

Evaluation of certain veterinary drug residues in food

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



Food and Agriculture
Organization of
the United Nations



World Health
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Seventy-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Rome, 8–17 November 2011

Members

Professor A. Anadón, Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain

Dr D. Arnold, Consultant, Berlin, Germany (*Vice-Chairman*)

Professor A.R. Boobis, Centre for Pharmacology & Therapeutics, Department of Experimental Medicine, Division of Medicine, Faculty of Medicine, Imperial College London, London, England (*Chairman*)

Dr R. Ellis, Consultant, Myrtle Beach, SC, United States of America (USA) (*Joint Rapporteur*)

Dr A. Fernández Suárez, Ciencias Veterinarias, Universidad del Salvador, Buenos Aires, Argentina

Dr L.G. Friedlander, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA

Dr K.J. Greenlees, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA (*Joint Rapporteur*)

Professor J. Palermo-Neto, Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

Dr L. Ritter, Canadian Network of Toxicology Centres, Professor Emeritus, School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

Dr P. Sanders, National Reference Laboratory for Veterinary Drug Residues and Antimicrobial Resistance, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Fougères, France

Professor G.E. Swan, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa¹

Secretariat

Dr J. Boison, Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada (*FAO Expert*)

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

¹ Dr Swan was invited but unable to attend the meeting.

- Dr C.E. Cerniglia, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, AR, USA (*WHO Temporary Adviser*)
- Dr P.L. Chamberlain, Office of the Chief/Office of the Chief Scientist/Office of Counterterrorism and Emerging Threats, Food and Drug Administration, Department of Health and Human Services, Silver Spring, MD, USA (*WHO Temporary Adviser*)
- Dr S. Ghimire, Veterinary Drugs Directorate, Health Canada, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)
- Dr N. Jarrett, European Medicines Agency, London, England (*WHO Temporary Adviser*)
- Professor S.H. Jeong, Department of Applied Biotoxicology, Hoseo University, Hoseo Toxicology Research Centre, Asan City, Chungnam, Republic of Korea (*WHO Temporary Adviser*)
- Professor B. Le Bizec, Laboratoire d'Étude des Résidus et des contaminants dans les aliments (LABERCA), École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique (ONIRIS), Nantes, France (*FAO Expert*)
- Dr K. Ogawa, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan (*WHO Temporary Adviser*)
- Dr F. Ramos, Bromatology, Pharmacognosy and Analytical Sciences Group, Pharmacy Faculty, Coimbra University, Coimbra, Portugal (*FAO Expert*)
- Dr G. Roberts, Consultant, Preston, Victoria, Australia (*WHO Temporary Adviser*)
- Ms M. Sheffer, Orleans, Ontario, Canada (*FAO/WHO Editor*)
- Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)
- Dr P. Verger, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary to JMPPR*)
- Dr A. Wennberg, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

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. 66, 2012, in press.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 12, 2012, in press.

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Most of the evaluations and summaries contained in this publication are based on unpublished proprietary data submitted to JECFA for use when making its assessment. A registration authority should not consider granting a registration based on an evaluation published herein unless it has first received authorization for such use from the owner of the data or any second party that has received permission from the owner for using the data.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 8 to 17 November 2011. The meeting was opened by Mr Berhe Tekola, Director of the Animal Production and Health Division of the Agriculture and Consumer Protection Department of the Food and Agriculture Organization of the United Nations (FAO), on behalf of the Directors-General of the World Health Organization (WHO) and FAO. Mr Tekola provided information to the Committee on the reform process in FAO, in particular in relation to the introduction of a corporate results-based approach to programme planning and measuring achievements to better meet the demands of countries for improved efficiency. The current medium-term plan of programmes and resources has been aligned with defined strategic objectives and outcomes. The provision of scientific advice on food safety is part of the strategic objective named “Improved quality and safety of food at all stages of the food chain”. When it comes to food security and food safety, this strategic approach provides for new opportunities for cooperation between units in FAO and with WHO and other United Nations agencies involved in the farm-to-table food production to consumption continuum, as well as in food safety control and standard setting.

Mr Tekola emphasized that consumers around the world expect the food supply to be safe and that the two organizations recognize the important contribution by the experts of the Committee in providing scientific advice on human health risks of residues of veterinary drugs used in food production animals. He expressed his sincere appreciation to the experts for taking time from their very busy schedules to prepare for and participate in this meeting. He also noted the need by countries and the Codex Alimentarius Commission to have access to objective advice on food safety matters and that this remains a high priority for the organizations.

Eighteen 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 and 193) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984

(I). The present meeting¹ 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 (see [section 2](#));
- To evaluate the safety of residues of certain veterinary drugs (see [section 3](#) and [Annex 2](#)); and
- To discuss proposals for methodologies for estimation of acute and chronic dietary exposure to residues of veterinary drugs (see [section 2.5](#)).

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the seventy-fifth meeting had completed declaration of interest forms. The following declared interests were discussed. Dr Pamela Chamberlain was employed by a laboratory conducting two genotoxicity studies on derquantel; however, she had no involvement in these studies. Dr Richard Ellis prepared the data submission on amoxicillin using the United States Food and Drug Administration (USFDA) data with permission of the pharmaceutical company. Consequently, the Committee decided that Dr Ellis should abstain from participation in the discussion on this compound. Dr Kevin Greenlees, while overseeing this data submission, was not involved in the actual data compilation, and hence this was not considered a conflict. Dr Gladwin Roberts was, in the past, a consultant for the pharmaceutical producer on toxicological aspects of derquantel. As this could be interpreted as a potential conflict, Dr Roberts abstained from discussions on this compound.

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-four previous meetings of JECFA ([Annex 1](#)).

2. General considerations

2.1 Report from the Eighteenth and Nineteenth Sessions of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)

The Codex Secretariat informed the Committee about the principal outcomes and discussions of the Eighteenth and Nineteenth Sessions of CCRVDF, which had been held since the seventieth meeting of the Committee.

CCRVDF finalized work on the majority of the MRLs recommended by the seventieth meeting of the Committee, which were subsequently adopted by the Codex Alimentarius Commission at its Thirty-second and Thirty-fourth Sessions. The MRLs for narasin in cattle tissues will be considered by the Twentieth Session of CCRVDF (May 2012) in light of the outcomes of the current meeting of the Committee. The Thirty-fourth Session of the Codex Alimentarius Commission also adopted the MRLs for melengestrol acetate and held the MRLs for ractopamine for further discussion at its Thirty-fifth Session in July 2012. CCRVDF also finalized the *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), which were adopted by the Thirty-second Session of the Codex Alimentarius Commission (2009).

With regard to methods of analysis, CCRVDF agreed to start new work on performance criteria for multi-residue analytical methods and further recommended that, in future evaluations, the Committee take into account the guidelines on single-laboratory validation (CAC/GL 71-2009).

Following the recommendations of the seventieth meeting of the Committee, CCRVDF collected information regarding honey and noted that a limited number of active ingredients were authorized for honey bee treatment (tymol being the most common) and that data collected on honey consumption seemed to support JECFA's proposed revised figure (50 g/day). CCRVDF agreed to develop a risk assessment policy for the establishment of MRLs in honey, based on data and criteria that have been used by national competent authorities.

CCRVDF agreed that the estimated daily intake (EDI) approach was an improvement over the current methodology for assessing the risk from chronic exposure, provided that adequate data were made available. CCRVDF supported the general approach and further work of JECFA on the development of the “decision-tree approach”.

CCRVDF agreed that communication between JECFA and CCRVDF needs to be improved and recommended that draft agendas of meetings of the Committee be published by the Secretariat in advance of the meetings to allow for input on matters for general consideration by the Committee. The Secretariat agreed to this for those general topics for consideration that are known before the meeting.

CCRVDF agreed to start work on the revision and updating of its risk analysis principles (see [section 2.2](#) below) with a special focus on revision of section 3.2 (“Evaluation of risk management options”) and the development of risk management and risk communication recommendations for veterinary drugs with no ADI and/or MRLs.

CCRVDF agreed on a priority list of veterinary drugs for evaluation (re-evaluation) by JECFA, the majority of which were considered by the current JECFA meeting.

The Codex Secretariat also informed the Committee of the adoption of the *Guidelines for risk analysis of foodborne antimicrobial resistance* (CAC/GL 77-2011) and the establishment of a new ad hoc Codex Task Force on Animal Feeding to develop guidance on application of risk assessment to feed and a prioritized list of hazards in feed.

2.2 **Comments on documents under elaboration for CCRVDF**

The Committee reviewed the draft report of the *Electronic working group on the revision of the risk analysis principles applied by the CCRVDF and risk assessment policy for the setting of MRLVDs* (no date), especially as it relates to the work of JECFA. The Committee commented on some of the proposed revisions, for the JECFA Secretariat to bring into the discussion at the upcoming Twentieth Session of CCRVDF (May 2012).

Regarding the draft *Report of the electronic working group to develop risk management options for veterinary drugs for which no ADI and/or MRL has been recommended by JECFA due to specific human health concerns* (8 July 2011), the Committee emphasized that any requests for further assessments on such compounds to JECFA needed to be accompanied by a clear description of the specific request from CCRVDF and formulation of the risk management needs.

Regarding the draft report of the *CCRVDF electronic working group on extrapolation of MRLs for veterinary drugs to additional species and tissues* (17 October 2011), the Committee provided comments on proposed risk analysis policy aspects, for the JECFA Secretariat to bring into the discussion at the upcoming Twentieth Session of CCRVDF.

2.3 Information on registration/approval status of veterinary drugs

Nationally approved good practices in the use of veterinary drugs make an important contribution to the risk profile of a drug. For JECFA, it is important that all related information relevant for the risk assessment is available to the Committee when it evaluates substances with a view to recommending MRLs. In the past, information on registration/approval status of veterinary drugs and on approved conditions of use was not always available to the Committee in time, leading to unnecessary difficulties in its discussions. The Committee therefore requests:

- that CCRVDF provide the Secretariat with information on registration/approval status and the use pattern of veterinary drugs whenever it requests an evaluation by JECFA;
- that the JECFA Secretariat always include a request for submission of such information by the sponsors of the data into future calls for data. The Secretariat should also verify that such information is contained in the data submission of sponsors before it gives work assignments to the experts of the Committee.

2.4 Extrapolation of MRLs

The Committee recognized the importance of using good science when extrapolating between food animal species to support the development of MRLs in additional food animal species and commodities. In addition, the Committee recognized the ongoing CCRVDF electronic working group that is collecting and evaluating information and developing recommendations on a risk analysis policy for use by CCRVDF when extrapolating MRLs.

The Committee agrees that it is important to develop minimum criteria for information upon which to base extrapolation between food animal species and commodities. In view of the foregoing, the Committee recommended that the Secretariat establish an electronic working group to continue work commenced at the current meeting and to develop proposed minimum criteria for consideration at the next JECFA meeting for veterinary drugs.

2.5 Dietary exposure assessment methodologies

Explanation

At its seventieth meeting, following discussion of the decision-tree approach¹ as well as consideration of the output of the workshop on MRLs in pesticides and veterinary drugs (2), the Committee identified that further work was required on approaches for exposure assessments for veterinary drug residues, in particular for chronic and acute exposures ([Annex 1](#), reference 193).

At its Eighteenth (3) and Nineteenth Sessions (4), CCRVDF responded with a request to FAO and WHO to convene an expert consultation on exposure assessment methodologies for residues of veterinary drugs in foods. CCRVDF requested that FAO and WHO address the following: review of the current model diet (so-called market basket approach) applied by JECFA; possible simplification of the current model diet; possible development of several model diets to reflect regional differences in consumption patterns; and development of approaches for acute and subchronic dietary exposure assessment.

To help address this need for updated methodology, FAO and WHO issued a call for data on consumption of foods of animal origin in 2010. In response, food consumption data from 47 countries were submitted, and a submission from an interested party was also received. To provide an opportunity for stakeholders and interested parties to present their views, FAO and WHO held an open stakeholder meeting in Rome on 7 November 2011. The stakeholder meeting was attended by members of an expert meeting convened to review and update the principles and methodology to assess dietary exposure to residues of veterinary drugs in food, held from 7 to 11 November 2011, as well as participants at the seventy-fifth meeting of JECFA.

The experts on exposure assessment prepared a draft report outlining their proposed new approaches for acute and chronic² dietary exposure assessment for veterinary drug residues, taking the key findings, concerns and recommendations of the stakeholders into consideration. Discussions and exchanges were organized between participants at both the meeting on dietary exposure assessment methodologies and the seventy-fifth JECFA.

Examples to compare the current model with proposed models were collaboratively developed.

¹ A concept paper entitled “A hypothesis-driven decision tree approach for the safety evaluation of residues of veterinary drugs” was developed by a small working group and discussed at the seventieth meeting.

² Includes subchronic for the purposes of this exercise.

JECFA considerations for acute dietary exposure assessments

Acute dietary exposure estimates should cover a time period of food consumption over a single meal or 1 day and are intended to be used for comparison with acute reference dose (ARfD) values in a risk assessment process (5). The present Committee emphasized that, depending on the health end-points for acute risk, acute exposure should be estimated for both the general population and children.

JECFA considerations for chronic dietary exposure assessments

Chronic dietary exposure estimates cover food consumption over the long term and are intended to be used for comparison with a health-based guidance value based on chronic toxicity, such as an ADI, in a risk assessment process (5). At its seventieth meeting, the Committee confirmed the use of the median residue level from depletion studies, with a correction for marker residue to total residue, instead of the MRL for long-term dietary exposure estimates, when supported by the available data.

Main outputs of the expert meeting on dietary exposure assessment methodologies

Models were proposed to estimate both acute and chronic exposure to residues of veterinary drugs in food. The Committee noted that, compared with the current model, the proposed models use more detailed consumption data. The exploration of new approaches to the assessment of dietary exposure to veterinary drug residues is part of the ongoing process of ensuring that evaluations undertaken by the Committee incorporate available data as well as recent advances in methodology and scientific knowledge. When finalized, the proposed models should be considered as tools for potential use in the assessment of dietary exposure to residues of veterinary drugs.

The report of the expert meeting will include the proposed new models for assessing acute and chronic dietary exposure to residues of veterinary drugs, the data on food consumption received and evaluated for use in the models and a summary of the input and views expressed at the stakeholder meeting.

Further steps

Comments on the draft report of the expert meeting will be solicited from participants of the seventy-fifth meeting of the Committee soon after the meeting. Following consideration of these comments, a revised draft report will be prepared for public comments. The final report will be presented to CCRVDF at its Twentieth Session (May 2012) for discussion and comments. The dietary exposure models will then be discussed at a future meeting of

the Committee, and other worked examples will be performed based on the proposed models to gain more experience.

2.6 **Decision-tree approach to the evaluation of residues of veterinary drugs**

The Committee gave further consideration to the proposal for a hypothesis-driven decision-tree approach for the safety evaluation of residues of veterinary drugs discussed at its seventieth meeting ([Annex 1](#), reference 193), following up on the recommendations from the discussion at that meeting. The JECFA Secretariat informed the Committee that the expert meeting on dietary exposure assessment methodologies conducted in parallel to this meeting was convened to address the recommendation to develop methods for acute and chronic exposure assessment. The other recommendations remain to be addressed.

In discussing the recommendations of the seventieth meeting, the Committee recommended that the JECFA Secretariat establish an electronic working group to elaborate principles to establish 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 the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). A draft guidance document will be prepared for discussion at the next meeting.

In discussing the application of a threshold of toxicological concern (TTC) approach to veterinary drugs, the Committee agreed that this is a valid approach and relevant for veterinary drugs in some circumstances; however, it requires additional specific consideration for veterinary drugs with respect to their possible pharmacological and microbiological effects. The Committee recommended that the JECFA Secretariat establish an electronic working group to develop the scope of work for application of the TTC approach to veterinary drugs and to develop a project plan to address this work.

The Committee also noted that a step for providing a preliminary risk assessment, covered under the problem formulation step in the decision-tree, is mentioned in the risk analysis policy as applied by CCRVDF. However, it seems that this step is currently not being implemented, and the Committee recommends that CCRVDF, when updating its risk analysis policy, develop guidance on how to implement this step in the future.

2.7 **Guidance for JECFA experts**

At its present meeting, the WHO group identified a number of inconsistencies in style or convention in its working documents, in addition to those

identified at recent previous meetings, reflecting the fact that the present guidance to experts was prepared some time ago. It was agreed that the guidance to experts for the preparation of working documents should be updated and include clear advice on those issues identified at recent meetings.

It was further agreed that the guidance to FAO experts for the preparation of meeting documents should be consolidated and updated.

The Committee requests the JECFA Secretariat to undertake this work. The importance of interaction when updating the WHO and FAO guidance was emphasized, as there are several issues requiring common agreement.

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 Amoxicillin

Explanation

Amoxicillin (Chemical Abstracts Service [CAS] No. 26787-78-0) is a moderate-spectrum, semi-synthetic β -lactam antimicrobial agent used to treat bacterial infections in animals and humans. It exerts bactericidal effects by inhibiting the transpeptidase that catalyses the cross-linking of bacterial cell wall peptidoglycan. It is susceptible to degradation by β -lactamase and is often combined with clavulanic acid (a β -lactamase inhibitor) to improve its effectiveness against β -lactamase-producing bacteria.

In animals, amoxicillin is approved for use in cats, dogs, pigs, pre-ruminating calves, including veal calves, cattle, sheep, horses, chickens, ducks, pigeons and turkeys to treat a variety of Gram-positive and Gram-negative bacterial infections. Not all jurisdictions, however, have approved the drug in all species of animals listed above. Amoxicillin, either as amoxicillin trihydrate or as sodium salt, has been used extensively in humans to treat a variety of infections. WHO has categorized it as a critically important antimicrobial agent in human medicine.

In food-producing animals, amoxicillin is approved for use as amoxicillin trihydrate in oral suspensions equivalent to 40 mg of amoxicillin for baby pigs under 4.5 kg; as a soluble powder of amoxicillin trihydrate at 400 mg/45.5 kg body weight (bw) for pre-ruminating calves, including veal calves, administered by drench or by mixing in milk replacer; as amoxicillin trihydrate boluses containing 400 mg of amoxicillin for pre-ruminating calves; as a sterile amoxicillin trihydrate powder for use as a suspension, administered by intramuscular or subcutaneous injection in cattle; as a sterile intramuscular injection suspension containing amoxicillin at 50 mg/ml at a dose rate of

7 mg/kg bw for sheep; as a 150 mg/ml long-acting amoxicillin trihydrate oily intramuscular injection suspension for sheep; and as a 200 mg/ml intramuscular injection of amoxicillin for sheep, cattle and pigs.

Amoxicillin has not previously been reviewed by the Committee. The Committee carried out the present evaluation of amoxicillin to establish an ADI and to recommend MRLs in cattle, sheep and pig tissues and cattle and sheep milk at the request of the Nineteenth Session of CCRVDF (4).

Toxicological and microbiological evaluation

Amoxicillin is an old drug with a long history of use. Studies were performed prior to implementation of Good Laboratory Practice (GLP) guidelines, but consistent with standards existing at the time of study. Published journal articles reviewed often did not declare the GLP compliance status of their studies.

Data supporting the evaluation of amoxicillin were provided by the USFDA with the permission of the pharmaceutical sponsor. In addition, a literature search was conducted, which identified a number of additional pharmacokinetic and epidemiological studies that were used in this evaluation.

Biochemical data

Amoxicillin is rapidly absorbed through oral and parenteral routes of administration and distributed in various tissues. Peak plasma concentrations of amoxicillin greater than 10 µg/ml are observed generally within an hour after oral administration in mice (50 mg/kg bw), rats (100 mg/kg bw), dogs (100 mg/kg bw) and humans (500 mg/person). Oral bioavailability is approximately 50% in rats, about 65% in dogs and greater than 80% in humans. There is a dose-dependent saturability of amoxicillin absorption. Partial degradation of amoxicillin may occur in the intestine, thereby reducing its bioavailability. Plasma protein binding is not high (<15%). Amoxicillin has a short plasma elimination half-life (~1 hour), and the absorbed drug is quickly eliminated unchanged from the body, principally via urinary excretion through both glomerular filtration and active secretion. Hepatic first-pass metabolism is not observed. Within the normal dose range, amoxicillin pharmacokinetics is not influenced by the presence of clavulanic acid. Only the parent compound has microbiological activity.

