# 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 veterinary drugs within the terms of reference of JECFA, including compounds without an ADI or MRL; recommendations on principles and methods in derivation of MRLs, including a new procedure for estimating chronic dietary intakes; the use of a spreadsheet-based procedure for the statistical evaluation of residue depletion data; a revised approach for the derivation of microbiological ADIs; and the Committee's review of and comments on documents provided by the Codex Committee on Residues of Veterinary Drugs. Summaries follow of the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: three antimicrobial agents (colistin, erythromycin, flumequine), two production aids (melengestrol acetate, ractopamine hydrochloride), an insecticide (trichlorfon (metrifonate)) and an anthelminthic (triclabendazole). In addition, the attempt by the Committee to use tylosin as an example to investigate if evaluations are possible based on published data in the absence of data submissions from sponsors is described. Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes and proposed maximum residue limits.

Sixty-sixth report of the
Joint FAO/WHO Expert Committee on
Food Additives

EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

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Sixty-sixth report of the
Joint FAO/WHO Expert Committee on
Food Additives

World Health
Organization

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Sixty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Rome, 22–28 February 2006

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. Boobis, Experimental Medicine and Toxicology, Division of Medicine, Faculty of Medicine, Imperial College, London, England

Dr R. Ellis, Consultant, Myrtle Beach, SC, USA

Dr A. Fernández Suárez, Instituto Nacional de Tecnología Agropecuaria, Instituto de Tecnología de los Alimentos, Buenos Aires, Argentina[1]

Dr K. Greenlees, Toxicologist, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA

Dr J. MacNeil, Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon Laboratory, Saskatoon, Saskatchewan, Canada

Dr J.G. McLean, Professor Emeritus, Camberwell, Victoria, Australia *(Chairman)*

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

Dr J.L. Rojas Martínez, Laboratorio Nacional de Servicios Veterinarios, Ministerio de Agricultura y Ganadería, Barreal de Heredia, Heredia, Costa Rica

Dr P. Sanders, Directeur, Agence Française de Sécurité Sanitaire des aliments, Laboratoire d'études et de Recherches sur les médicaments vétérinaires et les disinfectants, Fougères, France

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

Professor J. Wongtavatchai, Faculty of Veterinary Science, Department of Medicine, Chulalongkorn University, Bangkok, Thailand[2]

Secretariat

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

Dr C.E. Cerniglia, Director, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, AR, USA *(WHO Temporary Adviser)*

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[1] Dr A. Fernández Suárez was unable to attend the meeting but prepared the first draft of one of the residue monographs.
[2] Professor J. Wongtavatchai was unable to attend the meeting but prepared the first draft of one of the residue monographs.

Dr M. de Lourdes Costarrica, Senior Officer, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Staff Member)*

Dr L.G. Friedlander, Leader, Residue Chemistry Team, Division of Human Food Safety, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA *(FAO Consultant)*

Dr N. Iseki, Senior Standards Officer, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Staff Member)*

Dr Sang-Hee Jeong, Senior Researcher, Toxicology and Chemistry Division, National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang City, Republic of Korea *(WHO Temporary Adviser)*

Dr J. Lewicki, Division of Pharmacology and Toxicology, Department of Preclinical Sciences, Warsaw Agricultural University, Warsaw, Poland *(FAO Consultant)*

Professor S. McEwen, Professor and Interim Chair, Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada *(WHO Temporary Adviser)*

Dr S. Ozawa, Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan *(WHO Temporary Adviser)*[1]

Dr P. Reeves, Australian Pesticides and Veterinary Medicines Authority, Kingston, ACT, Australia *(FAO Consultant)*

Professor L. Ritter, Executive Director, Canadian Network of Toxicology Centres, Professor, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada *(WHO Temporary Adviser)*

Dr G. Roberts, Consultant Toxicologist, Greenway, ACT, Australia *(WHO Temporary Adviser)*

Ms M. Sheffer, Ottawa, Ontario, Canada *(WHO Editor)*

Dr S. Sundlof, Director, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA *(WHO Temporary Adviser)*

Dr A. Tritscher, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland *(WHO Joint Secretary)*

Dr A. Wennberg, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Joint Secretary)*

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[1] Dr S. Ozawa was invited but unable to attend the meeting.

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. 57, in preparation

Residue monographs are issued separately by FAO under the title:

Residues of some veterinary drugs in animals and foods. FAO JECFA Monographs, No. 2, 2006.

1. Introduction

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held at the Food and Agriculture Organization of the United Nations (FAO) Headquarters, Rome, from 22 to 28 February 2006. The meeting was opened by Ms Louise Fresco, Assistant Director-General, FAO, on behalf of the Directors-General of FAO and the World Health Organization (WHO). Ms Fresco informed the Committee of the recent decisions on the reform of FAO taken by the FAO Conference to better meet the demands of Member countries for improved efficiency in the achievement of the objectives of the organization. Consequent to the decisions taken, the Food and Nutrition Division, renamed the Nutrition and Consumer Protection Division, which hosts the FAO JECFA Secretariat, has been moved to the Agriculture, Biosecurity, Nutrition and Consumer Protection Department in line with the farm-to-table approach to issues of food safety and quality. Ms Fresco emphasized that expert scientific advice is one of the cornerstones in the process to develop and apply food safety standards, and the work of JECFA and other international expert bodies that provide scientific advice remains a high priority for FAO.

In reference to the task of JECFA at the present meeting, Ms Fresco referred to the recommendations of a FAO/WHO workshop held in Bangkok, Thailand, in 2004 (*1*) on substances used in veterinary medicine for which at the present time no international risk assessment has been concluded. Ms Fresco emphasized that the recommendations from the Committee will be highly valuable in the continued work of the Codex Alimentarius Commission and countries around the world, in particular developing countries.

Sixteen 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* and *169*) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984 (*2*). The present meeting[1] was convened to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

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

— To elaborate further 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 provide recommendations on possible alternative risk assessment principles and approaches for substances without established ADIs and/or MRLs in the international context (see section 2.1).

1.1 Declarations of interest

The Secretariat informed the Committee that all experts participating in the sixty-sixth JECFA meeting have completed declaration of interest forms and that no conflict of interest has been identified.

2. General considerations

2.1 General principles regarding the evaluation of veterinary drugs within the terms of reference of JECFA, including compounds without ADI or MRL

The Committee considered in detail the recommendation from the Bangkok workshop (*1*) and the draft paper prepared by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) working group to address recommendations from this workshop in relation to veterinary drugs with no JECFA ADI or MRL (*3*). In addition, other relevant parts of the 2005 Bilthoven MRL Workshop final report (*4*) were considered. In this context, the Committee discussed a number of closely linked issues, including data availability for compounds to be evaluated and the general terms of reference of the Committee, which are reported together because of their close linkage.

Response to recommendations of the 2004 Bangkok workshop and the 2005 report of the Codex Working Group on Residues of Veterinary Drugs without ADI/MRL

Considering the recommendations of the 2004 *Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/ MRL*, the Committee noted that there existed a potential misunderstanding conveyed by the report of the data requirements for certain risk assessment tools. Mathematical modelling tools such as the

benchmark dose offer alternative approaches to the traditional no-observed-effect level (NOEL) approach, but still require qualitatively similar dose–response data. The threshold of toxicological concern, while offering an alternative to the compound-specific data needed for an ADI, requires a significant number of physicochemical, pharmacological and toxicological data on the compound class, as well as chemical structure and exposure data on the compound of interest. Similarly, analysis of the risk presented to consumers by residues of a veterinary drug in the absence of an ADI calls for much the same kinds of data as are necessary to establish an ADI and MRL. The Committee also noted the usefulness of alternative approaches to the evaluation of veterinary drugs and the potential to provide meaningful information to risk managers responsible for mitigating this risk, particularly when it is not possible to set an ADI.

The role and relationship of risk management and risk assessment in the evaluation process

The risk analysis paradigm sets out specific roles for risk management and risk assessment. One of the roles of risk management is to formulate requests for specific information to be developed through a scientific risk assessment process. It is important that the specific information requests be clearly articulated to ensure that the risk assessment response will properly address the problem identified by risk management. The evaluation of residues of veterinary drugs by JECFA is designed to provide answers to a series of information requests from the Codex risk managers. While seldom spelled out, these questions typically take the following form:

JECFA is requested to develop the following scientific information regarding the veterinary drug:

1. Characterize the hazard for human consumption presented by residues of the drug in edible tissues, milk and eggs.
2. Establish an ADI for residues of the drug.
3. Recommend a maximum limit for residues of the drug in the edible tissues, milk and eggs of target species that will not result in an exposure of the human consumer in excess of the ADI.
4. In the event that an ADI or MRL cannot be determined:
   a. Define the scientific basis that prevents the determination of an ADI or MRL, identify the data gaps and characterize the hazard for human consumption presented by the drug.
   b. Characterize the exposure of the human consumer to residues of the veterinary drug in the edible tissues, milk and eggs of treated animals.

  c. Recommend analytical methods and concentrations derived from the performance characteristics of the method that could be used to manage the risk presented by residues in food.
5. Provide advice on the characterization of the health risk of compounds from specific exposure scenarios.

The Committee further noted that the nature of the risk assessment determines the data needed for an adequate evaluation of the veterinary drug. In particular, it noted that the development of the MRL is dependent upon information related to, and developed in accordance with, good practice in the use of the veterinary drug of interest. The critical impact of this veterinary drug use information underscores the need for provision of information resulting from the registration process in competent national authorities for the intended use of the veterinary drug.

Criteria for compounds to come on the JECFA agenda

The Committee considered the current criteria established by Codex for veterinary drugs to be evaluated by JECFA. These criteria are:

> In order to be placed on the CCRVDF priority list for the development of an MRL, the candidate veterinary drug, when used in accordance with good veterinary practices, should meet some, but not necessarily all, of the following criteria:

> 1. Use of the drug will have potential to cause public health and/or trade problems;
> 2. Drug available as commercial product;
> 3. Commitment that a dossier will be available.

The Committee considered that the process of prioritization of veterinary drugs for evaluation by Codex and the process of risk assessment of the veterinary drug by JECFA would be greatly improved by adherence to these criteria and provision of the information to the JECFA Secretariat.

The Committee expressed concern regarding recent experience with veterinary drugs submitted for evaluation where data relevant to the risk assessment were either inadequate or not available to the Committee. The Committee suggested that the request for evaluation by a member country be accompanied by evidence of the nature and extent of the available data. While there are a number of ways to provide this information, the Committee suggested that a table of contents of the material to be provided would be a valuable tool in assessing the availability of data for evaluation. In addition, the Committee noted that document CX/RVDF 06/16/10 (5), dated October

2005, contains an annex entitled "Template for the Establishment of a Preliminary Risk Profile" (see section 2.2), which may be useful in this context. The information identified in this annex would be extremely useful in the risk assessment of veterinary drugs if provided to JECFA with the initial request for evaluation of the compound.

Issues relating to data availability

In reaching its conclusions on ADIs and MRLs, the Committee evaluates the available data, including those submitted by the sponsor and those identified in a search of the open literature. The Committee's decisions depend on consideration of the primary data. Limited reliance is placed on summary or review data alone, if not supported by relevant primary data. On a number of occasions, limited or at times no data are available for evaluation of compounds on the meeting agenda. Hence, in these instances, the Committee is unable to complete its evaluation because of significant gaps in the database. On such occasions, the Committee will identify the critical gaps and will suggest those additional data that should enable the evaluation to be concluded. The Committee is concerned that even after a reasonable time interval, appropriate data are not being either generated or submitted to the Committee. It is important to note that JECFA is not a regulatory body and has no means to compel data submission. Hence, possible strategies to help resolve these issues were sought.

The Committee proposes that two lists of veterinary drugs of public health concern be introduced. These lists would include the following categories of veterinary drugs:

i) Veterinary drugs for which significant concerns had been identified, either because of incomplete information or pending resolution of a problem identified in the evaluation;
ii) Veterinary drugs for which the significant concerns noted in i) were not addressed, despite requests for data to resolve the outstanding issues. It is recommended that these compounds should not be used in food-producing animals until outstanding data are provided and evaluated by JECFA.

Compounds would remain in category i) for a specified period and then either would be removed from the list because of resolution of the concerns or would be moved to category ii). The Committee recommends that CCRVDF take an active role in establishing and supporting such lists and should emphasize the need for Codex members and commercial entities to fulfil their responsibility in submitting relevant data in a timely manner.

Considerations related to the terms of reference of JECFA

Information on approved uses

Assessment of efficacy is not within the mandate of the Committee. However, since one of the criteria for scheduling a compound for JECFA evaluation is that the veterinary product containing the active compound is currently registered by a national or regional authority, confirmation of its authorization, including approved dosages and conditions of use, should be provided in the data submission.

Risk–benefit comparison

The Committee recognizes that CCRVDF may use risk–benefit considerations in prioritizing compounds for evaluation. The number of veterinary drugs available and approved for certain therapeutic indications is very limited, and there is general concern that loss of a compound may have significant impact on food animals and derived products. Consideration of the relative benefit provided by the availability of such a drug is outside the scope of the Committee, which has neither the mandate nor the expertise to address such questions. Hence, JECFA will continue to restrict its considerations to the human health risks of the compound.

Considerations related to flexibility in the scientific process of the JECFA risk assessment

The Committee discussed the rapid developments in science typified by the fields of genomics, proteomics, analytical chemistry, mathematical modelling and toxicological testing, together with the need to be able to bring to bear the most appropriate tools in the evaluation of veterinary drugs. The Committee recognized the continued need for flexibility in its approach and the importance of balancing this flexibility with consistency. The Committee also recognized that some of these new tools and technologies may require validation.

JECFA risk assessment should not be tied to specific approaches. JECFA will continue to apply the necessary flexibility to bring to bear the most appropriate science and risk assessment techniques.

A decision-tree approach in the evaluation of veterinary drugs by JECFA

The Committee recommended that the JECFA Secretariat convene a working group to develop a general decision-tree for the evaluation of veterinary drugs, which would identify different options for hazard identification, hazard characterization and exposure assessment. The proposed approach will then be discussed at the next JECFA meeting dedicated to the assessment of veterinary drugs. The decision-tree

would be anticipated to provide a tool to assist in assessing different options in the evaluation of the veterinary drug, including the determination of a "traditional" ADI and recommended MRL. The decision-tree is envisioned as a flexible document that will be adapted to advancement in science and in response to the nature of the compounds under evaluation. The working group will be expected to develop possible branches to the decision-tree to make use of the best science available. Other options that may be considered are the use of a threshold of toxicological concern as an alternative to an ADI and recommendations for analytical methods for the detection of residues of the drug in the absence of a formal MRL.

2.2 Comments on the CCRVDF document "Risk management methodologies, including risk assessment policies in the Codex Committees on Residues of Veterinary Drugs in Foods"

The Committee discussed the document (*5*), in particular Appendix 1, and provides the following comments to CX/RVDF 06/16/10:

General remarks

The document has changed significantly from previous versions that JECFA had commented on, not only by title and content, but also in scope. The current title does not match the actual content, which covers both risk assessment and risk management within the context of Codex and the respective roles of CCRVDF and JECFA. Hence, it is recommended that the title be changed to reflect the fact that the document covers risk analysis principles: "Risk Analysis Principles Applied by the Codex Committee on Residues of Veterinary Drugs in Food".

The current document introduces terminology — for example, risk profile and level of protection — that is used in microbiological risk analysis and currently is not used by JECFA and CCRVDF in the evaluation of veterinary drug residues. Overall, it is not clear if the document is describing current procedures or a way of working that should be achieved in the future.

It was brought to the Committee's attention by the Secretariat that the corresponding document by the Codex Committee on Pesticide Residues (CCPR) is significantly different in level of detail and scope, as well as in the terminology used. The Committee noted that JECFA and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) have undertaken efforts to harmonize their procedures. Although JECFA and JMPR are independent scientific expert bodies, the main

users of their scientific advice are the respective Codex Committees, CCRVDF and CCPR. It is therefore desirable that the Codex Committees also harmonize their procedures as appropriate.

Specific comments

Appendix 1
1. Purpose — Scope
The current text does not give sufficient explanation of the purpose of the document.

3. Risk Management in CCRVDF
It is not clear if this part describes what CCRVDF understands to be current practice or how risk management activities should be undertaken in the future. Once the responsibilities of risk assessors and risk managers are clearly defined in the document, the process in general as described would greatly facilitate the interaction between CCRVDF as the risk management body and JECFA as the risk assessment body.

In the current text in this section of Appendix 1, there is no clear description or separation of the roles and responsibilities of CCRVDF and JECFA. To this end, it would be useful to separate the roles of CCRVDF and JECFA and to separate out risk management and risk assessment activities.

The need for a clear request from the risk manager, CCRVDF, to the risk assessor, JECFA, is implicit in several places, but should be stated more explicitly, as should the importance of dialogue between the two to ensure that the form of the risk assessment meets the requirements of the risk manager. As an example, JECFA might be asked to consider the consequences for human health of a number of risk management options.

Some of the suggestions have significant logistical and resource implications. Hence, some distinction needs to be made between what is desirable and what is essential. Some consideration needs to be given to how these logistical and resource limitations can be overcome.

Some of the proposals would require significant changes in risk assessment practice. Such changes would have implications far beyond the activities of JECFA. Hence, consideration should be given to the need to ensure harmonization, for example through the International Programme on Chemical Safety (IPCS).

The document should provide guidance as to the basis for requesting JECFA to reconsider an evaluation, to ensure the integrity of the process.

Annex to Appendix 1: Template for the establishment of a preliminary risk profile
The Committee concluded that this document would be very useful in the preliminary evaluation of veterinary drugs and in prioritizing the compounds for evaluation (although, as indicated above, the term "risk profile" should be reconsidered).

