to estimated dietary exposure from lasalocid residue. Poultry fat and skin contributed only negligible amounts to overall dietary exposure estimates.

Estimated dietary exposure to monepantel residues

The EDI of monepantel was calculated based on median residues found in muscle, liver, kidney and fat of sheep, determined after a 7-day withdrawal period. The estimated dietary exposure was 446 µg/person per day, which represents approximately 37% of the upper bound of the ADI of 0–20 µg/kg bw per day.

In comparison, the GECDE was calculated from the consumption of sheep and other ovine muscle, mammalian liver, mammalian kidney and mammalian trimmed fat. For the general population, exposure was estimated to be 481 µg/person per day (8.0 µg/kg bw per day). The GECDE for children was 13.2 µg/kg bw per day. Exposure of infants was estimated to be 11.5 µg/kg bw per day. The median residue found in liver was assigned to all mammalian offal, because liver consumption and kidney consumption were not reported separately for this population.

None of the dietary exposure estimates using the new methodology exceeded the upper bound of the ADI; the GECDEs were 40% (general population), 66% (children) and 57% (infants) of the upper bound of the ADI. For the general population, mammalian fat was the major contributor to estimated dietary exposure from monepantel residue. For children, mammalian liver contributed substantially more than fat to exposure. For infants, no consumption of fat was reported. Therefore, almost all of the estimated dietary exposure came from mammalian offal.

Evaluation

Overall, moving from an EDI, model diet–based approach to a GECDE and GEADE approach allows dietary exposure assessments of veterinary drug residues to be based on more accurate consumption data and so provides an improved estimate of dietary exposure. The use of more detailed consumption

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data and the calculation of exposure on a body weight basis across a wider range of population groups increase the breadth and flexibility of the risk assessment process. Care must be taken in the interpretation of chronic dietary exposure information expressed per kilogram of body weight in children and infants, as their consumption per kilogram of body weight will change over time, and the ADI is based on lifetime exposure.

The outcomes of the chronic dietary exposure assessments (i.e. EDI and GECDE) were mostly similar. However, exposures for children were usually higher, reflecting the higher consumption per kilogram body weight of this population group. In addition, the high consumption of offal reported for children who are consumers of these foods contributed to higher GECDEs in some cases. As offal is often a major contributor to dietary exposure, a higher level of confidence is required in the consumption of these foods. However, consumer numbers are often small, and deriving a reliable 97.5th percentile of consumption can be difficult.

Estimates of exposure to emamectin residues in fish were substantially higher using the new method for chronic exposure assessment. This was due to the comparatively low consumption amount for fish used in the model diet to calculate the EDI. In contrast, the GECDE is based on more accurate high-level chronic consumption amounts (based on large numbers of consumers), which are more than double the amount used for fish in the food basket. However, it should be noted that the assumption that all fish consumed comes from aquaculture rather than wild catches is highly conservative.

As the Committee derived an "ARfD" for emamectin benzoate, it was possible to carry out an acute exposure assessment for that compound. The outcome was that the "ARfD" was not exceeded for any of the population groups. As the assumptions made in the assessment were robust and conservative for both consumption and residue concentration, the exposure assessment should be suitable to assist in formulating MRLs that are sufficiently protective of consumers.

The Committee drew the following general conclusions from the pilot study of the new approach to carry out dietary exposure assessment:

- The new approach for dietary exposure assessment is preferable to the model diet approach because it moves from a food basket to consumption amounts derived from surveys. For future meetings of the Committee, the new approach should continue to be used in parallel with the model diet approach until more experience has been obtained in the interpretation of the results with the new approach.

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- Like the EDI, the GECDE and GEADE rely on realistic and reliable median and 95th percentile residue data in all foods that are contributors to exposure to a veterinary drug residue. It should be communicated to sponsors that such data form an essential part of the data package needed by the Committee to establish MRLs.
- Global food consumption data change over time. The latest and best quality consumption data available should be used in all dietary exposure assessments.
- Exposure can be estimated for population groups other than the general population, such as children and infants.
- For future assessments, it would be better practice to express dietary exposure on a body weight basis rather than a per person basis to allow for easier comparison with the ADI across population groups. Care should be taken in interpreting the comparisons.
- The GEADE approach is suitable for deriving an estimate of acute dietary exposure.
- The GECDE and GEADE use higher consumption amounts to calculate exposure to residues of veterinary drugs from fish. This would result in higher estimates of dietary exposure to veterinary drug residues in fish compared with the EDI.
- In some categories, such as mammalian muscle, the exposure estimates following the new methodology may include residues from more than one species (e.g. from cattle as well as poultry). When estimating the GECDE, there is only a single major contributor to exposure: that food that is the highest contributor to exposure using the chronic 97.5th percentile of consumption. In most cases, this makes it unlikely that including additional foods in the exposure assessment would substantially increase chronic exposure estimates.

A number of areas were identified that should be investigated further to improve dietary exposure assessment methodology for residues of veterinary drugs. The Committee recommends that a working group should be set up to investigate the following:

- The EDI, GECDE and GEADE assign residue concentrations to specific tissues. If, for example, the concentration of residues in liver is higher in pigs than in sheep, the higher level is used in the estimate. If, at a later date, higher residue data are submitted to establish MRLs for additional species, the dietary exposure estimate (and consequently the MRL) that was derived previously may be affected. A process should be elaborated for assessing the need to re-evaluate MRLs.

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- Some veterinary drugs (e.g. emamectin benzoate) produce residues that are also found in plant-based agricultural commodities. There is a need to estimate total dietary exposure from all foods. The methods and data requirements for this need to be further explored.
- Finfish, molluscs and crustaceans are different from most other animal products, as they are consumed from wild as well as aquacultural sources. When calculating the GECDE and GEADE, options should be investigated to obtain data from Member countries that would assist in estimating the proportion of farmed fish and other seafood in the food supply.
- The highest contributor of dietary exposure to veterinary drug residues is typically the residues in organ meats (offal). Many of the data for these foods that underpin the GECDE and GEADE are based on small numbers of consumers. Guidance should be elaborated on the use of these figures.

A monograph was prepared.[4]

2.4 Antimicrobial resistance: update on activities relevant to JECFA

Dr Awa Aidara-Kane from the Department of Food Safety and Zoonoses presented a review of ongoing activities within WHO, addressing the issue of antimicrobial resistance related to the veterinary use of antimicrobial agents in food animal production and its impact on the therapeutic use of these drugs in human medicine. The WHO list of critically important antimicrobials for human medicine was prepared in 2005 and is regularly updated. It is important that all relevant sectors involved in agricultural and human use of antimicrobials work together to tackle the challenging problem of antimicrobial resistance.

Dr Carl E. Cerniglia described the complex interactions of the mechanisms involved in the emergence and spread of antimicrobial resistance. His overview focused on the use of antimicrobial agents in food animals and what is currently known about the potential of antimicrobial resistance development. Two types of antimicrobial resistance were outlined: 1) the incidence of antimicrobial-resistant bacterial veterinary pathogens, zoonotic bacterial pathogens and commensal microorganisms in food-producing animals through the veterinary use of antimicrobial agents; and 2) the selection and emergence of resistant microorganisms in the human

[4] Available in FAO JECFA Monographs 15 (2014) and on the WHO website at http://www.who.int/foodsafety/chem/jecfa/publications/monographs/en/index.html.

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gastrointestinal tract through exposure to residues present in commodities from food-producing animals treated with antimicrobial agents. Whereas both types of resistance have implications for human health, only that arising from human exposure to residues of antimicrobial agents in edible foods is relevant to the work of JECFA. The Committee has established procedures for evaluating this, as well as the possible impact of such residues on disruption of the colonization barrier function of the gastrointestinal microbiota.

The Committee will continue to monitor developments in the area of antimicrobial resistance and apply those relevant to its work, as appropriate.

2.5 Review of the need to update *Principles and methods for the risk assessment of chemicals in food* (EHC 240)

JECFA, like other expert groups advising WHO and FAO, has codified the general principles by which it evaluates residues of veterinary drugs for their possible risk to consumers from dietary exposure. These were published in reports of the JECFA meetings, as they were developed. WHO sought to consolidate these evolving principles and to harmonize, to the extent possible, the approaches used by the various expert groups (JECFA, JMPR, etc.). This culminated in the publication, in 2009, of EHC 240: *Principles and methods for the risk assessment of chemicals in food (7)*. Even at the time of publication, it was recognized that regular updating would be necessary, and it was envisaged that this could be done by providing updates online.

The present Committee agreed that a review of EHC 240 should be a standing item on its agenda from its next meeting onwards and that any sections or chapters requiring updating would be identified. In such cases, the Committee would make specific recommendations on how this might be achieved.

2.6 Feedback from JMPR on ongoing work on general criteria for interpretation of toxicological data

The Committee was informed that JMPR is developing guidance on the interpretation of minor and adaptive changes observed in studies in experimental animals. This work started with publication of "Guidance on the interpretation of hepatocellular hypertrophy" in 2006. JMPR is extending this guidance to cover a variety of additional end-points. This guidance will also have relevance to JECFA, and it will form a discussion document for eventual inclusion as a section of EHC 240: *Principles and nethods for the risk assessment of chemicals in food (7).*

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2.7 Extrapolation of MRLs to minor species

CCRVDF request for additional considerations concerning extrapolation of MRLs to minor species

The Twentieth Session of CCRVDF in 2012 *(2)* posed several questions to JECFA concerning the extrapolation of MRLs from major to minor species. The JECFA Secretariat engaged a consultant to prepare a draft working paper to review the background and JECFA practices regarding extrapolation of MRLs from major to minor species, to review available guidance from other sources and to prepare responses to each of the questions forwarded from the Twentieth Session of CCRVDF. The working paper was then circulated to members of an electronic working group of JECFA residue experts[5] for comment and discussion. Responses to the questions were finalized by the electronic working group and were presented to the Twenty-first Session of CCRVDF in 2013 *(4)*.

The Twenty-first Session of CCRVDF then addressed additional comments and questions to JECFA concerning the extrapolation of MRLs to additional (minor) species. JECFA's responses are provided below.

1. While JECFA's position is scientifically sound, in practice "compounds should be present in quantitatively similar proportions" could be unnecessarily restrictive for MRL extrapolation. Many jurisdictions do not require radiolabel studies (and hence MR:TR) in extrapolated species.

JECFA response: JECFA does not generally require data from a radiolabel study when considering a request for extrapolation of MRLs. Such data are requested only when available data do not enable JECFA to make a determination that the distribution and depletion of the drug residues in a minor species are comparable to those observed in a representative major species. This can apply particularly when the calculated exposure approaches the ADI and there is evidence of variability in marker residue to total residues (MR:TR) adjustment factors used in the dietary exposure calculations. JECFA has been reluctant to place quantitative limits on the "similar proportions" so as not to be unnecessarily restrictive when considering extrapolation. However, if there are large differences in the pattern of metabolites observed in two species, this may have a significant impact on the relationships between the marker residue and total residues and therefore also a significant impact on the dietary exposure calculations. These concerns must be reflected in advice provided by JECFA.

[5] The electronic working group consisted of Drs Joe Boison, Alan Chicoine (Health Canada), Holly Erdely, Lynn Friedlander, Fernando Ramos, Pascal Sanders, Stefan Scheid (German Federal Office of Consumer Protection and Food Safety) and Zonghui Yuan.

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2. For comparative metabolism data assessment in a major species, JECFA does not consider that metabolites in target animals should be present in "quantitatively similar proportions" to those observed in laboratory animals (from which the ADI is derived); rather, the compounds are required to be qualitatively similar (i.e. the same major metabolites should appear in the metabolite profile). Also, in many cases, estimated exposure to residues at the MRL represents only a fraction of the ADI. Consequently, the extrapolated MRLs would not exceed the ADI even if the MR:TR ratio is several-fold different.

JECFA response: Only metabolites of toxicological concern identified in studies in food animals are considered by JECFA when comparing metabolism in laboratory animals and food animal species for the recommendation of MRLs. It is correct that JECFA does not consider the quantitative relationships between metabolites observed in laboratory animals and those observed in food animal species, as the focus for recommendation of MRLs is on the behaviour of the residues in food animal species. Although it is also correct that there are instances in which only a small fraction of the ADI is represented in the dietary exposure calculation, there are also instances in which the estimated dietary exposure approaches the ADI. The Committee would have to be satisfied that there was adequate exposure of toxicological species to metabolites of toxicological concern observed in food animal species. This is one reason why JECFA has taken a case-by-case approach in responding to requests for recommendations on extrapolation. When the dietary exposure calculation represents only a small fraction of the ADI, there is less risk that estimated exposure will exceed the ADI when MRLs are extrapolated to a minor species, and therefore fewer data for the minor species should suffice. When the calculated exposure approaches the ADI, more information on residues that occur in foods derived from the minor species may be required to ensure that dietary exposure through consumption of edible tissues, milk or eggs from the minor species does not exceed the ADI. When the marker residue is the only residue of toxicological concern, then extrapolation of MRLs to a minor species should result in the same calculated dietary exposure.

3. JECFA may consider being flexible in defining the "reasonable limits" to define the comparative metabolic profile and in metabolism data requirements in extrapolated species based on the overall safety profile of the drug (e.g. proportion of ADI used). Alternatively, the MR:TR ratio from physiologically related species could be used for MRL extrapolation.

JECFA response: JECFA follows the procedures for assessment of metabolites as described in VICH guidance documents. As stated in response to a previous point, JECFA considers that it may at this point be

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unwise to specify "reasonable limits" in concise numerical terms. Given the variability in the quantity and quality of data available to support requests for extrapolation, it is better to assess each case based on the available data, taking into account the uncertainties in the data provided. A review of past JECFA recommendations of MRLs for the "related" species cattle and sheep reveals several instances in which differences in residue concentrations in tissues have resulted in differences in the MRLs recommended for one or more tissues from these species. In such cases, there may be a concern about using the MR:TR ratio from one major species for extrapolation.

4. We note that the EU has extensively extrapolated MRLs of veterinary drugs to all food-producing species. No serious public health issues have been reported because of public exposure to residues of veterinary drugs in extrapolated species.

JECFA response: While JECFA is aware of the practices followed in the European Union (EU) for extrapolation of MRLs, the situations are not necessarily directly comparable. JECFA follows principles established within CCRVDF and JECFA, consistent with principles and methods for the risk assessment of chemicals in food given in EHC 240. The species that are defined as minor within the regulatory context in the EU may not reflect the consumption patterns and potential exposure to veterinary drug residues within the global context. It is a requirement under current procedures followed by both CCRVDF and JECFA that there is evidence of an approved use of a drug (GVP) in a Member State before MRLs can be considered. JECFA risk assessments consider potential effects following established risk assessment principles. JECFA considers that a review based on a lack of reported health problems would be an insufficient means of ensuring public health protection and would be inconsistent with practices followed by the Codex Alimentarius Commission and JECFA to ensure protection of consumer health.

5. Absence of metabolites or residues of toxicological concern in extrapolated species can generally be substantiated by data from a radiolabel study. In practice, if radiolabel studies are available, MRLs can be established by routine procedure (i.e. extrapolation is not required).

JECFA response: The metabolites found in a major species are typically determined from radiolabel studies, where fractions are chromatographically separated from tissue extracts and then further characterized using spectroscopic and mass spectrometric techniques. Once such studies have been completed in a major species, the same metabolites, if present, may be identified in extracts of tissues from minor species that have been treated

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with the unlabelled drug using chromatographic separation combined with spectrometric techniques. The same approach may be taken to identify the metabolites formed in in vitro experiments. As noted in the CCRVDF comment, MRLs can be established following the routine procedures used for major species when complete radiolabel studies are available for the minor species. In such cases, extrapolation is not required. In addition, as noted in JECFA's response to the questions posed to JECFA by the Twentieth Session of CCRVDF and provided to the Twenty-first Session of CCRVDF, MRLs can be and have been recommended for minor species when there are no or very limited data from radiolabel studies conducted in the minor species, but data from a depletion study with unlabelled drug are available. In such cases, JECFA will usually apply MR:TR ratios from a physiologically related major species, if required, in the intake calculation. For example, the seventieth meeting of JECFA (Annex 1, reference *193*) used MR:TR ratios for chicken to recommend common MRLs for chicken and turkey.

6. Radiolabel studies are generally not available when extrapolation is requested. Rather than asking to demonstrate the absence of metabolites of toxicological concern, could a practical approach be taken to ascertain, based on available data and public literature, whether there is any evidence suggesting that metabolites or residues of toxicological concern occur in extrapolated species (i.e. absence of evidence, rather than evidence of absence)?

JECFA response: The information available on residues of a drug in a minor species typically includes either no data from a radiolabel study or else very limited data from a study that is not compliant with good laboratory practice (GLP). In these situations, JECFA considers other information on metabolism that may be available, such as the identification of known metabolites found in the major species in tissues from the minor species by chemical analysis techniques, the body of information available on the metabolism of the drug in other laboratory and food animal species and data from in vitro studies, if available. Information on the metabolism of chemically related drugs or on common metabolic pathways may also be considered. It is difficult to prove a negative (no novel unknown metabolites of potential toxicological concern in tissues of the minor species), so JECFA will consider the available information and assess the potential for the occurrence of a previously unknown metabolite to occur in the minor species. This has not typically been a major source of uncertainty when JECFA has considered requests for extrapolation. The key issues are commonly lack of information on distribution profiles, depletion profiles and/or MR:TR ratios in the minor species. Lack of such information means that there is a greater level of uncertainty associated with the MRL recommendations, and this uncertainty will be outlined in the JECFA evaluations.

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7. Could a well designed marker residue depletion study further substantiate this?

JECFA response: A review of past JECFA decisions indicates that JECFA has been more likely to recommend the extrapolation (extension) of MRLs from a major to a minor species when data from a residue depletion trial with unlabelled drug are available for the minor species. For example, the extrapolation of MRLs for moxidectin from cattle to deer was based on in vitro comparative metabolism data and a residue depletion study with unlabelled drug in deer. MRLs for phoxim were extrapolated from cattle and sheep to goats based on metabolic data for cattle and sheep and a residue depletion study with unlabelled drug in goats. MRLs for other drugs, such as colistin and erythromycin, were extrapolated from chicken to turkey based on metabolic data for chicken and evidence of a common marker residue and a validated analytical method for turkey tissues. MRLs for deltamethrin in salmon have been recommended by JECFA based primarily on data from pharmacokinetic and depletion studies with unlabelled drug, using information from metabolic, pharmacokinetic and depletion studies in major food animal species to supplement the information. The problems faced by JECFA when asked to recommend extrapolation typically involve an absence of data from "well designed" studies.

8. Could JECFA consider extrapolation to all aquatic animals instead of just finfish, provided minimum criteria are met?

JECFA response: In principle, the extrapolation of MRLs to all food-producing animals once similar MRLs have been established in a major species representative of each class seems a practical solution. However, current procedures within CCRVDF and JECFA require that evidence of an approved use of the drug in a Member State (GVP) must be provided for any food-producing species for which extrapolation of MRLs is requested. JECFA will consider whether the extrapolation of the MRLs is valid, taking into consideration all the data available to the Committee in the classes of animals exposed by the treatment, including the existence of MRLs in a species considered as physiologically related to the species to which extrapolation of MRLs has been requested. A future JECFA may, for example, consider recommending extension of MRLs from one species of fish to related species of fish. However, this will be considered only when there is evidence of approved usage of the drug in multiple species and GVP conditions are available for review.

9. JECFA may also wish to consider other in-built safety (e.g. human exposure to residues at MRL in species in which MRLs are established often represents only a fraction of the ADI, which could compensate for any

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differences in MR:TR ratio) inherent in the MRL establishment procedure in future extrapolation work.

JECFA response: Under current procedures, when data to establish an MR:TR ratio in tissues of a minor species to which extrapolation of MRLs has been requested are not available, JECFA will consider assigning surrogate values based on the most conservative MR:TR ratios determined for other species to which MRLs have been assigned. The new GECDE calculations assessed in the pilot study of alternative approaches to dietary exposure assessment by the current Committee may also provide additional flexibility in the consideration of future requests for the extrapolation of MRLs.

JECFA guidance for the extrapolation of MRLs to minor species

The following is guidance on the criteria/assumptions currently used by JECFA for interspecies extrapolations, including minimum data required to support such extrapolations among physiologically related species and extrapolation to additional minor species.

When requested to consider the extrapolation of MRLs to another species, JECFA must address certain issues related to the toxicology of the residues and the dietary exposure calculations, based on the MRLs, to ensure consumer safety. Basically, it must be determined from the available information whether there is a significant risk that the ADI will be exceeded if the MRLs previously established for the major species are extrapolated to the additional minor species. It is inherent, when considering extrapolation, that a complete data package is not available for use of the drug under GVP in the minor species to which extrapolation of MRLs has been requested. Past JECFA reports have also used the term *extension* when making such recommendations, particularly when the recommendation is based on data from a residue depletion study in the minor species. However, in those cases, there have been other gaps in the available data, typically data obtained from a radiolabel study to determine total residues and their relationship to the marker residue. JECFA will use the term *extension* when sufficient depletion data are available for the minor species to permit the derivation of MRLs for tissues of that species from the depletion curves. The term *extrapolation* will be used when insufficient depletion data are available in that species to derive MRLs for tissues from that species.

The minimum requirements for extrapolation identified in EHC 240, which include data on metabolism in the minor species, a common marker residue and the availability of an analytical method suitable for application to foods derived from the minor species, should be satisfied. In addition, JECFA should determine if there is a significant risk that the extrapolation will result in an exposure that exceeds the ADI. The fifty-second meeting of

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JECFA (Annex 1, reference *140*) made a distinction between major species (cattle, pigs, sheep and chickens), for which a full residue data package is required for the establishment of MRLs, and minor species, which are considered to include all other food animal species. JECFA will continue to use these designations when addressing requests for the extrapolation of MRLs to additional species, so that a full data package will continue to be required to support a recommendation of MRLs for a species designated as a major species. When a complete data package is available for the minor species, the usual evaluation procedures will be followed to recommend MRLs for the minor species, based on the residue data for use in the minor species. When a full residue data package is not available for the minor species, then the available information will be considered to determine if the MRLs previously established for a relevant (i.e. physiologically related) major species may be extrapolated to the minor species, consistent with estimates of potential dietary exposure for consumers.

Two factors that are used in the dietary exposure calculation could affect the outcome of that calculation. First, the MR:TR ratio is considered to determine whether available information suggests a significant risk that the MR:TR ratio in the tissues of the minor species differs significantly from that observed in the major species and whether this could result in a higher estimate of the dietary exposure, which could exceed the ADI. There typically is not a large difference in MR:TR ratios in the comparable edible tissues of the various food animal species. When the information is not available to confirm that the ratios are the same in the major and minor species being compared, the ratio for any species for which MRLs have been established that will result in the highest estimate of exposure will typically be considered by JECFA.

The second factor that has sometimes been used in the dietary exposure calculation is a bioavailability factor, which adjusts the exposure to the fractional amount of the residue that may be absorbed during the digestive process, based on experimental data. It is preferable that this factor should be confirmed as equivalent for tissues from multiple species.

The available information on GVP use in the additional (minor) species must also be assessed to determine if the extrapolated MRLs are practical; that is, it must be determined if the MRLs are consistent with the withdrawal period established as a condition of the GVP use in the minor species. When this information is not available, that will be indicated in the risk assessment provided by JECFA to CCRVDF.