Toxicological data

Acute toxicity results in mice, rats and dogs suggest that the drug is well tolerated at high doses following oral exposure (median lethal dose [LD₅₀] > 5000 mg/kg bw).

Short-term toxicity studies were conducted in rats, cats and dogs. In one study, rats of both sexes were administered amoxicillin by gavage at 500 mg/kg bw per day for 21 days. The absolute and relative liver weights in treated groups were significantly lower than those in the controls, but there were no histological changes that were treatment related. The no-observed-adverse-effect level (NOAEL) from this study was 500 mg/kg bw per day, the only dose tested.

In another study, male and female rats were treated with amoxicillin by gavage at 500 mg/kg bw per day for 21 days. Histopathological examination revealed minimal, statistically non-significant fatty changes in the livers of treated females. The NOAEL for this study was 500 mg/kg bw per day, the only dose tested.

Groups of male and female cats were treated orally with amoxicillin at 100, 300 or 500 mg/animal per day, 5 days/week, for 28 days. In the highest-dose males, there was an increase in the level of renal tubular lipid, but it was not considered to be treatment related. No other treatment-related lesions were identified. The NOAEL for this study was the highest dose tested, 500 mg/day per animal, equal to 149 mg/kg bw per day based on the group mean animal body weight of 3.36 kg at the initiation of the study.

One male and one female dog were orally administered amoxicillin at a dose of 250 mg/kg bw per day for 14 days. Gross examination during necropsy and histopathological examination of major organs did not reveal any significant changes. The no-observed-effect level (NOEL) for this study was 250 mg/kg bw per day, the only dose tested.

In a 6-month study, male and female rats were dosed with amoxicillin by gavage at 0, 200, 500 or 2000 mg/kg bw per day, 6 days/week, for 26 weeks. At 13 weeks, some males and females from each group were necropsied; the remaining animals were necropsied at the end of the study. At the interim necropsy, moderate enlargement of the caecum in the highest-dose group was the only treatment-related change. The only change noticed at termination was elevated relative liver weights in the highest-dose males, but it was considered not to be of toxicological relevance. The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

In a 6-month study, male and female dogs were treated orally with amoxicillin at a dose of 200, 500 or 2000 mg/kg bw per day for 6 months. Control dogs were left untreated (no placebo administration). At the end of 3 months, half of the males and females from each group were necropsied; the remaining animals were necropsied at the end of 6 months. Some vomiting occurred either immediately or within 1–4 hours of dosing in some animals in the highest-dose group. Grey-coloured faeces were observed mainly in the first

3 weeks in a dose-related manner in dogs receiving 500 or 2000 mg/kg bw per day. The weight gain in the highest-dose group was decreased compared with controls. At termination of the study, relative liver weights were increased for the highest-dose dogs, but no associated histology was observed. The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

Amoxicillin was negative *in vitro* for sister chromatid exchange, chromosomal aberration and the induction of micronuclei in human peripheral lymphocytes with and without exogenous metabolic activation. In studies reviewed by the USFDA,¹ but not available to the Committee, the combination of amoxicillin–clavulanic acid gave negative results for genotoxicity *in vitro* in an Ames test, a human lymphocyte cytogenicity assay and a yeast recombination assay. Amoxicillin–clavulanic acid was weakly positive in a mouse lymphoma assay only at cytotoxic concentrations. It was negative *in vivo* in a mouse micronucleus test and a dominant lethal test. As with other β -lactam antimicrobial agents, amoxicillin caused deoxyribonucleic acid (DNA) damage *in vitro* in cultured cells at high concentrations. There is some evidence that this is an indirect effect due to the formation of reactive oxygen species.

The Committee concluded that it was unlikely that amoxicillin would be genotoxic *in vivo* following exposure to residues arising from its use as a veterinary drug.

Data from studies of long-term toxicity and carcinogenicity were not available for the Committee to review. However, the long history of use of amoxicillin in a wide range of species, including humans, has not identified an association between the use of amoxicillin and carcinogenicity. Similarly, there were no indications of potential carcinogenicity effects (e.g. increased incidence of tumours or preneoplastic lesions) in the 6-month rat study. These findings, together with the genotoxicity data reviewed, led the Committee to conclude that amoxicillin is unlikely to be carcinogenic to humans at levels of exposure to residues in food-producing animals.

In a reproductive toxicity study, rats were treated orally with amoxicillin at 200 or 500 mg/kg bw per day for several weeks before mating, during cohabitation and thereafter for the study duration, and animals were followed through first and second matings. No significant differences were noted between treated and control animals or their pups in most parameters measured after both the first and second matings, except reduced weight gain in treated males. The NOAEL from this reproductive study was 500 mg/kg bw per day, the highest dose tested.

¹ Based on the USFDA's label on the approved human drug amoxicillin–clavulanate potassium.

Groups of pregnant mice were treated with amoxicillin at 0, 200, 500 or 2000 mg/kg bw per day by gavage between days 6 and 15 of pregnancy and killed on day 17. Fetal losses of 4.8%, 13.9%, 15.2% and 13.0%, respectively, were observed. No other treatment-related abnormalities were noted in either the dams or the pups. Although the difference in fetal losses between the control and treated groups was significant, these values were within the historical control range of 3.8–14.3% (mean 7.9%) for the laboratory, and there was no dose–response relationship. These results were considered equivocal, and a NOAEL for developmental toxicity in mice could not be identified.

Groups of pregnant rats were treated with amoxicillin at doses up to 2000 mg/kg bw per day by gavage between days 6 and 15 of pregnancy and killed on day 20. No significant effects were observed in either the dams or the pups from treated animals. The NOEL from this developmental toxicity study was 2000 mg/kg bw per day, the highest dose tested.

In another rat study, animals were treated with amoxicillin at 200 or 500 mg/kg bw per day by gavage from day 15 of gestation through to lactation day 21. There was no effect on dams, pup mortality or growth, and no abnormal pups were observed. The NOAEL for perinatal and postnatal toxicity was 500 mg/kg bw per day, the highest dose tested.

Epidemiological studies that investigated the risk of teratogenicity of amoxicillin in humans have not identified any increased risk. Based on the overall data reviewed, the Committee considered that amoxicillin is unlikely to cause reproductive or developmental toxicity in humans.

In humans, the use of amoxicillin–clavulanic acid has been reported to be associated with hepatotoxicity. However, hepatotoxicity is rarely associated with the use of amoxicillin alone. Information in the published literature suggests that clavulanic acid is likely to be responsible for this toxicity.

Hypersensitivity is considered to be the most relevant toxicological end-point for the safety assessment of amoxicillin. Individuals who are hypersensitive to amoxicillin are also sensitive to other β -lactams, including benzylpenicillin. In recent studies, amoxicillin was often the most frequent positive allergen among several β -lactams tested in human patients, and a high proportion of patients who are reactive to amoxicillin (~80%) were also allergic to other penicillins. Although published literature indicates that hypersensitivity in sensitized individuals could be evoked by oral exposure to a small quantity of amoxicillin, no reports were found on hypersensitivity reactions occurring in humans from exposure to the residues of amoxicillin in foods of animal origin. The Committee previously evaluated benzylpenicillin and procaine benzylpenicillin, which dissociates to procaine and benzylpenicillin in the body ([Annex 1](#), references 91 and 134), and concluded that allergy was the

determining factor in the safety evaluation of these residues, recommending that daily intake of benzylpenicillin from food be kept as low as practicable, and in any event below 30 µg of the parent drug.

Limited information available suggests that, compared with benzylpenicillin, amoxicillin is chemically less reactive with proteins to form haptens. Additionally, the recommended starting dilution of amoxicillin used in the diagnosis of hypersensitivity in sensitized individuals by the skin prick test is more than 2500 times higher than that of benzylpenicillin.

Microbiological data

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181) and that complies with Guideline 36 of VICH (VICH GL36) (7) was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for amoxicillin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated. Amoxicillin was active against *Escherichia coli* (minimum inhibitory concentration required to inhibit the growth of 50% of organisms [MIC_{50}] = 5 µg/ml), *Bifidobacterium* (MIC_{50} = 0.06 µg/ml), *Clostridium* (MIC_{50} = 0.1 µg/ml), *Bacteroides* (MIC_{50} = 0.5 µg/ml), *Lactobacillus* (MIC_{50} = 0.25 µg/ml), *Fusobacterium* (MIC_{50} = 0.1 µg/ml), *Eubacterium* (MIC_{50} = 0.1 µg/ml) and *Peptostreptococcus* (MIC_{50} = 0.1 µg/ml).

Amoxicillin residues may be present at low levels in meat products consumed by humans; therefore, amoxicillin-related residues could enter the colon of a person ingesting edible tissues from treated animals. Although amoxicillin was rapidly absorbed after oral administration in animals, a considerable amount of the administered amoxicillin was excreted through faeces. In the absence of data to support faecal inactivation, it is considered that amoxicillin residues entering the human colon will remain microbiologically active.

There is potential for disruption of the colonization barrier in the human gastrointestinal tract, as MIC values for the most relevant and predominant bacteria in the gastrointestinal tract indicated that they were susceptible to amoxicillin. Because the majority of amoxicillin residue levels detected in target tissue were below the lowest MIC_{50} of any of the representative human intestinal microbiota tested, it is unlikely that the development of resistance to amoxicillin residues would occur.

The formula for calculating the microbiological ADI is as follows:

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

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} 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 *Escherichia coli* ($MIC_{50} = 5 \mu\text{g/ml}$), *Bifidobacterium* ($MIC_{50} = 0.06 \mu\text{g/ml}$), *Clostridium* ($MIC_{50} = 0.1 \mu\text{g/ml}$), *Bacteroides* ($MIC_{50} = 0.5 \mu\text{g/ml}$), *Lactobacillus* ($MIC_{50} = 0.25 \mu\text{g/ml}$), *Fusobacterium* ($MIC_{50} = 0.1 \mu\text{g/ml}$), *Eubacterium* ($MIC_{50} = 0.1 \mu\text{g/ml}$) and *Peptostreptococcus* ($MIC_{50} = 0.1 \mu\text{g/ml}$), the MIC_{calc} is $0.10 \mu\text{g/ml}$.

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. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. The lowest amoxicillin urinary recovery data from the human studies was 43.4%. Therefore, the fraction of oral dose available would be $1 - 0.434 = 0.566$.

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

The upper bound of the microbiological ADI for amoxicillin is calculated as indicated below:

$$\begin{aligned} \text{Upper bound of ADI} &= \frac{0.10 \mu\text{g/ml} \times 220 \text{ g}}{0.566 \times 60 \text{ kg bw}} \\ &= 0.65 \mu\text{g/kg bw} \end{aligned}$$

Therefore, a microbiological ADI of 0–0.7 $\mu\text{g/kg bw}$ (rounded to one significant figure), or 42 μg for a 60 kg adult, was derived from in vitro MIC susceptibility testing.

Evaluation

As hypersensitivity is frequently encountered in the therapeutic use of penicillins, including amoxicillin, in human medicine, this was considered to be the toxicological effect of concern. The thirty-sixth meeting of the Committee based its toxicological guidance value of 30 µg/person for benzylpenicillin on only four case-studies of allergy to oral exposure to residues of penicillins, in the absence of information related specifically to benzylpenicillin.

Available limited evidence indicates that, compared with benzylpenicillin and possibly other penicillins, amoxicillin is less chemically reactive with proteins to form haptens. While the Committee recognizes that adverse reactions are generally under-reported, it was unable, through a search of the published literature, to identify any cases of allergy following oral exposure to residues of amoxicillin in food. The Committee therefore concluded that the value of 30 µg/person previously established by the thirty-sixth meeting to protect against allergic reactions from residues of penicillins would be unnecessarily conservative in protecting against residues of amoxicillin.

A microbiological ADI of 0–42 µg/person could be established on the basis of disruption of the colonization barrier of the gastrointestinal tract. The Committee therefore concluded that this ADI is sufficient to ensure that allergic reactions would be very unlikely to occur following oral exposure to residues of amoxicillin in food.

The Committee therefore established an ADI of 0–0.7 µg/kg bw on the basis of microbiological effects.

A toxicological monograph was prepared.

Residue evaluation

The Committee reviewed studies on the pharmacokinetics and metabolism of amoxicillin as well as a large number of non-radiolabelled amoxicillin 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

No absorption, distribution, metabolism and excretion (ADME) studies in cattle, pigs or sheep using radioactive isotopic labelled amoxicillin were provided. Amoxicillin is unstable in solution.

Amoxicillin studies have been evaluated in pre-ruminating and ruminating cattle, lactating dairy cows, pigs and sheep, including lactating dairy sheep.

Amoxicillin was administered orally to 16 pre-ruminant calves at a dose of 7 mg/kg bw. Only a small proportion of the dose was absorbed. Peak serum concentrations of 0.7–1.0 µg/ml were found at 4 hours post-dosing and declined to 0.3–0.4 mg/ml at 8 hours. Peak urine concentrations occurred at 8 hours. Concentrations in excess of serum levels were found in kidney, liver, bile and urine.

In a study with eight veal calves, an amoxicillin dose of 7 mg/kg bw was administered intramuscularly. Peak amoxicillin serum levels of 2.0–2.5 µg/ml were obtained at 1 hour and were sustained for 6 hours, declining to 1.5 µg/ml at 8 hours. About 50–60% of the drug was recovered from the urine in the 24 hours following intramuscular administration, with the majority of the excreted dose (48–52%) recovered in the first 8 hours.

Several pharmacokinetic studies were conducted in pigs. Animals were treated with amoxicillin by the intravenous, intramuscular or oral route. For intramuscular administration, the area under the plasma concentration–time curve (AUC) ranged from 21 mg·h/l to 33 mg·h/l for doses ranging from 7.5 to 20 mg/kg bw. For long-acting amoxicillin formulations containing aluminium stearate, the maximum AUC was approximately 44 mg·h/l. Intramuscular bioavailability was approximately 1. Absorption of amoxicillin after oral administration was about 30% in pigs.

Comparative pharmacokinetic studies were evaluated in which sheep received amoxicillin at a dose of 10 mg/kg bw either intravenously or intramuscularly. In the intramuscular study, sheep received sodium amoxicillin or amoxicillin trihydrate. The $AUC_{0-\infty}$ was greater for intravenous than for intramuscular administration and was comparable for intramuscular sodium amoxicillin and intramuscular amoxicillin trihydrate. The mean residence time was 8 and 16 times longer for amoxicillin trihydrate compared with intramuscular and intravenous sodium amoxicillin, respectively. Bioavailability following intramuscular administration of amoxicillin trihydrate was 0.95.

Metabolism studies are limited. Two identified metabolites of amoxicillin have been described in pigs: amoxicilloic acid and amoxicillin piperazine-2',5'-dione (DIKETO). In two studies, the pharmacokinetics of amoxicilloic acid and DIKETO were evaluated after intravenous and oral administration at 20 mg/kg bw in pigs. In these studies, amoxicilloic acid was detected in plasma for a longer duration than the parent amoxicillin. In tissues of pigs following oral administration, amoxicillin concentrations were less than the limit of detection (LOD) (1.7, 3.5, 1.5 and 1.7 µg/kg for kidney, liver, muscle and fat, respectively) at 48 hours post-treatment. Amoxicilloic acid was below the limit of quantification (LOQ) (25 µg/kg) in edible tissues through 72 hours post-treatment.

Residue data

No residue depletion studies using radioactive isotopic labelled amoxicillin were provided in cattle, pigs or sheep.

Pre-ruminating calves: Five studies were evaluated by the Committee that used a microbiological growth inhibition assay not validated according to current standards for quantitative methods, but the methods still provide useful information on residues. In three of the studies, amoxicillin was administered orally at 400 mg/calf twice daily for 5 days. In the fourth study, amoxicillin was administered orally at 500 mg/calf twice daily for 5 days. In the final study, pre-ruminating calves were treated with an amoxicillin suspension by deep intramuscular injection (250 mg/ml suspension) at a dose rate of 17.6 mg/kg bw once a day for 7 days. In all animals receiving oral formulations of amoxicillin, residues in muscle were less than 0.01 mg/kg at all sampling times. Residues in liver were low but detectable for 1 day following treatment with two 400 mg boluses. In contrast, the calves treated twice daily with 400 mg as soluble powder had no detectable residues in liver. Amoxicillin residues were detected in kidney for up to 18 days post-treatment (0.02–0.21 mg/kg). In the intramuscular study, at 1 day post-treatment, residues were detected in all sampled tissues (range from 6.4 mg/kg in injection site muscle to 0.2 mg/kg in fat). Residues were below the LOD (0.01 mg/kg) in liver, fat and non-injection site muscle at 9 days post-treatment. Residues were detected in kidney through day 12 and in injection site muscle through day 18.

Ruminating calves: Amoxicillin was administered intramuscularly to ruminating calves in four studies.

In a GLP-compliant study, calves were treated for 5 consecutive days with an intramuscular dose of 7 mg amoxicillin per kilogram body weight. Residues were detected in all tissues (LOD = 1.0, 3.9, 2.1 and 1.4 µg/kg for muscle, liver, kidney and fat, respectively) except liver at 2 days post-treatment. By 6 days post-treatment, amoxicillin residues were detected in injection site tissues. Injection site residue concentration declined from about 71 000 µg/kg at 2 days to not detectable at 45 days post-treatment.

In another study, calves were treated at 17.6 mg/kg bw once a day for 7 days. Residues were detected in all sampled tissues for 3 days (>0.16 mg/kg in injection site muscle to 0.01 mg/kg in fat). By 8 days post-treatment, detectable residues were measured only in liver and injection site muscle. By 11 days post-treatment, all samples were below the LOD (0.01 mg/kg). In the two other studies, calves were treated at 17.6 mg/kg bw once daily for 7 days either intramuscularly or subcutaneously. In the subcutaneous dosing study, no results were available because of problems with the analysis. Following intramuscular administration, residues were detected in all sample

tissues through 22 days post-treatment. There were no detectable residues at 25 days post-treatment (LOD = 0.01 mg/kg).

Lactating dairy cows: In a GLP-compliant study, cows were treated intramuscularly at 7 mg amoxicillin per kilogram of body weight once daily for 5 days. There was no evidence of significant excretion in milk (LOQ = 1 µg/kg) and, following cessation of treatment, amoxicillin residues in milk declined from an average ($n = 4$) of 5.8 µg/kg at 12 hours to 0.46 µg/kg at 156 hours post-treatment.

In an intramammary study, a single cow was treated with 62.5 mg amoxicillin per quarter (total dose 250 mg). Milk residues declined from about 1 µg/ml at 8 hours post-treatment to less than the LOD (1 ng/ml) at 72 hours.

Two other studies were evaluated with cows treated intramuscularly or subcutaneously at 11 mg/kg bw once daily for 5 days. Residues were detected in milk at all sampling times following intramuscular administration (range 0.01–1.57 mg/l at 60 hours) and through 84 hours following subcutaneous administration.

Pigs: Six amoxicillin residue studies were evaluated.

In two studies, piglets were treated orally by syringe at 22 mg/kg bw twice daily for 5 days. Residues were detected occasionally (0.01–0.16 mg/kg) in all tissues up to 7 days, but by 9 days post-treatment, all samples were below the LOD (0.01 mg/kg).

In another study, pigs were treated for 5 consecutive days with an intramuscular dose of 7 mg/kg bw. Amoxicillin residues were detected only in kidney (45 µg/kg) and in injection site tissues at 2 days post-treatment. Thereafter, residues were detected only in injection site tissues, declining from more than 11 000 µg/kg to below the LOQ (25 µg/kg) at 43 days post-treatment.

In two non-GLP studies, pigs were treated intravenously or orally by gavage at 20 mg/kg bw. Amoxicillin, amoxicilloic acid and DIKETO were measured in edible tissues. In these studies, amoxicillin and DIKETO were detected only for 12 hours post-treatment. Amoxicilloic acid was detected in kidney and liver tissues of pigs through 60 hours post-treatment (LOQ = 25 µg/kg).

Finally, in a study performed in pigs treated by gavage with daily oral amoxicillin doses of 20 mg/kg bw for 5 days, the mean concentration ($n = 4$) of amoxicillin residues in kidneys 6 days after the last dose was 21.4 µg/kg, and in the liver, it was 12.3 µg/kg; no amoxicillin could be detected in fat or muscle (LOQ = 10 µg/kg).