Appendix 2: Proposed draft risk assessment policy for the setting of MRLVDs [maximum residue limits for veterinary drugs] in food
As in Appendix 1, this appendix needs to distinguish clearly between the roles of CCRVDF as risk manager and JECFA as risk assessor.

Consideration needs to be given to how best to balance the scientific integrity and expertise of the risk assessment with other issues related to membership of JECFA, such as geographical distribution. In addition, to aid the development of capacity, the training of experts needs consideration.

The document should reflect and build upon previous international consensus, for example on core principles for the provision of scientific advice.

There is a lack of clarity with respect to the issue of intake assessment. It is not clear whether a major change in approach is being recommended; if so, the feasibility of this needs to be considered.

2.3 Expression of the ADI and derivation of the MRL

Introduction

CCRVDF at its 15th session (*6*) discussed rounding practices when establishing ADIs and recommending MRLs for veterinary drug residues and requested JECFA to comment on certain practices suggested by CCRVDF.

The Committee considered the expression of the ADI at its thirty-sixth meeting in 1990 (Annex 1, reference *91*). The Committee decided to express the ADI numerically to only one significant figure. If an ADI is calculated from a NOEL that has more than one significant figure, the ADI would therefore be rounded to one significant figure, consistent with accepted rounding procedures.

In the past, JECFA has applied its rounding practice to the derivation of ADIs for 25 veterinary drugs, resulting in 14 ADIs that have been rounded down and 11 ADIs that have been rounded up. Most of the veterinary drugs that have been reviewed by JECFA resulted in a calculated ADI of one significant figure without rounding.

The present Committee noted that the recommendation from CCRVDF in its report of the 15th session (*6*) suggests a misunderstanding of the relationship between the ADI and the derivation of the MRL.

General considerations at the current meeting

One of the functions of JECFA is to establish health-based guidance values for residues of veterinary drugs, most often an ADI. The ADI is an output of a risk assessment of the compound, following application of the first two steps of the risk assessment paradigm: hazard identification and hazard characterization. As such, it represents a health-based guidance value, where exposure is considered to represent a negligible risk to consumers if it does not exceed this value. The ADI has a number of uses in risk assessment and risk management, only one of which is in helping to derive the recommended MRLs.

The MRL and the ADI are separate outputs of the risk assessment process and serve different purposes.

The ADI is derived from the NOEL or lowest-observed-effect level (LOEL) from the appropriate toxicological studies, using a safety factor. Given that there are assumptions and uncertainties in deriving the ADI, such as the use of safety factors, the use of a range of doses in toxicological studies and normal biological variation, it is more meaningful to express the ADI to only one significant figure to avoid any inference of inappropriate precision.

The general rounding rule for mid-way values (x.5) is to round up, in line with common convention (see, for example, Australian Standard AS 2706-2003 (*7*)). Examples for rounding to one significant figure are as follows: 1.25 becomes 1, 0.73 becomes 0.7 and 1.5 becomes 2.

The MRL recommendation procedure is an iterative process. The MRL is not derived directly from the ADI. If the ADI is based on toxicological end-points, all residues of toxicological relevance are considered; if the ADI is based on microbiological end-points, all residues of microbiological relevance are considered. The MRL recommendation procedure also takes into account the conditions of use (e.g. use of the veterinary drug according to good practice in the use of veterinary drugs, or GPVD) and the residues that result from such use (e.g. residue depletion studies). It also considers results of radiolabel residue studies, the bioavailability of bound residues, the identification of target tissues and a marker residue, the availability of practical analytical methods, estimated exposure resulting from recommended MRLs and consideration of extension of the MRLs to tissues, eggs and milk of other species.

The initial consideration in recommending an MRL is whether it is sufficiently protective of human health. If the use of the veterinary drug yields an estimated intake of veterinary drug residues consistent with the ADI, the recommended MRLs may then be adjusted accordingly when taking into account the other factors noted above. As a general principle, the Committee will not normally recommend an MRL that results in residue levels that lead to dietary intake exceeding the ADI based on toxicological or microbiological considerations.

To protect consumers in all segments of the population, historically the Committee has based its recommendations on intakes estimated using a conservative model diet consisting of 300 g of muscle, 100 g of liver, 50 g of kidney and fat, 1.5 kg of milk and 100 g of eggs. Previously, the Committee estimated intakes by using MRLs to derive a theoretical maximum daily intake (TMDI). At the current meeting, the Committee modified this procedure and is now using the median residue levels to derive an estimated daily intake (EDI) to better reflect estimates of chronic (lifetime) exposure (see section 2.4.1).

Figure 1 is an update of the figure prepared during the Bilthoven MRL workshop (4).

Figure 1
The JECFA residue evaluation process

JECFA Residue Evaluation
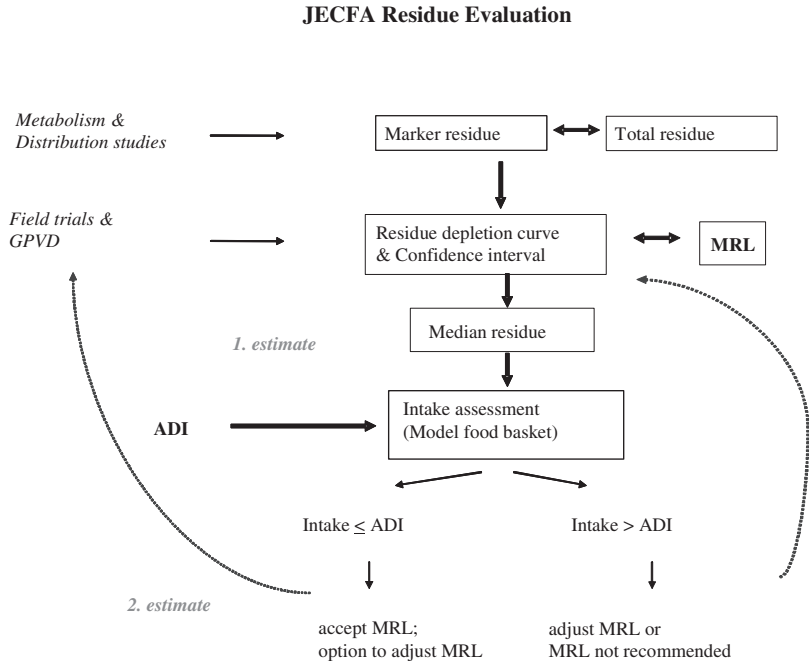
Conclusions

The Committee confirmed that the rounding practices used in expressing the ADI are scientifically and mathematically sound. In addition, since the ADI is not directly used in the derivation of the MRL, the JECFA rounding practices have no direct consequence on the MRL.

2.4 Recommendations on principles and methods in derivation of MRLs

FAO, the Netherlands National Institute for Public Health and the Environment (RIVM) and WHO organized a joint workshop on "Updating the Principles and Methods of Risk Assessment: Maximum Residue Levels (MRLs) for Pesticides and Veterinary Drugs", within the framework of the Project to Update the Principles and Methods for the Risk Assessment of Chemicals in Food. The main objective of this workshop was to review principles and procedures used by JECFA and JMPR in recommending MRLs, to reaffirm those that remain valid in view of current scientific knowledge and to harmonize to the extent appropriate.

The workshop resulted in a number of recommendations, several of them addressed to JECFA. The sixty-sixth Committee considered these recommendations, and its conclusions are listed below (in italics).

The risk assessment framework for proposing MRLs

1. JECFA should consider the use of the concept of the acute reference dose (ARfD), in addition to the ADI, when a veterinary drug being considered exhibits acute toxicity. JECFA should develop procedures to discriminate between ADI and ARfD for cases where it would be appropriate to estimate short-term (acute) intakes.
   The Committee recommended that a paper should be prepared by an expert for the next meeting that considers compounds for which ARfD considerations are necessary and proposes a procedure for establishment of such values, taking previous JECFA guidance and the ARfD guidance developed by JMPR into account. The paper also needs to consider the impact on intake assessment methods.

Identification and description of residues and methods

2. The workshop concluded that the definitions of a pesticide residue and a veterinary drug residue are essentially the same. The definition for "residues of veterinary drugs" could be

made more consistent with the definition for "pesticide residue" by the addition of the phrase "considered to be of toxicological significance".

The Committee agreed to amend the definition of veterinary drug residues to: Parent compounds and/or their metabolites, including associated impurities of the veterinary drug concerned, in any edible portion of the animal product, which may be of significance to human health.

3. The workshop recommended that FAO prepare a guidance manual to define, in detail, data needs and evaluation procedures for residue definitions and the derivation of MRLs for veterinary drugs.

The Committee recommended that FAO develop a guidance manual for submission and evaluation of data.

4. JECFA should recommend MRLs for fat-soluble dual-use substances only for the trimmable fat from the meat.

JECFA has considered this in the past, and the Committee reaffirmed the existing practice.

5. Partitioning of residues in milk into the fat is influenced by the molecular structure of the compound. Furthermore, the fat content of milk is variable. JECFA proposes MRLs for whole milk. JMPR now recommends two MRLs for fat-soluble compounds, one based on whole milk and one on milk fat. This is necessary to estimate residues in processed dairy commodities. The workshop recommended that JECFA and JMPR consider harmonizing this practice.

JECFA agrees to recommend MRLs for whole milk and for milk fat in the future. The Committee requested the Secretariat to reflect this in future calls for data.

Criteria for selecting data, species and commodities

6. For dual-use substances, the evaluation of the application as a pesticide/drug to animals should be undertaken using the same principles. This can be achieved by several means that require coordination between JECFA and JMPR and also CCRVDF and CCPR (risk assessment policy) and will involve the adoption of mutual notification and coordination of procedures.

The Committee agreed on the importance of the coordination between JECFA and JMPR for dual-use substances and requested the Secretariat to take this into consideration when scheduling compounds for evaluation.

7. JMPR and JECFA should carry out a comprehensive review of all commodity and tissue definitions, as appropriate: harmonizing

meat and muscle tissue definitions, combining definitions of poultry and poultry meat, avoid subdivision into specific commodities for milk and eggs, harmonize definitions of animal fat to be equivalent and to exclude dairy milk, harmonize definitions for aquatic species and consider whether JECFA MRLs for liver and kidney should include other offal. Subsequently, amending instructions on the portion of commodity to which the MRL applies is recommended.

The Committee recommended that the Secretariat convene a working group to address this issue.

Extrapolation issues

8. National governments are encouraged to submit good agricultural practices (GAP) information, particularly on "minor crops", during the data and information call-in process for JMPR.
   No action necessary.

9. JMPR should continue to evaluate extrapolation of pesticide residues data between geographic zones.
   No action necessary.

10. JECFA should investigate a specific approach for MRLs in honey.
    The Committee recommended that a paper be prepared by an expert with experience in beekeeping and honey production for the next meeting to consider if a separate approach for honey is warranted and in such case develop a draft recommendation for consideration at the next meeting.

11. Procedures for extrapolation from one species of animal having a full data set and recommended MRLs to another species need to be agreed upon and harmonized guidance documents prepared. This should be based on past experience with specific cases.
    The Committee concluded that extrapolation may not be the appropriate term, but rather extension of the MRL. This was applied at the present meeting to the MRL of flumequine for shrimp. The Committee noted that there is no formal procedure for extending MRLs, and further action on this is necessary.

12. A general principle on recommending group MRLs in wider circumstances should be considered in an attempt to cover more uses where national authorizations exist.
    The Committee has set group MRLs in the past and continues this practice, but JECFA needs to develop a definition of "group MRL".

Dietary risk assessment of residues

13. To improve the international food consumption information database, national governments should be encouraged to submit their consumption data to FAO and WHO.
No action necessary.

14. JECFA should consider using the median value of the distribution of residue concentrations from which the MRL is derived for the calculation of conservative estimates of long-term (chronic) intakes.
The Committee considered this recommendation and adopted the approach, as described below in section 2.4.1.

2.4.1 **New procedure for estimating chronic dietary intakes**

The estimation of long-term (chronic) dietary intakes of residues of veterinary drugs by the Committee was in the past closely linked to the determination of the MRLs recommended by the Committee. The Committee used a calculated figure of total residue of toxicological or microbiological concern, the "theoretical maximum daily intake" (TMDI), for comparison with the ADI. The new procedure uses the same formula as used previously for the calculation of the TMDI, including factors such as the ratio of marker to total residue concentrations, the only exception being that the median concentration replaces the MRL as the point estimate of the residue concentration in the formula.

The MRL and the median concentration are derived from the same time point of the depletion data of the marker residue. The MRL is a point on the curve describing the upper one-sided 95% confidence limit over the 95th percentile. The median is the corresponding point on the regression line for the same time point. Both figures are obtained from a statistical evaluation of the data (see Figure 2).

In developing this new calculation procedure, the present Committee concluded that the TMDI was no longer the most suitable estimate of chronic intake, because the MRL was a single concentration representing the estimated upper limit of a high percentile of the distribution of marker residue present in a given tissue of the treated animals. The Committee concluded that it was not realistic to use an extreme value of the distribution in a scenario describing chronic intakes. In such a scenario, all concentrations of the distribution of residues should be considered. The median concentration represents the best point estimate of a central tendency over a prolonged period of time, because the concentration of residues in a given tissue consumed varies from day to day, as reflected in the distribution.

Figure 2

Explanation of the relationship between MRL and the median concentration used for the calculation of the estimated daily intake (EDI)
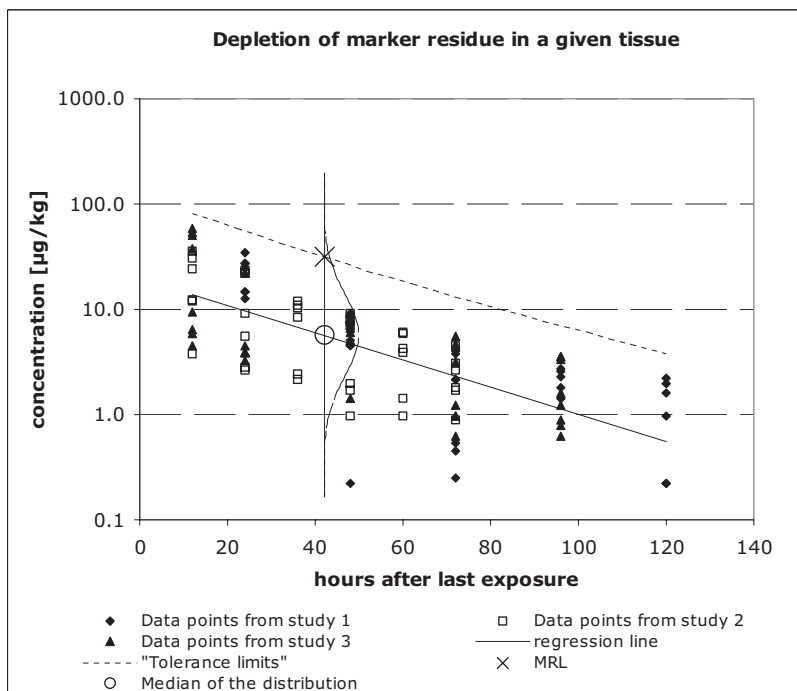
Therefore, the Committee decided to use the median of the residue distribution to substitute for the MRL in the intake estimate. The new estimate of intake is called "estimated daily intake" (EDI). In calculating the median from an array of results, including values below the limit of quantification (LOQ) or below the limit of detection (LOD), half of the respective limit is used for the calculation of median concentrations of residues.

2.5 Use of spreadsheet-based procedure for statistical evaluation of residue depletion data

The Committee has on several occasions used a statistical approach for the evaluation of marker residue depletion data when estimating MRLs. The approach is primarily based on linear regression analysis and statistical estimation of one-sided upper tolerance limits for the marker residue depletion in the individual target tissues (see also Figure 2 of section 2.4.1 above). An iterative procedure is then used to calculate, for different time points on the depletion curve, the intake of residues of concern in the food basket. The calculated intake of

residues is compared with the ADI, and the time point of depletion below the ADI is selected to determine the MRLs.

At the sixty-second meeting of JECFA (Annex 1, reference *169*), the FAO Joint Secretariat proposed to the Committee an Excel-based workbook facilitating the complex calculations required to use this approach. The Committee investigated the workbook and recommended that the Secretariat should continue with its development. In order to take the necessary steps, the Secretariat requested comments from interested parties on both the features and the documentation of the tool. Comments were received from Canada, the European Medicines Agency (EMEA), the International Federation for Animal Health (IFAH) and Argentina. The Committee reviewed all comments and noted that all respondents agreed that the mathematical/ statistical approach was scientifically sound. Some comments analysed the advantages and the limitations of use of the workbook in a very objective manner. The comments from Canada, EMEA and Argentina supported the use of the statistical approach and of the tool in cases where it was appropriate. IFAH indicated that it "does not support use of this programme by JECFA in any of its reviews of veterinary medicinal products" because the organization considered that it was a tool to calculate withdrawal times, which falls outside the terms of reference of JECFA. The Canadian comment suggested that JECFA should use the approach whenever possible and explain the reasons if it is not used.

The Committee concluded that the workbook would primarily be of value in helping the experts to statistically evaluate available depletion data during the development of MRL recommendations. The Committee also concluded that it would use the statistical approach in future whenever it was appropriate and that the experts drafting the working documents should explain to the Committee the reasons when not using it.

2.6 Revised approach for the derivation of a microbiological ADI

The Committee considered the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guideline entitled *Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI* (*8*). The document provides guidance on the assessment of human food safety for residues of antimicrobial veterinary drugs with regard to effects on the human intestinal microflora. The guideline provides recommendations for a harmonized approach for establishing microbiological

ADIs. A decision-tree approach for the evaluation of antimicrobial veterinary drugs was introduced by JECFA at its forty-fifth meeting in 1995 (Annex 1, reference *119*) and later adopted at its fifty-second meeting in 1999 (Annex 1, reference *140*). Similar approaches have been subsequently developed and used by several regulatory authorities. In the interest of harmonization of methods, VICH developed a guideline, which was recently finalized.