The above issues are addressed by consideration of the following questions in the review of a request for extrapolation of MRLs by JECFA:

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- Have MRLs previously been established in a relevant major species (i.e. a physiologically related species)? In the absence of such MRLs, a data package is required for the species under consideration. If an ADI has not previously been established for the compound, then a full toxicological evaluation is required to establish an ADI.
- Do the residues found in the species to which extrapolation of MRLs is under consideration contain any metabolites or bound residues of unknown toxicity that are not present in the major species for which MRLs have been established? This is addressed by the requirement that the metabolic profiles are comparable in the two species. The requirement therefore may be satisfied by either a minimum set of comparative metabolism data, which might be provided through in vitro methods, or a sound scientific argument as to why there should be no expected difference in the metabolic profiles (e.g. based on information available for related compounds).
- Does the marker residue include all residues of toxicological concern? When the marker residue is the only residue of toxicological concern, it is not necessary to consider total residues in the dietary exposure assessment. However, when the total residue is considered to be of toxicological concern, then the ratio between marker residue and total residues must be considered.
- Is an adjustment factor for bioavailability used in the dietary exposure calculation? When a bioavailability factor has been applied in the dietary exposure estimates, it must be determined whether the same factor should also be applied to the additional species.
- Are the dietary consumption quantities used in the dietary exposure calculation appropriate? JECFA is considering alternatives to the previously used TMDI and EDI dietary exposure calculations to better refine estimates of consumer exposure.
- Are data available to demonstrate that the extrapolated MRLs are consistent with the GVP conditions of use (withdrawal period) established for the use of the drug in the minor species? When data from a depletion study conducted in the additional species are available, it can usually be determined whether the extended MRLs are consistent with the approved conditions of use (GVP). In the absence of residue depletion data for the minor species, pharmacokinetic data may provide an indication of similarities or differences in rates of absorption and elimination in the major and minor species, which may suggest similarities or differences in depletion rates from tissues. The absence of depletion data for the minor species should not preclude a recommendation that the MRLs established for the major species may

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be extrapolated to the minor species. However, such a recommendation should include the information that it could not be confirmed that the MRLs are consistent with the withdrawal periods that may have been established by national authorities that have approved the use of the drug in the minor species.

To support an extrapolation, some of the required data must be obtained from experimental in vitro and in vivo studies. Extrapolation requires a case-by-case approach, as many factors are drug dependent.

Based on the above considerations, the following principles have been established, to be applied by JECFA when considering the extrapolation of MRLs from major to minor species:

- There should be evidence of approved use of the drug under GVP (label or equivalent information) in the minor species in one or more Member States of Codex.
- MRLs should already have been established by Codex in one or more species considered as relevant for extrapolation of MRLs to the "minor" species (e.g. chicken to turkey, cattle or sheep to goat).
- Information should be available to enable the Committee to determine that the metabolic profiles are qualitatively and quantitatively similar in the two species, with parent drug and major metabolites present in edible tissues in similar proportions, although the concentrations of the residues may differ in the two species due to factors such as dosage and pharmacokinetics.
- Strict numerical limits should not be applied when assessing the quantitative relationships, but the parent compound and major metabolites should be in similar proportions in the species compared to provide some evidence of similar residue distribution and composition in the major and minor species.
- The sum of the minor metabolites and bound residues should constitute equivalent proportions of the total residue in both species.
- The same marker residue designated for the major species should be appropriate for monitoring residues in edible tissues of the minor species.
- When data are not available to establish the relationship between the marker residue and total residues and total residues are of toxicological concern, the MR:TR ratios observed in other relevant species may be considered and used as surrogate values for the minor species. The same considerations also apply to bioavailability factors that may be used in the dietary exposure calculations.
- When residue depletion data are incomplete or unavailable for the drug in the minor species, other sources of information, such as data from

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metabolic and pharmacokinetic studies, may be used to compare the behaviour of the drug in the major and minor species.

- When residue depletion data are not available for the minor species to confirm that an MRL extrapolated from a major to a minor species is consistent with the GVP use in the minor species, this should be noted with any recommendations of MRLs for the minor species.
- A validated analytical method used for the determination of residues of the drug in edible tissues of the major species should be considered suitable for extension to the analysis of residues of the drug in tissues of the minor species. When an expert review of the available methodology does not consider such an extension to be likely, a validated analytical method for the determination of residues of the drug in edible tissues of the minor species is required.

Fig. 1 contains a decision-tree for the process to be followed by JECFA in determining whether extrapolation of MRLs from a major species to a minor species may be recommended. The risk assessment provided by JECFA to CCRVDF should indicate additional uncertainties associated with the dietary exposure calculations if the MRLs are extrapolated to the minor species (e.g. MR:TR ratio could not be confirmed for the minor species, so the highest MR:TR ratio observed for another species for which MRLs have been established was used) or the data did not enable JECFA to confirm that the MRLs are consistent with the withdrawal period established by a Member State for use of the drug in the minor species under GVP.

Use of models in the extrapolation of data

The Committee noted the recent publications in the peer-reviewed literature on the use of predictive models for residue distribution in tissues based on pharmacokinetics and considered that developments in this area should continue to be monitored and discussed at future meetings of the Committee, as this has the potential to be a useful approach when considering extrapolation of MRLs.

2.8 MRLs for veterinary drug residues in honey

CCRVDF request to JECFA for additional considerations concerning the establishment of MRLs for honey

Is it possible to establish MRLs for honey using monitoring data from national authorities, similar to the approaches used by JMPR for setting MRLs for spices?

JECFA response: Alternative approaches to obtaining residue data to support MRLs for honey other than the traditional dose administration under controlled situations were discussed by the seventieth meeting of the Committee (Annex 1, reference *193*) and also by the electronic working

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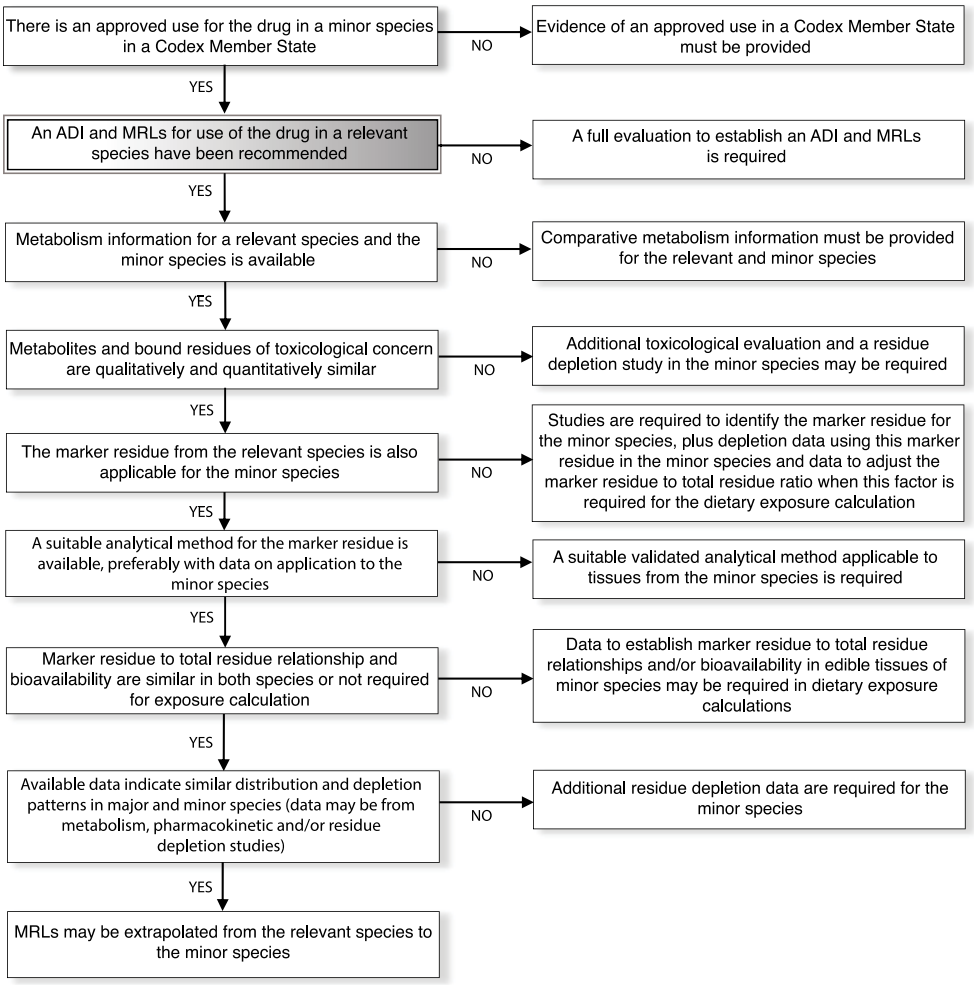
Fig. 1

Decision-tree for extrapolation of MRLs to minor species



group of JECFA,[6] which provided responses to the Twenty-first Session of CCRVDF *(4)* in answer to questions posed to JECFA by the Twentieth Session of CCRVDF *(2)*. Given the difficulty in obtaining reliable residue depletion data from a limited study (i.e. it appears that multiple hives at multiple locations and times may be required to derive a representative picture), JECFA agrees that the consideration of alternative approaches to

[6] The electronic working group consisted of Drs Joe Boison, Alan Chicoine (Health Canada), Holly Erdely, Lynn Friedlander, Fernando Ramos, Pascal Sanders, Stefan Scheid (German Federal Office of Consumer Protection and Food Safety) and Zonghui Yuan.

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the standard dose administration trials conducted for most uses of veterinary drugs is indicated.

JECFA is aware that JMPR has used information from surveys (monitoring data) in the recommendation of MRLs for spices and that monitoring data are also used in establishing maximum limits for some contaminants. An approach using data from statistically based surveys of product in the marketplace could provide some assurance that the MRLs established in this manner for veterinary drug residues in honey are consistent with GVP and provide appropriate protection to consumers.

As an alternative, a supervised field trial approach, such as that taken by JMPR for residue evaluation of pesticides, may be more appropriate for the establishment of MRLs for veterinary drug residues in honey than the conventional residue study approach used in the evaluation of veterinary drug residues in animal tissues, milk and eggs. This could perhaps be achieved by structuring residue studies so that composite samples taken at each of four or five geographically separated locations are analysed to provide a representative set of data for typical field conditions. Further guidance on the design of residue studies for veterinary drugs used in honey is being developed by VICH and will be considered as guidance by JECFA when it is complete.

JECFA guidance for the establishment of MRLs in honey

The discussion paper prepared for the seventieth meeting of JECFA (Annex 1, reference *193*) described the various issues related to the establishment of MRLs for honey and some particular problems associated with the evaluation of residues for the establishment of MRLs. Extensive variability can be observed in the concentrations of the residue found in samples collected from different areas of the same hive or from different hives. For large-scale production, where products from various sources are blended in bulk, samples from multiple hives at multiple locations and times may be required to derive a representative picture for the typical bulk product in international trade. In addition, any reduction in residue concentration is typically a result of dilution or chemical degradation of the parent drug over time from sources such as moisture, heat and light exposure, rather than from metabolic processes. Furthermore, as the depletion pathway in honey is different from the typical metabolic pathways in animals treated with drugs, the marker residue designated for tissues, milk and/or eggs may not be appropriate for honey.

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Data requirements

Honey is generally sold internationally not in small quantities collected from a single hive or producer, but rather as a bulk commodity that contains honey from multiple sources. Thus, MRLs established within the Codex Alimentarius system must reflect residue concentrations expected to be found in bulk honey from multiple producers with hives treated under GVP. The Committee noted that, as stated in EHC 240, MRLs for honey cannot be recommended based on extrapolation from MRLs for tissues, eggs or milk and considered that MRLs derived using extrapolation may result in MRLs for honey that are not consistent with the approved GVP use in Member States. If practices followed by JECFA in recommending MRLs for veterinary drugs used in honey production are to be consistent with those followed for recommending MRLs for edible tissues, milk and eggs from food-producing animals treated with veterinary drugs, the following information is required:

- all available information on approved uses in a Codex Member State;
- an existing ADI or the availability of toxicological data to establish an ADI;
- data to establish a marker residue in honey;
- evidence of a validated analytical method for the determination of residues in honey;
- data on the nature of residues in honey, typical concentrations found and the stability of these residues.

It must be noted that the evaluation of drug residues in honey differs from the evaluation of residues of drugs used in other species of food-producing animals, as there are no pharmacokinetic depletion data or metabolic pathways to consider. The reduction of concentrations of residues in honey is from dilution and/or environmental factors. In addition, the use of veterinary drugs in honey production is usually considered as a minor use in a minor species, and therefore a policy on risk assessment requires some flexibility. Data on the depletion of residues in honey will therefore be considered by JECFA from statistically based field trials (which should be conducted according to guidance from VICH when this has become available) or from other sources, such as data from statistically based national monitoring programmes. Three potential situations are envisaged by CCRVDF and JECFA:

1. the establishment of an MRL for honey for substances with an ADI, typically established by JECFA or JMPR, and/or a Codex MRL in a food-producing animal or food commodity;
2. the establishment of an MRL for honey for substances for which an ADI has not previously been established by JECFA or JMPR; or

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3. the establishment of an MRL for honey for substances that are not approved for use in food animals.

The manner in which each of these situations may be assessed and the data requirements for such assessments differ, based on the information that is already available from prior evaluations of the safety of the substance.

1) Substances with an established ADI and/or MRL in a food-producing animal or food commodity

The main groups of substances that typically leave residues in honey are antibiotics and persistent lipophilic acaricides. Of the products known to be used for treatment of bee diseases, most, but not all, have a national registration and a JECFA or JMPR evaluation with an ADI and/or MRL for either a food-producing animal or other food commodity. The MRLs recommended by JECFA must be consistent with GVP to protect consumer health while ensuring that the veterinary product can be used effectively. It is proposed that the data requirement for compounds with an existing ADI and/or Codex MRLs would be limited to residue depletion studies in honey, which could be used to establish Codex MRLs in honey and by national authorities to also establish withdrawal periods following treatment.

While available information suggests that the parent drug is expected to be the marker residue in honey in most situations, this should be confirmed before residue studies are conducted. Residue studies using the marker residue compound in honey may then be used to provide data for the recommendation of MRLs consistent with GVP, which are therefore practical for monitoring residues in products in international trade. Further details of the factors to consider in developing MRLs relating to the use of veterinary drugs for bees are contained in the report of the seventieth meeting of JECFA (Annex 1, reference *193*).

2) Substances for which an ADI has not previously been established by JECFA or JMPR

In the case of a new substance not previously considered for registration by national authorities, substances would have to be evaluated as new animal drugs or pesticides and subject to a full food safety risk assessment. This issue was discussed at the seventieth meeting of JECFA (Annex 1, reference *193*).

3) The establishment of an MRL for honey for substances that are not approved for use in food animals

In the situation in which it has been recommended by the Committee that a substance should not be used in food-producing animals (e.g. chloramphenicol or nitrofurans), no exception for honey would be applied. This issue was also discussed at the seventieth meeting of JECFA (Annex 1, reference *193*).

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Fig. 2

Decision-tree for establishment of MRLs for veterinary drug residues in honey

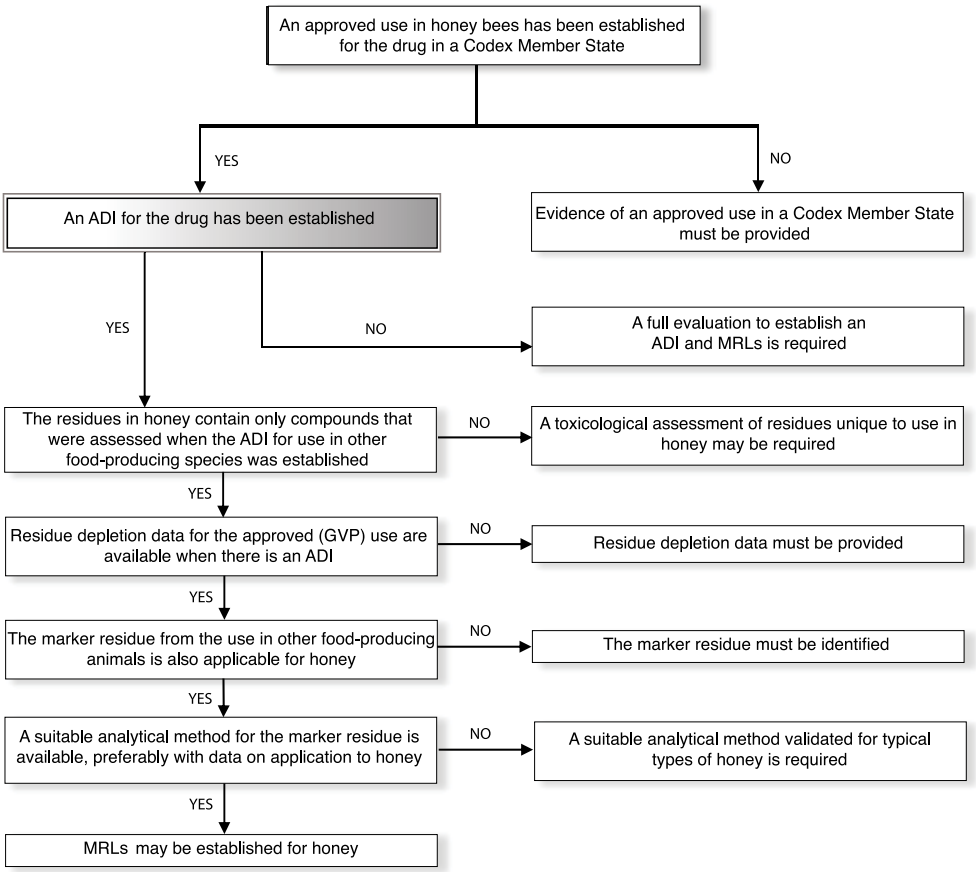

Fig. 2 illustrates the decision-tree approach to establishing MRLs for veterinary drug residues in honey to be followed by JECFA at future meetings.

Dietary consumption

A dietary portion size of 50 g/person per day was recommended for honey by the Twenty-first Session of CCRVDF, consistent with the recommendation of the seventieth meeting of JECFA (Annex 1, reference *193*). This quantity will be used in dietary exposure estimates (EDI or TMDI) performed during risk assessments by JECFA, replacing the 20 g of honey per person per day used in exposure calculations conducted prior to the current meeting of JECFA. JECFA is currently improving its dietary exposure methodology and may use an updated alternative value based on updated food consumption data.

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2.9 Scope of MRLs established by JECFA relating to fish and fish species

The Committee noted that some previous recommendations for MRLs have been for specific species of fish, such as salmon and trout, whereas others have been for "fish", which could be interpreted to include shellfish. To more accurately reflect the species for which MRL recommendations are made, the Committee recommends, consistent with the terminology used in the report of the Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs *(8)*, that the term "fish" should be used when an MRL recommendation applies to multiple species of finfish. For other "seafood", the term "mollusc" should be used for species such as clams, oysters and scallops, and the term "crustacean" should be used when MRLs are recommended for species such as shrimp, prawn and crayfish. When the recommendation of an MRL is for a specific species of fish or seafood, this will be reflected in the MRL recommendation. In this regard, the Committee considered that it may be appropriate to also identify some representative species of fish, such as salmon, and of seafood, such as shrimp (crustacean), as "major species" of fish and seafood. It is recommended that this matter should be further discussed at a future meeting of the Committee.

2.10 JECFA analytical method validation requirements

Current JECFA guidelines for the validation of analytical methods were adopted at the fifty-second meeting of the Committee in 1999 (Annex 1, reference *140*) and subsequently published as Annex 3 of Food & Nutrition Paper 41/14. The present Committee noted that a new Codex guideline, *Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals* (CAC/GL 71-2009), includes detailed updated information on criteria for the selection and validation of analytical methods suitable for use in regulatory programmes for the control of veterinary drug residues in foods. The Committee agreed that the method selection and validation criteria contained in CAC/GL 71-2009 and subsequent revisions to these guidelines will be applied when assessing the suitability of methods proposed to JECFA as regulatory methods to support recommended MRLs. The Committee also agreed that in view of developments in method validation criteria that have occurred since the adoption of the current JECFA method validation requirements in 1999 *(9)*, the criteria for validation of methods used in the pharmacokinetic, metabolism and depletion studies submitted to the Committee should be reviewed and updated at a future meeting of the Committee.

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2.11 Guidance for the evaluation of veterinary drug residues in food by JECFA

The Committee reiterated the decision made at the seventy-fifth meeting (Annex 1, reference *208*) to update the guidance for both FAO and WHO experts for the preparation of working documents. The Committee requests the JECFA Secretariat to undertake this work in collaboration with WHO and FAO experts.

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3. Comments on residues of specific veterinary drugs

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

3.1 Derquantel

Explanation

Derquantel (Chemical Abstracts Service [CAS] No. 187865-22-1), a broad-spectrum anthelminthic agent with activity against the adult and larval stages of gastrointestinal nematodes in sheep, was evaluated at the seventy-fifth meeting of JECFA in 2011 (Annex 1, reference *208*). Derquantel is available only as a combination product with abamectin. At the seventy-fifth meeting, an ADI of 0–0.3 µg/kg bw was established. Although deficiencies were identified in the residue dossier, MRLs were recommended, determined as derquantel, in sheep tissue at 0.2 µg/kg in muscle, 0.2 µg/kg in kidney and 0.7 µg/kg in fat. In addition, an MRL of 0.2 µg/kg in liver was estimated by the Committee; however, due to an error, this MRL was presented in the report as 2 µg/kg. There were insufficient data to calculate an EDI, and the TMDI approach was used.

At the Twentieth Session of CCRVDF *(2)*, concern was raised regarding the ADI, and a proposal for an alternative approach to the derivation of the MRLs for derquantel in sheep tissues was submitted. CCRVDF requested that JECFA (i) review the ADI in light of a possible different interpretation of the toxicological data, (ii) review the calculation of the marker to total radiolabelled residue and (iii) revise the recommended MRLs, if appropriate.

Review of the ADI

Previous JECFA evaluation

The Committee at the seventy-fifth meeting considered the acute clinical observations in two 90-day toxicity studies in dogs, which were consistent with the antagonistic activity of derquantel on nicotinic acetylcholine receptors,

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to be the most relevant toxicological effect for the establishment of an ADI for derquantel. The lowest-observed-adverse-effect level (LOAEL) for this effect was 0.1 mg/kg bw per day in both studies. The Committee established an ADI of 0–0.3 µg/kg bw based on the LOAEL and an uncertainty factor of 300. An uncertainty factor of 3 was used in addition to the default uncertainty factor of 100 for interspecies and intraspecies variability to account for the use of a LOAEL instead of a no-observed-adverse-effect level (NOAEL). The Committee noted that the dog is appreciably more sensitive than the rat to the anti-nicotinergic effects of derquantel, but had no information to allow a relative comparison with humans. The Committee further noted that it may be possible to refine the ADI with additional studies, in particular on the comparative sensitivity of the nicotinic acetylcholine receptors to derquantel among experimental animals and humans.

The Committee performed benchmark dose (BMD) modelling to better define a point of departure (POD) for nictitating membrane protrusion, which was among the most sensitive effects seen in the dog. There were only limited data available to model a dose–response relationship for the elicitation of protrusion of the nictitating membrane in the dog, and there existed a high degree of uncertainty at the low end of the dose–response curve. Therefore, in order to conduct the BMD analysis, the Committee considered combining the results from the two 90-day toxicity studies in dogs. However, the two studies from which the data were derived differed in the age of dogs on study (juvenile versus adult) and mode of test article administration (capsule versus gavage). Therefore, the Committee decided that it was inappropriate to combine the data from the two toxicology studies. In view of the above, the Committee concluded that it was not possible to apply the BMD approach to an evaluation of this compound.

Concern from sponsor

The sponsor submitted a report containing a BMD analysis of two critical end-points from the two 90-day dog studies with derquantel, which were conducted at different laboratories. The two end-points, based on cage-side observations, were protruding nictitating membranes and dilated pupils, which are related to the mode of action of derquantel (a neuronal nicotinic acetylcholine receptor antagonist). Both effects were observed in both studies, but with marked differences in the dose–response relationship. Possible reasons for the differences were discussed in the report, including differences in the concentration of an impurity (paraherquamide) in the test materials used in the studies.