Sheep: A single GLP-compliant study was evaluated. In this study, sheep were treated once daily for 5 days intramuscularly at an amoxicillin dose of 7 mg/kg bw. Residues of amoxicillin in liver, kidney, muscle and fat depleted

rapidly, and at 48 hours post-dosing, all amoxicillin concentrations were lower than the LOQ (25 µg/kg). Injection site residues declined to below the LOQ (25 µg/kg) at and beyond 49 days post-treatment.

Lactating dairy sheep: In a GLP-compliant study, sheep were treated once daily for 5 days intramuscularly at a dose of 7 mg/kg bw. Milk was collected at 12-hour intervals for 10 days. Amoxicillin residues in milk increased from 23.1 µg/kg at 12 hours after the first dose to 33.0 µg/kg 12 hours after the second treatment. These mean residue concentrations (i.e. approximately 33 µg/kg) were maintained following doses 3–5. Amoxicillin residues in milk declined steadily and were less than 4 µg/kg at 60 hours following cessation of treatment. There was no evidence of bioaccumulation in sheep milk with repeated dosing.

Analytical methods

Suitably validated analytical methods for amoxicillin residues were used in the GLP-compliant depletion studies evaluated.

A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method with an LOQ for amoxicillin of 25 µg/kg for liver, kidney, fat and muscle and 2 µg/kg for milk was validated in a GLP-compliant study for sheep tissue and milk. The intra-day accuracy at 25, 50 and 100 µg/kg for tissues ranged from 80% to 104%, with precision below 16%. For milk, intra-day accuracy was 64–78% at 2, 4 and 8 µg/kg for milk, with precision below 23%. The LOQ was 25 µg/kg for all edible tissues, and the LODs for amoxicillin were 3, 5, 2, 10 and 0.14 µg/kg for liver, kidney, muscle, fat and milk, respectively.

In a GLP-compliant method validation study for pigs, the LOQ for amoxicillin was 25 µg/kg for swine liver, kidney, muscle and skin with fat. The matrix-corrected mean recoveries were all between 67% and 102%, with coefficients of variation of 1–15%. The inter-day accuracy at 25, 50 and 100 µg/kg ranged between 70% and 90%, and the corresponding precisions were all less than or equal to 15%. The LODs of the method for amoxicillin were 6, 2, 2 and 4 µg/kg for liver, kidney, muscle and skin with fat, respectively. In a third GLP-compliant method validation study in cattle tissues and milk, the LOQ for amoxicillin was 25 µg/kg in cattle tissues and 1.0 µg/kg in milk. At 25, 50 and 100 µg/kg, the intra-day accuracy for muscle tissue ranged from 73% to 103%, with a corresponding precision of 4–14%. The intra-day accuracy for milk at 1, 2, 4 and 8 µg/kg ranged from 77% to 86%, with a corresponding precision of 9–14%.

Maximum residue limits

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

- An ADI of 0–0.7 µg/kg bw was established by the Committee based on a microbiological end-point, equivalent to an upper bound of 42 µg for a 60 kg person.
- Amoxicillin is primarily metabolized to amoxicilloic acid and DIKETO, which have no microbiological activity.
- Amoxicillin is the only microbiologically active residue and is suitable as a marker residue.
- Amoxicillin residues are consistently highest in kidney, and kidney is a suitable target tissue.
- Suitable validated routine analytical methods were available for monitoring purposes.
- The MRLs were based on twice the LOQ of 25 µg/kg for amoxicillin in edible tissues (including skin plus fat in pigs) and of 2 µg/kg for amoxicillin in sheep milk.

The Committee recommended MRLs for amoxicillin in cattle, sheep and pig tissues of 50 µg/kg and in cattle and sheep milk of 4 µg/kg, determined as amoxicillin parent compound.

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

A residue monograph was prepared.

3.2 **Apramycin**

Explanation

Apramycin (CAS No. 65710-07-8) is a broad-spectrum aminocyclitol antibiotic produced by a strain of *Streptomyces tenebrarius*. It is effective against both Gram-positive and Gram-negative bacteria, including isolates of *Escherichia coli* and *Salmonella* species, and some strains of mycoplasma from farm animals and human origin. Apramycin is extracted from the fermentation medium as apramycin sulfate at a purity of at least 85%, and activity is expressed as equivalents of apramycin base using a microbiological assay.

Apramycin exerts its antibacterial effect by inhibiting protein synthesis at the level of peptidyl translocation. It is used for the treatment of a variety of enteropathogenic infectious diseases in target species such as cattle, swine,

poultry and rabbits. Apramycin is marketed as the sulfate in soluble powder and premix formulations. Apramycin is not used in human medicine.

Apramycin has not been previously evaluated by the Committee. The Committee evaluated apramycin to establish an ADI and recommend MRLs in cattle, pig and chicken tissues at the request of the Nineteenth Session of CCRVDF (4).

Toxicological and microbiological evaluation

In addition to a submission from the sponsor, a literature search was conducted, which identified only one further study report. The Committee considered the results of studies on pharmacokinetics, acute, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, pharmacology, ototoxicity, kidney toxicity and microbiological safety. A number of critical studies, namely the 2-year studies in mice and rats, the multigeneration reproductive toxicity study in rats, two of the four genotoxicity studies and the microbiology studies, were carried out according to appropriate standards. The majority of the other studies were performed prior to the establishment of standards for study protocol and conduct.

Biochemical data

In a preliminary study in rats, an oral dose of apramycin was poorly absorbed from the gastrointestinal tract, and approximately 99.5% was excreted unchanged in the faeces. Primary data were not supplied for this study.

In 6-month and 12-month toxicity studies in dogs given daily oral doses, absorption was low and was proportional to the dose. Essentially no apramycin was detected in serum at 24 hours, and there was no evidence of accumulation following repeated administration. Between 0.3% and 10.5% of the administered dose was excreted in urine, which represents the absorbed fraction. The primary route of excretion was in the faeces.

Toxicological data

Apramycin sulfate exhibited low acute toxicity by the oral route, which was probably a reflection of low absorption. The LD₅₀ values (as apramycin activity) were greater than 4000 mg/kg bw in mice and rats, greater than 1250 mg/kg bw in guinea-pigs, greater than 832 mg/kg bw in rabbits and greater than 520 mg/kg bw in dogs. High doses in mice, rats, guinea-pigs, rabbits and dogs resulted in gastrointestinal effects that were presumably related to antimicrobial activity on the intestinal microflora. Renal injury was also observed in guinea-pigs. In mice and rats, single intravenous doses of apramycin base were more toxic than oral doses, and animals showed signs of central nervous system toxicity and renal damage.

In a 3-month study in which mice were fed diets containing 0, 5000, 10 000, 20 000 or 50 000 mg of apramycin activity per kilogram of feed, dietary assays generally revealed apramycin concentrations well below the target level. Virtually no apramycin was present in the feed containing a nominal concentration of 10 000 mg/kg. Alopecia was observed in males given 20 000 and 50 000 mg/kg feed. Body weight gain was reduced in both sexes given 50 000 mg/kg feed and in males given 20 000 mg/kg feed. Slightly decreased lymphocyte counts and increased neutrophil counts were observed at the highest dose. Absolute and relative liver and kidney weights were lower in males and females given 50 000 mg/kg feed. Because of the uncertainty in the administered doses, a NOAEL could not be determined.

In a 1-month study, rats were fed diets containing 0, 10 000, 25 000 or 50 000 mg apramycin activity per kilogram feed. During the last week of the study, rats from the 25 000 and 50 000 mg/kg feed groups excreted dark to black faeces. Body weight gain was depressed and blood glucose and blood urea nitrogen were increased in males and females given 50 000 mg/kg feed. Also at this dose level, there were slight decreases in serum levels of creatinine in females and in alkaline phosphatase activity in males. Haematological parameters were not examined, and only limited pathology was undertaken. The caeca of all high-dose rats were 2–3 times normal size. Because only a limited range of parameters was investigated, a NOAEL was not determined.

In a 3-month study, rats were fed diets containing 0, 200, 400 or 1000 mg apramycin sulfate per kilogram feed. Another group received apramycin sulfate in the drinking-water at a concentration of 10 mg/ml. Mild diarrhoea in the drinking-water group was the only finding. The NOEL was the highest dietary dose of 1000 mg apramycin sulfate per kilogram feed, equal to 26 mg of apramycin activity per kilogram body weight per day.

In another 3-month study, rats were fed diets containing 0, 1800, 2750, 6200 or 10 000 mg apramycin activity per kilogram feed. Feed consumption in all treated groups and body weight gains in females given 6200 and 10 000 mg/kg feed were slightly elevated, but not in a dose-related manner. Mild nephrosis was observed in some male rats at the highest dose. Based on kidney toxicity, the NOAEL was 6200 mg/kg feed, equal to 460 mg of apramycin activity per kilogram body weight per day.

In a 6-month study, rats were fed diets containing 0, 1000, 2500 or 5000 mg apramycin activity per kilogram feed. Darker, softer faeces with increased moisture were found in all treated groups in a dose-related manner and were associated with a concomitant increase in water intake. Erythrocyte counts, haemoglobin and haematocrit were lower in males given 5000 mg/kg feed

for 1 month, but not at the end of the study. Serum glutamate dehydrogenase activity was increased in animals given 5000 mg/kg feed for 6 months. Absolute and relative kidney weights were lower in high-dose males. Based on haematological and biochemical findings, the NOAEL was 2500 mg/kg feed, equal to 170 mg apramycin activity per kilogram body weight per day.

In a 3-month study, dogs were administered 0, 5, 10 or 25 mg apramycin sulfate per kilogram body weight per day in capsules. There were no treatment-related effects. The NOEL was the highest dose of 25 mg apramycin sulfate per kilogram body weight per day, equal to 13 mg apramycin activity per kilogram body weight per day.

In a 6-month study, dogs received oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Body weight gain was lower in both sexes given 100 mg/kg bw per day. One dog out of eight at the high dose had decreased appetite and developed anorexia; as a result of weight loss and weakness, this animal was killed. During months 3–5, one dog of eight given 50 mg/kg bw per day and four dogs of eight given 100 mg/kg bw per day did not respond to noise stimuli, but all responded at 6 months. Soft stools occurred in all treated dogs, which is likely to be a consequence of an effect on the bacterial flora rather than a toxic effect in the dog. Erythrocyte counts were consistently lower in both sexes during treatment with 50 and 100 mg/kg bw per day, but not always in a dose-related manner and rarely statistically significantly; consequently, they were considered unrelated to treatment. Based on reduced body weight gain, the NOAEL was 50 mg apramycin activity per kilogram body weight per day.

In a 1-year study, dogs were given oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Adrenal weights were increased in males at the highest dose, but were not associated with pathological alterations. The NOAEL was the highest dose of 100 mg apramycin activity per kilogram body weight per day.

In a long-term study in which mice were fed diets containing 0, 1500, 5000, 15 000 or 45 000 mg apramycin activity per kilogram feed for 2 years, body weight gains were lower in males and females given 5000 mg/kg feed and above. At a dose of 45 000 mg/kg feed, haemoglobin and haematocrit were increased in both sexes, erythrocyte counts were increased in females and males showed slightly decreased lymphocyte counts and increased neutrophil counts. Serum alkaline phosphatase activity was increased in females given 5000 mg/kg feed and higher. Serum glucose level was decreased and blood urea nitrogen was increased in both sexes at the highest dose. Cytoplasmic basophilia involving the renal cortical tubular epithelium was observed in both sexes at doses of 5000 mg/kg feed and higher. The incidences of

tumours were not increased. Based on reduced body weight gain and kidney pathology, the NOAEL was 1500 mg/kg feed, equal to 189 mg apramycin activity per kilogram body weight per day. There was no evidence for carcinogenicity in mice.

In a study in which rats were given diets containing 0, 2500, 5000, 10 000 or 50 000 mg apramycin activity per kilogram feed for 2 years, body weight gains and liver and kidney weights were reduced in both sexes at the highest dose. The incidences of tumours were not influenced by treatment. Based on reduced body weight gain and reduced organ weights, the NOAEL was 10 000 mg/kg feed, equal to 488 mg apramycin activity per kilogram body weight per day. There was no evidence for carcinogenicity in rats.

Apramycin was evaluated for potential genotoxicity in two in vitro assays for the induction of gene mutations in strains of *Salmonella typhimurium* and a strain of *Escherichia coli*, an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells and an in vitro assay for the induction of DNA repair in primary cultures of rat hepatocytes. The results were negative in all cases.

Because the carcinogenicity and genotoxicity studies were negative, the Committee concluded that apramycin is unlikely to pose a carcinogenic risk to humans.

In a multigeneration reproductive toxicity study, rats were fed diets containing 0, 2500, 5000 or 10 000 mg apramycin activity per kilogram feed continuously over four generations. No adverse effects on fertility and reproductive indices or on growth and survival of offspring were found. The NOEL was the highest dose of 10 000 mg/kg feed, equal to 785 mg apramycin activity per kilogram body weight per day.

In a developmental toxicity study, pregnant female rats were given a gavage dose of 0, 250, 500 or 1000 mg apramycin activity per kilogram body weight per day on gestation days 6–15. There were no effects on maternal toxicity, fetal growth and development or the incidences of fetal abnormalities. The NOEL was the highest dose of 1000 mg apramycin activity per kilogram body weight per day.

In another developmental toxicity study, pregnant female rabbits were given a gavage dose of 0, 2, 8 or 32 mg apramycin activity per kilogram body weight per day on gestation days 6–18. Maternal feed consumption and body weight gains were markedly reduced, and the numbers of animals aborting were increased, in a dose-related manner. Most of the dams aborting showed an empty gastrointestinal tract. The number of resorptions was increased at 32 mg/kg bw per day. Fetal body weight was depressed in a dose-related manner, and the incidence of bilateral 13th ribs was increased at the highest

dose. The incidences of fetal malformations and external and visceral abnormalities were unaffected. The maternal toxicity was likely related to the particular sensitivity of the gut flora in rabbits given certain antimicrobial agents (5), and therefore it was not possible to identify a NOEL. The fetal toxicity was probably secondary to the severe toxicity in the dams.

In a special study to assess renal toxicity in rats, urine volume was decreased (38%) by a single oral apramycin sulfate dose of 50 mg/kg bw. However, the absolute amounts of urinary electrolytes and creatinine excreted were unchanged. Other special studies indicated that ototoxicity and intrinsic pharmacological activity would not be expected.

No data could be identified relating to effects in humans.

The most relevant study for determining a toxicological ADI was the 6-month study in dogs. The NOAEL was 50 mg/kg bw per day, based on reduced body weight gain. A safety factor of 100 was considered appropriate. Therefore, an ADI of 0–0.5 mg/kg bw could be established on the basis of the toxicological data.

Microbiological data

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181) and that complies with VICH GL36 (7) was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for apramycin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated. Apramycin was active against *Escherichia coli* ($MIC_{50} = 4 \mu\text{g/ml}$). Apramycin exerted no measurable antibacterial activity (MIC values $> 128 \mu\text{g/ml}$) against *Bifidobacterium*, *Clostridium*, *Bacteroides fragilis* and “non-*fragilis*” *Bacteroides* strains. Apramycin exerted relatively poor activity against *Lactobacillus* ($MIC_{50} = 64 \mu\text{g/ml}$), *Enterococcus* ($MIC_{50} = 32 \mu\text{g/ml}$), *Fusobacterium* ($MIC_{50} = 16 \mu\text{g/ml}$), *Eubacterium* ($MIC_{50} = 16 \mu\text{g/ml}$) and *Peptostreptococcus* ($MIC_{50} = 16 \mu\text{g/ml}$).

Apramycin residues may be present at low levels in meat products consumed by humans; therefore, apramycin-related residues could enter the colon of a person ingesting edible tissues from treated animals. As the data submitted for evaluation did not contain measurements of the amount of drug residue in the intestinal tract, the Committee used the pharmacokinetic studies to determine the fraction of the oral dose available to the human intestinal microbiota. Apramycin was poorly absorbed after oral administration in animals, and a considerable amount of the administered apramycin was detected as unmetabolized parent compound in faecal samples. Therefore, apramycin residues entering the human colon will remain microbiologically active.

There is potential for disruption of the colonization barrier of the human gastrointestinal tract. Diarrhoea occurred in some toxicity studies in animals after oral administration of apramycin, suggesting adverse effects of apramycin on the intestinal microbiota. Because the majority of apramycin residue levels detected in target tissue were below the lowest MIC₅₀ of any of the representative human intestinal microbiota tested and the clinical breakpoint for *E. coli* is 32 µg/ml, it is unlikely that the development of resistance to apramycin residues would occur.

The formula for calculating the microbiological ADI is as follows:

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

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC₅₀ for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a MIC₅₀ of 32 µg/ml or less (i.e. *Escherichia coli*, *Enterococcus*, *Fusobacterium*, *Peptostreptococcus* and *Eubacterium* species), the MIC_{calc} is 8.3 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. Apramycin is poorly absorbed and is excreted in faeces primarily in unchanged form; therefore, the value is 1.

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

Therefore, the upper bound of the microbiological ADI for apramycin is calculated as indicated below:

$$\begin{aligned}
 \text{Upper bound of ADI} &= \frac{8.3 \mu\text{g/ml} \times 220 \text{ g}}{1 \times 60 \text{ kg bw}} \\
 &= 30.4 \mu\text{g/kg bw}
 \end{aligned}$$

Therefore, a microbiological ADI of 0–30 $\mu\text{g/kg bw}$ (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing.

Evaluation

The Committee considered that microbiological effects were more appropriate than toxicological effects for the establishment of an ADI for apramycin. Therefore, the Committee established an ADI of 0–30 $\mu\text{g/kg bw}$ on the basis of the data for disruption of the colonization barrier.

A toxicological monograph was prepared.

Residue evaluation

The Committee reviewed studies on the pharmacokinetics and metabolism of apramycin as well as a large number of non-radiolabelled apramycin 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

In calves, pigs, chickens and rabbits, apramycin is rapidly and poorly absorbed by the oral route and quickly eliminated. Maximum concentrations in serum, ranging from 0.38 to 0.88 $\mu\text{g/ml}$ for cattle, pigs and chickens, are found a few hours after treatment (until 6 hours). The drug is undetectable after 36 hours. Pharmacokinetic studies indicate bioavailability of approximately 3% in pigs and 2% in chickens after oral administration. Oral doses are extensively excreted in faeces (more than 82% in pigs).

In one ^{14}C radiolabel study in each of the three species, cattle, pigs and chickens, levels of radioactivity were highest in kidney, followed by liver, and very low quantities were found in muscle and fat. Apramycin metabolism was similar across the three species. Biotransformation ranged from 36% to 85% of the absorbed dose across the three species and the corresponding tissues. The drug remained in tissues mostly as unchanged apramycin. In general, tissues contained insufficient residues for further characterization of chemical structures.

Residue data

Two GLP-compliant depletion studies in calves using non-radiolabelled apramycin were provided. In one study, 24 male calves were given oral

apramycin doses of 40 mg/kg bw (maximum recommended dose) once daily for 5 days. Groups of four calves were killed at 4 hours and at 7, 14, 21, 28 and 35 days. Apramycin residues were determined using a sponsor-validated high-performance liquid chromatography (HPLC) method. Apramycin residues in kidneys were less than 20 mg/kg at 14 days and were mainly below the LOQ (5 mg/kg) at 21 days. Residues in liver were always less than the LOQ (5 mg/kg) and were detected in only one of the eight samples collected at 28 and 35 days' withdrawal. Apramycin was not detected in fat at or beyond 14 days (LOD = 0.13 mg/kg). Residues were not detected in muscle (LOD = 0.27 mg/kg) except for one sample at 21 days (<1 mg/kg).

In the second study, 24 male and 24 female calves were given oral apramycin doses of 40 mg/kg bw (maximum recommended dose) once daily for 5 days. Groups of four calves were killed at 7, 14, 21, 28, 35 and 42 days. Apramycin was detected at all withdrawal times in kidney and liver. In kidney, three of the four samples collected at 7 days' withdrawal contained apramycin above the LOQ (5 mg/kg), at concentrations between 6.5 and 7.2 mg/kg, but no residues above the LOQ were found at 14 days or later. Residues in liver were all below the LOQ (5 mg/kg). A limited number of muscle and fat samples contained residues above the LOQ (0.5 mg/kg) at 7 and/or 14 days. Residues were undetectable in muscle from 35 days and in fat from 28 days.