The VICH guideline is a refinement of the current JECFA approach. The Committee, in recognition of the importance of international harmonization, agreed to incorporate the VICH guideline in future assessments to ensure consistency and transparency in the determination of microbiological ADIs.

2.7 Antimicrobial resistance risk assessment

The Committee was informed on the conclusions of a Joint FAO/OIE (Office International des Epizooties)/WHO expert risk assessment workshop on non-human antimicrobial usage and antimicrobial resistance, a WHO working group consultation on critically important antibacterial agents for human medicine to be used in risk management strategies for non-human use, and an OIE ad hoc group on critically important veterinary antimicrobials. The Committee was also informed on general qualitative and quantitative approaches for pre- and post-registration risk assessment of antimicrobials for antimicrobial resistance from non-human antimicrobial usage and emerging methodological issues in risk assessment of antimicrobial resistance.

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 Colistin

Explanation

Colistin is an antibacterial agent with activity against a range of Gram-negative organisms. It is a cyclopeptide antibiotic, also known as polymyxin E, produced as a fermentation product of *Bacillus polymyxa* var. *colistinus*. Colistin is composed of a mixture of colistin A and colistin B, which differ only in the length of the acyl side chain. One unit of colistin is defined as the minimum concentration of colistin that can inhibit the growth of *Escherichia coli* 95 I.S.M. in 1 ml of

broth at pH 7.2. Pure colistin base has been assigned a potency of 30 000 IU/mg. An International Standard for colistin sulfate is prepared to contain 20 500 IU/mg. Sodium colistin methanesulfonate should not have a potency less than 11 500 IU/mg.

Colistin has bactericidal activity as a result of binding to the outer cell membrane with subsequent disruption of the cell envelope. Colistin is used for the prevention and treatment of various infectious diseases caused by colistin-sensitive bacteria in target species such as swine, poultry, cattle, sheep, goats and rabbits. It is also used in human medicine. Colistin is available as the sulfate and the methanesulfonate. In both humans and animals, colistin is used orally (colistin sulfate) and parenterally (colistin sulfate, colistin methanesulfonate).

Colistin has not previously been evaluated by the Committee. Colistin was placed on the agenda because it was on the priority list of the CCRVDF.

The Committee considered the results of studies on pharmacokinetics, acute and short-term toxicity, genotoxicity, fertility and developmental toxicity, renal toxicity, microbiological safety and effects in humans. Some of the genotoxicity 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. Several Good Laboratory Practice (GLP)-compliant residue depletion studies and method validation data were also submitted for the Committee's evaluation.

Toxicological and microbiological evaluation
Biochemical data
Colistin, when administered orally as either the sulfate or methanesulfonate, was very poorly absorbed in humans. Drug concentrations in serum were either very low or non-detectable. Following oral administration, colistin was excreted in faeces. Following an injection of sodium colistin methanesulfonate, the drug hydrolysed to form colistin base. Excretion was principally in the urine.

Similarly, in rats, rabbits and dogs, colistin compounds were poorly absorbed in the gastrointestinal tract. When colistin compounds were injected into rats, there was wide distribution; with the exception of the kidney, however, drug concentrations were lower in tissues than in serum. The majority of injected drug was excreted in urine within a 24-h period. In rabbits, approximately 7% of the dose was recovered in bile.

In humans, intramuscular administration of colistin methanesulfonate resulted in therapeutic drug concentrations in serum that persisted for 6 h. The elimination half-life for colistin sulfate was approximately twice that for colistin methanesulfonate, 4 h vs 2 h, following intravenous administration of the drugs to patients with cystic fibrosis.

After parenteral administration, there was some evidence for metabolism in rabbits and dogs. In rabbits injected with sodium colistin methanesulfonate, 8.4% of the dose was excreted as colistin N-glucuronide in urine and bile. Dogs injected with either the sulfate or methanesulfonate excreted an unquantified proportion of drug material in faeces, in a form that had no microbiological activity, suggesting biotransformation of colistin.

Toxicological data
The acute oral toxicities of both colistin sulfate and sodium colistin methanesulfonate were low in mice. The lowest median lethal dose (LD_{50}) values were 432 mg colistin base/kg body weight (bw) for the sulfate and 766 mg colistin base/kg bw for the methanesulfonate. The sulfate was more toxic than the methanesulfonate when parenterally administered to mice. The LD_{50} values were generally below 50 mg/kg bw for the sulfate given as subcutaneous, intramuscular, intraperitoneal or intravenous injection and between 126 and 405 mg/kg bw for the methanesulfonate given as subcutaneous, intraperitoneal or intravenous injection. At lethal doses, toxic signs included muscular incoordination, respiratory distress and occasionally convulsions. Death occurred within 3 h from asphyxia.

Colistin sulfate was administered orally to rats at doses of 6.67, 20 or 60 mg colistin base/kg bw per day for 90 days. It was reported that there were no adverse effects on growth, behaviour, haematology, urinalysis, liver function or pathology. The design of the study was inadequate to allow the full toxicological potential of colistin sulfate to be evaluated.

Rats were fed diets containing colistin sulfate at concentrations of 0, 40, 200 or 1000 mg/kg for a period of 26 weeks. The dietary concentrations were equivalent to 0, 20.2, 101 and 505 mg colistin base/kg. There were no toxicologically significant changes as a result of treatment. The NOEL was the highest dose, 505 mg/kg in feed, equivalent to 50.5 mg colistin base/kg bw per day.

Colistin sulfate was administered orally to dogs at doses of 6.67, 20 or 60 mg colistin base/kg bw per day for 90 days. It was reported that there were no adverse effects on growth, behaviour, haematology,

urinalysis, liver and kidney function or pathology. The design of the study was inadequate to allow the full toxicological potential of colistin sulfate to be evaluated.

Mice were given intraperitoneal injections of sodium colistin methanesulfonate at 0, 50, 100 or 300 mg/kg bw per day for 30 days. At 300 mg/kg bw per day, reduced body weight gain, reduced spontaneous activity, convulsions and death of some animals of each sex were observed. The number of erythrocytes, haematocrit and haemoglobin were depressed and liver weight was increased in males at this dose. The authors reported that "reactive symptoms" were noted in liver, spleen and kidney.

In three separate studies, rats were given intraperitoneal injections of sodium colistin methanesulfonate. When rats were administered doses of 0, 5, 10, 20 or 60 mg/kg bw per day for 30 days, reduced body weight gain, reduced spontaneous activity and convulsions were observed at higher doses, and all high-dose animals died within 3 days. The number of erythrocytes, haematocrit and haemoglobin were depressed in males given 20 mg/kg bw per day, and alanine aminotransferase was increased in females at this dose. In a second study, at doses of 0.83, 2.5 or 7.5 mg colistin base/kg bw per day for 60 days, the only observed effect was hyperaemia of the kidneys, which occurred frequently at the highest dose and infrequently at the lower doses. In the third study, at doses of 0, 5, 10, 20 or 30 mg/kg bw per day for 6 months, body weight gain was depressed in males at 30 mg/kg bw per day and in females at 10 mg/kg bw per day and above. Mortality was dose-related at 10 mg/kg bw per day and above, and reduced spontaneous activity and convulsions were observed. Decreased aspartate aminotransferase and alanine aminotransferase and increased blood urea nitrogen were seen in males at 20 and 30 mg/kg bw per day, and albumin/globulin ratios were increased in females at 30 mg/kg bw per day. Albuminuria was seen in both sexes at 20 and 30 mg/kg bw per day. It was reported that "pathological changes" were noted in kidney, liver and spleen at 20 and 30 mg/kg bw per day.

Dogs received intramuscular injections of sodium colistin methanesulfonate at doses of 0.83, 2.5 or 7.5 mg colistin base/kg bw per day for 60 days. It was reported that there were no significant adverse effects on growth, behaviour, haematology, urinalysis or pathology.

Assays covering an adequate range of genotoxic end-points were conducted with colistin. An equivocal result was claimed in the SOS chromotest for deoxyribonucleic acid (DNA) damage in *E. coli* strains. Colistin was tested as one of a range of compounds in a series of screening assays. However, it was not possible to assess the validity

of this finding, as no primary data were provided. A concentration-related increase in chromosomal damage was observed in cultured human lymphocytes associated with decreases in the mitotic index and in the rate of cell division. Bromodeoxyuridine was added at the initiation of cultures in order to visualize sister chromatid exchanges. This is not normal practice in assays for the detection of chromosomal aberrations, and it is not known if there may have been an interaction with the added bromodeoxyuridine. Assays for reverse mutation in *Salmonella typhimurium* and *E. coli*, DNA repair in *Bacillus subtilis*, forward mutation in cultured Chinese hamster cells, sister chromatid exchange formation in cultured human lymphocytes and micro-nucleus formation in bone marrow of treated mice were clearly negative. The Committee concluded that, on the weight of evidence, colistin is unlikely to pose a genotoxic hazard.

No long-term studies of toxicity, including carcinogenicity, have been reported for colistin. Colistin has no significant genotoxic activity and is not chemically related to known carcinogens. Furthermore, colistin is poorly absorbed from the gastrointestinal tract, and no neoplastic or preneoplastic lesions were observed in 26-week studies in rats given repeated oral or parenteral doses. Despite the absence of studies on carcinogenicity, the Committee concluded that colistin compounds are unlikely to pose a carcinogenic risk.

Reproductive toxicity was evaluated in three separate studies in mice that were given intraperitoneal injections of sodium colistin methanesulfonate. The administration of 24–100 mg colistin base/kg bw per day, from prior to mating through the first 7 days of gestation, caused no detrimental effects on reproductive capacity or on the fetuses. The administration of 15–150 mg/kg bw per day during the period of organogenesis slightly increased resorptions at the highest dose. The administration of 125–500 mg/kg bw per day during the period of organogenesis resulted in delayed ossification in fetuses at 250 and 500 mg/kg bw per day. Postnatal monitoring identified a reduction in spontaneous activity in offspring of the 250 and 500 mg/kg bw per day groups. Maternal toxicity and fetal abnormalities were not observed in any study in mice.

Rats received colistin sulfate at doses of 0, 2.6, 65 or 130 mg colistin base/kg bw per day by gavage on gestation days 7–17. There were no toxicologically significant findings on dams or fetuses. Therefore, the NOEL was the highest dose, 130 mg colistin base/kg bw per day.

Reproductive toxicity was investigated in four separate studies in rats that were given parenteral doses of sodium colistin methanesulfonate.

The intravenous administration of 2.5–10 mg colistin base/kg bw per day, from prior to mating through the first 7 days of gestation, caused no detrimental effects on reproductive capacity, but delayed ossification was apparent in fetuses at the highest dose. The intraperitoneal administration of 5–40 mg/kg bw per day during the period of organogenesis slightly decreased fetal body weight at 20 and 40 mg/kg bw per day. The intravenous administration of 2.5–10 mg colistin base/kg bw per day during the period of organogenesis resulted in reduced ossification in fetuses at 10 mg/kg bw per day. Postnatal monitoring of offspring revealed reduced survival in litters of the 10 mg/kg bw per day group. Maternal toxicity and fetal abnormalities were not observed in any study. The intravenous administration of 6.25–25 mg/kg bw per day, from after organogenesis through the end of lactation, caused no effect on growth and development of the offspring in rats.

Rabbits received sodium colistin methanesulfonate at doses of 0, 20, 25 or 32 mg colistin base/kg bw per day by intravenous injection on gestation days 6–18. There were no significant effects on dams or fetuses.

In a special study to assess renal toxicity in rats, blood urea nitrogen concentrations and kidney pathology were unaffected by single subcutaneous injections of sodium colistin methanesulfonate at 8 or 24 mg/kg bw.

In humans, systemic toxicity following oral doses of colistin sulfate has not been observed, presumably because of its negligible absorption. Parenteral administration of colistin sulfate or sodium colistin methanesulfonate has been associated with kidney toxicity, such as elevated blood urea nitrogen, and neurotoxicity, such as numbness, tingling, dizziness and ataxia. Renal tubular necrosis and neuromuscular blockade have also occurred at lower frequencies. These toxic effects were generally observed following the use of higher than recommended doses or in persons with already impaired renal function, and in most cases they were reversible. Gastrointestinal disturbances have not been reported.

The most relevant study for determining a toxicological ADI is the 26-week study in rats given colistin sulfate in the diet. Studies in which colistin salts were given by parenteral injection are less relevant for assessing the acceptable intake of colistin in food. The NOEL for toxicity was 50.5 mg colistin base/kg bw per day, the highest dose used in this study. A safety factor of 100 was considered appropriate in view of the absence of toxicity after oral administration and the poor absorption through the gastrointestinal tract in all species tested.

Therefore, an ADI of 0–500 μg/kg bw could be established on the basis of the toxicological data.

Microbiological data
Colistin sulfate has been tested for its inhibitory activity against microorganisms representative of the human colonic microflora. The most sensitive organism was *E. coli*, with a minimum inhibitory concentration for 50% of strains (MIC_{50}) of 0.1 μg colistin base/ml in one study and 1 μg colistin base/ml in a second, more comprehensive study. Other predominant human intestinal microflora were not susceptible to colistin sulfate.

Using anaerobic chemostat systems, human faecal cultures were exposed to colistin sulfate at concentrations of 2.5, 25 or 250 μg colistin base/ml. Reductions in total anaerobes and the *Bacteroides fragilis* group were observed at 250 μg/ml. *E. coli* levels were non-detectable at concentrations of 25 and 250 μg/ml. The susceptibility of the bacteria to colistin sulfate before and after addition of the drug to the cultures was unchanged for the anaerobes, but a decrease in susceptibility was observed in the total aerobe cultures at all three concentrations. An increase in resistant *E. coli* was seen at a concentration of 2.5 μg/ml. Reductions in glucosidase and glucuronidase activities were seen at the highest concentration, and a transient increase in azoreductase activity was observed at all three concentrations. Some modest changes in short-chain fatty acid ratios were also demonstrated. A no-observed-effect concentration (NOEC) was not identified in this study.

Colistin sulfate was administered in drinking-water at concentrations of 0, 50 or 500 μg/ml to human flora-associated (HFA) mice for 3 weeks, commencing 10 days after the inoculation of human faecal flora. These concentrations correspond to 0, 3.1 and 31 mg colistin base/kg bw per day, respectively. Reductions in *E. coli* numbers at both doses were significant, but other bacterial population changes were not significant. No change was observed in susceptibility to colistin sulfate. A small decrease in glucuronidase activity was observed at both doses, whereas azoreductase levels were initially higher, followed by a drop to below the control levels. No changes in short-chain fatty acid ratios were observed. A NOEL was not identified in this study.

In a study of the effects of colistin sulfate on human faecal microflora, a daily oral dose of 0.45 g colistin base was given to six healthy adult volunteers for 3 consecutive days. There was no decrease in the total numbers of anaerobes in faeces; however, the enterobacteriaceae

were eliminated in all volunteers between 24 and 48 h after treatment started, with the exception of one individual carrying *Proteus mirabilis*, which persisted throughout the treatment. All six volunteers were progressively recolonized by colistin-sensitive enterobacteriaceae in the days that followed the withdrawal of treatment. A NOEL was not identified in this study.

A decision-tree for evaluating the potential effect of veterinary drug residues on human intestinal microflora was developed by the Committee at its fifty-second meeting (Annex 1, reference *140*). At its present meeting, the Committee used the decision-tree to answer the following questions in its assessment of colistin:

1. *Does the ingested residue have antimicrobial properties?*
   Yes, but the spectrum of activity against the major groups of intestinal microflora is limited. The mechanism of action of colistin involves binding with the anionic lipopolysaccharide molecules by displacing calcium and magnesium from the outer cell membrane of Gram-negative bacteria, leading to permeability changes in the cell envelope, leakage of cell contents and cell death. Colistin is active against many Gram-negative aerobic bacilli, including *Acinetobacter* species, *Pseudomonas aeruginosa*, *Klebsiella* species, *Enterobacter* species, *E. coli*, *Salmonella* species, *Shigella* species, *Citrobacter* species, *Yersinia pseudotuberculosis*, *Morganella morganii* and *Haemophilus influenzae*, and it has considerable activity against *Stenotrophomonas* species. Colistin is not active against some Gram-negative aerobic bacilli (including *Pseudomonas mallei*, *Burkholderia cepacia*, *Proteus* species, *Providencia* species, *Serratia* species, *Edwardsiella* species and *Brucella* species), Gram-negative and Gram-positive aerobic cocci, Gram-positive aerobic bacilli, anaerobes, fungi and parasites.
2. *Does the drug residue enter the lower bowel?*
   Yes. Colistin is very poorly absorbed from the intestinal tract of humans and laboratory animals after oral administration. Studies in humans given oral doses of colistin sulfate have shown high concentrations of colistin in faeces. Therefore, oral doses of colistin compounds are available to the gastrointestinal tract microflora.
3. *Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, by metabolism mediated by the host or intestinal microflora in the bowel and/or by binding to intestinal contents?*
   No specific information was available on the metabolism of colistin by intestinal microflora. However, it has been reported that a significant proportion of the drug excreted in human faeces is in

bound form, which may result in inactivation of microbiological activity.

4. *Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal flora?*

No. Colistin is very poorly absorbed from the gastrointestinal tract, and therefore the compound exhibits low toxicity in humans and laboratory animals. Microbiological studies have indicated that colistin sulfate may have adverse effects on the intestinal microflora.

5. *Do clinical data from the therapeutic use of the class of drugs in humans or data from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal tract?*

Yes. In vitro MIC data indicated that *E. coli* was sensitive to colistin sulfate. In addition, in a short-term, high-dose study in humans, the enterobacteriaceae were eliminated within 48 h of treatment. All subjects in the study group were recolonized by colistin-sensitive enterobacteriaceae in the days that followed the withdrawal of treatment. It was noted that, based on the spectrum of activity and mechanism of action of colistin, gastrointestinal effects such as nausea, vomiting, abdominal cramps and diarrhoea would not be expected and are not commonly reported.