The sponsor conducted the BMD analysis with guidance from external experts, using the United States Environmental Protection Agency's (USEPA)

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BMD software (BMDS version 2.2) and following USEPA guidance for BMD analysis. For each of the two critical end-points in each study, there was one observation per day post-dosing for 91 consecutive days for each individual animal (four of each sex per group); thus, combining the sexes, there were in total 728 observations for each dose group per study over the course of the 91 days. These data were transformed into continuous data by calculating for each dose group the ratio of positive observations (sum of clinical signs observed with any animal in a specific dose group at any day during the study) to total observations (n = 728). As such, the effects were expressed as fractions ranging from 0 to 1.

The transformed continuous datasets were then analysed with the BMD software using the models for continuous data. Because there were differences between the two studies, but no marked differences between sexes with regard to the two end-points observed, data from both studies were modelled separately, whereas data from both sexes within each study were combined for modelling. A benchmark response corresponding to a 5% or 10% change in proportional response from the control was selected, and BMDs and the lower 95% confidence limits on the BMDs (BMDLs) were determined. The lowest $BMDL_{05}$ of 0.096 mg/kg bw per day for one of the critical effects (dilated pupils) obtained from the dataset from the study with the higher impurity concentration (1462N-60-05-703) was the most conservative estimate and was proposed as the POD for the risk assessment.

Comments by the present Committee

Following an expert review,[7] the Committee concluded that the sponsor's proposed methodology for dose–response modelling of derquantel was statistically valid. The Committee found the sponsor's proposed BMD modelling approach to be an unusual one. No information was available to demonstrate that the approach of converting dichotomous clinical observational data to continuous data has been applied previously. The Committee therefore considered that, in the absence of independent verification of the scientific reliability of the BMD approach proposed by the sponsor, it would not be appropriate to use this approach for the purpose of determining the critical POD for establishing an ADI. The Committee concluded that the LOAEL approach for the determination of the ADI for derquantel remains appropriate, and the ADI as established by the Committee at the seventy-fifth meeting was maintained.

[7] The review was conducted by Klaus Schneider and Eva Kaiser, Forschungs- und Beratungsinstitut Gefahrstoffe GmbH, Freiburg, Germany, upon request from the JECFA Secretariat.

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Residue evaluation

No new data or studies were provided for the current evaluation. Details of the concerns that had been expressed during the Twentieth Session of CCRVDF were provided in a submission from a Member State. Additionally, an alternative approach to determining the ratio of marker residue to total residues was proposed by the sponsor.

Concern from Member State

The concern identified is that the ratios of marker residue to total residues (MR:TR) used by JECFA are not appropriate given the time point selected for recommending MRLs. As a result, the selected MR:TR ratios may lead to an underestimation of exposure. The request for clarification included an outline of concerns over the interpretation of the MR:TR ratios used in the risk assessment together with an interpretation of the total residue data. The conclusion reached in the analysis included in the submission is that the proposed JECFA MRLs will lead to a TMDI estimate that exceeds the established ADI.

Analyses provided to the Committee suggest that the marker residue will be much lower during the slow terminal phase of elimination, resulting in lower MR:TR ratios. Supporting scenarios for MR:TR ratio interpretation were provided. The submission noted that the samples may have been stored at a temperature insufficiently low to maintain sample integrity, resulting in a reduction in derquantel concentrations. The MR:TR ratio adjusted for reduction during storage of the MR would be less than 0.01 (< 1%) at day 6 and would be expected to be even lower at day 8, the time point used for the JECFA MRL recommendations. Finally, the submission noted that the changes in MR:TR ratios over time are also illustrated using results from the marker residue study and a separate total residue study. However, the submission did not propose that this approach be used for the MRL estimation or the dietary exposure calculation.

Alternative approach from sponsor

The alternative approach proposed does utilize the marker residue depletion data and the combined total residue data to calculate an MR:TR ratio to be used for the MRL estimation and the dietary exposure calculation. The focus remains on early time points (≤ 6 days of withdrawal), the only time points for which there are total residue data. Because the animals in the residue depletion study received a dose 50% higher than that used in the radiolabel study, the residue values from the radiolabel study were multiplied by 1.5 to allow comparison of radiolabelled residues with the marker residues (i.e. normalization of residues to 1.5-fold dosing across studies).

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Analytical methods

Two validated analytical methods, based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) principles, were reviewed previously. These methods remain appropriate for the determination of derquantel residues in sheep tissues.

Maximum residue limits

The current Committee reconsidered the appropriateness of the MRLs recommended by the seventy-fifth meeting of the Committee in light of the new proposed interpretation of the existing data.

The Committee reviewed the comments provided. A reassessment of the residue depletion data indicated that residues at day 6 are consistent with a total exposure below the TMDI. Thus, the day 6 time point can be used for the recommendation of MRLs, rather than the day 8 time point used for the initial assessment. Data through day 6 were used to determine the MR:TR ratios.

Regarding the alternative approach, the Committee concluded that determining the MR:TR ratio from the radiolabel study was customary and preferred practice. This customary approach is compatible with MR:TR ratios through day 6.

In recommending MRLs for derquantel, the Committee considered the following factors:

- An ADI of 0–0.3 µg/kg bw was established previously by the Committee (Annex 1, reference *208*) and confirmed at this meeting, based on an acute toxicological end-point. The upper bound of this ADI is equivalent to 18 µg/day for a 60 kg person.
- Derquantel is extensively metabolized; derquantel represents 6% of total residues in muscle, 3% in liver, 7% in kidney and 15% in fat. Derquantel, although constituting a small percentage of total residues, is suitable as the marker residue in tissues. No data are provided for residues in sheep milk.
- Liver contains the highest concentration of total radiolabelled residues at all sampling times. Fat contains the highest concentrations of derquantel residues in the unlabelled residue depletion studies at early sampling points. At times beyond the day 4 sampling time, derquantel residues are highest in liver. Derquantel residue concentrations are variable. The highest concentration of the proposed marker residue, derquantel, at time points relevant to recommending MRLs is found in liver, followed by fat, then kidney and then muscle. Liver and fat can serve as the target tissues.

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- A validated analytical procedure for the determination of derquantel in edible sheep tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.
- The MRLs recommended for sheep tissues are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile (the "upper tolerance limit 95/95" or UTL 95/95) for the day 6 post-treatment data from the unlabelled residue depletion study.

Based on these new assessments, the Committee proposed the following revised MRLs in sheep tissues: 0.3 µg/kg in muscle, 0.4 µg/kg in kidney, 0.8 µg/kg in liver and 7.0 µg/kg in fat. There were insufficient data to calculate an EDI, and the TMDI approach was used.

Using the model diet and the MT:TR approach, these MRLs result in an estimated dietary exposure of 6.8 µg/person, which represents approximately 38% of the upper bound of the ADI.

A residue monograph was prepared.

Summary and conclusions

ADI

The ADI of 0–0.3 µg/kg bw established by the Committee at the seventy-fifth meeting was maintained.

MRLs

The Committee proposed the following revised MRLs: 0.3 µg/kg in muscle, 0.4 µg/kg in kidney, 0.8 µg/kg in liver and 7.0 µg/kg in fat.

Dietary exposure

There were insufficient data to calculate an EDI, and the TMDI approach was used. Using the model diet and the MT:TR approach, these MRLs result in an estimated dietary exposure of 6.8 µg/person, which represents approximately 38% of the upper bound of the ADI.

3.2 Emamectin benzoate

Explanation

Emamectin benzoate (CAS No. 155569-91-8) is a macrocyclic lactone insecticide derived from the avermectin series isolated from fermentation of *Streptomyces avermitilis*. Emamectin benzoate contains a mixture of at least 90% emamectin B1a benzoate and at most 10% emamectin B1b benzoate. Emamectin benzoate acts by stimulating the release of γ-aminobutyric acid,

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an inhibitory neurotransmitter, thus causing insect paralysis within hours of ingestion and subsequent insect death 2–4 days later.

Emamectin benzoate is authorized for use as a pesticide on fruits, vegetables, cereals, tree nuts, oilseeds, herbs and tea. It is also registered for use as a veterinary drug in the treatment of sealice infestations in Salmonidae and other finfish in several countries. Emamectin benzoate is used as a premix coated onto non-medicated fish feed pellets to achieve an intended dose of 50 µg/kg of fish biomass per day for 7 days. It can be used up to 3 times per year with a maximum of five treatments in any 2-year growth cycle.

Emamectin benzoate has not previously been evaluated by the Committee. The Committee evaluated emamectin benzoate at the present meeting at the request of the Twentieth Session of CCRVDF *(2)*, with a view to establishing an ADI and recommending MRLs in salmon and trout. Other avermectins, such as ivermectin, eprinomectin and doramectin, have previously been evaluated by JECFA (Annex 1, references *92*, *105*, *120*, *135* and *158*). Although no data were submitted to JECFA by the sponsor for the evaluation of emamectin benzoate, JECFA decided to undertake an evaluation based on the recent JMPR evaluation and published literature.

Toxicological and microbiological evaluation

Because a recent toxicological evaluation of emamectin benzoate by JMPR *(10)* was available to the Committee, it was decided to use this as the basis for the evaluation. The Committee reviewed the full JMPR evaluation of emamectin benzoate, which included the establishment of both an ADI and an ARfD.

JMPR established an ADI of 0–0.0005 mg/kg bw for emamectin benzoate on the basis of an overall NOAEL of 0.25 mg/kg bw per day in 1- and 2-year studies in rats for increases in body weight gain, serum triglyceride concentrations and relative kidney weight at 1.0 mg/kg bw per day and on the basis of an overall NOAEL of 0.25 mg/kg bw per day in 14- and 53-week studies in dogs for histopathological changes in the brain, spinal cord and sciatic nerve and clinical signs of neurotoxicity at 0.5 mg/kg bw per day. An additional uncertainty factor of 5 was applied to the default uncertainty factor of 100 for interspecies and intraspecies variability, as a number of studies in mice, rats and dogs showed steep dose–response curves and irreversible histopathological effects in neural tissues at the LOAEL. JMPR noted that a NOAEL based predominantly on such histopathological changes is considered to be less sensitive than the observation of clinical signs. Moreover, in the 1-year dog study, animals were killed in extremis at doses that were only 3 times higher than the NOAEL in this study.

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JECFA confirmed that the critical NOAEL for establishing an ADI for emamectin benzoate was 0.25 mg/kg bw per day, identified in long-term studies in rats and in short-term studies in dogs. However, the Committee considered the neurotoxic effects in dogs, typical of this family of drugs, as the most relevant critical effects in establishing the ADI. It was therefore concluded that it would be appropriate to base the ADI on the overall NOAEL of 0.25 mg/kg bw per day for neurotoxicity from the 14- and 53-week studies in dogs, supported by the overall NOAEL of 0.25 mg/kg bw per day from the 1- and 2-year studies in rats.

In addition, JECFA considered the need to establish a microbiological ADI. Emamectin, like other related avermectin compounds, has neither antibacterial nor fungicidal properties, and effects on the intestinal microflora have not been reported. Therefore, the Committee determined that a microbiological ADI for emamectin benzoate was not necessary.

"ARfD" for use in pilot study of assessment of the acute dietary exposure of consumers to veterinary drug residues

JECFA does not routinely establish ARfDs for veterinary drugs. However, because JMPR established an ARfD for emamectin benzoate, and because the availability of an ARfD would facilitate the pilot testing of JECFA's new approach for the assessment of the acute dietary exposure of human consumers to veterinary drug residues, the Committee considered deriving an "ARfD" for emamectin benzoate. JMPR based its ARfD of 0.03 mg/kg bw on a NOAEL of 5 mg/kg bw for clinical signs of neurotoxicity (tremors and irritability) observed at 10 mg/kg bw in an acute neurotoxicity study in rats. JMPR applied an uncertainty factor of 200, which included a 100-fold factor for interspecies and intraspecies variability and a 2-fold factor for serious histopathological observations of degeneration of neurons in brain, spinal cord and sciatic nerve at 25 mg/kg bw.

JECFA considered that dogs were more sensitive than rats to the neurotoxic effects of emamectin benzoate. In the 14-week study in dogs, the high dose was decreased from the original dose of 1.5 mg/kg bw per day to 1.0 mg/kg bw per day in week 2 because of clinical signs of such severity that some of the dogs had to be killed prematurely for humane reasons. In the 53-week study in dogs, clinical signs were first observed in the high-dose group (1.0 mg/kg bw per day) during week 2, and all dogs in this group were killed at day 23 owing to clinical signs of severe neurotoxicity. The Committee could not exclude the possibility that at higher doses, clinical signs of neurotoxicity would occur after a single dose. It therefore considered the occurrence of clinical signs in dogs as the critical effect upon which to base an ARfD. The highest dose tested of 1.0 mg/kg bw per day was

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identified as the likely NOAEL for any acute clinical signs of neurotoxicity from a single dose in the dog.

JECFA concluded that an "ARfD" of 0.01 mg/kg bw could be derived on the basis of a NOAEL of 1.0 mg/kg bw per day (the highest dose tested) for possible acute signs of neurotoxicity in the 14-week and 53-week studies in dogs. The Committee applied an uncertainty factor of 100 for interspecies and intraspecies variability. The Committee did not apply any additional uncertainty factor because of the conservative assumption that acute clinical signs could occur in dogs administered a single dose above 1.0 mg/kg bw.

Evaluation

JECFA confirmed the ADI of 0–0.0005 mg/kg bw established by JMPR, based on an overall NOAEL of 0.25 mg/kg bw per day for neurotoxicity from the 14- and 53-week studies in dogs, supported by an overall NOAEL of 0.25 mg/kg bw per day from the 1- and 2-year studies in rats, with application of an uncertainty factor of 500. This includes an additional uncertainty factor of 5 to account for the steep dose–response curve and irreversible histopathological effects in neural tissues at the LOAEL in dogs, as used by JMPR and confirmed by this Committee.

The Committee recommended that JMPR re-evaluate emamectin benzoate at a future meeting in view of the above considerations with respect to the ARfD.

A toxicological monograph was not prepared.

Residue evaluation

The present evaluation was performed on the basis of available published peer-reviewed scientific papers, evaluations from national agencies and the JMPR evaluation. Despite the request of the Committee, the sponsor of a marketed authorized emamectin benzoate formulation for sealice control did not send the dossier used by national authorities for risk assessment.

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

Data on pharmacokinetics and metabolism

In salmon, trout and cod, emamectin benzoate is absorbed by the oral route and slowly eliminated, with a terminal half-life of 11 days. In cod, oral absolute bioavailability was calculated to be 38%. A linear relationship was established between dose administered through the intraperitoneal or oral route and muscle and skin concentrations. In salmon, the drug is detectable

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90 days after oral administration. Parent drug and metabolites are slowly excreted in faeces post-dosing.

In a pharmacokinetic and residue depletion study in salmon in which a nominal dose of 50 µg (actual dose 33 µg) of [^{3}H]emamectin benzoate per kilogram body weight was administered daily to salmon in feed for 7 consecutive days, the metabolite profile was analysed and compared with previously identified metabolites. Several minor components were observed in pooled faeces. The metabolism of emamectin benzoate was very similar to that reported for rat. The drug remained in tissues mostly as unchanged emamectin B1a.

Residue data

In a pharmacokinetic and residue depletion study, tissues (liver, kidney, muscle, skin) were collected at time points ranging from 3 hours to 30 days post-dosing. Collected samples were analysed for the total radioactive residue concentrations, expressed as equivalent emamectin benzoate. Emamectin B1a was quantified in muscle and skin using a validated high-performance liquid chromatography (HPLC)–fluorescence method based on extraction and derivatization of emamectin B1a, and the results were compared with those obtained by HPLC with radiometric detection.

The concentrations of radiolabelled emamectin benzoate quantified in the tissue matrices decreased in the order kidney > liver > skin > muscle. Total radioactive residues expressed as emamectin benzoate equivalent were compared with emamectin B1a concentrations in muscle and skin at different time points from 3 hours to 30 days to calculate the ratio of the concentration of marker residue to the concentration of total radioactive residue. The ratio of the mean concentration of the marker residue emamectin B1a to that of the total residue was calculated as 0.9 for muscle and fillet (muscle + skin) and 0.8 for skin.

In three depletion studies in salmon, data were obtained from groups of animals administered a 50 µg/kg bw dose of emamectin benzoate daily for 7 days. In the first study, salmon were farmed at two different ambient water temperatures (15 °C, 19 °C). Groups of 10 fish were killed at days 7, 14, 21, 35, 49, 56 and 63 after the start of treatment. In the second study, groups of 20 fish were killed at days 8, 10, 11, 17 and 22 and groups of 10 fish were killed at days 27, 37 and 52 after the start of treatment. In the third study, groups of 10 fish were killed at days 7, 14 and 35 after the start of treatment.

In the first study, emamectin B1a in muscle was determined by a validated method based on emamectin derivatization and quantified by HPLC using a fluorescence detector (limit of quantification [LOQ] = 5 µg/kg; limit of

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detection [LOD] = 1 µg/kg). The analytical method for quantification of emamectin B1a was not reported in the peer-reviewed paper describing the second study. The concentrations of drug in muscle and skin were quantified by a validated LC-MS/MS method (LOQ = 5 µg/kg; LOD = 2.5 µg/kg) in the third study. In all three studies, groups of untreated animals served as controls. Mean residue concentration data from the three studies were graphically compared with the value obtained from the radioactive residue depletion study.

In a GLP-compliant study, the depletion of emamectin B1a in the edible tissues (fillet as muscle + skin in natural proportion, muscle only, skin only) of rainbow trout was studied at two temperatures (6 ± 1 °C, 15 ± 1 °C) following treatment with emamectin benzoate in feed at a nominal dose rate of 50 µg/kg bw per day. Groups of 15 fish farmed at 6 °C and 10 fish farmed at 15 °C were killed on days 6, 7, 9, 13, 27 and 41 after the start of treatment at both temperatures and on days 62 and 83 for the 6 °C study and on day 56 for the 15 °C study. Emamectin concentrations in fillet, muscle and skin were determined by a method based on emamectin derivatization and quantified by HPLC using a fluorescence detector (LOQ = 20 µg/kg).

Emamectin B1a residues decreased in muscle with different half-lives as a function of water temperature. On average, emamectin B1a concentrations in skin and fillet were, respectively, approximately 1.8 and 1.25 times higher than in muscle.

Strict control of treatment conditions, rate of feed ingestion and a residue monitoring programme are recommended for this compound because of its wide range of terminal half-lives reported in several studies and the variation in feed intake according to local living conditions of fish.

Analytical methods

An analytical method was developed and validated to determine the concentration of emamectin B1a residue in muscle, skin and intact muscle plus skin in natural proportion from Atlantic salmon. The method is based on solid-phase extraction, followed by derivatization involving trifluoroacetic anhydride in the presence of *N*-methylimidazole. Emamectin B1a is analysed by HPLC with fluorescence detection. Calibration curves were obtained with fortified tissue over a range of 50–800 µg/kg. The LODs were 2.6, 8.3 and 3.8 µg/kg as emamectin B1a for muscle, skin and intact fillet (muscle + skin), respectively. The LOQ was 50 µg/kg for muscle, skin and intact fillet. Recoveries were 94 ± 7% for muscle, 88 ± 5% for skin and 88 ± 4% for intact fillet.

The method was adapted and validated for trout muscle, skin and intact fillet.

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Upper tolerance limits (UTL 95/95) were calculated using the logarithmic transformed emamectin B1a concentrations and the total residue expressed as equivalent emamectin benzoate obtained from the pivotal radiolabel study in salmon published in a peer-reviewed paper. On the basis of these data, the daily dietary exposure to total residue equivalent as emamectin B1 benzoate was estimated to be 11.2 µg. This median estimate means that the UTL 95/95 of the estimated dietary exposure derived for this dataset is below the upper bound of the ADI of 30 µg/person and at the same time point that the largest upper tolerance limit (UTL 99/99) of the emamectin B1a residue depletion curves was below 100 µg/kg. A large UTL was chosen to take into account the high variability in conditions of exposure and farming in fish production.

Maximum residue limits

In recommending MRLs for emamectin B1a in salmon and trout, the Committee considered the following factors:

- Emamectin benzoate is authorized for use in salmon and trout. For salmon, the maximum recommended dose is 50 µg/kg fish per day for 7 days, administered through medicated feed.
- An ADI for emamectin benzoate of 0–0.5 µg/kg bw was established by the Committee, corresponding to an upper bound of acceptable intake of 30 µg/day for a 60 kg person.
- Emamectin B1a is predominantly unmetabolized.
- Emamectin B1a is the marker residue in tissues.
- The ratio of the concentration of marker residue to the concentration of total residue is 0.9 in muscle and fillet of salmon.
- Residue data were provided using a validated analytical method to quantify emamectin B1a in tissue.
- Residue data in trout were available.
- A validated analytical method for the determination of emamectin B1a in edible tissue of salmon and trout is available and may be used for monitoring purposes.

MRLs were calculated on the basis of the upper limit of the one-sided 99% confidence interval over the 99th percentile (UTL 99/99) of total residue concentrations in salmon derived from the pivotal study used for this assessment.

The time point at which the MRLs were set was based on the approach described at the sixty-sixth meeting of the Committee (Annex 1, reference *181*).

The Committee recommended MRLs for emamectin B1a in salmon of 100 µg/kg in muscle and fillet and extended these MRLs to trout.

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The EDI is 11.2 µg/person per day, which represents approximately 37% of the upper bound of the ADI.

A residue monograph was prepared.

Summary and conclusions

NOAEL

Overall NOAEL of 0.25 mg/kg bw per day for neurotoxicity from 14- and 53-week studies in dogs, supported by an overall NOAEL of 0.25 mg/kg bw per day from 1- and 2-year studies in rats

Uncertainty factor

500 (100 for interspecies and intraspecies variability, 5 to account for the steep dose–response curve and irreversible histopathological effects in neural tissues in dogs)

ADI

0–0.0005 mg/kg bw

Residue definition

Emamectin B1a

MRLs

The Committee recommended MRLs for emamectin B1a in salmon of 100 µg/kg in muscle and fillet and extended these MRLs to trout.

Dietary exposure

The EDI is 11.2 µg/person per day, which represents approximately 37% of the upper bound of the ADI.

3.3 Gentian violet

Explanation

Gentian violet (CAS No. 548-62-9) has many common names, including CI Basic Violet 3, crystal violet and methyl violet 10B. It is a triphenylmethane dye with antibacterial, antifungal and anthelminthic properties. Gentian violet has been used for the treatment of fungal and parasitic infections in fish and topically for skin and eye infections in livestock. It was previously used in poultry feeds to inhibit the growth of mould and fungus; however, several countries have withdrawn approval or registration of this use.

In humans, gentian violet has been used as a hair dye, to treat gut parasites and for topical fungal treatment. It has also been used in human medicine

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to treat blood held for transfusions in order to prevent the transmission of Chagas disease caused by *Trypanosoma cruzi*. It also has activity as a topical antiviral agent.

Gentian violet is used in industrial processes for wood, leather, silk, nylon, paper and ribbon tapes and also as a biological stain. Contamination of the environment can occur, as about 10–15% of all dyes are lost directly to wastewater in the dyeing process. Gentian violet in water originating from contamination as a result of its industrial applications or from its illegal use in aquaculture is efficiently taken up from the water by fish.

Gentian violet has not previously been evaluated by the Committee. It was evaluated by the Committee at the current meeting at the request of the Twentieth Session of CCRVDF *(2)*, which asked for advice as to whether an ADI can be established and whether the continued use of gentian violet in food-producing animals is safe for humans.

Toxicological evaluation

The Committee reviewed studies submitted by a Member State as well as additional papers available in the published literature.

Gentian violet is structurally related to malachite green. The Committee evaluated malachite green in 2009 (Annex 1, reference *193*) and concluded that the use of malachite green in food-producing animals could not be supported. This was because its major metabolite, leucomalachite green, induces hepatocellular adenomas and carcinomas in female mice, and it could not be ruled out that this was by a genotoxic mode of action.