One GLP-compliant depletion study in pigs using non-radiolabelled apramycin was provided. Twenty-four pigs were medicated with a single daily dose of apramycin in water at 20 mg/kg bw (160% of maximum recommended dose) by gavage using a stomach tube for 7 days. Tissues were sampled at 1, 4, 7, 14, 21 and 28 days' withdrawal, and residues were determined using a sponsor-validated HPLC method. Residues in kidney at 4 days after treatment were between 3.60 and 6.40 mg/kg, declining to below the LOQ (0.5 mg/kg) by 7 days' withdrawal. No residues were detected at any withdrawal interval for muscle, liver or fat samples (LODs of 0.28, 0.20 and 0.05 mg/kg, respectively).

One GLP-compliant depletion study using non-radiolabelled apramycin was provided for pigs treated with apramycin premix at 200 mg/kg feed, with estimated daily doses based on feed consumption at 103–134% of the maximum recommended dose expressed per kilogram body weight. In this study, 16 pigs were dosed for 28 days. Samples of muscle, liver, kidney and skin with fat were collected at withdrawal periods of 3, 6, 9 and 12 days. Residues were determined using the sponsor-validated HPLC method. Highest residue concentrations were found in liver (LOQ = 0.5 mg/kg) at all withdrawal times. This is the only residue depletion study in the four species in which residues were highest in liver tissue. At 3 days' withdrawal time, residues in liver were 1.3–1.4 mg/kg; at day 6, 1.4–1.6 mg/kg; at day 9, 1.1–1.4 mg/kg;

and at day 12, 1.0–1.2 mg/kg. Kidney, muscle and fat residues were less than the LOQ (2.5 mg/kg in kidney and 0.5 mg/kg in muscle and fat) at all times post-treatment.

Most of the studies in pigs lack sufficient sampling times at short withdrawal times for the measurement of residues, compromising extensive assessment of the residue depletion studies.

One GLP-compliant depletion study using non-radiolabelled apramycin was provided for chickens treated with apramycin in drinking-water at 500 mg/l. Forty-eight 4-week-old broiler chickens were treated for 5 days. Based on the total pen bird weight, the average dose was estimated to be 118 mg/kg bw per day (approximately 150% of the maximum recommended dose expressed per kilogram body weight per day). Tissues from 10 birds were taken at 3, 6, 9 and 12 days following withdrawal from the medicated drinking-water. Tissues were analysed for apramycin using a sponsor-validated HPLC method. The LOQ was 0.5 mg/kg for all tissues. Kidney residues were between 0.6 and 1.1 mg/kg at 3- and 6-day withdrawal times in 12 samples and were below the LOQ in the remaining 8 samples. Two samples at the 9-day withdrawal time had residues at 0.6 mg/kg, and the remaining eight samples had residue concentrations below the LOQ. Two skin/fat samples at the 3-day withdrawal time had residue levels of 0.6 mg/kg, and the remaining eight samples had residue concentrations below the LOQ. In liver and muscle, residues were below the LOQ or non-detectable at all sampling times.

For calves, pigs and chickens, nine old, non-GLP-compliant non-radiolabel studies were provided (two for cattle feed, three for pigs treated with drinking-water, two for pigs treated with medicated feed and two for chickens treated with drinking-water). Residues in these nine studies were analysed by qualitative microbiological assays (bioautography). However, allowing for the differences in withdrawal times and the precision of the assays, the results of these studies are in general agreement with the new GLP-compliant studies using HPLC.

In all but 1 of these 14 studies, the majority of the positive residue values were in kidney tissues. Liver tissue contains the second highest concentration of residues in tissue, and in one pig study, liver contained the highest amounts of apramycin. Residue levels in muscle and fat or fat/skin were universally very low. In four calf studies, considering all time points and the LOQs provided by the sponsor's data, 20 residue values were reported at or above the LOQ. For the eight pig studies, 22 residue values were reported at or above the LOQ. In two chicken studies, 52 residue values were at or above the LOQ.

Analytical methods

Most of the preliminary studies on apramycin residues were based on microbiological assays and used *Bacillus subtilis* ATCC 6633 as the test organism, employing a bioautographic procedure that measured zones of inhibition. This type of assay is used to produce qualitative data.

Most of the other studies used an LC/fluorescence detection method. Tissue is treated with ammonium hydroxide/methanol solution to release apramycin. Apramycin is determined by HPLC with fluorescence detection after precolumn derivatization with *o*-phthalaldehyde. No internal standard is used in the method. The specificity is considered to be weak. LODs were calculated from the noise observed in blank tissue samples plus 3 standard deviations. Twenty chromatograms of negative control tissue were used to determine the LODs in each tissue and species. Results were generally good in fat or skin/fat tissue, whereas the LODs for kidney and muscle were less satisfactory. For liver extracts, owing to the complex nature of the matrix, the cleanliness of the extracts is usually poorer.

The approach for the determination of the LOQ was based on the lowest concentration for which the method has been validated. This approach is not compatible with the definition of LOQ as stated in most international standards. For this reason, the LOQ values are high and probably significantly different from the true values. Repeatability (recovery) was assessed through intra- and inter-batch variations, but reproducibility was not carried out in the validation process. Thus, the analytical method combines a nonspecific purification (co-extraction of amino interfering compounds) with a weak specific detection system that may generate results that are more difficult to interpret. The only identification criterion is the chromatographic retention time. Internal standards are not used for the identification and quantification of apramycin.

The consequence of the strategy used by the sponsor is that there are very significant differences in the ratios of the LOQ to the LOD, with values ranging across species and tissues from 1 to 100 in the multiplicity of method validation studies reported.

The Committee considered that the data generated with the microbiological assays were of limited value. The data generated by the LC/fluorescence detector method were of medium quality. A limited set of quality criteria was applied to each sample batch (retention time, repeatability of the standard signal, linearity of the calibration curve and recovery). The Committee recalculated the LOQs. The signal to noise ratios of individual tracings for all the studies were reanalysed, and a factor of 3 times the LODs was applied to recalculate and estimate the LOQs where the quality of data supported this approach. This resulted in some modified LOQs with which to assess the

residue findings that were greater than the LOQs originally calculated in the 14 studies. As a consequence, the Committee was in a position to consider additional depletion data.

The only combinations of matrices and species for which the statistical model developed by the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181) may be applied are cattle kidney and chicken kidney. For all the other combinations of matrix and species, this approach is not possible because the set of quantified data is too limited. In the latter case, there was an option to base the calculation of the MRLs on twice the LOQs recalculated by the Committee. However, this approach led in some cases to an unrealistic overestimation of the MRL because of the weakness of the method performances. As an illustration, the apramycin MRL for cattle liver would be calculated as 10 mg/kg ($= 2 \times 5 \text{ mg/kg}$), whereas for cattle kidney, the model would extrapolate the MRL to a value of 5 mg/kg. This is inconsistent with the fact that kidney contains the higher tissue residues, which is in line with current knowledge—i.e. that the concentrations of aminoglycoside residues are significantly higher in kidney than in liver (8). For this reason, the Committee did not retain this option to recommend MRLs on “matrix/species” combinations besides cattle/kidney and chicken/kidney.

Maximum residue limits

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

- A microbiologically based ADI was established at 0–30 µg/kg bw, equivalent to an upper-bound exposure of 1800 µg/day for a 60 kg individual.
- Apramycin is produced as a fermentation product, and its acceptable purity is at least 85%.
- Apramycin is poorly absorbed orally in calves, pigs and chickens.
- LOQs revised by the Committee were used to identify the new method values that can be used in the analysis of depletion studies.
- Considering the revised LOQs, in four calf studies, 3 muscle, 24 kidney and 5 fat values were greater than the LOQ; in eight pig studies, 16 liver and 15 kidney samples were greater than the LOQ, with almost all at doses of 1.6–2.3 times the recommended dose; in chicken, 24 kidney samples were above the LOQ.
- Statistical tolerance limits could be calculated only for calf kidney and chicken kidney. There were too few reported values above the respective LOQs to enable estimation of statistical tolerance limits in all other species and tissues.

- Residues are consistently highest in kidney tissues in the residue depletion studies with the exception of one study. Kidney is the appropriate target tissue.
- Apramycin remains mostly unchanged and is therefore the appropriate marker residue.

The Committee recommended temporary MRLs of 5 mg/kg only in cattle and chicken kidney, measured as apramycin, based on statistical approaches. Because of data limitations, the Committee was unable to recommend MRLs in tissues and species other than cattle and chicken kidney.

Using the LOQs calculated by the Committee as residues for muscle, fat and liver, together with the proposed MRL for kidney, the theoretical intake in the worst-case scenario would be around 1400 µg/day and would not exceed the upper bound of the ADI.

The sponsor is requested to provide improved analytical methods with better performance and lower LOQs and residue depletion studies with appropriate sampling points close to the zero withdrawal periods for all tissues and species. The validated analytical methods and residue depletion studies are requested by the end of 2014.

3.3 **Derquantel**

Explanation

Derquantel (CAS No. 187865-22-1), also known as 2-desoxoparaherquamide, 2-deoxy paraherquamide, 2-DOPH, PF-00520904 and PNU-141962, is a semi-synthetic spiroindole characterized by an indole or oxindole moiety fused with a cyclopentyl ring at position C-3 of the indole. Derquantel is a broad-spectrum anthelmintic agent with activity against the adult and larval stages of gastrointestinal nematodes in sheep.

Derquantel acts as a nicotinic cholinergic receptor antagonist. Its mode of action is blockage of cation channels in nematode muscle cell membranes.

When used as an anthelmintic agent for sheep, derquantel is combined with abamectin to control infections by nematodes that are resistant to macrocyclic lactone-, levamisole-, benzimidazole- and closantel-based drenches (and combinations of these). The combination product is composed of derquantel at a concentration of 10 mg/ml and abamectin at a concentration of 1 mg/ml. It is administered to sheep and lambs 3–4 times per year as a single-dose oral drench at a dose rate of 2 mg/kg bw for derquantel and 0.2 mg/kg bw for abamectin.

Abamectin was previously evaluated by the Committee at its forty-seventh meeting ([Annex 1](#), reference 125), at which MRLs for cattle fat, liver and

kidney were proposed. An ADI of 0–1 µg/kg bw was established by the 1995 JMPR for abamectin used as a veterinary drug (9).

Derquantel has not previously been evaluated by the Committee. Derquantel was placed on the agenda of the current meeting at the request of the Nineteenth Session of CCRVDF (4). The Committee was asked to establish an ADI and recommend MRLs for derquantel in sheep tissues.

Toxicological and microbiological evaluation

The Committee considered the results of studies on receptor pharmacology, pharmacokinetics, acute and short-term toxicity, in vitro, in vivo and in silico genotoxicity, reproductive toxicity and microbiological safety. The majority of the studies were performed in accordance with GLP standards. In addition, a literature search in Embase database was performed. No additional information relevant to this evaluation was identified.

Biochemical data

In rats dosed orally with derquantel at 200 mg/kg bw per day for 4 days, peak plasma concentrations (C_{\max}) of approximately 20 µg/ml occurred at 2 hours post-dosing (T_{\max}). When rats were administered daily oral doses of derquantel ranging from 0.01 to 150 mg/kg bw per day for a longer duration (90 days, 1 year or multigeneration), the mean concentration of derquantel in plasma showed a dose-dependent increase, with no accumulation. T_{\max} was mainly 2 hours (ranging from 0.5 to 2 hours), according to the dose administered. Exposure, as measured by AUC and C_{\max} , was consistently higher in females than in males.

In rats dosed once daily by oral gavage for 7 days with the combination product containing a 10:1 ratio of derquantel and abamectin at doses up to 20/2 mg/kg bw per day, the plasma concentration of derquantel and abamectin increased with increasing doses of the combination product. Derquantel appeared not to accumulate, whereas abamectin showed accumulation. T_{\max} was 2 hours post-dosing for derquantel and 6 hours post-dosing for abamectin.

When pregnant rabbits were given derquantel orally by stomach tube once daily on days 7 through 19 of gestation at doses up to 10 mg/kg bw per day, the $AUC_{0-t(\text{last})}$ and C_{\max} values increased with increasing dose, and T_{\max} was consistently 0.5 hour post-dosing.

When dogs were given daily oral doses of derquantel of up to 10 mg/kg bw per day for 28 days, the AUC_{0-24} and C_{\max} values increased at a rate greater than proportional to increasing dose. Accumulation was found on day 27 only at 10 mg/kg bw per day. T_{\max} ranged from 0.5 to 2 hours, and the elimination half-life was 2–6 hours.

In two studies, derquantel was given to dogs as a single oral dose via capsule at dose levels up to 10 mg/kg bw per day or by oral gavage at dose levels up to 5 mg/kg bw per day for 3 months. The C_{\max} values for derquantel indicated no accumulation over time for doses of 0.1, 0.5, 1 and 5 mg/kg bw per day. Accumulation was seen at 10 mg/kg bw per day. T_{\max} was generally between 0.5 and 2 hours in the three lowest-dose groups, but ranged from 1 to 9 hours at 5 and 10 mg/kg bw per day. Half-lives generally increased with dose; however, all were relatively short, less than 7 hours.

One male and one female horse were given a single oral dose of derquantel at 2 mg/kg bw. Maximum plasma concentrations occurred at 1 hour post-dosing, with C_{\max} values of 0.157 $\mu\text{g/ml}$ and 0.075 $\mu\text{g/ml}$ and AUC values of 0.48 $\mu\text{g}\cdot\text{h/ml}$ and 0.27 $\mu\text{g}\cdot\text{h/ml}$ for the male and female, respectively. Additionally, a mono-hydroxylated metabolite of derquantel, previously not seen in other species, was identified at higher apparent plasma concentrations than for the parent derquantel.

Derquantel (1 $\mu\text{mol/l}$) was metabolized extensively in dog liver microsomes, with only 2.8% of parent detected after a 1-hour incubation. In rat, sheep and human liver microsomes, derquantel was metabolized moderately, with approximately half of the derquantel unchanged. Among 18 metabolites occurring in microsomal cultures, M3, M4, M5a and M5b were predominant in dog liver microsomes, whereas M1, M9 and M10 were found as predominant metabolites in rat, sheep and human liver microsomes. The M8 metabolite was another predominant metabolite in rats.

Hepatocytes from rat, sheep, dog and human metabolized 49–81% of derquantel (1 $\mu\text{mol/l}$) over a 4-hour incubation. Unchanged derquantel and 26 radioactive metabolites were detected in rat, sheep, dog and/or human hepatic incubations. Hepatocytes from rats, dogs, sheep and humans exhibited similar patterns of metabolites, as M1, M8, M10, M12 and M19/20 commonly occurred as predominant metabolites, with variations in the relative levels of each. Also, M18 was a prominent metabolite, accounting for 8% of the total in rat and human hepatocytes. M15 was found as a predominant metabolite only in dogs, comprising 11% of the total.

Rats (one of each sex) were given a single oral dose of [^{14}C]derquantel at 100 mg/kg bw (14.8 MBq/kg bw). Most of the radiolabel (85% and 67% for the male and female, respectively) was recovered in the faeces in the first 2 days. Approximately 95% of the radioactive dose was recovered in urine and faeces during the 7-day post-dosing period, with 87–90% of the dose in the faeces and 5.5–7.6% in the urine. Negligible amounts of the dose were detected in the carcass and terminal blood samples. The formation of approximately 11 metabolites was observed during 7 days post-dosing. The

molecular weights of some of the metabolites indicated metabolism by glucuronidation and hydroxylation.

When cannulated rats were dosed orally with [¹⁴C]derquantel at 50 mg/kg bw to identify metabolite profiles in urine, faeces, bile and tissues, the majority (69–75%) of the radioactive dose was recovered in faeces, and small amounts (3–4% and 1%, respectively) were recovered in urine and tissues. The majority of the radioactive dose (64–70%) was excreted in bile. The results showed that derquantel has high oral bioavailability. Large numbers of metabolites were found in urine, faeces and bile, indicating a high degree of biotransformation in the rat. Metabolic profiles in males and females were similar, although the rate of metabolism was more rapid in males than in females. Faeces contained mainly the parent drug at the 0- to 24-hour collection interval, whereas only small amounts of parent derquantel along with a large number of metabolites were found at the 144- to 150-hour collection interval. Parent derquantel and metabolites M1, M2, M4 and M5 are the most common components found in the tissues. In rat liver, derquantel comprised 6–8% of the total radioactivity. In rat muscle and fat, derquantel comprised higher percentages of the total residues in tissues of females (21% and 53%, respectively) relative to the males (5% and 31%, respectively).

Toxicological data

The acute oral toxicity of derquantel varies between species. Derquantel was extremely toxic to horses, but showed low toxicity in rats ($LD_{50} > 2000$ mg/kg bw). Clinical signs of acute toxicity in mice, rats and dogs were attributable to the pharmacological activity of derquantel as an antagonist of nicotinic acetylcholine receptors. Whether higher sensitivity in horses was due to a toxic metabolite or greater sensitivity to the pharmacological activity of derquantel could not be determined from the data. The primary clinical signs of acute toxicity included various effects on the nervous system, such as prostration, seizures, hypoactivity and ataxia, and various ocular effects, including mydriasis, ptosis and relaxation of the nictitating membrane. Derquantel was not a skin sensitizer in guinea-pigs or a skin or ocular irritant in rabbits.

In a non-GLP-compliant, 3-day escalating oral dose range-finding study, 3-month-old rats (two of each sex per group) received derquantel via gastric intubation at a dose of 0, 25, 50, 100, 200, 300, 400 or 600 mg/kg bw per day. Dose volumes increased with dose up to a maximum volume of 12 ml/kg bw for the 600 mg/kg bw per day group. Because of clinical signs of excessive toxicity, dosing of the 400 mg/kg bw per day group was interrupted, and the animals were allowed a 2-week washout period. Dosing then resumed at a dose level of 300 mg/kg bw per day for 2 days. The 600 mg/kg bw per

day dose was given only once due to excessive toxicity. Clinical signs of toxicity included death, hypoactivity, loss of righting reflex, ataxia, prostration, jerky movements, abnormal posture, laboured respiration, tremors and ptosis. There were no clinical signs of toxicity at doses up to and including 300 mg/kg bw per day. There was no microscopic examination of tissues. The NOAEL for this study was 300 mg/kg bw per day.

In a 12-day oral dose range-finding study, 6- to 8-week-old rats (two of each sex per group) received derquantel via gastric intubation at a dose of 0, 30, 50, 100, 150, 200, 250, 400, 600 or 800 mg/kg bw per day. Clinical signs of toxicity included death (≥ 250 mg/kg bw per day), prostration, paralysis, convulsions, jerky movements, ataxia, hypoactivity, hunched or tilted posture, piloerection, porphyrin staining around the nose and excessive salivation. No test article-related clinical signs or effects on body weight were seen up to and including 150 mg/kg bw per day. Test article-related macroscopic findings included a dose-related increase in mean liver weight of males and females in all test article-treated groups. Microscopic examinations were limited to livers of animals in the 30 and 150 mg/kg bw per day groups. Test article-related microscopic findings included peri-acinar hepatocyte swelling and hepatic cytoplasmic vacuolation with a few scattered necrotic hepatocytes at 150 mg/kg bw per day. There were no microscopic findings in the liver to correspond with increased liver weight at 30 mg/kg bw per day. Based on a slight increase in mean liver weight with no associated histopathological findings, the NOAEL for this study was 30 mg/kg bw per day.

In a 28-day oral toxicity study, 6- to 8-week-old rats received derquantel via gastric intubation at doses of 0, 20, 100 or 200 mg/kg bw per day. Clinical signs of toxicity included death (200 mg/kg bw per day), ataxia, prostration, convulsions, hypoactivity, piloerection, laboured breathing, paralysis, tilted posture, jerky movements, tremors, splayed hindlimbs and porphyrin staining around the nose. Decreases in body weight gain and feed consumption occurred in males and females at 200 mg/kg bw per day. Mean relative liver weights were significantly increased in males and females in the 100 and 200 mg/kg bw per day groups. Elevated serum gamma-glutamyltransferase (GGT) levels were seen in males and females at 200 mg/kg bw per day. Microscopically, bile duct hyperplasia of minimal severity was seen in males and females at 20 mg/kg bw per day. The severity of this finding increased to minimal to mild in males and females at 100 mg/kg bw per day and to minimal to severe in males and females at 200 mg/kg bw per day. Based on the significant increases in mean liver weights at 100 and 200 mg/kg bw per day with an associated dose-related increase in the incidence and severity of bile duct hyperplasia and elevated serum GGT levels and mortality at 200 mg/kg bw, the NOAEL for this study was 20 mg/kg bw per day.