6. *Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora.*

An increase in resistant *E. coli* was seen in an in vitro study. However, emergence of resistance does not appear to be the microbiological end-point of concern for colistin in the case of the predominant anaerobes in the human gastrointestinal tract. The available data indicate that disruption of the colonization barrier may be a minor concern with colistin compounds, but it is the most sensitive adverse effect on the human gastrointestinal microflora.

7. *If disruption of the colonization barrier is the concern, determine the MIC of the drug against 100 strains of predominant intestinal flora and take the geometric mean MIC of the most sensitive genus or genera to derive an ADI using the formula discussed at the forty-seventh meeting of the Committee (Annex 1, reference 125). Other model systems may be used to establish a NOEC to derive an ADI.*

The Committee considered that the MIC data were the most appropriate to use in determining a microbiological ADI. An evaluation of the MIC_{50} values for relevant gastrointestinal microflora provides a figure of 1 µg colistin base/ml for *E. coli*. This value can be used to calculate a microbiological ADI, as follows:

$$\text{Upper limit of ADI} = \frac{\text{MIC}_{50} \times \text{MCC}}{\text{FA} \times \text{SF} \times \text{BW}}$$

where:

MIC$_{50}$ = minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism. The MIC$_{50}$ for the most sensitive relevant genus of the gut flora was 1 µg colistin base/ml (1 µg/g) for *E. coli*.

MCC = mass of colonic contents: a value of 220 g, determined by the Committee at its forty-seventh meeting (Annex 1, reference *125*), was used in the calculation.

FA = available fraction of the dose: the microbiologically active residue is colistin. Colistin is very poorly absorbed from the gastrointestinal tract in humans and laboratory animals. However, measurement of colistin concentrations in human faeces did not account for the administered dose. It has been reported that a significant proportion of the drug excreted in human faeces is in bound form. Therefore, a conservative estimate of the fraction of the dose available to the gastrointestinal microflora is 50%. Hence, the value in the equation is 0.5.

SF = safety factor: the magnitude of the safety factor depends on the quality and quantity of the microbiological data available. A value of 1 is appropriate when extensive relevant microbiological data are available, as is the case in the current assessment. Thus, the safety factor should be 1.

BW = body weight: a value of 60 kg has been adopted for an adult.

Hence,

$$\text{Upper limit of ADI} = \frac{1\,\mu g/g \times 220\,g}{0.5 \times 1 \times 60\,kg}$$

$$= 7\,\mu g/kg\ bw$$

Evaluation

The Committee considered that microbiological effects were more relevant than toxicological effects for the establishment of an ADI for colistin. Therefore, the Committee established an ADI of 0–7 µg/kg bw on the basis of the MIC$_{50}$ for *E. coli*. The upper bound of this ADI is significantly lower than the upper bound of the toxicological ADI of 500 µg/kg bw.

A toxicological monograph was prepared.

Residue evaluation

Data on pharmacokinetics and metabolism

Pharmacokinetic data are available for several animal species. Oral doses of colistin sulfate were very poorly absorbed in cattle, pigs and chickens. In general, low concentrations were detected in serum, with trace amounts in kidneys and liver. Drug concentrations in other tissues were below the LOD.

Milk-fed calves were administered colistin sulfate intravenously. A peak serum concentration of approximately 16 µg/ml, a volume of distribution of 1.3 litres/kg and renal clearance of approximately 3.4 ml/min per kilogram were observed. Colistin was significantly bound to tissues. The elimination half-life was 4–6 h. Following intramuscular administration of colistin methanesulfonate to lactating dairy cows and calves, residues persisted in serum for several hours. Peak serum concentrations were reached between 0.5 and 3 h (cows) and between 1 and 2 h (calves) after dosing. The elimination half-life was nearly double in cows compared with calves. Concentrations in cows' milk were generally low and were detectable through two milkings. Oral administration of colistin sulfate in veal calves resulted in no detectable residues.

In ewes, intramuscular administration of colistin methanesulfonate resulted in higher serum concentrations than did intramuscular administration of colistin sulfate. Serum protein binding was higher for colistin sulfate than for colistin methanesulfonate.

Plasma protein binding of colistin in cattle and sheep is 40% and 70%, respectively.

In pigs, peak serum concentrations were reached at 1 h after intravenous administration of colistin sulfate. Serum concentrations were undetectable at sampling times thereafter. In another study, no colistin residues were detected in the serum of gnotobiotic piglets fed colistin sulfate, 40 mg/kg, in sterilized milk. In a more recent study, pigs were treated intramuscularly and intravenously with colistin sulfate. Peak plasma concentrations were reached 30 min after dosing. The elimination half-life for all doses and routes was approximately 4–4.5 h, and the clearance rate was approximately 3 ml/min per kilogram.

Residue data

The Committee reviewed several recent GLP-compliant residue studies that used high-performance liquid chromatography (HPLC) with fluorescence detection for the detection and quantification of residues. The studies are described below.

Cattle: Four calves, treated twice daily for 7 days with milk replacer containing colistin sulfate to provide 100 000 IU/kg bw, were slaughtered 6 h after the final treatment, and samples of muscle, liver, kidney and fat were collected for analysis. Only one kidney sample contained quantifiable residues (139 µg/kg) when analysed with the validated HPLC method.

Pigs: Four pigs, treated orally twice daily for 5 days with colistin sulfate administered in a small quantity of feed to provide 100 000 IU/ kg bw, were slaughtered 6 h after the final treatment, and samples of muscle, liver, kidney and skin + fat were collected and analysed using the validated HPLC method. No quantifiable residues were found in any tissues.

Chickens: Six chickens, treated orally twice daily for 5 days with colistin sulfate administered in a small quantity of feed to provide 100 000 IU/kg bw, were slaughtered 6 h after the final treatment, and samples of breast muscle, liver, kidney and skin + fat were collected and analysed using the validated HPLC method. Only one kidney sample contained quantifiable residues (184 µg/kg).

Turkeys: Six turkeys, treated orally by gavage twice daily for 5 days with colistin sulfate administered in a small quantity of water to provide 100 000 IU/kg bw, were slaughtered 6 h after the final treatment, and samples of breast muscle, liver, kidney and skin + fat were collected and analysed using the validated HPLC method. Quantifiable residues were found in the kidney (194.5 µg/kg) and skin + fat (98 µg/kg) from one animal.

Rabbits: Twelve rabbits, treated orally by gavage twice daily for 5 days with colistin sulfate administered in a small quantity of water to provide 100 000 IU/kg bw, were slaughtered in groups of four rabbits each at 6, 24 and 48 h after the final treatment. Samples of muscle, liver, kidney and fat were collected and analysed using the validated HPLC method. All the residues in muscle and liver samples were below the LOQ for the method. Two kidney samples collected 6 h after the final treatment contained quantifiable residues, 1021 µg/kg and 239 µg/kg. Low but quantifiable concentrations were detected in all of the fat collected 6 h after the final treatment (range 75–90 µg/kg). Fat samples collected from the two male rabbits at 24 h after the final treatment contained quantifiable residues (227 µg/kg and 76 µg/kg), as did the fat sample from one male rabbit slaughtered 48 h after the final dose (87 µg/kg). Residues in the fat samples from the female rabbits slaughtered at 24 and 48 h after the final treatment were below the LOQ.

Cows' milk: Ten cows were treated intramuscularly once daily for 5 days with colistin sulfate to provide 25 000 IU/kg bw. Cows were milked morning and evening, and pooled milk samples from each of the four quarters were mixed and analysed using the validated HPLC method. Quantifiable residues (range = 21–109 µg/kg) were detected in all milk samples collected during dosing and in the morning milk collected 1 day after the last treatment (range = 26–81 µg/kg). Thereafter, the number of samples containing quantifiable residues declined.

Sheep's milk: In a non-GLP study, colistin residues were determined in sheep's milk following single intramuscular administrations of colistin sulfate or colistin methanesulfonate at doses of 3.5 mg/kg bw or 7.5 mg/kg bw. Colistin concentrations in milk resulting from the administration of colistin methanesulfonate were approximately 3 µg/ml and 10 µg/ml, respectively, whereas the concentrations produced by the corresponding doses of colistin sulfate were approximately 1 µg/ml and 1.5 µg/ml, respectively. Peak concentrations were reached 2–3 h after treatment. Residues resulting from administration of colistin sulfate declined more rapidly than did the residues resulting from colistin methanesulfonate administration.

Hens' eggs: Fifteen laying hens were treated orally twice daily for 5 days with colistin sulfate administered in a small quantity of feed to provide 100 000 IU/kg bw. Eggs were collected from treated hens on treatment (day 3), on the day of treatment withdrawal (day 5) and following a 1-day withdrawal (day 6). Yolk and albumen were mixed for each egg and analysed using the validated HPLC method. No detectable residues of colistin were found in any eggs at any of the sampling times.

Effects on starter cultures
No data were presented.

Analytical methods
A validated HPLC method with fluorescence detection has been used in tissue residue studies in cattle, pigs, chickens, turkeys and rabbits and for cows' milk and hens' eggs. Quantification is achieved by summation of the colistin A and colistin B peaks. The colistin sulfate standard used in the analyses contains 20 935 IU/mg. The LOQ is 75 mg/kg for muscle, liver and fat (or skin + fat, as appropriate) and 100 mg/kg for kidney. The LOQs for milk and eggs are 10 mg/kg and 150 mg/kg, respectively. The LODs are as given in Table 1.

Acceptable linearity, precision, recovery and accuracy were demonstrated for the method.

Table 1

Limits of detection for colistin residues in tissues, milk and eggs of various species

Species	Limits of detection (µg/kg)					
	Muscle	Liver	Kidney	Fat	Milk	Eggs
Cattle	60	45	45	32	3	
Sheep					NP	
Pigs	51	30	49	45		
Chickens	51	30	49	45		47
Turkeys	6	33	49	31		NP
Rabbits	34	58	37	30		

NP = not provided

The method uses readily available reagents and materials and can be implemented easily under normal laboratory conditions.

The Committee was aware that suitable microbiological assays are available for screening, but these methods were not submitted for evaluation.

Maximum residue limits
In recommending MRLs for colistin, the Committee considered the following factors:

— Residues of colistin following oral administration generally were below the LOQ for the method of analysis in most tissues of most species, even at very short withdrawal periods. Low but quantifiable residues were detected in the fat of orally treated rabbits and in eggs of hens treated by intramuscular injection. Quantifiable residues of colistin were also found in cows' milk following intramammary infusion and intramuscular injection.
— Colistin A+B is considered a suitable marker residue in tissues, eggs and milk and represents approximately 80% of the microbiologically active residues. This information is incorporated in the calculation of the intake estimates to ensure that they correctly reflect residues of microbiological concern.
— The validated HPLC method, used to measure residues of colistin in the more recently conducted studies submitted for the Committee's review, is suitable for monitoring residues for regulatory purposes. The assay measures colistin A (polymyxin E_1) and colistin B (polymyxin E_2).
— The MRLs recommended for all edible tissues in all species and for hens' eggs are twice the LOQ for the HPLC method. Because

detectable residues were found in these tissues, the theoretical intake values for all the edible tissues are included in the calculation of the TMDI.

— EDI values were determined using median residue values for each tissue from each food-producing species for which data were available. Where residue values were below the LOD or LOQ of the validated method, values of one half the LOD and one half the LOQ, respectively, were used in the calculations.

— The MRL recommended for cows' milk takes into consideration the potential use of colistin by both the intramuscular and intramammary routes of administration.

— An ADI of 0–7 µg/kg bw was established by the Committee based on a microbiological end-point. This ADI is equivalent to up to 420 µg for a 60-kg person.

The Committee recommended MRLs, measured as colistin A+B, in cattle, sheep, goats, pigs, chickens, turkeys and rabbits of 150 µg/kg in liver, muscle and fat (including skin + fat, where applicable), 200 µg/kg in kidney, 300 µg/kg in hens' eggs and 50 µg/kg in cows' and sheep's milk, determined with the validated HPLC method.

The MRLs recommended would result in a TMDI of 229 µg, or 55% of the ADI, based on the model daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, 100 g of eggs and 1.5 kg of milk.

The calculated EDI values represent 4% (for chickens) to 9% (for cattle) of the upper bound of the ADI.

The EDI is calculated using the highest median values from among the tissues and food-producing species (Table 2). This EDI represents 14% of the upper bound of the ADI.

A residue monograph was prepared.

Table 2
Calculation of EDI using highest median values

Tissue	Adjusted median (µg/kg)	Food basket (kg)	EDI (µg)
Muscle (turkey)	48	0.3	14.3
Liver (pigs)	48	0.1	4.8
Kidney (rabbits)	181	0.05	9.1
Fat[a] (rabbits)	103	0.05	5.1
Milk (cattle)	14	1.5	20.6
Eggs (chickens)	30	0.1	3.0
Total			56.9

[a] Skin + fat, where applicable.

3.2 Erythromycin

Explanation

Erythromycin is a 14-membered macrocyclic lactone produced by the streptomycete *Saccharopolyspora erythraea*, a soil-borne organism. It is a mixture of three compounds, named A, B and C, produced during fermentation. The principal product is erythromycin A, with small portions of B (≤5%) and C (≤5%). It has been widely used as an antimicrobial compound against a variety of infectious microorganisms in animals and humans. In veterinary medicine, erythromycin is used for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases due to erythromycin-sensitive bacteria in cattle, sheep, swine and poultry and for the treatment of chronic diseases due to mycoplasma in poultry. The maximum recommended therapeutic dose in veterinary use is 20 mg/kg bw per day as erythromycin base.

Erythromycin was previously evaluated by the Committee at its twelfth meeting in 1968 (Annex 1, reference *17*). At this meeting, no ADI was established, but acceptable levels of residues were defined for milk (0–0.04 mg/ml) and meat and eggs (0–0.3 mg/kg). Erythromycin was re-evaluated on priority request of CCRVDF. The Committee was requested to establish an ADI and recommend MRLs in poultry tissues only.

The present Committee considered data on the pharmacokinetics, metabolism, acute toxicity, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity, immunotoxicity, epidemiological findings and microbiological effects of erythromycin. Many of the studies were conducted prior to the development of GLP. Three new non-radiolabelled residue depletion studies in chickens, laying hens and turkeys treated with erythromycin and the description and validation of the analytical procedures employed were provided. Two previous residue studies in calves and chickens were also provided. Data and publications from the open literature on pharmacokinetic and metabolic studies in experimental and target animals and humans were submitted for evaluation by the Committee.

Toxicological and microbiological evaluation

Biochemical data

Erythromycin administered orally to rats at a dose of 25 mg/kg bw was absorbed mainly in the small intestine. The peak serum concentration was reached after 1–2 h. Twenty hours after an intravenous administration of 10 mg of erythromycin (*N*-methyl-^{14}C-erythromycin;

0.3 MBq), approximately 37–43% of the radiolabelled dose was recovered in the intestinal tract and faeces, 27–36% in the urine and 21–29% in the expired air. Erythromycin was rapidly metabolized in the liver, mainly through a demethylation process, and excreted into the bile mainly as des-N-methyl-erythromycin; the major metabolite was present only in the bile and in the intestinal contents. The isotopic methyl group was eliminated in the expired air as carbon dioxide.

In dogs (route of administration and dose not stated), peak erythromycin concentrations in most tissues exceeded the serum concentration, except for saliva, pancreatic secretion, cerebrospinal fluid, muscle and fetal tissues. After intravenous administration of 10 mg/kg bw, approximately 5% of the dose was excreted into the bile after 8 h. The elimination half-life was 60 min, and the apparent volume of distribution in the body was higher than 2 litres/kg.

In humans, erythromycin is slowly absorbed after oral administration. Peak serum concentrations occurred 1–6.3 h after dosing and ranged from 0.1 to 4.8 μg/ml according to the formulation of erythromycin administered. Erythromycin is sensitive to degradation by gastric acid, and the oral absorption is less than 50%. An estimated 0.1% of a daily dose appears in breast milk. Fetal blood levels are less than 10% of those present in the maternal circulation. Erythromycin is absorbed in the small intestine as erythromycin base.

Toxicological data
The acute oral toxicity of erythromycin was very low. The LD$_{50}$ in mice and rats was greater than 2000 mg/kg bw. Signs of toxicity included clonic convulsion, prostration and respiratory depression.

Groups of mice given dietary erythromycin stearate at a dose of 0, 580, 1160, 2300, 2800 or 5000 mg/kg bw per day for 14 days showed decreased feed consumption and lethargy, rough coat and hydration of cornea beginning at a dose of 1160 mg/kg bw per day. The NOEL was 580 mg/kg bw per day.

Rats were given erythromycin stearate in the diet at doses of 0, 360, 720, 1160, 1400 or 2250 mg/kg bw per day for 14 days. Rats showed decreased body weight gain and feed consumption, lethargy, rough coat and hyperaemic intestine at 1160, 1400 or 2250 mg/kg bw per day. The NOEL was 720 mg/kg bw per day.

Rats were given erythromycin stearate orally in the diet at doses of 0, 60, 120, 240, 480 or 1000 mg/kg bw per day for 13 weeks. All rats receiving more than 60 mg/kg bw per day were lethargic. At the highest dose, rats exhibited multinucleated syncytial hepatocytes. The NOEL was 60 mg/kg bw per day.

There were increased mortalities in rats given oral erythromycin base or salts at a dose of 800 mg/kg bw per day for 6 weeks.

Rats receiving an oral dose of 0, 0.12, 1.2 or 12 mg/kg bw per day for 68 weeks showed no compound-related adverse effects. The NOEL for the study was 12 mg/kg bw per day, the highest dose tested.

In four studies in dogs, erythromycin was administered either orally as a capsule or intravenously for up to 12 months at doses up to at least 100 mg/kg bw per day. No adverse effects were reported in any of these studies.