Biochemical data

Gentian violet is metabolized to leucogentian violet by isolated gut microflora from rats, chickens and humans. Strong binding of gentian violet to isolated gut bacteria and microsomal fractions of liver was demonstrated, and this is likely to affect the bioavailability of gentian violet. In studies in mice and rats using radiolabelled gentian violet, most of the administered dose is excreted in faeces, with urinary excretion being much less important. In mice, the excretion of gentian violet and its metabolites in urine is greater than in rats, but still represents less than 10% of the dose. Demethylation is the major metabolic pathway of biotransformation in liver microsomes, with mouse microsomes in vitro being less active than those from other rodents or chickens. In both rats and mice, the parent compound (gentian violet), its major metabolite leucogentian violet and their demethylated metabolites are found in tissues, urine and faeces.

Absorption of gentian violet from the gut is higher than that of other triphenylmethane dyes. Dosing mice and rats over 7 days demonstrated its distribution to fat, particularly in females.

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Toxicological data

There were few data available on the acute and short-term toxicity of gentian violet, but the reported range of median lethal doses (LD_{50}s), from 100 to 800 mg/kg bw, shows that it is of moderate acute oral toxicity. The most common sign of toxicity was lethargy, followed by anorexia and, in some animals, diarrhoea, excessive thirst, emesis and weight loss. In 90-day studies in rats and dogs, the only reported signs were slight body weight loss and a liver weight increase, respectively.

In a 24-month study, gentian violet was given to mice in the feed at a concentration of 0, 100, 300 or 600 mg/kg (equal to 0, 10.7–14.3, 32.1–35.7 and 64.3 mg/kg bw per day for males and 0, 14.3, 35.7–39.3 and 71.4 mg/kg bw per day for females, respectively). Few dose-related non-neoplastic lesions were reported, but there were statistically significant dose-related increases in erythropoiesis in the spleen and atrophy of the ovaries in females at 24 months. The LOAEL for non-carcinogenic effects was 14.3 mg/kg bw per day, the lowest dose tested. Significant, dose-related increases in neoplastic lesions were observed in both sexes, with the female mice being more sensitive. Hepatocellular adenomas and carcinomas were the most common lesions, with significant, dose-related increases found at 24 months in males and at both 18 and 24 months in females. Mortality due to liver neoplasms showed positive trends in both males and females, and there was a dose-related decrease in the time for the onset of liver neoplasms. The females also showed statistically significant dose-related increases in adenoma of the Harderian gland and in type A reticulum cell sarcoma in the urinary bladder, uterus, ovaries and vagina. The data clearly indicate that gentian violet is a multisite carcinogen in the mouse.

In a long-term study of toxicity, rats were exposed to gentian violet in the feed at a concentration of 0, 100, 300 or 600 mg/kg (equal to approximately 0, 30, 80 and 160 mg/kg bw per day for males and 0, 40, 100 and 200 mg/kg bw per day for females, respectively). Gentian violet exposure of these animals was achieved by dosing the parents of the study animals prior to and during mating, with the same dose fed to the offspring from weaning up to 24 months of age. There was a statistically significant increase in liver regeneration in all dose groups and statistically significant dose-related increases in eosinophilic foci in the liver in both sexes in both the mid- and high-dose groups. For liver centrilobular necrosis, there was a dose-related increase, but statistical significance was seen only in the 300 mg/kg feed group in males and in the 600 mg/kg feed group in females. As in mice, female rats appeared to be more sensitive than males. The incidence of thyroid adenocarcinoma was increased in males, with statistical significance at the top dose only at 24 months. Females showed a statistically significant

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dose–response relationship for thyroid adenocarcinoma at 24 months. The incidence of hepatocellular adenomas showed a small but significant dose–response relationship in males and a significant increase in females at 300 mg/kg feed, but not at other doses. The data indicate a carcinogenic response to gentian violet in rats, although much weaker than the response in mice.

The data show that gentian violet binds to DNA, and this, together with the cellular toxicity of gentian violet, complicates both the testing of gentian violet in vitro and the interpretation of the results. The results are somewhat varied in *Salmonella typhimurium*, with positive responses in some strains but not in others. Gentian violet was clastogenic in vitro and positive in indicator tests for DNA damage. There are few in vivo tests on gentian violet. A single in vivo test for clastogenicity (mouse bone marrow assay) showed no evidence of clastogenic activity, but the Committee noted that the gentian violet was given via the drinking-water at lower doses (4 and 8 mg/kg bw per day) than those used in the mouse cancer bioassay (ranging from 10 to 70 mg/kg bw per day). Similarly, the other in vivo test on DNA damage in mouse lymphocytes using single intravenous doses up to 6 mg/kg bw showed no effect. The Committee concluded that, overall, the data show that gentian violet is genotoxic.

In view of the carcinogenicity of gentian violet in the mouse and rat and evidence showing genotoxicity in a number of tests, the Committee concluded that gentian violet should be considered a carcinogen acting by a genotoxic mode of action.

In a multigeneration reproductive toxicity study, rats were given gentian violet in the feed at a concentration of 0, 100, 300 or 600 mg/kg (equivalent to 0, 5, 15 and 30 mg/kg bw per day, respectively) over three generations. There were significant reductions in body weight in the top dose group in all generations. The NOAEL for parental toxicity was 15 mg/kg bw per day. In the F_{3a} generation, examined for histopathological effects, a dose-related trend for focal dilatation of the renal cortex and tubules, a statistically significant dose-related trend for necrosis of the thymus and an inverse dose–response relationship for red pulp haematopoietic cell proliferation of the spleen were seen. The effects in the F_{3a} generation were present in all dose groups, and a NOAEL for offspring toxicity could not be determined. Gentian violet had no effect on the number of pups per litter, fertility index, pup survival, sex ratio or number of stillborn animals. The NOAEL for reproductive toxicity was 30 mg/kg bw per day, the highest dose tested.

Two developmental toxicity studies were conducted in rats. In the first study, CD rats were given gentian violet at 0, 2.5, 5 or 10 mg/kg bw per day by oral gavage on days 6–15 of gestation. In the second study, the three-

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generation study in Fischer 344 rats described above, the F_{3b} generation was examined for teratogenic effects. In that study, gentian violet was given in the feed at a concentration of 0, 100, 300 or 600 mg/kg (equivalent to 0, 5, 15 and 30 mg/kg bw per day, respectively). CD rats appeared to be more sensitive than Fischer 344 rats to the toxicity of gentian violet, with dose-related reductions in maternal weight gain at 5 and 10 mg/kg bw per day and increased clinical signs of toxicity, significant at 10 mg/kg bw per day and limited at 5 mg/kg bw per day (maternal toxicity NOAEL of 2.5 mg/kg bw per day). In Fischer 344 rats, reduction in body weight was seen only at 30 mg/kg bw per day and not at lower doses of 5 and 15 mg/kg bw per day (maternal toxicity NOAEL of 15 mg/kg bw per day). It was also noted that malformations (hydroureter, hydronephrosis and short ribs) were seen only in the CD rats. Effects on the fetus were seen only at doses that also caused maternal toxicity. The NOAEL for embryo and fetal toxicity in CD rats was 5 mg/kg bw per day.

In a developmental toxicity study, rabbits were given gentian violet at 0, 0.5, 1 or 2 mg/kg bw per day by oral gavage on days 6–19 of gestation. Maternal mortality was increased in a dose-related manner, and maternal body weight gain was decreased in all treated groups compared with controls. Fetal weights were significantly reduced in all treated groups compared with controls. There was no evidence of teratogenic effects. Owing to the presence of maternal toxicity and significantly reduced fetal weights in all dosed groups, NOAELs could not be identified for maternal or embryo/fetal toxicity.

In humans, case reports have shown that gentian violet has been associated with dermal irritation/sensitization, ocular irritation, mucosal irritation and bladder irritation following topical or employment-related exposure, but these are not relevant to the evaluation of the safety of gentian violet in food.

Evaluation

The Committee concluded that it is inappropriate to set an ADI for gentian violet because it is genotoxic and carcinogenic. Gentian violet is widely used in various ways other than as an authorized veterinary drug, and there may be residues in fish from unauthorized use or from environmental exposures. Therefore, irrespective of whether it is used as a veterinary drug, the Committee agreed that some further guidance to risk managers was needed.

The Committee determined that the pivotal study for the evaluation of gentian violet is the carcinogenicity study in mice. Although it was not possible to add the adenomas and carcinomas in liver, the dose–response relationship for the two tumour types was very similar. Accordingly, a BMD evaluation was conducted using the data for the female mouse malignant liver neoplasms at the 24-month sacrifice.

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The USEPA's BMD software (BMDS, version 2.2) was used for modelling the dose–response relationship for malignant liver neoplasms in gentian violet–treated female mice. The following dose–response models were fitted to the dose–incidence data and resulted in an acceptable fit: gamma, logistic, log-logistic, multistage, multistage cancer, probit, log-probit and Weibull. The BMD and BMDL values for an extra 10% risk compared with the modelled background incidence (BMD_{10} and $BMDL_{10}$) were estimated by performing 250 iterations.

The BMD_{10} values from the accepted models ranged from 19.9 to 25.2 mg/kg bw per day, and the $BMDL_{10}$ values ranged from 16.8 to 19.8 mg/kg bw per day. In order to be prudent, the Committee decided to use the more conservative lower end of this range of values for the evaluation and chose a $BMDL_{10}$ value of 16.8 mg/kg bw per day as the reference point for a margin of exposure (MOE) calculation.

The Committee estimated MOEs assuming a residue level of 0.5 µg/kg, which is a typical LOQ for gentian violet residues in foods, and a residue level of 5 µg/kg, which is 10 times the typical LOQ, as a hypothetical scenario. Assuming a daily consumption of 300 g of fish contaminated with gentian violet and its metabolites, the estimated theoretical exposures to gentian violet for a 60 kg person were 0.0025 and 0.025 µg/kg bw per day for the two residue levels, respectively. Comparison of these estimated exposures with the $BMDL_{10}$ of 16.8 mg/kg bw per day indicates MOEs of about 6.7×10^6 and 6.7×10^5, respectively. Based on considerations discussed at the sixty-fourth meeting of the Committee for unintended contaminants (Annex 1, reference *176*), these MOEs would be considered to be of low concern for human health.

However, the Committee noted that there were a number of uncertainties associated with the risk assessment, some of which were substantial. The uncertainties relate to two aspects of the data available for risk assessment. Firstly, there were insufficient residue data in food-producing animals or the environment from which to estimate dietary exposure to gentian violet, and hence assumptions had to be made. Secondly, there is very little information on the proportion of gentian violet and its metabolites in the total residue and on the carcinogenicity of the metabolites. For example, there is a published report that one of the possible metabolites of gentian violet, demethylated leucopararosaniline, is carcinogenic in rats, but no information is available on its potency. In addition, there is no information on the carcinogenicity of the major metabolite, leucogentian violet. The structure of gentian violet is similar to that of malachite green, and it is known that leucomalachite green is a more potent carcinogen than malachite green; therefore, it is possible that leucogentian violet is similarly a more potent carcinogen than gentian violet.

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Gentian violet and leucogentian violet are readily interconvertible in the body, and so it is likely that exposure to gentian violet will also result in exposure to leucogentian violet. Thus, there is inadequate information to determine the overall carcinogenicity of the metabolites of gentian violet (demethylated gentian violet, leucogentian violet and its demethylated metabolites).

A toxicological monograph was prepared.

Residue evaluation

A risk assessment was provided by a national authority. In addition a supplementary literature search was performed.

Data on pharmacokinetics and metabolism

In chickens, radiolabelled gentian violet was detected in blood, tissues and eggs, demonstrating absorption of gentian violet following oral administration. Gentian violet and demethylated products were identified in excreta, but gentian violet was the predominant excretion product.

A metabolism study has not been conducted in fish.

Residue data

Chickens. In a GLP-compliant study, chickens were treated with[phenyl-U-^{14}C]gentian violet orally by capsule for 7 days. Samples of liver, kidney, gizzard, breast, thigh, heart and skin were analysed for total ^{14}C residue 6, 24, 48, 120 and 240 hours after the last treatment. Highest residue concentrations were detected in the liver. The depletion of total residues was biphasic in each tissue. Gentian violet and identified demethylated metabolites were analysed by HPLC with ultraviolet (UV) detection and detected in all tissues analysed at the 6-hour withdrawal period. Unidentified metabolites were present at subsequent time points. Non-extractable residues in tissues represent a substantial portion of the total residue in most tissues at all depletion times.

Finfish. Atlantic salmon, with an approximate weight of 100 g, were exposed to gentian violet at an initial concentration of 1 mg/L delivered at a flow rate of 1 L/min as a bath in a tank with continuous flushing for 5 hours. Fish were sampled at 1, 7, 14, 28, 63 and 91 days post-dosing. Samples were analysed either for combined gentian violet and leucogentian violet residues (by oxidizing leucogentian violet back to gentian violet) or for the parent and the leucogentian violet separately using LC-MS/MS. Gentian violet was rapidly metabolized to leucogentian violet within 24 hours post-dosing. The mean leucogentian violet and gentian violet concentrations on day 1 post-dosing were 134 ± 36 µg/kg and 2.4 ± 0.9 µg/kg, respectively (ratio of leucogentian

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violet to gentian violet of 56:1). Gentian violet was not detected (LOD = 2 µg/kg) by 14 days post-dosing. Leucogentian violet was detected at all times post-treatment, with 8 µg/kg detected on day 91 post-treatment.

Channel catfish were exposed to water containing gentian violet at 100 µg/L for 1 hour. The fish were then transferred to gentian violet–free water for 79 days to study the depletion of gentian violet residues. Gentian violet was rapidly (approximately 2 hours) converted to leucogentian violet. Mean leucogentian violet residues were approximately 17 and 3 µg/kg after 2 hours and 79 days, respectively (LOD = 0.2 µg/kg).

No absorption or depletion data for topical administration of gentian violet in terrestrial species were available.

Analytical methods

Two approaches are currently available for gentian violet and leucogentian violet determination in finfish, molluscs and crustaceans. The first is based on the measurement of each compound separately, and the second is based on the measurement of the compounds together after conversion of gentian violet and leucogentian violet by chemical oxidation. Owing to the rapid metabolism of gentian violet to leucogentian violet and the persistence of leucogentian violet residues in fish tissue, a method utilizing detection of leucogentian violet independently may be preferred for monitoring of residues in fish. Methods were usually developed to detect other triphenylmethane dyes in addition to gentian violet and leucogentian violet.

Gentian violet and leucogentian violet are generally extracted from tissue with an acetonitrile buffer mixture and then purified on solid-phase extraction cartridges. HPLC coupled to a UV or fluorescence detector has been used, but it has not reached the performances of mass spectrometry (MS). Post-column oxidation (e.g. with lead dioxide) of leucogentian violet to gentian violet is often reported when UV is used as a detector. Methods are generally validated according to internationally recognized requirements. When LC-MS/MS with electrospray ionization is used, the LOD and LOQ for gentian violet are in the 0.01–2.0 µg/kg range, and the LOD and LOQ for leucogentian violet are in the 0.1–7.5 µg/kg range.

Maximum residue limits

MRLs for gentian violet could not be recommended by the Committee, as it was not considered appropriate to establish an ADI. The Committee also noted that there was limited information on residues.

A residue monograph was prepared.

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Summary and conclusions

Studies relevant to risk assessment

Species / study type (route)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Mouse				
Two-year study of toxicity and carcinogenicity (dietary)	Females: 0, 14.3, 35.7–39.3, 71.4	Erythropoiesis in spleen, atrophy of ovaries	–	14.3[a]
		Benign and malignant liver neoplasms (females)	–	**$BMDL_{10}$: 16.8***
Rat				
Two-year study of toxicity and carcinogenicity (dietary)	Males: 0, 30, 80, 160 Females: 0, 40, 100, 200	Increase in liver regeneration	–	30[a]
		Thyroid follicular cell adenocarcinoma (both sexes) and hepatocellular adenoma (males)	–	–
Three-generation study of reproductive toxicity, including developmental toxicity (dietary)	0, 5, 15, 30	Reproductive toxicity: No effects seen	30[b]	–
		Parental toxicity: Decreased body weight	15	30
		Offspring toxicity: Necrosis of thymus, focal dilatation of renal cortex and tubules, lowered red pulp haematopoietic cell proliferation in spleen	–	5[a]
Developmental toxicity study (gavage)	0, 2.5, 5, 10	Maternal toxicity: Reduced body weight gain, clinical signs	2.5	5
		Embryo and fetal toxicity: Increased hydroureter, hydronephrosis and short ribs	5	10
Rabbit				
Developmental toxicity study (gavage)	0, 0.5, 1, 2	Maternal toxicity: Increased mortality, decreased body weight gain, clinical signs	–	0.5[a]
		Embryo and fetal toxicity: Reduced fetal weight	–	0.5[a]

* **Pivotal study value *(11, 12)***
[a] Lowest dose tested.
[b] Highest dose tested.

ADI

The Committee concluded that it is inappropriate to set an ADI for gentian violet because it is genotoxic and carcinogenic.

MRLs

MRLs for gentian violet and leucogentian violet could not be recommended by the Committee, as no ADI was established and there is a lack of residue data.

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3.4 Ivermectin

Explanation

Ivermectin (CAS No. 70288-86-7) is a macrocyclic lactone that is a member of the avermectin series and is widely used as a broad-spectrum antiparasitic drug against nematode and arthropod parasites in food-producing animals. In human medicine, it is used mainly for the treatment of onchocerciasis. Ivermectin was previously considered by the Committee at its thirty-sixth, fortieth, fifty-eighth and seventy-fifth meetings (Annex 1, references *91*, *104*, *157* and *208*). At its fortieth meeting, the Committee established an ADI of 0–1 mg/kg bw and recommended MRLs of 100 µg/kg for liver and 40 µg/kg for fat as ivermectin B1a. The Twenty-first Session of CCRVDF *(4)* asked that the Committee at its present meeting advise on whether it was possible to establish an MRL for bovine muscle.

Residue evaluation

An examination of the JECFA monographs on ivermectin from the thirty-sixth and fortieth meetings at the present meeting led to the conclusion that it might be possible for the Committee to recommend an MRL based on the existing summarized data. In addition, an analytical method for ivermectin B1a monitoring in bovine muscle, which has been implemented in an ISO 17025–accredited laboratory, was received and reviewed.

Analytical methods

One accredited laboratory has provided JECFA with a validated method for ivermectin B1a based on LC-MS/MS. The sample is prepared for analysis using a modified QuEChERS (*Quick Easy Cheap Effective Rugged Safe*) method. The sample is extracted by shaking in acetonitrile, magnesium sulfate and sodium chloride before being cleaned up by dispersive solid-phase extraction, using C18 and magnesium sulfate, concentrated, filtered and transferred to an HPLC vial. Ivermectin residues are determined by ultra performance liquid chromatography on a reverse-phase C18 column, then ionized in an electrospray interface before measurement in a triple quadrupole mass spectrometer. Two independent transitions are monitored for ivermectin B1a. An internal standard (selamectine, structural analogue) is used.

Method performance

The method has been validated for selectivity/specificity, linearity of the calibration curve, working range, LOD, limit of decision, detection capability, recovery, within-laboratory repeatability and within-laboratory reproducibility. The selectivity/specificity of the method was demonstrated

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by the analysis of repeated non-fortified muscle samples. The LOD was 0.8 µg/kg. At 2 µg/kg, the overall recoveries calculated on 3 days by three different operators were 99.2% and 105.2%, respectively. At 2 µg/kg, 3 µg/kg and 4 µg/kg, the accuracy and precision of the method were determined and were shown to be fit for purpose.

The Committee assessed the validation data against the analytical requirements as published in the Codex guidelines for analytical methods for residue control (CAC/GL 71-2009). The Committee reviewed information on the LOD (0.8 µg/kg) and LOQ (2 µg/kg) of the submitted LC-MS/MS method for the determination of ivermectin B1a in muscle. The Committee concluded that the analytical method can be recommended for regulatory monitoring of muscle samples for ivermectin.

Maximum residue limits

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

- A new, compliant, fully validated LC-MS/MS method complete with adequate performance factors and method validation was provided that was considered suitable for routine monitoring of ivermectin B1a as marker residue.
- The analytical method has been validated for use in cattle muscle, with an LOQ of 2 µg/kg.
- The radiolabel study considered by the thirty-sixth meeting of the Committee demonstrated that the total residue of ivermectin in muscle at 28 days was 1 µg/kg.
- The depletion study considered by the fortieth meeting of the Committee based on which MRLs were recommended for bovine fat and liver demonstrated that residues of the marker residue in bovine muscle at 28 days, the time point at which MRLs were recommended for bovine fat and liver, were approximately 1 µg/kg, using an analytical method with an LOD of 1 µg/kg.

The Committee at the present meeting recommended an MRL of 4 µg/kg for cattle muscle determined as ivermectin B1a, based on 2 × LOQ of the analytical method. The dietary exposure calculation prepared by the fortieth meeting of the Committee included an estimate of the potential intake from muscle, based on the concentrations of total residue reported from the radiolabel study. No further assessment of dietary exposure was undertaken.

An addendum to the residue monograph was prepared.

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Summary and conclusions

Residue definition

Ivermectin B1a

MRLs

The Committee recommended an MRL of 4 µg/kg for cattle muscle.

Dietary exposure

No assessment of dietary exposure was undertaken.

3.5 Lasalocid sodium

Explanation

Lasalocid sodium (CAS No. 25999-20-6) is produced by *Streptomyces lasaliensis* and is a mixture of several closely related homologues: A, B, C, D and E. Lasalocid homologues B, C, D and E make up no more than a total of 10% of the total weight of the active substance.

Lasalocid sodium, a divalent polyether ionophore antibiotic, is approved for continuous use to control coccidiosis in poultry species at concentrations of 7.5–125 mg/kg feed. It is approved to protect against *Eimeria* species in broilers and replacement pullets, turkeys, pheasants and quails.

The mechanism of action of lasalocid and other ionophores has been extensively investigated and reported. Like other carboxylic polyether ionophores, lasalocid disturbs ionic homeostasis, leading to osmotic lysis of coccidia.

Lasalocid sodium has not previously been evaluated by the Committee. The Committee evaluated lasalocid sodium at the present meeting at the request of the Twentieth Session of CCRVDF *(2)* with a view to establishing an ADI and recommending MRLs in poultry tissues and eggs.

Toxicological and microbiological evaluation

The Committee considered data on pharmacodynamics, pharmacokinetics, short-term and long-term toxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity and microbiological safety. In addition to the sponsor's submission, a number of studies were retrieved from the published literature. Although most of the studies submitted to the Committee pre-date GLP implementation, the overall package of data was sufficient to allow the derivation of a robust ADI. Those studies that were not performed to GLP standards are identified in this report.

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Biochemical data

Following oral administration of a single radiolabelled dose of lasalocid sodium to mice, radioactivity was rapidly absorbed and excreted. Peak concentrations of radiolabelled material were seen in whole blood 15 minutes after administration, and levels had declined to background within 24 hours. The half-life of elimination of radioactivity in whole blood was 3 hours. Radioactivity was widely distributed to tissues, with the highest concentrations seen in liver, where they peaked 1 hour after administration. Approximately 95% of radioactivity was excreted in the faeces, and approximately 1% in urine, within 24 hours. A similar pattern was seen following multiple oral administrations, with radioactivity peaking in whole blood 30 minutes after the last dose and declining to background levels by 24 hours. Tissue levels were highest in the liver, where they remained detectable 48 hours after administration. Seventy-seven per cent of radioactivity was excreted in faeces within 4 hours of the last dose, and 95% within 24 hours. Excretion was observed to be more rapid in female mice than in male mice, with radioactivity in faeces peaking between 4 and 8 hours in females and between 8 and 12 hours in males.