In a 28-day oral toxicity study, 7- to 8-week-old rats received derquantel enriched with 4% *N*-oxide, a degradation product of derquantel seen during accelerated stability testing of the derquantel/abamectin drug product. Treatment was given by gastric intubation at dose levels of 0, 0.5, 5, 25 and 175 mg/kg bw per day. As a result of excessive toxicity and deaths in females, the 175 mg/kg bw per day dose level was lowered to 150 mg/kg bw per day in females only beginning on day 9. Significantly lower rearing counts were seen in females at 5 mg/kg bw per day and in males and females at 25 and 175/150 mg/kg bw per day. Additional treatment-related effects included significantly increased liver weight and hepatocellular hypertrophy at 25 mg/kg bw per day and elevations of serum GGT, alanine aminotransferase (ALT) and sorbitol dehydrogenase enzymes in males and females and significantly increased thyroid weight and follicular cell hypertrophy in females at 175/150 mg/kg bw per day. Based on lower rearing counts in females at 5 mg/kg bw per day and higher, the NOAEL for this study was 0.5 mg/kg bw per day.

In a 90-day oral toxicity study, rats less than 9 weeks of age received derquantel by gastric intubation at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day. The quality and reliability of the histopathology data were limited due to the poor quality of the slides. There were also concerns about whether the timing of the clinical observations was adequate to capture the potential neurobehavioural effects of derquantel. As a result, the Committee could not determine a NOAEL for this study.

In a 1-year oral toxicity study, 8-week-old rats received derquantel by gastric intubation at a dose of 0, 1, 5 or 50 mg/kg bw per day. There were no test article-related deaths and no clinical signs of toxicity. Evidence of toxicity in the serum chemistry data included elevated mean levels of GGT at 50 mg/kg bw per day in both males and females. In 50 mg/kg bw per day females, mean bilirubin level was significantly elevated, and mean cholesterol and triglyceride levels were increased. In males at 50 mg/kg bw per day, a significant increase in mean globulin resulted in a significant increase in mean total protein and a lower albumin to globulin ratio compared with controls. In females at 50 mg/kg bw per day, mean globulin levels were increased at all dose levels compared with controls. The difference was significant, resulting in a significant ($P < 0.01$) decrease in the albumin to globulin ratio and significantly increased total protein at this dose level. In addition, mean total protein values were significantly increased in all test article-treated groups. Cataracts seen in males at 50 mg/kg bw per day were treatment related. Treatment-related effects on the liver included enlarged livers and increased mean liver weight. Increased incidence and severity of age-related biliary hyperplasia were also seen starting at 1 mg/kg bw per day. Macroscopic and microscopic liver effects were considered adverse at 50 mg/kg bw

per day in males and females due to associated elevations in serum chemistry parameters indicative of hepatocellular injury. The severity of spontaneous, chronic progressive nephropathy was increased in males and females at 50 mg/kg bw per day. Mild to marked hypospermia and degeneration of the seminiferous tubules were adverse effects seen in males at 50 mg/kg bw per day. Minimal degeneration of the seminiferous tubules was a non-adverse test article-related finding in males at 5 mg/kg bw per day.

A second 1-year oral toxicity study in the rat was conducted with lower dose levels, which allowed for a better evaluation of the hepatobiliary effects of derquantel seen in the previous study. In this study, 8-week-old rats received derquantel by gastric intubation at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day. There were no test article-related deaths or clinical signs of toxicity. Test article-related effects in this study were limited to histopathological findings of an increased incidence and severity of biliary hyperplasia and fibrosis in the liver in males at and above 0.03 mg/kg bw per day. These were considered to be non-adverse and age-related spontaneous findings, the incidence or severity of which was accentuated by the test article. There were no associated changes in liver weights or in the serum chemistry data.

Biliary hyperplasia occurs spontaneously in rats and has not been correlated with liver carcinogenicity or neoplasia. A statistical analysis of the biliary hyperplasia response in the two chronic rat studies with derquantel comparing any positive response in each dose group with the control response showed that the incidence of biliary hyperplasia was significantly elevated relative to controls only at 50 mg/kg bw per day. In addition, this dose level was associated with significant increases in liver weight and changes in GGT levels consistent with hepatotoxicity and is therefore a clear adverse effect level. There was no statistically detectable difference in the incidence of biliary hyperplasia seen at lower dose levels, nor were there significant increases in liver weights or changes in serum chemistry parameters indicative of hepatocellular injury. Thus, 5 mg/kg bw per day would be considered the NOAEL on the basis of this analysis. This NOAEL is further supported by benchmark dose (BMD) modelling of the pooled data from both 1-year toxicity studies in the rat using various models. This modelling was performed by the sponsor and reviewed by the Committee. The lower bound of the confidence interval for a benchmark dose representative of 10% extra risk compared with placebo ($BMDL_{10}$) derived from these models ranged from 2.0 to 7.6 mg/kg bw per day, which was in close agreement with the previously statistically verified NOAEL of 5 mg/kg bw per day. On the basis of these two analyses, the Committee concluded that the NOAEL for biliary hyperplasia in the rat was 5 mg/kg bw per day. The Committee further noted that this NOAEL is consistent with the overall NOAEL that the Committee

determined for both 1-year studies, which was also 5 mg/kg bw per day based on testicular effects.

In a non-GLP-compliant 28-day preliminary oral toxicity study, dogs received derquantel as neat test article in gelatine capsules at a dose of 0, 1, 5 or 10 mg/kg bw per day. Control animals were dosed with an empty gelatine capsule. Clinical signs of dry conjunctiva, dry eyes, relaxed nictitating membrane, ptosis, red eyes or mydriasis were seen in every dog dosed with derquantel during the first week of dosing, but the incidence of dry conjunctiva and dry eyes was lower in weeks 3 and 4 (compared with the first 2 weeks of the study). There was no associated pathology. The lowest-observed-adverse-effect level (LOAEL) for this study was 1 mg/kg bw per day, based on test article-related clinical observations seen at all dose levels.

In a 28-day oral toxicity study, dogs received derquantel by gavage at a dose of 0, 0.01, 0.03 (0.01 mg/kg bw per dose, 3 times per day), 0.03 or 0.1 mg/kg bw per day. Owing to the absence of clinical signs at 0.03 mg/kg bw per day given once daily or at 0.01 mg/kg bw given 3 times daily, dosing and data collection were suspended in the 3 times daily group after 7 days of dosing. There were no test article-related effects at any dose tested. Therefore, the NOAEL for this study was 0.1 mg/kg bw per day, the highest dose tested.

In a 90-day oral toxicity study, adolescent dogs received neat derquantel by capsule at a dose of 0, 0.1, 0.5, 1 or 10 mg/kg bw per day. Clinical signs seen in all animals in all treatment groups included dry mouth, mydriasis and relaxed nictitating membranes. Dilated pupils were seen in all treated animals at both 2 and 5 hours post-dosing at one point or another in the study. The incidence increased with dose. At 24 hours post-dosing, the pupils were still dilated, with incidences of 4%, 7% and 47% at 0.5, 1 and 10 mg/kg bw per day, respectively. Ptosis was also seen in all treatment groups, with a dose-related increase in the number of animals affected and the incidence of observations in individual animals. Dry conjunctiva, dry eyes, red eyes and tremors were also treatment related in some groups based on increased numbers of animals affected and increased incidence of observations in individual animals relative to controls. Test article-related decreases in mean body weight and feed consumption of dogs at 10 mg/kg bw per day were also noted. On the basis of observed test article-related clinical signs at all dose levels, the LOAEL for this study was 0.1 mg/kg bw per day.

In a second 90-day oral toxicity study, mature dogs received derquantel by gavage at doses of 0, 0.1, 0.5, 1 or 5 mg/kg bw per day. Test article-related clinical signs included relaxed nictitating membrane in 0/0, 1/1, 2/4, 4/3 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day and mydriasis in 0/0, 1/1, 3/1, 4/4 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw

per day. In affected animals, the incidence of observations in individual animals increased with increasing dose. An increased incidence of eye redness was seen at 1 and 5 mg/kg bw per day for males and females. There was no pathology associated with the clinical signs observed. Based on treatment-related observations consistent with the known pharmacological activity of the test article at all dose levels, the LOAEL for derquantel was 0.1 mg/kg bw per day.

The Committee performed and considered the results of BMD modelling to better define a point of departure for nictitating membrane protrusion, which was among the most sensitive effects seen in the dog. The Committee concluded that the BMDL₁₀ approach was not appropriate for the purpose of this evaluation. This conclusion was based on the high degree of uncertainty at the low end of the dose–response curve, the limited data available with which to model a dose–response relationship for elicitation of protrusion of the nictitating membrane in the dog and the fact that 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).

In an adequate range of in vitro and in vivo genotoxicity studies, derquantel produced positive results in an in vitro chromosomal aberration assay in human peripheral lymphocytes, without metabolic activation, at dose levels that produced at least 50% cytotoxicity. Derquantel was not genotoxic in in vitro tests for bacterial mutagenicity or in in vivo studies in mouse bone marrow or rat hepatocytes. The Committee concluded that derquantel was weakly clastogenic in vitro, but was unlikely to be genotoxic in vivo. Derquantel had no structural alerts for mutagenicity, carcinogenicity or clastogenicity as determined by in silico analysis using the computer program DEREK.

No carcinogenicity studies were performed. Based on the absence of genotoxicity in vivo, the absence of structural alerts for carcinogenic, mutagenic or clastogenic potential by in silico analysis and lack of evidence of carcinogenicity or preneoplastic changes in rats dosed up to 1 year, the Committee concluded that derquantel is unlikely to pose a carcinogenic risk to humans at exposure levels likely to occur in food.

In a two-generation (one litter per generation) reproduction study, male and female rats were dosed with derquantel by oral gavage at dose levels of 0, 1, 5 or 25 mg/kg bw per day. Derquantel was well tolerated, with no evidence of reproductive impairment or adverse effects on progeny at 0, 1, 5 or 25 mg/kg bw per day. At 25 mg/kg bw per day, an increase in liver weight was seen in P₁ males, and P₁ females had increased thyroid weight and increased incidence of thyroid follicular epithelial cell hypertrophy and hyperplasia. Terminal body weight, liver weight and adrenal weight were also increased

in 25 mg/kg bw per day P₁ females. At 25 mg/kg bw per day, increased liver weights were also seen in F₁ males and females, and terminal body weight was increased in F₁ females. Increased incidence and severity of thyroid follicular epithelial hyperplasia were seen in F₁ males and females at 25 mg/kg bw per day. The NOAEL for reproductive effects was 25 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, derquantel was administered orally by gavage to pregnant rats at doses of 0 (vehicle), 20, 70 or 120 mg/kg bw per day on gestation days 5 through 20. The dose levels were established in a previously conducted pilot study in pregnant rats using dose levels of 0, 2, 10, 50 or 100 mg/kg bw per day. No signs of maternal toxicity were observed at the lowest dose of 20 mg/kg bw per day. Test article-related increased incidences of porphyrin discharge and piloerection were seen at 70 and 120 mg/kg bw per day. At the highest dose of 120 mg/kg bw per day, test article-related signs of toxicity included increased porphyria, subdued behaviour, laboured breathing, thin appearance, piloerection and a significant reduction in body weight gain correlating with significantly reduced feed intake. There were no biological differences in the number of corpora lutea, number of implantation sites, number of live fetuses and percentage of resorptions between treated groups and the vehicle control group. A lower trend in uterus weight was observed in dams in the 120 mg/kg bw per day group. Male fetus weight was significantly lower in the 120 mg/kg bw per day group than in vehicle control-treated animals. Ossification retardation was observed in fetuses in the 120 mg/kg bw per day group and was attributed to nonspecific inhibition or retardation of physiological growth due to the effects of maternal toxicity at this dose. Skeletal development was related to the reduced body weight of the fetuses. No soft tissue abnormalities were identified in any treatment group. Based on the results of this study, derquantel was not teratogenic. The NOAEL for maternal toxicity was 20 mg/kg bw per day, and the NOAEL for developmental toxicity was 70 mg/kg bw per day.

In another developmental toxicity study, derquantel was administered orally by gavage to pregnant rabbits at doses of 0 (vehicle), 0.1, 1 or 10 mg/kg bw per day on gestation days 7 through 19. Dose levels were determined by a previously conducted pilot study in pregnant rabbits given derquantel at dose levels of 0, 0.1, 1, 10 or 100 mg/kg bw per day. There was one death attributable to test article toxicity at 10 mg/kg bw per day. Other clinical findings at 10 mg/kg bw per day included low feed consumption, resulting in scant faeces, significant body weight loss and reduced body weight gains. At 10 mg/kg bw per day, an increase in total resorptions was seen, and fetal body weights were significantly reduced. Developmental toxicity characterized by an increased incidence of supernumerary thoracic ribs with associated

Table 1

Toxicity comparison of derquantel/abamectin combination product, derquantel and abamectin

Study type	NOAEL		
	Derquantel/abamectin	Derquantel	Abamectin
Acute oral rat	88.72/8.872 mg/kg bw ^a	>2000 mg/kg bw ^a	8–2.8 mg/kg bw ^a
Acute dermal rat	No dermal toxicity	No dermal toxicity	>330 mg/kg bw ^a
Acute (4 h) inhalation rat	>5.04 mg/l ^b	>2.4 mg/l ^b	ND
Short-term oral rat	3/0.3 mg/kg bw per day ^c 20/2 mg/kg bw per day ^d	0.5 mg/kg bw per day ^c 30 mg/kg bw per day ^e	1.5 mg/kg bw per day ^f

ND, no data

^a Estimated LD₅₀.^b LC₅₀.^c Twenty-eight-day study.^d Seven-day study.^e Twelve-day study.^f Eight-week study.

increases and decreases in the numbers of thoracic and lumbar vertebrae, respectively, at 10 mg/kg bw per day was attributable to maternal toxicity and the decreased fetal weights observed in this group. Based on the findings at 10 mg/kg bw per day, the NOAEL for developmental toxicity was 1 mg/kg bw per day.

The Committee observed that adverse effects on developmental parameters occurred only at dose levels that were maternally toxic. Therefore, the Committee concluded that derquantel had no direct reproductive or developmental toxicity potential.

For comparative toxicity purposes, results of toxicity studies with the 10 mg/ml derquantel and 1 mg/ml abamectin combination product, derquantel and abamectin are shown in [Table 1](#).

The Committee concluded that there was no indication that the derquantel/abamectin combination showed any greater toxicity than the more toxic component, abamectin. It was further noted that the pharmacokinetic profile for derquantel when given in the combination product was similar to the pharmacokinetic profile of derquantel given alone in the laboratory species tested.

When the functional antagonistic potency of derquantel on $\alpha 3$ nicotinic acetylcholine receptors endogenously expressed in rat and canine dorsal root ganglion neuron cell cultures was evaluated using whole-cell patch clamp assays, derquantel displayed more potent antagonist action against acetylcholine at canine $\alpha 3$ nicotinic acetylcholine receptors (median inhibitory

concentration [IC_{50}] of 1 $\mu\text{mol/l}$) than at rat $\alpha 3$ nicotinic acetylcholine receptors (IC_{50} of 30 $\mu\text{mol/l}$).

In an in vitro study of functional agonistic and antagonistic potency using a fluorescent imaging plate reader assay to measure calcium flux, derquantel showed no agonist activity in human $\alpha 3$, muscle-type or $\alpha 7$ nicotinic acetylcholine receptor models. Antagonist activity of derquantel was demonstrated by inhibiting the activity of nicotine at $\alpha 3$ nicotinic acetylcholine receptors of humans with an IC_{50} of 9 $\mu\text{mol/l}$. In addition, in vitro antagonistic potency of derquantel at human muscle-type nicotinic acetylcholine receptors was reported with an IC_{50} value of 10 $\mu\text{mol/l}$, but derquantel was inactive with an IC_{50} value of $>100 \mu\text{mol/l}$ at the central nervous system $\alpha 7$ nicotinic acetylcholine receptor subtype. Direct comparison of the results of this study with those from studies in dogs and rats was not possible because of differences in study design.

Microbiological data

A JECFA decision-tree approach that was adopted at the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181) and complies with VICH GL36 (7) was used by the Committee to determine the need to establish a microbiological ADI for derquantel. The decision-tree approach initially seeks to determine if there may be microbiologically active derquantel residues entering the human colon.

Derquantel is not classified as an antimicrobial agent and is not structurally related to antimicrobial agents used in animal or human medicine.

The Committee considered an in vitro evaluation of derquantel against 62 strains representing a wide range of Gram-positive and Gram-negative bacteria of veterinary importance. Derquantel showed limited strain-dependent activity against the staphylococci (MIC values ranging from 64 to 128 $\mu\text{g/ml}$ for all strains). Against the streptococci, derquantel showed very limited activity (MIC values $\geq 128 \mu\text{g/ml}$). Against *Pasteurella* spp., derquantel showed limited activity (MIC values ranging from 32 to $>128 \mu\text{g/ml}$). Derquantel was not active against the enteric organisms tested.

The Committee concluded that a microbiological ADI for derquantel is not necessary.

Evaluation

The Committee considered the acute clinical observations in dogs, which were consistent with the antagonistic activity of derquantel on nicotinic acetylcholine receptors, as the most relevant toxicological effect for the establishment of an ADI for derquantel. The LOAEL for this effect was 0.1

mg/kg bw per day. The Committee established an ADI of 0–0.3 µg/kg bw by applying an uncertainty factor of 300, using the default uncertainty factor of 100 and an additional uncertainty factor of 3 to account for setting the ADI on the basis of a LOAEL instead of a 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 receptors to derquantel.

A toxicological monograph was prepared.

Residue evaluation

The Committee reviewed studies on the pharmacokinetics and metabolism of derquantel as well as a non-radiolabelled derquantel residue depletion study in the target species. The analytical methods used in the residue depletion study were also assessed. Some of these studies were performed in compliance with GLP guidelines.

Data on pharmacokinetics and metabolism

Sheep: Two studies evaluated the pharmacokinetics of derquantel in the combination product in sheep.

For the commercial combination product, the half-life for derquantel in the combination product was 9.3 hours (range 6.1–19.3 hours) in the first study and 13.1 hours (range 9.82–19.6 hours) in the second study. The $AUC_{0-\infty}$ was 1790 ng·h/ml (range 1360–2360 ng·h/ml) in the first study and 1250 ng·h/ml (range 1000–1570 ng·h/ml) in the second study. The C_{max} was 108 ng/ml (range 80.8–145 ng/ml) in the first study and 92.8 ng/ml (range 68.2–126 ng/ml) in the second study. The T_{max} was 4.17 hours (range 3.25–5.10 hours) in the first study and 2.60 hours (range 1.05–4.16 hours) in the second study.

Two disposition studies using radioisotope-labelled derquantel in sheep were evaluated.

In the non-GLP-compliant pilot disposition study, sheep were treated orally via capsule with [¹⁴C]derquantel to achieve a target derquantel dose of 2 mg/kg bw. The animals were slaughtered 3, 6, 12, 24 and 48 hours after dosing. Faeces, urine and cage rinse samples were collected beginning on day –1 and continuing until slaughter. Blood was collected immediately prior to slaughter. In the other disposition study, sheep were treated orally via gastric tube with [¹⁴C]derquantel. All animals received a single oral derquantel dose of approximately 2.10 mg/kg bw with specific activity of the test article adjusted so that animals in the later slaughter groups received derquantel

with the higher specific activity. Animals were slaughtered approximately 6, 12, 24, 48, 96 and 144 hours after dosing. Urine, faeces, plasma, blood, bile and cage rinses were collected from each sheep daily, beginning on day -1 and continuing through slaughter.

In both studies, the majority of the radioactivity was eliminated in the faeces at each sacrifice interval after 12 hours. Approximately 50% of the radioactivity was recovered in the excreta in the first 24 hours. By 48 hours, approximately 85% of the radioactivity had been recovered in the excreta.

Two in vitro studies were conducted to evaluate the metabolism of derquantel in rats, sheep, humans and dogs.