Monkeys given erythromycin orally at a dose of 75 mg erythromycin base/kg bw per day for 64 days exhibited no compound-related adverse effects.

Long-term (2-year) studies were carried out in mice and rats with dietary erythromycin stearate.

Mice receiving doses of 250 or 500 mg/kg bw per day (female) or 270 or 545 mg/kg bw per day (male) for 2 years showed no treatment-related carcinogenic effects. Inflammation of glandular stomach in male mice and lymphoid hyperplasia of urinary bladder in female mice were found with increased frequency at both doses.

Rats receiving doses of 180 or 370 mg/kg bw per day (male) or 210 or 435 mg/kg bw per day (female) for 2 years showed no treatment-related carcinogenic effects. Non-neoplastic effects observed included granulomas of the liver at 210 and 435 mg/kg bw per day in females and at 370 mg/kg bw per day in males and reticulum cell hyperplasia of the bone marrow at 210 and 435 mg/kg bw per day in females. The LOEL was 210 mg/kg bw per day based on the granulomas of the liver and reticulum cell hyperplasia of the bone marrow in female rats.

Assays covering an adequate range of genotoxic end-points were conducted with erythromycin stearate. Erythromycin stearate was not mutagenic in the *Salmonella typhimurium* assay for strains TA 98, TA 100, TA 1535 or TA 1537, in the sister chromatid exchange test or in the chromosomal aberrations test (Chinese hamster ovary cells) in either the presence or absence of metabolic activation. In the mouse L5178Y lymphoma assay for mutagenicity, it was noted that the response was marginal, that it occurred at levels close to those resulting in precipitation of the compound and that the increase in mutant fraction was not dose-related. The Committee concluded that, on the weight of evidence, erythromycin is unlikely to pose a genotoxic hazard.

Two reproductive toxicity studies were performed in rats. In one study, animals were given about 1 mg erythromycin thiocyanate/kg bw per day in the diet for 100 days prior to mating. Further treatment was not given, and reproductive indices were examined over three generations. In a second study, animals received 125 mg erythromycin base/kg bw per day over two generations. While no adverse effects were reported, both studies are of limited use due to the study design and limited availability of the original data.

Rats receiving oral doses of 3.3 mg erythromycin/kg bw per day showed a decreased frequency of mitotic division in testes. In vitro, high concentrations of erythromycin impaired the motility and viability of sperm from humans (as well as ram, bull, rabbit and horse). The Committee concluded that, on the weight of the available evidence, there was no evidence of developmental or reproductive effects in rats.

Mice treated with 2000 mg erythromycin/kg bw per day orally by gavage during gestation days 8–13 showed a significant decrease in maternal body weight at gestation day 19 and in fetal body weight, but no fetal malformations.

Epidemiological studies in the published literature identify a potential prenatal and postnatal effect following therapeutic administration of erythromycin to women. There was one report, with inadequate statistical power to reach adequate conclusions, of cardiovascular malformations in the fetuses from women receiving therapeutic treatment with erythromycin. Other studies have found no effect on fetal malformation in fetuses from treated women. The Committee determined that there was insufficient evidence to draw a conclusion at this time concerning effects on the fetus. Infantile hypertrophic pyloric stenosis has been identified as a possible effect in developing children following early postnatal exposure to erythromycin in breast milk. The Committee assumed a human therapeutic dose of 500 mg per person and 60 kg bw, or 8 mg/kg bw per day.

In humans, high doses above 2 g/day induced signs of gastrointestinal disturbance (nausea, vomiting, diarrhoea) in 5–30% of the patients. Hepatotoxicity was reported in patients after 10–16 days of treatment at a dose of 1 g erythromycin estolate/day and in children treated with 1.2 g/day. Epidemiological data in humans indicate that hypersensitivity reactions to erythromycin are rare and generally mild.

Microbiological data
Several studies evaluated the activity of erythromycin against anaerobic clinical isolates and bacterial species representative of

those found in the human gastrointestinal tract. Erythromycin inhibited most of the *Eubacterium*, *Bifidobacterium* and *Lactobacillus* at 0.5 μg/ml or less. In *Clostridium* species, 64% and 84% of the strains were sensitive to 0.5 and 1.0 μg/ml, respectively. Erythromycin was less active against *Bacteroides fragilis* and other *Bacteroides* species. Antimicrobial susceptibility for 37 strains of *Bifidobacterium*, including *B. bifidum*, *B. longum* and *B. infantis*, to erythromycin indicated that most of the bifidobacteria showed an MIC value below 0.19 μg/ml.

The lowest relevant MIC_{50} for erythromycin was 0.1 μg/ml for *Bifidobacterium* species.

A decision-tree for evaluating the potential effect of veterinary drug residues on human intestinal microflora was developed by the Committee at its fifty-second meeting (Annex 1, reference *140*). The present Committee used the decision-tree to answer the following questions in its assessment of erythromycin:

1. *Does the ingested residue have antimicrobial properties?*
   Yes, erythromycin is a macrolide antibiotic commonly used in poultry, livestock and human clinical practice to treat infections due to Gram-positive bacteria, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and enterococci. In veterinary medicine, it is used for the treatment of mastitis in cows, chronic respiratory diseases due to mycoplasmas in poultry and infectious diseases due to erythromycin-sensitive bacteria. Anaerobes are relatively sensitive to erythromycin. Erythromycin is not active against Gram-negative bacilli.
2. *Does the drug residue enter the lower bowel?*
   Yes. Erythromycin is poorly absorbed from the gastrointestinal tract in humans. Following oral administration of erythromycin, 50% of the dose was absorbed in humans. In addition, 20 h after oral administration of 10 mg of radiolabelled erythromycin to rats, approximately 37–43% of the administered radioactivity was recovered in the intestinal tract and faeces. Therefore, oral doses of erythromycin are available to the gastrointestinal tract microflora.
3. *Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, by metabolism mediated by the host or intestinal microflora in the bowel and/or by binding to intestinal contents?*
   No. There is no specific information on the ability of intestinal microflora to metabolize erythromycin. However, the metabolism of erythromycin by cytochrome P450-mediated *N*-demethylation

reactions occurs in the liver of various species of rodents, ruminants and humans. Des-*N*-methyl-erythromycin is the major metabolite present in the bile and intestinal contents of rats after oral administration of 10 mg of erythromycin. The antimicrobial activity of des-*N*-methyl-erythromycin is presumably low, and the only form of erythromycin known to be active is the free base.

4. *Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal flora?*

   No. A number of in vitro and in vivo studies have demonstrated the potential for adverse effects of erythromycin on the intestinal microflora. Studies of toxicity have not identified adverse findings at low oral doses, and thus the toxicological ADI would not be expected to provide adequate protection for the intestinal microflora.

5. *Do clinical data from the therapeutic use of the class of drugs in humans or data from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal tract?*

   Yes. Gastrointestinal effects are the most commonly reported adverse reactions to therapeutic use of erythromycin in humans. The effects include abdominal pain, nausea, vomiting and diarrhoea. In humans, doses of 2 g/day or more can induce signs of gastrointestinal disturbance.

6. *Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora.*

   The available data indicate that oral exposure to erythromycin is associated with disruption of the colonization barrier, rather than emergence of resistance. There are no studies available on the emergence of resistance to erythromycin in human intestinal microflora. It was concluded that disruption of the colonization barrier is the most appropriate end-point for the determination of a microbiological ADI.

7. *If disruption of the colonization barrier is the concern, determine the MIC of the drug against 100 strains of predominant intestinal flora and take the geometric mean MIC of the most sensitive genus or genera to derive an ADI using the formula discussed at the forty-seventh meeting of the Committee (Annex 1, reference 125). Other model systems may be used to establish a NOEC to derive an ADI.*

   Using all relevant data acquired in studies conducted in vitro and in vivo, the Committee considered that MIC studies for erythromycin against a range of bacterial species representative of those typically found in the human gastrointestinal tract were the most appropriate to use in determining a microbiological ADI.

An evaluation of the MIC$_{50}$ values for relevant gastrointestinal microflora provides a figure of 0.1 µg/ml for *Bifidobacterium*. This value can be used to calculate a microbiological ADI, as follows:

$$\text{Upper limit of ADI} = \frac{\text{MIC}_{50} \times \text{MCC}}{\text{FA} \times \text{SF} \times \text{BW}}$$

where:

MIC$_{50}$ = minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism. The MIC$_{50}$ for the most sensitive relevant genus of the gut flora was 0.1 µg/ml (0.1 µg/g) for *Bifidobacterium*.

MCC = mass of colonic contents: a value of 220g, determined by the Committee at its forty-seventh meeting (Annex 1, reference *125*), was used in the calculation.

FA = available fraction of the dose; the microbiologically active residue is erythromycin. Erythromycin is poorly absorbed in humans. In addition, in rats, approximately 37–43% of an oral dose of erythromycin was recovered in the intestinal tract and faeces. Therefore, a conservative estimate of the fraction of the dose available to the gastrointestinal microflora is 50%. Thus, the value in the equation is 0.5.

SF = safety factor: the magnitude of the safety factor depends on the quality and quantity of the microbiological data available. A value of 1 is appropriate when relevant microbiological data on disruption of the colonization barrier are available, as is the case in the current assessment. Thus, the safety factor should be 1.

BW = body weight: a value of 60kg has been adopted for an adult.

Hence,

$$\text{Upper limit of ADI} = \frac{1\,\text{µg/g} \times 220\,\text{g}}{0.5 \times 0.1 \times 60\,\text{kg}}$$

$$= 0.7\,\text{µg/kg bw}$$

Evaluation

The Committee considered that microbiological effects were more relevant than toxicological effects for the establishment of an ADI for erythromycin. Due to the deficiencies and uncertainties in the toxicological database, the Committee was unable to establish a toxicological ADI with any confidence. However, the microbiological data did

permit calculation of a robust microbiological ADI. To help compare the microbiological ADI with potentially relevant toxicological end-points, the Committee adopted a margin of exposure approach. This involved obtaining the ratio of the respective LOEL to the upper bound of the microbiological ADI.

The most relevant animal study for the evaluation of the safety of residues of erythromycin is the 2-year study in rats given erythromycin stearate in the diet. A NOEL was not established in this study, and the LOEL for non-tumorigenic effects was 210 mg/kg bw per day in female rats based on granulomas of the liver and reticulum cell hyperplasia of the bone marrow. The upper bound of the microbiological ADI, 0.7 μg/kg bw, provides a 300 000-fold margin of exposure over the LOEL in rats. The Committee concluded that, although based on a LOEL, this margin of exposure is such that residues of erythromycin are unlikely to pose a risk of systemic toxicity to humans.

Infantile hypertrophic pyloric stenosis has been identified as a possible effect in developing children following early postnatal exposure to erythromycin. The Committee assumed a human therapeutic dose of 500 mg per person and 60 kg bw, or 8 mg/kg bw per day. While a potential might exist for a postnatal effect in infants exposed through the breast milk from mothers receiving therapeutic erythromycin, the upper bound of the microbiological ADI, 0.7 μg/kg bw, provides a greater than 10 000-fold margin of exposure over the dose associated with postnatal effects. The Committee concluded that residues of erythromycin are unlikely to pose a risk to breast-fed infants.

Therefore, the Committee established an ADI of 0–0.7 μg/kg bw on the basis of the MIC_{50} of 0.1 μg/g for *Bifidobacterium*.

A toxicological monograph was prepared.

Residue evaluation

Data on pharmacokinetics and metabolism
Erythromycin is rapidly metabolized in the liver, mainly through an *N*-demethylation process, in several species of ruminants (as well as rodents and humans). Des-*N*-methyl-erythromycin was the major metabolite and the only microbiologically active metabolite of erythromycin. However, its antimicrobial activity is low, and the only form of erythromycin known to be active in vivo is erythromycin free base.

In cattle, the elimination half-life ranged from approximately 1 to 4 h following intravenous or intramuscular administration. Following a

single intramuscular dose of 5 mg erythromycin/kg bw, a plasma concentration of 0.652 µg/ml was reached after 2 h. In cows, after intramammary infusion, the elimination half-life in the milk was 2 h.

In chickens, 30 min after the beginning of a repeated administration of erythromycin via drinking-water at a dose of 25 000 IU/kg bw per day for 3 days (approximate1y 27 mg/kg bw), the average serum levels were 0.11–0.22 µg/ml. After the last administration, concentrations quickly declined to about 0.04 µg/ml. Erythromycin appeared in the lungs within 6 h of the last administration, and the concentration was higher in the lungs than in serum. Twelve hours after its administration, pulmonary levels were less than the LOD.

Residue data

Two original studies were performed in 1988 in calves and poultry using erythromycin thiocyanate. As only evaluation in poultry was requested, studies in cattle are not reported in this summary. In poultry, six new residue studies were submitted:

— Three erythromycin residue studies after administration in drinking-water, one for 3 days at 20 mg/kg bw, one for 8 days at 20 mg/kg bw and one for 5 days at 50 mg/kg bw;
— Two studies for residues of erythromycin in eggs of laying hens treated with 20 mg/kg bw, one for 3 days and one for 7 days; and
— One residue study in turkeys treated with 20 mg/kg bw for 3 days.

The new residue depletion studies with unlabelled erythromycin were performed using the treatments indicated in Table 3. These studies were performed in accordance with GLP procedures.

Table 3
Dosing regimen in new residue depletion studies

Species	Dosage
Chickens	20 mg/kg bw per day for 3 days
	20 mg/kg bw per day for 8 days
	50 mg/kg bw per day for 5 days
Laying hens	20 mg/kg bw per day for 3 days
	20 mg/kg bw per day for 7 days
Turkeys	20 mg/kg bw per day for 3 days

In the residue depletion studies in broiler chickens, laying hens and eggs, the concentration of the erythromycin declined to values below the LOD 3 days after the end of treatment in chicken tissues and 6 days after the end of treatment in the whole eggs.

In the first two residue studies, 36 chickens were treated by oral administration of erythromycin via the drinking-water at 20 mg/kg bw per day for 3 and 8 consecutive days, respectively. The veterinary drug was administered as erythromycin thiocyanate 20% oral powder. Erythromycin A and the metabolite des-*N*-methyl-erythromycin A were measured in liver, muscle, kidney and fat/skin by the liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method. Results showed that from day 1 to day 3 after the end of the treatment period, low concentrations of des-*N*-methyl-erythromycin A (<LOQ) were measured only in two samples of liver. No erythromycin A was detected. In the second residue study, similar results were obtained. These studies showed that, regardless of the duration of administration, an erythromycin dose of 20 mg/kg bw per day leads to concentrations of residues in edible tissues below the LOQ at all time points sampled. The median residue concentrations at 12, 24 and 48 h, respectively, were as follows: muscle, 1.5 µg/kg at all time points; liver, 15 µg/kg at all time points; kidney, 12.5 µg/kg at all time points; skin + fat, 50 µg/kg at 12 h and 2.5 µg/kg at all subsequent time points.

The third study was performed with erythromycin administered as a 5.5% thiocyanate powder in edible tissues of broiler chickens at a dose of 50 mg/kg bw (two and a half times the recommended dose) in drinking-water for 5 days. Erythromycin A was assayed with the LC/MS/MS method. Median residues at 6, 10, 24 and 48 h, respectively, were as follows: muscle, 50 µg/kg, 50 µg/kg, 1.5 µg/kg and 1.5 µg/kg; liver, 3162 µg/kg, 310 µg/kg, 113 µg/kg and 1.5 µg/kg; kidney, 270 µg/kg, 90 µg/kg, 12.5 µg/kg and 12.5 µg/kg; fat + skin, 116 µg/kg at 6 h and 50 µg/kg at all subsequent time points.

A residue study to assess the depletion of erythromycin A and its metabolite (des-*N*-methyl-erythromycin A) in eggs of laying hens after 3 consecutive days of repeated administration was done using an oral administration of erythromycin thiocyanate as a 20% wettable powder via drinking-water at 20 mg/kg bw per day. All eggs produced were collected daily during the treatment and 10 days after the end of the treatment. Erythromycin A and des-*N*-methyl-erythromycin A were measured with the LC/MS/MS method. The total antimicrobial activity of residues was also measured. Taking into account the standard deviation, there are no significant

differences in residue concentration between days. Residues of erythromycin were at or above the LOD in only 25% of the eggs at day 1 and 12.5% of the eggs at day 2 following the end of the treatment period. The median concentration of residues of erythromycin in eggs for the first 3 days after removal of treatment was below the LOD. For days 4–9 after the end of the treatment, the median residues were 25 µg/kg; from day 10 to day 28, residues were not detectable.

In a residue depletion study for 7 days using a 20 mg/kg bw treatment in drinking-water, residues could be quantified only at the 1-day withdrawal time. At all subsequent sampling times, residues were below the LOQ in all samples and below the LOD at 6 days in all eggs.

A new residue study was reported with 34 turkeys treated by oral administration of erythromycin thiocyanate 20% oral powder via drinking-water at 20 mg/kg bw per day for 3 days. The LC/MS/MS method for residues in turkey was adapted from the residue method validated for chickens. At 3 days after the end of the treatment, residues were found in only two liver samples and one sample each of muscle, kidney and fat + skin. Four days post-treatment, all concentrations of residues were below the LOQ or the LOD. Median residue concentrations at days 1, 2, 3 and 4, respectively, were as follows: muscle, 50 µg/kg, 50 µg/kg, 1.5 µg/kg and 1.5 µg/kg; liver, 50 µg/kg, 50 µg/kg, 1.5 µg/kg and 1.5 µg/kg; kidney, 50 µg/kg at 1 day and 1.5 µg/ kg for all subsequent time points; fat + skin, 50 µg/kg, 25.8 µg/kg, 2.5 µg/kg and 2.5 µg/kg.