The pattern of pharmacokinetic behaviour in rats following a single oral administration of radiolabelled lasalocid sodium was comparable to that seen in mice, with rapid absorption and excretion and a wide distribution of radioactivity in tissues. Whole blood radioactivity peaked at 3 hours, and the half-life of elimination was 4.8 hours. Radioactivity was widely distributed to tissues, with the highest levels seen in the liver, where it peaked at approximately 6 hours after administration. Approximately 85% of the administered dose was excreted in faeces within 24 hours, and approximately 1% was excreted in urine over the same period. Similar results were seen after seven daily oral doses.

In bile duct–cannulated male rats administered a single oral dose of radiolabelled lasalocid, approximately 61% of the dose was absorbed. Approximately 59% of the dose was excreted in bile within 48 hours.

In a comparative metabolism study in pig, dog, rat, mouse, chicken and turkey, the radioactive metabolite profile was similar in the faecal and liver fractions, although the relative proportions varied. The only component identified was lasalocid A, which represented the major component of the total radioactive residues in the faeces and liver in all species.

Although other residues were not identified, they were present at low levels and are considered to be minor.

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Toxicological data

The acute toxicity of lasalocid sodium has been investigated in a number of species. Oral LD_{50} values were 146, 122, 33 and 40 mg/kg bw in the mouse, rat, neonatal rat and rabbit, respectively. The increased sensitivity of the rabbit may be due to the increased sensitivity of this species to effects of antimicrobial drugs on the intestinal microflora.

Lasalocid sodium was not irritating to the skin of rabbits but caused corneal irritation, conjunctival redness and chemosis when applied to the eyes.

Lasalocid sodium did not cause skin sensitization in the guinea-pig maximization test.

In a non-GLP 13-week study in rats, lasalocid sodium was administered in the diet at concentrations adjusted to achieve doses of 0, 2, 5 and 20 mg/kg bw per day. Based on reduced feed consumption, increased liver to body weight ratios and increased haemosiderin in the liver in females, the LOAEL was 5 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a non-GLP 13-week study in weanling rats, lasalocid sodium was administered in the diet at concentrations adjusted to achieve doses of 0, 1, 2, 3 and 10 mg/kg bw per day. Based on increased alkaline phosphatase levels seen in males at all doses at week 13, the LOAEL was 1 mg/kg bw per day. No NOAEL could be established. It is noted, however, that the low-dose effect on alkaline phosphatase seen in this study was not seen in other rat studies.

In a non-GLP 13-week study performed in weanling rats that had been exposed to lasalocid sodium in utero (parents were exposed prior to and during mating, gestation and lactation), the substance was administered in the diet at concentrations adjusted to achieve doses of 0, 1, 2, 3 and 10 mg/kg bw per day. Based on increased haemosiderin seen in the liver of males and (predominantly) females, the LOAEL was 3 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a non-GLP 13-week toxicity study in dogs, lasalocid sodium was administered in gelatine capsules at doses of 0, 2, 5 and 10 mg/kg bw per day. Transient muscle weakness involving primarily the hindlimbs was noted in animals at the top dose only. Based on decreased serum chloride levels, increased spleen weights, increased congestion in the spleen and increased hepatocyte vacuolation, the LOAEL was 5 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a 2-year toxicity study in dogs, lasalocid sodium was administered in the diet at concentrations of 0, 10, 35 and 180 mg/kg feed (equivalent to 0, 0.25, 1 and 5 mg/kg bw per day, respectively). Based on the transient

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intermittent paralysis of limbs occurring on a single day and on increased alkaline phosphatase levels, the LOAEL was 180 mg/kg feed (equivalent to 5 mg/kg bw per day), and the NOAEL was 35 mg/kg feed (equivalent to 1 mg/kg bw per day).

In a 24-month carcinogenicity study, mice were administered lasalocid sodium in feed at a concentration of 0, 10 (low-dose animals were dosed with 20 mg/kg feed for the first 5 weeks of the study, after which the dose was adjusted downward), 35 (mid-dose animals were dosed with 60 mg/kg feed for the first 5 weeks, after which the dose was adjusted downward) or 120 mg/kg feed (equivalent to 0, 1.5, 5.25 and 18 mg/kg bw per day, respectively, after week 5). Lasalocid sodium did not show evidence of tumorigenic potential. The NOAEL was 120 mg/kg feed (equivalent to 18 mg/kg bw per day), the highest dose tested.

In a 30-month toxicity and carcinogenicity study, rats were administered lasalocid sodium in feed at a concentration of 0, 10, 35 or 120 mg/kg (equal to mean doses of 0, 0.5, 1.8 and 6.2 mg/kg bw per day for males and 0, 0.6, 2.2 and 8.1 mg/kg bw per day for females, respectively). The animals used in this study were weanlings bred from parental animals administered the same doses of lasalocid sodium during breeding, gestation and lactation. Lasalocid sodium did not demonstrate tumorigenic properties in this study. Based on a transient impairment of righting and grasping reflexes seen in females between weeks 31 and 49, the LOAEL was 120 mg/kg feed (equal to 8.1 mg/kg bw per day), and the NOAEL was 35 mg/kg feed (equal to 2.2 mg/kg bw per day).

Lasalocid sodium did not show evidence of genotoxic potential in a range of in vitro tests covering the end-points of gene mutation and chromosomal aberration. Although there was no in vivo test for chromosomal effects, the Committee considered that this was unnecessary in view of the existing genotoxicity and carcinogenicity data.

In a multigeneration reproductive toxicity study incorporating a teratology arm, rats were administered lasalocid sodium in feed at a concentration of 0, 10, 35 or 120 mg/kg (equivalent to 0, 0.5, 1.75 and 6 mg/kg bw per day, respectively). At weaning, F_1 animals were randomly selected to become the parents of the F_2 generation; at weaning of F_2 animals, these were randomly selected to become parents of the F_3 generation. F_0 animals and F_2 animals were mated more than once in order to allow for evaluations of teratology. In the high-dose group (120 mg/kg feed), reduced body weights were seen in parental females. The NOAEL for parental toxicity was 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day). The mean numbers of corpora lutea and implantations per pregnant dam were reduced in both the high-dose (120 mg/kg feed) and mid-dose (35 mg/kg feed) groups, resulting in

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decreased implantation efficiency in these groups. The high-dose group also showed decreased pregnancy and fertility rates. Based on these effects, the NOAEL for reproductive toxicity was 10 mg/kg feed (equivalent to 0.5 mg/kg bw per day). In the high-dose group (120 mg/kg feed), the number of pups surviving to weaning and the body weights of pups surviving to weaning were reduced. In the high-dose group of the teratogenicity arm, fetal weights were slightly reduced, and the incidence of visceral and skeletal variants was increased. The NOAEL for offspring and embryo/fetal toxicity was therefore 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day).

In a developmental toxicity study in rabbits, lasalocid sodium was administered by oral gavage over days 6–28 of gestation at a dose of 0, 0.5, 1 or 2 mg/kg bw per day. A NOAEL for maternal effects could not be established, as soft stools and effects on body weight gain and feed consumption were seen at all doses. This is likely the result of the known sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract, and consequently it is not considered appropriate to consider the maternal toxicity in relation to the derivation of an ADI. The LOAEL for embryo and fetal toxicity was 1 mg/kg bw per day, based on decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants at this dose. Although the Committee acknowledges the possibility that these effects may have been secondary to maternal toxicity, it considers the NOAEL for embryo and fetal toxicity to be 0.5 mg/kg bw per day.

No original studies dedicated specifically to the evaluation of the neurotoxic potential of lasalocid sodium were provided. Literature data indicate that polyether ionophores, including lasalocid, do have neurotoxic potential. In line with this, a number of the repeated-dose studies summarized above did include examination of neurological end-points. Evidence of neurotoxicity, consisting of transient patterns of muscle weakness involving primarily the hindlimbs, was seen in the 13-week and 2-year dog studies. These effects were seen only at the highest dose and resolved spontaneously, despite continued administration of the drug. In addition, in the 30-month rat study, impairment of the righting and grasping reflexes was seen. A clear effect was evident only at the top dose and, as with the effects seen in the dog, resolved spontaneously, despite continued administration of the drug.

No observations in humans were identified.

Microbiological data

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and which complies with VICH Guideline 36 (GL36) *(13)* was used by the Committee to determine the need

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for, and to establish, if necessary, a microbiological ADI for lasalocid sodium. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated.

The microbiological ADI was derived from in vitro minimum inhibitory concentration (MIC) data as described in VICH GL36. The strains needed to determine the MIC_{calc}, which is the minimum inhibitory concentration derived from the lower 90% confidence limit for the mean minimum concentration required to inhibit the growth of 50% of organisms (MIC_{50}) of the relevant genera for which the drug is active, were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. The genera with a MIC_{50}, including *Eubacterium* (0.125 µg/mL), *Bacteroides* (32 µg/mL), *Bifidobacterium* (0.25 µg/mL), *Fusobacterium* (1 µg/mL), *Peptostreptococcus* (2 µg/mL), *Clostridium* (0.125 µg/mL), *Enterococcus* (0.5 µg/mL) and *Lactobacillus* (0.125 µg/mL), were used to determine the MIC_{calc}.

Lasalocid sodium residues may be present at low levels in meat products consumed by humans; therefore, lasalocid sodium–related residues could enter the colon of a person ingesting edible tissues from treated animals. The Committee used pharmacokinetic studies and faecal binding studies to determine the fraction of the oral dose available to the human intestinal microflora. Lasalocid sodium was poorly absorbed after oral administration in animals and also binds extensively (> 90%) to faecal contents. Therefore, low levels of lasalocid sodium residues entering the human colon will remain biologically active. There is potential for disruption of the colonization barrier in the human gastrointestinal tract, as MIC values for some of the most relevant and predominant genera in the gastrointestinal tract were susceptible to lasalocid sodium. Lasalocid sodium does not appear to select for resistance in bacteria, and carboxylic polyether ionophores are not used in human medicine.

The formula for calculating the microbiological ADI is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (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}

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were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the MIC_{50} values for *Eubacterium* (0.125 µg/mL), *Bacteroides* (32 µg/mL), *Bifidobacterium* (0.25 µg/mL), *Fusobacterium* (1 µg/mL), *Peptostreptococcus* (2 µg/mL), *Clostridium* (0.125 µg/mL), *Enterococcus* (0.5 µg/mL) and *Lactobacillus* (0.125 µg/mL), the MIC_{calc} is 0.228 µg/mL.

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to 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. Lasalocid sodium is poorly absorbed and is excreted in faeces of experimental animals, primarily in unchanged form. Lasalocid sodium binds rapidly and extensively (> 90%) to faecal contents; therefore, the fraction of oral dose available to microorganisms would be 0.10.

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

The upper bound of the microbiological ADI for lasalocid sodium is therefore calculated as follows:

$$\text{Upper bound of the ADI} = \frac{0.228\ \mu g/mL \times 220\ g}{0.10 \times 60\ kg\ bw} = 8.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–8.4 µg/kg bw could be derived from in vitro MIC susceptibility testing and bioavailability studies.

Evaluation

The Committee considered the toxicological effects of lasalocid sodium to be the most relevant for the purpose of establishing an ADI. A toxicological ADI of 0–5 µg/kg bw was established based on the NOAEL of 0.5 mg/kg bw per day from the developmental toxicity study in rabbits and the multigeneration reproductive toxicity study in rats, with application of an uncertainty factor of 100 for interspecies and intraspecies variability.

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Residue evaluation

The Committee reviewed studies on pharmacokinetics and metabolism of lasalocid as well as a number of radiolabelled and unlabelled lasalocid residue depletion studies in the relevant species. The analytical methods used in the residue depletion studies were also assessed. Some of these studies were performed in compliance with GLP guidelines.

Data on pharmacokinetics and metabolism

Studies with radiolabelled lasalocid sodium in chicken and turkey as well as unlabelled lasalocid sodium in chicken have been evaluated.

Chicken. In a GLP-compliant study, 7-week-old broilers received [^{14}C] lasalocid sodium at 127 mg/kg in the diet for 7 days following a 7-day pretreatment phase. Three quarters (77.5%) of the total radioactivity was recovered in combined excreta within 8 days after withdrawal of the test diet. In a non-GLP-compliant mass balance study, broilers received unlabelled lasalocid sodium at 75 mg/kg in the feed (equivalent to 5 mg/day) for 16 days, after which birds were treated with [^{14}C]lasalocid sodium via oral capsules at 5 mg/day for 3 days. Systemic absorption and elimination of lasalocid were rapid. A peak blood concentration of 5.62 µg/mL was observed at 2 hours after dosing, and a blood elimination half-life of about 3 hours was calculated.

In a GLP-compliant study, broilers received [^{14}C]lasalocid sodium via twice-daily capsule administration at doses equivalent to dietary supplementation at 125 mg/kg for 7 days. A mean of 89.7% of total radioactivity was recovered from excreta and cage wash by 7 days after the first dose. By 14 days after the first dose, 90.6% of the administered dose was recovered in the excreta, cage wash and feather wash. The major component in excreta from birds at both the 1-day and 7-day sampling times was lasalocid A, constituting 9.6–10.6% of the administered radioactivity and comprising approximately 75–83% of the total radioactive residues. Up to three components believed to be homologues of lasalocid as well as up to five more polar unidentified components were separated by thin-layer chromatography. No residues in excess of 20 µg/kg equivalents were found in liver, kidney or skin plus fat samples after a 7-day withdrawal period. No residues greater than 20 µg/kg equivalents were found in muscle samples at any time except at 0-day withdrawal. Up to seven unidentified components were detected in tissues. Lasalocid A was the major residue in all tissue extracts investigated using HPLC. Lasalocid A represented 21.4–23.4% of the total radioactive residues in liver, 31.1–50.3% in kidney, 53.7–55.7% in muscle and approximately 50% in skin plus fat. This study confirmed that lasalocid A was the appropriate marker compound.

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Turkey. In a GLP-compliant study, 10-week-old turkeys received [^{14}C] lasalocid sodium in diet at a concentration of 127 mg/kg for 14 days. More than three quarters (80–84%) of the dose was excreted within 120 hours of administration. The concentration of radioactivity derived from [^{14}C]lasalocid sodium and its metabolites found in whole blood reached a plateau and was higher in females than in males. The higher concentration of radioactivity in the female turkeys may reflect the higher daily [^{14}C]lasalocid sodium intake on a body weight basis. Mean concentrations of radioactivity declined rapidly during withdrawal, from 0.50 mg/L at 8 hours down to 0.04 mg/L at 120 hours in the female turkeys.

Residue data

A number of residue depletion studies using radiolabelled and unlabelled lasalocid sodium were provided in chicken and turkey.

Chicken. In a GLP-compliant study to establish metabolism in broilers (as outlined above) at the 8-hour withdrawal, the concentration of total radioactive residues was 2010 µg/kg equivalents, and the concentration of lasalocid A was 94 µg/kg fresh tissue in the pooled liver samples. Using these pooled values, the calculated marker residue to total residue ratio was about 4.7% at the 8-hour withdrawal. Two non-GLP-compliant studies confirmed the depletion profiles.

In a GLP-compliant study, Ross broilers received lasalocid sodium in feed for 6 weeks at a dose level of 130 mg/kg to determine the residues of lasalocid A in tissues. The mean lasalocid A concentrations were above 400 µg/kg in all tissues collected at 0-day withdrawal and below 150 µg/kg in muscle, kidney and skin plus fat at 1 day of withdrawal and in liver at 2 days of withdrawal. In an earlier GLP-compliant study, chickens were fed a starter diet containing lasalocid sodium at a concentration of approximately 138 mg/kg for 21 days, followed by 21 days of finisher diet containing lasalocid sodium (approximately 130 mg/kg). One group of birds was then sacrificed at each of 0, 24, 72, 120 and 168 hours following withdrawal from the test diet. Concentrations of lasalocid A in liver were less than 100 µg/kg in all individual birds at the 24-hour withdrawal. However, two birds had higher values (171.8 and 141.5 µg/kg) at the 72-hour withdrawal. Residues of lasalocid A in kidney, muscle and skin plus fat were less than 50, 20 and 100 µg/kg, respectively, in all individual birds by the 24-hour withdrawal.

In a non-GLP-compliant study, chickens were fed lasalocid sodium at 125 mg/kg from day 18 to day 59 and then subjected to a 5-day withdrawal interval. Mean lasalocid A concentrations at 0-day withdrawal in both internal fat and skin plus fat were approximately 260 µg/kg and decreased to about 50 µg/kg after 1 day of withdrawal. In another non-GLP-compliant study, broilers were fed lasalocid sodium at a level of 125 g/kg for 42 days

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followed by a drug withdrawal period. Lasalocid A residues depleted rapidly between 3 hours and 24 hours of withdrawal in both skin plus fat and muscle, the only tissues assayed. Subsequently, residue depletion was much slower, and lasalocid A was still detectable at the 10-day withdrawal in both tissues. All skin plus fat samples were below 300 µg/kg, and all muscle samples were below 60 µg/kg by 1 day of withdrawal.

Turkey. In a GLP-compliant study, 10-week-old turkeys received 127 mg/kg of [^{14}C]lasalocid sodium in feed for 14 days. The bile and liver had the highest levels of radioactivity at all time points. At 8 hours, the concentrations of radioactivity were 2590–4180 µg/kg in liver, 360–510 µg/kg in kidney, 20–50 µg/kg in muscle and 150–460 µg/kg in skin plus fat. After 5 days of withdrawal, concentrations were 850–890 µg/kg in liver, 70–90 µg/kg in kidney, less than 20 µg/kg in muscle and 70–110 µg/kg in skin plus fat. Concentrations of parent lasalocid in muscle, abdominal fat and liver were less than 25 µg/kg at all sacrifice times.

Another GLP-compliant study was performed in growing turkeys fed lasalocid sodium at 130 mg/kg for 112 days followed by a withdrawal period. Tissues were analysed for lasalocid A using a validated LC-MS/MS method with an LOQ of about 50 µg/kg for liver and skin plus fat, about 25 µg/kg for kidney and about 10 µg/kg for muscle. At the 0-hour withdrawal, mean residues of lasalocid A were highest in skin plus fat (159 µg/kg), followed by liver (155 µg/kg), kidney (108 µg/kg) and muscle (25.3 µg/kg). Lasalocid A residues in liver, kidney, muscle and skin plus fat were below the LOQ in each tissue after a 3-day withdrawal.

Other poultry. A non-GLP-compliant residue depletion study was conducted in quail given lasalocid at a dietary concentration of 90 mg/kg for 27 days. Birds were maintained on an unmedicated feed for a 9-day withdrawal period. At the 0-hour withdrawal, the concentration of lasalocid A in muscle samples was 39.5 µg/kg. By the 3-day withdrawal, only one bird had a residue above 20 µg/kg, at 25 µg/kg. The concentration of lasalocid in skin plus fat was 298 µg/kg at the 0-hour withdrawal, but rapidly decreased to 30.8 µg/kg by 6 days of withdrawal.

Analytical methods

LC-MS/MS methods were developed and validated in accordance with GLP for the quantitative determination of lasalocid in the muscle, liver, kidney and skin plus fat of chicken and turkey. LOQs of the methods for lasalocid A, the marker residue, were 5 µg/kg for all tissues of chicken and 50 µg/kg for liver and skin plus fat, 25 µg/kg for kidney and 10 µg/kg for muscle of turkey. The intra-day recovery at 5 µg/kg for all tissues ranged from 87% to 108%, with precision below 20%.

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An HPLC method using fluorescence detection in ISO 78/2 format was developed and validated for assay of the liver, kidney, muscle and skin plus fat samples from chicken. The LOQs were 20 µg/kg for all tissues. The intra-day assay recoveries at 20, 40, 100 and 400 µg/kg for the tissues were all between 71% and 93%, with coefficients of variation of 2.4–21.9%.

Maximum residue limits

In recommending MRLs of lasalocid sodium in poultry food commodities, the Committee considered the following factors:

- An ADI of 0–5 µg/kg bw for lasalocid sodium was established by the Committee. The upper bound of the ADI is equivalent to 300 µg lasalocid sodium for a 60 kg person.
- Where information on approved veterinary uses was provided, withdrawal times were in the range 0–7 days.
- Lasalocid sodium is extensively metabolized in poultry, although the metabolites were not identified.
- Lasalocid A is a suitable marker residue in all edible tissues of poultry.
- Lasalocid A represents 22% of the total radioactive residues in liver, 41% in kidney, 55% in muscle and 52% in skin plus fat in chicken.
- The extension of MRLs to turkey and quail and the extrapolation of MRLs to pheasant are appropriate, as depletion data were available, the marker residue has been demonstrated and information was available on authorized uses.
- Validated LC-MS/MS and HPLC methods were provided and considered suitable for routine monitoring of lasalocid A as marker residue in poultry tissues.

The Committee recommended MRLs for lasalocid determined as lasalocid A in chicken, turkey, quail and pheasant tissues.

The MRLs recommended for chicken, turkey, quail and pheasant tissues are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile (UTL 95/95) for the 1-day post-treatment data from the unlabelled residue depletion study.

The recommended MRLs for chicken, turkey, quail and pheasant are 1200 µg/kg in liver, 600 µg/kg in kidney, 400 µg/kg in muscle and 600 µg/kg in skin plus fat. An EDI of 80 µg/person per day was calculated, based on median residues, which represents approximately 27% of the upper bound of the ADI. The MRLs and median residues are based on the data for a 1-day withdrawal.

No information was available for duck, including on approved uses. According to the sponsor, the compound is not registered for use in laying hens. Therefore, it is not appropriate to recommend MRLs for eggs.

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A residue monograph was prepared.

Summary and conclusions

Studies relevant to risk assessment

Species / study type (route)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Mouse				
Two-year study of toxicity and carcinogenicity (dietary)	0, 1.5, 5.25, 18	No relevant findings	18[a]	–
Rat				
Thirty-month study of toxicity and carcinogenicity (dietary)	Males: 0, 0.5, 1.8, 6.2 Females: 0, 0.6, 2.2, 8.1	Increased incidence of impaired righting and grasping reflexes in females	2.2	8.1
Multigeneration reproductive toxicity study, including teratogenicity study (dietary)	0, 0.5, 1.75, 6	Parental toxicity: Reduced body weights	1.75	6
		Reproductive toxicity: Decreased numbers of corpora lutea and implantations, decreased implantation efficiency	**0.5***	1.75
		Offspring toxicity: Decreased number of pups surviving to weaning, decreased body weight of pups surviving to weaning	1.75	6
		Embryo and fetal toxicity: Decreased fetal weights, increased incidence of visceral and skeletal variants	1.75	6
Rabbit				
Developmental toxicity study (gavage)	0, 0.5, 1, 2	Maternal toxicity: Decreased body weight gain, decreased feed consumption and altered faecal output	–	0.5[b,c]
		Embryo and fetal toxicity: Decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants	**0.5***	1
Dog				
Two-year toxicity study (dietary)	0, 0.25, 1, 5	Transient intermittent paralysis of limbs and increased serum alkaline phosphatase	1	5

* **Pivotal study value *(14, 15)***
[a] Highest dose tested.
[b] Lowest dose tested.
[c] Maternal toxicity was likely due to the sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract. It is not considered appropriate to consider the maternal toxicity in relation to derivation of an ADI.

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Uncertainty factor

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

Toxicological effects

A toxicological ADI of 0–5 µg/kg bw could be derived.

Microbiological effects

A microbiological ADI of 0–8.4 µg/kg bw could be derived.

ADI (based on toxicological effects)

0–5 µg/kg bw

Residue definition

Lasalocid A

MRLs

The recommended MRLs for chicken, turkey, quail and pheasant are 1200 µg/kg in liver, 600 µg/kg in kidney, 400 µg/kg in muscle and 600 µg/kg in skin plus fat.