In these metabolism studies, [¹⁴C]derquantel was incubated with rat, sheep, dog and human liver microsomes or with cryopreserved hepatocytes from rats, dogs and humans and freshly prepared hepatocytes from sheep. Enzymatic activity of the microsomes and metabolic integrity of the hepatocytes were confirmed with an appropriate positive control (non-radiolabelled 7-ethoxycoumarin). [¹⁴C]Derquantel was extensively metabolized by dog microsomes and moderately metabolized by rat, sheep and human microsomes. In addition to unchanged derquantel, 18 metabolites were detected in the microsome test system. [¹⁴C]Derquantel was extensively metabolized by hepatocytes from rats, sheep and dogs and moderately metabolized by human hepatocytes. In addition to unchanged derquantel, 26 metabolites were detected in the hepatocyte test system. In both test systems, more than 92% of the radioactivity was recovered. The studies demonstrate comparable metabolism in the tested species.

In a non-GLP-compliant study, tissues, urine, faeces and bile from sheep treated in the total [¹⁴C]derquantel residue study were evaluated for derquantel-related metabolites. Selected urine and bile samples and tissue and faecal extracts were analysed to obtain structural information on derquantel and its metabolites. Parent derquantel was detected in sheep liver, muscle, fat, urine, faeces and bile. Eight major metabolites were identified in sheep tissues.

Residue data

Sheep: One non-GLP-compliant and two GLP-compliant studies were evaluated to assess total residues of [¹⁴C]derquantel in sheep tissues.

In the non-GLP-compliant pilot disposition study, sheep were treated orally via capsule with [¹⁴C]derquantel to achieve a target derquantel dose of 2 mg/kg bw. Animals were slaughtered at 3, 6, 12, 24 and 48 hours after dosing; samples were stored frozen at an unspecified temperature pending analysis. The highest residues in each of the tissues were seen at the 6-hour sampling point. At all sampling times, total radiolabelled tissue residues were highest

in liver, followed by fat and kidney. Residues were consistently lowest in muscle. The ratio of the concentration of the marker residue to the concentration of the total residues was not determined in this pilot study.

In the first GLP-compliant disposition study, 20 crossbred sheep were treated orally via gastric tube with [¹⁴C]derquantel to deliver a single derquantel dose of approximately 2.10 mg/kg bw. Radiopurity was greater than 98.5% for all batches used. Animals were slaughtered approximately 6, 12, 24, 48, 96 and 144 hours after dosing. Samples were stored below -10 °C until analysed. As in the pilot study, total radiolabelled residues were highest in liver and lowest in muscle. Residues in kidney and fat were similar at the early sampling times. At later sampling times, residues were generally higher in kidney than in fat.

In the second study, intended to confirm the ratio of the concentration of the marker residue to the concentration of the total residues, six sheep were treated once orally via gastric tube with 2 mg [¹⁴C]derquantel per kilogram body weight. The radiopurity was approximately 92.5%. Animals were slaughtered 12, 24, 48, 96 and 672 hours (0.5, 1, 2, 4 and 28 days) post-dosing. Samples were stored below -70 °C until analysed. As in the pilot and previous GLP-compliant studies, radiolabelled total residues were highest in liver and lowest in muscle. Residues in kidney were greater than residues in fat at all sampling times.

The ratio of the concentration of the marker residue to the concentration of the total residues determined for derquantel is variable, and neither of the GLP-compliant studies provides the ideal data for determining the ratio of the concentration of the marker residue to the concentration of the total residues. The major difference in the studies was the storage temperature for the tissue samples.

To evaluate the effect of storage temperature on analyte stability, two sets of data were available. In one study, samples fortified with derquantel were stored for 1 month at -20 °C. When assayed, the derquantel concentrations were 50% of the theoretical derquantel concentrations. In a second study, conducted to support analytical method development, incurred samples were analysed initially in Australia, shipped to the USA, and reanalysed 4–5 months later using a more sensitive analytical method. Throughout, the samples were maintained at temperatures below -70 °C. In the single overlapping time point (96 hours), concentrations were comparable in both analyses. Derquantel does not appear to be stable in tissue samples of sheep under standard storage temperature (-20 °C), and samples containing derquantel require storage at temperatures of or below -70 °C to ensure stability pending

analysis. When stored at this lower temperature, tissue samples containing derquantel demonstrate stability for 4–5 months.

In recommending the final ratio of the concentration of the marker residue to the concentration of the total residues for derquantel, the results of the two GLP-compliant [¹⁴C]derquantel studies and the supportive information on derquantel stability under storage were considered. Derquantel is extensively metabolized, and derquantel represents, conservatively, 6% of total residues in muscle, 3% of total residues in liver, 7% of total residues in kidney and 15% of total residues in fat.

In the only residue depletion study evaluated, 38 sheep and 38 lambs were allocated to 12 slaughter groups. Animals were treated orally at 150% of the labelled dose of the commercial combination product: 3 mg derquantel per kilogram body weight and 0.3 mg abamectin per kilogram body weight. Animals were slaughtered at 12 hours and 1, 2, 4, 6, 8, 10, 14, 17, 21, 28 and 35 days after dosing. Samples were collected from liver, kidney, muscle, and perirenal and subcutaneous fat. Samples were stored at temperatures of –70 °C or below until analysed. Samples were analysed in Australia with an LC-MS/MS method having an LOQ of 1 µg/kg, shipped frozen to the USA, and reanalysed using an ultraperformance liquid chromatographic (UPLC-MS/MS) procedure with an LOQ of 0.1 µg/kg. Residue concentrations were highest in fats, followed by liver and kidney. Residues were consistently low in muscle. Residue concentrations depleted rapidly. In the Australian analyses, residues were below the method LOQ (1.0 µg/kg) by 6 days post-treatment. In the reanalysis, as a result of the improved sensitivity, residues above the LOQ were detected in liver and perirenal fat samples collected through 35 days. Residues were below the LOQ in muscle by 6 days post-treatment, in kidney by 8 days post-treatment and in subcutaneous fat by 17 days post-treatment. In the single overlapping time point (4 days), concentrations were comparable in both analyses.

Analytical methods

Two analytical methods are available for the determination of derquantel in sheep tissues. There are slight differences in sample preparation and in the method of detection and a 10-fold difference in LOQ. The methods are based on LC-MS/MS and UPLC-MS/MS principles.

In the original LC-MS/MS method, the data provided in the validation study showed an appropriate accuracy and precision over the range of concentrations tested (1–500 µg/kg) for derquantel quantification in sheep tissues. Intra-day accuracy of the validation samples was 84.3–99.7% for liver, 88.6–103.6% for kidney, 72.3–117.7% for muscle and 88.4–111.2% for fat. The imprecision (% coefficient of variation) for each tissue ranged between 2.4%

and 6.9% for liver, 3.2% and 11.5% for kidney, 3.2% and 24.0% for muscle and 2.3% and 29.3% for fat. The inter-day accuracy was between 86.1% and 108.6% across all tissue types and validation concentrations. The inter-day imprecision ranged from 3.6% to 25.0% but was generally less than 15% across all tissue types and validation concentrations. The LOD for the original method in tissues was between 0.04 and 0.07 µg/kg. The LOQs have been set at the lowest concentration used for the validation (i.e. 1.0 µg/kg).

The original method was further evaluated and refined as a UPLC-MS/MS method. The validation data provided demonstrated a suitable accuracy and precision for derquantel quantification over the range of concentrations tested (0.1–1 µg/kg) in sheep tissues. Intra-day accuracy of the validation samples was 52.0–139.0% in liver, 48.4–113.6% in kidney, 58.4–92.9% in muscle and 73.8–123.0% in fat. The imprecision (% coefficient of variation) for each tissue ranged between 8.5% and 16.9% in liver, 4.6% and 19.9% in kidney, 4.3% and 11.3% in muscle and 3.5% and 6.8% in fat. The inter-day accuracy was between 68.3% and 102.3% across all tissue types and validation concentrations. The inter-day imprecision ranged from 5.5% to 23.6% but was generally below 20% across all tissue types and validation concentrations. The LOD for the UPLC-MS/MS method in tissues was between 0.007 and 0.022 µg/kg. The LOQs have been set at the lowest concentration used for the validation (i.e. 0.1 µg/kg).

Maximum residue limits

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

- An ADI of 0–0.3 µg/kg bw was established by the Committee based on an acute toxicological end-point. This ADI is equivalent to up to 18 µg for a 60 kg person.
- Derquantel is extensively metabolized; derquantel represents, conservatively, 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 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 4-day sampling time, residues are highest in liver. 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.

- 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 liver and fat are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile for the 8-day post-treatment data from the unlabelled residue depletion study. The MRLs recommended for sheep muscle and kidney are twice the LOQ of the UPLC-MS/MS method.
- No MRLs were recommended for sheep milk, as no residue data were provided for milk.

The Committee recommended MRLs for derquantel in sheep tissues of 0.2 µg/kg in muscle, 2.0 µg/kg in liver, 0.2 µg/kg in kidney and 0.7 µg/kg in fat, determined as derquantel. No MRLs are recommended for sheep milk.

Because the ADI was based on an acute toxicological end-point, the Committee decided to derive a TMDI. Using the model diet and the ratio of the concentration of the marker residue to the concentration of the total residues noted above, these MRLs result in a TMDI of 8 µg/person, which represents 45% of the upper bound of the ADI.

A residue monograph was prepared.

3.4 Ivermectin

Explanation

At its Nineteenth Session, CCRVDF requested the re-evaluation of the ADI of ivermectin and, if necessary, the recommendation of new MRLs (4). Ivermectin was previously evaluated by the Committee at its thirty-sixth and fortieth meetings for toxicology ([Annex 1](#), references 91 and 104) and at its fifty-eighth meeting for the proposal of MRLs ([Annex 1](#), reference 157). The ADI established at the fortieth meeting, the Committee's most recent review of the toxicology of ivermectin, was based on effects observed in the CF-1 mouse.

Following a public request for data by the JECFA Secretariat, no data were submitted for ivermectin. However, as the current ADI for ivermectin is based on effects in the CF-1 mouse, now known to be particularly sensitive to the effects of compounds like ivermectin (avermectins), the JECFA Secretariat suggested that the Committee should consider 1) whether it was likely that a new ADI would need to be established and 2) what sorts of information would be of value in any future toxicological and residue evaluations.

Toxicological evaluation

The Committee at its fortieth meeting, in reviewing the evaluation of the thirty-sixth meeting, concluded that ivermectin was a developmental toxicant rather than an overt teratogen and acknowledged the extreme sensitivity of the CF-1 mouse to these effects. However, it was concluded that as the CF-1 mouse was the most sensitive species studied, the ADI should continue to be based on the NOEL¹ of 0.1 mg/kg bw per day for maternal toxicity in that species. On this basis, an ADI of 0–1 µg/kg bw was established.

Since this evaluation of ivermectin at its fortieth meeting, the Committee as well as JMPR have evaluated other members of the same group of compounds (avermectins). A consistent observation was that the CF-1 mouse was uniquely sensitive to the toxicity of these compounds. In the absence of information to the contrary, the NOAEL in the CF-1 mouse was therefore regarded as the critical NOAEL and served as the basis for establishing the respective ADI. Advances in understanding of the membrane transport protein P-glycoprotein in the mid-1990s led to the realization that avermectins are substrates for this transporter and that their potency in the CF-1 mouse is a consequence of a natural mutation leading to lack of expression of this protein. Two other examples of genetic absence of P-glycoprotein are known, in subpopulations of Collie dogs and Murray red cattle; no other strain or species is known in which this occurs. Studies in humans have established that P-glycoprotein is expressed in all individuals and is expressed at near adult levels in the newborn. Although polymorphisms of P-glycoprotein are known in humans, their impact on P-glycoprotein activity is relatively modest.

Such knowledge led JECFA and JMPR in their recent evaluations of avermectins to discount effects observed in the CF-1 mouse in identifying critical effects for the establishment of ADIs². The Committee at its current meeting therefore concluded that there was a need to evaluate the toxicological information on ivermectin with a view to identifying a critical effect other than in the CF-1 mouse for the establishment of an ADI.

The Committee discussed the types of information that might be of value in future toxicological evaluation of ivermectin. It concluded that information on the following would be of particular value: 1) reports on the effects of ivermectin when used as a therapeutic agent in humans and 2) information from *in vitro* and/or *in vivo* studies evaluating the critical effects upon which recent ADIs for other avermectins have been established.

¹ At its sixty-eighth meeting (Annex 1, reference 187), the Committee decided to differentiate between NOEL and NOAEL. This NOEL would now be considered a NOAEL.

² Example abamectin: last JMPR evaluation in 1997: ADI 0–0.002 mg/kg bw for sum of abamectin and 8,9-*Z* isomer (10) (previous 1994 ADI: 0–0.001 mg/kg bw for abamectin when used as a veterinary drug and when residue does not contain 8,9-*Z* isomer) (9).

Residue evaluation

Residue depletion studies of ivermectin in food-producing animals were not provided to the Committee, and no relevant residue studies of the quality and detail required by the Committee are available in the open literature. The Committee is aware that early studies that were submitted for review to its earlier meetings would not reflect the current state of scientific knowledge.

Before it could re-evaluate the residue depletion of ivermectin and propose updated MRLs, the Committee would need a submission indicating the animals and products for which MRLs are requested, marker residue depletion studies in support of proposed withdrawal times and/or in support of applications for MRLs, and pharmacokinetics and metabolism studies in food-producing animals that might enable interspecies extrapolations. A complete up-to-date list of approved products on the market together with a documentation of approved uses, including withdrawal times and all relevant parts of proprietary studies directly or indirectly supporting the approved uses, and an expert report summarizing the above content of the submission and additional relevant published data are also requested.

Methods of analysis

No information on methods of analysis was provided to the Committee.

The Committee has conducted an extensive literature search and concluded that there were suitably validated analytical methods available for the regulatory control of ivermectin residues in milk, fat, liver, kidney and muscle tissues. Suitably validated analytical methods should be provided for regulatory control based on contemporary analytical techniques for any future re-evaluation of ivermectin.

3.5 Monensin

Explanation

The Committee at its seventieth meeting evaluated the safety of residues of monensin in different animal species ([Annex 1](#), reference 193). In the evaluation, the Committee considered monensin A to be a suitable marker residue in both milk and tissues. Monensin A constitutes 98% of the monensin analogue mixture. The Committee recommended MRLs for monensin for poultry (chicken, turkey and quail) of 10 µg/kg in liver, kidney and muscle and 100 µg/kg in fat and MRLs for monensin for ruminant cattle, sheep and goat of 10 µg/kg in kidney and muscle, 20 µg/kg in liver, 100 µg/kg in fat and 2 µg/kg in milk, all of which were subsequently adopted by the Codex Alimentarius Commission. The Nineteenth Session of CCRVDF (4) requested a re-evaluation of the MRL for liver of cattle, as additional data from studies

using other treatment modalities and analytical methods had become available, showing that the existing MRL for cattle liver could be exceeded. In addition, the sponsor requested that any proposed change to the cattle liver MRL be extended to other ruminants (i.e. to goat and sheep).

The Committee at its present meeting reviewed two new residue depletion studies in cattle as well as the previously assessed residue depletion studies in cattle, in particular those using a combination of intraruminal controlled-release capsule and administration via feed. Monensin can be administered by feed to non-lactating goats and to sheep. A validated method for the determination of monensin A in cattle liver was also assessed. The original assessment of the monensin MRL for liver in cattle was based on the existing claims and warnings about the use of controlled-release capsule with feed premix in cattle, including lactating cows. The sponsor provided new information about the simultaneous use of controlled-release capsule and feed premix without warnings in certain countries. The sponsor also expressed the dose as milligrams per kilogram of body weight instead of dose per animal or dose rate in feed. The maximum dose used for monensin in cattle is now estimated by the sponsor to be 2 mg/kg bw per day.

Residue evaluation

Data on pharmacokinetics and metabolism

The metabolism and pharmacokinetics of monensin in cattle were previously reviewed by the Committee at its seventieth meeting. The data showed that monensin is extensively metabolized and rapidly eliminated.

Residue data

In cattle, total residues at zero withdrawal time were highest in the liver. In a previously assessed residue depletion study in lactating dairy cows, monensin was delivered by intraruminal controlled-release capsules (2 × 32 g monensin delivered), in a diet containing 24 mg monensin per kilogram for 10 days, followed by a diet containing 36 mg monensin per kilogram for 21 days. Using the individual daily feed intakes and animal weights, the dose was equivalent to 2.4–3 mg/kg bw per day, which is higher than the recommended maximum dose. At zero withdrawal time, monensin A was quantifiable in four of six liver samples, at concentrations ranging from 45.8 to 84.5 µg/kg (HPLC analytical method, LOQ = 25 µg/kg). The concentrations of monensin in liver were between the LOD and the LOQ in the two other liver samples. In another previously assessed residue depletion study, lactating dairy cows were treated twice daily for 7 days with gelatine capsules corresponding to a daily monensin dose of 0.9 mg/kg bw. Using an HPLC-MS/MS method (LOQ = 1 µg/kg), mean residues in liver were

9.1 µg/kg, 5.5 µg/kg and 3.1 µg/kg at 6, 18 and 30 hours, respectively, after the final dosing.

In one new study, using two controlled-release capsules and administration via the diet (24 mg monensin per kilogram feed for 21 days), the dose range was estimated to be between 1 and 2.4 mg/kg bw per day, using the individual daily feed intakes and animal weights. Monensin was quantified in one of the six liver tissue samples at zero withdrawal time. The concentration found was 25.8 µg/kg, just above the LOQ (25 µg/kg).

In a new GLP-compliant study, residues of monensin in tissues and milk of dairy cows were analysed following a single oral administration of monensin in a controlled-release capsule (32 g monensin delivered over a period of 95 days), corresponding to a mean daily dose of 0.53 mg/kg bw. Ten animals were slaughtered 14 days after administration of the controlled-release capsule, and tissue samples were collected and analysed by HPLC-MS/MS (LOQ = 0.75 µg/kg for liver). At zero withdrawal time, the highest mean residue levels were found in liver (14.9 µg/kg; $n = 10$), and the highest concentration reported was 26.3 µg/kg.

Upper tolerance limits (UTL 95/95) were calculated using the logarithmic-transformed monensin concentrations obtained in the different studies at zero withdrawal time. The low number of animals with quantified residue concentrations in liver led to UTL 95/95 values higher than 100 µg/kg.

Analytical methods

An analytical method for liver residue of monensin A based on HPLC-MS/MS was reviewed. The method of analysis involved repeated liquid extractions of liver in isooctane/ethyl acetate followed by solid-phase extraction cleanup of a portion of the extract. Nigericin was used as internal standard. The method, which has been validated at 30 µg/kg, has an LOD of 0.08 µg/kg and an LOQ of 0.75 µg/kg. Accuracy, precision and specificity have been assessed. The results of the validation study were acceptable and demonstrated that the method is fit for the purpose of monitoring monensin A levels in liver.

Maximum residue limits

In recommending a revised MRL for monensin in cattle liver, the Committee considered the following factors:

- An ADI of 0–10 µg/kg bw was established by the seventieth meeting of the Committee based on a chronic toxicological end-point. This ADI is equivalent to up to 600 µg monensin for a 60 kg person.
- Monensin A is a suitable marker residue in liver.

- Monensin A is extensively metabolized and represents conservatively 5% of total residues in tissues and 2.7% in milk.
- Different oral formulations of monensin and intraruminal controlled-release capsules are approved for use in cattle or lactating cows. Concomitant administration of these formulations and intraruminal controlled-release capsule would lead to a maximum daily dose regimen up to 2 mg/kg bw, according to the sponsor.
- At zero withdrawal time, one GLP study based on the administration of one controlled-release capsule to lactating cows showed that the existing MRL for liver originally set at 20 µg/kg was exceeded. The UTL 95/95 of monensin A in cattle liver was calculated to be slightly above 100 µg/kg. This value is explained partly by the uncertainty associated with the low number of animals ($n = 10$) slaughtered at the zero withdrawal time point.
- An HPLC-MS/MS method with adequate performance parameters and method validation was provided and was considered suitable for routine monitoring of monensin A as marker residue.
- Using the data issued from the over-dosage studies conducted with a combination of two controlled-release capsules and medicated feed, liver concentrations higher than 25 µg/kg were reported for a dose rate above 2 mg/kg bw. Under the assumption of dose linearity, for a maximum daily dose of 2 mg/kg bw, the UTL 95/95 led to a value significantly higher than 100 µg/kg. This value is explained by the uncertainty associated with the low number of reported values ($n = 5$) and lack of information on the residue concentrations below the method LOQ (25 µg/kg).

On the basis of the residue study performed with the controlled-release capsule administered alone to lactating cows, the Committee recommended a revision of the MRL for cattle liver to 100 µg/kg, determined as monensin A.