Analytical methods
The microbiological activity of erythromycin residues may be measured in chickens, laying hens and turkeys by a microbiological plate assay, using agar medium and *Micrococcus luteus* ATCC9341 as the test organism. The LOD was 50 µg/kg for residues in chicken tissues and eggs, and the LOQ was 100 µg/kg for residues in chicken and turkey tissues and eggs. The concentrations of erythromycin A may also be determined using an LC/MS/MS method. For residues in chicken tissues, the LODs for erythromycin A are 3 µg/kg for muscle, 30 µg/kg for liver, 25 µg/kg for kidney and 5 µg/kg for skin + fat; the LOQ is 100 µg/kg for residues in all tissues and 50 µg/kg for residues in eggs. For residues in turkey tissues, the LODs for erythromycin A are 3 µg/kg for muscle, liver and kidney and 4 µg/kg for skin + fat; the LOQ is 100 µg/kg for residues in all tissues. For residues of des-*N*-methyl-erythromycin A in chicken tissues, the LODs are 5 µg/kg for muscle, 48 µg/kg for liver, 25 µg/kg for kidney and 24 µg/kg for skin +

fat; the LOQ for residues is 100 μg/kg in all tissues. For eggs, the LOQ for erythromycin A is 50 μg/kg, and the LOD is 1 μg/kg. The reported LOQs are the lowest calibrated concentrations tested. It should be possible to attain lower LOQs for tissues and eggs based on the LOD. Typically, an LOQ is 3–5 times the LOD, not 20–30 times, as is the case for most of the tissues with the LC/MS/MS method. The method was validated according to internationally recognized requirements.

Maximum residue limits
Factors considered in recommending MRLs are:

— The marker residue is erythromycin A. Metabolites exhibited little or no microbiological activity in vivo.
— Only MRLs in poultry tissues and eggs were considered.
— For purposes of calculating the EDI, one half the limit of the LOD and one half the limit of the LOQ were used where residue values were below the LOD or LOQ, respectively, of the validated method.
— Residue depletion studies provided a very limited number of residue concentrations above the LOQ for all studies in chickens, turkeys and eggs.
— A validated LC/MS/MS method is available with LOQs of 100 μg/kg for all tissues and 50 μg/kg for eggs. The LOQ of the microbiological method is 100 μg/kg for all tissues and eggs.
— The ADI established based on a microbiological end-point for erythromycin was 0–0.7 μg/kg bw, equivalent to an upper limit of 42 μg for a 60-kg person.

Considering the factors noted above, the Committee recommended MRLs of 100 μg/kg, measured as erythromycin A, for muscle, liver, kidney and fat/skin and 50 μg/kg for eggs at the LOQ of the LC/MS/MS method.

For estimating the TMDI, the MRLs were used and applied with the model diet noted below. The TMDI calculation is 55 μg/day, equivalent to 130% of the upper bound of the ADI.

Applying the estimated median residue concentrations of 50 μg/kg at 1 day post-treatment in turkeys, the time point with the highest median residues, and 25 μg/kg for eggs at 4 days post-treatment, the time point with the highest median residues, the EDI for erythromycin residues in 300 g of muscle, 100 g of liver, 50 g each of kidney and fat + skin and 100 g of eggs is 27.5 μg/day, equivalent to approximately 65% of the upper bound of the ADI.

A residue monograph was prepared.

3.3 Flumequine

Flumequine is a fluoroquinolone compound with antimicrobial activity against Gram-negative organisms. It is used in the treatment of enteric infections in food animals and in the treatment of bacterial infections in farmed fish. Flumequine also has limited use in humans for the treatment of urinary tract infections.

Flumequine has previously been evaluated by the Committee (Annex 1, references *110*, *125*, *146*, *163* and *167*). The sixty-second meeting (Annex 1, reference *167*) established an ADI of 0–30 µg/kg bw, based on the overall NOEL for hepatotoxicity of 25 mg/kg bw per day observed in a 13-week study in mice and a safety factor of 1000, and recommended MRLs for cattle, pigs, sheep, chickens and trout. In addition, a temporary MRL of 500 µg/kg for muscle of the black tiger shrimp (*Penaeus monodon*) was recommended, and the following information was requested by 2006:

1. A detailed description of a regulatory method, including its performance characteristics and validation data; and
2. Information on the approved dose for the treatment of black tiger shrimp and the results of residue studies conducted at the recommended dose.

Analytical methods

The present meeting noted that the Government of Thailand had submitted an analytical method based on HPLC analysis with fluorescence detection. The method, which had been validated in a single laboratory study, has an LOQ of 5 µg/kg and is suitable for regulatory use. An additional method reported in the published literature for the determination of flumequine in shrimps was evaluated. The method also determines flumequine using liquid chromatography with fluorescence detection and has an LOQ of 5 µg/kg. The results of a single laboratory validation study were acceptable, and the method is suitable for regulatory purposes.

Information on the approved dose for treatment of black tiger shrimp and the results of the residue studies conducted at the recommended dose

No new information had been provided to answer the second request, which related to the approved dose for the treatment of black tiger shrimp and the results of residue studies conducted at the recommended dose. The Committee noted the limited availability of approved veterinary drugs for the treatment of aquaculture species. This concern has been addressed by the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL,

which convened in Bangkok in August 2004 (*1*). In the absence of data, the present Committee could not apply any procedures to extrapolate between species; however, the Committee thought it was appropriate to assign the temporary MRL for flumequine to the muscle of all freshwater and marine species of shrimp based on known species similarity.

Conclusion

An ADI of 0–30 µg/kg bw has been established for flumequine. The Committee recommended maintaining the temporary MRL of 500 µg/kg for muscle of the black tiger shrimp (*Penaeus monodon*) and assigning the temporary MRL to all freshwater and marine species of shrimp. The TMDI of flumequine residues accounts for 92% of the upper bound of the ADI. No suitable data were available to calculate an EDI value. A suitable validated analytical method is available for regulatory purposes. The Committee confirmed its previous request for information on the approved dose for the treatment of diseases in shrimps and the results of residue depletion studies conducted at the recommended dose. This information is requested by the end of 2008.

3.4 **Melengestrol acetate**

Melengestrol acetate (MGA) is an orally active progestogen. It is used to improve feed conversion efficiency, promote growth and suppress estrus in female beef cattle fed for slaughter. The range of approved doses is 0.25–0.50 mg/heifer daily. MGA is fed for the duration of the fattening/finishing period, usually 90–150 days. The Committee previously evaluated MGA at its fifty-fourth, fifty-eighth and sixty-second meetings (Annex 1, references *146*, *157* and *167*). An ADI of 0–0.03 µg/kg bw was established, and MRLs of 8 µg/kg for fat and 5 µg/kg for liver in cattle were recommended. The Secretariat was made aware of an error in the calculation of the MRLs and placed MGA on the agenda for the purpose of recalculating the MRLs.

The sixty-second meeting reported the structure and progestogenic activity of the major metabolites of MGA. The progestogenic activity of the metabolites relative to MGA ranged from 0.09% to 12%. The percentage of the total progestogenic activity attributable to MGA and to its metabolites was estimated from the percentage of the total radioactive residue attributable to MGA and metabolites of MGA and by assuming that the relative progestogenic potency of all metabolites of MGA was 12% (Table 4).

The present Committee reconsidered data submitted to the fifty-fourth meeting. It recommended that the MRLs should be derived

Table 4
Bioactivity weighting factors for MGA-related residues in tissues

Tissue	% of total radioactive residue attributable to:		% of total progestogenic activity attributable to[b]:		
	MGA[a]	MGA metabolites	MGA	MGA metabolites	Sum of progestogenic residues
Fat	86	14	$\dfrac{86 \times 1 \times 100}{86 + (0.12 \times 14)}$	$\dfrac{14 \times 0.12 \times 100}{86 + (0.12 \times 14)}$	$98.08 + 1.92 = 100$
Liver	30	70	$\dfrac{30 \times 1 \times 100}{30 + (0.12 \times 70)}$	$\dfrac{70 \times 0.12 \times 100}{30 + (0.12 \times 70)}$	$78.12 + 21.88 = 100$
Muscle	40	60	$\dfrac{40 \times 1 \times 100}{40 + (0.12 \times 60)}$	$\dfrac{60 \times 0.12 \times 100}{40 + (0.12 \times 60)}$	$84.75 + 15.25 = 100$
Kidney	34	66	$\dfrac{34 \times 1 \times 100}{34 + (0.12 \times 66)}$	$\dfrac{66 \times 0.12 \times 100}{34 + (0.12 \times 66)}$	$81.11 + 18.89 = 100$

[a] % median [^{3}H]MGA data from 54th JECFA.
[b] The percentage of progestogenic activity of MGA-related residues is calculated by applying a weighting factor of 1 to MGA and of 0.12 (corresponding to the relative potency of the metabolite of MGA with the highest progestogenic activity) to all MGA metabolites in fat, liver, muscle and kidney.

from the 99th percentile of MGA concentrations in perirenal fat, collected either by biopsy or upon slaughter within short intervals after cessation of treatment, of feedlot animals treated with the highest recommended dose. The fifty-fourth meeting of the Committee had evaluated eight studies involving approximately 380 animals treated at different doses to obtain this information. The majority of the animals in these studies had been treated with the highest recommended dose. For those animals receiving the highest recommended dose, a 99th percentile of 18.5 µg/kg was derived for MGA residues in fat. For animals in all studies, dose–response interpolation was used to derive the 99th percentile of 16.3 µg/kg for MGA residues in fat.

The median concentration of the marker residue, obtained from a study with three animals treated with tritium-labelled MGA, was 6.6 µg/kg for fat, 3.6 µg/kg for liver, 0.2 µg/kg for muscle and 0.6 µg/kg for kidney (Table 5). The proportion of the concentration found in the other three tissues, compared with the perirenal fat that contains the highest residues, was derived from this study and was approximately 1:1.8 for liver, 1:11 for kidney and 1:33 for muscle.

Maximum residue limits

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

— Marker residue in edible tissue is parent MGA.
— Fat, which contains the highest residue, is the most suitable tissue

Table 5
Estimated daily intake of MGA residues

Tissue	Median MGA[a] (µg/kg)	Fraction of total progestogenic activity attributable to marker residue	Total residue (µg/kg)	Diet (kg)	Intake of residues (µg)
Fat	6.6	0.98	6.8	0.05	0.34
Liver	3.6	0.78	4.4	0.1	0.46
Muscle	0.2	0.85	0.24	0.3	0.07
Kidney	0.6	0.81	0.75	0.05	0.04
EDI					0.9

[a] Marker residue MGA.

for the purpose of monitoring residues of MGA. The highest of the 99th percentiles of MGA residues was 18.5 µg/kg.

— The median concentrations of the marker residue were 6.6 µg/kg for fat, 3.6 µg/kg for liver, 0.2 µg/kg for muscle and 0.6 µg/kg for kidney.

— The conversion of the marker to total residue was based on the fraction of total progestogenic activity attributable to marker residue; the fraction was 0.98 for fat, 0.78 for liver, 0.85 for muscle and 0.81 for kidney.

— A suitable validated analytical routine method was available for monitoring.

— The established ADI is 0–0.03 µg/kg bw, equivalent to 0–1.8 µg for a 60-kg person.

On the basis of the above considerations, the Committee recommended MRLs in cattle of 18 µg/kg in fat, 10 µg/kg in liver, 1 µg/kg in muscle and 2 µg/kg in kidney, expressed as MGA. The TMDI corresponding to these MRLs is 2.7 µg, or 150% of the upper bound of the ADI. The EDI (see Table 5) is 0.9 µg, or 50% of the upper bound of the ADI.

An addendum to the residue monograph on MGA was prepared.

3.5 **Ractopamine hydrochloride**

The sixty-second meeting of the Committee (Annex 1, reference *167*) established an ADI of 0–1 µg/kg bw, equivalent to 0–60 µg for a 60-kg person. The following MRLs were recommended for edible tissues of pigs and cattle, expressed as ractopamine base: muscle, 10 µg/kg; liver, 40 µg/kg; kidney, 90 µg/kg; fat, 10 µg/kg. The recommended MRLs resulted in a calculated TMDI of 50 µg, or 84% of the upper bound of the ADI, based on a daily intake of 300 g of muscle, 100 g of liver and 50 g each of kidney and fat.

The 15th Meeting of the CCRVDF noted that the ADI recommended by JECFA had been rounded down, resulting in a lower ADI than had been established by some member governments. The CCRVDF therefore requested the "recalculation of MRLs and TMDI taking decision of the 15th CCRVDF into account regarding rounding practices" for ractopamine hydrochloride. The sponsor submitted a proposal for adjustment of the MRL for liver.

Residue data

No new data were presented. The present Committee affirmed the practices used in establishment of an ADI. The data provided to the sixty-second Committee for cattle were further reviewed to determine if there were scientific grounds for recommending MRLs for cattle different from those that had been recommended by the sixty-second Committee for pigs and cattle. These MRLs were based primarily on the extensive data available for residue depletion in pigs. For recommending MRLs in cattle, the information from residue studies in pigs was taken into account together with the cattle residue data. The Committee noted that the data provided to the sixty-second Committee for cattle included two studies with administration of unlabelled ractopamine in feed, one at the maximum recommended dose of 30 mg/kg in feed and one at 33% in excess of the maximum recommended dose. Studies were also provided with radiolabelled ractopamine, including a study using administration via a capsule at a dose equivalent to 30 mg/kg in feed to establish marker-to-total residue relationships in liver and kidney at 12 h after last administration. In addition, a total residue depletion study with administration via capsule at a dose equivalent to 60 mg/kg in feed and total and marker residue depletion studies using administration via capsule at doses equivalent to 40 and 45 mg/kg in feed, respectively, were provided.

The Committee noted that there was an apparent effect on the distribution of ractopamine residues in cattle liver and kidneys, based on dose and mode of administration. Similarly, this affected the relationships of marker to total residue. The Committee confirmed its choice of factors for adjustment from marker to total residue based on the study conducted at the recommended dose rate with slaughter 12 h after the final administration. The Committee also noted that residues in cattle livers ranged from 30% to 85% of those found in kidneys in the various residue studies, but considered that the limited data from administration of unlabelled drug in feed demonstrated a relationship in the low or middle end of that range, or approximately 30%. The study conducted at a dose equivalent to 45 mg/kg in feed by rumen

insertion of a capsule containing the drug was the only study in which ractopamine parent residues were detected in muscle and fat (0.02 and 0.01 mg/kg, respectively). Residues of parent ractopamine were not detected in the other studies where tissues were tested. Total radiolabelled residues were 0.01–0.05 mg/kg in muscle and fat at 12 h post-administration and depleted rapidly to non-detectable levels within a few days, even with administration above the recommended dose. The recommendation of MRLs for muscle and fat of cattle based on the LOQ was therefore considered appropriate. The studies conducted at the recommended dose did not provide evidence that residues in liver and kidney of cattle would exceed the MRLs established for those tissues. The Committee therefore concluded that, based on the available data, the MRLs recommended by the sixty-second Committee for liver and kidney of pigs and cattle were appropriate.

Maximum residue limits

No change was made in the MRLs recommended by the sixty-second Committee. To reconsider the MRLs for cattle, the Committee requested a residue depletion study with unlabelled drug in feed at the maximum recommended dose. This study should provide sufficient additional data to determine if the relationship used in establishing the MRLs for cattle liver and kidney requires adjustment and provide median values for residues in these tissues.

The Committee also calculated an EDI using the median values from the residue data used in calculation of the MRLs for pigs at the sixty-second meeting of the Committee. The median residue concentrations calculated from the data were as follows: muscle, 5 µg/kg; liver, 8 µg/kg; kidney, 15 µg/kg; fat, 2.5 µg/kg. In addition, exact data-derived factors for the transformation of marker residue concentrations to total residue concentrations were used. The data-derived factors were: muscle, 1.0; liver, 6.3; kidney, 3.1; fat, 1.0. Based on the median values and the data-derived factors for pigs, the corresponding EDI is 9.0 µg, which represents 15% of the upper bound of the ADI.

An addendum to the residue monograph for ractopamine hydrochloride was prepared.

3.6 Trichlorfon (metrifonate)

Explanation

Trichlorfon (metrifonate) is an organophosphonate compound with insecticidal, acaricidal and anthelminthic properties. It was evaluated by the Committee at its fifty-fourth meeting (Annex 1, reference *146*; hereafter referred to as the 54th Committee), when it established an

ADI of 0–20 µg/kg bw on the basis of a LOEL of 0.2 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in humans treated orally and application of a safety factor of 10. The Committee re-evaluated the ADI at its sixtieth meeting (Annex 1, reference *163*; hereafter referred to as the 60th Committee), to reflect additional information received since its fifty-fourth meeting. It considered that an ADI of 0–2 µg/kg bw could be derived from this study by application of a 100-fold safety factor (10-fold for a human study and an additional 10-fold for use of a LOEL) to the LOEL of 0.2 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in humans treated orally. The 60th Committee did not request any additional toxicological data for further evaluation.

The present evaluation was undertaken at the request of CCRVDF to address specific concerns raised by the European Community at the 15th session of CCRVDF (*6*). The delegation of the European Community stated at the 15th session of CCRVDF that it could not accept the recommendations of the 60th Committee due to its concerns regarding the pharmacokinetics, teratogenicity, mutagenicity and neurotoxicity of trichlorfon. The present report addresses both new and previously submitted data since the evaluation by the 60th Committee that are specifically relevant to the concerns raised by the European Community at the 15th session of CCRVDF.