Estimated dietary exposure

An EDI of 80 µg/person per day was calculated, based on median residues, which represents approximately 27% of the upper bound of the ADI.

3.6 Monepantel

Explanation

Monepantel (CAS No. 887148-69-8) is a member of the amino-acetonitrile derivative anthelminthics. Monepantel causes a paralysis of gastrointestinal nematodes by binding to a unique receptor. It is administered as an oral drench to control gastrointestinal nematodes (roundworms) in sheep.

Monepantel was previously reviewed by the Committee at its seventy-fifth meeting (Annex 1, reference *208*), which established an ADI of 0–20 µg/kg bw, corresponding to an upper bound of acceptable intake of 1200 µg/day for a 60 kg person. The Committee recommended MRLs, determined as monepantel sulfone, in sheep tissue of 300 µg/kg in muscle, 700 µg/kg in kidney, 3000 µg/kg in liver and 5500 µg/kg in fat. The EDI was 201 µg/person per day, which represents 17% of the upper bound of the ADI.

At the Twentieth Session of CCRVDF *(2)*, concerns were raised that the recommended MRLs were significantly lower than those already established in some countries and could create trade problems. It was also noted that

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the recommended MRLs were not consistent with the withdrawal times in some countries.

CCRVDF requested that JECFA evaluate the safety of the proposed higher MRLs in light of the information provided by CCRVDF. JECFA was asked to consider if higher MRLs (muscle, 700 µg/kg; liver, 5000 µg/kg; kidney, 2000 µg/kg; fat, 7000 µg/kg) are compatible with the ADI and consistent with the JECFA MRL derivation process.

Residue evaluation

No new data or studies were provided for the current evaluation. A summary of global approvals, the MRLs assigned by regulatory authorities and associated withdrawal periods was provided. The withdrawal times reported to the Committee ranged from 7 to 14 days.

Analytical methods

Validated analytical methods (HPLC/UV for screening and LC-MS/MS for confirmation) were reviewed previously. These methods remain appropriate for the monitoring of monepantel residues in sheep tissues.

Maximum residue limits

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

- An ADI of monepantel of 0–20 µg/kg bw was previously established by the Committee (Annex 1, reference *208*), corresponding to an upper bound of acceptable intake of 1200 µg/day for a 60 kg person.
- Monepantel is extensively metabolized.
- The metabolite monepantel sulfone is the marker residue in tissues.
- Fat contains the highest concentration of monepantel sulfone at all sampling times, followed by liver, then kidney and muscle. Liver and fat can serve as the target tissues.
- The ratios of the concentration of marker residue to total residues are 1.0 in muscle and 0.66 in fat, liver and kidney.
- A validated analytical method for the determination of monepantel sulfone in edible sheep tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.
- MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations (UTL 95/95).

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Consistent with the shortest withdrawal time assigned in Member States with an approved use of monepantel, the Committee recommended MRLs determined as monepantel sulfone, expressed as monepantel, in sheep tissue of 500 µg/kg in muscle, 1700 µg/kg in kidney, 7000 µg/kg in liver and 13 000 µg/kg in fat. Using the model diet and marker residue to total residue ratio of 1.00 for muscle and 0.66 for fat, liver and kidney, and applying a correction factor of 0.94 to account for the mass difference between monepantel sulfone (the marker residue) and monepantel, the EDI is 446 µg/person per day, which represents approximately 37% of the upper bound of the ADI.

A residue monograph was prepared.

Summary and conclusions

Residue definition

Monepantel sulfone, expressed as monepantel

MRLs

The Committee recommended MRLs (expressed as monepantel) in sheep tissue of 500 µg/kg in muscle, 1700 µg/kg in kidney, 7000 µg/kg in liver and 13 000 µg/kg in fat.

Estimated dietary exposure

The EDI is 446 µg/person per day, which represents approximately 37% of the upper bound of the ADI.

3.7 Recombinant bovine somatotropins

Explanation

Somatotropins are proteins secreted by the anterior pituitary gland that stimulate growth, cell regeneration and reproduction in humans and animals. Most anabolic and growth-promoting effects of somatotropins are mediated through insulin-like growth factor-I (IGF-I). Bovine somatotropins produced by recombinant DNA techniques (rbSTs) are used in lactating dairy cows to increase milk production. Four analogues of bovine somatotropin (bST), somagrebove, sometribove, somavubove and somidobove, were previously evaluated by the Committee at its fortieth meeting (Annex 1, reference *104*) and further evaluated at its fiftieth meeting (Annex 1, reference *134*). Only two of these (sometribove and somavubove) are currently registered for use. Although the chemical properties of the recombinant products vary slightly from those

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of pituitary bST, the Committee considered the recombinant products to be biologically and toxicologically similar, as they all act by binding with high affinity to the bST receptor.

The Committee at its fortieth meeting established an ADI and MRLs "not specified" for these four rbSTs. The term "not specified" was used because of the lack of bioactivity following oral intake of rbSTs and IGF-I and the low concentrations and non-toxic nature of the residues of these compounds. The ADI and MRLs "not specified" were reaffirmed by the Committee at its fiftieth meeting.

Draft Codex standards for rbSTs have been held at the final step (before adoption) for more than a decade. When considering the adoption of these standards, the Codex Alimentarius Commission at its Thirty-fifth Session *(3)* requested a re-evaluation of the four analogues of natural bST, somagrebove, sometribove, somavubove and somidobove, by JECFA, noting that the scientific assessment of bST dated back to the 1990s. In particular, the Commission requested that JECFA (i) update the toxicological evaluation, (ii) update the exposure assessment based on any new occurrence data in food, (iii) evaluate potential adverse health effects and (iv) consider the need to revise or maintain the ADI and MRLs for rbSTs. The Commission further requested that JECFA consider new data and information related to other factors pertaining to human health, including (i) the possible increased use of antimicrobials to treat mastitis in cows, (ii) the possibility of increased levels of IGF-I in the milk of cows treated with rbSTs, (iii) the potential effects of rbSTs on the expression of certain viruses in cattle and (iv) the possibility that exposure of human neonates and young children to milk from rbST-treated cows increases health risks (e.g. the development of insulin-dependent diabetes mellitus). JECFA was also asked to consider aspects of antimicrobial resistance associated with the use of rbSTs in relation to human health.

In response to JECFA's call for data, data were submitted to the Committee by a sponsor and two Member countries. Additionally, the Committee undertook a systematic review to address the following questions:

- What are the hormone levels in the milk and/or meat of cattle, goats or sheep treated with rbSTs compared with untreated animals?
- Are the incidences of clinically relevant mastitis different between cattle, sheep and goats treated with rbSTs compared with untreated animals? Are there differences in antimicrobial residue levels in the milk and meat products from treated compared with untreated animals?
- Are retroviral/lentiviral levels and serotype distributions different between cattle, sheep and goats treated with rbSTs compared with untreated animals?

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- Are prion levels in meat and milk and prion infectivity different between cows treated with rbSTs compared with untreated animals?
- Is consumption of milk or meat from rbST-treated cattle, sheep or goats associated with increased rates of morbidity and mortality in infants or in the general population compared with the equivalent age groups consuming meat or milk from untreated animals?

Details of the search strategy and databases used are available on the WHO website as supplementary information to the meeting report at http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/index.html.

In addition, PubMed and Web of Knowledge databases were searched for toxicity studies of rbSTs in laboratory animals, bioavailability/bioactivity of oral IGF-I and analytical methods.

Biochemical data

The Committee at its fortieth and fiftieth meetings concluded that human and bovine somatotropins are structurally different and have species-specific receptor binding activity. Furthermore, the total concentration of bST detected in tissues and milk of rbST-treated cattle is similar to that from untreated cattle, and bST is denatured by high temperatures (e.g. by cooking or pasteurization) and biodegradation processes in the gut. No new biochemical data on rbSTs were available since the previous evaluation of the compound by the Committee at the fiftieth meeting. The Committee evaluated a part of a study submitted to previous JECFA meetings, but not specifically discussed in the respective monographs. This study investigated the serum level of anti-rbST antibodies as a surrogate measure for oral absorption/bioavailability in rats administered an rbST by gavage for 90 days. The results indicated increased levels of circulating anti-rbST antibodies in 20% and 30% of rats treated with the rbST at 5 and 50 mg/kg bw per day, respectively, and in one animal (3%) treated with the rbST at 0.1 mg/kg bw per day. The experimental design, however, did not allow an assessment as to whether the antibody response was a result of absorption of intact rbST or only an immunologically active peptide fragment (epitope or antigenic determinant) of the rbST into the systemic circulation or due to mucosal immunity in the gut. Also, there were no systemic effects on growth or feed intake in orally treated rats. These data, together with the data evaluated at previous meetings of the Committee, confirm the absence of the biological activity of rbSTs following oral intake.

Toxicological data

The Committee at its fortieth meeting evaluated the toxicity of different rbSTs. Acute oral toxicity studies in rats with rbST doses up to 5 g/kg bw,

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two 2-week oral feeding studies in rats with rbST doses up to 10 mg/kg bw per day and two 4-week oral feeding studies in rats with rbST doses up to 50 mg/kg bw per day caused no effects up to the highest dose tested. Similarly, no treatment-related effects were observed in two 90-day oral feeding studies in rats at rbST doses up to 100 mg/kg bw per day and a 90-day oral feeding study in dogs at rbST doses up to 10 mg/kg bw per day, the highest doses tested. No new toxicity studies on rbSTs were available since the previous evaluation of rbSTs by the Committee at the fiftieth meeting.

The present Committee evaluated long-term carcinogenicity studies in rats and mice using related, but distinct, compounds (i.e. recombinant rat and mouse somatotropins). Daily subcutaneous administration of recombinant rat and mouse somatotropins to groups of rats and mice, respectively, for 2 years did not show any carcinogenic effects. Although the Committee considered these data not directly relevant to the risk assessment of rbSTs, these observations do illustrate that other somatotropins are not potential carcinogens.

Concentration of rbSTs and IGF-I in milk and tissues

Previous meetings of the Committee have concluded that owing to the structural dissimilarity between bovine and human somatotropins and species-specific receptor binding, rbSTs are not biologically active in humans. Also, similar concentrations of total bST are detected in milk and tissues of rbST-treated and untreated cows. Very few new publications investigating the concentrations of bST in milk and tissues following treatment with rbSTs were available in the literature since the fiftieth meeting of the Committee. Available information supports the conclusions of the previous Committee that there is no significant change in the concentrations of total bST detected in milk and tissues of rbST-treated cows when compared with untreated controls.

Available new information supports previous conclusions that the IGF-I concentration in milk varies widely in lactating cows and is influenced by parity, stage of lactation, nutritional status, season and somatic cell counts (an indication of udder health) of the milk. IGF-I concentrations measured in colostrum are substantially higher than concentrations in milk produced subsequently. Treatment of cows with rbSTs transiently increased the mean IGF-I concentration in milk by up to 50%, but such increases were within the physiological variations observed in untreated cows.

A new cross-sectional study of retail milk in the USA suggests that the IGF-I levels in retail milk labelled as conventional, which includes milk from both rbST-treated and untreated cows (3.1 ± 0.1 ng/mL), were not different from levels in milk labelled to be from rbST-free cows (3.0 ± 0.1 ng/mL). However, the percentage of conventional milk that comes from cows treated with rbSTs is not known.

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The fiftieth meeting of the Committee considered that some milk-borne IGF-I may escape degradation by gastrointestinal tract enzymes and get absorbed from the gastrointestinal tract. In vitro digestion studies indicated that IGF-I is rapidly degraded by gastrointestinal tract enzymes. However, subsequent studies in experimental animals showed that the rate of degradation could be reduced by the components in milk/colostrum. In vivo studies in laboratory animals suggested that up to 25% of IGF-I fed with milk could be absorbed from the gastrointestinal tract, although only a fraction of it would reach the systemic circulation. Studies in infants showed that feeding a formula supplemented with a 20-fold higher concentration of IGF-I did not increase the serum IGF-I concentrations compared with feeding a standard formula. Randomized trials in active adult athletes did not detect any difference in plasma IGF-I concentrations in an intervention group fed up to 120 000 ng IGF-I per person per day from bovine colostrum for up to 8 weeks when compared with controls fed whey protein during pretreatment, treatment or post-treatment periods.

The literature suggests that the concentration of IGF-I in serum in humans is influenced by a number of factors, including age, physiological stage and nutritional status. Consumption of milk per se was associated with increased blood IGF-I concentrations in humans. There is evidence that orally administered IGF-I has some local bioactivity in the gastrointestinal tract. However, given the large quantity of IGF-I secreted in the digestive tract of humans, the small additional quantity of IGF-I in milk from cows treated with rbSTs is unlikely to make a biologically relevant contribution to the effects of endogenous IGF-I. The endogenous IGF-I production in humans will be more influenced by the consumption of milk per se, irrespective of whether it is from rbST-treated or untreated cows.

The present Committee concluded that some milk-borne IGF-I may not be degraded by gastrointestinal enzymes. However, even if some of the IGF-I in milk were absorbed, the incremental human exposure would be negligible when compared with total daily human production of IGF-I of 10 mg/day, as reported by the Committee at the fiftieth meeting. This is consistent with the previous conclusion of the Committee.

Expression of retroviruses and prion proteins

The fiftieth meeting of the Committee concluded that the available studies provided no evidence that rbSTs affect the expression of retroviruses in cattle. The Committee also concluded that the possibility of a link between rbST treatment and bovine spongiform encephalopathy was highly speculative, as there was no evidence for a direct link. No new information

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on the role of rbSTs in the expression of retroviruses or prion proteins in ruminants was available from the literature.

Risk of type 1 diabetes in genetically susceptible infants

There is evidence that in infants genetically susceptible to type 1 diabetes, exposure to cow's milk early in infancy, when an infant's gastrointestinal tract is not fully developed, may stimulate the production of antibodies that can cross-react with pancreatic islet β-cell surface antigens. This may be a risk factor for the development of type 1 diabetes. Stimulation of aberrant immune response in infancy, however, is not limited to milk components alone, as infants genetically predisposed to type 1 diabetes also have a generalized aberrant immune response to several other proteins (e.g. cereals, fruits, bacteria, viruses).

Animal and human studies suggest that IGF-I is unlikely to have an adverse impact on the pathogenesis of diabetes in humans. The composition of milk from cows treated with rbSTs did not differ materially from that of untreated cows, and therefore consumption of milk from rbST-treated cows would not pose an additional risk for the development of diabetes.

Risk of cancer

The Committee also considered the potential cancer risk in humans associated with the consumption of milk from rbST-treated cows. The Committee concluded that any carcinogenic risk from rbSTs themselves was negligible, because they are not absorbed from the gastrointestinal tract, they are not bioactive in humans and the respective orthologues did not cause cancer in rats or mice when administered subcutaneously.

As stated above, the IGF-I exposure from consumption of milk from cows treated with rbSTs represented a small fraction of the physiological amounts produced in humans, and endogenous IGF-I production in humans will be influenced more by the consumption of milk per se than by whether the milk is from rbST-treated or untreated cows. Circulating IGF-I concentrations at the higher end of the normal physiological range were observed in some cancer patients, although these were inconsistent between studies and between different types of cancers. Moreover, these observations came from epidemiological studies in which the impact of reverse causation cannot be excluded.

Risk to human health from use of antimicrobial agents

The fiftieth Committee concluded that the use of rbSTs would not result in a higher risk to human health due to the use of antimicrobial agents to treat mastitis and that increased potential for drug residues in milk could

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be managed by practices currently in use within the dairy industry and by following the directions for use.

The potential risk to human health due to the potential for increased use of antimicrobial agents to treat mastitis or increased incidence of non-compliant residues in milk of cows treated with rbSTs was also considered by the present Committee. A meta-analysis published in 1998 observed that cows treated with rbSTs had a higher incidence (up to 25%) of mastitis compared with untreated cows. A systematic review of the literature published since the fiftieth meeting of the Committee did not find any significant difference in the incidence of mastitis between rbST-treated and untreated cows. However, the Committee did not have data to determine the use of antimicrobial agents to treat mastitis on farms using rbSTs.

The fiftieth meeting of the Committee had assessed the data from a post-approval monitoring programme established in the USA to monitor the effects on animal health, including mastitis and non-compliant drug residues in milk. Additional monitoring data for 1996–2012 from the same programme were assessed for the long-term trend in antimicrobial residues in bulk milk. Since 1996, there has been a consistent decrease in the number of bulk milk samples positive for non-compliant antimicrobial residues, with only 0.017% of samples testing positive in 2012, compared with 0.1% in 1996. Several factors could influence the observed results, including adherence to GVP and improved animal husbandry practices. Moreover, the available data did not provide individual animal-level data to correlate with the use of rbSTs. Nonetheless, the Committee considered that the available evidence suggested that in the USA, the approval of rbSTs did not lead to an increased incidence of non-compliant antimicrobial residues in bulk milk. The Committee found no relevant monitoring data from other jurisdictions where rbSTs are authorized for use.

Although the Committee was aware of the concern regarding potential antimicrobial resistance, its systematic review of the literature did not find specific studies correlating the use of rbSTs with the development of antimicrobial resistance in mastitis pathogens.

Based on the data reviewed, the Committee concluded that there was no evidence to suggest that the use of rbSTs would result in a higher risk to human health due to the possible increased use of antimicrobial agents to treat mastitis or the increased potential for non-compliant antimicrobial residues in milk.

Evaluation

Based on the above assessment, the Committee's responses to the issues raised by the Codex Alimentarius Commission are as follows:

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(i) update the toxicological evaluation

No new toxicological studies were available. Owing to structural differences between bovine and human somatotropins, species-specific receptor binding of somatotropins and lack of oral activity of rbSTs, the Committee concluded that if any rbST residues are present in milk or tissues, they would pose a negligible risk to human health.

(ii) update the exposure assessment based on any new occurrence data in food

The Committee concluded that similar concentrations of total bST were present in milk and tissues of rbST-treated and untreated cows.

(iii) consider new data and information related to the possibility of increased levels of IGF-I in the milk of cows treated with rbSTs

There is a transient increase in IGF-I concentrations in milk of rbST-treated cows, which fall within the normal physiological range. IGF-I is substantially, if not completely, degraded in the gut and is unlikely to be absorbed from the gut and be bioavailable at biologically relevant exposures. Therefore, the contribution of exogenous IGF-I resulting from the ingestion of milk from rbST-treated cows is extremely low in comparison with endogenous production.

(iv) evaluate potential adverse health effects, including the possibility that exposure of human neonates and young children to milk from rbST-treated cows increases health risks (e.g. the development of insulin-dependent diabetes mellitus)

Exogenous IGF-I from milk makes no significant contribution to circulating levels of IGF-I in humans, and there are no significant differences in the composition of milk from rbST-treated cows when compared with the milk from untreated cows. The Committee concluded that there was no additional risk for the development of type 1 diabetes due to the consumption of milk from rbST-treated cows. The Committee also concluded that the literature did not support a link between exposure to IGF-I in milk from rbST-treated cows and an increased risk of cancer.

(v) consider new data and information related to the potential effects of rbSTs on the expression of certain viruses in cattle

There was no new information on the link between rbST use and either potential stimulation of retrovirus expression or prion protein expression in cattle. The present Committee considers that the position expressed by the previous Committee remains valid.

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(vi) consider new data and information related to the possible increased use of antimicrobials to treat mastitis in cows and aspects of antimicrobial resistance associated with the use of rbSTs in relation to human health

The Committee concluded that there was no evidence to suggest that the use of rbSTs would result in a higher risk to human health due to the possible increased use of antimicrobials to treat mastitis or the increased potential for non-compliant antimicrobial residues in milk. The Committee did not find specific studies linking the use of rbSTs with the development of antimicrobial resistance. The present Committee considers that the position expressed by the previous Committee remains valid.

(vii) consider the need to revise or maintain the ADI and MRLs for rbSTs

The Committee reaffirmed its previous decision on ADIs and MRLs "not specified" for somagrebove, sometribove, somavubove and somidobove.

Summary and conclusions

ADI and MRLs

The Committee reaffirmed its previous decision on ADIs and MRLs "not specified" for somagrebove, sometribove, somavubove and somidobove.

3.8 Zilpaterol hydrochloride

Explanation

Zilpaterol hydrochloride (zilpaterol HCl, CAS No. 119520-06-8) is a β_2-adrenoceptor agonist used for promoting body weight gain, feed efficiency and carcass muscle ratio in cattle fed in confinement before slaughter. There are four enantiomers of zilpaterol HCl: (6*R*,7*R*), (6*R*,7*S*), (6*S*,7*R*) and (6*S*,7*S*). The product in use, which has the code name RU 42173, is racemic *trans* zilpaterol HCl, a mixture of the (6*R*,7*R*) and (6*S*,7*S*) enantiomers; it will be referred to as zilpaterol HCl in this report.

The only use of zilpaterol HCl is as a veterinary drug in cattle. The recommended dose added to cattle feed is 7.5 mg/kg (on a 90% dry matter basis) of the total daily ration during the last 20–40 days of the feeding period before slaughter. This level in the feed is equivalent to approximately 0.15 mg/kg bw per day or 60–90 mg/animal per day. Where use is authorized, a withdrawal period ranging from 2 to 4 days is applied.

Zilpaterol HCl has not previously been evaluated by the Committee. The Committee evaluated zilpaterol HCl at the current meeting at the request

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of the Twentieth Session of CCRVDF *(2)*. The Committee was asked to establish an ADI and recommend MRLs for zilpaterol HCl in cattle tissue.

Toxicological evaluation

The Committee considered the results of studies on blood protein binding in vitro, pharmacokinetics, pharmacological effects in vitro and in vivo, acute, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity and relay pharmacology, as well as observations in humans. The majority of the studies were performed in accordance with GLP.

Biochemical data

In rats, dogs and humans, the binding of zilpaterol HCl to serum albumin was low (14–15%). In human red blood cells, the bound fraction was 55%, irrespective of the concentration and the presence of plasma. Zilpaterol HCl was rapidly absorbed via the oral route, with almost 100% bioavailability in rats and dogs. In rats, peak blood concentrations were reached within 1 hour of a single oral gavage dose and by 3–23 hours when given in the diet. Total area under the plasma concentration–time curve correlated approximately with dose. The half-life in plasma ranged from 2.4 to 5.5 hours. After repeated dosing, the pharmacokinetic profile in rats was similar to that following a single dose. Systemic exposure was slightly higher in females than in males. In a study in human male volunteers in which zilpaterol HCl was given as a single dose in drinking-water, the time to reach the peak concentration was 1 hour, and the half-life in plasma was 4–5 hours, independent of the dose.

In studies in rats using radiolabelled zilpaterol HCl, approximately 50–55% of the dose was eliminated in the urine and 40–42% in the faeces over 8 days. In urine, unchanged zilpaterol predominated, accounting for at least half of the radioactivity, with the metabolites deisopropyl zilpaterol, acetylated deisopropyl zilpaterol, hydroxy-zilpaterol, a glucuronide conjugate of hydroxy-zilpaterol and two unidentified metabolites each accounting for between 2% and 20% of the radioactivity. Deisopropyl zilpaterol was the main metabolite present. In faeces, parent compound accounted for 10–45% of the radioactivity present and hydroxy-zilpaterol for 60–80%, with a small fraction present as deisopropyl zilpaterol. After 8 days, residues in liver and carcass were less than 0.1% and less than 1%, respectively, of the administered dose; radioactivity could not be detected in fat or muscle.

In studies using rat hepatic microsomal fractions, unchanged zilpaterol was present, together with deisopropyl zilpaterol and hydroxy-zilpaterol. The metabolites were shown to be largely the products of cytochromes P450 (CYP); deisopropyl zilpaterol was formed preferentially by members of the CYP1A subfamily, whereas hydroxy-zilpaterol was formed less specifically,

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possibly involving members of the CYP2B subfamily. Zilpaterol HCl did not induce drug-metabolizing enzymes.