The combined use of the controlled-release capsule and feed premix in cattle at the highest dosage reported by the sponsor will likely result in residues in liver in excess of the revised MRL of 100 µg/kg.

For goat and sheep, no additional residue data were provided by the sponsor. Without any additional data, the Committee was unable to revise the current MRLs.

Using the revised MRL, the TMDI from the seventieth meeting was recalculated, resulting in a value of 481 µg/day per person, which represents 80% of the upper bound of the ADI.

An addendum to the residue monograph was prepared.

3.6 Monepantel

Explanation

Monepantel (CAS No. 887148-69-8), also known as AHC 2102225, is an anthelmintic of the amino-acetonitrile derivative class, indicated for the treatment of nematodes in sheep. The recommended dose is 2.5 mg/kg bw, and the maximum dose used is 3.75 mg/kg bw. Monepantel is the *S*-enantiomer of an optically active molecule. It exerts its nematocidal action through activation of a nematode-specific subfamily of nicotinic acetylcholine receptors.

Monepantel has not previously been evaluated by the Committee. It was included on the agenda of the current meeting of the Committee at the request of the Nineteenth Session of CCRVDF (4).

Toxicological and microbiological evaluation

The Committee considered data from studies on pharmacodynamics, pharmacokinetics, including metabolism, short-term toxicity, genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity submitted by the sponsor. A supplementary literature search was performed that revealed only one additional study considered to be of relevance. All pivotal studies reported were conducted in line with GLP and other relevant standards.

Biochemical data

Pharmacokinetic studies were performed in rats, dogs and sheep, as well as in vitro.

Following oral administration of monepantel to rats, uptake and elimination were rapid. Absorption was approximately 30%, whereas bioavailability of unchanged monepantel was slightly less than 10%, indicating significant first-pass metabolism. The peak concentration of radiolabelled material in blood was reached between 2 and 8 hours after oral administration. Radioactivity was poorly distributed to tissues, with highest levels in liver and fat, followed by adrenals, pancreas and ovaries. Levels in kidney were intermediate, and those in muscle were lowest. Residue levels in organs and tissues were generally higher in females than in males. There was little evidence of accumulation of radioactivity in tissues after seven daily doses. The terminal elimination half-life of radioactivity from blood was 55–60 hours. More than 90% of orally administered monepantel was eliminated in the faeces by 168 hours after dosing, with less than 5% excreted in urine.

In a non-GLP-compliant study in the dog using the racemic mixture of monepantel and the corresponding *R*-enantiomer, absorption after oral

administration was rapid, with maximal blood concentrations occurring 1–2 hours after administration. The half-life of elimination was 44 hours. The oral bioavailability of combined parent substance and sulfone metabolite was 24%. Blood levels of the sulfone metabolite far exceeded those of the parent compound.

Protein binding of radiolabelled monepantel was investigated in vitro using rat, dog, sheep and bovine plasma. The results showed a high level of protein binding (96.2–99.5%), independent of monepantel concentration and the species from which the plasma was obtained.

Following oral administration of radiolabelled monepantel in the rat, the predominant compounds seen in blood were the sulfone derivative and the parent compound. The main components in faeces were M3 (an oxidized metabolite of the sulfone), the parent compound and the sulfone derivative. Parent compound was not present in bile or urine. The predominant compound seen in muscle, fat, liver and kidney was the sulfone metabolite, although parent compound was also seen in significant amounts. All significant residues seen in edible sheep tissues were also detected in rat tissues.

In in vitro studies, intrinsic clearance rates for monepantel were lower in mouse and dog liver microsomes than in rat and human liver microsomes.

In liver microsomal fractions from rats administered daily doses of monepantel for 4 weeks, there was an increase in total cytochrome P450 (CYP) content, a slight increase in 7-ethoxyresorufin *O*-deethylase (CYP1A1) activity, as well as a slight increase in lauric acid 11- and 12-hydroxylase (CYP4A1) activities. CYP1A2- and CYP2B1-dependent activities were not increased. Microsomal glucuronidation of 3-methyl-2-nitrophenol was increased. Plasma concentrations of thyroid stimulating hormone, thyroxine and triiodothyronine were not affected. It is concluded that there is some evidence that monepantel is a weak inducer of xenobiotic metabolizing enzymes, but there is little evidence that it resembles phenobarbital in its induction profile.

Toxicological data

The LD₅₀ of monepantel in rats following oral or dermal administration has been reported to be greater than 2000 mg/kg bw. Monepantel has been reported to be non-irritating to the skin of rabbits and slightly irritating to the rabbit eye.

In a 13-week study in mice with dietary concentrations of 0, 30, 120, 600 and 6000 ppm, liver was the target organ. Increased total bilirubin was seen at 600 and 6000 ppm, and a possible effect on lipid metabolism was demonstrated by slightly increased cholesterol levels at 600 and 6000 ppm, corroborated by

increased incidences of fatty liver change at all doses. At 600 and 6000 ppm, females had increased levels of focal necrosis and lymphoid cell infiltrates. It is notable that histological effects of the test substance were seen predominantly in females. Liver enzyme effects (increased mean plasma aspartate aminotransferase [AST], ALT and alkaline phosphatase activities) were seen at a range of doses, but often without a clear dose–response relationship. The LOAEL was 30 ppm (equal to 5.27 mg/kg bw per day), the lowest dose tested, based on an increased incidence of fatty change in the liver of females.

In a 4-week study in rats with dietary concentrations of 0, 1000, 4000 and 12 000 ppm, disturbed fat metabolism was suggested by increased cholesterol, triglyceride and phospholipid levels in females at all doses and increased cholesterol and phospholipid levels in males at 4000 and 12 000 ppm. Increased absolute and relative liver weights were seen in females at all doses and in males at 4000 and 12 000 ppm. Centrilobular hepatocellular hypertrophy (males and females) and diffuse follicular hypertrophy of the thyroid (males only) were seen at all doses. The LOAEL was 1000 ppm (equal to 86 mg/kg bw per day), the lowest dose tested.

In a 13-week study in rats with dietary concentrations of 0, 50, 200, 1000 and 12 000 ppm, the main target organ was the liver, reflected by increased absolute and relative liver weights and centrilobular hepatocellular hypertrophy at 1000 and 12 000 ppm in females. Biochemical findings indicative of liver effects included elevated levels of cholesterol and phospholipids (females at 1000 and 12 000 ppm) and triglycerides (males and females at 12 000 ppm), indicative of effects on lipid metabolism, and increased total protein (males at 12 000 ppm) and albumin levels (males at 1000 and 12 000 ppm) in blood. In addition, absolute and relative weights of adrenals and spleen were increased at the highest dose level in females, although without associated histological findings. High-dose findings also included hypospermatogenesis in males and sex cord stromal hypertrophy or hyperplasia in females. Histological findings were largely reversible, although some evidence of hypospermatogenesis was still present at the end of the 4-week recovery period. The NOAEL was 200 ppm (equal to 15.20 mg/kg bw per day), based on effects on the liver (centrilobular hypertrophy and increased cholesterol and phospholipid levels) in females at 1000 ppm (equal to 81.45 mg/kg bw per day).

In a 52-week study in rats with dietary concentrations of 0, 50, 200, 1000 and 12 000 ppm, an effect on lipid metabolism was evident from increased cholesterol, phospholipid and triglyceride levels at 12 000 ppm. Increases in absolute and relative liver and relative kidney weights were also seen at this dose. The NOAEL was 1000 ppm (equal to 54.45 mg/kg bw per day), based on increased absolute and relative liver weights and increased cholesterol,

triglyceride and phospholipid levels, indicative of effects on the liver, at 12 000 ppm (equal to 656.08 mg/kg bw per day).

A 4-week dose range-finding study in dogs with dietary concentrations of 0, 5000, 15 000 and 40 000 ppm (equal to 0, 161.2, 566.1 and 1216.6 mg/kg bw per day in males and 0, 183.6, 561.0 and 1472.4 mg/kg bw per day in females) identified the liver, thymus, adrenal gland and thyroid as targets of toxicity. Alkaline phosphatase activity was increased at all test doses, although not in a clearly dose-dependent manner. Absolute and relative thymus weights were reduced at all doses, and increased severity of thymus involution was seen at 40 000 ppm. Absolute and relative adrenal gland, liver and thyroid weights (females only) were also altered, but with no associated histological effects. Body weight gain and feed consumption were reduced in males at 40 000 ppm.

In a 13-week study in dogs with dietary concentrations of 0, 300, 3000 and 30 000 ppm, the main target of toxicity was the liver, with increased alkaline phosphatase activity seen at all doses, increased absolute and relative liver weights seen at all doses and histological findings, which were reversible, also seen in the liver (hepatocellular hypertrophy in males at 30 000 ppm and in females at all doses, biliary proliferation at 3000 and 30 000 ppm and brown pigment in Kupffer cells and hepatocytes at 30 000 ppm in males and at 3000 and 30 000 ppm in females). Other effects of note included reduced activated partial thromboplastin time in males and females at 3000 and 30 000 ppm. Liver effects seen at 300 ppm were considered to be non-adverse, as these consisted of biochemical changes that did not reach statistical significance and the severity of which did not increase over time, hepatocellular hypertrophy in a single female animal and increased relative liver weights that were not associated with consistent histological effects and were not statistically significantly different from control values. The NOAEL was 300 ppm (equal to 9.9 mg/kg bw per day), based on hepatocellular hypertrophy, biliary hyperplasia, increased alkaline phosphatase activity and reduced activated partial thromboplastin time at 3000 ppm (equal to 96.8 mg/kg bw per day).

In a 52-week study in dogs with dietary concentrations of 0, 100, 300 and 3000 ppm, alkaline phosphatase activity was statistically significantly increased at 300 ppm (females only) and 3000 ppm (both sexes). Other effects on the liver included effects on blood protein (3000 ppm, both sexes), decreased plasma albumin to globulin ratios (females only at 300 ppm, both sexes at 3000 ppm), increased ALT activity (3000 ppm, both sexes), increased GGT activity (3000 ppm, males only), increased absolute and relative liver weights at all doses (statistically significant in females at 300 ppm and in males at 3000 ppm), hepatocellular hypertrophy (all doses, both sexes),

brown pigment corresponding to lipofuscin in hepatocytes, Kupffer cells and macrophages (300 and 3000 ppm, both sexes) and bile duct hyperplasia (3000 ppm, both sexes). Increased absolute and relative thyroid weights (with no histological correlate) were seen at 300 ppm (females only) and 3000 ppm (both sexes). Electron microscopy of the liver revealed smooth endoplasmic reticulum proliferation, consistent with induction of xenobiotic metabolizing enzymes. The NOAEL was 100 ppm (equal to 2.96 mg/kg bw per day), based on increased alkaline phosphatase activity, decreased albumin to globulin ratio, increased thyroid weights and increased pigmentation in liver at 300 ppm (equal to 8.21 mg/kg bw per day).

Single-dose and repeated-dose target species tolerance studies were performed in sheep using oral administration. The single-dose study used a dose of 37.5 mg/kg bw (10 times the recommended dose), whereas the repeated-dose study used doses of 3.75, 11.25 and 18.75 mg/kg bw administered every 3 weeks, on a total of eight occasions. No findings considered to be toxicologically significant were seen in either study.

In an 18-month carcinogenicity study in mice, animals received monepantel in the diet at a concentration of 0, 10, 30, 120 or 500 ppm. At the top dose, slightly increased mortality in females was noted, as well as some changes in haematological parameters (decreased red blood cell count, increased white blood cell count, decreased eosinophil count). Increased absolute and relative liver weights were seen in females at 120 and 500 ppm. The only test item-related effects noted in the histological examinations consisted of an increased incidence of fatty change in liver at all doses. The effect demonstrated a dose-response relationship in females and was statistically significant at 120 and 500 ppm in males and females. Although the effect did not reach statistical significance at 10 and 30 ppm, the Committee considered that the effect in females at 30 ppm could not be dismissed. A statistically significant increase in the incidence of hepatocellular hypertrophy was noted at all test substance doses except for the top dose in females. Although the hypertrophy was clearly increased in all treated groups, there was no dose-response relationship. There was no indication of carcinogenic potential of monepantel in this study. The NOAEL was 10 ppm (equal to 1.8 mg/kg bw per day), based on increased incidence of fatty change in liver of females seen at 30 ppm (equal to 5.5 mg/kg bw per day) and supported by increased hepatocellular hypertrophy.

In a 2-year carcinogenicity study in rats, animals received monepantel in the diet at a concentration of 0, 100, 1000 or 12 000 ppm. Decreased body weights in females relative to controls were seen at 12 000 ppm. Increased absolute and relative liver, heart and kidney weights were seen in females at 1000 and 12 000 ppm, and absolute and relative mean thymus weights were

increased at all doses in males, although the effect did not reach statistical significance. The other finding of note was an increase in macroscopic foci in adrenal glands in females (present in 2% of controls, 4% at 100 ppm, 6% at 1000 ppm and 16% at 12 000 ppm) that reached statistical significance at 12 000 ppm. No evidence of carcinogenicity was seen in the rat. The NOAEL was 100 ppm (equal to 4.63 mg/kg bw per day), based on organ weight changes and macroscopic observations in the adrenal glands at 1000 ppm (equal to 47.40 mg/kg bw per day).

The genotoxicity of monepantel was investigated in vitro in mutation studies in *Salmonella typhimurium* (Ames test) and a chromosomal aberration test in cultured human peripheral blood lymphocytes and in vivo in a mouse bone marrow micronucleus test. No evidence of genotoxicity was seen.

The racemic sulfone metabolite AHC 2092404 was also tested in a screening version of the Ames test, with no evidence of mutagenicity.

In light of the negative results in genotoxicity studies and the lack of carcinogenicity in mice and rats, monepantel is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats, animals received monepantel in the diet at a concentration of 0, 200, 1500 or 12 000 ppm. The main effects seen on parental animals were consistent with those seen in the repeated-dose studies (increased liver weights associated with hepatocellular hypertrophy, increased adrenal gland weights associated with cortical cell hypertrophy). Increased relative liver weights were also noted in F₁ and F₂ pups, but no associated histopathology was seen in F₂ pups (F₁ pups were not examined histologically). The other notable effect was an increased incidence of hyperplasia of the sex cord stromal cells in the ovary at 12 000 ppm in F₁ parental animals. No evidence of reproductive toxicity was seen in this study, and the NOAEL for reproductive toxicity was therefore 12 000 ppm (equal to 647 mg/kg bw per day), the highest dose tested. The NOAEL for general effects in the P and F₁ generation parental animals was 200 ppm (equal to 13.5 mg/kg bw per day), based on macroscopically observed enlarged livers in P generation females, increased absolute and relative liver weights associated with centrilobular hepatocellular hypertrophy in P and F₁ generation females, increased absolute and relative adrenal gland weights in F₁ generation females and cortical cell hypertrophy in P and F₁ generation females seen at 1500 ppm (equal to 103 mg/kg bw per day). The NOAEL for offspring toxicity was 1500 ppm (equal to 103 mg/kg bw per day), based on increased relative liver weights seen at 12 000 ppm (equal to 863 mg/kg bw per day) in F₁ and F₂ generation pups.

Developmental toxicity studies were performed in rats and rabbits with doses of up to 1000 mg/kg bw per day from day 6 post-coitum to day 20 (in rats) or 27 (in rabbits). Monepantel was well tolerated in maternal animals, and no findings considered to be test substance related were seen in maternal or fetal animals. The NOAEL for maternal toxicity and for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested, in both rats and rabbits.

The Committee concluded that monepantel was not teratogenic or otherwise developmentally toxic in rats or rabbits.

Special studies were carried out in rats to investigate the effects of monepantel on cardiovascular and respiratory end-points, behavioural end-points (modified Irwin screen test), intestinal motility and delayed contact hypersensitivity. Monepantel did not induce notable effects on any of these end-points.

No data were identified on the effects of monepantel in humans.

On the basis of the toxicological findings, the Committee considered liver to be the primary target of toxicity for monepantel, as demonstrated by effects on relevant biochemical variables, absolute and relative liver weights and histological findings. The Committee observed that, although, in some cases, effects on the relevant end-points failed to demonstrate a clear dose–response relationship, there was a consistent effect of monepantel on the liver across studies and species.

The Committee considered the most appropriate point of departure for the derivation of the toxicological ADI to be the NOAEL of 10 ppm (equal to 1.8 mg/kg bw per day) in a 78-week oral dosing study in mice, based on an increased incidence of fatty change in the liver of females seen at 30 ppm (equal to 5.5 mg/kg bw per day) and supported by increased hepatocellular hypertrophy.

Microbiological data

No data on microbiological effects of monepantel were available for evaluation. In the absence of any evidence to suggest that the substance has a microbiological effect, such data are not required.

Evaluation

The Committee established an ADI of 0–20 µg/kg bw on the basis of a NOAEL of 1.8 mg/kg bw per day in a 78-week oral dosing study in mice, based on an increased incidence of fatty change in the liver of females seen at 5.5 mg/kg bw per day, application of a safety factor of 100 and rounding to one significant figure.

A toxicological monograph was prepared.

Residue evaluation

Data on pharmacokinetics and metabolism

A pharmacokinetic GLP-compliant study was performed. Thirty-six sheep were allocated to five groups. In the first and second groups, respectively, monepantel and monepantel sulfone were administered intravenously at a dose rate of 1 mg/kg bw. In the remaining groups, monepantel was administered at 1, 3 or 10 mg/kg bw as an oral drench. Blood samples were collected at 1, 2, 4, 7, 14, 21 and 28 days post-dosing. Monepantel and monepantel sulfone concentrations in blood were quantified by a validated HPLC method with an LOQ of 3 ng/ml for both analytes. In blood and plasma, the monepantel concentration decreased rapidly after intravenous administration for 48 hours, at which time it was no longer detected. Mean total body clearance was 1.49 l/kg bw per hour, and the volume of distribution was 7.4 l/kg bw. The monepantel sulfone concentration in blood declined for 4 days, at which time it was no longer detected. The mean total body clearance (0.28 l/kg bw per hour) for monepantel sulfone was lower than that for monepantel, and a higher volume of distribution (31 l/kg bw) was estimated for this compound. The oral bioavailability of monepantel was 31%. The AUC for monepantel sulfone after oral administration of monepantel at a dose of 1 mg/kg bw was close to the value obtained after intravenous administration of monepantel sulfone, demonstrating an almost complete first-pass effect and a total absorption of the oral dose of monepantel.

In an ADME and residue depletion GLP-compliant study in which a single oral dose of 5 mg [¹⁴C]monepantel per kilogram body weight as a 2.5% weight per volume (w/v) solution was administered to sheep, approximately 50% of the administered radioactivity was recovered in faeces and 30% in urine after 14 days. Two different positions of the ¹⁴C label in monepantel were used to assess other possible modes of metabolism for the compound. Groups of sheep received the radiolabel on either ring or an equimolar mixture of each labelled substance. The position of the radiolabel on either ring did not influence the interpretation of the total radioactivity or the metabolic profile in tissue and excreta. The metabolite profile was analysed, and seven metabolites were identified. After oxidation, monepantel was metabolized to a sulfoxide and a sulfone, which was identified as the predominant metabolite. A second metabolic pathway involved cleavage to yield the phenol metabolite together with its sulfate conjugate. Monepantel sulfone was the major metabolite found in blood and tissue and represented 100% of radioactivity in blood. Fat was the tissue with the highest concentration of radioactivity,

followed by liver, kidney and muscle. The approximate proportions of total radioactive residues were 10 (fat) : 5 (liver) : 2 (kidney) : 1 (muscle).

Residue data

In the ADME and residue depletion GLP-compliant study, edible tissues (fat, muscle, kidney and liver) were collected at time points ranging from 2 to 35 days post-dosing. Collected samples were analysed for the total radioactive residue concentrations and expressed as monepantel equivalent. Monepantel and monepantel sulfone were quantified in tissue using a validated HPLC/ultraviolet (UV) method with a chiral column and an LOQ of 50 µg/kg, and the results were compared.

The concentrations of radiolabelled monepantel quantified in the tissue matrices decreased in the order fat > liver > kidney > muscle. The corresponding concentrations of monepantel sulfone residues decreased in the same order. After administration, monepantel residues were quantifiable (LOQ = 50 µg/kg) for 14 days in fat, 7 days in muscle and only 2 days in kidney and liver, whereas monepantel sulfone was still quantifiable 35 days post-dosing in fat, liver, kidney and muscle tissues.