Biochemical data

The European Community submitted that differences in pharmacokinetics between laboratory animals and humans comprise orders of magnitude and that toxic effects in humans may be expected at trichlorfon doses that are several orders of magnitude lower than those inducing the corresponding effects in laboratory animals. The 60th Committee evaluated the pharmacokinetics of trichlorfon, comparing the results in healthy human volunteers and in patients with various levels of renal impairment. It was concluded that renal function had no significant effect on the pharmacokinetics of trichlorfon and that doses need not be adjusted for patients with renal impairment. The 60th Committee also concluded that concomitant administration of magnesium or aluminium hydroxide containing antacids had no effect on the pharmacokinetics of trichlorfon or dichlorvos, the major metabolite of trichlorfon, in a healthy volunteer population. Moreover, the 60th Committee also opined that the higher NOELs observed in studies in which trichlorfon was administered in feed rather than in tablets or by gavage may reflect differences in the bioavailability of the administered dosage form, rather than interspecies differences in pharmacokinetics per se.

The present Committee could find no basis to conclude that there was a marked interspecies difference in the pharmacokinetics of trichlorfon. The present Committee also noted that a number of publications in the open scientific literature relating to the pharmacokinetics of trichlorfon that were suggested by the European Community as not having been considered by the 60th Committee were all included in the deliberations of the 60th Committee and are included in the references cited in the evaluation of trichlorfon by that Committee (Annex 1, reference *164*). No additional relevant studies have been submitted since the evaluation by the 60th Committee. Hence, the present Committee confirmed the previous assessment of the pharmacokinetics of trichlorfon.

Toxicological data

Developmental toxicity

The present Committee re-evaluated the assessment of potential teratogenicity related to trichlorfon exposure. In its submission to the 15th session of CCRVDF, the European Community expressed the view that trichlorfon is fetotoxic and teratogenic in a number of laboratory animal species. The European Community noted that NOELs for developmental toxicity could be identified in the mouse, rat, hamster and rabbit, but that NOELs for severe teratogenic effects in guinea-pigs and pigs could not be identified. The 60th Committee evaluated a study of developmental toxicity in guinea-pigs that was designed to evaluate brain hypoplasia in offspring exposed in utero to trichlorfon given to dams either by stomach tube or by subcutaneous injection. In this study, trichlorfon was administered in oral doses ranging from 25 to 200 mg/kg bw per day to groups of guinea-pigs on days 40–44 of gestation. Clinical signs that typically follow ingestion of this substance were observed in dams given trichlorfon at 100, 150 or 200 mg/kg bw. A NOEL of 50 mg/kg bw for brain hypoplasia in offspring was identified. The present Committee noted that this NOEL was 25 000 times greater than the ADI for trichlorfon established by the 60th Committee. The present Committee also noted that the LOEL in pigs of about 50 mg/kg bw per day provides for a large margin of exposure relative to the ADI.

The present Committee also evaluated the results of a study in which pregnant female mice were treated intraperitoneally with a single trichlorfon dose of 0, 100 or 200 mg/kg bw during the early preimplantation period (*9*). This study was not carried out in accordance with international guidelines for reproductive toxicity or mutagenicity testing and was reported as a brief two-page summary of an original Japanese published report. Cholinesterase activity was not deter-

mined in the study. The authors concluded that while the number of embryos with micronuclei in both treatment groups was increased relative to controls, neither treatment group demonstrated any evidence of a reduction of implantation or adverse impact on normal development of embryos. The Committee concluded that while both doses used in this study produced increased micronuclei, these doses were 50 000 and 100 000 times greater, respectively, than the upper bound of the ADI of 2 µg/kg bw established for trichlorfon by the 60th Committee.

The 60th Committee reviewed developmental toxicity studies with trichlorfon conducted in four animal species. In these studies, teratogenic effects were seen only at very high doses that were maternally toxic.

The present Committee also evaluated the results of a developmental neurotoxicity study that had been conducted in compliance with United States Environmental Protection Agency (USEPA) and Health Canada guidelines (10). Groups of Wistar rats were administered trichlorfon at doses of 0, 150, 500 or 1750 mg/kg in feed, corresponding to 0, 13, 49 and 146 mg/kg bw per day during gestation and doses of 0, 33, 103 and 265 mg/kg bw per day during lactation. Mated females were treated from gestation day 0 through lactation day 21. On postnatal day 4, litters were culled to yield four females and four males per group. Erythrocyte, plasma and brain cholinesterase activities were determined in offspring on postnatal days 4 and 21 and in dams on lactation day 21. Subsets of surviving offspring were also assessed for neurobehavioural effects, preputial separation or vaginal patency, body weight, food consumption, automated measures of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance after weaning and a water maze task on postnatal day 60); an ophthalmological examination was also performed. Brains were collected for histopathology and morphometry from 10 rats per sex and per dose group on postnatal day 21 or at the study termination.

Trichlorfon had no effect on reproductive parameters, including fertility index and gestation length. Clinical signs of toxicity were not observed in dams at any dose level during gestation or lactation, but two females of the high-dose group were found moribund during lactation. Neither body weight nor food consumption was significantly affected at any dose level during gestation, although body weight was reduced by 7% during lactation in the high-dose group. Food consumption was also reduced at 49 and 103 mg/kg bw per day and above for the last 2 weeks of lactation.

No neurobehavioural effects in dams were observed. The cholinesterase activities in dams were determined on lactation day 21. Inhibition of plasma, erythrocyte and brain cholinesterase activities occurred at 49 and 103 mg/kg bw per day (43%, 66% and 48%, respectively) during gestation and at 146 and 265 mg/kg bw per day (55%, 71% and 72%, respectively) during lactation. The only effects at 13 and 33 mg/kg bw per day were slight, but statistically significant, inhibition of erythrocyte (26%) and brain (16%) cholinesterase activities. The Committee considered that the brain was the relevant target and that inhibition of cholinesterase activity of less than 20% was not toxicologically significant.

There were many deaths of offspring during lactation and a few shortly after weaning in the high-dose group. No clinical signs related to the compound were observed. At 49 and 103 mg/kg bw per day and above, reduced body weight and body weight gain as well as reduced food consumption were observed. At the high dose level, delays in preputial separation and vaginal patency were observed and were interpreted to be due to the marked decrease in body weight.

Neonatal cholinesterase activity was slightly inhibited at the high dose level at postnatal days 4 and 21 and at the middle dose level at postnatal day 21. In the 146 and 265 mg/kg bw per day dose group, about 25% inhibition of plasma cholinesterase activity and 28% inhibition of erythrocyte cholinesterase activity were found on postnatal day 4, whereas a slight (7–11%) inhibition of brain cholinesterase activity was determined on postnatal day 21. No neurobehavioural effects in offspring were observed. In the figure-eight maze, a higher activity was observed in the high dose group. The acoustic startle habituation was considered to be decreased in the middle and high dose groups on postnatal day 22, but not later. Startle latency and habituation were not affected. No significant effects were seen in passive avoidance, water maze, ophthalmology, gross pathology, brain weight or histopathology. The slight differences in brain weights and morphometry at postnatal day 21 were related to the lower body weight. NOELs of 13 and 33 mg/kg bw per day were identified for dams and offspring, respectively, on the basis of inhibition of brain cholinesterase activity.

Genotoxicity
The European Community stated that trichlorfon was associated with clear evidence of mutagenicity both in vivo and in vitro. The European Community further stated that there was no evidence of a NOEL for these effects and no new information suggesting that these data were not valid.

The 54th Committee considered extensive data related to the potential genotoxicity of trichlorfon. The only information on genotoxicity that was not reviewed by the 54th Committee, but was included in the evaluation of the 60th Committee, was the results of two tests for sister chromatid exchange, both reported in the same paper (*11*). In a study conducted in human lymphocytes in vitro, trichlorfon at a concentration of 10, 20, 30, 40, 50 or 60 μg/ml did not induce sister chromatid exchange; however, sister chromatid exchange was observed in bone marrow cells in mice that were given trichlorfon at a dose of 30, 60 or 120 mg/kg bw. The 60th Committee considered and confirmed the conclusions of the 54th Committee. The 60th Committee commented that trichlorfon has been tested in a large number of studies for genotoxicity covering a wide range of end-points, with considerable variation in the results for most end-points. Both positive and negative results were obtained in tests for bacterial mutations and for gene mutation in mammalian cells in vitro, but the results of studies of effects on chromosomes in mammalian cells in vitro were uniformly positive. Mostly negative results were found in assays for germ cell mutagenicity in vivo. The 54th Committee concluded that since the tests conducted in vivo produced mostly negative results when trichlorfon was administered orally, the weight of evidence suggests that trichlorfon is unlikely to represent a genotoxic risk. The present Committee confirmed this conclusion.

The 54th Committee noted that bioassays for carcinogenicity in rats and mice gave negative results, and the 60th Committee concluded that trichlorfon residues in foods of animal origin would not present a carcinogenic hazard to consumers. Dichlorvos, the major trichlorfon metabolite, causes compound-related tumours only of the forestomach of mice, and only at a dose of 20 mg/kg bw per day and above. Hence, tumours are a consequence of the unique exposure conditions in the rodent forestomach — conditions that could never arise in humans following dietary exposure. The present Committee noted that the 20 mg/kg bw per day dose is 10 000 greater than the upper bound of the ADI for trichlorfon established by the 60th Committee.

The 60th Committee evaluated a study involving pregnant women that suggested that exposure to trichlorfon residues of uncertain concentration in fish may have contributed to the appearance of trisomy 21 in their offspring as a result of germ cell aneugenicity. The 60th Committee noted that the mothers of all the affected infants reported having eaten fish during pregnancy and that several of the ponds around the village used for fish farming had been treated with trichlorfon, either measured or estimated to be in the range of 0.15–100 mg/kg in the types of fish consumed. The 60th Committee

observed that the results of this study provided only limited evidence of a possible association between birth defects in humans and oral exposure to trichlorfon residues in food. Particularly noteworthy was the fact that the published report did not include information on the frequency of intake of fish potentially contaminated with trichlorfon or confirmation of whether exposure had occurred at all. The 60th Committee concluded that this was the only report of possible reproductive effects associated with oral exposure to trichlorfon, despite its widespread use as an anthelminthic, and that this study would not significantly affect its risk assessment of trichlorfon.

The 60th Committee evaluated the effects of trichlorfon on fertilization, spindle morphology and chromosomal segregation in mouse oocytes exposed in vitro to a concentration of $50\,\mu g/ml$. The Committee noted that effects on spindles leading to aneuploidy have thresholds; assuming 100% bioavailability, systemic exposure leading to an intake in the order of the upper bound of the ADI would be orders of magnitude lower than the dose used in this study. The 60th Committee therefore concluded that exposure to trichlorfon at the ADI would pose a negligible risk to human oocytes. The 60th Committee also reviewed results from a study in which aneuploidy induction was investigated in sperm cells collected from groups of male mice 22 days after a single intraperitoneal injection of trichlorfon at a dose of 200, 300 or $400\,mg/kg\,bw$. While a significant, dose-related increase in the percentage of sperm cells with an extra chromosome was observed at all doses, the Committee noted that these effects resulted from intraperitoneal injection of doses that were at least 5 orders of magnitude higher than the current ADI for trichlorfon. Similarly, the 60th Committee reviewed a study in which chromosomal effects were studied in the lymphocytes of 31 humans who had attempted suicide by the ingestion of unknown doses of trichlorfon. While there was an increase in the rate of chromatid- and chromosome-type aberrations, the 60th Committee concluded that the intake that resulted in these effects was far in excess of any exposure that might be expected from the consumption of a dose at the ADI. The present Committee noted that three published reports of the induction of aneuploidy, which were identified by the delegation of the European Community and not included in the 60th Committee assessment, did not alter the conclusions of the present Committee regarding the aneugenicity of trichlorfon.

Acute neurotoxicity
The present Committee evaluated results of an acute neurotoxicity study performed according to USEPA/Federal Insecticide, Fungicide,

and Rodenticide Act (FIFRA) guidelines (*12*). In this study, groups of 18 rats per sex per dose group were administered technical-grade trichlorfon at doses of 0, 10, 50 or 200 mg/kg bw by gavage. Observations and measurements included clinical signs, mortality, automated measurements of activity in a figure-eight maze, neurobehaviour, cholinesterase activity, brain weight, gross necropsy and microscopic examination of skeletal muscle, peripheral nerves, eyes and central nervous system tissues. Severe toxicity and mortality were observed in both sexes at a dose of 200 mg/kg bw. Compound-related decreases in motor and locomotor activity were observed in the figure-eight maze in high-dose males and females and in females in the 50 mg/kg bw group. Complete recovery was observed after 7 days. No gross lesions or effects on brain weight, histopathology or neuropathology were seen in the surviving animals of either sex. Significant depression of brain, plasma and erythrocyte cholinesterase activities was observed in male animals that survived the 200 mg/kg bw dose and in both sexes at the 50 mg/kg bw dose. The Committee identified a NOEL of 10 mg/kg bw for this study based on depression of cholinesterase activity in brain and erythrocytes.

The present Committee considered recent additional acute neurotoxicity studies in rats (*13*, *14*) designed to further investigate the sensitivity of pups in comparison with adults. These studies, carried out in a complex and non-conventional protocol, added little new information, in that they investigated the same end-points as had already been investigated in several other acute neurotoxicity studies (cholinesterase activity inhibition) and provided information similar to that already available in the developmental neurotoxicity study described above in this report. Moreover, the benchmark doses for a 10% response (BMD_{10}) determined in these studies were in the range of 10 mg/kg bw and were therefore 2 orders of magnitude greater than the dose from which the 60th Committee determined its ADI; hence, they would not result in any amendment to the evaluation of the 60th Committee. These studies also do not directly address the issues of concern identified by the European Community and are therefore not described further in this report.

Delayed neurotoxicity
The European Community expressed concerns regarding the potential delayed neurotoxicity of trichlorfon in hens and primates. While the European Community noted that delayed neurotoxicity in these species was observed only at toxic doses, the European Community submitted that it regarded delayed neurotoxicity as a non-threshold effect. The 60th Committee evaluated the maximal effects

of trichlorfon on soluble neuropathy target esterase. While peak inhibition of neuropathy target esterase activity was observed to range from 15% to 44% 6 h after dosing, there were no signs of delayed neuropathy in the four hens during the 28-day observation period. The 54th Committee was aware of a study in which a monkey was given a single oral dose of 250 mg/kg bw. The monkey was reported to have impaired nerve conduction 4 weeks after treatment, as well as histological evidence of demyelination of nerves and axonal degeneration. Noting that only a single dose was utilized in this study and that this was a toxic dose 125 000 times greater than the upper bound of the current ADI for trichlorfon, the present Committee considered the evidence from a single monkey to have only limited relevance in the evaluation of potential risks to consumers. The present Committee also noted that there was no evidence for neuropathy in monkeys treated for 10 years with a dose equivalent to 2500 times the upper bound of the ADI, suggesting that if a very high dose does have an effect in the monkey, there is a clear threshold for this effect.

The present Committee also evaluated results from a study, not included in the evaluation by the 60th Committee, in which inhibition of the activities of plasma and brain acetylcholinesterase and neuropathy target esterase was measured in three different strains of chicken after treatment with a single dose of tri-*ortho*-cresyl phosphate (Babcock chicken: 800 mg/kg bw; Hy-line w36 and Isabrown chicken: 1600 mg/kg bw) or a single dose of trichlorfon (Babcock or Isabrown chicken: 80 mg/kg bw) (*15*). In all cases, tri-*ortho*-cresyl phosphate treatment resulted in 80% inhibition of brain acetylcholinesterase activity and more than 70% inhibition of neuropathy target esterase activity, and Isabrown chickens showed classical signs of organophosphate-induced delayed neuropathy, including complete paralysis. In contrast, treatment with trichlorfon resulted in inhibition of brain neuropathy target esterase activity of less than 20%, and signs of organophosphate-induced delayed neuropathy were not evident. The present Committee concluded that significant differences regarding the induction of delayed neuropathy exist between chicken strains, noting that trichlorfon did not induce delayed neuropathy in the most sensitive strain in which brain acetylcholinesterase activity was inhibited by 80%. The potential of trichlorfon to induce delayed neuropathy has been evaluated in a large number of studies that were reviewed by the 54th and 60th Committees, both of which concluded that trichlorfon does not cause delayed neuropathy in hens. The study evaluated by the present Committee further supports this conclusion.

Comments

The European Community expressed concern regarding the approach that had been utilized for the derivation of an ADI. The European Community submitted that it is inappropriate to base an ADI on a LOEL from clinical data derived from an aged subset of the human population. The 60th Committee carefully reconsidered the basis for the ADI derived by the 54th Committee, which was 0–20 µg/kg bw, based on a NOEL of 0.2 mg/kg bw per day in a study with volunteers with Alzheimer disease and the application of a safety factor of 10. The 60th Committee concluded that the dose identified by the 54th Committee was effective in maintaining the steady-state inhibition of erythrocyte cholinesterase activity and was therefore more appropriately considered a LOEL, a reinterpretation that was also suggested by the European Community in its submission to the 15th session of CCRVDF. The 60th Committee considered that an ADI of 0–2 µg/kg bw could be derived from this study by application of an additional safety factor of 10 to the LOEL of 0.2 mg/kg bw per day. The present Committee concluded that deriving ADIs from studies in diseased human populations, where such data are available, likely represents a worst-case scenario for establishing ADIs. Available data indicate that the pharmacokinetics of trichlorfon were not appreciably different between the study population and healthy subjects.

Evaluation

The present Committee confirmed the ADI of 0–2 µg/kg bw for trichlorfon previously established by the 60th Committee. The Committee could find no basis for revising the ADI.

No addendum to the toxicological monograph was prepared.

3.7 Triclabendazole

Explanation

Triclabendazole is a benzimidazole anthelminthic used for the control of liver fluke, *Fasciola hepatica* and *Fasciola gigantica*, in cattle, sheep and goats. It is related by both chemical structure and pharmacological activity to other benzimidazole compounds, such as fenbendazole and thiabendazole.