Many in vitro, ex vivo and in vivo studies have clearly demonstrated the β_2-agonist effect of zilpaterol HCl and its main metabolite, deisopropyl zilpaterol (as free base or hydrochloride form). These effects are manifested as contraction of cardiac muscle and relaxation of the smooth muscles of the vasculature and the bronchi. There is little or no affinity of zilpaterol or its main metabolite for α_1- or α_2-adrenoceptors or for dopaminergic receptors D_1 and D_2. Studies on guinea-pig lung membranes have demonstrated that zilpaterol HCl and deisopropyl zilpaterol are only partial agonists at the β_2-adrenoceptor in terms of adenylyl cyclase activation.

In rat studies, zilpaterol HCl given intravenously to anaesthetized, pithed animals induced a dose-dependent decrease in diastolic blood pressure from 0.01 mg/kg bw, with a maximum effect observed at 0.1 mg/kg bw, and an increase in heart rate from 0.3 mg/kg bw. Deisopropyl zilpaterol (in either its free base or hydrochloride form) caused a dose-dependent decrease in diastolic blood pressure from 0.1 mg/kg bw, with a steady-state maximum effect observed at 0.3 mg/kg bw, but had no effect on the heart rate. These data indicate that deisopropyl zilpaterol has 10-fold lower β_2-agonist activity on the cardiovascular system, compared with the parent compound. Use of specific antagonists for β_1- and β_2-adrenoceptors demonstrated that the effect of the compounds on blood pressure was mediated by β_2-adrenoceptors, whereas the activity of zilpaterol HCl on the heart was associated with the β_1-adrenoceptor.

Toxicological data

A comprehensive set of toxicological studies was performed, mainly in compliance with GLP, in both rodent (mice, rat) and non-rodent species (dog, Cynomolgus monkey and microswine). The purity of the test item was higher than 90% in all studies.

Zilpaterol HCl and deisopropyl zilpaterol were of relatively low acute toxicity by the oral route. The oral LD_{50} values of zilpaterol HCl were about 1100 mg/kg bw in rats and about 500 mg/kg bw in mice. The main metabolite, deisopropyl zilpaterol free base, had an oral LD_{50} value in mice of about 1000 mg/kg bw, indicating that its acute toxicity is about half that of the parent compound. Zilpaterol HCl is not irritating to skin and is slightly irritating to eyes. It is not a skin sensitizer in guinea-pigs.

In short-term toxicity studies performed in mice, rats, dogs, microswine and Cynomolgus monkeys, the main effects observed were those classically associated with β-adrenoceptor agonists, such as cardiovascular effects

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(increased heart rate and decreased diastolic blood pressure). In parallel, increased body weight associated with increased feed consumption was also observed.

In mice, zilpaterol HCl administered orally for 4 weeks at a dose of 0, 0.2, 2, 4 or 40 mg/kg feed (equal to 0, 0.046, 0.46, 0.895 and 8.867 mg/kg bw per day for males and 0, 0.047, 0.483, 0.986 and 9.601 mg/kg bw per day for females, respectively) induced no treatment-related effects except body weight change. There was a small but statistically significant increase in body weight in female mice at the highest dose, but body weight had returned to control values by the end of treatment. The NOAEL was 4 mg/kg feed (equal to 0.986 mg/kg bw per day), based on increased body weight in female mice.

In a 30-day study, rats were given zilpaterol HCl at a dose of 0, 1, 10 or 100 mg/kg bw per day by oral gavage. Body weight was increased at 10 and 100 mg/kg bw per day in males and at 100 mg/kg bw per day in females, with no change in feed consumption. A dose-dependent decrease in heart rate was observed at all doses in female rats. In male rats, total leukocyte count was increased at the highest dose, and activated partial thromboplastin time was slightly decreased at all doses. Decreased prothrombin time was observed at 10 and 100 mg/kg bw per day in females. Microscopic examination revealed no treatment-induced lesions. The LOAEL was 1 mg/kg bw per day, the lowest dose tested, based on decreased heart rate in females and decreased activated partial thromboplastin time in males.

In a 90-day study, rats were given zilpaterol HCl at a dose of 0, 1, 10 or 100 mg/kg bw per day by oral gavage. At 10 and 100 mg/kg bw per day, dose-dependent hypersalivation in both sexes was observed. Statistically significant increases in body weight gain were seen in females at 10 and 100 mg/kg bw per day and in males at 100 mg/kg bw per day, and an increase in feed consumption in males was observed at 10 and 100 mg/kg bw per day. There was a slight increase in blood urea at all doses in both sexes, but it was not accompanied by histopathological changes. Increased plasma creatinine was found at 10 and 100 mg/kg bw per day in males and females on week 5, but levels returned to normal at 10 mg/kg bw per day in males and at 100 mg/kg bw per day in females on week 13. The LOAEL was 1 mg/kg bw per day, the lowest dose tested, based on increased blood urea. In a follow-up study, when zilpaterol HCl was administered to rats at 0, 0.05, 0.5 or 1 mg/kg bw per day for 4 weeks, body weight was significantly increased at the highest dose without significant change in feed consumption in female rats. The NOAEL was 0.5 mg/kg bw per day, based on body weight change in females.

In a further short-term study, when rats were given zilpaterol HCl for 13 weeks at 0, 0.05, 0.5 or 1 mg/kg bw per day by oral gavage, increased body weight was observed in males and females at 0.5 and 1 mg/kg bw per day.

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Significantly lower mean heart rates were observed at all doses in females and at the highest dose in males on week 3, 4, 8 or 13. This was associated with longer PQ intervals at all doses in females and at 0.5 or 1 mg/kg bw per day in males. Lower systolic blood pressure was observed at 0.5 or 1 mg/kg bw per day on week 3 or 4 in males. A LOAEL of 0.05 mg/kg bw per day, the lowest dose tested, was derived from the results of this study.

In a 7-day study, dogs were given zilpaterol HCl at 100 mg/kg bw per day in a capsule by oral gavage. Clinical signs included frequent vomiting, reduced motor activity, apathy, vasodilatation and diarrhoea, accompanied by reduced body weight, changes in haematology and blood biochemistry, and morphological changes in the liver.

In a 30-day study, dogs were given zilpaterol HCl at 0, 0.5, 5 or 50 mg/kg bw per day in a capsule by oral gavage. A dose-dependent peripheral vasodilatation was observed at all doses. Reduced blood pressure and increased heart rate were observed at 1 hour, but blood pressure and heart rate had returned to normal values by 24 hours after dosing at all doses. Irregular vomiting and reduced motor activity were found at 5 and 50 mg/kg bw per day. The LOAEL was 0.5 mg/kg bw per day, the lowest dose tested, based on peripheral vasodilatation, increased heart rate and decreased blood pressure.

Microswine (one of each sex per group) were given increasing doses of zilpaterol HCl of 0.05, 0.5, 1, 5 and 10 mg/kg bw per day by oral gavage, each dose being given for 4 days with a 3-day withdrawal period between doses. A slight increase in heart rate associated with a decreased QT interval, without any change in blood pressure, was found from 0.5 mg/kg bw per day in a male and at 10 mg/kg bw per day in a female. In a 4-week toxicity study in which microswine were given zilpaterol HCl at 0, 0.005, 0.01, 0.05 or 5 mg/kg bw per day by oral gavage, the NOAEL was 5 mg/kg bw per day, the highest dose tested, as no treatment-related adverse effects were found. In a 13-week toxicity study in which microswine were given zilpaterol HCl at 0, 0.001, 0.05, 1 or 10 mg/kg bw per day by oral gavage, non-statistically significant increases in body weight were observed only in males at 1 and 10 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day, the highest dose tested.

Cynomolgus monkeys (one of each sex per group) were given increasing doses of zilpaterol HCl of 0.05, 0.5, 1, 5 and 10 mg/kg bw per day by oral gavage, each dose being given for 4 days with a 3-day withdrawal period between doses. A slight increase in heart rate associated with a decreased QT interval, without any change in blood pressure or dose–response relationship, was found at all doses.

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In a 4-week toxicity study in which Cynomolgus monkeys were given zilpaterol HCl at 0, 0.005, 0.01, 0.05 or 5 mg/kg bw per day by oral gavage, a reduction in blood pressure, accompanied by an increased heart rate with an associated decrease in QT interval, was observed at 0.05 and 5 mg/kg bw per day. The NOAEL was 0.01 mg/kg bw per day, based on cardiovascular effects.

In a chronic toxicity and carcinogenicity study in female mice, zilpaterol HCl was given orally by gavage at doses of 0, 10, 20, 50 and 250 µg/kg bw per day for 18 months. Significantly increased haemoglobin, associated with significantly increased red blood cell counts and haematocrit, was found at 50 and 250 µg/kg bw per day. There were also significantly decreased numbers of platelets, absolute neutrophil counts and monocyte numbers at 50 and 250 µg/kg bw per day. There were no neoplastic or preneoplastic findings. The NOAEL was 20 µg/kg bw per day, based on haematological changes.

In a chronic toxicity study in rats, zilpaterol HCl was given in the feed at concentrations adjusted to achieve doses of 0, 25, 50, 125 and 250 µg/kg bw per day for 52 weeks. There were slight decreases in heart rate, without impact on waveform traces, at 125 and 250 µg/kg bw per day and slight increases in systolic blood pressure at the highest dose in both sexes. These effects were fully reversible after a 4-week recovery period. The NOAEL was 50 µg/kg bw per day, based on the decrease in heart rate.

In a chronic toxicity and carcinogenicity study in rats, zilpaterol HCl was given in the feed at concentrations adjusted to achieve doses of 0, 25, 50, 125 and 250 µg/kg bw per day for 104 weeks. Slightly reduced body weight gains were found in males and females at the highest dose. The number of surviving male animals was significantly reduced at the highest dose. A marked increase in the weight of ovaries with increased incidence of ovarian cysts was observed at 125 and 250 µg/kg bw per day. An increased incidence of ovarian leiomyomas of the suspensory ligament was also found in the two highest dose groups. The NOAEL was 50 µg/kg bw per day, based on the increased ovarian weight with increased incidence of cysts and the increased incidence of leiomyomas.

Ovarian leiomyomas are benign tumours known to be related to the use of β_2-adrenoceptor agonists in rodents. The proliferation of the mesovarian smooth muscle is considered to be an adaptive physiological response to prolonged stimulation of the β-receptors, with muscle relaxation as a consequence. The occurrence of this neoplastic lesion in rodents was reported to be blocked by concomitant treatment with propranolol, a β_2-adrenoceptor antagonist. Little or no relaxant response to β_2-agonists can be demonstrated in the uteri of non-pregnant women. There is no evidence in humans of any increased incidence of smooth muscle tumours such as leiomyomas among

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users of β-agonists. Thus, the occurrence of ovarian leiomyomas in rats treated with zilpaterol HCl may represent a species-specific effect, and it is considered unlikely that oral exposure in humans would result in ovarian leiomyomas.

In an adequate range of tests of genotoxic activity, mainly performed under GLP-compliant conditions, zilpaterol HCl showed no evidence of genotoxic potential in microorganisms, in cultured mammalian cells or in vivo in mice. Like the parent compound, its main metabolite deisopropyl zilpaterol showed no evidence of genotoxicity in an adequate range of tests.

Considering the absence of any evidence of genotoxicity in vitro and in vivo, together with the likelihood that induction of mesovarian leiomyomas observed with zilpaterol HCl is associated with species-specific pharmacological β_2-adrenergic activity, the Committee concluded that zilpaterol HCl is unlikely to pose a carcinogenic risk to humans.

In a one-generation reproductive toxicity study in rats, zilpaterol HCl was given in the feed at a concentration of 0, 0.9, 3.6 or 14.4 mg/kg (equal to 0, 0.06, 0.23 and 0.94 mg/kg bw per day for males and 0, 0.10, 0.40 and 1.61 mg/kg bw per day for females, respectively) from 15 days prior to and during mating, pregnancy and lactation. There were no effects attributable to the treatment on F_0 reproduction or F_1 litters, apart from significant increases in body weight in all treated males, mainly during the 1st week of treatment, and in females during the entire treatment period at the highest dose. Based on body weight increases, the LOAEL for parental toxicity was 0.9 mg/kg feed (equal to 0.06 mg/kg bw per day in F_0 males), the lowest dose tested. The NOAEL for offspring and reproductive toxicity was 14.4 mg/kg feed (equal to 0.94 mg/kg bw per day), the highest dose tested.

In a two-generation reproductive toxicity study in rats, zilpaterol HCl was given in the feed at a concentration of 0, 0.9, 3.6 or 14.4 mg/kg from 71 days prior to mating and until weaning in F_0 or F_1 males and from 15 days before mating until the end of lactation for F_0 or F_1 females (equal to 0, 0.06, 0.23 and 0.94 mg/kg bw per day for F_0 males; 0, 0.10, 0.40 and 1.61 mg/kg bw per day for F_0 females; 0, 0.08, 0.32 and 1.26 mg/kg bw per day for F_1 males; and 0, 0.12, 0.45 and 1.77 mg/kg bw per day for F_1 females). There were no effects attributable to the treatment on F_0 or F_1 reproduction or on F_1 and F_2 litters, apart from slightly higher feed consumption and body weight gain in both F_0 and F_1 parents at all doses. The LOAEL for parental toxicity was 0.9 mg/kg feed (equal to 0.06 mg/kg bw per day), the lowest dose tested, for effects on feed consumption and body weight gain. The NOAEL for offspring and reproductive toxicity was 14.4 mg/kg feed (equal to 0.94 mg/kg bw per day), the highest dose tested.

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In a limited but GLP-compliant developmental toxicity study in mice, zilpaterol HCl was given at a dose of 0, 300 or 450 mg/kg bw per day by oral gavage from days 6 to 18 of pregnancy. There were no effects on general condition or behaviour of the dams or on body weight. No litter parameters were affected, and there were no malformations attributable to zilpaterol HCl. The NOAEL for maternal and embryo/fetal toxicity was 450 mg/kg bw per day, the highest dose tested.

In a series of three developmental toxicity studies in rats, zilpaterol HCl was given at doses ranging from 30 to 750 mg/kg bw per day by oral gavage from days 6 to 15 of gestation. A dose-dependent increase in maternal salivation was seen in all three studies (from 50 mg/kg bw per day). At the two highest doses used (600 and 750 mg/kg bw per day), there was an increase in postimplantation loss and a reduction in fetal weight, respectively. In two of the studies, there were significant increases in the numbers of fetuses with skeletal or visceral anomalies and/or variants, reaching statistical significance at the highest doses (450 and 750 mg/kg bw per day).

In a developmental toxicity study in rats using lower doses, zilpaterol HCl was given at a dose of 0, 0.2, 2, 10 or 50 mg/kg bw per day by oral gavage from days 6 to 15 of pregnancy. Hypersalivation was observed at 2, 10 and 50 mg/kg bw per day, with a more marked incidence in the high-dose group. Maternal body weight gains were increased in a dose-dependent manner from 2 mg/kg bw per day. Feed consumption was increased at 2 mg/kg bw per day; at 10 and 50 mg/kg bw per day, feed consumption decreased transiently and then increased. Water consumption was increased at 50 mg/kg bw per day. The incidences of delayed ossification of some bones (5th and 6th sternebrae, skull) and of wavy ribs were higher at 50 mg/kg bw per day. The NOAEL for maternal toxicity was 0.2 mg/kg bw per day, based on hypersalivation and increased body weight gain. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on delayed ossification.

In a developmental toxicity study in rabbits, zilpaterol HCl was given at a dose of 0, 20, 60 or 180 mg/kg bw per day by oral gavage from days 6 to 18 of pregnancy. Maternal body weights as well as final body weights (excluding gravid uterus) were slightly higher at all doses, with statistical significance at 60 mg/kg bw per day. The incidence of skeletal anomalies was increased in all treated groups, reaching statistical significance in the low- and high-dose groups. The LOAEL for maternal and embryo/fetal toxicity was 20 mg/kg bw per day, the lowest dose tested.

In a relay pharmacology study in dogs, one male and one female were fed daily for 5 days with either 200 g liver or 200 g muscle from steers treated

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orally with a zilpaterol HCl dose of 0.2 mg/kg bw per day for 50 days and then slaughtered immediately after the end of the treatment or 7 days later. A positive control group was fed with 200 g muscle from untreated cattle together with zilpaterol HCl (by oral capsule) at 3 µg/kg bw per day. The achieved doses of zilpaterol HCl were 1742–1985 ng/kg bw per day for dogs fed with 200 g liver from steers killed immediately after the 50 days of treatment and 252.5–275.6 ng/kg bw per day for dogs fed with 200 g muscle from steers killed immediately after the 50 days of treatment. The achieved doses of zilpaterol HCl were 11.3 ng/kg bw per day for dogs fed with 200 g liver and 1.5–1.7 ng/kg bw per day for dogs fed with 200 g muscle from steers killed 7 days after the 50 days of treatment. There was no effect of this treatment regimen on either heart rate or blood pressure. In the positive controls treated with 3 µg/kg bw per day, a marginal effect on heart rate, but not on blood pressure, was observed.

Observations in humans

Studies were conducted in which human volunteers, either healthy or asthmatic, were administered zilpaterol HCl. The study protocols were approved by ethics committees, and the studies were carried out according to the guidelines in the Declaration of Helsinki (revised Tokyo version).

In a four-way cross-over design, single-blind and placebo-controlled study involving eight healthy male volunteers, ascending single doses of 0, 0.25, 0.50, 1.00 and 2.00 mg/person (equal to 0, 3.6, 7.1, 14.3 and 28.6 µg/kg bw, respectively) were given orally in aqueous vehicle. The volunteers fasted before dosing. The dose of 0.25 mg/person slightly increased heart rate, and higher doses provoked significant increases in heart rate. Airway calibre was significantly increased at 0.50, 1.00 and 2.00 mg/person, and systolic blood pressure was significantly increased at 1.00 and 2.00 mg/person. Haematology remained unaffected, but blood glucose was increased in a dose-dependent manner at all dose levels. Tremor frequency was significantly increased from 0.50 mg/person (equal to 7.1 µg/kg bw). Based on this study, the LOAEL was 0.25 mg/person (equal to 3.6 µg/kg bw), the lowest dose tested, for cardiovascular effects and higher blood glucose levels.

In a double-blind, comparative placebo-controlled study, 13 healthy male volunteers were given placebo or zilpaterol HCl at a dose of 0.25 mg/person (equal to 3.68 µg/kg bw), 3 times a day for 7 consecutive days (i.e. a daily dose of 0.75 mg/person or about 11.04 µg/kg bw). This dosing regimen had significant effects on the cardiovascular system, tremor and bronchodilatation.

A double-blind, randomized, placebo-controlled cross-over trial was performed on 12 adult asthmatic volunteers given a single oral dose of 0 or 0.25 mg

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zilpaterol HCl per person (equal to 0 or 3.85 µg/kg bw). The volunteers fasted before dosing. Zilpaterol HCl significantly increased bronchodilatation (measured by forced expiration volume in 1 second) up to 4 hours after dosing. Heart rate was slightly but significantly increased up to 1.5 hours post-dosing, with no major palpitation or electrocardiographic changes. Diastolic blood pressure was slightly but significantly decreased at 0.5 and 1 hour after dosing. Short-lasting finger tremors were seen in 2/12 patients after treatment. The LOAEL was 0.25 mg/person (equal to 3.85 µg/kg bw), the only dose tested, for bronchodilatation, tremors and cardiovascular effects.

In a double-blind, randomized, placebo-controlled, four-way cross-over trial involving 11 asthmatic volunteers, three single oral zilpaterol HCl doses of 0, 0.05, 0.10 or 0.25 mg/person (equal to 0, 0.76, 1.52 and 3.79 µg/kg bw, respectively) were tested. The volunteers fasted before dosing. Bronchodilatation was observed at 0.10 and 0.25 mg/person, remaining slight and transient, although only the highest dose induced a slight but significant change in heart rate. Blood pressure remained unaffected at all doses. Mild tremor was seen when evaluated clinically by a physician (two patients at 0.05 and 0.10 mg/person and eight patients at 0.25 mg/person). When the tremor was assessed by drawing a line between two parallel sinusoids, no significant differences were observed at any time between the three doses of zilpaterol HCl and the placebo. The LOAEL was 0.05 mg/person (equal to 0.76 µg/kg bw), based on the clinical assessment of tremor.

In all studies involving human volunteers (16 healthy and 23 asthmatic), the observed effects after an oral dose were slight and transitory and typically related to the β_2-adrenergic agonist activity of zilpaterol HCl. Considering all the human data, the LOAEL for zilpaterol HCl was 0.05 mg/person (equal to 0.76 µg/kg bw). An overall NOAEL could not be identified.

Evaluation

The Committee considered tremors observed in humans, which were consistent with the compound's β_2-adrenergic agonist activity, as the most relevant adverse effect for establishing an ADI for zilpaterol HCl. The LOAEL for tremor was 0.05 mg/person (equal to 0.76 µg/kg bw); the effect was slight at this dose. The Committee established an ADI of 0–0.04 µg/kg bw per day by applying an uncertainty factor of 20, comprising a default uncertainty factor of 10 for human individual variability and an additional uncertainty factor of 2 to account for use of a LOAEL for a slight effect instead of a NOAEL. The Committee noted that the ADI is based on an acute effect. The Committee also noted that the upper bound of the ADI provides a margin of safety of at least 1250 with respect to the NOAEL of 50 µg/kg bw per day for the formation of leiomyomas in rats.

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Residue evaluation[8]

The Committee considered results of pharmacokinetic and metabolism studies conducted in laboratory animals (see above) and food animals together with residue depletion studies and methods of analysis. Some of the studies were performed in accordance with GLP standards.

Pharmacokinetics and metabolism in food-producing animals

Pigs. In a GLP-compliant study, pigs weighing about 120 kg were administered a single oral (gavage) dose of 0.3 mg/kg bw of [^{14}C]zilpaterol HCl. Plasma, urine and faeces were collected at different time points, and animals were killed at 24 hours. Tissues were collected for analysis of total radioactivity by HPLC coupled with a radiometric detector. The maximum plasma concentration was observed at 1 hour after administration. More than 85% of the administered dose was eliminated in urine, and about 3% in faeces. In urine and faeces, unchanged zilpaterol accounted for about 90% of the radioactivity, the remainder being deisopropyl zilpaterol and hydroxy-zilpaterol. In liver, about 80% of the administered radioactivity was extractable, although this was about 90% in other tissues. In all tissues, zilpaterol represented about 90% of the extractable radioactivity, followed by deisopropyl zilpaterol and hydroxy-zilpaterol.

Cattle. A GLP-compliant study was conducted with cattle having a mean weight of 295 kg. Animals were given a single 0.3 mg/kg bw dose by gavage of [^{14}C]zilpaterol HCl and were killed at 12 hours, 48 hours and 8 days, respectively. The radioactivity concentration in plasma samples showed a rapid increase and peaked around 11 hours following drug administration. Depletion of plasma radioactivity occurred with a biphasic profile. The half-life for the first phase was about 12 hours. The second phase corresponded to a very slow decrease of radioactivity, but could not be accurately described. Liver, kidneys, muscle and fat were collected at the kill points for analysis. Total radioactive residues decreased rapidly, but were still detected at 8 days in liver. Unchanged zilpaterol represented more than 60% of the radioactivity in urine, with the remainder comprising four metabolites. In edible tissues, unchanged zilpaterol was the main residue, and one major metabolite was identified by gas chromatography–mass spectrometry analysis as deisopropyl zilpaterol, representing about 20% of the extractable residue in tissues and about 13% of the radioactive residue in urine. Over 90% of the dose was excreted over the 8 days. The material was excreted primarily in the urine (86%) and to a lesser extent (9%) in faeces.

[8] Dr Alan Chicoine of Health Canada and Stefan Scheid of the German Federal Office of Consumer Protection and Food Safety acted as peer reviewers of the residue monograph.