Total radioactive residues expressed as monepantel equivalent were compared with monepantel and monepantel sulfone tissue concentrations at different time points from 2 to 35 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 to that of the total residue was calculated as 1 for muscle and 0.66 for fat, liver and kidney.

Three depletion studies were evaluated. In the first study, 32 lambs were administered a 3.8 mg/kg bw dose of monepantel. Groups of eight animals were terminated at 7, 18, 29 and 40 days after treatment. In the second study, 47 lambs were administered a 3.9 mg/kg bw dose of monepantel. Groups of eight animals were terminated at 7, 19, 29, 40, 70 and 77 days after treatment. In the third study, 52 sheep were administered a 3.8 mg/kg bw dose of monepantel. Animals were terminated at 7, 18, 29, 35, 70, 120 and 127 days after treatment. In all three studies, a group of four untreated animals served as control. Monepantel sulfone was quantified in tissue samples collected from these studies using a validated analytical method with an LOQ of 10 µg/kg.

Residue concentration data from the three single oral dose administration studies were statistically compared. An analysis of variance study of the data indicated that there was no significant difference in observed residues among the three studies at the significance level of 5%. Therefore, the three data sets were pooled and used for the statistical analysis according to the

procedure established at the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181). The median ($n = 24$) concentrations of monepantel sulfone measured in the animal tissues 7 days post-dosing were 2620 µg/kg in fat, 1295 µg/kg in liver, 406 µg/kg in kidney and 152 µg/kg in muscle.

Analytical methods

A validated method based on acetonitrile extraction, centrifugation and quantification on a two-column switching HPLC/UV system was available and was used for the analysis of incurred residues of monepantel as its sulfone metabolite in edible sheep tissues. The method provided fit-for-purpose performances for muscle, kidney, liver and fat samples. Concentrations in tissue samples were determined by reference to non-matrix-matched, external standard calibration curves. The method is not enantiomer specific and will determine both enantiomers of monepantel sulfone. Specificity and selectivity of the method were assessed. LODs and LOQs were estimated as the mean detector response plus 3 times and 10 times the standard deviation of the mean for each tissue type, respectively. The estimated LOQs of 51 µg/kg for fat, 56 µg/kg for liver, 13 µg/kg for kidney and 15 µg/kg for muscle tissue were validated by replicated fortified samples. Accuracy estimation was based on recovery calculation at different concentrations starting from 50 µg/kg. The overall mean recoveries ranged from 91% to 106%, and intra-day recoveries were within the 70–110% range. The intra-day and inter-day precision measured at 50 µg/kg expressed as coefficients of variation were $\leq 20\%$ and $\leq 25\%$, respectively.

Maximum residue limits

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

- Monepantel is registered for use in sheep at a maximum recommended single oral dose of 3.75 mg/kg bw.
- An ADI for monepantel of 0–20 µg/kg bw was established by the Committee, corresponding to an upper bound of acceptable intake of 1200 µg/day for a 60 kg person.
- Monepantel is extensively metabolized.
- 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 the concentration of total residues are 1 in muscle and 0.66 in fat, liver and kidney.

- Residue data were determined using a validated analytical method to quantify monepantel sulfone in tissue.
- 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.
- The time point at which the MRLs were set was based on an EDI < ADI approach described at the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181).
- No data are provided for sheep milk.

The Committee recommended MRLs for 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. Using the food basket model diet and a ratio of marker residue to total residue of 100% for muscle and 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 201 µg/person per day, which represents 17% of the upper bound of the ADI.

A residue monograph was prepared.

3.7 **Narasin**

Explanation

The Committee evaluated the safety of residues of narasin in several species of food-producing animals at its seventieth meeting ([Annex 1](#), reference 193). In its evaluation, the Committee considered narasin A to be a suitable marker residue for narasin in animal tissues of cattle, pigs and chickens. The Committee recommended temporary MRLs for narasin A in cattle tissues, as the available analytical method was not considered fit for purpose. The temporary MRLs for cattle were based on the LOQ values for the HPLC/UV methods.

Additional information and validation data on an HPLC-MS/MS method for analysis of narasin in cattle tissues were received and reviewed.

Residue evaluation

Analytical methods

The sponsor provided a new GLP-compliant HPLC-MS/MS method. In this method, muscle, liver or kidney test samples are initially processed from

sample material at approximately $-20\text{ }^{\circ}\text{C}$. Processing involves solvent extraction, sample cleanup using silica solid-phase purification and reconstitution in methanol for LC-MS/MS analysis. Quantification is from a matrix-matched calibration line and is based on narasin A. Fat samples use an additional step (refrigeration at $4\text{ }^{\circ}\text{C}$ for 15 minutes). The alternative is employed to minimize particulates forming a suspension. Nigericin ($\text{C}_{40}\text{H}_{67}\text{NaO}_{11}$, molecular weight 746.94) is used as an internal standard.

Analytical measurement employs HPLC separation using a C18 column and elution at $40\text{ }^{\circ}\text{C}$ using a gradient mixed phase of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The MS/MS analysis employs selected reaction monitoring acquisition mode using a positive electrospray ion source. Transitions monitored for quantitative determination of narasin are $787.5>431.3$, and confirmation, $787.5>531.3$ and $787.5>279.2$. For the internal standard, nigericin, the transition monitored is $746.6>729.6$.

Method performance

System suitability was demonstrated based upon column efficiency, peak width at half height, tailing factor and system precision for each test item. System linearity was demonstrated over the concentration range of 0.5–100 $\mu\text{g}/\text{kg}$ (all tissues) for matrix-matched calibration standards prepared in extracted control samples for each matrix. Assay LODs and LOQs were reported. The LOQ in muscle, liver and kidney was 0.75 $\mu\text{g}/\text{kg}$, and in fat, 1.0 $\mu\text{g}/\text{kg}$.

Accuracy was measured as per cent recovery and precision as the coefficient of variation at the LOQ of 0.75 $\mu\text{g}/\text{kg}$. For muscle, accuracy was 75%, with a coefficient of variation of 2.9%. Corresponding values for liver were 94% and 6.3%; kidney, 96% and 5.4%; and fat, 88% and 2.4%. Inter-day accuracy and precision were determined at $\frac{1}{2}$ MRL, MRL and $2 \times$ MRL. Accuracy and precision for all four tissues were uniformly consistent for each tissue across all three tissue concentrations. System suitability was measured by column efficiency, peak width at half height and tailing factor for the test items and internal standards. Performance factors were established by 10 replicate injections.

The system LOQ for each test item was determined by the extraction and analysis of six replicate aliquots of control matrix fortified with decreasing concentrations of each test item and assaying these samples with the standard method. The LOQ was determined to be 0.75 $\mu\text{g}/\text{kg}$ for muscle, liver and kidney tissues and 1.0 $\mu\text{g}/\text{kg}$ for fat tissue. The target intra-day assay accuracy at the LOQ was 70–110%. The precision at each concentration was less than or equal to 20%.

The specificity of the assay for each test item and the internal standard was examined by extraction and analysis of aliquots of each matrix with and without the addition of the test item. The assay requirement for each test item was no significant interfering substances greater than 20% of the peak area at the LOQ. Several analytes were used to evaluate the potential for interference: penicillin, tylosin, tilmicosin, tetracycline, lasalocid, ceftiofur, ractopamine and ketoprofen. This study was limited to injection of solution standards.

The reports documented acceptable system suitability, system linearity, accuracy and precision, LODs and LOQs, but did not specify the limit of identification; however, it is expected to be consistent with the LOQ. Other performance factors demonstrating method performance and method validation include intra- and inter-day accuracy and precision performance, analytical specificity with a number of veterinary antimicrobial drugs, analyte-fortified storage stability, freeze/thaw and extended frozen storage stability, autosampler stability and solution stability. The method description, reagents, equipment, mass spectrometry settings and conditions are adequately described. Data provided should enable a regulatory laboratory to develop specific quality control and quality assurance for regulatory programme use.

Maximum residue limits

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

- A new HPLC-MS/MS method validated in a GLP-compliant study and exhibiting adequate performance characteristics was provided that was considered suitable for routine monitoring of narasin A as marker residues.
- The analytical method has been validated for use in cattle tissues and is also appropriate for chicken and pig tissues.

The seventieth meeting of the Committee recommended temporary MRLs of 50 µg/kg for cattle liver and fat and 15 µg/kg for cattle muscle and kidney, determined as narasin A. The LOQs for the new analytical method for cattle tissues are more than adequate to accommodate the MRLs recommended at the seventieth meeting of the Committee for other animal species and tissues.

The Committee recommended full MRLs for narasin of 15 µg/kg for cattle muscle and kidney and 50 µg/kg for liver and fat tissues, determined as narasin A.

An addendum to the residue monograph was prepared.

3.8 Triclabendazole

Explanation

The Committee previously reviewed triclabendazole at its fortieth, sixty-sixth and seventieth meetings ([Annex 1](#), references 104, 181 and 193). At its seventieth meeting, the Committee recommended revised MRLs for muscle, liver, kidney and fat of cattle and sheep. The present Committee reviewed triclabendazole again at the request of the Nineteenth Session of CCRVDF (4), which had asked whether MRLs for goat tissues could be established by extrapolation considering data used for recommending MRLs for cattle and sheep tissues.

The sponsor resubmitted the data used by the Committee at its seventieth meeting, together with an addendum to the previous expert report and a literature search, which was extended by the Committee.

The Committee specifically re-evaluated pharmacokinetic, metabolism and residue data that were considered relevant for investigating the possibility of extrapolating MRLs from sheep to goat. Many approved commercial triclabendazole products used in sheep are also recommended for use in goat, and the recommended doses are typically the same (with some exceptions, more or less uniformly oral doses of 10 mg/kg bw). Most studies conducted in cattle were not evaluated again because the Committee at its seventieth meeting had already concluded that the kinetic behaviour of triclabendazole was distinctly different in cattle and sheep and that there was no basis for establishing MRLs of identical numerical values for the two species.

Pharmacokinetics

No state-of-the-art comparative pharmacokinetic study conducted in the same laboratory and using a commercial product or equivalent formulation in a sufficient number of animals of both species was available. A study carried out with ¹⁴C-labelled triclabendazole in one goat and in one sheep was of limited value. Kinetics of radioactivity in plasma was qualitatively similar in the goat and the sheep, but quantitatively different. The cumulative excretion pattern of the radioactivity was very similar in both animals in this study, and the metabolites identified were the same. A study in the open literature (11) reported no statistically significant differences in the values for T_{max} , C_{max} and AUC of the two major metabolites of triclabendazole (the sulfoxide and the sulfone) in the two species of animal. However, in reviewing this publication, the Committee concluded that there was a statistically significant difference between the results obtained for the AUC in the two species.

Evaluation of residues

Kinetic residue depletion studies were not performed in the goat.

Approximately 2.4% of the radioactivity administered in the above-mentioned study was calculated to be present in blood and tissues of the goat on day 10 after treatment (3.03% in the similarly treated sheep). Radioactivity in most tissues was higher in the sheep than in the goat. Residues in goat tissues were convertible to the marker residue used for cattle and sheep, and the analytical method was also validated for tissues of goats. However, as the comparison between cattle and sheep had already shown at the previous evaluations, these similarities are insufficient to conclude that residue kinetics would also be the same or quantitatively similar.

Muscle is the only one of the four edible standard tissues of the JECFA food basket for which the ratio of marker residue concentration to total residue concentration in the goat is known for day 10 after treatment. The numerical value was similar in muscle of sheep on day 10 and in calves and sheep on day 28 after treatment.

Exposure assessment

The results of the exposure modelling that was performed by the Committee at its seventieth meeting have shown that the bioavailability of residues must be taken into account. The factor developed by the Committee at that meeting for incurred residues in liver of cattle (13%) needs to be used for all tissues of the three ruminants in the absence of data for the other tissues and species.

Maximum residue limits

The procedure for deriving MRLs adopted at the sixty-sixth meeting of the Committee could not be used, because the necessary data were not available.

The Committee concluded that the available database on the residues of tri-clabendazole in goat did not allow a scientifically justifiable extrapolation of MRLs to this species of animal.

An addendum to the residue monograph was prepared.

4. Future work

Information on registration/approval status of veterinary drugs

The Committee requests that the JECFA Secretariat always include a request for submission of information on registration status of veterinary drugs and on approved conditions of use by the sponsors of the data into future calls for data. The Secretariat should also verify that such information is contained in the data submission of sponsors before it gives work assignments to the experts of the Committee.

Extrapolation of MRLs

The Committee agrees that it is important to develop minimum criteria for information upon which to base extrapolation between food animal species and commodities and recommends that the JECFA Secretariat establish an electronic working group to continue work commenced at the current meeting and to develop proposed minimum criteria for consideration at the next JECFA meeting for veterinary drugs.

Decision-tree approach to the evaluation of residues of veterinary drugs

The Committee recommends that the JECFA Secretariat establish an electronic working group to elaborate principles to establish ARfDs for residues of veterinary drugs, taking the guidance developed by JMPR into account as well as ongoing efforts by VICH. A draft guidance document will be prepared for discussion at the next meeting of JECFA for veterinary drugs.

The Committee recommends that the JECFA Secretariat establish an electronic working group to develop the scope of work for application of the TTC approach to veterinary drugs and to develop a project plan to address this work.

Guidance for JECFA Experts

The Committee requests that the JECFA Secretariat update the guidance to experts for the preparation of meeting documents, including clear advice on those issues identified at recent meetings.

5. Recommendations

Recommendations relating to specific veterinary drugs, including ADIs and proposed MRLs, are given in [section 3](#) and [Annex 2](#). Recommendations relating to future work by the JECFA Secretariat are given in [section 4](#).

1. With respect to the CCRVDF electronic working group to develop risk management options for veterinary drugs for which no ADI and/or MRL has been recommended by JECFA due to specific human health concerns, the Committee emphasized that any requests to JECFA for further assessments on such compounds need to be accompanied by a clear description of the specific request from CCRVDF and formulation of the risk management needs.
2. The Committee requests that CCRVDF provide the JECFA Secretariat with information on registration/approval status and the use pattern of veterinary drugs whenever it requests an evaluation by JECFA.
3. The Committee noted that a step for providing a preliminary risk assessment, covered under the problem formulation step in the hypothesis-driven decision-tree approach for the safety evaluation of residues of veterinary drugs, is mentioned in the risk analysis policy as applied by CCRVDF, but that this step is currently not being implemented. The Committee recommends that CCRVDF, when updating its risk analysis policy, develop guidance on how to implement this step in the future.

Acknowledgement

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Annex 1

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

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. II. *Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
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 11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
 12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases*. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
 13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
 14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
 15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents*. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
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Annex 2

Recommendations on compounds on the agenda and further information required

Amoxicillin (antimicrobial agent)

Acceptable daily intake: The Committee established an acceptable daily intake (ADI) of 0–0.7 µg/kg body weight (bw) on the basis of microbiological effects.

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

Residue definition: Amoxicillin

Recommended maximum residue limits (MRLs)

Species	Fat ^a (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)	Milk (µg/kg)
Cattle	50	50	50	50	4
Sheep	50	50	50	50	4
Pigs	50	50	50	50	

^a Includes skin plus fat in pigs.

Apramycin (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–30 µg/kg bw on the basis of microbiological effects.

Estimated dietary exposure: Using the limits of quantification (LOQs) of the analytical methods as calculated by the Committee as residue levels for muscle, fat and liver, together with the proposed MRL for kidney, the theoretical intake in the worst-case scenario would be around 1400 µg/day and would not exceed the upper bound of the ADI.

Residue definition: Apramycin

Recommended maximum residue limits (MRLs)

Species	Kidney (µg/kg)
Cattle	5000 ^a
Chickens	5000 ^a

^a The MRLs are temporary. Because of data limitations, the Committee was unable to recommend MRLs in tissues and species other than cattle kidney and chicken kidney. The sponsor is requested to provide improved analytical methods with better performance and lower LOQs and residue depletion studies with appropriate sampling points close to the zero withdrawal periods for all tissues and species. The validated analytical methods and residue depletion studies are requested by the end of 2014.

Derquantel (anthelmintic)

Acceptable daily intake: The Committee established an ADI of 0–0.3 µg/kg bw on the basis of a lowest-observed-adverse-effect level (LOAEL) of 0.1 mg/kg bw per day for acute clinical observations in dogs, consistent with antagonistic activity on the nicotinic acetylcholine receptors. A safety factor of 300 was applied to the LOAEL.

Estimated dietary exposure: As the ADI was based on an acute effect, the Committee did not calculate an EDI. Using the model diet of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 litres of milk with the MRLs recommended, the

TMDI is 8 µg/person, which represents 45% 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	0.7	0.2	2.0	0.2

The Committee was not able to recommend an MRL for sheep milk, as no residue data were provided.

Ivermectin (anthelmintic)

No data on ivermectin were submitted following a public request for data.

The Committee previously established an ADI of 0–1 µg/kg bw at its fortieth meeting (WHO TRS No. 832, 1993). The Committee concluded at the current meeting that there was a need to evaluate the toxicological information on ivermectin with a view to identifying a critical effect other than in the CF-1 mouse for the establishment of an ADI. Information that would be of value in a future toxicological evaluation of ivermectin includes reports on the effects of ivermectin when used as a therapeutic agent in humans and information from in vitro and/or in vivo studies evaluating the critical effects upon which recent ADIs for other avermectins have been established.

MRLs were proposed at the fifty-eighth meeting (WHO TRS No. 911, 2002). Before it could re-evaluate the residue depletion of ivermectin and propose updated MRLs, the Committee would need a submission indicating the animals and products for which MRLs are requested, marker residue depletion studies in support of proposed withdrawal times and/or in support of applications for MRLs, and pharmacokinetics and metabolism studies in food-producing animals that might enable interspecies extrapolations. A complete up-to-date list of approved products on the market together with documentation of approved uses, including withdrawal times and all relevant parts of proprietary studies directly or indirectly supporting the approved uses, and an expert report summarizing the above content of the submission and additional relevant published data are also requested. Suitably validated analytical methods should be provided for regulatory control based on contemporary analytical techniques for any future re-evaluation of ivermectin.

Monensin (antimicrobial agent and production aid)

Acceptable daily intake:	The Committee established an ADI of 0–10 µg/kg bw at its seventieth meeting (WHO TRS No. 954, 2009).
Estimated dietary exposure:	Using the revised MRL, the exposure estimate (TMDI) from the seventieth meeting of the Committee was recalculated, resulting in a value of 481 µg/person, which represents 80% of the upper bound of the ADI.
Residue definition:	Monensin A

Recommended maximum residue limits (MRLs)

Species	Liver (µg/kg)
Cattle	100

The Committee was unable to revise the current MRLs for goats and sheep, as no additional residue data were provided.

Monepantel (anthelmintic)

Acceptable daily intake:	The Committee established an ADI of 0–20 µg/kg bw on the basis of a no-observed-adverse-effect level (NOAEL) of 1.8 mg/kg bw per day, considering liver effects in mice, and a safety factor of 100, with rounding to one significant figure.
Estimated dietary exposure:	Using the model diet and a ratio of marker residue to total residue of 100% for muscle and 66% for fat, liver and kidney, and applying a correction factor of 0.94 to account for the mass difference between the marker residue and monepantel, the EDI is 201 µg/person, which represents 17% of the upper bound of the ADI.
Residue definition:	Monepantel sulfone

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Sheep	5500	700	3000	300

The Committee was unable to propose an MRL for sheep milk, as no data were provided.

Narasin (antimicrobial agent and production aid)

Acceptable daily intake: The Committee established an ADI of 0–5 µg/kg bw on the basis of a NOAEL of 0.5 mg/kg bw per day and a safety factor of 100 at its seventieth meeting (WHO TRS No. 954, 2009).

Residue definition: Narasin A

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	50	15	50	15

The Committee recommended full MRLs, as a validated analytical method for residue control purposes is available and was evaluated as satisfactory for the purpose.

Triclabendazole (anthelmintic)

The Committee concluded that the available database on the residues of triclabendazole in goat was too limited to allow a scientifically justifiable extrapolation of MRLs for cattle and sheep tissues to this species of animal.

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 comments on documents under elaboration for the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), information on registration/approval status of veterinary drugs, extrapolation of maximum residue limits (MRLs), 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 antimicrobial agents (amoxicillin, apramycin), four anthelmintics (derquantel, ivermectin, monepantel, triclabendazole) and two antimicrobial agents and production aids (monensin and narasin). Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes (ADIs) and proposed MRLs.