Triclabendazole was first evaluated by the Committee at its fortieth meeting (Annex 1, reference *104*), which established an ADI for triclabendazole of 0–30 µg/kg bw based on the lowest NOEL of 0.27 mg/kg bw per day observed in the long-term study in mice and using a safety factor of 100. The following MRLs, expressed as

triclabendazole equivalents, were recommended for cattle tissues: muscle, 200 µg/kg; liver, 300 µg/kg; kidney, 300 µg/kg; and fat, 200 µg/kg. MRLs for sheep tissue were as follows: muscle, 100 µg/kg; liver, 100 µg/kg; kidney, 100 µg/kg; and fat, 100 µg/kg.

The Committee at its fortieth meeting concluded that more accurate estimates of total residues in edible tissues and of the ratio of total residue concentrations to marker residue concentrations would be required before the MRLs for triclabendazole in sheep could be reconsidered. Since the recommended MRLs result in intake estimates based on a TMDI calculation at 28 days post-treatment that approaches the ADI, the fortieth Committee concluded that bioavailability studies on the bound residue of triclabendazole may be needed to adjust the marker to total residue conversion factors for sheep tissues.

The sponsor submitted new studies on metabolism, bioavailability, residue depletion and analytical methods for consideration by the present Committee.

Data on pharmacokinetics and metabolism

Results of the new studies of triclabendazole metabolism in food-producing animals (cattle and sheep) and laboratory animals (rats and dogs) were consistent with the metabolic pattern reviewed by the fortieth meeting of the Committee. Biotransformation and excretion of triclabendazole were very rapid and qualitatively similar in all species studied (cattle, sheep, rats and dogs). The majority of an oral dose of triclabendazole was eliminated in faeces (>80%), with minimal urinary excretion. The major triclabendazole metabolites in cattle faeces, which are sulfone, sulfoxide and hydroxylated metabolites, are the same as those found in rats. Residue concentrations in cattle and sheep tissues 28 days after dosing were highest in liver, followed by muscle and kidney, while residues in fat tissue were negligible.

Three new GLP studies of bioavailability of triclabendazole residues were conducted in rats. In the first study, bioavailability was investigated by routes of administration that included i) intravenous, ii) orally by gavage, iii) addition to powdered diet or iv) dietary dose of [^{14}C]triclabendazole-derived residues from cattle tissues. Measurements of the area under the blood concentration–time curve showed that bioavailability of triclabendazole was approximately 70% when given by gavage or included in powdered diet, but only 6.4% and 9.8% from dietary intake of lyophilized cattle muscle and liver containing incurred triclabendazole residues, respectively. In the two other stud-

ies, bioavailability was defined as the sum of the radioactivity in urine, bile, tissues and carcass of rats following administration. In the second study, in which tissues from cattle and sheep containing incurred residues were lyophilized and fed to bile duct–cannulated male rats, the bioavailability was 14%, 9% and 4% for cattle kidney, liver and muscle, respectively, for incurred triclabendazole residues. The corresponding bioavailability values from incurred residues in sheep tissues were 7%, 8% and 5%. In the third study, bile duct–cannulated male rats were fed freeze-dried tissues from cattle containing incurred residues. Bioavailability was found to be 3.3%, 20% and 18% for incurred triclabendazole residues in cattle kidney, liver and muscle, respectively. These studies demonstrated that the bioavailability of the incurred triclabendazole residues from lyophilized cattle and sheep tissues fed to rats was ≤20% for all tissues tested.

Residue data

Three residue depletion studies in cattle considered by the fortieth Committee demonstrated that at 28 days following a single oral dose of 12 mg/kg bw, mean triclabendazole residue concentrations were 0.12 mg/kg in liver, 0.07 mg/kg in kidney, 0.11 mg/kg in muscle and 0.05 mg/kg in fat, representing, respectively, 50%, 66%, 84% and >100% of the total residues in these tissues.

Two new studies using [^{14}C]triclabendazole administered orally at 12 mg/kg bw provided additional information on the relationship between the marker and the total residues in cattle. In the first study, two ruminating calves were slaughtered 28 days after administration of a single oral dose (12 mg/kg bw) of [^{14}C]triclabendazole. Residues measured after conversion to the marker residue, keto-triclabendazole, corrected for analytical recovery, accounted for 24% of the total residue in liver, 27% of the total residue in kidney and 32% of the total residue in muscle at 28 days post-administration. Subsequently, residues were measured in tissues from a single calf killed 28 days after receiving a single oral dose (12 mg/kg bw) of [^{14}C]triclabendazole. Residues measured as keto-triclabendazole accounted for the following percentages of the total residue in each tissue: liver, 26.5%; kidney, 29.4%; and muscle, 34.9%. The new studies, in which both total and marker residues were measured in the same tissues, yield different conversion factors for marker to total residue than those calculated by the fortieth Committee, which compared results from separate studies with labelled and unlabelled triclabendazole.

The fortieth Committee also calculated that marker residue accounted for 24%, 23%, 33% and 51% of the total residue in liver,

kidney, muscle and fat, respectively, from sheep, based on a comparison of studies conducted with equivalent doses of ^{14}C-labelled and unlabelled triclabendazole. These studies demonstrated that highest residues in sheep are found in liver and that residues deplete more slowly in muscle, so that residues in muscle are higher than those found in kidney or fat at 21 days after treatment and at longer periods post-treatment. In a new study in which muscle and liver tissues were collected from two sheep slaughtered 28 days after administration of a single oral dose (10 mg/kg bw) of [^{14}C]triclabendazole, residues measured as keto-triclabendazole, corrected for analytical recovery, accounted for 39% of the total residues in muscle and 24% of the residues in liver. These results are consistent with those found for incurred residues in tissues from cattle and with the relationships calculated for liver and muscle of sheep by the fortieth Committee. The relationship for marker to total residues in sheep kidney calculated by the fortieth Committee is consistent with the relationship seen in cattle kidneys in the new studies, suggesting that the same factors may be used for adjustment of marker to total residue for muscle, liver and kidney from both cattle and sheep.

Based on the results from the new GLP studies, factors for conversion of recovery-corrected residues measured as keto-triclabendazole to total residues are 4.3 for liver, 4.2 for kidney and 2.7 for muscle. No conversion factor was considered necessary for fat, given the limited distribution and rapid depletion of residues in that tissue.

The new GLP depletion studies in cattle and sheep gave results consistent with the studies reviewed by the fortieth Committee. Unlike the earlier studies, however, the new GLP study in cattle was conducted under conditions of repeat treatment, 28 days subsequent to the initial treatment, and used a dose of 18 mg/kg bw, 1.5 times the recommended dose. Highest residues measured as keto-triclabendazole were found in liver at all time points (14, 28, 42 and 56 days), but residues in muscle were similar to those found in liver at 56 days after the second treatment. Residues were below the LOQ of 0.05 mg/kg in kidney at 56 days and were detectable in fat only at 14 days post-treatment.

The three residue depletion studies in sheep that were considered by the fortieth Committee, in which sheep were orally dosed with triclabendazole (single dose of 10 mg/kg bw or 15 mg/kg bw), demonstrated that residues depleted to below the LOD in fat (0.03 mg/kg) within 14–21 days after administration, but remained detectable in liver, kidney and muscle for 28 days. Highest residues were reported in liver at 21 days post-treatment, but results were similar in liver,

kidney and muscle at 28 days post-treatment. In the new GLP study considered by the present Committee, sheep received a single oral dose of 10–13 mg/kg bw. Residues were detectable in muscle from 50% of the animals at 56 days post-treatment. Residues were not detected in liver or kidney samples at 42 days post-treatment or in fat samples at 14 days post-treatment.

The results of the studies demonstrate that muscle is the preferred target tissue for monitoring purposes.

Analytical methods

A suitable validated liquid chromatographic method with ultraviolet detection was available for regulatory use to detect and quantify residues as keto-triclabendazole. Tissues are initially digested with hot alkali solution to release bound residues; after further cleanup, the extracted residues are oxidized to keto-triclabendazole, the marker residue (identified as 5-chloro-6-(2′,3′-dichlorophenoxy)-benzimidazole-2-one in the report of the fortieth Committee). The demonstrated method LOQ was 0.05 mg/kg for the various tissues. Analytical recoveries ranged from 79% to 102%.

Maximum residue limits

In recommending MRLs, the Committee took into account the following factors:

— The marker residue is keto-triclabendazole.
— The appropriate target tissue is muscle.
— A validated analytical method is available for analysis of triclabendazole residues in edible tissues of cattle, sheep and goats.
— The bioavailability of incurred triclabendazole residues in tissues fed to rats did not exceed 20%.
— The factors calculated for conversion of marker residue to total residue in cattle tissues by the fortieth meeting of the Committee, based on the then-available studies, have been demonstrated to be incorrect by data from the more recent GLP studies, which provided both total residue and marker residue concentrations from the same tissues.
— The factors to convert from marker residue to total residue, derived from mean results of the new GLP studies in cattle and sheep, are 4.3 for liver, 4.2 for kidney and 2.7 for muscle, calculated at 28 days after a single administration at the recommended dose. When multiple doses are used, these factors are sufficiently conservative to apply to the various time points where residues

are at the MRLs. The same factors are applicable to goats, based on the available information. A factor was required for kidney, as detectable residues were present below the LOQ at the time point used for calculation of the MRLs for liver and muscle, requiring their inclusion in the intake estimate. Owing to the rapid depletion of residues in fat, a factor to convert marker residues to total residues was not required, as the intake estimate based on one half the LOQ of the analytical method provides a conservative estimate.

— MRLs for liver and muscle of cattle, sheep and goats were based on the mean residue concentrations plus 3 standard deviations from the new GLP studies in cattle and sheep. The time point for which the MRLs for cattle were calculated is 56 days following the second treatment at 1.5 times the recommended dose. Residues deplete more rapidly in cattle following a single treatment at the recommended dose. These MRLs will be achieved in sheep at a time point intermediate between 28 and 42 days after a single treatment at the recommended dose.

— MRLs for kidney and fat were based on twice the LOQ.

— An ADI of 0–3 µg/kg bw was established by the fortieth meeting of the Committee, equivalent to 0–180 µg for a 60-kg person.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, sheep and goats, expressed as the marker residue, keto-triclabendazole: muscle, 150 µg/kg; liver, 200 µg/kg; kidney, 100 µg/kg; and fat, 100 µg/kg.

The MRLs recommended above would result in a TMDI of 230 µg/day, based on a daily food intake of 300 g of muscle, 100 g of liver and 50 g each of kidney and fat, or 128% of the upper bound of the ADI.

The Committee calculated an EDI using the median values from the residue data used to derive the MRLs. The median residue concentrations calculated from the data were: muscle, 88.5 µg/kg; liver, 99.5 µg/kg; kidney, 25 µg/kg; fat, 25 µg/kg. In the absence of quantifiable residues in kidney and fat at the 56-day time point in the cattle study from which the MRLs were derived, a value equal to one half the LOQ was assigned as a conservative estimate of intake from kidney and fat. The median residue concentrations above would result in an EDI of 121 µg, based on a daily food intake of 300 g of muscle, 100 g of liver and 50 g each of kidney and fat, or 67.2% of the upper bound of the ADI.

A residue monograph was prepared.

3.8 Tylosin

Tylosin is a macrolide antibiotic that is active against certain Gram-positive and Gram-negative bacteria, especially different species of *Mycoplasma*. Tylosin is extensively and exclusively used in veterinary medicine and is primarily directed against the chronic respiratory disease complex in chickens and infectious sinusitis in turkeys. It is also effective for bovine respiratory and swine dysentery diseases. In some countries, tylosin is also used as a growth promoter for poultry, pigs and cattle.

Tylosin was previously evaluated at the twelfth meeting of the Committee in 1968 (Annex 1, reference *17*) and thirty-eighth meeting of the Committee in 1991 (Annex 1, references *97*, *98* and *99*). The thirty-eighth meeting of the Committee was not able to set MRLs for tylosin because no ADI was established. Before reviewing the compound again, the Committee requested the following:

1. Detailed information from the reported reproduction and teratogenicity studies.
2. Studies designed to explain the positive result that was obtained in the mouse lymphoma genotoxicity assay in the absence of metabolic activation.
3. Studies designed to test the hypothesis that the increased incidence of pituitary adenomas in male rats after the administration of tylosin was a consequence of the greater rate of body weight gain in these rats.
4. Studies from which a NOEL for microbiological effects in humans can be determined.
5. Additional studies of residues in eggs using more sensitive analytical methods.
6. Additional information on microbiologically active metabolites of tylosin.
7. Studies on the contribution of the major metabolites of tylosin to the total residues in edible tissues of cattle and pigs.

The substance was included on the agenda and call for data of the sixty-sixth meeting of the Committee, as a result of a request from the 15th meeting of CCRVDF (*6*). None of the requested information on tylosin was provided to the Committee, and therefore the Committee had recourse only to information available in the open literature.

A review of available toxicological data published in the scientific literature resulted in the conclusion that publicly available data are insufficient to perform a toxicological evaluation of tylosin. In consequence, tylosin could not be evaluated toxicologically at this meeting.

A comprehensive review of the available information in the open literature concerning analytical methods, pharmacokinetics and tissue residues of tylosin in different animal species was carried out, as a large number of scientific articles on tylosin have been published since the thirty-eighth meeting of the Committee. The available information was not suitable to perform a residue evaluation of the compound. Therefore, the document was not reviewed by the Committee according to JECFA procedure. However, the document will, after editing, be made available upon request from the FAO Joint Secretariat.

The Committee used this compound as an example to investigate if evaluations are possible based on published data in the absence of data submissions from sponsors. In the case of tylosin, the Committee concluded that the accessible data were not sufficient for an evaluation.

4. Future work

Several suggestions on future work of the Committee derived from the Committee's discussion of general considerations. The Committee recommended that:

— The JECFA Secretariat convene a working group to develop a general decision-tree for the evaluation of veterinary drugs.
— A paper be prepared that considers compounds for which ARfD considerations are necessary and proposes a procedure for establishment of such values, taking previous JECFA guidance and the ARfD guidance developed by JMPR into account.
— FAO develop a guidance manual for submission and evaluation of data.
— The Secretariat convene a working group to address the issue of harmonizing all commodity and tissue definitions.
— A paper be prepared to consider if a separate approach for honey is warranted and in such case develop a draft recommendation for consideration by the Committee.

5. Recommendations

1. Recommendations relating to specific veterinary drugs, including ADIs and proposed MRLs, are given in section 3 and Annex 2.
2. The Committee recommended that median residue levels be used to derive an estimated daily intake (EDI) to better reflect esti-

mates of chronic (lifetime) exposure. This will replace the theoretical maximum daily intake (TMDI) used previously.

3. The Committee recommended that the statistical approach for the evaluation of marker residue depletion data be used in future when estimating MRLs whenever it was appropriate and that the experts drafting the working documents should explain to the Committee the reasons when not using it.

4. The Committee recommended that CCRVDF take an active role in establishing and supporting lists of veterinary drugs of public health concern and should emphasize the need for Codex members and commercial entities to fulfil their responsibility in submitting relevant data in a timely manner.

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).
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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.

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

Colistin (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–7 μg/kg bw, on the basis of the MIC_{50} of 1 μg/g of colistin base for *E. coli.*

Residue definition: Sum of colistin A and colistin B

Recommended maximum residue limits (MRLs)

Species	Fat[a] (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)	Milk (µg/kg)	Eggs (µg/kg)
Cattle	150	200	150	150	50	
Sheep	150	200	150	150	50	
Goat	150	200	150	150		
Pig	150	200	150	150		
Chicken	150	200	150	150		300
Turkey	150	200	150	150		
Rabbit	150	200	150	150		

[a] The MRL includes skin + fat where appropriate.

Erythromycin (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–0.7 μg/kg bw, on the basis of the MIC_{50} of 0.1 μg/g for *Bifidobacterium.*

Residue definition: Erythromycin A

Recommended maximum residue limits (MRLs)

Species	Fat[a] (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)	Eggs (µg/kg)
Chicken	100	100	100	100	50
Turkey	100	100	100	100	

[a] The MRL includes skin + fat where appropriate.

Flumequine (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–30 μg/kg bw at its sixty-second meeting (WHO TRS No. 925, 2004).

Residue definition: Flumequine

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)
Black tiger shrimp (*Penaeus monodon*)	500[a]
Shrimp	500[a,b]

[a] The MRL is temporary. The following information is requested by the end of 2008: (1) Information on the approved dose for the treatment of diseases in shrimp and the results of residue depletion studies conducted at the recommended dose.
[b] The assignment of the temporary MRL applies to all freshwater and marine shrimp.

Melengestrol acetate (production aid)

Acceptable daily intake: The Committee established an ADI of 0–0.03 µg/kg bw at its fifty-fourth meeting (WHO TRS No. 900, 2001).

Residue definition: Melengestrol acetate

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	18	2	10	1

Ractopamine hydrochloride (production aid)

Acceptable daily intake: The Committee established an ADI of 0–1 µg/kg bw at its sixty-second meeting (WHO TRS No. 925, 2004).

Residue definition: Ractopamine

The Committee maintained the MRLs recommended at its sixty-second meeting (WHO TRS No. 925, 2004):

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	10	90	40	10
Pig	10	90	40	10

To reconsider the MRLs for cattle, the Committee requested a residue depletion study with unlabelled drug in feed at the maximum recommended dose. This study should provide sufficient additional data to determine if the relationship used in establishing the MRLs for cattle liver and kidney requires adjustment and provide median values for residues in these tissues.

Trichlorfon (Metrifonate) (insecticide)

Acceptable daily intake: The Committee confirmed the ADI of 0–2 µg/kg bw established at its sixtieth meeting (WHO TRS No. 918, 2003).

Residues: The MRLs that were recommended by the sixtieth meeting of the Committee were not reconsidered and were maintained.

Triclabendazole (anthelminthic)

Acceptable daily intake: The Committee established an ADI of 0–30 µg/kg bw at its fortieth meeting (WHO TRS No. 832, 1993).

Residue definition: Keto-triclabendazole

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	100	100	200	150
Sheep	100	100	200	150
Goat	100	100	200	150