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Comparative metabolism

Metabolism studies conducted in rats, swine and cattle demonstrated that the metabolites produced by the three species following oral administration are qualitatively and quantitatively comparable. Unchanged zilpaterol is the main compound excreted in the urine of both swine and cattle and is the main residue found in cattle tissues (liver, kidney and muscle). The main metabolite excreted is deisopropyl zilpaterol, which could exceed 10% of the total residue. On the basis of the experimental observations, a metabolic pathway was proposed.

Residue data

Cattle. A GLP-compliant study was performed with male and female steers (200–230 kg), assigned to six groups of three animals each. Group I served as control. Animals were treated with [^{14}C]zilpaterol HCl at 0.15 mg/kg bw per day for 12 days (or 15 days for group III) and killed 12 hours (groups II and III), 24 hours (group IV), 48 hours (group V) and 96 hours (group VI) after the last dose. Liver, kidney, fat, muscle and plasma samples were collected at the kill points and analysed for total radioactive residues. Zilpaterol and deisopropyl zilpaterol residues in the tissue samples were analysed by HPLC with radiometric detection and HPLC with fluorescence detection. Analysis of the total [^{14}C]zilpaterol-related residues showed that 40–58% of the residues in liver at 12 and 24 hours and 24–31% at 48 and 96 hours were extractable. In kidney, 85–93% of the residues were extractable at 12 and 24 hours and 38–74% at 48 and 96 hours, respectively. All of the residues (100%) in muscle were extractable at the 12- and 24-hour withdrawal periods. Low levels of total radioactive residues of zilpaterol were detected in fat at 12 hours. HPLC analysis with radiometric detection of the liver and kidney tissue extracts showed that the extractable radioactivity was mainly associated with unchanged zilpaterol and deisopropyl zilpaterol. The proportion of unchanged zilpaterol in the extractable residues was approximately 4–8 times higher than the proportion of deisopropyl zilpaterol. Depletion of total radioactive residues in liver followed a biphasic profile, whereas depletion of total radioactive residues in muscle and kidney was monophasic. The marker residue to total radioactive residue ratio (MR:TR) decreased as a function of time for liver and kidney, but was constant in muscle.

A GLP-compliant radiolabel study was conducted in rats to determine the oral bioavailability of non-extractable residue in liver obtained from cattle. It showed that the non-extractable residues from livers of cattle at all points were weakly absorbed, with a mean maximum of 3.3% of the dose being absorbed and therefore bioavailable.

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Residue depletion study (unlabelled drug) in cattle

Three GLP-compliant pivotal residue depletion studies were conducted in cattle with the commercial product over a period of 96 hours. The first study was conducted to measure the concentration of residues of zilpaterol in liver, muscle and kidney tissues of cattle. Animals weighing about 500 kg were fed the commercial premix medicated feed of zilpaterol HCl at a dose of 0.15 mg/kg bw daily for 12 consecutive days. Samples of liver, kidney and muscle were collected at 0.5, 1, 2 or 4 days after the last dose and assayed using a validated HPLC/fluorescence method with LOQs of 3, 1 and 1 µg/kg for liver, kidney and muscle, respectively. The mean concentrations of zilpaterol measured at 0.5, 1 and 2 days post-dosing were 28.3 ± 9.1 µg/kg, 11.4 ± 4.0 µg/kg and 4.5 ± 4.0 µg/kg in liver; 4.96 ± 1.9 µg/kg, 2.06 ± 0.47 µg/kg and < LOQ in muscle; and 50.8 ± 33.1 µg/kg, 1.29 ± 1.54 µg/kg and 5.67 ± 5.2 µg/kg in kidney.

In the second GLP-compliant study conducted with steers and heifers, cattle were administered the commercial product via component feeding at a dose of 0.15 mg/kg bw daily for 20 consecutive days. Samples (muscle and liver) were collected from animals killed 0.5, 1, 2, 3, 4, 5, 6 and 10 days after the last feeding and assayed using a validated HPLC/fluorescence method with LOQs of 1.5 µg/kg in muscle and 2.5 µg/kg in liver. The mean concentrations of zilpaterol measured in the liver 0.5, 1, 2 and 3 days after the last dose were 13.9 ± 7.3 µg/kg, 5.7 ± 2.4 µg/kg, 3.8 ± 1.0 µg/kg and 2.3 ± 0.4 µg/kg, respectively. Other than a concentration of 3.8 ± 0.5 µg/kg measured in muscle at 0.5 day, there were no measurable residues in any muscle samples collected beyond 0.5 day.

The third GLP-compliant study was conducted with steers and heifers, in which the cattle were administered the commercial product in feed at the recommended dosage regimen of zilpaterol HCl of 0.15 mg/kg bw per day for 20 consecutive days. Samples (muscle and liver) were assayed using the validated HPLC/fluorescence method used in the preceding depletion study. At 0.5 day post-dosing, the concentrations of zilpaterol in the liver and muscle were 12.9 ± 5.3 µg/kg and 3.0 ± 0.7 µg/kg, respectively. There were no measurable concentrations in muscle or liver samples collected 1 day post-dosing and beyond.

Analytical methods

Zilpaterol was measured in the depletion studies using a validated HPLC method with fluorescence detection with LOQs of 1.0–1.5 µg/kg, 1.0 µg/kg and 2.5–3.0 µg/kg for muscle, kidney and liver, respectively. An LC-MS/MS method also developed by the sponsor demonstrated comparable levels of performance. A published method in which zilpaterol residues in tissue

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and urine are extracted after hydrolysis with glucuronidase/arylsulfatase and protease, cleaned up on solid-phase extraction and analysed by LC-MS/MS was recently developed, validated and used to quantify incurred zilpaterol concentrations in muscle, kidney, retina and liver samples collected from cattle administered zilpaterol HCl at 0.15 mg/kg bw once daily for 14 days and killed 1 and 10 days post-dosing. The concentrations of zilpaterol measured in muscle, kidney and liver samples collected were 0.01 µg/kg, 0.03 µg/kg and 0.03 µg/kg at 10 days, respectively. This LC-MS/MS method demonstrated detection sensitivities 10–100 times better than those of the HPLC/fluorescence method used in the pivotal studies.

Maximum residue limits

In considering the recommendation of MRLs, the Committee considered the following factors:

- An ADI of 0–0.04 µg/kg bw for zilpaterol was established by the Committee, corresponding to an upper bound of acceptable intake of 2.4 µg/day for a 60 kg person.
- Zilpaterol HCl is registered to be mixed into feed at a level of 7.5 mg/kg on a 90% dry matter basis. This level is designed to treat animals with approximately 0.15 mg/kg bw or 60–90 mg zilpaterol HCl per animal per day.
- Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.
- Zilpaterol HCl is not permitted for use in lactating dairy cattle.
- Zilpaterol has two major metabolites: deisopropyl zilpaterol (tissues and urine) and hydroxy-zilpaterol (faeces).
- The Committee agreed that parent zilpaterol was an appropriate marker residue in muscle. Only limited data were available for tissues other than muscle, and the Committee was unable to determine a suitable marker residue in other edible tissues. Liver and kidney contained the highest concentration of zilpaterol at all sampling times, followed by muscle. The data provided are not sufficient to determine the total residue half-life in the liver after 96 hours. There are no measurable residues in adipose fat.
- The ratios of the concentration of zilpaterol to the concentration of the total residues for liver and for kidney over the 96-hour withdrawal period after the last drug administration could not be determined with any confidence due to the very limited data available and lack of sensitivity of the methods used.

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- The ratio of zilpaterol to total radioactive residues in muscle is approximately 50%.
- The analytical methods used in the depletion studies do not allow the characterization of the pharmacokinetics at times when, even at the LOQ, the concentrations are not compatible with dietary exposures below the ADI, particularly in liver.

A marker residue could not be established in any edible tissue other than muscle, and the Committee concluded that an appropriate marker residue for other tissues should be identified.

In the absence of an appropriate marker residue for liver and kidney, a marker residue to total residue ratio could not be established for these tissues.

The Committee used the highest concentrations of total residues to estimate dietary exposure, because no median residue levels could be determined and no marker residue in liver and kidney was defined. These highest concentrations of extractable radioactivity, expressed as zilpaterol equivalent, were 1.0, 28.6 and 5.4 µg/kg at 96 hours for muscle, liver and kidney, respectively. These calculations indicated that the dietary exposure was higher than the ADI for the withdrawal times for which data were provided. It was also noted that the ADI is based on an acute end-point and is applicable to both acute and chronic exposure.

The Committee concluded that it was not possible to recommend MRLs for zilpaterol. The following data are needed to establish MRLs:

- results from studies investigating marker residue in liver and kidney;
- results from studies determining marker residue to total residue ratios in liver and kidney;
- results from depletion studies to enable the derivation of MRLs compatible with the ADI.

All such studies should use sufficiently sensitive validated analytical methods capable of measuring zilpaterol and its major metabolites in edible tissues of cattle.

A residue monograph was prepared.

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Summary and conclusions

Studies relevant to risk assessment

Species / study type (route)	Doses (mg/kg bw per day[a])	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day[a])
Mouse				
Eighteen-month study of toxicity and carcinogenicity (gavage)	0, 0.01, 0.02, 0.05, 0.25	Increased haemoglobin, red blood cell counts, haematocrit; decreased numbers of platelets, absolute neutrophil counts and monocyte numbers	0.02	0.05
Rat				
Thirteen-week study of toxicity (gavage)	0, 0.05, 0.5, 1	Decreased mean heart rate	–	0.05[b]
Two-year study of toxicity and carcinogenicity (dietary)	0, 0.025, 0.05, 0.125, 0.25	Increased weight of ovaries with increased incidence of ovarian cysts	0.05	0.125
		Increased incidence of ovarian leiomyomas	0.05	0.125
Two-generation reproductive toxicity study (dietary)	0, 0.06, 0.23, 0.94 (F_0 males); 0, 0.10, 0.40, 1.61 (F_0 females); 0, 0.08, 0.32, 1.26 (F_1 males); 0, 0.12, 0.45, 1.77 (F_1 females)	Parental toxicity: Increased body weight and feed consumption	–	0.06[b]
		Reproductive and offspring toxicity: No effects	0.94[c]	–
Developmental toxicity study (gavage)	0, 0.2, 2, 10, 50	Maternal toxicity: Increased occurrence of hypersalivation, increased body weight gain	0.2	2
		Embryo and fetal toxicity: Delayed ossification	10	50
Rabbit				
Developmental toxicity study (gavage)	0, 20, 60, 180	Maternal toxicity: Increased body weight	–	20[b]
		Embryo and fetal toxicity: Increased incidence of skeletal anomalies	–	20[b]
Dog				
Thirty-day study of toxicity (capsule)	0, 0.5, 5, 50	Increased peripheral vasodilatation, increased heart rate, decreased blood pressure	–	0.5[b]
Microswine				
Thirteen-week study of toxicity (gavage)	0, 0.001, 0.05, 1, 10	No effects	10[c]	–

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Species / study type (route)	Doses (mg/kg bw per day[a])	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day[a])
Cynomolgus monkey				
Four-week study of toxicity (gavage)	0, 0.005, 0.01, 0.05, 5	Increased heart rate with a decreased QT interval	0.01	0.05
Human				
Four-way cross-over design, single-blind and placebo-controlled study (healthy adult)	0, 3.6, 7.1, 14.3, 28.6 µg/kg bw	Increased heart rate and blood glucose level	–	3.6 µg/kg bw[b]
Double-blind, randomized, placebo-controlled four-way cross-over study (asthmatic patient)	0, 0.76, 1.52, 3.79 µg/kg bw	**Increased incidence of tremor**	–	**0.76 µg/kg bw[b]***

*** Pivotal study value *(16)***
[a] Except where otherwise noted.
[b] Lowest dose tested.
[c] Highest dose tested.

Uncertainty factor

20 (10 for intraspecies variability, 2 for use of a LOAEL instead of a NOAEL)

ADI

0–0.04 µg/kg bw

Residue definition

Zilpaterol (in muscle)

MRLs

The Committee agreed that it was not possible to recommend MRLs for zilpaterol.

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4. Future work and recommendations

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

Decision-tree approach for the safety evaluation of residues of veterinary drugs in foods

- The Committee recommended that an electronic working group should be established to develop guidance on what would comprise a preliminary risk assessment, as envisaged in the decision-tree for the safety evaluation of residues of veterinary drugs in foods, taking into account the risk analysis principles applied by CCRVDF.
- The Committee recommended that an electronic working group should be established to perform a feasibility exercise on the application of the TTC approach to residues of veterinary drugs and, if appropriate, to make specific recommendations for developing such an application.
- The Committee recommended that an electronic working group should be established to develop guidance for establishing ARfDs for residues of veterinary drugs, addressing situations in which it would be necessary to establish an ARfD and how this would be done. Consideration should also be given to compounds for which the ADI is based on an acute effect (e.g. pharmacological effects, antimicrobial effects). The working groups should include an expert from JMPR who is experienced in the establishment of ARfDs.

Dietary exposure for veterinary drug residues

- The Committee recommended that the JECFA Secretariat communicate to sponsors that realistic and reliable median and 95th percentile residue data in all foods that are contributors to exposure to a veterinary drug residue form an essential part of the data package needed by the Committee to establish MRLs.

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- The Committee recommended that a working group be set up to further investigate a number of issues to improve the dietary exposure assessment methodology for residues of veterinary drugs. Issues include, for example, the estimation of total dietary exposure when a veterinary drug residue is also found in plant-based agricultural commodities and the need to estimate the proportion of farmed fish in the food supply.
- The Committee recommended that the new approach should continue to be used in parallel with the model diet approach at future meetings of the Committee until more experience has been obtained in the interpretation of the results with the new approach.

Review of the need to update *Principles and methods for the risk assessment of chemicals in food* (EHC 240)

- The Committee agreed that a review of EHC 240 should be a standing item on its agenda from its next meeting onwards and that any sections or chapters requiring updating would be identified. In such cases, the Committee would make specific recommendations on how this might be achieved.

Extrapolation of MRLs to minor species

- For the current and future meetings, JECFA will use the term *extension* when sufficient depletion data are available for the minor species to permit the derivation of MRLs for tissues of that species from the depletion curves. The term *extrapolation* will be used when insufficient depletion data are available in that species to derive MRLs for tissues from that species.
- The Committee noted the recent publications in the peer-reviewed literature on the use of predictive models for residue distribution in tissues based on pharmacokinetics and considered that developments in this area should continue to be monitored and discussed at future meetings of the Committee, as this has the potential to be a useful approach when considering extrapolation of MRLs.

MRLs for veterinary drug residues in honey

- A dietary portion size of 50 g/person per day will be used in dietary exposure estimates (EDI or TMDI) performed during risk assessments by JECFA, replacing the 20 g of honey per person per day used in exposure calculations conducted prior to the current meeting of JECFA.

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Scope of MRLs established by JECFA relating to fish and fish species

- The Committee recommended that the term "fish" should be used when an MRL recommendation applies to multiple species of finfish. For other "seafood", the term "mollusc" should be used for species such as clams, oysters and scallops, and the term "crustacean" should be used when MRLs are recommended for species such as shrimp, prawn and crayfish.
- The Committee considered that it may be appropriate to also identify some representative species of fish, such as salmon, and of seafood, such as shrimp (crustacean), as "major species" of fish and seafood. It is recommended that this matter should be further discussed at a future meeting of the Committee.

JECFA analytical method validation requirements

- In view of developments in method validation criteria that have occurred since the adoption of the current JECFA method validation requirements in 1999, the criteria for validation of methods used in the pharmacokinetic, metabolism and depletion studies submitted to the Committee should be reviewed and updated at a future meeting of the Committee.

Guidance for the evaluation of veterinary drug residues in food by JECFA

- The Committee reiterated the decision made at the seventy-fifth meeting to update the guidance for both FAO and WHO experts for the preparation of working documents. The Committee requests the JECFA Secretariat to undertake this work in collaboration with WHO and FAO experts.

Emamectin benzoate

- The Committee recommended that JMPR re-evaluate emamectin benzoate at a future meeting in view of JECFA's considerations with respect to the ARfD for emamectin benzoate.

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Acknowledgements

The Committee wishes to thank Ms M. Sheffer, Ottawa, Canada, for her assistance in the preparation of the report. The participation of Dr Alan Chicoine of Health Canada and Stefan Scheid of the German Federal Office of Consumer Protection and Food Safety in the electronic working group on extrapolation of MRLs to minor species and in reviewing the residue monograph for zilpaterol hydrochloride and the expert review of the sponsor's proposed BMD modelling approach for derquantel by Klaus Schneider and Eva Kaiser, Forschungs- und Beratungsinstitut Gefahrstoffe GmbH, Freiburg, Germany, are gratefully acknowledged.

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

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Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

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Annex 2
Recommendations on compounds on the agenda and further information required

Derquantel (anthelminthic agent)

Acceptable daily intake: The Committee maintained the ADI of 0–0.3 µg/kg body weight established at its seventy-fifth meeting (WHO TRS No. 969, 2011).

Estimated dietary exposure: There were insufficient data to calculate an estimated daily intake (EDI), and the theoretical maximum daily intake (TMDI) approach was used. Using the model diet and the marker residue to total residue ratio approach with the maximum residue limits (MRLs) recommended, the estimated dietary exposure is 6.8 µg/person, which represents approximately 38% of the upper bound of the ADI.

Residue definition: Derquantel

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Sheep	7.0	0.4	0.8	0.3

Emamectin benzoate (antiparasitic agent)

Acceptable daily intake: The Committee confirmed the ADI of 0–0.0005 mg/kg body weight established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in

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2011, based on an overall no-observed-adverse-effect level (NOAEL) of 0.25 mg/kg body weight per day for neurotoxicity from 14- and 53-week studies in dogs, supported by an overall NOAEL of 0.25 mg/kg body weight per day from 1- and 2-year studies in rats. An uncertainty factor of 500 was applied to the NOAEL, which includes an additional uncertainty factor of 5 to account for the steep dose–response curve and irreversible histopathological effects in neural tissues at the lowest-observed-adverse-effect level (LOAEL) in dogs, as used by JMPR and confirmed by the current Committee.

Estimated dietary exposure: The EDI is 11 µg/person per day, which represents approximately 37% of the upper bound of the ADI.

Residue definition: Emamectin B1a

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Fillet[a] (µg/kg)
Salmon	100	100
Trout	100	100

[a] Muscle plus skin in natural proportion.

The Committee extended the MRLs for muscle and fillet in salmon to trout.

Gentian violet (antibacterial, antifungal and anthelminthic agent)

Acceptable daily intake: The Committee concluded that it is inappropriate to set an ADI for gentian violet because it is genotoxic and carcinogenic.

Maximum residue limits: MRLs could not be recommended by the Committee, as it was not considered appropriate to establish an ADI. The Committee also noted that there was limited information on residues.

Ivermectin (antiparasitic agent)

Acceptable daily intake: The Committee established an ADI of 0–1 µg/kg body weight at its fortieth meeting (WHO TRS No. 832, 1993).

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Estimated dietary exposure: The fortieth meeting of the Committee (WHO TRS No. 832, 1993) included an estimate of the potential intake from muscle. No further assessment of dietary exposure was undertaken at the current meeting.

Residue definition: Ivermectin B1a

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)
Cattle	4

Lasalocid sodium (antiparasitic agent)

Acceptable daily intake: The Committee established an ADI of 0–5 µg/kg body weight on the basis of a NOAEL of 0.5 mg/kg body weight per day from a developmental toxicity study in rabbits and a multigeneration reproductive toxicity study in rats, with application of an uncertainty factor of 100 for interspecies and intraspecies variability.

Estimated dietary exposure: An EDI of 80 µg/person per day was calculated, which represents approximately 17% of the upper bound of the ADI.

Residue definition: Lasalocid A

Recommended maximum residue limits (MRLs)

Species	Skin + fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Chicken	600	600	1 200	400
Turkey	600	600	1 200	400
Quail	600	600	1 200	400
Pheasant	600	600	1 200	400

The Committee extended the MRLs in chicken to turkey and quail and extrapolated the MRLs in chicken to pheasant. No information was available for duck, including on approved uses. As the compound is not registered for use in laying hens, according to the sponsor, it is not appropriate to recommend MRLs for eggs.

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Monepantel (anthelminthic agent)

Acceptable daily intake: An ADI of 0–20 µg/kg body weight was established by the Committee at its seventy-fifth meeting (WHO TRS No. 969, 2012).

Estimated dietary exposure: Using the model diet and marker residue to total residue ratios of 1.00 for muscle and 0.66 for fat, liver and kidney, and applying a correction factor of 0.94 to account for the mass difference between monepantel sulfone (the marker residue) and monepantel, the EDI is 446 µg/person per day, which represents approximately 37% of the upper bound of the ADI.

Residue definition: Monepantel sulfone, expressed as moneantel

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Sheep	13 000	1 700	7 000	500

These MRLs are consistent with the shortest withdrawal time assigned in Member States with an approved use of monepantel.

Recombinant bovine somatotropins (production aid)

Acceptable daily intake: Based on a systematic review of the literature published since the last evaluation, the Committee reaffirmed its previous decision on ADIs “not specified” for somagrebove, sometribove, somavubove and somidobove, established at the fortieth meeting (WHO TRS No. 832, 1993).

Maximum residue limits: The Committee reaffirmed its previous decision on MRLs “not specified” for somagrebove, sometribove, somavubove and somidobove, established at the fortieth meeting (WHO TRS No. 832, 1993).

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Zilpaterol hydrochloride (adrenoceptor agonist, growth promoter)

Acceptable daily intake: The Committee established an ADI of 0–0.04 µg/kg body weight on the basis of a LOAEL of 0.76 µg/kg body weight for tremor in humans. An uncertainty factor of 20 was applied, comprising a default uncertainty factor of 10 for human individual variability and an additional uncertainty factor of 2 to account for the use of a LOAEL for a slight effect instead of a NOAEL. The Committee noted that the ADI is based on an acute effect. The Committee also noted that the upper bound of the ADI provides a margin of safety of at least 1250 with respect to the NOAEL of 50 µg/kg body weight per day for the formation of leiomyomas in rats.

Residue definition: Zilpaterol (in muscle). The Committee was unable to determine a suitable marker residue in other edible tissues.

Maximum residue limits: The Committee concluded that it was not possible to recommend MRLs for zilpaterol.

The following data are needed to establish MRLs:

- results from studies investigating marker residue in liver and kidney;
- results from studies determining marker residue to total residue ratio in liver and kidney;
- results from depletion studies to enable the derivation of MRLs compatible with the ADI.

All such studies should use sufficiently sensitive validated analytical methods capable of measuring zilpaterol and its major metabolites in edible tissues of cattle.

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SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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 (75 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Seventy-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 68, 2013 (335 pages)

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 (190 pages)

Safety Evaluation of Certain Food Additives
Seventy-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 67, 2012 (335 pages)

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 (108 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Seventy-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 66, 2012 (183 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Seventy-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 65, 2012 (825 pages)

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Evaluation of certain veterinary drug residues in food

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of residues of certain veterinary drugs in food and to recommend maximum levels for such residues in food.

The first part of the report considers general principles regarding the evaluation of residues of veterinary drugs within the terms of reference of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), including extrapolation of maximum residue limits (MRLs) to minor species, MRLs for veterinary drug residues in honey, MRLs relating to fish and fish species, dietary exposure assessment methodologies, the decision-tree approach to the evaluation of residues of veterinary drugs and guidance for JECFA experts.

Summaries follow of the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: two anthelminthic agents (derquantel, monepantel), three antiparasitic agents (emamectin benzoate, ivermectin, lasalocid sodium), one antibacterial, antifungal and anthelminthic agent (gentian violet), a production aid (recombinant bovine somatotropins) and an adrenoceptor agonist and growth promoter (zilpaterol hydrochloride). Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes (ADIs) and proposed MRLs.

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